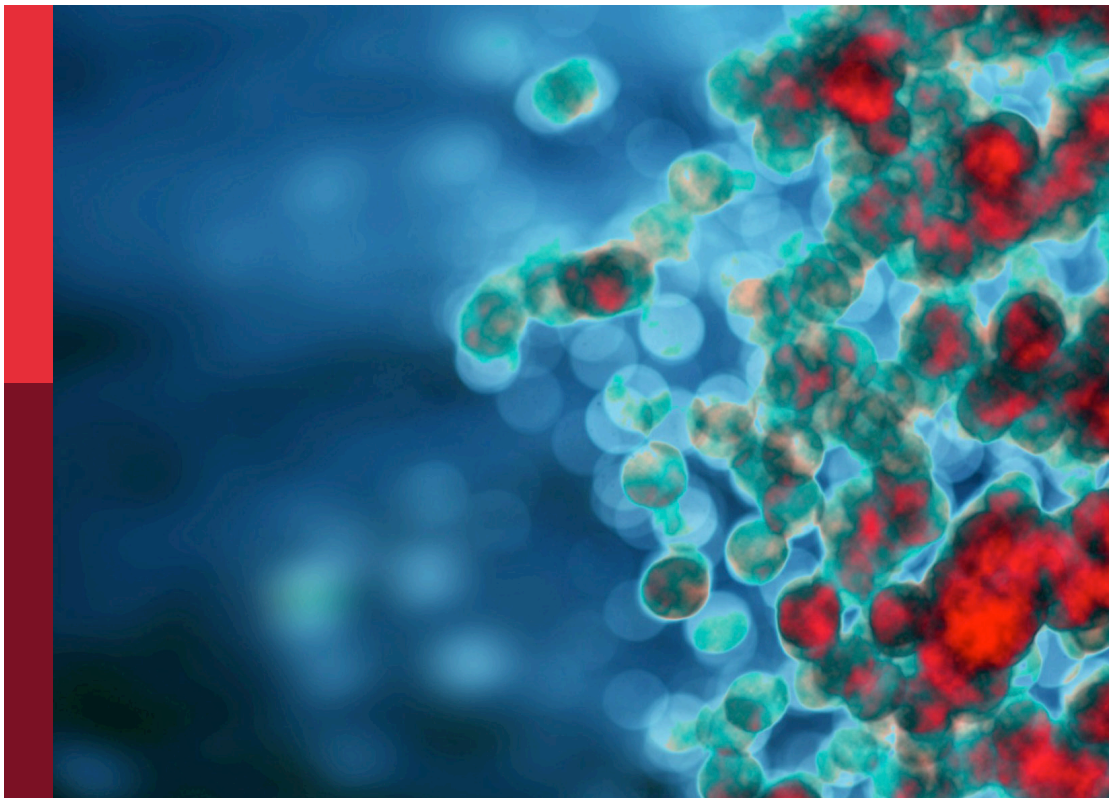
frontiers | Research Topics

Gluten-related disorders: pathogenesis, diagnosis, and treatment

Edited by
Simone Baldi, Amedeo Amedei, Isabel Comino, Ángela Ruiz-Carnicer and Carolina Sousa

Published in
Frontiers in Immunology
Frontiers in Nutrition



ISSN 1664-8714
ISBN 978-2-8325-6174-4
DOI 10.3389/978-2-8325-6174-4

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Gluten-related disorders: pathogenesis, diagnosis, and treatment

Topic editors

Simone Baldi — Department of Experimental and Clinical Medicine, Italy
Amedeo Amedei — University of Florence, Italy
Isabel Comino — Sevilla University, Spain
Ángela Ruiz-Carnicer — Sevilla University, Spain
Carolina Sousa — Sevilla University, Spain

Citation

Baldi, S., Amedei, A., Comino, I., Ruiz-Carnicer, Á., Sousa, C., eds. (2025). *Gluten-related disorders: pathogenesis, diagnosis, and treatment*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-6174-4

Table of contents

frontiers | Frontiers in Nutrition

TYPE Editorial
PUBLISHED 13 March 2025
DOI 10.3389/fnut.2025.1585381

OPEN ACCESS

EDITED AND REVIEWED BY
Willem Van Eden,
Utrecht University, Netherlands

*CORRESPONDENCE
Amedeo Amedei
✉ amedeo.amedei@unifi.it

RECEIVED 28 February 2025
ACCEPTED 03 March 2025
PUBLISHED 13 March 2025

CITATION
Baldi S, Ruiz-Carnicer Á, Comino I, Sousa C and Amedei A (2025) Editorial: Gluten-related disorders: pathogenesis, diagnosis, and treatment. *Front. Nutr.* 12:1585381. doi: 10.3389/fnut.2025.1585381

Editorial: Gluten-related disorders: pathogenesis, diagnosis, and treatment

Simone Baldi[1], Ángela Ruiz-Carnicer[2], Isabel Comino[2], Carolina Sousa[2] and Amedeo Amedei[1]*

[1]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy, [2]Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain

KEYWORDS

gluten related disorders, celiac disease, gluten free diet (GFD), celiac disease—diagnosis, diet therapy, epidemiology

Editorial on the Research Topic
Gluten-related disorders: pathogenesis, diagnosis, and treatment

Celiac disease (CD) is a systemic autoimmune disorder triggered by gluten peptides, which provoke a T-cell-mediated immune response, leading to villous atrophy and chronic inflammation in the small intestine (1, 2). It affects ~1.4% of the global population (3), with a highly variable clinical presentation that ranges from classic gastrointestinal symptoms to extraintestinal manifestations or even asymptomatic cases. The only effective treatment is lifelong adherence to a gluten-free diet (GFD) (4). Recent studies suggest that the loss of gluten tolerance may occur either upon initial gluten exposure or later in life, implying that additional environmental factors contribute to CD development. Challenges in diagnosis and poor adherence to the GFD can significantly impact health and quality of life, highlighting the need for early and accurate diagnosis to improve long-term disease management (5).

This Research Topic brings together nine comprehensive articles exploring the pathogenesis, diagnosis, and management of gluten-related disorders, aiming to deepen our understanding of these crucial aspects.

CD diagnosis relies on clinical presentation, serological markers, and histopathological findings from duodenal biopsies. However, discrepancies between serological and histological results, along with biopsy inaccessibility, often lead to inconclusive outcomes. Since most CD patients carry the HLA-DQ2 and/or DQ8 alleles, which trigger an autoimmune response, HLA testing serves as a valuable complementary tool, particularly in atypical or controversial cases where histological findings are inconclusive (Ruera et al.). Atypical CD presentations may include constitutional symptoms, dermatological and mucosal issues, bone abnormalities, neuropsychiatric symptoms, renal and reproductive complications, disturbances in biological markers, and associations with other autoimmune conditions. Recognizing this diverse clinical spectrum is crucial for optimal patient management, especially in ensuring proper growth and development in children (Lupu et al.). Given the heterogeneous presentation of CD, a multifaceted diagnostic approach is essential. Early and accurate diagnosis improves treatment effectiveness and quality of life, particularly for patients at risk of poor adherence due to unclear or delayed diagnoses.

Emerging research has identified gluten immunogenic peptides (GIP) in human breast milk, with significant interindividual variations in secretion. This discovery is particularly relevant, as early gluten exposure through breast milk may influence the immune system's development in genetically predisposed infants, potentially affecting CD risk. Understanding this mechanism is crucial for identifying early-life environmental factors that contribute to CD onset, providing insights for more effective prevention strategies (Ruiz-Carnicer et al.).

Additionally, GIP detection in urine offers a non-invasive method for assessing gluten exposure and gastrointestinal function following dietary challenges or fasting. This approach is particularly useful for identifying subclinical CD cases and monitoring treatment effectiveness. By tracking GIP levels in urine, it becomes possible to detect gluten ingestion in patients who may not exhibit obvious symptoms, enabling earlier diagnosis and better management. These findings, together with GIP detection in breast milk, emphasize the role of early gluten exposure in CD development and its potential for precise disease monitoring (Rodríguez-Ramírez et al.).

Regarding CD treatment, a strict lifelong GFD remains the cornerstone; however, adherence is often challenging due to high costs, dietary restrictions and social implications. Recent phase 2 clinical trials exploring non-dietary pharmacological therapies for CD underscore the need for more extensive research, as no proposed treatments have yet shown significant efficacy in preventing gluten-induced histological damage (Scalvini et al.). Hence, larger and more rigorous clinical trials are necessary to assess their long-term safety and effectiveness. A promising therapeutic approach involves exopeptidases, enzymes that break down gluten-derived peptides in the gastrointestinal tract, potentially reducing the immune response and alleviating CD symptoms. Although still under investigation, this strategy holds potential for improving current treatment options and enhancing quality of life for CD patients (Mourabit et al.).

The development of gluten-free food products, such as cookies, is being evaluated for their physicochemical and sensory properties ensuring that CD patients have access to safe, nutritious and palatable food options (Silva-Paz et al.). The availability of high-quality gluten-free foods plays a crucial role in promoting dietary adherence and improving patient's overall experience with GFD.

Beyond dietary restrictions, the psychosocial burden of CD is becoming increasingly apparent. The need to evaluate health-related quality of life (HRQoL) in CD patients is critical, as dietary limitations often contribute to social isolation, anxiety and psychological distress. These challenges are further exacerbated by diagnostic difficulties and the constant need to avoid gluten exposure.

Recent research comparing general and disease-specific HRQoL questionnaires have provided deeper insights into the psychosocial impact of CD (Falcomer et al.). A study conducted in Portugal highlighted the necessity of a comprehensive care model integrating both medical and psychosocial support. Such a holistic approach is essential for enhancing patient outcomes and ensuring that individuals with CD can maintain a fulfilling quality of life despite the challenges of their condition (Chaves et al.).

In conclusion, significant progress has been made in understanding the pathogenesis, diagnosis, and treatment of gluten-related disorders. While the GFD remains the primary treatment, emerging pharmacological therapies, such as exopeptidase-based approaches, offer potential alternatives for individuals struggling with gluten avoidance. Additionally, increasing awareness of the psychosocial impact of CD underscores the importance of a multidisciplinary care approach that addresses both physical and emotional wellbeing. Looking ahead, effective management of gluten-related disorders will require precise diagnostic tools, innovative therapies and a patient-centered, integrative approach to optimize clinical care and enhance quality of life.

Author contributions

SB: Writing – original draft. ÁR-C: Writing – original draft. IC: Writing – original draft. CS: Writing – original draft. AA: Writing – original draft.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Al-Toma A, Volta U, Auricchio R, Castillejo G, Sanders DS, Cellier C, et al. European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. *United Eur Gastroenterol J.* (2019) 7:583–613. doi: 10.1177/2050640619844125

2. Besser HA, Khosla C. Celiac disease: mechanisms and emerging therapeutics. *Trends Pharmacol Sci.* (2023) 44:949–62. doi: 10.1016/j.tips.2023.09.006

3. Mendia I, Segura V, Ruiz-Carnicer Á, Coto L, Negrete M, Long JCD, et al. Rapid anti-tTG-IgA screening test for early diagnosis of celiac disease in pediatric populations. *Nutrients.* (2023) 15:4926. doi: 10.3390/nu15234926

4. Cabanillas, B. Gluten-related disorders: celiac disease, wheat allergy, and nonceliac gluten sensitivity. *Crit Rev Food Sci Nutr.* (2020) 60:2606–21. doi: 10.1080/10408398.2019.1651689

5. Caio G, Volta U, Sapone A, Leffler DA, De Giorgio R, Catassi C, et al. Celiac disease: a comprehensive current review. *BMC Med.* (2019) 17:142. doi: 10.1186/s12916-019-1380-z

frontiers | Frontiers in Nutrition

TYPE Brief Research Report
PUBLISHED 05 January 2024
DOI 10.3389/fnut.2023.1304117

OPEN ACCESS

EDITED BY
Simone Baldi,
Department of Experimental and Clinical Medicine, Italy

REVIEWED BY
Richard Atinpoore Atuna,
University for Development Studies, Ghana
Iriani Rodrigues Maldonade,
Brazilian Agricultural Research Corporation (EMBRAPA), Brazil
Babatunde Olawoye,
First Technical University, Nigeria

*CORRESPONDENCE
Reynaldo J. Silva-Paz
✉ rsilva@upeu.edu.pe

RECEIVED 28 September 2023
ACCEPTED 29 November 2023
PUBLISHED 05 January 2024

CITATION
Silva-Paz RJ, Silva-Lizárraga RR, Jamanca-Gonzales NC and Eccoña-Sota A (2024) Evaluation of the physicochemical and sensory characteristics of gluten-free cookies. *Front. Nutr.* 10:1304117. doi: 10.3389/fnut.2023.1304117

Evaluation of the physicochemical and sensory characteristics of gluten-free cookies

Reynaldo J. Silva-Paz[1]*, Roxana R. Silva-Lizárraga[1], Nicodemo C. Jamanca-Gonzales[2] and Amparo Eccoña-Sota[1]

[1]EP Ingeniería de Industrias Alimentarias, Facultad de Ingeniería y Arquitectura, Universidad Peruana Unión, Lima, Peru, [2]Departamento de Ingeniería – Escuela de Ingeniería en Industrias Alimentarias, Universidad Nacional de Barranca, Lima, Peru

The increasing prevalence of celiac disease and gluten intolerance has led to an increased demand for gluten-free food products in Peru. The research objective was to develop gluten-free cookies from substitute flours, evaluating their physicochemical and sensory parameters. Eight formulations were developed using 100% broad bean, chickpea, pea, kiwicha, quinoa, lentil, corn, and bean flour. One hundred consumers participated in this evaluation (59% women and 41% men). A completely randomized design (CRD) and a randomized complete block design (RCBD) were used for physicochemical analysis and acceptability, respectively. To describe the sensory characteristics of the cookies, Cochran's Q-test and correspondence analysis (CA) were performed. From the results obtained, the lentil cookie presented the highest amount of protein and fiber but lower fat and carbohydrate contents compared to the other samples. In terms of color, the corn cookie was the lightest, with greater luminosity (L*), less redness (a*), and greater yellowness (b*). Regarding the sensory analysis, the CATA questions allowed us to form six groups, and the samples with the greatest acceptability were the corn and chickpea cookies, which were rated as "I like them." Lentil flour crackers are a nutritionally adequate option, and corn flour crackers are highly sensorially acceptable, suggesting commercial opportunities for softer and more flavorful gluten-free products. However, it is crucial to continue researching and developing innovative products to meet changing market demands and offer healthier and more attractive options to consumers.

KEYWORDS

substitute flours, gluten free, consumers, sensory evaluation, cookies

1 Introduction

Several disorders associated with gluten ingestion are now recognized, including celiac disease (CD), intolerance, and gluten allergy (1, 2). In particular, celiac disease is an autoimmune disorder that is triggered in individuals susceptible to the ingestion of gluten from wheat, barley, rye, and others (3). Various studies have identified that approximately 3% of the world population suffers from celiac disease, and until two decades ago it was considered rare, but it has now become widespread worldwide (4).

Gluten causes inflammation of the small intestine, atrophy of the villi of the mucosa, and poor absorption of nutrients, and the only treatment for this disease is to have a gluten-free diet for life (5). For this reason, there was a need to develop gluten-free products to meet the demand of celiac consumers who are intolerant or allergic to gluten (6, 7). Food companies that manufacture and supply gluten-free foods and beverages must work with various tools for the development and innovation of foods and decision-making that allow understanding of the success of the product in the market (8).

In recent years, a notable increase in the demand for gluten-free products has been observed, driven by the medical needs of some people suffering from celiac disease and by the conscious choice of consumers who opt for a healthy diet (1, 9). Among these gluten-free products that can be made are cookies, which can be consumed at any time of the day due to their practicality, long shelf life, availability in a presentable format, and having an affordable cost for the consumer (10). The shelf life of the biscuit is prolonged due to its low moisture content, which hinders microbial growth, allowing the product to retain its optimal characteristics for longer, provided it is properly stored (11). The inclusion of flours derived from legumes and pseudocereals in the preparation of cakes, breads, pastas, and cookies represents a technological option that allows us to offer products of nutritional quality, favoring acceptability by the consumer (7, 12, 13). Kaur et al. (6) indicated that the quality of cookies prepared with buckwheat flour and incorporating xanthan gum showed similar sensory profiles to those made with refined wheat flour. On the other hand, Silva et al. (11) mentioned that cookies made with rice and bean flour were rated as innovative products, achieving good acceptability and being recommended for celiac consumers. Similarly, Hamdani et al. (14) reported that cookies prepared with rice and chickpea flour and added gum karaya showed great acceptability by consumers and had a favorable impact on their characterization.

Legumes and pseudocereals have emerged as promising alternative ingredients in the formulation of gluten-free products due to their nutritional profiles, technological functionalities, and unique sensory properties. These ingredients not only offer a rich source of protein, fiber, and other essential nutrients but also present specific characteristics that improve the texture, flavor, and appearance of the final products (15–17). From a technological point of view, they have specific properties, such as the ability to form viscous gels, structural stability, the absence of gluten, a lack of elasticity, and gas retention, which are crucial aspects of achieving a pleasant texture in baked products. When considering consumer acceptance, it is essential to not only address dietary restrictions but also offer alternatives that do not compromise sensory pleasure and the dining experience (18, 19). The benefits of legumes and pseudocereals with the growth of the celiac population have motivated us to propose their exploration as a substitute option for conventional flours in the production of gluten-free products.

A tool that allows understanding of the development and consumption in the market of a product is sensory evaluation, a discipline that encompasses a series of tests and methods to evaluate the perception of food and beverages by the consumer (20, 21). The general acceptability of a product can be evaluated through a hedonic scale that consists of a list of responses with different degrees of satisfaction, where the consumer indicates the response based on their sensory perception (22). There are other methods that allow the description and understanding of the level of enjoyment of the product, such as the check all that applies (CATA) method, where consumers select the attributes that identify the samples evaluated and indicate their acceptability (23). Therefore, the objective of this research was to develop gluten-free cookies from substitute flours and evaluate their physicochemical and sensory parameters.

2 Materials and methods

2.1 Ingredients

Different substitute flours of lentils, peas, common beans, white corn, chickpeas, broad beans, kiwicha, and white quinoa, obtained in the central market of Lima, were used, in addition to other ingredients such as butter, brown sugar, sodium bicarbonate, egg, and water, where the percentages used for the different formulations are shown.

The formulation of the cookies consisted of G1 (broad bean, 53.80, 8.00, 16.10, 0.50, 10.80, and 10.80% of flour, butter, brown sugar, baking soda sodium, egg, and water, respectively), G2 (chickpea, 56.80, 8.50, 17.0, 0.60, 11.40, and 5.70%), G3 (pea, 56.80, 8.50, 17.00, 0.60, 11.40, and 5.70%), G4 (kiwicha, 55.20, 8.30, 16.60, 0.60, 11.00, and 8.30%), G5 (quinoa, 55.20, 8.30, 16.60, 0.60, 11.00, and 8.30%), G6 (lentil, 55.20, 8.30, 16.60, 0.60, 11.00, and 8.30%), G7 (corn, 53.80, 8.00, 16.10, 0.50, 10.80, and 10.80), and G8 (common bean, 56.80, 8.50, 17.00, 0.60, 11.40, and 5.70%,). All formulations were designed according to the proposal by the American Association for Clinical Chemistry (AACC) (24).

2.2 Gluten-free cookie-making process

To prepare the cookies, we followed the procedure outlined by Huatuco et al. (25), with some adjustments. Initially, we measured all the ingredients based on the formulations detailed in Section 2.1. For the creaming process, we combined butter and brown sugar in a KitchenAid mixer (Model: Artisan, United States) at 6 rpm for 5 min until a uniform mixture was achieved. Eggs were then added and beaten at 4 rpm for 5 min to form a smooth, creamy emulsion. Subsequently, the substitute flour was manually mixed with sodium bicarbonate in a stainless-steel container. The cream mixture was then added to this new blend and mixed for an additional 5 min. Gradually, water was incorporated until a homogeneous dough was attained. The dough was rolled to a thickness of 5 mm and molded to a diameter of 40 mm. The resulting products were baked in a rotary oven (Brand: Nova, Model: Max 600, Peru) at 140°C for 10 min, followed by cooling at room temperature for 20 min. They were then packaged in polyethylene bags and hermetically sealed. Finally, the cookies were stored at room temperature for subsequent physical, chemical, and sensory analyses.

2.3 Physicochemical analysis

2.3.1 Nutritional composition analysis

Moisture and ash contents were determined according to the AOAC analysis method (26), while crude fat, crude protein, and crude fiber were determined by the AACC analysis method (27), and the amount of carbohydrates was calculated by difference (28).

2.3.2 Color analysis

The color was determined using a portable colorimeter (3nh brand, model Nh310, China). The cookies were placed in direct contact to measure the color of the surface. This analysis was performed in triplicate using the CIEL*a*b* system. The parameters to be measured were L* (brightness) [(0) black / (100) white], a* [(+) red / (−) green], and b* [(+) yellow / (−) blue] (11, 22). In addition, the whiteness index (WI) was determined, $WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$ (29) and the browning index (BI) (30), BI = (100*((x-0.31)/0.17), where x is equal, x = (a + 1.75 L*)/(5.645 L* + a* - 3.012b*) using the parameters L*, a*, and b*. The color parameters of the control sample to quantify the ΔE were L* = 76.26 ± 0.24, a* = 4.42 ± 0.19, and b* = 40.45 ± 0.28. To determine the $\Delta E = \sqrt{(L^* - Lo^*)^2 + (a^* - ao^*)^2 + (b^* - bo^*)^2}$, where Lo *, a*, and b* correspond to the values of the control sample and L*, a*, and b* are the data of each sample.

2.4 Sensory analysis

2.4.1 Consumers

The evaluators were recruited from the Faculty of Engineering and Architecture of the Universidad Peruana Unión, with a total of 100 consumers, of whom 59% were women and 41% were men (aged 24 ± 6 years). Their participation was voluntary, and the study was carried out with informed consent approved by the Ethics Committee of the Faculty of Engineering and Architecture of the Universidad Peruana Unión (N° 2022-CEFIA-0006).

2.4.2 Check all that apply (CATA)

All participants received eight cookies randomly, coded with three digits, and delivered monadically. Previously, consumers gave informed consent to participate in the sensory tests and were provided with general instructions on the CATA methodology. Then, the evaluation sheet was provided with 13 sensory attributes, of which 7 described the texture (sticky, soft, crunchy, brittle, hard, greasy, and porous), 3 described the taste (bitter, sweet, and strange taste), 2 described the appearance (light color and dark color), and 1 described the aroma (strange smell). These terms were selected from previous studies (31–33). For the sensory test, participants were asked to select the terms they considered appropriate to describe the samples (34, 35). The samples were evaluated in a single session of approximately 30 min in the laboratory of the Food Science Research Center (CICAL) of the Universidad Peruana Unión. Participants were instructed to drink table water between each sample to cleanse the palate.

2.4.3 Overall liking

For the liking test, a 9-point hedonic scale was used, with the highest score being I like it very much (9 points) and the lowest being I dislike it a lot (1 point). Consumers were instructed to rate the samples according to their perception, as well as to drink table water between each sample to minimize the carryover effect and influence the evaluation from the first to the last sample (11, 36). Figure 1 describes the stages of the research process, from the use of flour to the preparation of cookies to the physicochemical and sensory tests carried out.

2.5 Statistical analysis

For the data of the physicochemical analysis, a completely randomized design (CRD) was applied (25, 37), and for the general acceptability, a completely randomized block design (RCBD) (38, 39) was carried out to meet the assumptions of normality and homogeneity of variance. When evaluating the analysis of variance (ANOVA) and identifying if there were significant differences, and in the case of finding significance ($p < 0.05$), the Tukey test was carried out at a confidence level of 95% (11, 40) using the statistical software R.

Correspondence analysis (CA) was applied to the data obtained by the check all that apply (CATA) method to obtain the association map between the samples and the sensory attributes (41, 42). In addition to Cochran's Q-test to identify significant differences ($p < 0.05$) between the samples and the constancy of use of each attribute (43, 44), the statistical program XLSTAT 2023 was used for these analyses.

3 Results

3.1 Physico-chemical analysis

3.1.1 Nutritional composition analysis

The proximal composition of the cookies is shown in Table 1, expressed in percentages (%). G7 and G5 presented a higher moisture value and did not show significant differences ($p > 0.05$) between both samples. Similarly, it was observed that G4 acquired a smaller amount of ashes compared to G8, which was statistically superior ($p < 0.05$). Regarding the fat content, G2 registered a higher content than the rest of the cookies. According to the amount of fiber, the highest values were G3, G6, and G8; these samples did not show significant differences ($p > 0.05$). A reduced content of protein was presented by G7; on the contrary, G6 obtained the highest amount, showing significant differences ($p < 0.05$). On the other hand, G7 presented a higher carbohydrate content, although similar to G1, G3, G4, and G5 ($p > 0.05$).

3.1.2 Color analysis

Table 2 shows the color parameters expressed in dimensionless units; this is an important property for the acceptance of cookies by consumers. Sample G7 was the sample with the highest luminosity, while G6 obtained the lowest value of coloration (dark). The samples presented significant differences ($p < 0.05$). In the a* parameter, the samples were between the values of 15.4 ± 0.32 and 30.4 ± 0.05, showing little redness. While in the b* parameter, the values ranged between 45 and 56, indicating greater yellowing. The whiteness index of sample G7 is significantly different from the other samples, and G8 showed lower whiteness. For the browning index, an inverse behavior was observed for these samples. Samples G7 and G2 showed lower values of delta E; that is, they did not show large differences with the control sample.

3.2 Sensory analysis

3.2.1 Consumers

In this study, 100 consumers participated, of whom 59% were women and 41% were men (aged 24 ± 6 years). Of all the women, 54%

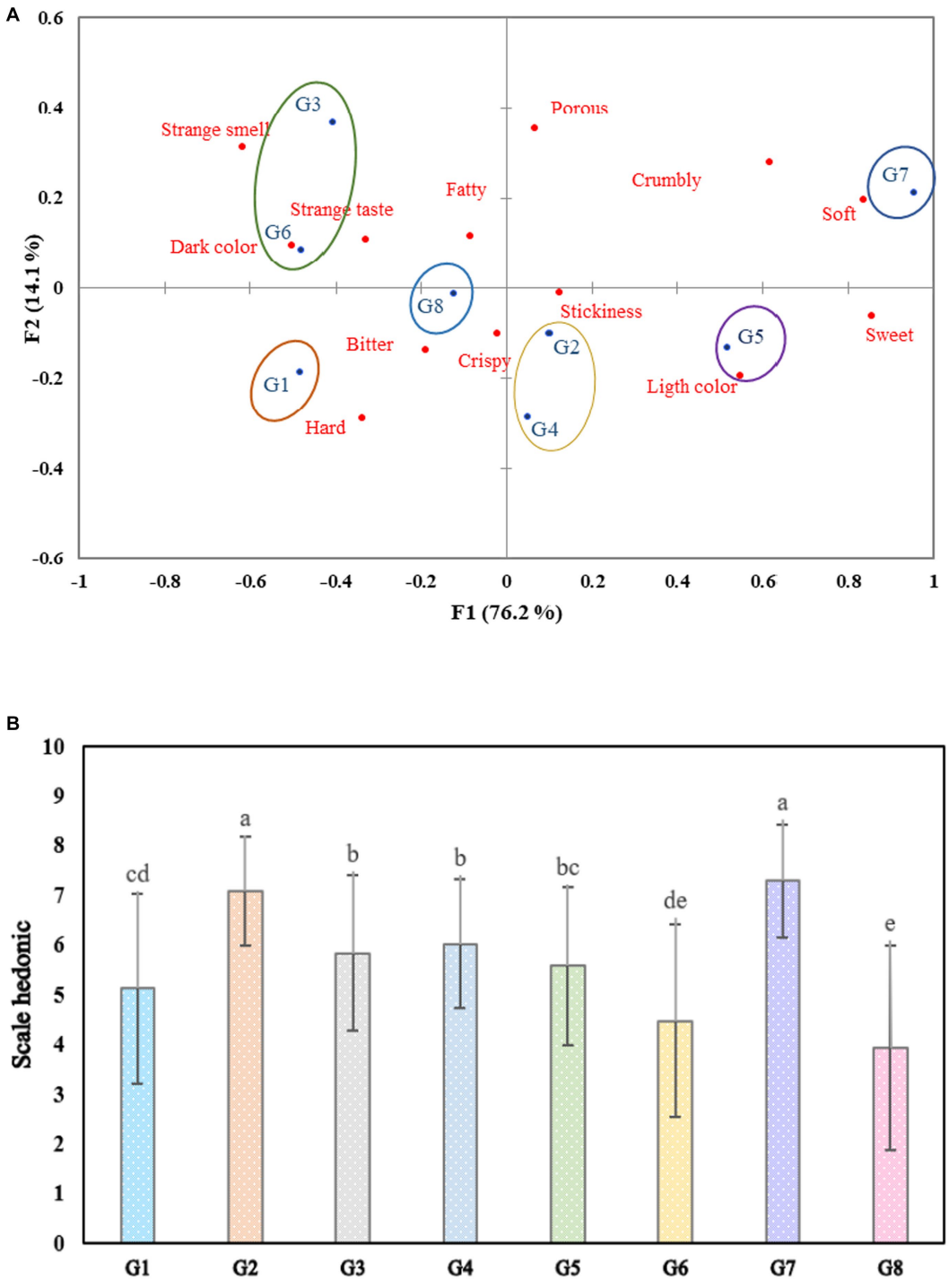


FIGURE 1
Map resulting from perception correspondence analysis (CA) using the CATA method **(A)** and cookie acceptability using a 9-point hedonic scale **(B)**. (Cookie, G1: broad bean, G2: chickpea, G3: pea, G4: kiwicha, G5: quinoa, G6: lentil, G7: corn, and G8: common bean).

were from the coast, 27% were from the mountains, and 19% were from the jungle. In addition, 63% preferred the chocolate flavor, 13% preferred vanilla, 14% preferred Andean grains, and 10% preferred salty; additionally, it was found that 67% eat cookies frequently, 19% sometimes, and 14% eat very few cookies. Of the participants, 79% were from the "Coast" region, 15% from the "Sierra," and 6% from the "Selva." Similarly, it was observed that 62% prefer the chocolate flavor, 20% prefer vanilla, 9% prefer Andean grains, and 9% prefer salty. On the other hand, it was found that 65% eat cookies frequently, 23% sometimes, and 12% eat very few cookies.

3.2.2 Check all that apply (CATA) method and overall acceptability

Cochran's Q-test shown in Table 3 shows that consumers found significant differences ($p < 0.05$) in 11 of the 13 attributes evaluated in the CATA questions, so the use of the CATA method allows the

TABLE 1 Proximate composition of biscuits on dry base (g/100 g).

Sample	Moisture content (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	Carbohydrates (%)
G1: Broad bean	5.17 ± 0.07^{c}	2.31 ± 0.19^{abc}	12.10 ± 0.13^{b}	10.60 ± 0.33^{d}	2.75 ± 0.30^{a}	67.10 ± 0.49^{ab}
G2: Chickpea	5.20 ± 0.08^{c}	2.89 ± 0.03^{a}	13.50 ± 0.05^{a}	10.30 ± 0.05^{d}	2.63 ± 0.47^{a}	65.50 ± 0.46^{cd}
G3: Pea	5.45 ± 0.27^{b}	2.46 ± 0.40^{ab}	9.58 ± 0.12^{de}	12.60 ± 0.41^{b}	3.46 ± 0.18^{a}	66.50 ± 0.32^{ab}
G4: Kiwicha	5.37 ± 0.10^{bc}	1.69 ± 0.09^{c}	10.40 ± 0.08^{cd}	12.20 ± 0.09^{bc}	2.67 ± 0.19^{a}	67.70 ± 0.20^{ab}
G5: Quinoa	5.96 ± 0.19^{ab}	1.91 ± 0.06^{bc}	10.50 ± 0.45^{cd}	11.50 ± 0.17^{c}	2.72 ± 0.04^{a}	67.50 ± 0.07^{ab}
G6: Lentil	5.54 ± 0.16^{b}	2.62 ± 0.11^{a}	9.19 ± 0.09^{e}	13.80 ± 0.11^{a}	3.60 ± 0.16^{a}	65.20 ± 0.09^{cd}
G7: Corn	6.13 ± 0.04^{a}	2.34 ± 0.08^{abc}	11.20 ± 0.40^{bc}	8.49 ± 0.16^{e}	3.31 ± 0.33^{a}	68.60 ± 0.46^{a}
G8: Common Bean	5.39 ± 0.26^{bc}	2.94 ± 0.05^{a}	10.30 ± 0.26^{cd}	12.80 ± 0.13^{b}	3.45 ± 0.06^{a}	65.10 ± 0.42^{cd}

$^{a, b, c, d, e}$ Different superscripts represent significant differences ($p < 0.05$), according to the Tukey method.

TABLE 2 Color parameters using the CIE Lab system for cookies.

Sample	L* (0) black/ (100) white	a* (+) red/(−) green	b* (+) yellow/(−) blue	Whiteness index (WI)	Browning index (BI)	ΔE
G1: Broad bean	48.7 ± 0.34^{e}	18.4 ± 0.43^{de}	47.1 ± 0.08^{bc}	27.9 ± 0.41^{e}	220.0 ± 3.99^{d}	31.6 ± 0.51^{d}
G2: Chickpea	55.2 ± 0.12^{b}	17.8 ± 0.33^{e}	47.9 ± 0.36^{b}	38.7 ± 0.44^{b}	135.6 ± 2.13^{f}	18.3 ± 0.45^{g}
G3: Pea	46.1 ± 0.45^{f}	25.5 ± 0.32^{b}	56.9 ± 0.41^{a}	17.6 ± 0.67^{g}	364.8 ± 14.2^{b}	40.3 ± 0.67^{b}
G4: Kiwicha	54.8 ± 0.39^{c}	19.3 ± 0.04^{d}	45.6 ± 0.26^{de}	33.0 ± 0.42^{c}	171.5 ± 3.37^{e}	26.6 ± 0.34^{f}
G5: Quinoa	51.0 ± 0.38^{d}	18.9 ± 0.33^{d}	46.3 ± 0.22^{cd}	29.9 ± 0.21^{d}	198.0 ± 1.46^{de}	29.7 ± 0.44^{e}
G6: Lentil	43.2 ± 0.40^{g}	20.9 ± 0.08^{c}	47.3 ± 0.18^{bc}	23.2 ± 0.42^{f}	281.5 ± 7.25^{c}	37.5 ± 0.42^{c}
G7: Corn	80.0 ± 0.34^{a}	15.4 ± 0.32^{f}	45.1 ± 0.10^{e}	48.3 ± 0.05^{a}	93.7 ± 0.01^{g}	12.5 ± 0.42^{h}
G8: Common Bean	44.0 ± 0.35^{g}	30.4 ± 0.05^{a}	57.4 ± 0.45^{a}	14.2 ± 0.55^{h}	415.7 ± 15.40^{a}	44.8 ± 0.45^{a}

$^{a, b, c, d, e, f, g, h}$ Different superscripts present significant differences ($p < 0.05$), according to the Tukey method.

description of similar and different characteristics of the cookies. Samples G4, G5, and G7 were similar to each other in the attributes of "dark color" and "strange smell," as well as G1, G3, G6, and G8, with G3 and G6 being considered more frequently as having a dark and strange smell. Regarding the attributes "porous" and "strange taste," samples G1, G2, G4, G5, G6, G7, and G8 showed similarity, presenting less porosity and a strange flavor, unlike G3. Regarding the "bitter" attribute, samples G1 and G8 were similar to each other, although they were described as bitter, unlike G2, G3, G4, G5, and G7. Samples G1, G3, G6, and G8 were similar to each other in the "light color" attribute, as were G2, G5, and G7, with G5 being the sample characterized as light. Regarding the attributes "soft," "crumbly," and "sweet," samples G1, G2, G3, G4, G6, and G8 were similar to each other, differing from G7, which was described as soft, crumbly, and sweet. For the "hard" attribute, samples G1, G4, G6, and G8 showed similarity, as did G2, G3, and G5, with G1 being the sample most frequently mentioned as hard, unlike the others.

Figure 1A shows eight cookie samples and the sensory attributes used to describe them in the first two dimensions. Where six defined subgroups are observed, the first group formed by G1 was characterized as hard. The second group, which is G3 and G6, had the characteristics of dark color and a strange smell, while G8, which represents the third group, was described as bitter and crunchy. The fourth group, made up of G2 and G4, was determined as crispy and adhesive. The fifth group, which is G5, was characterized by a light color. Finally, the sixth group formed by G7 was rated as soft. In Figure 1B, we can find that G7 and G2 reached the highest scores by not registering significant differences ($p > 0.05$) between both samples, being moderately evaluated as I like. However, G8 was the least accepted and evaluated as "Dislike moderately." This could be because it was characterized as bitter.

4 Discussion

4.1 Physicochemical analysis

4.1.1 Nutritional composition analysis

According to the results obtained from the proximal analysis in Table 1, G6 was lower than that reported by Gómez et al. (45) in biscuits partially substituted with legume flours. This could be because the lentil flour was previously induced to reach a moisture content of 10%. Soler et al. (46) found a humidity of 1.11 ± 0.05 in biscuits based on 100% bean flour, although this result is lower than that found in G8 of the present study. This difference could be due to the variety of the ingredients.

On the other hand, the highest ash content was obtained by G8, as well as the biscuit made with bean flour in the study carried out by Gómez et al. (45) in biscuits partially substituted with legume flour and as reported by Soler et al. (46) in the cookie with the 100% bean formulation. Millar et al. (47) mentioned that the high content of ashes in legume flours increases the intake of minerals in the diet. In

TABLE 3 Cochran's Q-test of the attributes evaluated by consumers.

Attribute	*p*-value	G1	G2	G3	G4	G5	G6	G7	G8
Dark color	0.00	0.68^{c}	0.35^{b}	0.70^{c}	0.27^{ab}	0.10^{ab}	0.78^{c}	0.07^{a}	0.69^{c}
Porous	0.00	0.13^{a}	0.12^{a}	0.40^{b}	0.13^{a}	0.21^{a}	0.20^{a}	0.28^{ab}	0.16^{a}
Bitter	0.00	0.51^{c}	0.20^{ab}	0.25^{ab}	0.26^{ab}	0.24^{ab}	0.29^{b}	0.16^{a}	0.56^{c}
Light color	0.00	0.17^{ab}	0.49^{cd}	0.09^{a}	0.42^{c}	0.63^{d}	0.11^{a}	0.52^{cd}	0.20^{ab}
Fatty	0.10	0.02^{a}	0.07^{a}	0.11^{a}	0.06^{a}	0.07^{a}	0.06^{a}	0.02^{a}	0.04^{a}
Soft	0.00	0.02^{a}	0.18^{ab}	0.10^{ab}	0.11^{ab}	0.23^{b}	0.05^{a}	0.51^{c}	0.11^{ab}
Stickiness	0.77	0.05^{a}	0.10^{a}	0.07^{a}	0.05^{a}	0.09^{a}	0.05^{a}	0.06^{a}	0.06^{a}
Crumbly	0.00	0.02^{a}	0.21^{ab}	0.17^{ab}	0.09^{a}	0.28^{b}	0.15^{ab}	0.51^{c}	0.21^{ab}
Hard	0.00	0.85^{d}	0.39^{b}	0.41^{b}	0.66^{cd}	0.36^{b}	0.70^{cd}	0.05^{a}	0.68^{cd}
Strange taste	0.00	0.24^{ab}	0.18^{ab}	0.35^{b}	0.20^{ab}	0.09^{a}	0.26^{ab}	0.09^{a}	0.23^{ab}
Sweet	0.00	0.09^{a}	0.23^{ab}	0.05^{a}	0.29^{ab}	0.44^{c}	0.05^{a}	0.65^{d}	0.12^{ab}
Strange smell	0.00	0.38^{cd}	0.27^{b}	0.56^{d}	0.16^{ab}	0.01^{a}	0.45^{cd}	0.04^{a}	0.40^{cd}
Crispy	0.00	0.38^{ab}	0.48^{ab}	0.35^{ab}	0.43^{ab}	0.40^{ab}	0.38^{ab}	0.28^{a}	0.52^{b}

$^{a, b, c, d, e}$ Different superscripts represent significant differences ($p < 0.05$), according to Cochran's Q-test.

another order of ideas, G2 presented the highest fat value compared to the rest of the cookies, as indicated by Gómez et al. (45) in the biscuit made with chickpea flour in the partial substitution of legume flour. Similarly, Foschia et al. (12) mentioned that chickpea flour has the highest lipid content compared to other legumes. Silva et al. (11) reported fat values ranging from 9.83 ± 0.98 to 11.61 ± 1.10 in crackers made with rice and beans, with the result obtained in G8 being within these values. In terms of fiber, G5 obtained a value of 3.45 ± 0.06, whereas Huatuco et al. (25) found values ranging from 6.3 to 11.3 in cookies made with wheat flour, granadilla, and quinoa; these results were superior to this research. On the other hand, G8 was similar to what was found by Soler et al. (46) in formulation F100 (3.38 ± 0.04) of the cookie made with 100% bean flour. A high fiber content is essential for celiac consumers since gluten-free products generally have a low fiber content, and their intake can induce obesity and other health risks (11). The protein content in G6 was similar to that reported by Gómez et al. (45) in biscuits partially substituted with legume flour, reporting a value of 14.3 ± 0.4% for biscuits made with lentil flour. Regarding the proteins in G8, it was higher than that reported by Silva et al. (11) in crackers made with rice and beans that obtained values from 7.99 ± 0.23 to 10.10 ± 0.48%; this may be due to the fact that some ingredients had a cooking process before. On the other hand, the amount of protein in G7 was lower than the rest of the cookies; this is due to the fact that corn flour has a low protein content (9). The carbohydrate content of G8 differed from that reported by Soler et al. (46) in the cookie made with 100% bean flour, which could be attributed to the type of grain and the method used to obtain the flour. The cookies made by Gómez et al. (45), the one substituted with chickpea flour, obtained a carbohydrate content of 59.5%, which was lower than that reported in G2. Foschia et al. (12) mentioned that, in general, the total content of carbohydrates in legumes constitutes between 45 and 66% of the dry weight.

4.1.2 Color analysis

The results of the chromatic parameters are shown in Table 2. L* (80.0 ± 0.34) in G7 was similar to that found by Gutiérrez et al. (48) in corn crackers, and different types of starch in treatment 1 were L* (84.48 ± 1), but the values of a* and b* differed. On the other hand, G8 presented a lower L* (44.0 ± 0.35) but a higher a* (15.4 ± 0.32) and b* (45.1 ± 0.10) compared to L* (91.13 ± 1.35), a* (−0.28 ± 0.02), and b* (6.16 ± 0.15) in crackers made from rice and beans, as reported by Silva et al. (11). This difference may be due to the fact that polished rice contains mostly starch. In another study by Hamdani et al. (14), there were higher values in L* (55 ± 1 to 56 ± 3) and lower values in a* (2 ± 0.3 to 4 ± 1) and b* (32 ± 0.2 to 35 ± 1) in cookies made with rice, chickpea, and gum flour compared to G2, which was found to be 55.2 ± 0.12 in L*, 17.8 ± 0.33 in a*, and 47.9 ± 0.36 in b*. This could be due to the speed at which the Maillard reaction occurs since it varies according to the type of sugar.

4.2 Sensory analysis

4.2.1 Check all that apply (CATA) method

In Table 3, consumers differentiated 11 of the 13 sensory attributes, similar to the research carried out by Rocha et al. (49) in sweet cookies, where they identified significant differences in 15 of the 21 sensory descriptors. They suggest that this method allows samples to be distinguished according to the perception of the evaluators.

The graphic representation of the samples and sensory attributes in Figure 1A explains 90.3% of the total variation, which agrees with Pramudya and Seo (43) and Rocha et al. (49), who presented total variations of 92.95 and 97.01%, respectively, where they illustrate the associations between the samples and the sensory descriptors in the first two dimensions of the correspondence analysis (CA).

The G7 sample based on corn flour was considered the softest, most crumbly, and sweetest. This is because starch has the functionality of improving the texture, decreasing the hardness, and increasing the characteristic flexibility of the products baked (48, 50).

4.2.2 Overall acceptability

The samples with the highest acceptability were based on corn flour (G7) and chickpea (G2), while the least admissible one was made with bean flour (G8), as observed in Figure 1B. This result was similar

to what was reported by Gómez et al. (45) when evaluating the effect of the partial substitution with legume flours. In another study, Gutiérrez et al. (48) found that treatment 3 (90% corn flour and 10% starch) obtained the highest acceptability from evaluators for corn crackers and different types of starch. Similarly, Hamdani et al. (14) mentioned that cookies made with rice flour and chickpea-added karaya gum were the most accepted by consumers because they showed the highest ratings in appearance, mouthfeel, flavor, and mainly texture, helping to reduce the hardness of cookies.

It is important to highlight the increasing demand for gluten-free diets due to the prevalence of celiac disease and gluten sensitivity. The research allowed us to develop specific formulations that improve the texture, flavor, and quality of gluten-free cookies, generating a direct impact on the formulation of commercial products and consumer preference. Furthermore, by addressing sensory attributes, it allows for the improvement of marketing and positioning strategies for gluten-free products, providing the industry with valuable information to adapt to constantly evolving market demands. This approach will help to highlight the practical importance of research and its contribution to knowledge in the field of gluten-free products.

5 Conclusion

Gluten-free cookies were developed, with significant differences in the physicochemical, colorimetric, and sensory parameters. Of the different formulations made, the lentil flour cookie had a higher protein and fiber content with reduced levels of fat and carbohydrates, which distinguished it from other cookies. The corn and chickpea flour cookies obtained the highest acceptability scores compared to the rest of the cookies, being described as soft, crunchy, and sticky. These findings highlight the viability of gluten-free cookies as an accessible and marketable option, especially aimed at people with celiac disease, gluten intolerant people, and those seeking a healthy diet. The research not only offers a solution to the dietary needs of this demographic but also presents a sensory-appealing product. However, it is recognized that there is a need for future research to delve into the optimization of the formulation, shelf life, and production quality to further improve commercialization and provide a more complete and robust alternative in the gluten-free product market.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

This study was approved by Ethics Committee of Facultad de Ingeniería y Arquitectura - Universidad Peruana Unión (N° 2022-CEFIA-0006).

Author contributions

RS-P: Conceptualization, Methodology, Software, Writing – original draft, Writing – review & editing. RS-L: Data curation, Investigation, Methodology, Writing – original draft. NJ-G: Conceptualization, Data curation, Formal analysis, Writing – original draft. AE-S: Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by the laboratory of the Ciencia de Investigación en Tecnología de Alimentos (CITAL) of the Universidad Peruana Unión – Perú.

Acknowledgments

This article was developed thanks to the support provided by the Universidad Peruana Unión through the financing of the project, which included financial support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Estévez V, Y Araya M. La dieta sin gluten y los alimentos libres de gluten. *Revista Chilena de Nutrición*. (2016) 43:428–33. doi: 10.4067/S0717-75182016000400014

2. Miranda Villa PP, Mufari JR, Bergesse AE, Planchuelo AMR, Calandri EL. Calidad nutricional y propiedades físicas de panes libres de gluten. *Nutrición Clínica y Dietética Hospitalaria*. (2018) 38:46–55. doi: 10.12873/383miranda

3. Deora NS, Deswal A, Mishra HN. Functionality of alternative protein in gluten-free product development. *Food Sci Technol Int*. (2015) 21:364–79. doi: 10.1177/1082013214538984

4. Castillo Hernández LF, Sánchez Mundo MD, Rayo García V, García Nieves S, González Miguel ME, Ramírez Higuera A. Sustitución de la harina de trigo por harinas compuestas e ingredientes funcionales para la elaboración de panes libres de gluten. *Revista Mexicana de Agroecosistemas*. (2019) 6:190–201. Available at:https://bit.ly/3DFqCxM

5. Ciacci C, Ciclitira P, Hadjivassiliou M, Kaukinen K, Ludvigsson J, McGough N, et al. The gluten-free diet and its current application in coeliac disease and dermatitis herpetiformis. *United European Gastroenterol J*. (2015) 3:121–35. doi: 10.1177/2050640614559263

6. Kaur M, Sandhu KS, Arora A, Sharma A. Gluten free biscuits prepared from buckwheat flour by incorporation of various gums: physicochemical and sensory properties. *LWT Food Sci Technol*. (2015) 62:628–32. doi: 10.1016/j.lwt.2014.02.039

7. Di Cairano M, Condelli N, Caruso MC, Marti A, Cela N, Galgano F. Functional properties and predicted glycemic index of gluten free cereal, pseudocereal and legume flours. *LWT Food Sci Technol*. (2020) 133:109860. doi: 10.1016/j.lwt.2020.109860

8. De Kock HL, Magano NN. Sensory tools for the development of gluten-free bakery foods. *J Cereal Sci*. (2020) 94:102990. doi: 10.1016/j.jcs.2020.102990

9. Pellegrini N, Agostoni C. Nutritional aspects of gluten-free products. *J Sci Food Agric*. (2015) 95:2380–5. doi: 10.1002/jsfa.7101

10. Ferreira da Silva T, Conti-Silva AC. Potentiality of gluten-free chocolate cookies with added inulin/oligofructose: chemical, physical and sensory characterization. *LWT Food Sci Technol*. (2018) 90:172–9. doi: 10.1016/j.lwt.2017.12.031

11. Wesley SD, André BHM, Clerici MTPS. Gluten-free rice & bean biscuit: characterization of a new food product. *Heliyon*. (2021) 7:e05956. doi: 10.1016/j.heliyon.2021.e05956

12. Foschia M, Horstmann SW, Arendt EK, Zannini E. Legumes as functional ingredients in gluten-free bakery and pasta products. *Annu Rev Food Sci Technol*. (2017) 8:75–96. doi: 10.1146/annurev-food-030216-030045

13. A Felker FC, Singh M, Byars JA, Berhow MA, Bowman MJ, Winkler-Moser JK. Comparison of composition and physical properties of soluble and insoluble navy bean flour components after jet-cooking, soaking, and cooking. *LWT Food Sci Technol*. (2020) 130:109765. doi: 10.1016/j.lwt.2020.109765

14. Hamdani AM, Wani IA, Bhat NA. Gluten free cookies from rice-chickpea composite flour using exudate gums from acacia, apricot and karaya. *Food Biosci*. (2020) 35:100541. doi: 10.1016/j.fbio.2020.100541

15. Boye J, Zare F, Pletch A. Pulse proteins: processing, characterization, functional properties and applications in food and feed. *Food Res Int*. (2010) 43:414–31. doi: 10.1016/j.foodres.2009.09.003

16. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. *Nat Protoc*. (2010) 5:725–38. doi: 10.1038/nprot.2010.5

17. Bengoechea C, Romero A, Villanueva A, Moreno G, Alaiz M, Millán F, et al. Composition and structure of carob (*Ceratonia siliqua L.*) germ proteins. *Food Chem*. (2008) 107:675–83. doi: 10.1016/j.foodchem.2007.08.069

18. Gómez M, Del Real S, Rosell CM, Ronda F, Blanco CA, Caballero PA. Functionality of different emulsifiers on the performance of breadmaking and wheat bread quality. *Eur Food Res Technol*. (2004) 2004:145–50. doi: 10.1007/s00217-004-0937-y

19. Miñarro B, Albanell E, Aguilar N, Guamis B, Capellas M. Effect of legume flours on baking characteristics of gluten-free bread. *J Cereal Sci*. (2012) 56:476–81. doi: 10.1016/j.jcs2012.04.012

20. Tuorila H. From sensory evaluation to sensory and consumer research of food: an autobiographical perspective. *Food Qual Prefer*. (2015) 40:255–62. doi: 10.1016/j.foodqual.2014.05.006

21. Galmarini MV. The role of sensory science in the evaluation of food pairing. *Curr Opin Food Sci*. (2020) 33:149–55. doi: 10.1016/j.cofs.2020.05.003

22. Cervini M, Frustace A, Duserm Garrido G, Rocchetti G, Gianluca G. Nutritional, physical and sensory characteristics of gluten-free biscuits incorporated with a novel resistant starch ingredient. *Heliyon*. (2021) 7:e06562. doi: 10.1016/j.heliyon.2021.e06562

23. Jaeger SR, Chheang SL, Jin D, Roigard CM, Ares G. Check-all-that-apply (CATA) questions: sensory term citation frequency reflects rated term intensity and applicability. *Food Qual Prefer*. (2020) 86:103986. doi: 10.1016/j.foodqual.2020.103986

24. American Association for Clinical Chemistry. *Baking quality of cookie. Flour-Micro method*. USA: (1999).

25. Huatuco Lozano M, Achulla Parco B, Flores Alarcón JE. Efecto de la sustitución parcial de harina de trigo (*Triticum aestivum*) por harina de granadilla (*Passiflora ligularis* juss) y harina de quinua (*Chenopodium quinoa*) en las características fisicoquímicas y sensoriales de galletas dulces. *Tayacaja*. (2020) 3:181–97. doi: 10.46908/rict.v3i2.129

26. AOAC. *Official methods of analysis of AOAC international (17a ed.)* AOAC International (2000) Available at:https://bit.ly/3x7eWBC.

27. AACC. *Approved methods of the American Association of Cereal Chemists (10a ed.)* Cereals & Grains Association (2000) Available at:https://bit.ly/3nyVhra.

28. Torres González JD, González Morelo KJ, Acevedo Correa D, Y Jaimes Morales J d. Efecto de la utilización de harina de Lens culinaris como extensor en las características físicas y aceptabilidad de una salchicha. *Tecnura*. (2016) 20:15–28. doi: 10.14483/udistrital.jour.tecnura.2016.3.a01

29. Tuncel NBB, Yılmaz N, Kocabiyik H, Uygur AA, Kocabıyık H, Yilmaz N, et al. The effect of infrared stabilized rice bran substitution on B vitamins, minerals and phytic acid content of pan breads: part II. *J Cereal Sci*. (2014) 59:162–6. doi: 10.1016/j.jcs.2013.12.005

30. Sakin-Yilmazer M, Kemerli T, Isleroglu H, Ozdestan O, Guven G, Uren A, et al. Baking kinetics of muffins in convection and steam assisted hybrid ovens. *J Food Eng*. (2013) 119:483–9. doi: 10.1016/j.jfoodeng.2013.06.019

31. Tarancón P, Salvador A, Sanz T, Fiszman S, Tárrega A. Use of healthier fats in biscuits (olive and sunflower oil): changing sensory features and their relation with consumers' liking. *Food Res Int*. (2015) 69:91–6. doi: 10.1016/j.foodres.2014.12.013

32. Antúnez L, Ares G, Giménez A, Jaeger S. Do individual differences in visual attention to CATA questions affect sensory product characterization? A case study with plain crackers. *Food Qual Prefer*. (2016) 48:185–94. doi: 10.1016/j.foodqual.2015.09.009

33. Lívia Silva SM, Lopes Almeida E, Melo L. Discrimination of sensory attributes by trained assessors and consumers in semi-sweet hard dough biscuits and their drivers of liking and disliking. *Food Res Int*. (2019) 122:599–609. doi: 10.1016/j.foodres.2019.01.031

34. Henrique NA, Deliza R, Rosenthal A. Consumer sensory characterization of cooked ham using the check-all-that-apply (CATA) methodology. *Food Eng Rev*. (2015) 7:265–73. doi: 10.1007/s12393-014-9094-7

35. Esmerino E, Tavares E, Thomas B, Ferraz J, Silva H, Pinto L, et al. Consumer-based product characterization using pivot profile, projective mapping and check-all-that-apply (CATA): A comparative case with Greek yogurt samples. *Food Res Int*. (2017) 99:375–84. doi: 10.1016/j.foodres.2017.06.001

36. Simanca Sotelo M, De Paula C, Domínguez Anaya Y, Pastrana Puche Y, Álvarez Badel B. Physico-chemical and sensory characterization of sweet biscuits made with Yacon flour (Smallanthus sonchifolius). *NFS J*. (2021) 22:14–9. doi: 10.1016/j.nfs.2020.12.001

37. Quintana Obregón E, San Martín Hernández C, Muy-Rangel M, Vargas Ortiz M. Valorization of mango (*Mangifera indica* L.) pericarp powders as an alternative for the generation of functional foods. *Revista Especializada en Ciencias Químico-Biológicas*. (2019) 22:1–5. doi: 10.22201/fesz.23958723e.2019.0.178

38. Navarro Flores JR, Vargas Rojas JC. Eficiencia relativa del diseño de bloques completos al azar para ensayos de arroz en Bagaces, Guanacaste, Costa Rica. *InterSedes*. (2015) 16:61–70. Available at:http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S2215-24582015000200061&lng=en&tlng=es

39. Olutayo Akinwale R, Kayode Odunlami L, Emmanuel Eze C, Samuel Oladejo A. Effectiveness of different alpha lattice designs in the evaluation of maize (*Zea mays* L.) genotypes in a rainforest agro-ecology. *Heliyon*. (2021) 7:e07414. doi: 10.1016/j.heliyon.2021.e07414

40. Santos FG, Aguiar EV, Centeno AC, Rosell CM, Capriles VD. Effect of added psyllium and food enzymes on quality attributes and shelf life of chickpea-based gluten-free bread. *LWT Food Sci Technol*. (2020) 134:110025. doi: 10.1016/j.lwt.2020.110025

41. Alexi N, Nanou E, Lazo O, Guerrero L, Grigorakis K, Byrne DV. Check-all-that-apply (CATA) with semi-trained assessors: sensory profiles closer to descriptive analysis or consumer elicited data? *Food Qual Prefer*. (2018) 64:11–20. doi: 10.1016/j.foodqual.2017.10.009

42. Vidal L, Antúnez L, Ares G, Cuffia F, Lee P-Y, Le Blond M, et al. Sensory product characterizations based on check-all-that-apply questions: further insights on how the static (CATA) and dynamic (TCATA) approaches perform. *Food Res Int*. (2019) 125:108510. doi: 10.1016/j.foodres.2019.108510

43. Pramudya RC, Seo H-S. Using check-all-that-apply (CATA) method for determining product temperature-dependent sensory-attribute variations: A case study of cooked rice. *Food Res Int*. (2018) 105:724–32. doi: 10.1016/j.foodres.2017.11.075

44. Machado N, Godoy T, Barone B, André A, Biasoto A, Jorge H. Sensory profile and check-all-that-apply (cata) as tools for evaluating and characterizing syrah wines aged with oak chips. *Food Res Int*. (2019) 124:156–64. doi: 10.1016/j.foodres.2018.07.052

45. Gómez Flores GA, Ramos Herrera OJ, Gómez Ruiz SE, Chávez Murillo CE. Estudio proximal y sensorial de galletas sustituidas parcialmente con harina de leguminosas nativas y modificadas. *Investigación y Desarrollo en Ciencia y Tecnología de Alimentos*. (2016) 1:95–101. Available at:https://bit.ly/3CIW9xS

46. Soler Martínez N, Castillo Ruíz O, Rodríguez Castillejos G, Perales-Torres A, González Pérez AL. Análisis proximal, de textura y aceptación de las galletas de trigo, sorgo y frijol. *Arch Latinoam Nutr*. (2017) 67:227–34. Available at:https://bit.ly/3FujKDW

47. Millar KA, Gallagher EB, McCarthy S, Barry-Ryan C. Proximate composition and anti-nutritional factors of fava-bean (*Vicia faba*), green-pea and yellow-pea (*Pisum sativum*) flour. *J Food Compos Anal*. (2019) 82:103233. doi: 10.1016/j.jfca.2019.103233

48. Gutiérrez Mendívil KL, Treto Padrón AL, Frías Escobar A, Pérez Carrillo E, Guajardo Flores S. Elaboración de galleta con maíz nixtamalizado y diferentes tipos de almidón. *Investigación y Desarrollo en Ciencia y Tecnología de Alimentos*. (2016) 1:127–33. Available at:https://bit.ly/3kXyAet

49. Rocha C, Ribeiro JC, Costa Lima R, Prista C, Raymundo A, Vaz Patto MC, et al. Application of the CATA methodology with children: qualitative approach on ballot development and product characterization of innovative products. *Food Qual Prefer*. (2021) 88:104083. doi: 10.1016/j.foodqual.2020.104083

50. Liendo Bastardo MC, Silva Chávez MV. Producto tipo galleta elaborado con mezcla de harina de quinchoncho (*Cajanus cajan L.)* y almidón de maíz (*Zea mays L.*). *Saber Revista Multidisciplinaria del Consejo de Investigación de la Universidad de Oriente*. (2015) 27:78–86. Available at:http://www.redalyc.org/articulo.oa?id=427739474010

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 04 March 2024
DOI 10.3389/fimmu.2024.1372369

OPEN ACCESS

EDITED BY
Ángela Ruiz-Carnicer,
Sevilla University, Spain

REVIEWED BY
Verónica Segura Montero,
University of Sevilla, Spain
Ivan Milos Stankovic,
University of Belgrade, Serbia
Diego Sánchez Muñoz,
Instituto Digestivo, Spain

*CORRESPONDENCE
António Raposo
antonio.raposo@ulusofona.pt
Edite Teixeira-Lemos
etlemos3@gmail.com

RECEIVED 17 January 2024
ACCEPTED 20 February 2024
PUBLISHED 04 March 2024

CITATION
Chaves C, Zandonadi RP, Raposo A, Nakano EY, Ramos F, Farage P and Teixeira-Lemos E (2024) Health-related quality of life among celiacs in Portugal: a comparison between general and specific questionnaires. *Front. Immunol.* 15:1372369. doi: 10.3389/fimmu.2024.1372369

Health-related quality of life among celiacs in Portugal: a comparison between general and specific questionnaires

Cláudia Chaves[1], Renata Puppin Zandonadi[2], António Raposo[3]*, Eduardo Yoshio Nakano[4], Fernando Ramos[5,6], Priscila Farage[7] and Edite Teixeira-Lemos[8]*

[1]ESSV, Centre for Studies in Education and Innovation (CI&DEI), Polytechnic University of Viseu, Viseu, Portugal, [2]Faculty of Health Sciences, Nutrition Department, University of Brasília, Brasília, Brazil, [3]CBIOS (Research Center for Biosciences and Health Technologies), Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal, [4]Department of Statistics, University of Brasília, Brasília, Brazil, [5]Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal, [6]Associated Laboratory for Green Chemistry (LAQV) of the Network of Chemistry and Technology (REQUIMTE), Porto, Portugal, [7]Faculty of Nutrition, Federal University of Goiás, Goiânia, Brazil, [8]CERNAS Research Centre, Polytechnic University of Viseu, Viseu, Portugal

Objective: This study aimed to compare the 36-Item Short Form Survey Instrument version 2 (SF-36-v2) (generic) and Celiac Disease Questionnaire (CDQ) (specific) questionnaires used to evaluate the quality of life (QoL) in celiac Portuguese adult individuals.

Methods: This cross-sectional study used non-probabilistic sampling based on Portuguese celiac patients who accessed the online survey in 2022. The online data collection used a self-reported instrument composed of three parts: (i) socioeconomic, health, and gluten-free diet (GFD) adherence questions; (ii) SF-36 v2 – Portuguese version (generic questionnaire) and (iii) Celiac Disease Questionnaire (CDQ) (specific questionnaire).

Results: A total of 234 individuals who accessed the survey completed the questionnaire. Seven of the eight SF-36 domains positively correlated to the specific questionnaire CDQ. The "General Health" domain (domain 4) showed a negative correlation with the CDQ. Differences in content between the two instruments might be able to explain this finding since the CDQ explores issues regarding the specificities of celiac disease (CD) and the lifelong GFD burden. About half of the sample from this study displayed poor diet adherence, it is possible that the SF-36 could not reflect the impact of CD treatment - the complete elimination of gluten from the diet - on patients' health. Therefore, this issue should be carefully evaluated in future research.

Conclusion: Specific validated questionnaires for CD individuals, such as the CDQ, contemplate social, economic, and clinical variables that permeate the patient's life context. Therefore, these instruments may be more suitable for

evaluating QoL in this public. However, using a general questionnaire such as the SF-36 would be indicated for comparing QOL between celiac patients and the general population or even between CD and other disease individuals. In this case, we recommend assessing GFD compliance for control parallelly.

KEYWORDS

celiac disease, gluten-free diet, Portugal, quality of life, questionnaire

1 Introduction

For Celiac disease (CD) is a permanent autoimmune disorder triggered by gluten ingestion by genetically predisposed individuals, affecting approximately 1% of the worldwide population (1, 2). CD is considered a public health problem and may cause malabsorption, leading to nutritional deficiencies, liver and bone diseases, gastrointestinal symptoms, growth deficiency, or several other consequences (1, 3, 4).

Until now, the only safe treatment for CD is a life-long gluten-free diet (GFD) (1, 3) and usually, GFD compliance improves the quality of life (QoL) in most of CD patients due to symptom remission, nutritional deficiencies and other CD-related health consequences avoidance, and mortality reduction. However, multiple factors influence GFD compliance, such as acceptance, access, availability, and cost of gluten-free products; dietary restrictions; socialization difficulties; and economic burden, among others, potentially negatively impacting CD QoL (5–9). In this sense, CD is considered a chronic condition that affects patients' QoL as other chronic diseases (5–8) and, to achieve optimal health, it is essential to understand the individual's perception of QoL (10). In chronic conditions, it is crucial to evaluate the impact of patients' health conditions on their ability to live a fulfilling life and promote public policies to minimize the physical, emotional, and social burden on the patient (11, 12).

Studies have explored CD patients' QoL perceptions using generic and specific questionnaires developed for celiac patients (13–22). The use of a specific questionnaire is important to comprehend aspects related to the celiacs' QoL, mental health, well-being, and the economic and social aspects caused by this chronic condition and their lifelong dietary and lifestyle changes (11, 23). However, the use of a general questionnaire such as Short Form-36 (SF-36) may allow comparison among individuals with different chronic diseases or healthy individuals (24–27). The SF-36 is a widely recognized questionnaire designed to assess an individual's health-related quality of life and functional abilities and is highly used as a generic instrument in gastroenterology (13, 28–30). Comprising 36 items that explore eight different aspects of QoL, it offers a detailed evaluation of physical functioning, limitations in daily activities due to physical health issues, pain levels, overall health perception, energy levels, social functioning, limitations in activities due to emotional problems, and mental health.

Considering the specific questionnaires to measure CD patients' QoL, the Celiac Disease Questionnaire (CDQ) is broadly applied (11, 23) that used SF-36 in its validation process (13). CDQ was developed, validated and applied in Germany (2006) and later, it was translated and applied in several European and Extra-European countries (5, 6, 15, 23, 31–43). In Portugal, a study translated and validated the CDQ into Portuguese (41) and our previous study evaluated the quality of life (QoL) perception among Portuguese celiac patients using this Portuguese version of CDQ (42). Furthermore, a separate study conducted in Portugal utilized the general questionnaire SF-36 to assess the perception of QoL in a sample of 195 Portuguese celiacs regarding compliance with a gluten-free diet (GFD) (44). However, no study has compared a generic (SF-36) and a specific (CDQ) questionnaire to evaluate the perception of QoL among Portuguese celiac patients.

Therefore, this study aimed to compare the SF-36 v2 (generic) and CDQ (specific) questionnaires used to evaluate the QoL in celiac Portuguese adult individuals. The study is justified by the need to understand the differences between specific and generic questionnaires and how they could impact the evaluation of QoL in CD.

2 Materials and methods

2.1 Study design and instruments

This cross-sectional study used non-probabilistic sampling based on Portuguese celiac patients who accessed the online survey in 2022. The online data collection method was chosen due to the pandemic caused by SARS-CoV-2, making it impossible to use face-to-face interviews. In addition, it is considered a productive and cheap method to enroll participants and reach a more extensive sample (45, 46). The instrument was composed of 3 parts: (i) socioeconomic, health and GFD adherence questions; (ii) the SF-36 v2– Portuguese version (generic questionnaire) and (iii) CDQ (specific questionnaire) (5). The CDQ is a specific questionnaire to evaluate CD patients' QoL. It was developed by Häuser et al. (5) and validated in Portugal by Lobão et al. (41). This

questionnaire comprises 28 items divided into 4 domains (emotions, gastrointestinal symptoms, concerns, and social) evaluated by 7-point scale (from "1" - worst QoL perception to "7" - best QoL perception). The QoL general instrument used was the SF-36 v2 Portuguese version, validated in Portugal. It is an adaptation of the SF-36, which generates a physical component summary (PCS) and a mental one (MCS). This questionnaire has 36 items divided into 8 domains (1. Physical functioning, 2. Role limitations due to physical health, 3. Pain, 4. General Health, 5. Energy/fatigue, 6. Social functioning, 7. Role limitations due to emotional problems, 8. Emotional well-being) (47). It is a widely used generic, coherent, and easily administered QoL questionnaire.

We also collected sociodemographic characteristics (gender, age, marital status, educational level) and clinical variables (age at CD diagnosis, GFD compliance, use of antidepressants). The GDF compliance was self-reported since we do not have a validated instrument to evaluate GFD compliance in Portugal. Considering data collection occurred during the COVID-19 pandemic, it was not possible to validate a new instrument to evaluate it since the laboratory tests were limited. Therefore, we opt to use self-reported GFD compliance, as performed in other studies (34, 39, 48–52). Participants chose the option that best characterized their current diet regarding the question: "Do you follow a gluten-free diet?". The response options were: 1) Never; 2) Rarely; 3) Sometimes; 4) Almost always (most of the time); 5) Always. Strict GDF compliance was considered for those who self-reported always adhering to a GFD whereas all others considered "gluten-exposed". All the participants filled out both questionnaires.

2.2 Participants and ethics

The online instrument was inserted in the SurveyMonkey® online platform. Individuals were invited to participate in the study by the Portuguese Celiac Association (Associação Portuguesa de Celíacos - APC) or via social media posting the link from February to May 2022. The inclusion criteria were as follows: a) Individuals aged >18 years diagnosed with celiac disease (CD) underwent a comprehensive diagnostic process, including clinical, serological, and histopathological assessments (specifically high upper digestive endoscopy with duodenal biopsies), along with genetic testing (HLA DQ2 and DQ8 analysis), in line with the ESsCD guideline (53). This criterion encompasses adults initially diagnosed with CD during childhood, adhering to the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) criteria (54)) and b) Participants were residents of Portugal and affiliated with the Portuguese Celiac Association (Associação Portuguesa de Celíacos - APC). After reading all the information about the study, those diagnosed with CD who agreed to participate accessed the questionnaire items. Those who disagreed were driven to the final page, acknowledging their time. All 234 individuals who signed the consent form to participate in the study completed the questionnaire.

The research followed the American Psychological Association (APA) Ethical Guidelines for Research involving Human Subjects. The participants were informed about the study's scope, signed the informed consent form, and were not compensated for their participation. The Polytechnic University of Viseu Ethics Committee approved the ethical aspects of this study (n.° 59/SUB/2021 - 26th July 2021).

2.3 Statistical analysis

Data were extracted from the SurveyMonkey® platform and evaluated using International Business Machines Corporation (IBM) Statistical Package for the Social Sciences (SPSS) Statistics, version 22 (Armonk, NY: IBM Corp). The statistical analysis considered the CDQ and SF-36 scores.

Descriptive statistics were performed as mean and standard deviation for SF-36 subscales. Student's t-test, Analysis of Variance (ANOVA) and Tukey's posthoc test were used to compare the SF-36 and the variables of interest. The tests considered two-tailed hypotheses and a significance level of 5%. The association between the CDQ and SF-36 V2 was verified using Spearman's correlation.

3 Results

A total of 234 individuals accepted to participate in the study and completed the questionnaire. The questionnaire was virtually applied, and all individuals who accessed it completed it. Table 1 shows data from the SF-36 domains subcategorized by sex, age, age at diagnosis, education, marital status, and diet. Males showed better scores for SF-36 domain 1 (Physical functioning), domain 2 (Role limitations due to physical health) and domain 7 (Role limitations due to emotional problems), and lower scores for domains 4 (General Health) and 5 (Energy/fatigue). Age differed only for domain 2 (Role limitations due to physical health), in which those > 40 y/o had better scores. Age at diagnosis differed only for domains 5 (Energy/fatigue), in which > 20 y/o at CD diagnosis had better scores and 6 (Social functioning) in which up to 20 y/o at CD diagnosis had better scores. Considering the educational level, participants with the highest educational level presented lower scores for domains 1, 2, 3, 6 and 7. Patients living alone presented lower scores for domain 1. Those following a GFD presented lower scores for D1, 2, 6, and 7 and the best score for D5 (Energy/fatigue). The use of antidepressants did not influence the SF-36 domains.

The CDQ domains' maximum scores can be 49 and 196 in total. Table 2 shows that our sample presented the lowest score for social and gastrointestinal CDQ domains (23.03 ± 9.53 and 25.12 ± 8.81, respectively). Evaluating the associations, the SF-36 Domain 4 (general health) presented a negative association with all CDQ domains (Table 2). All the other domains showed positive associations with the CDQ.

4 Discussion

This study recently evaluated the QoL perception of Portuguese celiac patients using a general questionnaire (SF-36) and compared

TABLE 1 SF-36 domains analyzed with subcategories based on sex, age groups, age at diagnosis of the condition, educational attainment, marital status, and dietary habits (n=234).

	D1 Physical functioning	D2 Role limitations due to physical health	D3 Pain	D4 General Health	D5 Energy/ fatigue	D6 Social functioning	D7 Role limitations due to emotional problems	D8 Emotional well-being
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Gender*								
Female (n=162)	24.69 (23.22)[a]	34.72 (25.66)[a]	36.33 (24.13)[a]	51.93 (13.34)[b]	52.70 (19.32)[b]	38.35 (22.90)[a]	38.12 (25.36)[a]	46.94 (18.13)[a]
Male (n=66)	33.94 (24.50)[b]	46.97 (26.66)[b]	35.41 (23.04)[a]	47.47 (9.75)[a]	46.40 (11.13)[a]	43.37 (16.00)[a]	46.59 (24.76)[b]	46.36 (10.65)[a]
p	0.008	0.001	0.791	0.006	0.002	0.061	0.022	0.808
Age*								
Up to 40 y/o (n=132)	24.55 (23.89)[a]	37.36 (27.19)[a]	33.58 (22.67)[a]	50.02 (13.21)[a]	51.37 (18.26)[a]	39.77 (21.00)[a]	40.09 (26.81)[a]	47.73 (17.36)[a]
> 40 y/o (n=102)	32.45 (23.86)[b]	40.87 (25.52)[a]	39.39 (24.53)[a]	50.97 (11.58)[a]	49.88 (16.19)[a]	40.69 (21.45)[a]	42.40 (23.40)[a]	45.44 (14.28)[a]
p	0.013	0.315	0.062	0.567	0.515	0.744	0.490	0.282
Age at diagnosis*								
Up to 20 y/o (n=115)	30.65 (24.24)[a]	41.14 (26.58)[a]	35.89 (22.37)[a]	49.38 (10.85)[a]	47.72 (14.30)[a]	43.59 (19.05)[b]	44.49 (25.02)[a]	47.17 (13.77)[a]
> 20 y/o (n=116)	25.99 (23.90)[a]	37.66 (25.95)[a]	37.01 (24.78)[a]	50.93 (13.66)[a]	53.77 (19.64)[b]	37.39 (22.51)[a]	38.79 (24.92)[a]	46.77 (18.11)[a]
p	0.143	0.315	0.718	0.341	0.008	0.025	0.084	0.848
Educational level**								
Up to elementary school (n=35)	37.00 (22.00)[b]	50.18 (24.28)[b]	39.11 (20.09)[ab]	47.23 (11.40)[a]	48.04 (12.48)[a]	43.21 (17.24)[b]	50.24 (25.60)[b]	46.14 (11.89)[a]
High school (n=61)	41.15 (25.09)[b]	48.26 (26.10)[b]	39.97 (23.15)[b]	51.00 (10.30)[a]	50.20 (13.79)[a]	47.75 (18.61)[b]	46.86 (26.05)[ab]	50.41 (12.49)[a]
Undergraduate (n=89)	22.42 (22.02)[a]	34.06 (24.46)[a]	36.92 (25.53)[ab]	51.34 (14.28)[a]	52.04 (20.03)[a]	39.04 (23.14)[ab]	35.30 (22.30)[a]	45.45 (18.47)[a]
Post-graduation (n=49)	15.31 (17.63)[a]	27.93 (25.80)[a]	27.69 (21.42)[a]	50.39 (12.30)[a]	50.89 (19.31)[a]	30.61 (19.27)[a]	37.93 (26.85)[ab]	44.90 (17.69)[a]
p	0.000	0.000	0.035	0.411	0.707	0.000	0.004	0.219
Marital status*								
With partner (n=142)	30.53 (24.46)[b]	40.67 (25.71)[a]	37.56 (25.47)[a]	50.32 (12.90)[a]	50.92 (17.96)[a]	41.55 (21.81)[a]	42.02 (24.61)[a]	47.25 (16.42)[a]
(n=92)	24.08 (23.24)[a]	36.14 (27.53)[a]	33.88 (20.38)[a]	50.62 (11.94)[a]	50.41 (16.52)[a]	38.04 (20.04)[a]	39.67 (26.54)[a]	45.92 (15.64)[a]
p	0.046	0.202	0.246	0.857	0.825	0.216	0.491	0.538
Gluten-free diet*,***								
No (n=105)	37.67 (23.46)[b]	46.85 (24.58)[b]	38.95 (21.01)[a]	49.38 (10.23)[a]	47.32 (11.56)[a]	44.52 (17.84)[b]	46.75 (24.12)[b]	46.71 (12.38)[a]
Yes (n=129)	20.12 (21.79)[a]	32.41 (26.28)[a]	33.80 (25.40)[a]	51.29 (14.07)[a]	53.49 (20.58)[b]	36.63 (22.97)[a]	36.50 (25.50)[a]	46.74 (18.63)[a]

(Continued)

10.3389/fimmu.2024.1372369

TABLE 1 Continued

	D1 Physical functioning	D2 Role limitations due to physical health	D3 Pain	D4 General Health	D5 Energy/ fatigue	D6 Social functioning	D7 Role limitations due to emotional problems	D8 Emotional well-being
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Gluten-free diet*,* **								
p	0.000	0.000	0.091	0.230	0.004	0.003	0.002	0.989
Antidepressants*								
No (n=218)	27.50 (23.92)[a]	37.99 (26.50)[a]	35.91 (23.68)[a]	50.50 (12.27)[a]	50.37 (17.01)[a]	39.56 (20.89)[a]	39.91 (25.06)[a]	46.33 (15.81)[a]
Yes (n=16)	34.69 (26.99)[a]	51.17 (23.63)[a]	38.88 (23.43)[a]	49.63 (15.87)[a]	55.47 (21.76)[a]	48.44 (23.66)[a]	57.29 (24.51)[b]	52.19 (19.41)[a]
p	0.251	0.054	0.629	0.789	0.258	0.105	0.008	0.161

* Student's t-test.
**Anova with Tukey's posthoc. Groups with the same letters do not differ significantly.
***Compliance with a gluten-free diet was considered participants' responses of "always following the diet".

its association with the specific questionnaire CDQ since CD symptoms and a lifelong GFD may significantly impact celiacs' QoL. In our sample, about 45% of participants (n = 105) did not comply with the GFD, similar to data found in a previous study performed in Portugal in 2014 with 195 celiac patients, in which 47.7% did not comply with the GFD (44). The authors did not find an association between the QoL perception using the SF-36 and GFD compliance (44) and mentioned that it would be expected that GFD compliance would be positively associated with QoL. They list some potential explanations for their results: i) celiac patients who do not comply with the GFD were those who present milder symptoms, which do not significantly compromise their QoL; ii) those who did not comply with the GFD consider it less disruptive to their daily lives than that compliance with the GFD and iii) the possibility that the SF-36 was not sensitive enough to differentiate compliance with the GFD. In our study, celiacs not complying with the GFD showed the best scores for D1, D2, D6 and D7.

The D1(Physical functioning) scores were higher for males, > 40 y/o, those with the lowest educational levels, with partners and those not following the GFD. This SF-36 domain is important for identifying physical compromise in chronic diseases that impair common routine and exercise activities. A study (55) estimating the impact of chronic pain on patients' QoL and found that the participants presented significantly lower mean QoL scores across all domains of the SF-36. The score for the D1 domain among the 78 chronic pain subjects was 31.8 ± 27.2 in comparison to scores of 94.0 ± 12.4 and 90.2 ± 18.9 from the general population in studies from England and Wales, respectively (p=0.001). Regarding CD,

TABLE 2 Mean and SD of SF-36 V2 scores and correlation between CDQ scale subscores.

		Correlation with CDQ subscales									
	Mean	Emotion 28.35 (7.60)		Social 23.03 (9.53)		Worries 26.77 (8.78)		Symptoms 25.12 (8.81)		Total 103.28 (31.15)	
	(SD)	Corr*	p	Corr*	p	Corr*	p	Corr*	p	Corr*	p
D1	27.99 (24.15)	0.380	0.000	0.534	0.000	0.342	0.000	0.404	0.000	0.473	0.000
D2	38.89 (26.47)	0.370	0.000	0.537	0.000	0.297	0.000	0.366	0.000	0.441	0.000
D3	36.11 (23.62)	0.246	0.000	0.319	0.000	0.207	0.001	0.239	0.000	0.291	0.000
D4	50.44 (12.50)	-0.300	0.000	-0.358	0.000	-0.267	0.000	-0.310	0.000	-0.357	0.000
D5	50.72 (17.37)	0.353	0.000	0.213	0.001	0.161	0.014	0.206	0.002	0.237	0.000
D6	40.17 (21.15)	0.482	0.000	0.565	0.000	0.330	0.000	0.359	0.000	0.500	0.000
D7	41.10 (25.35)	0.376	0.000	0.417	0.000	0.201	0.002	0.289	0.000	0.351	0.000
D8	46.73 (16.10)	0.460	0.000	0.348	0.000	0.291	0.000	0.185	0.005	0.357	0.000

*Spearman's correlation coefficient.
D1. Physical functioning, D2. Role limitations due to physical health, D3. Pain, D4. General Health, D5. Energy/fatigue, D6. Social functioning, D7. Role limitations due to emotional problems, D8. Emotional well-being.

however, the impact of the condition on patients' physical functioning has been poorly studied. Tiredness/fatigue are common manifestations described in CD (56), but they do not severely compromise physical abilities. Nonetheless, some celiac patients may experience neurological manifestations (neuropathy and ataxia), which might affect the physical domain to a certain extent. Peripheral neuropathy usually manifests as tingling, pain, and numbness, primarily in the hands and feet (57).

Two dimensions measure the impact of health limitations due to role limitations arising from physical health (D2) or emotional problems (D7), considering the type and amount of work performed, the necessity to reduce work, or the challenges faced in carrying it out. D2 scores were higher for males, those with the lowest educational levels, and those not following the GFD. The role limitations due to emotional problems (D7) presented the lowest scores for females, those complying with the GFD and not using antidepressants.

The Pain dimension (D3) measures the intensity and discomfort caused by pain and how this interferes with normal work. D3 dimension was only affected by educational level, in which those with the highest educational level showed the worst score. Abdominal pain is a frequent symptom in celiac individuals, although more frequently found in childhood (58). Although a strict GFD improves CD clinical manifestations such as abdominal pain (59), participants from our study presented low scores in D3, possibly related to the poor diet compliance found in our sample.

Energy/fatigue dimension (D5) showed higher scores for females, those complying with the GFD, and those with the age of diagnosis > 20 y/o. Although fatigue is often reported among celiac individuals, it usually improves once the GFD is implemented by the patient (56), which is in accordance with our finding that those compliant with the diet had better scores for this domain.

Social functioning (D6) was higher in those with age at diagnosis up to 20 years old, with the lowest educational levels, and who did not comply with the GFD. The finding that participants who did not comply with the GFD had higher scores for the social functioning domain is not surprising. As mentioned above, although the restriction of gluten from the diet is essential to good health in celiac patients, it interferes with social situations in the patients' family, friends, and school/work environments (59). Wolf et al. (60) evaluated the association of QOL and GFD knowledge and adherence among 80 teenagers and adults. When asked about barriers to the GFD, 56% of adults and 70% of teens mentioned its adverse social impact. Feelings such as misunderstanding, embarrassment, stigma, exclusion, awkwardness, and guilt were expressed by participants (60).

The Emotional well-being dimension (D8) did not vary with sociodemographic data, or GFD compliance. Mental health problems have been documented in CD. Depression and/or depressive symptoms seem more frequent and/or severe in celiac patients than in healthy samples (61). Even though adherence to the GFD did not influence the D8 dimension in our sample, Sainsbury and Marques (61) suggest that poor diet adherence and self-reported depressive symptoms are associated, with the direction of causation being unclear. The authors mention that maintaining gluten on the diet may contribute to the appearance of a depressive state due to physiological mechanisms such as malabsorption of nutrients. On the other hand, being depressed compromises the individual's ability to provide self-care and implement a safe GFD.

Males presented better scores than females on D1, D2 and D7, and worse on D4 and D5. These data differ from the previous study performed in Portugal (44) in which gender differed in D3, D5 and D8 with best results from males. Interestingly, the "general health" domain of SF-36 showed a negative association with all CDQ domains, contrary to what the authors of this study would have anticipated. A Turkish study (23) performed in 2015 with 81 celiac participants who answered the CDQ and SF-36 questionnaires showed a correlation between both questionnaires for all domains, similar to what Hauser (13) found in a study performed with 463 German celiac patients and Marchese (31) in a study performed in Italy with 171 celiac patients. An important factor to consider analyzing our results is that nearly half of the subjects in the sample (45%, n = 105) did not adhere to the GFD. It might be possible that the SF-36 does not accurately capture the influence of the GFD on the QoL of celiac patients, as previously demonstrated in a study conducted in Portugal (40). Consequently, this limitation could potentially impact the interpretation of results for questions related to the GFD. It is expected that the complete elimination of gluten from the diet leads to the remission of symptoms, normalization of intestinal histology and reduced risk for other health complications associated with CD (53), which are necessary for good health status.

Another interesting point to consider in this regard is question 2 from the SF-36 v2. "Compared to one year ago, how would you rate your health in general now?". It might be reasonable to assume that this question, when applied to celiac individuals, would be influenced by diet compliance and time since the diagnosis. Patients who have received their diagnosis longer will probably have more tools to deal with difficulties related to the diet and the disease itself. There is evidence that more knowledge about CD and the diet, and support by health professionals and family improves the GFD compliance (53), all of which require time being diagnosed to be accomplished. Moreover, GFD effects on time until clinical improvement occurs and health depends on the length of time the patient remained undiagnosed due to the magnitude of intestinal mucosa damage (59).

This study presents some limitations. The sample comprised adult celiacs recruited using the snowball method by social media, leading to a possible selection bias due to a non-probabilistic sample. In this sense, our results may not represent the general Portuguese celiac people. In addition, despite the broad use of self-reported compliance to a GDF (34, 39, 48–52), we could not confirm the information (62), since data collection occurred online due to the COVID-19 pandemic restrictions, limiting the access to confirmation by laboratory tests. Despite the Portuguese Celiac Association has distributed the questionnaire to participants from all regions of Portugal to encompass the range of experiences and viewpoints of people living with CD in the country, the questionnaire did not ask for their exact location limiting the discussion about cultural and sociodemographic aspects.

5 Conclusions

In our study, seven out of the eight SF-36 v2 Portuguese Health Survey domains showed a positive correlation to the specific questionnaire CDQ. However, the "General Health" domain (domain 4) exhibited a negative correlation with the CDQ. Differences in content between the two instruments might be able to explain this finding, since the CDQ explores issues regarding specificities of CD and the lifelong GFD burden. Given that about half of the sample from this study displayed poor diet adherence, it is possible that the SF-36 could not reflect the impact of CD treatment - the complete elimination of gluten from the diet - on patients' health. This is a possible explanation for this result. Nonetheless, this issue should be carefully evaluated in future research.

Specific validated questionnaires for CD individuals, such as the CDQ, contemplate social, economic, and clinical variables that permeate the patient's life context. Therefore, these instruments may be more suitable for evaluating QOL in this public. However, using a general questionnaire such as the SF-36 would be indicated for comparing QOL between celiac patients and the general population or even between CD and other disease individuals. In this case, we recommend to parallelly assess GFD compliance for control.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The study was conducted in accordance with the American Psychological Association (APA) for research involving human subjects, and approved by the Ethics Committee of Polythecnic University of Viseu (59/SUB/2021). The studies were conducted in accordance with local and international laws and institutional guidelines. The participants provided their written informed consent to participate in this study.

Author contributions

CC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. RZ: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. AR: Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. EN: Formal analysis, Writing – review & editing. FR: Formal analysis, Funding acquisition, Investigation, Writing – review & editing. PF: Writing – original draft, Writing – review & editing. ET-L: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work is funded by National Funds through the FCT-Foundation for Science and Technology, I.P., within the scope of the projects Ref[a] UIDB/05507/2020 and UIDB/00681/202. The study was also supported by FAPDF N° 539/2022 and the Brazilian National Council for Scientific and Technological Development (CNPq -N° 302602/2021-6).

Acknowledgments

Authors would like to extend special acknowledgment to Associação Portuguesa de Celíacos (APC) and the students of the Health School of the Polytechnic University of Viseu, Ana Rita Santos, Carlota Oliveira, Carolina Nogueira, Christophe Oliveira, Dylan Oliveira. This work was supported by the FCT—Foundation for Science and Technology, Portugal. Furthermore, we would like to thank the Centre for Studies in Education and Innovation (CI&DEI), and the Institute Polytechnic of Viseu for their support. Renata Puppin Zandonadi acknowledges The University of Brasília, Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF) and the Brazilian National Council for Scientific and Technological Development (CNPq) for their scientific support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. McDermid JM, Almond MA, Roberts KM, Germer EM, Geller MG, Taylor TA, et al. Celiac disease: an academy of nutrition and dietetics evidence-based practice guideline. *J Acad Nutr Diet*. (2023) 123(12):1793–1807.e4. doi: 10.1016/j.jand.2023.07.018

2. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al. Global prevalence of celiac disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. (2018) 16:823–836.e2. doi: 10.1016/J.CGH.2017.06.037

3. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European society paediatric gastroenterology, hepatology and nutrition guidelines for diagnosing coeliac disease 2019. *J Pediatr Gastroenterol Nutr*. (2019) 70(1):141–56. doi: 10.1097/MPG.0000000000002497

4. Lohiniemi S, Mäki M, Kaukinen K, Laippala P, Collin P. Gastrointestinal symptoms rating scale in coeliac disease patients on wheat starch-based gluten-free diets. *Scand J Gastroenterol*. (2009) 35(9):947–9. doi: 10.1080/003655200750023002

5. Häuser W, Gold J, Stein J, Caspary WF, Stallmach A. CDQ Germany. *Eur J Gastroenterol Hepatol*. (2006) 18(7):747–53. doi: 10.1097/01.meg.0000221855.19201.e8

6. Pratesi CB, Häuser W, Uenishi RH, Selleski N, Nakano EY, Gandolfi L, et al. Quality of life of celiac patients in Brazil: questionnaire translation, cultural adaptation and validation. *Nutrients*. (2018) 10(9):1167. doi: 10.3390/nu10091167

7. Falcomer AL, Araújo LS, Farage P, Monteiro JS, Nakano EY, Zandonadi RP. Gluten contamination in food services and industry: A systematic review. *Crit Rev Food Sci Nutr*. (2018) 0:1–15. doi: 10.1080/10408398.2018.1541864

8. Itzlinger A, Branchi F, Elli L, Schumann M, Itzlinger A, Branchi F, et al. Gluten-free diet in celiac disease—Forever and for all? *Nutrients*. (2018) 10:1796. doi: 10.3390/nu10111796

9. Lobão ARF, Gonçalves RFLL, Monteiro RB, Castro FV. Qualidade de vida da pessoa celíaca adulta. *Int J Dev Educ Psychol*. (2010) 1:479–85. Available at: https://www.redalyc.org/pdf/3498/349832324051.pdf.

10. Fagerdahl A-M, Boström L, Ulfvarson J, Bergström G, Ottosson C. Translation and validation of the wound-specific quality of life instrument cardiff wound impact schedule in a swedish population. *Scand J Caring Sci*. (2014) 28:398–404. doi: 10.1111/scs.12050

11. Hauser W, Gold J, Stein J, Caspary WF, Stallmach A. Health-related quality of life in adult coeliac disease in Germany: results of a national survey. *Eur J Gastroenterol Hepatol*. (2006) 18:747–54. doi: 10.1097/01.meg.0000221855.19201.e8

12. Zingone F, Iavarone A, Tortora R, Imperatore N, Pellegrini L, Russo T, et al. The italian translation of the celiac disease-specific quality of life scale in celiac patients on gluten free diet. *Digestive Liver Dis*. (2013) 45:115–8. doi: 10.1016/j.dld.2012.10.018

13. Häuser W, Gold J, Stallmach A, Caspary WF, Stein J. Development and validation of the Celiac Disease Questionnaire (CDQ), a disease-specific health-related quality of life measure for adult patients with celiac disease. *J Clin Gastroenterol*. (2007) 41(2):157–66. doi: 10.1097/01.mcg.0000225516.05666.4e

14. Hughey JJ, Ray BK, Lee AR, Voorhees KN, Kelly CP, Schuppan D. Self-reported dietary adherence, disease-specific symptoms, and quality of life are associated with healthcare provider follow-up in celiac disease. *BMC Gastroenterol*. (2017) 17:1–8. doi: 10.1186/s12876-017-0713-7

15. Guennouni M, Admou B, Elkhoudri N, Bouchrit S, Ait Rami A, Bourrahouat A, et al. Quality of life of moroccan patients with celiac disease: arabic translation, cross-cultural adaptation, and validation of the celiac disease questionnaire. *Arab J Gastroenterol*. (2022) 23:246–52. doi: 10.1016/J.AJG.2022.06.009

16. Kurppa K, Collin P, Mäki M, Kaukinen K. Celiac disease and health-related quality of life. (2014) 5:83–90. doi: 10.1586/EGH.10.81

17. Real-Delor RE, Centurión-Medina IC. Quality of life in Paraguayan adults with celiac disease. *Duazary*. (2018) 15(1):61–70. doi: 10.21676/2389783X.2026

18. Leffler DA, Acaster S, Gallop K, Dennis M, Kelly CP, Adelman DCA. Novel patient-derived conceptual model of the impact of celiac disease in adults: implications for patient-reported outcome and health-related quality-of-life instrument development. *Value Health*. (2017) 20:637–43. doi: 10.1016/j.jval.2016.12.016

19. Casellas F, Rodrigo L, Molina-Infante J, Vivas S, Lucendo AJ, Rosinach M, et al. Transcultural adaptation and validation of the celiac disease quality of life transcultural adaptation and validation of the celiac disease quality of life (CD-QOL) survey , a specific questionnaire to measure quality of life in patients with celiac dis. *Rev Espanõla Enfermedades Digestivas*. (2013) 105:585–93. doi: 10.4321/S1130-01082013001000003

20. Casellas F, Rodrigo L, López Vivancos J, Riestra S, Pantiga C, Baudet JS, et al. Factors that impact health-related quality of life in adults with celiac disease: A multicenter study. *World J Gastroenterol*. (2008) 14:46–52. doi: 10.3748/wjg.14.46

21. Meyer S, Rosenblum S. Activities, participation and quality of life concepts in children and adolescents with celiac disease: A scoping review. *Nutrients*. (2017) 9:1–15. doi: 10.3390/NU9090929

22. Rodríguez-Almagro J, Hernández-Martínez A, Lucendo AJ, Casellas F, Solano-Ruiz MC, Siles-González J, et al. Health-related quality of life and determinant factors in celiac disease. A population-based analysis of adult patients in Spain. *Rev Esp Enferm Dig*. (2016) 108(4):181–9. doi: 10.17235/reed.2016.4094/2015

23. Aksan AAA, Mercanligil SM, Häuser W, Karaismailo E, Mercanlıgil SM, Häuser W, et al. Validation of the turkish version of the celiac disease questionnaire (CDQ). *Health Qual Life Outcomes*. (2015) 13:1–7. doi: 10.1186/s12955-015-0272-y

24. Al Nofaie N, Al Ahmadi J, Saadah O. Health related quality of life among saudi children and adolescents with celiac disease. *Saudi J Gastroenterol*. (2020) 26:26. doi: 10.4103/sjg.SJG_74_19

25. Ware JE, Kosinski M, Keller SD. *SF-36 Physical and Mental Health Summary Scales: A User's Manual*. Boston: The Health Institute. (1994).

26. Brazier JE, Harper R, Jones N, O'Cathain A, Thomas KJ, Usherwood T, et al. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. *Gen Pract*. (1992) 305:160–4. doi: 10.1016/S0140-6736(61)91704-4

27. Lins L, Carvalho FM. SF-36 total score as a single measure of health-related quality of life: scoping review. *SAGE Open Med*. (2016) 4:1–12. doi: 10.1177/2050312116671725

28. Burger JPW, Van Middendorp H, Drenth JPH, Wahab PJ, Evers AWM. How to best measure quality of life in coeliac disease? A validation and comparison of disease-specific and generic quality of life measures. *Eur J Gastroenterol Hepatol*. (2019) 31:941–7. doi: 10.1097/MEG.0000000000001432

29. Taft TH. When not to use a generic: measuring HRQoL in chronic digestive disease necessitates the use of disease-specific questionnaires. *Dig Dis Sci*. (2021) 66:3219–21. doi: 10.1007/S10620-020-06780-8/METRICS

30. Glise H, Wiklund I. Health-related quality of life and gastrointestinal disease. *J Gastroenterol Hepatol*. (2002) 17:S72–84. doi: 10.1046/J.1440-1746.17.S1.6.X

31. Marchese A, Klersy C, Biagi F, Balduzzi D, Bianchi PI, Trotta L, et al. Quality of life in coeliac patients: italian validation of a coeliac questionnaire. *Eur J Intern Med*. (2013) 24:87–91. doi: 10.1016/j.ejim.2012.09.015

32. Pouchot J, Despujol C, Malamut G, Ecosse E, Coste J, Cellier C. Validation of a french version of the quality of life "Celiac disease questionnaire". *PloS One*. (2014) 9:e96346. doi: 10.1371/journal.pone.0096346

33. Mahadev S, Gardner R, Lewis SK, Lebwohl B, Green PH. Quality of Life in Screen-detected Celiac Disease Patients in the United States. *J Clin Gastroenterol*. (2016) 50(5):393–7. doi: 10.1097/MCG.0000000000000433

34. Falcomer AL, Farage P, Pratesi CB, Pratesi R, Gandolfi L, Nakano EY, et al. Health-related quality of life and experiences of Brazilian celiac individuals over the course of the sars-cov-2 pandemic. *Nutrients*. (2021) 13:1582. doi: 10.3390/NU13051582

35. C D, Berry N, Vaiphei K, Dhaka N, Sinha SK, Kochhar R. Quality of life in celiac disease and the effect of gluten-free diet. *J Gastroenterol Hepatol*. (2018) 6(4):124–8. doi: 10.1002/jgh3.12056

36. Zysk W, Głąbska D, Guzek D. Social and emotional fears and worries influencing the quality of life of female celiac disease patients following a gluten-free diet. *Nutrients*. (2018) 10:1414. doi: 10.3390/NU10101414

37. Barzegar F, Pourhoseingholi MA, Rostami-Nejad M, Gholizadeh S, Malekpour MR, Sadeghi A, et al. Transcultural adaptation and validation of persian version of celiac disease questionnaire (CDQ); A specific questionnaire to measure quality of life of Iranian patients. *Galen Med J*. (2018) 7:e1106–6. doi: 10.31661/GMJ.V7I0.1106

38. Harnett JE, Myers SP. Quality of life in people with ongoing symptoms of coeliac disease despite adherence to a strict gluten-free diet. *Sci Rep*. (2020) 10:1–5. doi: 10.1038/s41598-020-58236-8

39. Selleski N, Zandonadi RP, Milde LB, Gandolfi L, Pratesi R, Häuser W, et al. Evaluation of quality of life of adult patients with celiac disease in Argentina: from questionnaire validation to assessment. *Int J Environ Res Public Health*. (2020) 17:7051. doi: 10.3390/IJERPH17197051

40. Henrietta S, Zsolt H, Gabriella dr V, Loránd Tudományegyetem E, és Pszichológiai Kar P, Doktori Iskola P. A szégyen mediációs szerepe a stigma és az életminőség kapcsolatában coeliakiában szenvedő Betegek körében.: A 8 tételes stigmatizáció Krónikus betegségekben kérdőív magyar adaptálása. *Orv Hetil*. (2021) 162:1968–76. doi: 10.1556/650.2021.32258

41. Lobão C, Gonçalves R, Monteiro BR. Development of the portuguese version of the celiac disease questionnaire. *Soc REVIEW. Int Soc Sci Rev / Rev Internacional Cienc Sociales*. (2013) 2:1–8. doi: 10.37467/GKA-REVSOCIAL.V2.1229

42. Chaves C, Raposo A, Zandonadi RP, Nakano EY, Ramos F, Teixeira-Lemos E. Quality of life perception among portuguese celiac patients: A cross-sectional study using the celiac disease questionnaire (CDQ). *Nutrients*. (2023) 15:2051. doi: 10.3390/nu15092051

43. Moreno M, de L, Sánchez-Muñoz D, Sousa C. Quality of life in teenagers and adults with coeliac disease: from newly spanish coeliac disease questionnaire validation to assessment in a population-based study. *Front Nutr*. (2022) 9:887573/BIBTEX. doi: 10.3389/FNUT.2022.887573/BIBTEX

44. Pimenta-Martins A, Pinto E, Gomes AMP. Perceção Do Estado de Saúde e Da Qualidade de Vida Numa Amostra de Celíacos Portugueses. *GE Jornal Português Gastrenterologia*. (2014) 21:109–16. doi: 10.1016/j.jpg.2013.09.006

45. Leighton K, Kardong-Edgren S, Schneidereith T, Foisy-Doll C. Using social media and snowball sampling as an alternative recruitment strategy for research. *Clin Simul Nurs*. (2021) 55:37–42. doi: 10.1016/j.ecns.2021.03.006

46. Webber-Ritchey KJ, Aquino E, Ponder TN, Lattner C, Soco C, Spurlark R, et al. Recruitment strategies to optimize participation by diverse populations. *Nurs Sci Q*. (2021) 34:235–43. doi: 10.1177/08943184211010471

47. Ferreira PL, Noronha Ferreira L, Nobre Pereira L. Medidas Sumário Física e Mental de Estado de Saúde Para a População Portuguesa. *Rev Portuguesa Saúde Pública*. (2012) 30:163–71. doi: 10.1016/j.rpsp.2012.12.007

48. Bulka CM, Davis MA, Karagas MR, Ahsan H, Argos M. The unintended consequences of a gluten-free diet. *Epidemiology*. (2017) 28:e24. doi: 10.1097/EDE.0000000000000640

49. Arámburo-Gálvez J, Carvalho Gomes I, André T, Beltrán-Cárdenas C, Macêdo-Callou M, Braga Rocha É, et al. Translation, cultural adaptation, and evaluation of a Brazilian portuguese questionnaire to estimate the self-reported prevalence of gluten-related disorders and adherence to gluten-free diet. *Medicina (B Aires)*. (2019) 55:593. doi: 10.3390/medicina55090593

50. Ontiveros N, Rodríguez-Bellegarrigue CI, Galicia-Rodríguez G, Vergara-Jiménez M, de J, Zepeda-Gómez EM, et al. Prevalence of self-reported gluten-related disorders and adherence to a gluten-free diet in salvadoran adult population. *Int J Environ Res Public Health*. (2018) 15:786. doi: 10.3390/IJERPH15040786

51. Silvester JA, Weiten D, Graff LA, Walker JR, Duerksen DR. Is it gluten-free? Relationship between self-reported gluten-free diet adherence and knowledge of gluten content of foods. *Nutrition*. (2016) 32:777–83. doi: 10.1016/J.NUT.2016.01.021

52. de Oliveira PM, Zandonadi RP, Cutrim AMV, Nakano EY, de Queiroz FLN, Botelho RBA, et al. Eating competence and aspects related to a gluten-free diet in Brazilian adults with gluten-related disorders. *Nutrients*. (2022) 14:2815. doi: 10.3390/nu14142815

53. Al-Toma A, Volta U, Auricchio R, Castillejo G, Sanders DS, Cellier C, et al. European society for the study of coeliac disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. *United Eur Gastroenterol J*. (2019) 7:583–613. doi: 10.1177/2050640619844125

54. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European society paediatric gastroenterology, hepatology and nutrition guidelines for diagnosing coeliac disease 2020. *J Pediatr Gastroenterol Nutr*. (2020) 70:141–56. doi: 10.1097/MPG.0000000000002497

55. Hadi MA, McHugh GA, Closs SJ. Impact of chronic pain on patients' Quality of life: A comparative mixed-methods study. *J Patient Exp*. (2019) 6:133–41. doi: 10.1177/2374373518786013

56. Jelsness-Jørgensen L-P, Bernklev T, Lundin K. Fatigue as an extra-intestinal manifestation of celiac disease: A systematic review. *Nutrients*. (2018) 10:1652. doi: 10.3390/nu10111652

57. Mearns E, Taylor A, Thomas Craig K, Puglielli S, Leffler D, Sanders D, et al. Neurological manifestations of neuropathy and ataxia in celiac disease: A systematic review. *Nutrients*. (2019) 11:380. doi: 10.3390/nu11020380

58. Leonard MM, Sapone A, Catassi C, Fasano A. Celiac disease and nonceliac gluten sensitivity. *JAMA*. (2017) 318:647. doi: 10.1001/jama.2017.9730

59. Bascuñán KA, Vespa MC, Araya M. Celiac disease: understanding the gluten-free diet. *Eur J Nutr*. (2017) 56:449–59. doi: 10.1007/s00394-016-1238-5

60. Wolf RL, Lebwohl B, Lee AR, Zybert P, Reilly NR, Cadenhead J, et al. Hypervigilance to a gluten-free diet and decreased quality of life in teenagers and adults with celiac disease. *Dig Dis Sci*. (2018) 63:1438–48. doi: 10.1007/s10620-018-4936-4

61. Sainsbury K, Marques MM. The relationship between gluten free diet adherence and depressive symptoms in adults with coeliac disease: A systematic review with meta-analysis. *Appetite*. (2018) 120:578–88. doi: 10.1016/j.appet.2017.10.017

62. Hall NJ, Rubin G, Charnock A. Systematic review: adherence to a gluten-free diet in adult patients with coeliac disease. *Aliment Pharmacol Ther*. (2009) 30:315–30. doi: 10.1111/j.1365-2036.2009.04053.x

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 23 April 2024
DOI 10.3389/fimmu.2024.1390755

OPEN ACCESS

EDITED BY
Simone Baldi,
Department of Experimental and Clinical Medicine, Italy

REVIEWED BY
Shailendra Katwal,
National Trauma Center, Nepal
Sadaf Dabeer,
Emory University, United States

*CORRESPONDENCE
Maria Oana Sasaran
oanam93@yahoo.com
Elena Jechel
elena.jechel@yahoo.com

†These authors have contributed equally to this work

RECEIVED 23 February 2024
ACCEPTED 03 April 2024
PUBLISHED 23 April 2024

CITATION
Lupu VV, Sasaran MO, Jechel E, Starcea IM, Ioniuc I, Mocanu A, Rosu ST, Munteanu V, Nedelcu AH, Danielescu C, Salaru DL, Knieling A and Lupu A (2024) Celiac disease - a pluripathological model in pediatric practice. *Front. Immunol.* 15:1390755. doi: 10.3389/fimmu.2024.1390755

Celiac disease - a pluripathological model in pediatric practice

Vasile Valeriu Lupu[1†], Maria Oana Sasaran[2*†], Elena Jechel[1*†], Iuliana Magdalena Starcea[1†], Ileana Ioniuc[1†], Adriana Mocanu[1†], Solange Tamara Rosu[1†], Valentin Munteanu[3†], Alin Horatiu Nedelcu[1†], Ciprian Danielescu[1†], Delia Lidia Salaru[1†], Anton Knieling[1†] and Ancuta Lupu[1†]

[1]Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania, [2]Faculty of Medicine, "George Emil Palade" University of Medicine, Pharmacy, Science and Technology, Targu Mures, Romania, [3]Faculty of Medical Bioengineering, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania

Being defined as an autoimmune, chronic pathology, frequently encountered in any age group, but especially in pediatrics, celiac disease (also called gluten enteropathy), is gaining more and more ground in terms of diagnosis, but also interest in research. The data from the literature of the last decades attest the chameleonic way of its presentation, there may be both classic onset symptoms and atypical symptoms. Given the impact played by celiac disease, especially in the optimal growth and development of children, the current narrative review aims to highlight the atypical presentation methods, intended to guide the clinician towards the inclusion of the pathology in the differential diagnosis scheme. To these we add the summary presentation of the general data and therapeutic lines regarding the underlying condition and the existing comorbidities. In order to place the related information up to date, we performed a literature review of the recent articles published in international databases. We bring forward the current theories and approaches regarding both classic celiac disease and its atypical manifestations. Among these we note mainly constitutional, skin or mucous, bone, neuro-psychic, renal, reproductive injuries, but also disorders of biological constants and association with multiple autoimmunities. Knowing and correlating them with celiac disease is the key to optimal management of patients, thus reducing the subsequent burden of the disease.

KEYWORDS

celiac disease, autoimmunity, child, atypical onset, associated pathologies, polymorphous clinical picture

1 Introduction

Representing almost a constant in pediatric cases of recent years, celiac disease (CD) is classified by specialists as being on a fine line between over- and under-diagnosis. The two variables have as a causal background the existence of atypical presentations of celiac disease (which turn out to be the same/more present than the classic form). Added to this is the tendency to diagnose exclusively on symptomatic criteria. In support of these two statements, we find the increase in recommendations for starting a gluten-free diet (GFD) (1). Trying to broadly define the disease, Holtmeier W. et al. notes the presence of objectified maldigestion and malabsorption in predisposed persons, in case of ingestion of gluten-based products (wheat, rye). The symptomatology is doubled by epigastric pain, abdominal flatulence, acceleration of intestinal transit, steatorrhea, weight loss, anemia, growth deficiency, osteo- articular, neurological diseases or infertility. In order to establish the diagnosis, digestive endoscopy with biopsy is performed, together with the dosage of anti-transglutaminase antibodies or, in particular cases, of other specific biomarkers. The histopathological examination shows the flattening of the jejunal mucosa and the infiltration with lymphoid cells (2, 3).

Although it represents the gold standard in the treatment of the condition, GFD seems not to be a universal "medicine", Veeraraghavan G. et al. emphasizing the existence of cases of children with non-receptive celiac disease. This is more common among girls, beginning with the slowing down of intestinal transit and abdominal pain, which cannot be controlled by an exclusion diet for at least 6 months (4). Among the risks of starting a GFD, we note the increase in the prevalence of nutritional deficiencies such as iron, ferritin, vitamin B12, folic acid and zinc among subjects exposed to this lifestyle (5). Adherence to the regimen is another key point, observed in only ¾ of the evaluated subjects (with a peak of failure among teenagers, caused by the absence of symptoms when ingesting small amounts of gluten), increasing compared to a decade ago (6).

Regarding the management, the Society for the Study of Celiac Disease reports as challenging the follow-up of the histological recovery of the damaged tissues due to the inconsistency with the clinical and serological presentation of the patient. Also, the hypothesis of a phenotypic division of the condition according to the clinical and immune characteristics is launched, the knowledge of which can improve the diagnosis rate and therapeutic efficiency (7). Screening is also important in the risk population (children with affected first-degree relatives), the curve of affect depending on sex, age and the identification of HLA-DQ (2/8) forms specific to the pathology. Meijer C. et al. noted the development of celiac disease among 135 out of 944 children included in this risk group. In them, a peak incidence was observed around the age of 4 years, with an increased ratio among girls compared to boys and homozygotes for the leukocyte antigen DQ2 (8).

Considering the wide spread of the pathology at the global level, but also the strong systemic noise triggered by it, we consider it opportune to know and raise awareness of the importance of early detection and adequate treatment of celiac disease. In this sense, we propose to develop the atypical forms of presentation among children and adolescents, along with general aspects of pathogenesis, diagnosis and therapeutic management.

We will therefore outline a general framework of signs and symptoms that should orient the clinician towards CD, by summarizing the data present in the specialized literature, obtained by accessing international databases: PubMed, EBSCO, Scholar, EMBASE. The selectedarticles will present current data, valid from a statistical point of view. To these are added future perspectives in CD therapy, intended to widen the horizons of interest in research. The text is mainly focused on the pediatric population. Since the pediatric population represents a vulnerable population, whose study imposes extremely strict ethical limits, and in order not to lose sight of particularly important aspects for the referenced pathology, we have enriched the reading with data on the adult population where there were not enough testimonies related to children. We are referring here mainly to the pathophysiological aspects of celiac disease or its manifestations. The data were therefore presented for illustrative purposes, to avoid the main reading biases that may arise from the omission of the theoretical data that are the basis of the acquisition of practical notions. Where this happened, we specified, avoiding to record percentages or definite statistical data due to their inconclusiveness with those in pediatrics. To create our database, we used general and specific terms related to "celiac disease", "diagnosis of celiac disease", "forms of celiac disease", "treatment of celiac disease" and a wide range of associated conditions. Finally, we reduced the risk of bias by including in the list of references both pro and con papers regarding the controversial information in the current scientific literature.

2 Epidemiology

Presenting two peaks of incidence (at 2 and 40 years respectively), depending on the onset of symptoms, CD is characterized by a real prevalence much higher than the estimated one. The average delay in diagnosis is approximately 4-10 years. Thus, it is recorded that, for each positively diagnosed patient, there are approximately 7-10 patients with a missed diagnosis (2, 9). Percentage, the prevalence is represented by 1% in the general population, 3.9% in the case of siblings of people with CD, 10-20% in the case of relatives and 75-80% for monozygotic twins. The gender ratio is 2:1 in favor of women. Although it is considered a frequent pathology among young children, research in recent years attests to the increase in the diagnosis rate among older children. This is partly due to the application of serological screening methods (IgA anti-tissue transglutaminase or endomysium antibodies) to those with atypical presenting symptoms or belonging to risk groups (9, 10). In adults, Llorente-Alonso MJ. et al. reports the high maintenance of CD cases among women (4:1 ratio in favor of men) (11). The diagnosis rate of CD is dependent on demographic indicators and ethnicity, presenting an increased density of cases in Europe, unlike in South America. The main theory underlying the demographic differences in the density of celiac disease cases mainly concerns the variability of the genetic factors involved in the pathogenesis [human leukocyte antigen

(HLA) and non-HLA genes]. On a secondary level, current medical literature also talks about the impact of environmental factors (e.g., wheat consumption, age at gluten intake, gastrointestinal infections, use of proton pump inhibitors or antibiotics, caesarean section rate and particularities of the breastfeeding process). All these aspects will be detailed further, from the perspective of extraintestinal manifestations (9).

The prevalence of extra-intestinal symptoms brings together, in descending order of occurrence, abdominal pain, poor weight curve, iron deficiency anemia, short stature, chronic constipation, skin manifestations (eczema) and reduction of bone mineral density (12, 13).

Regarding the factors that can precipitate the occurrence of autoimmunity and celiac disease, Andrén Aronsson C. et al. reports an increase in its risk proportional to each gram of gluten consumed per day (14). Another variable is represented by the type of birth, a physiological process that can leave an impression on the future course of the child in the sense of increasing the incidence of certain diseases (respiratory tract infections, asthma, obesity, disorders on the autistic spectrum, attention deficits or delays in neuro-psychic development). This aspect is generally attributed to an incomplete development of the infantile microbiota among infants born by caesarean section. To this are added particular aspects of the first years of life. However, the data regarding the impact of caesarean section, breastfeeding or the age of introduction of gluten in the diet on the increased risk of CD are contradictory (9, 15–17). Regarding the time of introducing gluten into the diet (6 versus 12 months), Lionetti E. et al. have objectified an increase in the incidence of autoimmunity related to celiac disease and CD in the first group. However, the difference was not maintained after the age of 5 years. The selective deficiency of immunoglobulin A (IgA) and particular HLA variants thus remain the most important predictors of the condition. However, genome-wide studies have found non-HLA risk factors common to other immune-related diseases (e.g., type 1 diabetes, rheumatoid arthritis, ulcerative colitis, and Crohn's disease) (18–20).

Similarly, a correlation was made between viral infections (e.g., rotavirus, adenovirus, enterovirus, hepatitis C or Epstein-Barr virus) and the development of CD, anticipating their possible involvement in modulating the individual response to gluten. This interrelation is possibly influenced by the existence of a sequence of 8-12 amino acids similar to the toxic gliadin fraction. Vaccination exerts protective effects among children with gluten intake before 6 months (9, 20).

In light of the recent Covid-19 pandemic, Cakir M. et al. demonstrates an increase in the diagnosis of CD individually or in association with diabetes in the period March 2020-June 2021, in contrast to the pre-pandemic period. In support of the positive correlation between the acute respiratory infection and the escalation of the CD incident, we find the positivity of the serological markers of passing through the disease in a percentage of 36.3% of the children diagnosed in the pandemic (21). In the opposite sense, Lionetti E. et al. report a similar prevalence of Covid-19 among CD subjects compared to the general population.

However, the development of more severe forms or burdened by complications is not noticeable in them (22). Similar findings in the reference period were also objectified in the case of other autoimmune pathologies, such as systemic lupus erythematosus, where there was a marked increase in the rate of relapse, diagnosis of new cases and even a decrease in therapeutic control in pre-existing cases (23).

3 Pathogenesis

The physio-pathogenic cascade of celiac disease originates, as we mentioned previously, in the triggering of the body's immune response as a result of the ingestion of gluten-based foods, in genetically susceptible individuals. The most common forms of human leukocyte antigen (HLA) associated with celiac disease are HLA-DQ2, HLA-DQ8 and, less commonly, HLA-DQ7. Despite the high degree of detection in the group of patients with symptomatic celiac disease, it is recorded that up to 20% of the general population possess these genes without manifesting a characteristic clinical picture during life. Thus, following the interaction between these and the deaminated peptide fragments, resulting from hard-to- degrade prolamins (gliadin, hordein, secalin, zein) under the action of tissue transglutaminase 2 (tTG2), CD4 T lymphocytes are activated. Following this, we encounter an increase in pro- inflammatory cytokines and chemokines, among which we distinguish interferon-gamma, interleukin 15 (IL-15), IL-2, IL-21 and tumor necrosis factor. The consequences are the infiltration of inflammatory cells and the promotion of cytotoxic CD8 T cells. Thus, atrophy of the villi of the mucosa, hyperplasia of the crypts and dysfunction of the intestinal barrier appear. These changes entail the promotion of pathogenic bacteria, the increase of translocation capacity and intestinal permeability, pathognomonic characteristics for celiac disease. In parallel, we aim to increase the production of antibodies directed at the import of tTG2 and gluten, important diagnostic markers, by B cells. Recently, Akbulut EU. et al. discuss the involvement of the IL-6 polymorphism (-572G/C) in dictating the susceptibility to the development of CD (2, 3, 7, 20, 24, 25). In addition to this mechanism, other factors that modulate the predisposition to CD include the premature cessation of breastfeeding and the gender of the patient, although the risk ratio for sexes in children is 1:1. The two exert their negative effect by decreasing the body's defense capabilities against exogenous infections, as well as by promoting a hormone-dependent pro-autoimmune status (3). Finally, we can also add the impact played by low birth weight, lack of *H. pylori* colonization, SARS-CoV-2 infection or smoking status (24). Focusing on *H. pylori* infection among patients with celiac disease, we notice that this is an intensely debated topic in the literature. Summarizing the significant data, we can conclude that the frequency of *H. pylori* infection is lower among CD patients compared to control groups, both for children and adults (26–28). Where the two entities coexist, it is noted that children show milder forms of enteropathy. Therefore, it has been hypothesized that *H. pylori* infection may confer some protection against the development of severe degrees of villous atrophy. The main pathophysiological mechanism of the protective effect exerted by *H. pylori* has been

attributed to a potential modulation of gluten immunogenicity among genetically susceptible patients (29).

In addition to the multitude of physiological processes that compete for the appearance of various pathologies, medical studies currently place the relationship between the disruption of the human microbiota and the escalation of the predisposition or the risk of associated comorbidities. In this sense, the balance of microorganisms, a constantly changing bioactive system, appears correlated with various diseases of the main organs (brain, heart, intestine, kidneys, lung, skin). The way in which the two entities communicate and influence each other is based on the existence of axes that connect the intestine with them. Thus, we found disturbances of the microbial flora in various atopies (asthma, dermatitis, food allergies), autoimmunity (systemic lupus erythematosus, CD, diabetes, autoimmune thyroiditis), organic insufficiency (cardiac, renal), neurological, oncological or metabolic disorders. To these are added inflammatory conditions (e.g., pancreatitis), respiratory infections, irritable bowel, gastroenteritis, esophagitis, gastroesophageal reflux disease or diverticular disease, pathologies frequently encountered in medical practice (30–36). The oral and intestinal microbiota of patients with CD is strongly influenced by a variety of factors, starting from the impact of genetic determinants, the environment (antibiotics, infections) and even the gluten-free diet. Their accumulation determines a state of microbial dysbiosis that affects the ability to take up and integrate peptides from food, being still under research if this is one of the causes or the effect of CD. The main microorganisms involved in the digestion of gliadin at the oral level are *Rothia, Actinomyces, Neisseria* and *Streptococcus*, while in the intestines of patients with active CD, an increase in *Proteobacteria* (e.g., *Neisseria*) was found in parallel with a decrease in *Firmicutes* and *Actinobacteria*. Thus, the hypothesis was outlined that in the small and large intestines of patients there are bacterial genera that influence the digestion of gliadin (e.g., *Lactobacillus, Streptococcus, Staphylococcus, Clostridium, Bifidobacterium*), their proteolytic activity being dictated by the amount of gluten ingested (20, 37). In this sense, current research focuses on the possible impact played by *Flavobacterium meningosepticum*, a bacterial endopeptidase that appears to be able to digest proline-rich peptides. However, the complete prevention of gluten toxicity by enzyme therapy is still controversial (2).

The systemic consequences of introducing a diet based on the exclusion of gluten are also worth mentioning. In this situation we can encounter deficiencies in vitamins, minerals or dietary fibers (source of short-chain fatty acids). Diet with excess fat can also precipitate an escalation of the risk of cardiovascular, metabolic diseases or body weight (in the sense of overweight or obesity) in the case of patients who do not benefit from adequate nutritional counseling. To this is added the influence played by nutrients such as vitamin A, vitamin E, selenium, calcium, iron, magnesium, zinc, omega-3 fatty acids, phytoestrogens or flavanols in the regulation of T cells and cytokine production. This balance is considered vital in the evolution of patients suffering from autoimmunity (38, 39).

4 Description of classic celiac disease

Due to the chameleonic ways of presentation, the diagnosis of celiac disease must be based on a complex and well-established protocol, made up of the corroboration of clinical and paraclinical data. The ultimate goal of following such protocols is primarily to reduce the risks of practitioners missing the correct diagnosis. Added to this are the benefits of early diagnosis and management of the pathology, aspects that improve the quality of life and "disabilities" of patients. In the following, we draw general lines in the recognition and certification of celiac disease in children, and then we draw attention to the particular forms of presentation.

Therefore, CD diagnosis starts gradually, from non-invasive to invasive, the choice of method being made according to the individual risk of each patient. Biologically, serology includes endomysial antibody (EMA), tissue transglutaminase (tTG) and deamidated gliadin peptide (DGP) assays, high sensitivity and specificity tests. Given the increased frequency in the general population of haplotypes associated with CD, despite the weak correlation with overt disease, HLA typing is considered to have a negative predictive value rather than a positive one. More invasive but considered the gold standard in difficult diagnostic cases (IgA deficiency, discrepant serology and the initiation of a gluten-free diet prior to testing), we find endoscopy doubled by intestinal biopsy (40).

Depending on the way of presentation and the response to the initiation of supportive therapy, children may be at risk of manifesting CD or may present the silent, with negative serology, refractory or non-responsive form. Cases with villous involvement but with characteristic negative serology require differential diagnosis with parasitic infection with *Giardia lamblia*, immunodeficiency, autoimmune or drug-induced enteropathy/in association with human immunodeficiency virus, Crohn's disease or intestinal lymphoma (2, 9). In support of these statements, Oliveira GN. et al. notes, following the analysis of 159 patients, a prevalence of the classic disease of 60%, while the non-classic form was found in 25% of children.

Regarding the subclinical or potential forms, their prevalence was estimated at 5, respectively 10% of the number of cases (41). Among these forms, we choose to detail refractory CD, defined as the persistence of clinical symptoms and histological changes, generally unaccompanied by the escalation of autoantibody titers, despite a GFD followed for at least 12 months. The importance of its awareness resides in the increased risk of association with malignancies (intestinal lymphoma with T cells). Its prevalence is approximately 5% of the total cases of CD, being subdivided into two forms. For an easier presentation of the two forms (I and II), we refer to the exhaustive descriptions of type I - normal intraepithelial lymphocytic phenotype and, respectively, type II - clonal intraepithelial lymphocytic phenotype. The latter can be detected by means of immunohistochemistry, polymerase chain reaction methods or, more recently, flow cytometry. The definition of the clonal phenotype includes the loss of the normal surface markers CD3, CD4 and CD8 with preserved expression of intracytoplasmic CD3 (CD3ϵ) in >50% of intraepithelial lymphocytes (on the sample

analyzed by immunohistochemistry) or >20%–25% (on the sample analyzed by cytometry in flow), doubled by the detection of T cell receptor chains (γ or δ) clonal rearrangement by polymerase chain reaction. The prognosis of type I is much better compared to type II, showing a better clinical/histological response to steroids or other immunosuppressive or biological drugs and less potential for lymphoma-malignancy (3, 9, 42).

4.1 Clinical

In celiac disease, the clinical picture of the pathology is in the first phase dependent on the age of diagnosis, without showing a significant correlation with the degree of damage to the intestinal mucosa. It should be noted that this has registered a significant increase in the last decade. The pathognomonic sign of CD is diarrhea with steatorrhea which, in small children, is doubled by anorexia, vomiting, flatulence and abdominal discomfort. Subsequently, these progress towards growth retardation, severe malnutrition, nutritional deficiencies, anemia or delay in the onset of puberty. It is noted that with advancing age, the clinical picture may become non-specific/atypical (2, 9, 24, 43). In this situation, the intestinal histology damage is more pronounced than in the classic/asymptomatic forms of the disease (44). The presence of age-related disease patterns was also studied by Tanpowpong P. et al. They demonstrated, through their analysis of 411 children and adolescents diagnosed with CD by biopsy, that age-dependent variations were present more frequently in the case of classic, gastrointestinal symptoms. On the other hand, age did not represent a significant variable in the case of non- specific symptoms (45).

Given the inclusion of the condition in the field of gastrointestinal pathologies, its initial extra-intestinal manifestations are considered atypical. Found in a large percentage of cases, the most important to know are small stature, skin-mucosal manifestations, osteoarticular, dental or skin appendages (hair) damage, endocrinopathies, hematological or neuro-psychic disorders. To these is added an increased risk of developing autoimmunity up to 10 times compared to the general population (3, 9). Being the basic theme of this article, all these manifestations will be detailed later.

4.2 Serological

The course of serological diagnosis of CD is well established based on protocols. Currently, the recommendations indicate the beginning of investigations by dosing IgA anti-tTG antibodies by enzyme-linked immunosorbent assay (ELISA) or, rarely, by radioimmunoassay (RIA). Due to the incrimination of gluten as the main trigger in the induction of symptoms, as well as the use of GFD as the current therapeutic gold standard, the necessity of testing during a normal diet, which includes gluten, is understood. This must precede the time of testing by at least 6 weeks. If it is impossible to achieve this, a gluten challenge test can be used (3-7.5 g/day, for 14 days). For the purpose of appropriate interpretation, the investigations must be completed by the evaluation of the serum IgA titer, to avoid cases of superimposition of normal values of anti-tTG over a selective immune deficiency. In order to obtain a reliable diagnosis in the case of a selective immune deficiency of IgA, the values of IgG antibodies are measured (9, 24, 46). However, it is necessary to clearly distinguish the selective IgA deficiency from the partial one. The latter is defined as total IgA with more than 2 standard derivations below the age average. In this case tTG IgA showed sensitivity up to 100% (47). A special recommendation is advanced in the case of children younger than 2 years where the immunoglobulin G (IgG) DGP test must be performed, due to the low sensitivity of tTG in this age group (9, 40). Liver damage and inflammation of the small intestine can also interfere with serological test results, precipitating a possible false-positive result (3).

The interpretation of positive values of anti-tTG IgA antibodies is reported according to a limit that exceeds 10 times the normal value. Depending on this, the need to perform a biopsy for diagnostic purposes is decided. Once this level is exceeded, the damage can be confirmed exclusively based on clinical-serological criteria (with the mandatory inclusion of anti-EMA antibodies in the protocol) with/without HLA typing. On the other hand, the positivity of IgG antibodies in the case of IgA deficiency does not exclude the biopsy, regardless of their value. In conclusion, the usefulness of serological testing in evaluating adherence to therapy should not be omitted. Thus, the markers become negative gradually, starting with the first half year after the initiation of the GFD (9, 46, 48).

4.3 Histopathologic

Although changes such as mucosal fissures, nodular mucosa (mosaicism), visible vascularization of the submucosa, bulbar atrophy or reduction of Kerckring folds are specific to CD, up to a third of newly diagnosed patients present a “clean” endoscopic image. Thus, for certainty, a puncture-biopsy with histopathological examination is recommended. The target biopsy area is represented by the duodenum. This is done in four quadrants doubled by collecting a sample from the level of the bulb. The preference for a multilocular pattern of analysis resides in the uneven intestinal touch encountered in CD. The main aspects that must be followed when objectify the diagnosis include the height of the intestinal villi, the depth of the crypts and the number of intraepithelial lymphocytes per 100 enterocytes (46, 49, 50). The most faithful way of classifying the histological forms of CD is according to the Marsh classification, brought up to date in the form of Marsh-Oberhuber and divided into six levels. Another way of classifying the intestinal damage is represented by the division into two groups (A – non-atrophic and B – atrophic). While group A targets an isolated increase of intraepithelial lymphocytes, group B (subdivided into B1 and B2) analyzes the inversion of the ratio of intestinal villi/crypts (normally over 3/1). However, the histological changes must be differentiated from other pathologies with similar manifestations. Among these we list autoimmune or chronic inflammatory conditions, *Helicobacter pylori* and other

gastrointestinal infections, use of non-steroidal anti-inflammatory drugs or proton pump inhibitors, hypogammaglobulinemia (3, 49). Regarding atypical forms, Semwal P. et al. they reiterate the increase in their prevalence with advancing age and the main forms of presentation. To this are added findings regarding the lower risk of objective damage with the help of upper digestive endoscopy/ histopathological examination in atypical forms, contrasting with classic ones. At the same time, histologically normal samples were found in increased numbers in the non-classical forms (51).

4.4 Pitfalls in diagnosis

Being faced with a complex pathology, with a variety of forms of presentation, CD evaluation can present a challenge for the clinician. In addition to this, the uncertainty hangs over the contexts in which screening is recommended, but also regarding the usefulness of classifying the disease in asymptomatic cases (7). Crossroads in the diagnosis and management of CD can refer to situations such as the young age of the patient, the discrepancy between the serological values and the histopathological changes, the objectification of increased intraepithelial lymphocytes (>25 IELs/100 enterocytes) without altering the villous structure or following a GFD at the time of investigation (40). Some of these may be attributed to uneven involvement of the duodenal mucosa, low gluten intake, or inappropriate biopsy orientation (9). Also, false negative results other than those stated above may appear in the case of the use of corticosteroids or immunosuppressive medication (48). In this sense, we discuss the findings of Kav T. et al. regarding the utility of enteroscopy in diagnosis. They emphasize its usefulness in the case of discordant serology-histopathology, in identifying locations of interest for biopsy, but also in the long-term follow-up regarding possible complications. To increase the efficiency of the method, the benefit of adding immersion in water is brought to the fore (52). His findings were also supported by subsequent studies, which emphasized the importance of monitoring intestinal changes in patients with CD with the help of the video capsule. The main arguments were represented by the ease of automating the investigation and thereby eliminating the possibility of bias (53, 54). In agreement with what was stated previously, in order to eliminate any diagnostic error, the initiation of a regular regimen, which includes gluten, is called for. Other minimally invasive ways to assess intestinal integrity in CD are double-contrast enema, small bowel ultrasound, MR enteroclysis and CT enterography. These are not currently included in the international guidelines as diagnostic methods of CD. However, they are useful and mainly used in follow-up, in the detection of intraluminal, mural (e.g., inversion of the jejunoileal fold pattern, transient intussusceptions, assessment of small intestine motility), mesenteric (e.g., enlarged mesenteric lymph nodes, mesenteric vascular engorgement) abnormalities mesenteric and transient intussusceptions) and intraabdominal (e.g., intestinal dilatation, increase in the volume of the gallbladder, free abdominal fluid) specific to CD and its complications (55–59). Thus, CD can most likely be excluded in the context of maintaining negative serological and histopathological results, despite a gluten-containing diet for 6-12 months (49).

Since most diagnostic variants are subject to bias, there may be false negative or false positive variations in interpretation in certain situations, Figure 1 details, in accordance with those previously presented, the optimal sequence to follow in order to obtain the most accurate diagnosis of CD.

5 Atypical forms of celiac disease

Comparing the classical form of presentation among young children, with the non-classical one encountered more frequently with advancing age, we conclude that, as researched and common in practice as this pathology has become in recent years, the more unclear it is for us still the whole range of manifestations that can accompany it. We can therefore say, without exaggerating, that the atypical form of CD is beginning to gain more and more prominence both in its description and in diagnosis (60, 61). Analyzing a group of 78 patients, Iwańczak B. et al. concludes that the prevalence of atypical celiac disease closely followed the classic form of the pathology. The dominant symptomatology was represented by recurrent abdominal pain, failure to thrive, short stature, anemia, altered liver enzymes, food allergy, and associated thyroid disorders (62). We can add to this long list of chameleon-like clinical manifestations, according to Cooney MJ. et al., intermittent, noncyclic fever, of unknown origin. The observation was raised in the case of a 16-year-old girl with serological and histological confirmed CD later. At the initiation of the GFD, the clinical symptoms and the changes in the paraclinical indices remitted (63). The literature records other accompanying pathologies, which later proved to mask celiac disease. Among these we note duodenal ulcer, recurrent (pseudocystic) pancreatitis, splenomegaly (important to differentiate from a lymphoproliferative disorder), urolithiasis, severe thrombocytosis, central retinal vein occlusion or Henoch-Schoenlein purpura (64–70). Regarding the variation in the level of tTG antibodies between the two types of the disease, Aleksandra B. et al. exclude this hypothesis based on a retrospective study (71). These represent only a part of the polymorphism characteristic of CD. Thus, the most accurate outline of the

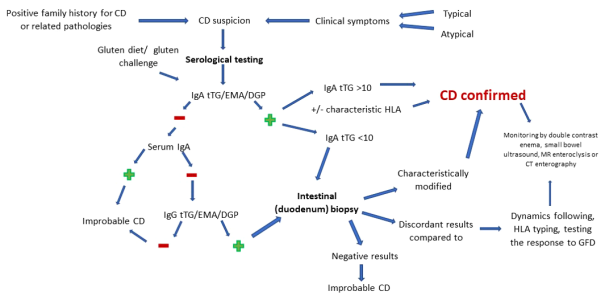


FIGURE 1
CD diagnostic scheme.

10.3389/fimmu.2024.1390755

systemic manifestations that can accompany celiac disease is vital in differentiating it from symptomatically similar conditions. Among these we note anorexia nervosa, autoimmune enteropathy/HIV, infectious/eosinophilic gastroenteritis, enteritis, lactose/soy intolerance, bacterial superinfection, Crohn's disease, hypogammaglobulinemia, Whipple's disease or Zollinger- Ellison syndrome (72). Finally, to make the distinction between the two forms of celiac disease (typical versus atypical), Table 1 compares their main clinical features.

Currently, given the multitude of studies in the field, the heterogeneous presentation of celiac disease should no longer represent an obstacle in the timely identification of the diagnosis. In order to facilitate this process, we detail below the main atypical forms of CD manifestation, summarized in Table 2 (adapted from subsection 5). At the same time, we outline the possible causes that determine their appearance, but also means of therapeutic approach where they can improve the prognosis or the quality of life.

5.1 Deficient development curve

Children's height is evaluated by comparing it with the anthropometric standards characteristic of each age group. Thus, the child is classified as small stature when his height falls below two standard deviations relative to the age-specific percentile. Disturbance of growth in height requires an extensive assessment carried out mainly with the aim of early diagnosis of the etiology and the initiation of appropriate treatment to be able to recover the deficit (73). Isolated short stature has been reported globally in 10% to 47.5% of celiac patients. The data are also supported by a study conducted on 104 Iranian children, who noted a prevalence of celiac disease of 33.6% among children with stature deficiency. The

TABLE 1 Clinical variations characteristic of typical versus atypical celiac disease.

Type of the condition	Typical celiac disease	Atypical celiac disease
Clinical manifestations	diarrhea with steatorrhea anorexia vomiting flatulence abdominal discomfort growth retardation severe malnutrition nutritional deficiencies anemia delay in the onset of puberty	small stature skin-mucosal manifestations osteoarticular, dental or skin appendages (hair) damage endocrinopathies hematological or neuro-psychic disorders increased risk of developing autoimmunity recurrent abdominal pain altered liver enzymes food allergy intermittent, noncyclic fever, of unknown origin accompanying pathologies: duodenal ulcer, recurrent (pseudocystic) pancreatitis, splenomegaly (important to differentiate from a lymphoproliferative disorder), urolithiasis, severe thrombocytosis, central retinal vein occlusion or Henoch-Schoenlein purpura

TABLE 2 Atypical manifestations of CD depending on the location of the damage (adapted from subsection 5).

Type	Associated manifestations
Constitutional features	– Stature deficit – Excess weight
Biological disorders	– Anemia (iron deficiency, inflammatory, aplastic thalassemia) – Elevated transaminases – Hypoalbuminemia – Coagulopathy – Severe thrombocytosis – Anti-phospholipid antibodies – Hydro-electrolytic imbalances
Systemic disorders	General: – Non-alcoholic steatohepatitis – Hypotension – Epistaxis/ecchymoses/ hemorrhages – Intermittent, non-cyclical fever – Duodenal ulcer – Recurrent (pseudocystic) pancreatitis – Splenomegaly – Central retinal vein occlusion – Henoch-Schoenlein purpura – Intestinal intussusception
	Cutaneous and mucous membranes: – Dermatitis herpetiformis – Atopic dermatitis – Pemphigus – Linear IgA bullous dermatosis – Chronic urticaria – Psoriasis – Vitiligo – Rosacea – Pellagra – Hereditary angioneurotic edema – Vasculitis – Nodosum/necrolytic migratory erythema – Behçet's disease – Dermatomyositis – Oral lichen planus – Hypertrichosis – Damage to the dentition (delayed tooth eruption, mineralization defects, dental caries) – Lesions of the oral mucosa (recurrent canker sores, ulcers, erythema, cheilitis, atrophic glossitis) – Dryness and burning sensation of the tongue

(Continued)

10.3389/fimmu.2024.1390755

TABLE 2 Continued

Type	Associated manifestations
	Renal: – Renal diabetes – IgA nephropathy – Glomerulonephritis – Renal damage with minimal damage – Focal and segmental glomerulosclerosis – Nephrotic syndrome – Urolithiasis – Urinary infection – Distal tubular renal acidosis
	Bone and joint damage: – Hypomotility – Proximal muscle weakness – Arthralgia – Osteoporosis – Osteopenia – Increased risk of fracture
	Neuropsychiatric disorders: – Gluten ataxia Peripheral neuropathy (Guillain-Barrè syndrome) – Headache – Migraine – Epilepsy – Depression – Anxiety – Cognitive-behavioral disorders (dementia, memory disorders, acalculia, lack of attention, executive or spatial orientation deficits)
	Reproductive: – Delayed puberty – Irregularity of menstruation – Amenorrhea – Early menopause – Risk of spontaneous abortion – Children with low birth weight – Reduced breastfeeding capacity – Stillborn children
Other associated diseases	Autoimmunities: – Diabetes mellitus – Hashimoto's thyroiditis – Graves' disease – Addison's disease (adrenal insufficiency) – Systemic lupus erythematosus – Secondary hyperparathyroidism – Ovarian insufficiency – Pituitary disorders – Autoimmune hemolytic anemia – Autoimmune hepatitis
	Notable: – Down syndrome – Asthma

authors also emphasize the important correlation between growth retardation and positivity of CD-specific serological screening tests and histological morphology (9, 74). Similar findings were reported in Saudi Arabia, Assiri AM. et al. supporting the link between small stature and CD, although their research objectified a prevalence of only 10.9% of the total number of included patients (75). Currently, it is considered that up to 1/3 of children with short stature may have associated CD, independent of the age and gender of the patients, but this is controversial (76, 77). In order to practically exemplify the usefulness of knowing the connection between the two entities, we bring into discussion the case of a 4-year-old girl, who was in the records since the age of 4 months for low growth rate, accompanied by malnutrition, without systemic damage (gastrointestinal, renal or endocrinological). In dynamics, serological values, HLA genotyping and histological examination finally confirmed the diagnosis. With the initiation of the GFD, the growth deficit experienced a significant improvement (78). Similar effects were observed by Soliman AT. et al. two years after starting the GFD, both in terms of waist and body mass index (79). As a result, we can conclude that the main factor that imprints the stature deficit among children with celiac disease is represented by dietary gluten. Extrapolating the findings, we can state that the wide range of short stature prevalence in CD, variable from a demographic point of view, can be largely attributed to the variability of the gluten concentration to which affected children are exposed.

Although this can be assimilated as a consequence of malnutrition, its detailed investigation is important especially among patients whose puberty has already been triggered. The literature attests to a height compensation 24 months after the initiation of GFD. However, results are poorly represented post-puberty. Pre-puberty, however, it is important to consider the differential diagnoses of small stature (e.g., growth hormone deficiency, inflammatory bowel disease, Turner syndrome). These can be solved by appropriate treatment, the mistake of assigning them exclusively CD can significantly decrease the chances of reaching an optimal final size (44). Newer theories regarding small stature associated with CD aim at the involvement of a dysfunction in the balance of insulin-like growth hormone (IGF1) and ghrelin. CD therefore appears to manifest itself by decreasing their levels, together with those of binding proteins 1 and 3. The theories underlying this dysfunction aim either at an association of autoimmunity directed against the pituitary gland, or at a disturbance supported by systemic inflammation (80). Giovenale D. et al. recommends, based on a large-scale retrospective study, the evaluation of serum GH levels among children who do not show an improvement in the growth curve despite GFD (81). Therefore, we can conclude based on the studies in the literature that CD and GH deficiency represent two key entities in pediatric practice, especially during the growing period. They can coexist, so the objectification of one should not stop the clinician from excluding the other. The optimal treatment in this case aims at both the supplementation with recombinant growth hormone and the therapy of the underlying gastrointestinal disorders (82, 83).

5.2 Excess weight

Although the body weight of patients with CD most often follows a decreasing trend, clearly attributed to nutritional and absorption deficiencies, the occurrence of weight gain or even obesity cannot be neglected. This manifestation can accompany the pathology from the beginning, or it can appear later in evolution. The physiological bases of this paradoxical clinical manifestation have tried to be explained by means of two theories. The "compensatory hypothesis" is defined as the increased absorption capacity of the distal intestine, a segment that undergoes a functional adaptation following duodenojejunal atrophy. Considering the fact that the intestinal compensatory surface increases with age, this theory is considered to be in agreement with the peculiarities of CD depending on the life stage in which the patient is. Another theory is that of gluten withdrawal. This presupposes the improvement of the intestinal absorption capacity due to the healing of the mucosa after initiation of GFD, as well as the inclination towards a diet with a high content of proteins and lipids, with a high content of sugars and low fiber among these patients (84). However, studies on adults do not attest to a direct correlation between the patient's nutritional status and the variability of gastrointestinal symptoms (85).

According to the latter theory, Rodrigues M. et al. report a more significant increase in weight in the first two years of initiation of therapy. Similar results were obtained by Radlović N. et al., although they emphasize the absence of a significant difference between adherent and non-adherent patients to therapy (86, 87). The specialized literature records that excess weight in newly diagnosed patients is more frequently associated with abdominal pain as an accompanying manifestation. The importance of screening among children with excess weight, with recurrent headaches of unspecified etiology, as well as close relatives of patients with CD who show excessive growth of body mass, is emphasized (88, 89).

5.3 Disorders of biological constants

Anemia is one of the most common extra-intestinal manifestations associated with CD. This can be the meeting in various forms, from iron deficiency anemia to thalassemia, inflammatory anemia or other types, as observed by Sanseviero MT. et al. From their reports, it appears that the gender distribution is approximately 2-1 in favor of girls. Also, cases of aplastic anemia associated with CD have been reported. The causality underlying it can be multiple, starting from nutritional deficiencies of iron, folate, vitamin B12 (due to villous atrophy or iron blocking in stores), blood loss and reaching the consequences of chronic pro-inflammatory status. In addition to these, in the case of CD, the polymorphism of the apical divalent transporter (DMT1). In case of massive iron loss due to villous atrophy and decreased absorption capacity, it may become unable to compensate despite its tendency to overexpress. Depending on these, the characteristics of erythrocytes vary (volume, color).

Regarding the serum level of iron and ferritin, we note that they are low, the value of hepcidin is variable, being able to even reach an increase in some cases. Similar findings are observed in the case of IL-2, which also recorded an increase (90–96). Other studies attest to an increase in ferroportin, doubled by the decrease in hephaestin, among children with celiac disease, compared to healthy ones (97).

Knowing and balancing these deficiencies is important especially at the pediatric age, when hyposideremia can negatively influence neuropsychological development. A known fact regarding the CD-anemia relationship is the disruption of the effective response to supplementation with oral preparations based on iron. In this case, the correction of the anemia will either follow the natural evolution of the CD therapy, or it will be done with intravenous preparations, depending on its severity. This interrelation was also demonstrated by Shahriari M. et al. in a study that targeted 184 children and adolescents. The authors thus emphasize the importance of early screening for the complications of celiac disease and their prevention (98, 99). Regarding the course of the underlying disease, the influence of anemia on the therapeutic response of GFD remains under investigation. However, the objectification of anemia since the CD diagnosis coincided with a more severe serological and histological profile. Also, the ability to return to normal hemoglobin was decreased, they recorded decreased values compared to the non-anemic group even after a year of GFD (100, 101).

In addition to affecting hemoglobin and nutritional components, CD has also been shown to be associated with the disruption of liver parameters and function. The main theory underlying this link is the interaction between toxic substances and the liver parenchyma.

These occur mainly due to the alteration of intestinal permeability that allows their passage into the portal circulation, inducing inflammation with liver lesions. Besides this, the involvement of immune factors cannot be neglected either, an aspect certified by the objectification of liver deposits of anti-tTG antibodies (80, 102). Also, in agreement with what was stated previously, the initiation of GFD can predispose to weight gain, especially in the first years. This disruption of body balance, together with the change in intestinal permeability, have been shown to be correlated with the increased risk of developing non-alcoholic steatohepatitis/fatty liver and metabolic syndrome. The stated pathological processes are chronic, with slow evolution and severity proportional to the duration. The risk was higher in the first years after the diagnosis but persisting up to 15 years after that. At the same time, it was higher among men compared to women and among children (103–105). From a biological point of view, it seems that elevated levels of liver enzymes (transaminases) are more common among young children with CD, increasing with intestinal alteration. They show a correlation with specific celiac disease antibodies, their increased titers being recorded in cases of hypertransaminasemia (106).

Therefore, the hepatic manifestations of CD should not be neglected. Ignoring this correlation leads to vices of etiological attribution. Severe insufficiency can be reached in time, with the need for grafting in order to restore the homeostasis of the internal

environment, consequences that could be avoided by excluding gluten at the right time (107–109). Other biological disorders include hypoalbuminemia and disturbances at different levels of the coagulation cascade, platelet abnormalities, the presence of anti-phospholipid antibodies or vitamin K deficiencies. All these disorders underlie the predisposition to generalized edema and hemorrhagic or thromboembolic events, characteristic of CD (110, 111). Cases of diffuse infiltration, hydro-electrolytic imbalances, hypotension, epistaxis, spontaneous ecchymoses and even intramuscular hemorrhage occurring in the context of coagulopathy or celiac crisis are recorded in adults and children (112–114).

5.4 Skin and skin appendages manifestations

We can say without exaggerating about the skin that it represents the map of the body. Thus, the relationship between the body and the skin is bidirectional. Both the disturbances of the internal environment are reflected at its level, and vice versa, the disturbance of its integrity determines consequences at the systemic level (115). Regarding the main mechanisms incriminated in the appearance of skin lesions in CD, it seems that they are represented by the generation of immunoglobulins, pro-inflammatory cytokines and circulating immune complexes in response to the reactions carried out in the intestinal submucosa (116). Many works have been written regarding the skin manifestations encountered in the atypical form of CD. Summarizing, Abenavoli L. et al. review the most important conditions that presented statistical significance based on the analysis of randomized studies, cohort studies, case series or systemic reviews. Among these we note vesicular diseases (pemphigus, dermatitis herpetiformis, bullous linear IgA dermatosis), urticaria, psoriasis, vitiligo, rosacea, pellagra, hereditary angioneurotic edema, atopic dermatitis, cutaneous vasculitis, nodosum/necrolytic migratory erythema or elevatum diutinum, Behçet's disease, dermatomyositis, oral lichen planus or acquired hypertrichosis (117). Similar findings were presented by Turjanmaa E. et al. They also emphasized that the severity of the skin manifestations did not vary between the study groups (118). Also, these conditions can overlap, there are forms of urticaria herpetiform dermatitis described in the literature (119). In order not to distract attention from the frequently encountered skin manifestations, we choose to detail them in the following.

Dermatitis herpetiformis (also called centrifugal annular erythema) is characterized by a vesicular, itchy rash, distributed mainly on the elbows, knees, sacral region and buttocks. Occasionally, the upper back, abdomen, scalp or face can also be affected. The reasoning behind this preference for extensor surfaces resides in the fact that they are much more prone to injury/inflammation due to the bending action of the joint. At the same time, during flexion, periarticular capillaries and nerves are stretched. Definitive diagnosis requires a skin biopsy, followed by analysis of the fragment by direct immunofluorescence. Thus, the deposition of granular immunoglobulin A (IgA) in the papillary dermis (dermo-epidermal junction) is objectify. The long-term prognosis is encouraging, the treatment of the condition being based on the administration of topical clobetasol or dapsone (diamino-diphenyl sulfone) (80, 116, 120, 121). The differential diagnosis must be performed with centrifugal annular erythema. It is presented by lesions with a polycyclic outline, erythematous or vesicular, which extend towards the periphery. They may or may not be accompanied by itching. The evolution of GFD pot is marked by the remission of skin lesions, with minimal residual hyperpigmentation (122).

Chronic urticaria (lasting more than 6 weeks) is a skin manifestation known to be associated with autoimmune diseases. The underlying mechanism is largely attributed to the existence of autoantibodies against the high-affinity immunoglobulin E (IgE) receptor (FcεRI). To this is added the existence of a fund of chronic systemic inflammation (123, 124). The relationship between the two manifestations is bidirectional, there is currently no unanimity regarding their sequence (125, 126). A particular case of its presentation was recorded in a boy aged 3 years and 8 months. He complained of hives and angioedema after exposure to low temperatures. The symptomatology was doubled by persistent iron deficiency anemia.

Investigations in dynamics have objectified CD with the help of serological and histological tests (127). Similar cases were also described later in the literature (128). In this case, in addition to GFD, preparations such as non-sedating/sedating antihistamines or oral steroids can be used. The latter must be used judiciously, by the allergist specialist, to avoid over- administration (129).

Rosacea is a chronic inflammatory condition manifested mainly in the central part of the face. The pathognomonic lesions are represented by persistent erythema, papules, pustules and telangiectasia. These may or may not be accompanied by eye involvement, oedema, burning/stinging or xerosis. Its pathophysiology brings together the disruption of epithelial barriers, genetic and environmental factors, immunological imbalances and neurovascular reactions. It shares common susceptibility loci with CD, having a more significant statistical association among females than males. In boys, the autoimmune disease that reached statistical relevance regarding the association with rosacea is rheumatoid arthritis (130–132).

The incidence rate of alopecia among newly diagnosed CD patients is estimated to be ~1%. Conversely, the prevalence of CD among children with alopecia is almost 50%. The importance of performing screening in the affected population is therefore explained. The gender ratio is in favor of girls, they more frequently present an association between alopecia and CD. The main pathogenic mechanism incriminated in its occurrence is the autoimmune one. In this sense, hair regeneration 1-2 years after GFD initiation attests to the hypothesis.

Depending on the type of lesions, the condition can be divided into alopecia areata, totalis and universalis (80, 133). The case reports attest to the correlation between the two entities. Also, the results of the gluten-free regimen on hair regeneration are reiterated (134, 135). To this, for an adjuvant purpose, we add the promising prospects of the Mediterranean diet, rich in protein and soy (136).

Affecting the components of the oral cavity (mucous membranes, dentition) can be identified as an accompanying

manifestation of celiac disease. Chronic CD can be associated with delayed tooth eruption, mineralization disorders and dental caries, recurrent canker sores, lesions of the oral mucosa (ulcerations, erythema, cheilitis, atrophic glossitis), dryness and burning sensation of the tongue. The main mechanism incriminated is oral dysbiosis. The consequences are an oral smear rich in leukocytes, a change in the salivary volume and the structure of the tests. Affecting the enamel can involve the initial dentition or the permanent dentition. They are frequently arranged symmetrically, at the level of the four quadrants of the dental arch, having a double prevalence among the celiac population, compared to the general population. The main drawback lies in the lack of response to the exclusion regime in the case of permanent dentition, an effect that does not occur in the case of stomatitis (44, 137–143). Multiple studies in the recent medical literature attest to the correlation (144–146).

Recurrent aphthous stomatitis specific to celiac disease is defined as the presence of multiple lesions on the non-keratinized oral mucosa, usually occurring in childhood or adolescence.

Broadly speaking, it is found in 4% to 41% of CD patients. The appearance is round or ovoid, bordered by an erythematous background, with a yellow or gray base. Their presenceinterferes especially with speech, mastication and swallowing, decreasing the quality of life due to pain. Regarding disturbing factors, it seems that HLA DQB1 has a protective role, while family predisposition, local trauma, stress, hormonal variations, nutritional deficiencies, food hypersensitivity or immune changes leave a negative impression on the patient's chances of developing the disease (147, 148). CD screening among children with recurrent aphthous stomatitis and nutritional deficiencies is vital, the incidence of the pathology being significantly increased compared to the general population (149). GFD together with the local, individualized treatment of the lesions has demonstrated beneficial results in terms of restoring the oral health of patients with CD. The exception is found in what concerns the damage to the dental enamel (150).

5.5 Systemic damage

Atypical celiac disease at the onset can involve various organic manifestations. Among these, we highlight intestinal intussusception. Although its clear frequency could not yet be established, the association between the two pathologies is certified by numerous reported cases (151–154). It is noted that the risk factors associated with this are age and low weight, diarrhea at presentation, abdominal distension, rickets, low serum albumin, severe villous atrophy and refeeding syndrome (155, 156). The risks in the dynamics are represented by the recurrence of symptoms, intestinal obstruction and acute surgical abdomen. The differential diagnosis must be made with tuberculosis and intestinal lymphoma. Since the manifestation is often transient, expectant management and the initiation of GFD are preferred over surgical resolution (157, 158). In conclusion, for an optimal approach to intestinal intussusception, especially in small children, it is recommended to perform CD screening even in the absence of nutritional deficits (159).

Intestinal disorders that accompany CD can represent a cause of kidney damage, mainly through the intestine-kidney axis. Thus, celiac disease can predispose and precipitate the appearance of conditions such as renal diabetes, IgA nephropathy (IgAN), glomerulonephritis, renal damage with minimal damage, focal and segmental glomerulosclerosis, nephrotic syndrome, urolithiasis, urinary infection or distal tubular renal acidosis (160, 161). Also, subclinical CD can be associated with hyperoxaluria and a dynamic evolution towards deterioration of renal function (162). By far the most particular form of kidney damage in CD that can exemplify the gut-kidney correlation is IgAN. A similar effect in which dysbiosis is blamed can also be observed in chronic kidney disease. Recent studies have demonstrated its genome-wide association with immune-mediated inflammatory bowel diseases, intestinal barrier functionality, and response to intestinal pathogens. This overlap has a negative impact on the prognosis (163–165). A peculiarity of the renal samples collected from celiac patients is represented by the objectification of IgA-tTG deposits only in the case of those who do not follow a gluten exclusion diet (166). In conclusion, Pérez- Sáez MJ. et al. attests to the beneficial impact played by GFD in the biological evolution of children with kidney damage (167).

The characteristic symptomatology of the osteoarticular apparatus is intensely evoked mainly in childhood. Its causality can be dictated both by physiological processes specific to the period, and by disturbances in the pathological sphere. Regarding the subject of the study, we discovered causal associations between CD and joint hypomotility, proximal muscle weakness, arthralgias, osteoporosis, osteopenia and increased fracture risk. All theseassociated diseases are included under the umbrella of the term "metabolic bone disease" (168–170). As the name indicates, metabolic bone disease has as its main substrate malnutrition following the alteration of intestinal absorption. Added to this are the consequences of the chronic inflammatory state. Certainly, for a proper approach, the exclusion of food gluten is not enough. Although it showed promising results, the GFD must be accompanied by supplementing the deficiencies (168). GFD led to an increase in vitamin D levels, improvement in bone mass content and bone mineral density, although the period required for its normalization cannot yet be estimated. However, its role in bone health cannot be neglected (171–173).

The main neurological conditions particularly associated with CD are gluten ataxia, peripheral neuropathy (Guillain-Barrè syndrome), headache, epilepsy, depression, anxiety and cognitive-behavioral disorders. The latter include various forms of dementia (Alzheimer's, vascular, frontotemporal), memory disorders, acalculia, lack of attention and deficits in execution or spatial orientation. Post-mortem examination in gluten ataxia objectifies as a distinctive sign the uneven loss of Purkinje cells in the cerebellar cortex.

Migraine is another manifestation possibly associated with celiac disease, the pathognomonic sign of the coexistence of the two entities being occipital and parieto-occipital calcifications. The hypotheses regarding the mechanism by which the central nervous system is affected are multiple. These can be briefly classified as cerebral hypoperfusion (due to perivascular inflammation) or

gluten-mediated mechanisms. The latter include cross-reaction of autoantibodies, deposition of immune complexes, direct toxicity or disturbances of the gut- microbiota-brain axis (147, 174–178). Predisposing factors for neurological manifestations are female sex, mild histopathological form and HLA-DQ2 heterozygosity (179).

Bashiri H. et al. records a prevalence of approximately 6% of celiac disease among children with epilepsy. This encourages through their study the GFD approach, noting promising results in the control of convulsive seizures (180). Although the prevalence of CD did not reach a statistical significance among children with autism spectrum disorders, compared to the general population, Prosperi M. et al. underlines the need for screening in this group of patients. The considerations on which they are based are given by the identification of some patients with asymptomatic CD in the target group, but especially by the frequency of the atypical clinical picture in this age group (181). In addition, Özbudak P. et al. describe the celiac disease-catatonia association, by exemplifying the case of a 15-year-old girl.

Characteristic manifestations include stupor, waxy flexibility, and muteness lasting more than 1 hour (182). Also, although the case concerns a man past the age of adolescence, we want to mention the possibility of the onset of CD through neurological symptoms specific to amyotrophic lateral sclerosis. In this situation, the elimination of gluten for a period of 4 months coincided with the stopping of the evolution of the neurological symptoms and the normalization of the gastrointestinal ones (183). Recent studies also attest to the association at the genomic level of the two conditions (184). In conclusion, the differential diagnosis of subclinical or clinical neurological manifestations in children must include CD. The benefits aim at both the prevention of progressive deterioration in adult life, as well as the ability to exploit the protective effects of GFD in evolution (185).

Puberty usually occurs around the age of 11 for girls and 12 for boys. The evolution of secondary sexual characters is monitored using the Tanner classification. The differential diagnosis must include investigations such as the assessment of bone age, based on growthnuclei. In the context of CD, the delay in the onset of puberty is attributed to malabsorption and the delay in maturation of the hypothalamic-pituitary-gonadal axis. If in girls this manifests itself through delayed menarche, in boys we observe a mode suggestive of androgen resistance (reduced serum level of dihydrotestosterone and increased by luteinizing hormone). Therefore, if we are faced with delayed puberty, it is necessary to perform the screening for CD and initiate the GFD. The lack of improvement after 1-2 years of the regimen requires the patient to be referred to endocrinology for the exclusion/objectification and treatment of coexisting conditions (44, 186–188). Bayrak NA. et al. attests the correlation between Tanner stages, GFD adherence, transferrin saturation, total iron binding capacity and vitamin D (189).

In evolution, untreated CD can lead to reproductive disorders among girls past the age of adolescence. These are manifested by irregular menstruation, amenorrhea, early menopause, risk of spontaneous abortion, children with low birth weight, reduced breastfeeding capacity or children stillborn. The severity of the consequences is directly proportional to the severity of malabsorption. They fade and even go away with GFD adherence (190, 191).

Consequently, CD screening among women with unexplained infertility is important, Remes- Troche JM. et al. noting a positivity rate of at least one specific marker for them of approximately 4.6% (192). Affecting male fertility was not observed (193).

5.6 Association of autoimmune diseases

It is known in the medical world that autoimmune diseases can form familiar aggregates. However, the possible association between multiple autoimmunities found in the same patient should not be neglected either. In this sense, we will present in the following the autoimmune associations that can camouflage celiac disease.

Broadly speaking, specialized literature correlates CD with endocrinopathies such as diabetes, Hashimoto's thyroiditis, Graves' disease, Addison's disease (adrenal insufficiency), secondary hyperparathyroidism, ovarian insufficiency or pituitary disorders (resulting in hypogonadism, prolactin level imbalances) (194, 195). Autoimmune hemolytic anemia or autoimmune hepatitis can also be added to this list (196). By studying a group of 228 patients, Varol Fİ. et col. notes the absence of predictive factors that can anticipate the risk of association of CD with other glandular autoimmunities. They analyzed age, sex, symptoms, tTG level, HLA haplotype and histopathological stage. They did not obtain statistical results except in terms of age (higher in the case of association) (197).

Exceptionally, the specialized literature records associations of CD with Down's syndrome or asthma (198–200). In this case, screening is encouraged, as only intestinal histological changes can be detected, without immunological disturbances or CD specific markers (201). The consequences are among the most diverse, being able to include sideroblastic anemia resistant to iron treatment. This subsided when GFD was introduced into the therapeutic regimen (202). The pathogenic bases of the association are most likely aimed at immune and microbial imbalances, common to the three pathologies (203, 204). Similar implications can be observed in the case of the association CD - systemic lupus erythematosus, a condition in the etiology of which we also find dysbiosis (205). Therefore, a number of autoimmunities can overlap in some situations, being included in the multiple autoimmune syndrome (MAS). MAS is divided into three types, depending on the coexisting pathologies. In this sense, Boccuti V. et al. presents the case of a known boy with CD atypical form in the antecedents (psychiatric symptoms at the age of 18). He was also diagnosed with Hashimoto's thyroiditis and systemic lupus erythematosus. The current association is included in MAS 3 (206).

Unlike the previous examples, where the connection with CD was explained from the perspective of similar pathogenesis, the overlap of CD - type 1 diabetes mellitus (T1DM) most likely refers to the common genetic predisposition (207). The prevalence estimated by Goh C. et al. it is approximately 6% if we refer to the positive serological testing for CD and 4% regarding the results of the characteristic jejunal biopsy (208). More recently, Joshi R. et al. notes the prevalence of CD in the group of those with diabetes

as being 15%, with a positivity rate of the biopsy of 7% and a characteristic symptomatology that affects 1/3 of the patients at the time of screening. Also, they place the T1DM - CD correlation in second place, after that between T1DM and autoimmune thyroiditis (209). Furthermore, Singh P. et al. emphasizes that the coexistence of the two conditions increases the patients' risk of developing hypothyroidism and small stature (210). Height retardation has not been shown to be correlated with gender, but it is dependent on the time of CD diagnosis (more important at younger ages) and following a GFD (211). Worthy of mention in this context is also the result of the study conducted by Delvecchio M. et al., who demonstrated an alteration of the absorption capacity of iodine among patients with CD, compared to the general population.

Also, this seems to be only partially corrected by the initiation of GFD (212). Finally, Bourhanbour AD. et al. discuss the prevalence of the CD-T1DM association in different areas of the world, by comparison with the Moroccan population. It also highlights the importance of CD screening in susceptible patients, especially due to the long-term risk of lymphoma or adenocarcinoma (213).

6 General therapeutic lines

The therapeutic gold standard of CD, respectively GFD, can be considered a necessary evil. In this context, some patients confess that they feel the burden of the gluten exclusion diet more acutely than that of the treatment for type 1 diabetes, intestinal or heart diseases. The exception was represented by patients with kidney disease in the dialysis stage, who felt the burden of the underlying disease more strongly. For this reason, current research focuses on the development of adjuvant therapeutic substances, intended to allow the inclusion of dietary gluten to be tolerated without triggering clinical symptoms and paraclinical manifestations. In this regard, we discuss prolyl endopeptidases, NKG2D antagonists, R-spondin-1, IL-15 blockers, glutenases (e.g., latiglutenase), the tight junction regulator (e.g., larazotide), nanoparticles that induce tolerance to gliadin, polysulfonated synthetic polymer (hydroxyethyl methacrylate-costyrene sulfonate), anti-gliadin antibodies from egg yolk and various immunoprophylaxis variants. However, their effectiveness remains controversial and open to future research (3, 47, 214–216). A benefit can be felt by patients when adding quinoa flour and malt to the diet. The results reside in the nutritional enrichment and increased food variability offered by the two components (217). Another adjuvant therapeutic approach is represented by the administration of steroids in recently diagnosed disease. However, this shows modest paraclinical results (218). To these is added the treatment of coexisting conditions and the supplementation of the main nutritional deficiencies of iron, calcium, magnesium, potassium, vitamin B12, A, D, K, folic acid or proteins (2).

Once CD is diagnosed and therapy initiated, a key point of management is the follow-up of adherence and its results. In this sense, it is important for the practitioner to know and avoidthe administration of medicines that contain traces of gluten in their composition.

Summarizing, we retain among its various preparations based on β-alanine, Acebutolol, Adenosine phosphate, Sulfadiazine, Cefaclor, Allopurinol, Phenobarbital, Trihexyphenidyl hydrochloride, Probenecid, Ketoprofen, Cholecalciferol, Ethambutol, Silymarin, Methotrexate and the list can go on (48). Of course, this specificity with possible implications for CD is dependent on the excipients and not the active substance. However, an alarm signal must be raised to raise awareness of the possible implications of some drugs commonly used in clinical practice, when their method of preparation is not fully understood by the clinician. The benefits of GFD regarding the reduction of celiac disease morbidity were most of the time indisputable, both for symptomatic and asymptomatic patients (219–221). The literature also notes superior results of GFD among children, compared to adults (222). Regarding the variability of the response to GFD, there is no data in the literature, to our knowledge, that attests different results in terms of efficiency between the typical and the atypical form. The distinction resides mainly in the manifestations that it diminishes/counteracts. Respectively, in the typical form of the disease, the aim is to obtain a histopathological remission at the level of the affected intestinal epithelium by using GFD. In contrast, taking into account the fact that extra-intestinal manifestations predominate in the atypical form, the desired in this situation is also the prevention of potential complications of the disease. Therefore, we can conclude that, while in classic CD GFD has mainly a therapeutic role, in the atypical form of CD it also plays a role in prevention. In both forms, the effectiveness of the GFD is rather influenced by the receptivity of the patients depending on their age (children show better results than patients whose therapy started at older ages) and the amount of gluten ingested "accidentally" (desirably below 10 mg/day). At the opposite pole, in the form of refractory celiac disease, we are mostly talking about a resistance to a GFD correctly followed and maintained for at least 12 months. This can be either primary or secondary (relapse after apparent results induced by GFD) (9, 72, 223). In this situation, GFD plays rather an adjuvant role, additional to nutritional therapy, pharmacological and surgical measures, rather than the main therapeutic one. It is noted that GFD reduces overall morbidity and mortality in CD. However, GFD alone may be an effective maintenance therapy in exceptional cases. Elemental diet showed promising results in a small heterogeneous group of patients with refractory enteropathy without clonal intraepithelial lymphocyte phenotype (refractory CD type 1) (42). Probiotics can represent adjunctive therapeutic means in the case of residual symptoms despite adherence to the GFD. For this purpose, preferably use preparations based on *Bifidobacterium* and *Lactobacillus*. The effects are marked by an increase in the *Firmicutes/Bacteroides* ratio and the abundance of *Actinobacteria*. To these is added the decrease of pro-inflammatory factors, acetic acid and total short-chain fatty acids, in parallel with the restoration of the microbiome (37). This clarification reinforces the conviction regarding the importance of early diagnosis and treatment of affected children, with the main aim of limiting the medium and long-term consequences.

In addition to GFD, adjuvant nutritional and pharmacological therapy, we also find an active lifestyle. In this sense, Nestares T. et al. argue that spending greater time in vigorous physical activity

was associated with higher lean mass and bone mineral density, regardless of the time they followed a GFD (224).

7 Conclusions

The current work is focused on the atypical, non-pathognomonic phenomena encountered in celiac disease. We tried to expose in a clear, concise and objective manner the main signs, symptoms or conditions that can be associated with CD. The subject is a topical one, mainly because of the objectification of the increase in the diagnosis rate of celiac disease at the global level. Added to this is the ease with which most of the imbalances correlated from a causal or associative perspective with CD can find their solution with the initiation and maintenance of a GFD. Especially in late childhood, adolescence and even later in adult life, the classic form of the pathology is only the tip of the iceberg. At its base there are numerous constitutional, biological and systemic disturbances whose exposure were made with the aim of emphasizing the importance of screening in risk groups. This awareness is vital because, compared to other diseases whose methods of diagnosis and treatment may still encounter cognitive biases, CD is well described from this point of view. The gold standard is represented by the GFD, doubled by adequate nutritional supplementation.

The therapeutic target is mainly maintaining the metabolic balance in balance given its role in the child's growth and development. There is currently no approved pharmacological treatment. In conclusion, the child with CD requires the formation around the disease of a multidisciplinary team, adequately trained in the knowledge, diagnosis and rapid countermeasures of the main associated symptoms. The period until the intervention dictates the long-term prognosis. However, it is not recommended to initiate a gluten exclusion regimen without clearly objectifying the pathology in order not to precipitate further consequences. Finally, we would like to draw attention to the wide range of pathologies entangled with CD, considering it necessary to deepen further research aimed at the physio- pathological bases of the interconnection. The main purpose of this initiative is the possible prevention of associated comorbidities (e.g., skin, kidney, brain damage by modulating the intestinal microbiota) and avoiding the exacerbation of the already existing ones.

Author contributions

VL: Conceptualization, Methodology, Writing – original draft. MS: Investigation, Software, Writing – original draft. EJ: Investigation, Visualization, Writing – original draft. IS: Validation, Writing – review & editing. II: Conceptualization, Visualization, Writing – original draft. SR: Investigation, Software, Writing – original draft. VM: Software, Validation, Writing – review & editing. AN: Validation, Writing – review & editing. CD: Validation, Writing – review & editing. DS: Validation, Writing – review & editing. AK: Validation, Writing – review & editing. AL: Investigation, Methodology, Writing – original draft. AM: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Gasbarrini G, Miele L, Malandrino N, Grieco A, Addolorato G, Gasbarrini A, et al. Celiac disease in the 21st century: issues of under- and over-diagnosis. *Int J Immunopathol Pharmacol.* (2009) 22:1–7. doi: 10.1177/039463200902200101

2. Holtmeier W, Caspary WF. Celiac disease. *Orphanet J Rare Dis.* (2006) 1:3. doi: 10.1186/1750-1172-1-3

3. Gujral N, Freeman HJ, Thomson AB. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. *World J Gastroenterol.* (2012) 18:6036–59. doi: 10.3748/wjg.v18.i42.6036

4. Veeraraghavan G, Therrien A, Degroote M, McKeown A, Mitchell PD, Silvester JA, et al. Non-responsive celiac disease in children on a gluten free diet. *World J Gastroenterol.* (2021) 27:1311–20. doi: 10.3748/wjg.v27.i13.1311

5. Kreutz JM, Heynen L, Vreugdenhil ACE. Nutrient deficiencies in children with celiac disease during long term follow-up. *Clin Nutr.* (2023) 42:1175–80. doi: 10.1016/j.clnu.2023.05.003

6. Czaja-Bulsa G, Bulsa M. Adherence to gluten-free diet in children with celiac disease. *Nutrients.* (2018) 10:1424. doi: 10.3390/nu10101424

7. Pinto-Sanchez MI, Silvester JA, Lebwohl B, Leffler DA, Anderson RP, Therrien A, et al. Society for the Study of Celiac Disease position statement on gaps and opportunities in coeliac disease. *Nat Rev Gastroenterol Hepatol.* (2021) 18:875–84. doi: 10.1038/s41575-021-00511-8

8. Meijer CR, Auricchio R, Putter H, Castillejo G, Crespo P, Gyimesi J, et al. Prediction models for celiac disease development in children from high-risk families: data from the preventCD cohort. *Gastroenterology.* (2022) 163:426–36. doi: 10.1053/j.gastro.2022.04.030

9. Sahin Y. Celiac disease in children: A review of the literature. *World J Clin Pediatr.* (2021) 10:53–71. doi: 10.5409/wjcp.v10.i4.53

10. Roma E, Panayiotou J, Karantana H, Constantinidou C, Siakavellas SI, Krini M, et al. Changing pattern in the clinical presentation of pediatric celiac disease: a 30- year study. *Digestion.* (2009) 80:185–91. doi: 10.1159/000227275

11. Llorente-Alonso MJ, Fernández-Acenero MJ, Sebastián M. Gluten intolerance: sex and age- related features. *Can J Gastroenterol*. (2006) 20:719–22. doi: 10.1155/2006/470273

12. Salarian L, Khavaran M, Dehghani SM, Mashhadiagha A, Moosavi SA, Rezaeianzadeh S. Extra-intestinal manifestations of Celiac disease in children: their prevalence and association with human leukocyte antigens and pathological and laboratory evaluations. *BMC Pediatr*. (2023) 23:8. doi: 10.1186/s12887-022-03826-w

13. Webster J, Vajravelu ME, Choi C, Zemel B, Verma R. Prevalence of and risk factors for low bone mineral density in children with celiac disease. *Clin Gastroenterol Hepatol*. (2019) 17:1509–14. doi: 10.1016/j.cgh.2018.10.035

14. Andrén Aronsson C, Lee HS, Hård Af Segerstad EM, Uusitalo U, Yang J, Koletzko S, et al. Association of gluten intake during the first 5 years of life with incidence of celiac disease autoimmunity and celiac disease among children at increased risk. *JAMA*. (2019) 322:514–23. doi: 10.1001/jama.2019.10329

15. Koletzko S, Lee HS, Beyerlein A, Aronsson CA, Hummel M, Liu E, et al. Cesarean section on the risk of celiac disease in the offspring: the teddy study. *J Pediatr Gastroenterol Nutr*. (2018) 66:417–24. doi: 10.1097/MPG.0000000000001682

16. Lupu VV, Miron IC, Raileanu AA, Starcea IM, Lupu A, Tarca E, et al. Difficulties in Adaptation of the Mother and Newborn via Cesarean Section versus Natural Birth—A Narrative Review. *Life*. (2023) 13:300. doi: 10.3390/life13020300

17. Pantazi AC, Balasa AL, Mihai CM, Chisnoiu T, Lupu VV, Kassim MAK, et al. Development of gut microbiota in the first 1000 days after birth and potential interventions. *Nutrients*. (2023) 15:3647. doi: 10.3390/nu15163647

18. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Tonutti E, Amarri S, et al. SIGENP (Italian Society of Pediatric Gastroenterology, Hepatology, and Nutrition) Working Group on Weaning and CD Risk. Introduction of gluten, HLA status, and the risk of celiac disease in children. *N Engl J Med*. (2014) 371:1295–303. doi: 10.1056/NEJMoa1400697

19. Poddighe D, Capittini C. The role of HLA in the association between igA deficiency and celiac disease. *Dis Markers*. (2021) 2021. doi: 10.1155/2021/8632861

20. Moerkens R, Mooiweer J, Withoff S, Wijmenga C. Celiac disease-on-chip: Modeling a multifactorial disease *in vitro*. *United Eur Gastroenterol J*. (2019) 7:467–76. doi: 10.1177/2050640619836057

21. Cakir M, Guven B, Issi F, Ozkaya E. New-onset celiac disease in children during COVID-19 pandemic. *Acta Paediatr*. (2022) 111:383–8. doi: 10.1111/apa.16173

22. Lionetti E, Fabbrizi A, Catassi C. Prevalence of COVID-19 in Italian children with celiac disease: A cross-sectional study. *Clin Gastroenterol Hepatol*. (2021) 19:1075. doi: 10.1016/j.cgh.2020.11.035

23. Lupu A, Miron IC, Gavrilovici C, Raileanu AA, Starcea IM, Ioniuc I, et al. Pediatric systemic lupus erythematous in COVID-19 era. *Viruses*. (2023) 15:272. doi: 10.3390/v15020272

24. Tamai T, Ihara K. Celiac disease genetics, pathogenesis, and standard therapy for Japanese patients. *Int J Mol Sci*. (2023) 24:2075. doi: 10.3390/ijms24032075

25. Akbulut UE, Çebi AH, Sağ E, İkbal M, Çakır M. Interleukin-6 and interleukin-17 gene polymorphism association with celiac disease in children. *Turk J Gastroenterol*. (2017) 28:471–5. doi: 10.5152/tjg.2017.17092

26. Bayrak NA, Tutar E, Volkan B, Sahin Akkelle B, Polat E, Kutluk G, et al. Helicobacter pylori infection in children with celiac disease: Multi-center, cross-sectional study. *Helicobacter*. (2020) 25:e12691. doi: 10.1111/hel.12691

27. Bayrak NA, Volkan B. Helicobacter pylori infection in children with concurrent celiac disease and type 1 diabetes mellitus. *Dig Dis*. (2021) 39:444–50. doi: 10.1159/000514276

28. Rostami-Nejad M, Javad Ehsani-Ardakani M, Assadzadeh H, Shahbazkhani B, Ierardi E, Losurdo G, et al. Pathological and clinical correlation between celiac disease and helicobacter pylori infection; a review of controversial reports. *Middle East J Dig Dis*. (2016) 8:85–92. doi: 10.15171/mejdd.2016.12

29. Maxim R, Pleşa A, Stanciu C, Girleanu I, Moraru E, Trifan A. Helicobacter pylori prevalence and risk factors among children with celiac disease. *Turk J Gastroenterol*. (2019) 30:284–9. doi: 10.5152/tjg.2018.18181

30. Lupu VV, Adam Raileanu A, Mihai CM, Morariu ID, Lupu A, Starcea IM, et al. The implication of the gut microbiome in heart failure. *Cells*. (2023) 12:1158. doi: 10.3390/cells12081158

31. Lupu VV, Ghiciuc CM, Stefanescu G, Mihai CM, Popp A, Sasaran MO, et al. Emerging role of the gut microbiome in post-infectious irritable bowel syndrome: A literature review. *World J Gastroenterol*. (2023) 29:3241–56. doi: 10.3748/wjg.v29.i21.3241

32. Lupu VV, Trandafir LM, Raileanu AA, Mihai CM, Morariu ID, Starcea IM, et al. Advances in understanding the human gut microbiota and its implication in pediatric celiac disease— A narrative review. *Nutrients*. (2023) 15:2499. doi: 10.3390/nu15112499

33. Pantazi AC, Mihai CM, Balasa AL, Chisnoiu T, Lupu A, Frecus CE, et al. Relationship between gut microbiota and allergies in children: A literature review. *Nutrients*. (2023) 15:2529. doi: 10.3390/nu15112529

34. Bozomitu L, Miron I, Adam Raileanu A, Lupu A, Paduraru G, Marcu FM, et al. The gut microbiome and its implication in the mucosal digestive disorders. *Biomedicines*. (2022) 10:3117. doi: 10.3390/biomedicines10123117

35. Pantazi AC, Kassim MAK, Nori W, Tuta LA, Mihai CM, Chisnoiu T, et al. Clinical perspectives of gut microbiota in patients with chronic kidney disease and end-stage kidney disease: where do we stand? *Biomedicines*. (2023) 11:2480. doi: 10.3390/biomedicines11092480

36. Lupu VV, Bratu RM, Trandafir LM, Bozomitu L, Paduraru G, Gimiga N, et al. Exploring the microbial landscape: gut dysbiosis and therapeutic strategies in pancreatitis—A narrative review. *Biomedicines*. (2024) 12:645. doi: 10.3390/biomedicines12030645

37. Wu X, Qian L, Liu K, Wu J, Shan Z. Gastrointestinal microbiome and gluten in celiac disease. *Ann Med*. (2021) 53:1797–805. doi: 10.1080/07853890.2021.1990392

38. de la Calle I, Ros G, Peñalver Miras R, Nieto G. Celiac disease: causes, pathology, and nutritional assessment of gluten-free diet. A review. *Nutr Hosp*. (2020) 37:1043–51. doi: 10.20960/nh.02913

39. Lupu VV, Jechel E, Mihai CM, Mitrofan EC, Lupu A, Starcea IM, et al. Connection between celiac disease and systemic lupus erythematosus in children—A development model of autoimmune diseases starting from what we inherit to what we eat. *Nutrients*. (2023) 15:2535. doi: 10.3390/nu15112535

40. Kaswala DH, Veeraraghavan G, Kelly CP, Leffler DA. Celiac disease: diagnostic standards and dilemmas. *Diseases*. (2015) 3:86–101. doi: 10.3390/diseases3020086

41. Oliveira GN, Mohan R, Fagbemi A. REVIEW OF CELIAC DISEASE PRESENTATION IN A PEDIATRIC TERTIARY CENTRE. *Arq Gastroenterol*. (2018) 55:86–93. doi: 10.1590/S0004-2803.201800000-17

42. Rubio-Tapia A, Murray JA. Classification and management of refractory coeliac disease. *Gut*. (2010) 59:547–57. doi: 10.1136/gut.2009.195131

43. Tapsas D, Hollén E, Stenhammar L, Fälth-Magnusson K. The clinical presentation of coeliac disease in 1030 Swedish children: Changing features over the past four decades. *Dig Liver Dis*. (2016) 48:16–22. doi: 10.1016/j.dld.2015.09.018

44. Jericho H, Guandalini S. Extra-intestinal manifestation of celiac disease in children. *Nutrients*. (2018) 10:755. doi: 10.3390/nu10060755

45. Tanpowpong P, Broder-Fingert S, Katz AJ, Camargo CA Jr. Age-related patterns in clinical presentations and gluten-related issues among children and adolescents with celiac disease. *Clin Transl Gastroenterol*. (2012) 3:e9. doi: 10.1038/ctg.2012.4

46. Al-Toma A, Volta U, Auricchio R, Castillejo G, Sanders DS, Cellier C, et al. European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. *United Eur Gastroenterol J*. (2019) 7:583–613. doi: 10.1177/2050640619844125

47. Silvester JA, Therrien A, Kelly CP. Celiac disease: fallacies and facts. *Am J Gastroenterol*. (2021) 116:1148–55. doi: 10.14309/ajg.0000000000001218

48. Ben Houmich T, Admou B. Celiac disease: Understandings in diagnostic, nutritional, and medicinal aspects. *Int J Immunopathol Pharmacol*. (2021) 35:1–22. doi: 10.1177/20587384211008709

49. Kowalski K, Mulak A, Jasińska M, Paradowski L. Diagnostic challenges in celiac disease. *Adv Clin Exp Med*. (2017) 26:729–37. doi: 10.17219/acem/62452

50. Adelman DC, Murray J, Wu TT, Mäki M, Green PH, Kelly CP. Measuring change in small intestinal histology in patients with celiac disease. *Am J Gastroenterol*. (2018) 113:339–347. doi: 10.1038/ajg.2017.480

51. Semwal P, Gupta RK, Sharma R, Garg K. Comparison of endoscopic and histological findings between typical and atypical celiac disease in children. *Pediatr Gastroenterol Hepatol Nutr*. (2018) 21:86–92. doi: 10.5223/pghn.2018.21.2.86

52. Kav T, Sivri B. Is enteroscopy necessary for diagnosis of celiac disease? *World J Gastroenterol*. (2012) 18:4095–101. doi: 10.3748/wjg.v18.i31.4095

53. Ciaccio EJ, Tennyson CA, Bhagat G, Lewis SK, Green PH. Methods to quantitate videocapsule endoscopy images in celiac disease. *BioMed Mater Eng*. (2014) 24:1895–911. doi: 10.3233/BME-140999

54. Parfenov AI, Akopova AO, Shcherbakov PL, Mikheeva OM, Gudkova RB. Is it necessary to use capsular endoscopy to diagnose celiac disease? *Ter Arkh*. (2018) 90:8–11. doi: 10.26442/terarkh20189048-11

55. Horton RK, Hagen CE, Snyder MR. Pediatric celiac disease: A review of diagnostic testing and guideline recommendations. *J Appl Lab Med*. (2022) 7:294–304. doi: 10.1093/jalm/jfab143

56. Koc G, Doganay S, Sevinc E, Deniz K, Chavhan G, Gorkem SB, et al. Magnetic resonance enterography in pediatric celiac disease. *J Pediatr (Rio J)*. (2017) 93:413–9. doi: 10.1016/j.jped.2016.11.003

57. Penizzotto A, Vespa F, López Grove R, Rendón O, Tsai R, Ocantos JA. CT and MR enterography in the evaluation of celiac disease. *Radiographics*. (2024) 44:e230122. doi: 10.1148/rg.230122

58. Masselli G, Picarelli A, Gualdi G. Celiac disease: MR enterography and contrast enhanced MRI. *Abdom Imaging*. (2010) 35:399–406. doi: 10.1007/s00261-009-9531-x

59. Rossi RE, Busacca A, Brandaleone L, Masoni B, Massironi S, Fraquelli M, et al. Small Bowel Imaging in Celiac Disease: Is there a role for Small Bowel Ultrasound? *Curr Gastroenterol Rep*. (2023) 25:430–9. doi: 10.1007/s11894-023-00907-3

60. Rostami Nejad M, Rostami K, Pourhoseingholi MA, Nazemalhosseini Mojarad E, Habibi M, Dabiri H, et al. Atypical presentation is dominant and typical for coeliac disease. *J Gastrointestin Liver Dis*. (2009) 18:285–91. doi: 10.1088/2058-7058/3/8/4

61. Imran, Cheema HA, Alvi MA, Rehman MU, Ali M, Sarwar HA. Spectrum of clinical presentation of celiac disease in pediatric population. *Cureus*. (2021) 13:e15582. doi: 10.7759/cureus.15582

62. Iwańczak B, Matusiewicz K, Iwańczak F. Clinical picture of classical, atypical and silent celiac disease in children and adolescents. *Adv Clin Exp Med*. (2013) 22:667–73.

63. Cooney MJ, El-Matary W. Celiac disease presenting as Fever of unknown origin. *Case Rep Gastrointest Med*. (2013) 2013. doi: 10.1155/2013/676327

64. Basha J, Appasani S, Vaiphei K, Singh K, Kochhar R. Celiac disease presenting as recurrent pancreatitis and pseudocyst. *JOP*. (2012) 13:533–5. doi: 10.6092/1590-8577/1091

65. Yarnell EL. A child with atypical celiac disease and recurrent urolithiasis. *Iran J Kidney Dis*. (2012) 6:146–8.

66. Lototskaya PS, Manina MA, Tertychnyy AS, Zamyatnin AA Jr, Erdes SI. Duodenal ulceration in a child with coeliac disease. *Diagnostics (Basel)*. (2020) 10:31. doi: 10.3390/diagnostics10010031

67. Panda A, McCarthy MR, Murray JA, Sharain RF, Shi M, Kendi AT. Incidentally detected celiac disease with splenomegaly on 18F FDG PET/CT: A potential lymphoma mimic. *Asia Ocean J Nucl Med Biol*. (2021) 9:51–5. doi: 10.22038/AOJNMB.2020.49000.1333

68. Voigt W, Jordan K, Sippel C, Amoury M, Schmoll HJ, Wolf HH. Severe thrombocytosis and anemia associated with celiac disease in a young female patient: a case report. *J Med Case Rep*. (2008) 2:96. doi: 10.1186/1752-1947-2-96

69. Jomni T, Bellakhal S, Abouda M, Abdelaali I, Douggui H. An atypical presentation of celiac disease: central retinal vein occlusion. *Pan Afr Med J*. (2015) 22:300. doi: 10.11604/pamj.2015.22.300.8196

70. Soylu A, Öztürk Y, Doğan Y, Özmen D, Yılmaz Ö, Kuyum P, et al. Screening of celiac disease in children with Henoch-Schoenlein purpura. *Rheumatol Int*. (2016) 36:713–7. doi: 10.1007/s00296-016-3425-3

71. Aleksandra B, Ivana K, Ivica S, Prokic D. Profile of typical and atypical celiac disease in Serbian children. *Indian Pediatr*. (2013) 50:1061–2. doi: 10.1007/s13312-013-0279-6

72. Admou B, Essaadouni L, Krati K, Zaher K, Sbihi M, Chabaa L, et al. Atypical celiac disease: from recognizing to managing. *Gastroenterol Res Pract*. (2012) 2012. doi: 10.1155/2012/637187

73. Polidori N, Castorani V, Mohn A, Chiarelli F. Deciphering short stature in children. *Ann Pediatr Endocrinol Metab*. (2020) 25:69–79. doi: 10.6065/apem.2040064.032

74. Hashemi J, Hajiani E, Shahbazin HB, Masjedizadeh R, Ghasemi N. Prevalence of celiac disease in Iranian children with idiopathic short stature. *World J Gastroenterol*. (2008) 14:7376–80. doi: 10.3748/wjg.14.7376

75. Assiri AM. Isolated short stature as a presentation of celiac disease in Saudi children. *Pediatr Rep*. (2010) 2:e4. doi: 10.4081/pr.2010.e4

76. Muhammad A 2nd, Arif N, Wajid KK, Rehman K, Sardar N, Khan P, et al. Short stature and celiac disease in children (5 to 16 years) presenting at a tertiary care hospital in peshawar. *Cureus*. (2022) 14:e26099. doi: 10.7759/cureus.26099

77. Aggarwal N, Dwarakanathan V, Singh A, Agarwal A, Khuttan A, Ahmed A, et al. Spectrum of height in patients with celiac disease. *Indian J Gastroenterol*. (2021) 40:604–12. doi: 10.1007/s12664-021-01173-9

78. Bozzola M, Bozzola E, Pagani S, Mascolo A, Porto R, Meazza C. Late diagnosis of celiac disease in an asymptomatic infant with growth failure. *Ital J Pediatr*. (2014) 40:4. doi: 10.1186/1824-7288-40-4

79. Soliman AT, Laham M, Jour C, Shaat M, Souikey F, Itani M, et al. Linear growth of children with celiac disease after the first two years on gluten- free diet: a controlled study. *Acta BioMed*. (2019) 90:20–7. doi: 10.23750/abm.v90i8-S.8515

80. Nardecchia S, Auricchio R, Discepolo V, Troncone R. Extra-intestinal manifestations of coeliac disease in children: clinical features and mechanisms. *Front Pediatr*. (2019) 7:56. doi: 10.3389/fped.2019.00056

81. Giovenale D, Meazza C, Cardinale GM, Sposito M, Mastrangelo C, Messini B, et al. The prevalence of growth hormone deficiency and celiac disease in short children. *Clin Med Res*. (2006) 4:180–3. doi: 10.3121/cmr.4.3.180

82. Saadah OI. Short children with impaired growth hormone secretion. Do they have celiac disease? *Saudi Med J*. (2020) 41:68–72. doi: 10.15537/smj.2020.1.24785

83. Witkowska-Sędek E, Labochka D, Majcher A. Pyrżak B. The pre-treatment characteristics and evaluation of the effects of recombinant human growth hormone therapy in children with growth hormone deficiency and celiac disease or inflammatory bowel disease. *Cent Eur J Immunol*. (2018) 43:69–75. doi: 10.5114/ceji.2018.74875

84. Diamanti A, Capriati T, Basso MS, Panetta F, Di Ciommo Laurora VM, Bellucci F, et al. Celiac disease and overweight in children: an update. *Nutrients*. (2014) 6:207–20. doi: 10.3390/nu6010207

85. Parada AC, Méndez C, Aguirre C. Excess weight and gastrointestinal symptoms in Chilean celiac patients at the time of diagnosis. *Rev Esp Enferm Dig*. (2019) 111:384–7. doi: 10.17235/reed.2019.5251/2017

86. Rodrigues M, Yonamine GH, Fernandes Satiro CA. Rate and determinants of non-adherence to a gluten-free diet and nutritional status assessment in children and adolescents with celiac disease in a tertiary Brazilian referral center: a cross-sectional and retrospective study. *BMC Gastroenterol*. (2018) 18:15. doi: 10.1186/s12876-018-0740-z

87. Radlović N, Mladenović M, Leković Z, Zivanović D, Brdar R, Radlović V, et al. Effect of gluten-free diet on the growth and nutritional status of children with coeliac disease. *Srp Arh Celok Lek*. (2009) 137:632–7. doi: 10.2298/SARH0912632R

88. Calcaterra V, Regalbuto C, Madè A, Magistrali M, Leonard MM, Cena H. Coexistence of excessive weight gain and celiac disease in children: an unusual familial condition. *Pediatr Gastroenterol Hepatol Nutr*. (2019) 22:407–12. doi: 10.5223/pghn.2019.22.4.407

89. Calcaterra V, Regalbuto C, Manuelli M, Klersy C, Pelizzo G, Albertini R, et al. Screening for celiac disease among children with overweight and obesity: toward exploring celiac iceberg. *J Pediatr Endocrinol Metab*. (2020) 33(8): 995–1002. doi: 10.1515/jpem-2020-0076

90. Bel'mer SV, Mitina EV, Karpina LM, Smetanina NS. [Iron deficiency anemia and anemia in chronic celiac disease in children]. *Eksp Klin Gastroenterol*. (2014) 1:23–9.

91. Pasricha SR, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. *Lancet*. (2021) 397:233–48. doi: 10.1016/S0140-6736(20)32594-0

92. Sanseviero MT, Mazza GA, Pullano MN, Oliveiro AC, Altomare F, Pedrelli L, et al. Iron deficiency anemia in newly diagnosed celiac disease in children. *Minerva Pediatr*. (2016) 68:1–4.

93. Martín-Masot R, Nestares MT, Diaz-Castro J, López-Aliaga I, Alférez MJM, Moreno- Fernandez J, et al. Multifactorial etiology of anemia in celiac disease and effect of gluten-free diet: A comprehensive review. *Nutrients*. (2019) 11:2557. doi: 10.3390/nu11112557

94. Tolone C, Bellini G, Punzo F, Papparella A, Miele E, Vitale A, et al. The DMT1 IVS4 + 44C>A polymorphism and the risk of iron deficiency anemia in children with celiac disease. *PloS One*. (2017) 12:e0185822. doi: 10.1371/journal.pone.0185822

95. Irfan O, Mahmood S, Nand H, Billoo G. Celiac disease associated with aplastic anemia in a 6-year-old girl: a case report and review of the literature. *J Med Case Rep*. (2018) 12:16. doi: 10.1186/s13256-017-1527-5

96. von Graffenried T, Rizzi M, Russo M, Nydegger A, Kayemba-Kay's S. Atypical hematological manifestation of celiac disease: A case report of aplastic anemia in a 2-year-old child and review of the literature. *Pediatr Investig*. (2021) 5:159–62. doi: 10.1002/ped4.12267

97. Repo M, Hannula M, Taavela J, Hyttinen J, Isola J, Hiltunen P, et al. Iron transporter protein expressions in children with celiac disease. *Nutrients*. (2021) 13:776. doi: 10.3390/nu13030776

98. Subramaniam G, Girish M. Iron deficiency anemia in children. *Indian J Pediatr*. (2015) 82:558–64. doi: 10.1007/s12098-014-1643-9

99. Shahriari M, Honar N, Yousefi A, Javaherizadeh H. Association of potential celiac disease and refractory iron deficiency anemia in children and adolescents. *Arq Gastroenterol*. (2018) 55:78–81. doi: 10.1590/S0004-2803.201800000-15

100. Rajalahti T, Repo M, Kivelä L, Huhtala H, Mäki M, Kaukinen K, et al. Anemia in pediatric celiac disease: association with clinical and histological features and response to gluten-free diet. *J Pediatr Gastroenterol Nutr*. (2017) 64:e1–6. doi: 10.1097/MPG.0000000000001221

101. Saukkonen J, Kaukinen K, Koivisto AM, Mäki M, Laurila K, Sievänen H, et al. Clinical characteristics and the dietary response in celiac disease patients presenting with or without anemia. *J Clin Gastroenterol*. (2017) 51:412–6. doi: 10.1097/MCG.0000000000000556

102. Scapaticci S, Venanzi A, Chiarelli F, Giannini C. MAFLD and celiac disease in children. *Int J Mol Sci*. (2023) 24:1764. doi: 10.3390/ijms24021764

103. Reilly NR, Lebwohl B, Hultcrantz R, Green PH, Ludvigsson JF. Increased risk of non- alcoholic fatty liver disease after diagnosis of celiac disease. *J Hepatol*. (2015) 62:1405–11. doi: 10.1016/j.jhep.2015.01.013

104. Agarwal A, Singh A, Mehtab W, Gupta V, Chauhan A, Rajput MS, et al. Patients with celiac disease are at high risk of developing metabolic syndrome and fatty liver. *Intest Res*. (2021) 19:106–14. doi: 10.5217/ir.2019.00136

105. Motazedian N, Sayadi M, Mashhadiagha A, Moosavi SA, Khademian F, Niknam R. Metabolic syndrome in celiac disease: what does following a one-year gluten-free diet bring? *Middle East J Dig Dis*. (2023) 15:185–9. doi: 10.34172/mejdd.2023.342

106. Cherkasova EA, Klimov LY, Kuryaninova VA, Yagupova AV, Ivenskaya TA, Gliva AV. Liver damage in children and adolescents with newly diagnosed celiac disease: clinical and anamnestic, serological and morphological patterns. *Ter Arkh*. (2023) 95:158–63. doi: 10.26442/00403660.2023.02.202112

107. Vajro P, Fontanella A, Mayer M, De Vincenzo A, Terracciano LM, D'Armiento M, et al. Elevated serum aminotransferase activity as an early manifestation of gluten-sensitive enteropathy. *J Pediatr*. (1993) 122:416–9. doi: 10.1016/s0022-3476(05)83430-4

108. Pavone P, Gruttadauria S, Leonardi S, Sorge G, Minervini MI, Greco F, et al. Liver transplantation in a child with celiac disease. *J Gastroenterol Hepatol*. (2005) 20:956–60. doi: 10.1111/j.1440-1746.2005.03223.x

109. Demir H, Yüce A, Caglar M, Kale G, Kocak N, Ozen H, et al. Cirrhosis in children with celiac disease. *J Clin Gastroenterol*. (2005) 39:630–3. doi: 10.1097/01.mcg.0000170734.49725.53

110. Balaban DV, Popp A, Ionita Radu F, Jinga M. Hematologic manifestations in celiac disease- A practical review. *Medicina (Kaunas)*. (2019) 55:373. doi: 10.3390/medicina55070373

111. Meena DS, Kumar D, Bohra GK, Choudhary S. Hypoalbuminemia and generalized edema as an atypical presentation of celiac disease. *J Family Med Prim Care*. (2020) 9:1206–8. doi: 10.4103/jfmpc.jfmpc_1116_19

112. Chen CS, Cumbler EU, Triebling AT. Coagulopathy due to celiac disease presenting as intramuscular hemorrhage. *J Gen Intern Med*. (2007) 22:1608–12. doi: 10.1007/s11606-007-0297-y

113. Djuric Z, Zivic S, Katic V. Celiac disease with diffuse cutaneous vitamin K-deficiency bleeding. *Adv Ther*. (2007) 24:1286–9. doi: 10.1007/BF02877775

114. Mazman Dİ, Dereci S, Hızlı Ş, Doğan HT, Ateş BB, Hızal G, et al. Celiac crisis with thrombocytopenia and coagulopathy in a child. *Turk J Pediatr*. (2022) 64:1156–60. doi: 10.24953/turkjped.2022.216

115. Akdis CA. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions? *Nat Rev Immunol*. (2021) 21:739–51. doi: 10.1038/s41577-021-00538-7

116. Rodrigo L, Beteta-Gorriti V, Alvarez N, Gómez de Castro C, de Dios A, Palacios L, et al. Cutaneous and mucosal manifestations associated with celiac disease. *Nutrients*. (2018) 10:800. doi: 10.3390/nu10070800

117. Abenavoli L, Dastoli S, Bennardo L, Boccuto L, Passante M, Silvestri M, et al. The skin in celiac disease patients: the other side of the coin. *Medicina (Kaunas)*. (2019) 55:578. doi: 10.3390/medicina55090578

118. Turjanmaa E, Hervonen K, Huhtala H, Arnala S, Reunala T, Kaukinen K, et al. Patient- reported burden of skin disorders in coeliac disease. *Scand J Gastroenterol*. (2023) 21:1–7. doi: 10.1080/00365521.2023.2236263

119. Sally R, Kim R, Lo Sicco K, Caplan AS. Urticarial dermatitis herpetiformis: A rare presentation of an uncommon disorder. *JAAD Case Rep*. (2022) 26:27–9. doi: 10.1016/j.jdcr.2022.05.037

120. Antiga E, Maglie R, Quintarelli L, Verdelli A, Bonciani D, Bonciolini V, et al. Dermatitis herpetiformis: novel perspectives. *Front Immunol*. (2019) 10:1290. doi: 10.3389/fimmu.2019.01290

121. Görög A, Antiga E, Caproni M, Cianchini G, De D, Dmochowski M, et al. S2k guidelines (consensus statement) for diagnosis and therapy of dermatitis herpetiformis initiated by the European Academy of Dermatology and Venereology (EADV). *J Eur Acad Dermatol Venereol*. (2021) 35:1251–77. doi: 10.1111/jdv.17183

122. Votto M, De Filippo M, Licari A, Caimmi S, Marseglia GL, Davidovich S, et al. Atypical erythema annulare centrifugum in a child with celiac disease. *Clin Case Rep*. (2021) 9:e04441. doi: 10.1002/ccr3.4441

123. Fraser K, Robertson L. Chronic urticaria and autoimmunity. *Skin Ther Lett*. (2013) 18:5–9.

124. Confino-Cohen R, Chodick G, Shalev V, Leshno M, Kimhi O, Goldberg A. Chronic urticaria and autoimmunity: associations found in a large population study. *J Allergy Clin Immunol*. (2012) 129:1307–13. doi: 10.1016/j.jaci.2012.01.043

125. Ludvigsson JF, Lindelöf B, Rashtak S, Rubio-Tapia A, Murray JA. Does urticaria risk increase in patients with celiac disease? A large population-based cohort study. *Eur J Dermatol*. (2013) 23:681–7. doi: 10.1684/ejd.2013.2158

126. Peroni DG, Paiola G, Tenero L, Fornaro M, Bodini A, Pollini F, et al. Chronic urticaria and celiac disease: a case report. *Pediatr Dermatol*. (2010) 27:108–9. doi: 10.1111/pde.2010.27.issue-1

127. Pedrosa Delgado M, Martín Muñoz F, Polanco Allué I, Martín Esteban M. Cold urticaria and celiac disease. *J Investig Allergol Clin Immunol*. (2008) 18:123–5.

128. Méndez Sánchez A, Pascual Pérez AI, Vázquez Piñera MA, Fernández González P. Cold urticaria and coeliac disease in a paediatric patient. *Pediatr (Engl Ed)*. (2019) 91:410–1. doi: 10.1016/j.anpedi.2019.01.018

129. Williams PV. Pharmacologic management of chronic urticaria in pediatric patients: the gap between guidelines and practice. *Paediatr Drugs*. (2020) 22:21–8. doi: 10.1007/s40272-019-00365-3

130. Wang FY, Chi CC. Rosacea, germs, and bowels: A review on gastrointestinal comorbidities and gut-skin axis of rosacea. *Adv Ther*. (2021) 38:1415–24. doi: 10.1007/s12325-021-01624-x

131. Egeberg A, Hansen PR, Gislason GH, Thyssen JP. Clustering of autoimmune diseases in patients with rosacea. *J Am Acad Dermatol*. (2016) 74:667–672.e1. doi: 10.1016/j.jaad.2015.11.004

132. Egeberg A, Weinstock LB, Thyssen EP, Gislason GH, Thyssen JP. Rosacea and gastrointestinal disorders: a population-based cohort study. *Br J Dermatol*. (2017) 176:100–6. doi: 10.1111/bjd.14930

133. Ertekin V, Tosun MS, Erdem T. Screening of celiac disease in children with alopecia areata. *Indian J Dermatol*. (2014) 59:317. doi: 10.4103/0019-5154.131468

134. Corazza GR, Andreani ML, Venturo N, Bernardi M, Tosti A, Gasbarrini G. Celiac disease and alopecia areata: report of a new association. *Gastroenterology*. (1995) 109:1333–7. doi: 10.1016/0016-5085(95)90597-9

135. Real Delor RE, López Esquivel NG, Real Aparicio NE. Silent celiac disease in an obese patient with alopecia areata. *Rev Fac Cien Med Univ Nac Cordoba*. (2020) 77:49–51. doi: 10.31053/1853.0605.v77.n1.25193

136. Pham CT, Romero K, Almohanna HM, Griggs J, Ahmed A, Tosti A. The role of diet as an adjuvant treatment in scarring and nonscarring alopecia. *Skin Appendage Disord*. (2020) 6:88–96. doi: 10.1159/000504786

137. Krzywicka B, Herman K, Kowalczyk-Zając M, Pytrus T. Celiac disease and its impact on the oral health status - review of the literature. *Adv Clin Exp Med*. (2014) 23:675–81. doi: 10.17219/acem/37212

138. Kalvandi G, Shahramian I, Farmany A, Yadegari S, Parooie F. Serological study of celiac disease in children with dental caries. *Hum Antibodies*. (2021) 29:237–41. doi: 10.3233/HAB-210445

139. Kuklik HH, Cruz ITSA, Celli A, Fraiz FC, AssunÇÃo LRDS. MOLAR INCISOR HYPOMINERALIZATION AND CELIAC DISEASE. *Arq Gastroenterol*. (2020) 57:167–71. doi: 10.1590/s0004-2803.202000000-31

140. Macho VMP, de Barros Menéres Manso MCA E, Silva DMV, de Andrade DJC. The difference in symmetry of the enamel defects in celiac disease versus non-celiac pediatric population. *J Dent Sci*. (2020) 15:345–50. doi: 10.1016/j.jds.2020.02.006

141. Pastore L, Carroccio A, Compilato D, Panzarella V, Serpico R, Lo Muzio L. Oral manifestations of celiac disease. *J Clin Gastroenterol*. (2008) 42:224–32. doi: 10.1097/MCG.0b013e318074dd98

142. Macho VMP, Coelho AS, Veloso E Silva DM, de Andrade DJC. Oral manifestations in pediatric patients with coeliac disease - A review article. *Open Dent J*. (2017) 11:539–45. doi: 10.2174/1874210601711010539

143. Erriu M, Canargiu F, Orrù G, Garau V, Montaldo C. Idiopathic atrophic glossitis as the only clinical sign for celiac disease diagnosis: a case report. *J Med Case Rep*. (2012) 6:185. doi: 10.1186/1752-1947-6-185

144. Souto-Souza D, da Consolação Soares ME, Rezende VS, de Lacerda Dantas PC, Galvão EL, Falci SGM. Association between developmental defects of enamel and celiac disease: A meta- analysis. *Arch Oral Biol*. (2018) 87:180–90. doi: 10.1016/j.archoralbio.2017.12.025

145. Villemur Moreau L, Dicky O, Mas E, Noirrit E, Marty M, Vaysse F, et al. Oral manifestations of celiac disease in French children. *Arch Pediatr*. (2021) 28:105–10. doi: 10.1016/j.arcped.2020.11.002

146. Ludovichetti FS, Signoriello AG, Girotto L, Del Dot L, Piovan S, Mazzoleni S. Oro-dental lesions in pediatric patients with celiac disease: an observational retrospective clinical study. *Rev Esp Enferm Dig*. (2022) 114:654–9. doi: 10.17235/reed.2022.8422/2021

147. Durazzo M, Ferro A, Brascugli I, Mattivi S, Fagoonee S, Pellicano R. Extra-intestinal manifestations of celiac disease: what should we know in 2022? *J Clin Med*. (2022) 11:258. doi: 10.3390/jcm11010258

148. Ferraz EG, Campos Ede J, Sarmento VA, Silva LR. The oral manifestations of celiac disease: information for the pediatric dentist. *Pediatr Dent*. (2012) 34:485–8.

149. Yılmaz S, Tuna Kırsaçlıoğlu C, Şaylı TR. Celiac disease and hematological abnormalities in children with recurrent aphthous stomatitis. *Pediatr Int*. (2020) 62:705–10. doi: 10.1111/ped.14155

150. Cervino G, Fiorillo L, Laino L, Herford AS, Lauritano F, Giudice GL, et al. Oral health impact profile in celiac patients: analysis of recent findings in a literature review. *Gastroenterol Res Pract*. (2018) 2018. doi: 10.1155/2018/7848735

151. Mínguez Rodríguez B, Montells Fuster S, Lomba Estévez M, Molera Busoms C. Martín de Carpi J.Intestinal intussusception as a key sign of Celiac Disease. A pediatric case report. *Arch Argent Pediatr*. (2020) 118:e188–90. doi: 10.5546/aap.2020.e188

152. Fernandes VP, Lomazi EA, Bellomo-Brandão MA. A rare association of intussusception and celiac disease in a child. *Sao Paulo Med J*. (2016) 19. doi: 10.1590/1516-3180.2016.0092220616

153. Shoshany C, Fahey LM. Intussusception as rare presenting sign of pediatric celiac disease: A case series. *J Pediatr Gastroenterol Nutr*. (2023) 76:343–6. doi: 10.1097/MPG.0000000000003699

154. Goyal P, Nohria S, Singh Grewal C, Sehgal R, Goyal O. Celiac disease and intussusception - a rare but important association. *Acta Gastroenterol Belg*. (2022) 85:111–2. doi: 10.51821/85.1.7959

155. Borkar VV, Poddar U, Thakral A, Agarwal J, Srivastava A, Yachha SK, et al. Intussusception in celiac disease: Is it a common feature in children? *J Gastroenterol Hepatol*. (2018) 33:380–4. doi: 10.1111/jgh.13865

156. Iacob D, Fufezan O, Farcău D, Samaşcă G, Slavcovici A, Gheban D. Celiac disease in toddler with atypical onset. Case report. *Med Ultrason*. (2016) 18:116–9. doi: 10.11152/mu.2013.2066.181.ffz

157. El Qadiry R, Lalaoui A, Nassih H, Aitsab I, Bourrahouat A. Atypical presentation of celiac disease: recurrent acute small bowel obstruction. *Clin Med Insights Case Rep*. (2021) 14:1–3. doi: 10.1177/1179547620986152

158. Makay O, Kazimi M, Doğanavşargil B, Osmanoğlu N, Yilmaz M. Acute abdomen in adult Celiac disease: an intestinal intussusception case. *Turk J Gastroenterol*. (2007) 18:103–6.

159. Reilly NR, Aguilar KM, Green PH. Should intussusception in children prompt screening for celiac disease? *J Pediatr Gastroenterol Nutr*. (2013) 56:56–9. doi: 10.1097/MPG.0b013e31826a1099

160. Vega J, Díaz R, Méndez GR, Goecke H. Nephrotic syndrome and acute kidney injury associated with celiac disease: report of one case. *Rev Med Chil*. (2013) 141:381–7. doi: 10.4067/S0034-98872013000300015

161. Wijarnpreecha K, Thongprayoon C, Panjawatanan P, Thamcharoen N, Pachariyanon P, Nakkala K, et al. Celiac disease and the risk of kidney diseases: A systematic review and meta-analysis. *Dig Liver Dis*. (2016) 48:1418–24. doi: 10.1016/j.dld.2016.08.115

162. Capolongo G, Abul-Ezz S, Moe OW, Sakhaee K. Subclinical celiac disease and crystal- induced kidney disease following kidney transplant. *Am J Kidney Dis*. (2012) 60:662–7. doi: 10.1053/j.ajkd.2012.02.342

163. Coppo R. The gut-renal connection in igA nephropathy. *Semin Nephrol*. (2018) 38:504–512. doi: 10.1016/j.semnephrol.2018.05.020

164. Mocanu A, Bogos RA, Lazaruc TI, Trandafir LM, Lupu VV, Ioniuc I, et al. Exploring a complex interplay: kidney–gut axis in pediatric chronic kidney disease. *Nutrients*. (2023) 15:3609. doi: 10.3390/nu15163609

165. Stanley JC, Deng H. Progress in pathogenesis of immunoglobin A nephropathy. *Cureus*. (2020) 12:e8789. doi: 10.7759/cureus.8789

166. Nurmi R, Korponay-Szabó I, Laurila K, Huhtala H, Niemelä O, Mustonen J, et al. Celiac disease-type tissue transglutaminase autoantibody deposits in kidney

biopsies of patients with igA nephropathy. *Nutrients*. (2021) 13:1594. doi: 10.3390/nu13051594

167. Pérez-Sáez MJ, Uffing A, Leon J, Murakami N, Watanabe A, Borges TJ, et al. Immunological impact of a gluten-free dairy-free diet in children with kidney disease: A feasibility study. *Front Immunol*. (2021) 12:624821. doi: 10.3389/fimmu.2021.624821

168. Sag E, Demir F, Sag S, Guven B, Kalyoncu M, Cakir M. Prevalence of celiac disease in children with joint hypermobility. *Acta Reumatol Port*. (2021) 46:134–9.

169. Priyadarshini S, Asghar A, Shabih S, Kasireddy V. Celiac disease masquerading as arthralgia. *Cureus*. (2022) 14:e26387. doi: 10.7759/cureus.26387

170. Rastogi A, Bhadada SK, Bhansali A, Kochhar R, Santosh R. Celiac disease: A missed cause of metabolic bone disease. *Indian J Endocrinol Metab*. (2012) 16:780–5. doi: 10.4103/2230-8210.100674

171. Kotze LM, Skare T, Vinholi A, Jurkonis L, Nisihara R. Impact of a gluten-free diet on bone mineral density in celiac patients. *Rev Esp Enferm Dig*. (2016) 108:84–8. doi: 10.17235/reed.2015.3953/2015

172. Usta M, Urganci N. Does gluten-free diet protect children with celiac disease from low bone density? *Iran J Pediatr*. (2014) 24:429–34.

173. Verma A, Lata K, Khanna A, Singh R, Sachdeva A, Jindal P, et al. Study of effect of gluten-free diet on vitamin D levels and bone mineral density in celiac disease patients. *J Family Med Prim Care*. (2022) 11:603–7. doi: 10.4103/jfmpc.jfmpc_1190_21

174. Pacitto A, Paglino A, Di Genova L, Leonardi A, Farinelli E, Principi N, et al. Celiac disease presenting with peripheral neuropathy in children: A case report. *Int J Environ Res Public Health*. (2017) 14:785. doi: 10.3390/ijerph14070785

175. Urban-Kowalczyk M, OEmigielski J, Gmitrowicz A. Neuropsychiatric symptoms and celiac disease. *Neuropsychiatr Dis Treat*. (2014) 10:1961–4. doi: 10.2147/NDT.S69039

176. Arzani M, Jahromi SR, Ghorbani Z, Vahabizad F, Martelletti P, Ghaemi A, et al. School of Advanced Studies of the European Headache Federation (EHF-SAS). Gut-brain Axis and migraine headache: a comprehensive review. *J Headache Pain*. (2020) 21:15. doi: 10.1186/s10194-020-1078-9

177. Hadjivassiliou M, Duker AP, Sanders DS. Gluten-related neurologic dysfunction. *Handb Clin Neurol*. (2014) 120:607–19. doi: 10.1016/B978-0-7020-4087-0.00041-3

178. Makhlouf S, Messelmani M, Zaouali J, Mrissa R. Cognitive impairment in celiac disease and non-celiac gluten sensitivity: review of literature on the main cognitive impairments, the imaging and the effect of gluten free diet. *Acta Neurol Belg*. (2018) 118:21–7. doi: 10.1007/s13760-017-0870-z

179. Cavusoglu D, Olgac Dundar N, Oztekin O, Arican P, Gencpinar P. Baran M. A neurological appearance of celiac disease: is there any associated factor? *Pediatr Emerg Care*. (2021) 37:303–7. doi: 10.1097/PEC.0000000000001918

180. Bashiri H, Afshari D, Babaei N, Ghadami MR. Celiac disease and epilepsy: the effect of gluten-free diet on seizure control. *Adv Clin Exp Med*. (2016) 25:751–4. doi: 10.17219/acem/43585

181. Prosperi M, Santocchi E, Brunori E, Cosenza A, Tancredi R, Muratori F, et al. Prevalence and clinical features of celiac disease in a cohort of Italian children with autism spectrum disorders. *Nutrients*. (2021) 13:3046. doi: 10.3390/nu13093046

182. Özbudak P, Karaduman AE, Menderes DK, Öztürk H, Gücüyener K. Celiac disease and catatonia: more than a coincidence? *Turk J Pediatr*. (2023) 65:144–8. doi: 10.24953/turkjped.2022.411

183. Ham H, Lee BI, Oh HJ, Park SH, Kim JS, Park JM, et al. A case of celiac disease with neurologic manifestations misdiagnosed as amyotrophic lateral sclerosis. *Intest Res*. (2017) 15:540–2. doi: 10.5217/ir.2017.15.4.540

184. Lee JE, Ryu DW, Kim JS, An JY. Celiac disease presenting as motor neuron disease. *Neurol India*. (2018) 66:1810–2. doi: 10.4103/0028-3886.246268

185. Aksoy E, Tıraş-, Kansu A, Deda G, Kartal A. Neurological findings spectrum in Celiac disease. *Turk J Pediatr*. (2016) 58:233–40. doi: 10.24953/turkjped.2016.03.001

186. Bona G, Marinello D, Oderda G. Mechanisms of abnormal puberty in coeliac disease. *Horm Res*. (2002) 57:63–5. doi: 10.1159/000058103

187. Abaci A, Esen I, Unuvar T, Arslan N, Bober E. Two cases presenting with pubertal delay and diagnosed as Celiac disease. *Clin Pediatr (Phila)*. (2008) 47:607–9. doi: 10.1177/0009922808316185

188. Gaudino R, De Filippo G, Bozzola E, Gasparri M, Bozzola M, Villani A, et al. Current clinical management of constitutional delay of growth and puberty. *Ital J Pediatr*. (2022) 48:45. doi: 10.1186/s13052-022-01242-5

189. Bayrak NA, Volkan B, Haliloglu B, Kara SS, Cayir A. The effect of celiac disease and gluten- free diet on pubertal development: a two-center study. *J Pediatr Endocrinol Metab*. (2020) 33:409–15. doi: 10.1515/jpem-2019-0378

190. Bykova SV, Sabel'nikova EA, Parfenov AI, Gudkova RB, Krums LM, Chikunova BZ. Reproductive disorders in women with celiac disease. Effect of the etiotropic therapy. *Eksp Klin Gastroenterol*. (2011) 3:12–8.

191. Stazi AV, Mantovani A. A risk factor for female fertility and pregnancy: celiac disease. *Gynecol Endocrinol*. (2000) 14:454–63. doi: 10.3109/09513590009167719

192. Remes-Troche JM, Sánchez-Vargas LA, Ríos-Gálvez S, Cano-Contreras AD, Amerena-Abreu J, Cruz-Patiño E, et al. Celiac disease seroprevalence in patients with infertility. A case-control study. *Gac Med Mex*. (2023) 159:142–6. doi: 10.24875/GMM.M23000762

193. Zugna D, Richiardi L, Akre O, Stephansson O, Ludvigsson JF. Celiac disease is not a risk factor for infertility in men. *Fertil Steril*. (2011) 95:1709–1713.e1-3. doi: 10.1016/j.fertnstert.2011.01.132

194. Sange I, Mohamed MWF, Aung S, Mereddy N, Hamid P. Celiac disease and the autoimmune web of endocrinopathies. *Cureus*. (2020) 12:e12383. doi: 10.7759/cureus.12383

195. Minelli R, Gaiani F, Kayali S, Di Mario F, Fornaroli F, Leandro G, et al. Thyroid and celiac disease in pediatric age: a literature review. *Acta BioMed*. (2018) 89:11–6. doi: 10.23750/abm.v89i9-S.7872

196. Khan SA, Imran M, Ali Q, Malik MI. Celiac disease with autoimmune hemolytic anemia and autoimmune hepatitis in a young child: case report and literature review. *Clin Med Insights Pediatr*. (2022) 16:1–4. doi: 10.1177/11795565221120565

197. Varol Fİ, Çamtosun E, Selimoğlu MA, Güngör Ş. Is there a predictive factor for an association with autoimmune glandular disease in children diagnosed with celiac disease? *J Clin Res Pediatr Endocrinol*. (2022) 14:409–14. doi: 10.4274/jcrpe.galenos.2022.2022-2-14

198. Costa Gomes R, Cerqueira Maia J, Fernando Arrais R, André Nunes Jatobá C, Auxiliadora Carvalho Rocha M, Edinilma Felinto Brito M, et al. The celiac iceberg: from the clinical spectrum to serology and histopathology in children and adolescents with type 1 diabetes mellitus and Down syndrome. *Scand J Gastroenterol*. (2016) 51:178–85. doi: 10.3109/00365521.2015.1079645

199. Szaflarska-Popławska A, Soroczyńska-Wrzyszcz A, Barg E, Józefczuk J, Korczowski B, Grzybowska-Chlebowczyk U, et al. Assessment of coeliac disease prevalence in patients with Down syndrome in Poland - a multi-centre study. *Prz Gastroenterol*. (2016) 11:41–6. doi: 10.5114/pg.2016.57794

200. Patel B, Wi CI, Hasassri ME, Divekar R, Absah I, Almallouhi E, et al. Heterogeneity of asthma and the risk of celiac disease in children. *Allergy Asthma Proc*. (2018) 39:51–8. doi: 10.2500/aap.2018.39.4100

201. Uibo O, Teesalu K, Metskula K, Reimand T, Saat R, Sillat T, et al. Screening for celiac disease in Down's syndrome patients revealed cases of subtotal villous atrophy without typical for celiac disease HLA-DQ and tissue transglutaminase antibodies. *World J Gastroenterol*. (2006) 12:1430–4. doi: 10.3748/wjg.v12.i9.1430

202. Pavlović M, Radlović N, Leković Z, Berenji K, Stojsić Z, Radlović V. Coeliac disease as the cause of resistant sideropenic anaemia in children with Down's syndrome: case report. *Srp Arh Celok Lek*. (2010) 138:91–4. doi: 10.2298/sarh1002091p

203. Malle L, Patel RS, Martin-Fernandez M, Stewart OJ, Philippot Q, Buta S, et al. Autoimmunity in Down's syndrome via cytokines, CD4 T cells and CD11c+ B cells. *Nature*. (2023) 615:305–14. doi: 10.1038/s41586-023-05736-y

204. Lupu A, Jechel E, Mihai CM, Mitrofan EC, Fotea S, Starcea IM, et al. The footprint of microbiome in pediatric asthma—A complex puzzle for a balanced development. *Nutrients*. (2023) 15:3278. doi: 10.3390/nu15143278

205. Lupu VV, Butnariu LI, Fotea S, Morariu ID, Badescu MC, Starcea IM, et al. The disease with a thousand faces and the human microbiome-A physiopathogenic intercorrelation in pediatric practice. *Nutrients*. (2023) 15:3359. doi: 10.3390/nu15153359

206. Boccuti V, Perrone A, D'Introno A, Campobasso A, Sangineto M, Sabbà C. An unusual association of three autoimmune disorders: celiac disease, systemic lupus erythematosus and Hashimoto's thyroiditis. *Auto Immun Highlights*. (2016) 7:7. doi: 10.1007/s13317-016-0079-9

207. Lauret E, Rodrigo L. Celiac disease and autoimmune-associated conditions. *BioMed Res Int*. (2013) 2013. doi: 10.1155/2013/127589

208. Goh C, Banerjee K. Prevalence of coeliac disease in children and adolescents with type 1 diabetes mellitus in a clinic based population. *Postgrad Med J*. (2007) 83:132–6. doi: 10.1136/pgmj.2006.049189

209. Joshi R, Madvariya M. Prevalence and clinical profile of celiac disease in children with type 1 diabetes mellitus. *Indian J Endocrinol Metab*. (2015) 19:797–803. doi: 10.4103/2230-8210.167555

210. Singh P, Seth A, Kumar P, Sajjan S. Coexistence of celiac disease & type 1 diabetes mellitus in children. *Indian J Med Res*. (2017) 145:28–32. doi: 10.4103/ijmr.IJMR_199_15

211. Simmons JH, Foster NC, Riddlesworth TD, DuBose SN, Redondo MJ, Liu E, et al. T1D Exchange Clinic Network. Sex- and age-dependent effects of celiac disease on growth and weight gain in children with type 1 diabetes: Analysis of the type 1 diabetes Exchange Clinic Registry. *Pediatr Diabetes*. (2018) 19:741–8. doi: 10.1111/pedi.12629

212. Delvecchio M, Bizzoco F, Lapolla R, Gentile A, Carrozza C, Barone M, et al. Iodine absorption in celiac children: A longitudinal pilot study. *Nutrients*. (2021) 13:808. doi: 10.3390/nu13030808

213. Bourhanbour AD, Ouadghiri S, Benseffaj N, Essakalli M. Serological tests for celiac disease in Moroccan patients with type 1 diabetes. *Pan Afr Med J*. (2016) 24:103. doi: 10.11604/pamj.2016.24.103.8555

214. Murray JA, Syage JA, Wu TT, Dickason MA, Ramos AG, Van Dyke C, et al. CeliacShield study group. Latiglutenase protects the mucosa and attenuates symptom severity in patients with celiac disease exposed to a gluten challenge. *Gastroenterology*. (2022) 163:1510–1521.e6. doi: 10.1053/j.gastro.2022.07.071

215. Murray JA, Kelly CP, Green PHR, Marcantonio A, Wu TT, Mäki M, et al. CeliAction study group of investigators. No difference between latiglutenase and placebo in reducing villous atrophy or improving symptoms in patients with symptomatic celiac disease. *Gastroenterology*. (2017) 152:787–798.e2. doi: 10.1053/j.gastro.2016.11.004

216. Lähdeaho ML, Kaukinen K, Laurila K, Vuotikka P, Koivurova OP, Kärjä-Lahdensuu T, et al. Glutenase ALV003 attenuates gluten-induced mucosal injury in patients with celiac disease. *Gastroenterology*. (2014) 146:1649–58. doi: 10.1053/j.gastro.2014.02.031

217. Miranda-Villa PP, Mufari JR, Bergesse AE, Calandri EL. Effects of whole and malted quinoa flour addition on gluten-free muffins quality. *J Food Sci*. (2019) 84:147–53. doi: 10.1111/1750-3841.14413

218. Newnham ED, Clayton-Chubb D, Nagarethinam M, Hosking P, Gibson PR. Randomised clinical trial: adjunctive induction therapy with oral effervescent budesonide in newly diagnosed coeliac disease. *Aliment Pharmacol Ther*. (2021) 54:419–28. doi: 10.1111/apt.16446

219. Mearin ML, Agardh D, Antunes H, Al-Toma A, Auricchio R, Castillejo G, et al. ESPGHAN special interest group on celiac disease. ESPGHAN position paper on management and follow-up of children and adolescents with celiac disease. *J Pediatr Gastroenterol Nutr*. (2022) 75:369–86. doi: 10.1097/MPG.0000000000003540

220. Hota D, Bhalla K, Nanda S, Gupta A, Mehra S. Beneficial effects of gluten free diet on IgA tissue transglutaminase levels and various growth parameters in celiac disease patients. *J Family Med Prim Care*. (2019) 8:823–7. doi: 10.4103/jfmpc.jfmpc_56_19

221. Kurppa K, Paavola A, Collin P, Sievänen H, Laurila K, Huhtala H, et al. Benefits of a gluten-free diet for asymptomatic patients with serologic markers of celiac disease. *Gastroenterology*. (2014) 147:610–617.e1. doi: 10.1053/j.gastro.2014.05.003

222. Sansotta N, Amirikian K, Guandalini S, Jericho H. Celiac disease symptom resolution: effectiveness of the gluten-free diet. *J Pediatr Gastroenterol Nutr*. (2018) 66:48–52. doi: 10.1097/MPG.0000000000001634

223. Aljada B, Zohni A, El-Matary W. The gluten-free diet for celiac disease and beyond. *Nutrients*. (2021) 13:3993. doi: 10.3390/nu13113993

224. Nestares T, Martín-Masot R, de Teresa C, Bonillo R, Maldonado J, Flor-Alemany M, et al. Influence of mediterranean diet adherence and physical activity on bone health in celiac children on a gluten-free diet. *Nutrients*. (2021) 13:1636. doi: 10.3390/nu13051636

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 29 April 2024
DOI 10.3389/fimmu.2024.1396589

OPEN ACCESS

EDITED BY
Ángela Ruiz-Carnicer,
Sevilla University, Spain

REVIEWED BY
Ezequiel Pinto,
University of Algarve, Portugal
Esteban Pérez,
University of Las Palmas de Gran Canaria, Spain

*CORRESPONDENCE
Ana Luísa Falcomer
ana.falcomer@aluno.unb.br
António Raposo
antonio.raposo@ulusofona.pt

RECEIVED 05 March 2024
ACCEPTED 15 April 2024
PUBLISHED 29 April 2024

CITATION
Falcomer AL, de Lima BR, Farage P, Fabris S, Ritter R, Raposo A, Teixeira-Lemos E, Chaves C and Zandonadi RP (2024) Enhancing life with celiac disease: unveiling effective tools for assessing health-related quality of life. *Front. Immunol.* 15:1396589. doi: 10.3389/fimmu.2024.1396589

Enhancing life with celiac disease: unveiling effective tools for assessing health-related quality of life

Ana Luísa Falcomer[1,2*], Bernardo Romão de Lima[1,2], Priscila Farage[3], Samantha Fabris[1], Ruth Ritter[1], António Raposo[4*], Edite Teixeira-Lemos[5], Cláudia Chaves[6] and Renata Puppin Zandonadi[1]

[1]Department of Nutrition, Faculty of Health Sciences, University of Brasília, Brasília, Brazil, [2]Department of Nutrition, Centro Universitário IESB, Brasília, Brazil, [3]Faculty of Nutrition (FANUT), Federal University of Goiás, Goiânia, Brazil, [4]CBIOS (Research Center for Biosciences and Health Technologies), Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal, [5]CERNAS Research Centre, Polytechnic University of Viseu, Viseu, Portugal, [6]ESSV, Centre for Studies in Education and Innovation (CI&DEI), Polytechnic University of Viseu, Viseu, Portugal

Celiac disease (CD) is an autoimmune chronic enteropathy provoked by gluten ingestion in genetically predisposed individuals. Considering it´s only safe treatment is a lifelong gluten-free diet, the burden of living with the disease becomes evident, as well as the need to assess CD health-related quality of life (HRQOL). This review aims to identify and analyze the instruments used to evaluate the HRQOL of adults with CD. This integrative review using a systematic approach was designed to achieve high scientific standards. Accordingly, the search strategy was developed and executed as recommended by the guideline of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Detailed individual searches were developed to Pubmed, Science Direct, Scopus, Web of Science, and Google Scholar. After careful analysis of the papers, 43 studies were included, in which seven instruments were identified: Celiac Disease Questionnaire (CDQ) (n=21), Celiac Disease Specific Quality of Life Instrument (CD-QOL) (n=17), Celiac Disease Assessment Questionnaire (CDAQ) (n=4), CeliacQ-7 (n=1), CeliacQ-27 (n=1), Black and Orfila´s self-developed instrument (n=1) and the Coeliac Disease Quality of Life Questionnaire (CDQL) (n=1). The CDQ and CD-QOL were the two most applied instruments. Since the first focuses on the physical and mental symptoms related to the disease and the second focuses on the emotional repercussions of adhering to the GFD treatment for life (dysphoria), the CDQ application is an interesting option for countries that struggle with public policies for CD patients and patients with active CD. The CD-QOL could be used for countries with strict regulations for CD and gluten-free products and populations in remission. When comparing results among different populations, it is preferable to utilize culturally validated instruments, which have been applied across multiple countries, providing greater comparability between study findings.

KEYWORDS

Celiac disease, gluten-free, quality of life, questionnaire, systematic review

Introduction

Celiac disease (CD) is an autoimmune chronic enteropathy by the ingestion of gluten in genetically predisposed individuals. It affects approximately 1% of the world´s population (1). As CD is linked to small bowel mucosa damage, its classic form main feature includes gastrointestinal malabsorption syndrome. Its clinical picture usually includes chronic diarrhea, abdominal distention and pain, weight loss, and failure to grow in infants (2, 3).

However, patients also usually face a wide range of extraintestinal symptoms and disorders that might include chronic fatigue, depression, anxiety, osteoporosis, compromised fertility, and libido, especially in women (4, 5). Although CD ordinarily combines a vast number of symptoms, some individuals are asymptomatic, even in cases in which mucosal damage is present (6). Those patients have a heightened risk of complications since they do not recognize the clinical aggravation of CD and tend to be more resistant to the treatment (7, 8).

Until now, the only safe and effective treatment for CD is the adoption of a gluten-free diet (GFD), characterized by the exclusion of cereal grains (wheat, rye, barley, and, in some cases, oats) and all their derivatives from the diet (9). When following a strict GFD, most patients experience remission of the disease's physical manifestations and normalization of small bowel mucosa (10, 11). Nonetheless, compliance with the GFD is challenging since it requires changes in lifelong dietary habits, which are accompanied by the lack of information and guidance for the preparation of healthy gluten-free meals among the general population, the high cost of gluten-free products, the risk of gluten cross-contamination, and even social exclusion (12). The combination of the physical, emotional, and social burdens and worries related to the GFD experienced by celiac patients is directly related to how they perceive their quality of life (4, 9).

Quality of life (QOL) is a multidimensional concept that includes subjective evaluations of both positive and negative aspects of life regarding individuals' goals, expectations, standards, and concerns (13). In the past years, worries about QOL in CD have increased and many questionnaires have been developed, adapted to different cultures, and validated to explore patients' perception of well-being (9, 14, 15).

However, instruments used to measure the QOL of celiac individuals must be carefully elaborated to comprise the specificities of CD, from its clinical manifestations to the overall difficulties faced regarding compliance with the GFD (16, 17). Thus, it is important to highlight that questionnaires whose domains do not address these particularities may present limitations (18, 19). Therefore, CD population-specific validated questionnaires are the most reliable ones since they include the patients' struggles and CD specificities (20, 21).

To our knowledge, no studies compare the existing instruments that measure celiac individuals' QOL, nor the main domains used to evaluate it. In this sense, this review aimed to identify and analyze the instruments used to evaluate the health-related quality of life of adults with celiac disease. The findings of this study may guide researchers in studies related to QOL and assist the development of public policies for celiac individuals, reducing the impact on health assistance and the costs of treatment of CD and its consequences.

Methods

This integrative review using a systematic approach was designed to achieve high scientific standards. Accordingly, the search strategy was developed and executed as recommended by the guideline of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (22).

Eligibility criteria

This review included quantitative studies that developed; translated and culturally adapted; or validated questionnaires to measure the QOL of adults with CD. Studies that evaluated QOL in the population mentioned using instruments designed for people with CD were also included.

All studies that analyzed the QOL of celiac patients using instruments designed for the general population were excluded. Additionally, qualitative studies, studies in which the population evaluated was under eighteen years old, reviews, letters, conference summaries, case reports, short communications, and books were excluded.

Information sources and search strategy

Detailed individual search strategies were developed for the following databases: Pubmed, Science Direct, Scopus, and Web of Science. Partial gray literature research was conducted using Google Scholar. The final search in all databases was performed on September 7th, 2023. Additionally, a manual examination of the reference lists of the full-text studies included was performed to ensure that possible relevant studies that could have been lost during the electronic search of databases were identified.

The literature search was conducted in English using the following terms, their mesh terms, and synonyms: "quality of life" AND ("celiac disease" OR "coeliac disease") AND ("questionnaire" OR "instrument") AND "adults". The appropriate combinations of truncation and words were selected and adapted to the search specificities of each database (Supplementary Table S1 - Supplementary File). No limitations of language or date of publication were applied; therefore, all studies published until the final search were included.

All references were managed by Endnote Web. After removing duplicate hits, the references were transferred to Rayyan, where the authors performed the selection of titles and abstracts.

Study selection and data collection processes

Calibration exercises were conducted before starting the review to ensure consistency among reviewers. The selection was conducted in two phases. In phase 1, two reviewers (SF, RR) independently reviewed the titles and abstracts of all references identified from databases. Articles that did not meet the eligibility

criteria were discarded. In phase 2, the same reviewers (SF, RR) applied the eligibility criteria to the full texts of the selected articles. In cases of disagreement, the two reviewers discussed until a consensus was obtained. A third reviewer (ALF) made the final decision when there was no consensus. These data were synthesized by the three reviewers (SR, RR, ALF) using a standardized table. The final selection was always based on the complete text of the publication. The list of references from the selected studies was critically evaluated by the BRL examiner. Additional studies were added by the experts (PF and RPZ). Figure 1 demonstrates the search and study selection processes through a flow diagram.

Data extraction

The following characteristics were collected from the selected articles and synthesized using a standardized table containing authors and year of publication, the country where the research was conducted, the aim of the study, methods, participants and sample size, instrument(s) used to measure the quality of life, and main findings. The complete table with collected results is available in Table 1.

Results

After a systematic literature search and subsequent peer analysis, 43 studies, published between 2006 and 2023, were included in this review. Table 1 presents the studies' general characteristics.

The 43 included studies were conducted in a total of 21 countries. Spain had the highest number of studies with 16.28% (n=7), followed by the United Kingdom (UK) (13.95%, n=6), the United States of America (USA), and Italy (9.3% each, n=4). In South America, Brazil accounted for 6.98% (n=3) of the studies, while Argentina, Chile, and Paraguay each contributed one study (2.33%). Iran, Portugal, France, and Germany each had two studies (4.65%, n=2 per country). Additionally, Canada, Hungary,

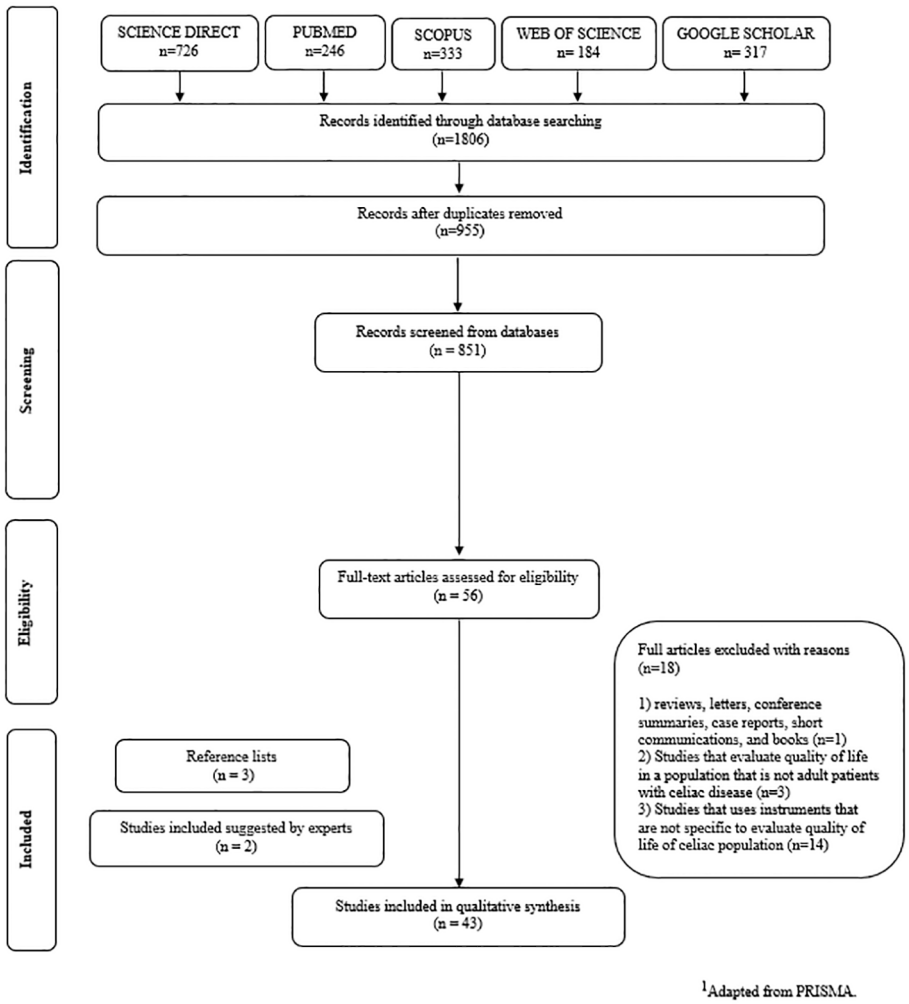


FIGURE 1
Flow Diagram of Literature Search and Selection Criteria.[1]

10.3389/fimmu.2024.1396589

TABLE 1 Summary of descriptive characteristics and instruments included in the studies.

Author	Year	Country	Title	Aim	Study design	Instrument (s) to measure of CD quality of life–
Häuser et al. (17)	2006	Germany	Development and Validation of the Celiac Disease Questionnaire (CDQ), a Disease-specific Health-related Quality of Life Measure for Adult Patients with Celiac Disease.	To develop and validate a disease-specific questionnaire to measure HRQOL in adult patients with Celiac Disease.	Development and validation of questionnaire	CDQ
Häuser et al. (15)	2007	Germany	Predictors of reduced health-related quality of life in adults with coeliac disease.	To test predictors of reduced health-related quality of life, described in the literature, by a multivariate approach	Cross-sectional study	CDQ
Dorn et al. (19)	2010	United States of America	The development and validation of a new coeliac disease quality of life survey (CD-QOL)	To develop and psychometrically validate a new coeliac disease-specific instrument, the CD-QOL	Development and validation of questionnaire	CD-QOL
Zampieron et al. (23)	2011	Italy	Quality of life in adult celiac disease in a mountain area of Northeast Italy	The aim of this study was to evaluate the health-related quality of life in patients diagnosed as having celiac disease and to study the factors involved in its impairment of quality of life	Cross-sectional study	CDQ
Black et al. (24)	2011	United Kingdom	Impact of coeliac disease on dietary habits and quality of life	The study aimed to investigate the effect of CD and a GFD on dietary habits and quality of life of a cohort of adult biopsy diagnosed coeliac patients who reside in England.	Cohort	Self-developed questionnaire
Zingone et al. (25)	2013	Italy	The Italian translation of the celiac disease-specifc quality of life scale in celiac patients on gluten free diet.	To assess the validity and reliability of the Italian translation of the Celiac Disease-specific Quality of Life Scale	Translation and cultural validation of a valid questionnaire	Italian version of CD-QoL
Marchese et al. (26)	2013	Italy	Quality of life in coeliac patients: Italian validation of a coeliac questionnaire.	To translate, cultural adapt and perform validation of the CDQ for use in Italy.	Translation and cultural validation of a valid questionnaire	Italian version of CDQ
Casellas et al. (27)	2013	Spain	Transcultural adaptation and validation of the Celiac Disease Quality of Life (CD-QOL) survey, a specific questionnaire to measure quality of life in patients with celiac disease	To translate and validate in Spanish the specific celiac disease questionnaire CD-QOL.	Translation and cultural validation of a valid questionnaire	Spanish version of CD-QOL
Lobão et al. (28)	2013	Portugal	Development of the Portuguese Version of the Celiac Disease Questionnaire	To develop the Portuguese version of the Celiac Disease Questionnaire - CDQ (developed by Dr. Winfried Häuser team in 2007	Translation and cultural validation of a valid questionnaire	Portuguese version of CDQ
Pouchot et al. (29)	2014	France	Validation of a French Version of the Quality of Life "Celiac Disease Questionnaire"	The objectives of this study were to provide a cross-cultural adaptation of the specific quality of life "Celiac Disease Questionnaire" (CDQ) and to analyze its psychometric properties	Translation and cultural validation of a valid questionnaire	French Version of CDQ (F-CDQ)
Casellas et al. (20)	2015	Spain	Benefit on health-related quality of life of adherence to gluten-free diet in adult patients with celiac disease	To examine the effect of adherence to the GFD on health perception of celiac patients measured using a specific questionnaire.	Cross-sectional study	Spanish version of CD-QOL

(Continued)

TABLE 1 Continued

Author	Year	Country	Title	Aim	Study design	Instrument (s) to measure of CD quality of life–
Castilhos et al. (5)	2015	Brazil	Quality of live evaluation in celiac patients from southern Brazil	This study aimed to evaluate the quality of life of patients with celiac disease on a capital in Southern Brazil	Cross-sectional study	CD-QOL
Aksan et al. (30)	2015	Turkey	Validation of the Turkish version of the Celiac Disease Questionnaire (CDQ)	The aim of the study was to translate, adapt and validate the Celiac Disease Questionnaire (CDQ), which was developed in Germany, for use in Turkey	Translation and cultural validation of a valid questionnaire	Turkish Version of CDQ
Lee et al. (31)	2016	United States of America	Coeliac disease: the association between quality of life and social support network participation	To exam the association between participation in different types of social support networks and quality of life in adults with CD	Cross-sectional study	CD-QOL
Rodríguez-Almagro et al. (32)	2016	Spain	Health-related quality of life and determinant factors in celiac disease. A population-based analysis of adult patients in Spain	To determine the health-related quality of life in a representative sample of Spanish adults with celiac disease along with its determinant factors.	Cross-sectional study	Spanish version of CD-QOL
Mahadev et al. (33)	2016	United States of America	Quality of Life in Screen-detected Celiac Disease Patients in the United States	To determine if differences exist between screen-detected and symptom-detected CD patients with regard to measures of QOL and dietary adherence.	Cross-sectional study	CD-QOL
Lee and Clarke (34)	2017	United States of America	Effect of clinical and laboratory parameters on quality of life in celiac patients using celiac disease-specific quality of life scores	To investigate the association between HR-QOL and clinical, laboratory findings using the previously validated CD-QOL (celiac disease-specific quality of life) instrument in patients with celiac disease.	Cross-sectional study	CD-QOL
Dowd and Jung (35)	2017	Canada	Self-compassion directly and indirectly predicts dietary adherence and quality of life among adults with celiac disease	To examine self-compassion in relation to celiac specific quality of life (CQoL) and adherence to a GFD among adults with celiac disease.	Cross-sectional study	CD-QOL
Skjerning et al. (11)	2017	Denmark and Ireland	A comprehensive questionnaire for the assessment of health-related quality of life in coeliac disease (CDQL)	To develop the Coeliac Disease Quality of Life questionnaire (CDQL): a comprehensive CD-specific HRQoL measure that can be completed by children, adolescents, and adults or by proxy.	Development and validation of questionnaire	CDQL
Real-Delor R. E. and Centurion-Medina I. C.	2017	Paraguay	Quality of life in adults from paraguay with celiac disease	The objectives of this research were to determine the quality of health-related life in adolescents and adults with celiac disease and to investigate conditions that they affect it.	Cross-sectional study	Spanish version of CD-QOL
Zysk et al. (9)	2018	Poland	Social and Emotional Fears and Worries Influencing the Quality of Life of Female Celiac Disease Patients Following a Gluten-Free Diet	The aim of the study was to analyze the social and emotional fears and worries influencing the QoL of female CD patients following a gluten-free (GF) diet, as well as to indicate the sociodemographic interfering factors.	Cross-sectional study	CDQ
Crocker et al. (36)	2018	United Kingdom	Quality of life in coeliac disease: qualitative interviews to develop candidate items for the Coeliac Disease Assessment Questionnaire	To gain indepth understanding of the impact of CD on HRQoL from the perspective of adults with the condition.	Questionnaire development	CDAQ

(Continued)

TABLE 1 Continued

Author	Year	Country	Title	Aim	Study design	Instrument (s) to measure of CD quality of life–
Crocker et al. (37)	2018	United Kingdom	Quality of life in coeliac disease: item reduction, scale development and psychometric evaluation of the Coeliac Disease Assessment Questionnaire (CDAQ)	To develop a questionnaire in accordance with best practice guidelines, capturing all aspects of quality of life important to adults with coeliac disease	Development and validation of questionnaire	CDAQ
Pratesi et al. (21)	2018	Brazil	Quality of Life of Celiac Patients in Brazil: Questionnaire Translation, Cultural Adaptation and Validation	The study aimed to translate, culturally adapt and validate a celiac disease quality of life questionnaire and apply it to a representative number of Brazilian CD patients	Translation and cultural validation of a valid questionnaire	Brazilian version of CDQ
Barzegar et al. (38)	2018	Iran	Transcultural Adaptation and Validation of Persian Version of Celiac Disease Questionnaire (CDQ); A Specific Questionnaire to Measure Quality of Life of Iranian Patients	The aim of this study was to validate a Persian version of Celiac Disease Questionnaire (CDQ) for Celiac disease (CD) among Iranian patients.	Translation and cultural validation of a valid questionnaire	Persian Version of CDQ
Burger et al. (39)	2019	Netherlands	How to best measure quality of life in coeliac disease? A validation and comparison of disease-specific and generic quality of life measures	To search for a brief, reliable, and valid tool to accurately assess the relevant quality of life domains in patients with coeliac disease. In addition, to investigate whether a disease-specific HRQoL questionnaire would add relevant information to a generic HRQoL questionnaire to better identify patients experiencing problems.	Translation and cultural validation of a valid questionnaire; Development and validation of questionnaire.	Dutch version of CD-QOL - CD-QOL-NL; self-developed instrument - CeliacQ-27; and its shorted version - CeliacQ7.
Crocker, H; Jenkinson, C; Peters, M;	2020	United Kingdom	Healthcare experiences and quality of life of adults with coeliac disease: a cross-sectional study	To investigate patients´s experiences of healthcare services in coeliac disease, from before diagnosis to the time of the survey, as well as explore the relationship between experiences of healthcare and quality of life.	Cross-sectional study	CDAQ
Harnett and Myers (40)	2020	Australia	Quality of life in people with on going symptoms of coeliac disease despite adherence to a strict gluten-free diet	To report on the quality of life in this specifc group of patients, with CD who have persistent symptoms despite adherence to a gluten free diet.	Cross-sectional study	CDQ
Fueyo-Diaz et al. (41)	2020	Spain	The effect of self-efficacy expectations in the adherence to a gluten free diet in celiac disease	To investigate the role of general and specific self-efficacy and their relationship with other psychosocial variables that can affect adherence to a GFD in patients with CD.	Cross-sectional study	Spanish version of CD-QOL
Casellas et al. (42)	2020	Spain	National survey on the experiences of people with celiac disease in Spain. The CELIAC-SPAIN project.	To know the opinion of patients and relatives regarding different aspects related to celiac, the unmet needs in the diagnosis and management of the disease, as well as the difficulties that patients have in following the diet.	Cross-sectional study	Spanish version of CD-QOL
Selleski (43)	2020	Argentina	Evaluation of Quality of Life of Adult Patients with Celiac Disease in Argentina: From Questionnaire Validation to Assessment	To translate, culturally adapt, validate, and apply the CDQ to a representative sample of the celiac population in Argentina.	Translation and cultural validation of a valid questionnaire	Argentinian version of CDQ
Fueyo-Díaz et al. (41)	2020	Spain	Influence of Compliance to Diet and Self-Efficacy Expectation on Quality of	To study the adherence to the GFD and HRQoL in patients with CD in Spain.	Cross-sectional study	Spanish version of CD-QOL

(Continued)

TABLE 1 Continued

Author	Year	Country	Title	Aim	Study design	Instrument (s) to measure of CD quality of life–
			Life in Patients with Celiac Disease in Spain.			
Muhammad et al. (44)	2021	United Kingdom	Telephone clinic improves gluten-free dietary adherence in adults with coeliac disease: sustained at 6 months	To evaluate the effect of a telephone clinic on GFD knowledge and GF dietary adherence in adults with CD.	Case-control	CDAQ
Falcomer et al. (45)	2021	Brazil	Health-Related Quality of Life and Experiences of Brazilian Celiac Individuals over the Course of the Sars-Cov-2 Pandemic.	To evaluate Brazilian celiac patients' QoL during the pandemic caused by the outbreak, rapid spread, and subsequent restrictive measures caused by COVID-19, in addition to the dietary restrictions and other burdens caused by CD.	Cross-sectional	Brazilian version CDQ
Schiepatti et al. (46)	2021	Italy	Long-Term Adherence to a Gluten-Free Diet and Quality of Life of Celiac Patients After Transition to an Adult Referral Center.	The aim of the study is threefold (1): to provide an overview on the clinical features, long-term GFD adherence, QOL, and continuity of follow-up of patients diagnosed with CD during childhood/adolescence and then followed-up at an adult tertiary referral center for CD over a twenty-year period (2); to evaluate whether timing of transition impacts long-term GFD adherence, QOL, and continuity of follow-up; and (3) to identify predictors of long-term GFD adherence in adulthood.	Prospective cohort	Italian version of CDQ
Szőcs et al. (47)	2021	Hungary	Shame mediates the relationship between stigma and quality of life among patients with coeliac disease.	The main aim of the study was the adaptation of the SSCI-8 and the necessary psychometric testing among celiac women. In addition, the study also aimed to investigate the relationship between stigmatization and different well-being variables among celiac women.	Cross-sectional study	CDQ
Dimidi et al. (8)	2021	United Kingdom	Predictors of adherence to a gluten-free diet in celiac disease: Do knowledge, attitudes, experiences, symptoms, and quality of life play a role?	To identify the relationship between adherence to a GFD and demographic characteristics, knowledge, attitudes, and beliefs regarding CD and a GFD, experiences of following a GFD, symptoms, and QoL.	Cross-sectional study	CDQ
Parada et al. (48)	2021	Chile	Adherence to a gluten-free diet and quality of life in Chilean celiac patients	To evaluate adherence to GFD and its relationship with quality of life in Chilean celiac patients.	Cross-sectional study	Spanish version of CD-QOL
Nikniaz et al. (49)	2021	Iran	The Persian Translation and validation of the celiac disease quality of life questionnaire (CDQOL)	To translate CDQOL into Persian and evaluate the psychometric properties of the Persian version.	Translation and cultural validation of a valid questionnaire	Persian version of CD-QOL
Moreno et al. (50)	2022	Spain	Quality of Life in Teenagers and Adults with Coeliac Disease: from Newly Spanish Coeliac Disease Questionnaire Validation to Assessment in a Population-based Study	To translate, culturally adapt, validade, and apply the Spanish version and estimate the HRQoL, using the EQ-5D in a representative sample of the Spanish teenagers and adults with CD.	Translation and cultural validation of a valid questionnaire	Spanish version of CDQ
Enaud et al. (51)	2022	France	Compliance with Gluten Free Diet Is Associated with Better Quality of Life in Celiac Disease	To determine the disease and clinical factors associated with better QOL in a large cohort of French CD patients.	Cross-sectional study	French Version of CDQ (F-CDQ)

(Continued)

TABLE 1 Continued

Author	Year	Country	Title	Aim	Study design	Instrument (s) to measure of CD quality of life–
Guennouni et al. (52)	2022	Morocco	Quality of life of Moroccan patients with celiac disease: Arabic translation, cross-cultural adaptation, and validation of the celiac disease questionnaire.	to translate, cross-culturally adapt, and validate the items of the CDQ and eventually evaluate the QoL among adults with CD in Morocco	Translation and cultural validation of a valid questionnaire	Morrocan version of CDQ
Chaves et al. (53)	2023	Portugal	Quality of Life Perception among Portuguese Celiac Patients: A Cross-Sectional Study Quality of Life Perception among Portuguese Celiac Patients: A Cross-Sectional Study Using the Celiac Disease Questionnaire (CDQ)	To assess Portuguese celiac patients' quality of life (QoL) perception.	Cross-sectional study	Portuguese version of CDQ

Netherlands, Poland, Morocco, Australia, and Turkey contributed one study each (2.33% each). A collaboration between Denmark and Ireland resulted in a joint research contribution (2.33%, n=1). A choropleth map regarding the distribution of the frequencies of included studies by different countries is available in Figure 2.

This article comprises information on studies published in a 17-year period, with the majority (53.33%, n=24) published in the last six years (2018-present). As evidenced in Figure 3, the first instrument developed to measure celiac QOL, the CDQ, was developed in 2006 (17) and set an important precedent for researchers in the field, since then, it became evident that the assessment of celiac QOL should be performed using tools designed to the celiac population specificities.

Among the studies that applied validated methods and questionnaires for analyzing QOL of CD patients, following instruments were identified: (i) Celiac Disease Questionnaire (CDQ) (n=21) (ii) Celiac Disease Specific Quality of Life Questionnaire (CD-QoL) (n=17); (iii) Celiac Q27 (n=1); (iv) Celiac Q7(n=1); (v) Coeliac Disease Assessment Questionnaire (CDAQ) (n=4); and (vi) Celiac Disease Quality of Life Questionnaire (CDQL) (n=1). It is imperative to emphasize the importance of cultural adaptation when using validated instruments like the ones mentioned in this review in different sociocultural backgrounds since the experiences and challenges faced by individuals with celiac disease can vary across different countries and cultural backgrounds. Studies carried out without cultural adaptation may fail to accurately capture the unique factors influencing the quality of life for celiac patients in the countries′ specific contexts.

Concerning the domains in the included instruments, a total of fifteen domains were observed when all instruments were

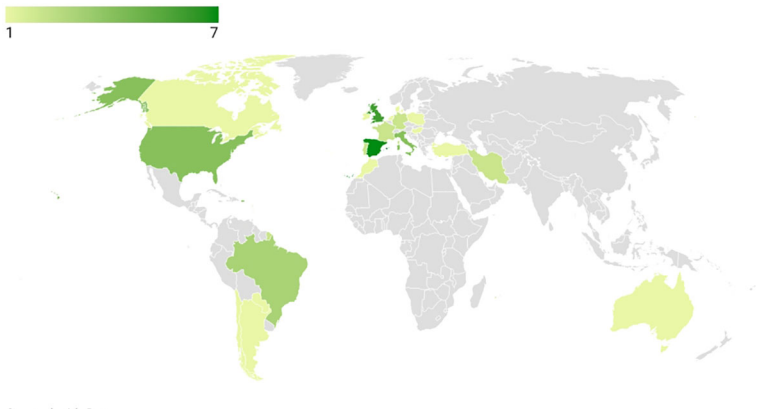


FIGURE 2
Chroropleth map generated from the number of studies according to their origin. Higher frequencies are represented with darker tones of green.

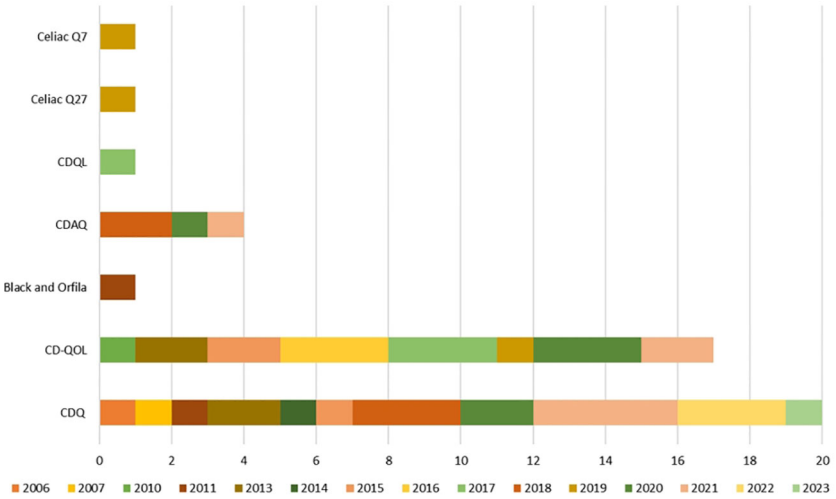


FIGURE 3
Use of specific questionnaires to measure celiac patients' quality of life throughout time.

analyzed together Clique ou toque aqui para inserir o texto (14, 17, 21, 27, 32, 34, 52). Figure 4 graphically represents the domains of the seven instruments included, it also represents intersections between domains with similar nomenclatures/subjects in different questionnaires.

It is important to note that similar denominations of the domains present in different questionnaires can evaluate different constructs depending on the questionnaire. Also, domains with different nomenclatures evaluate similar constructs.

Discussion

As displayed in Figure 2, the countries that have executed more research on the topic are Spain, followed by the UK, Italy, and the USA. It can be inferred that developed countries tend to have more preoccupation regarding health-related quality of life (HRQOL), more access to CD diagnoses and deal with less misdirection from CD diagnosis due to infectious diseases, which represents a struggle to control acute gastrointestinal cases, and the fact that there used to be a misconceived association of CD with populations exclusively of Caucasian origin (54, 55). This may explain the range of countries where studies regarding the quality of life of celiac patients were conducted.

In addition, it is essential to emphasize the importance of investigating celiacs´ health dimensions in nations that have not yet done so, even though CD has been reported in them, such as India and Russia, both of which rank among the world's ten most populous countries, along with several others (54, 56). The recent increase in studies reflects a growing awareness about how celiac disease impacts patients' quality of life. This heightened focus may stem from either an escalating prevalence of CD over time or an increased recognition of its importance as a global public health issue in the past two decades (54, 55).

CD specific instruments to assess QOL

Celiac disease questionnaire

The CDQ was designed in Germany in 2007 through the prism of patients, experts in the CD field and scientific literature to evaluate celiac individuals' HRQOL, being a pioneer in the subject (17, 18). It is a quantitative 10-minute self-administered questionnaire composed of four subscales: emotional issues, social problems, disease-related worries, and gastrointestinal symptoms. Each subscale has seven sub-items each, resulting in a total of 28 questions (18, 30).

The CDQ domains consider the patient´s feelings and perceptions concerning the challenges the disease and the GFD impose in their lives (29).The subcategories comprehend the dimensions of HRQOL, which are physical, emotional and social, and were related to domains of other disease-specific instruments like the Chronic Liver Disease Questionnaire (57) and the Inflammatory Bowel Disease Questionnaire (58).

The participants' answers are evaluated using a 7-point Likert scale that varies in crescent punctuation, from always, most of the time, often, now and then, rarely, almost never, and never (18). The CDQ final evaluation score is a result of the addition of each question´s points, and therefore ranges from a total of 0 to 196 points; the score varies from 0 to 49 in all domains (18, 30). Lower scores indicate reduced HRQOL, hence higher values indicate high HRQOL (18). Although the CDQ score does not have a cut-off point, its development included a group of people who do not present CD-related disease as a comparison (18).

Throughout various studies conducted in different countries, the CDQ was used for measuring HRQOL in its original version, developed and validated in Germany (17), and has also been translated, culturally adapted, and validated to a diverse range of

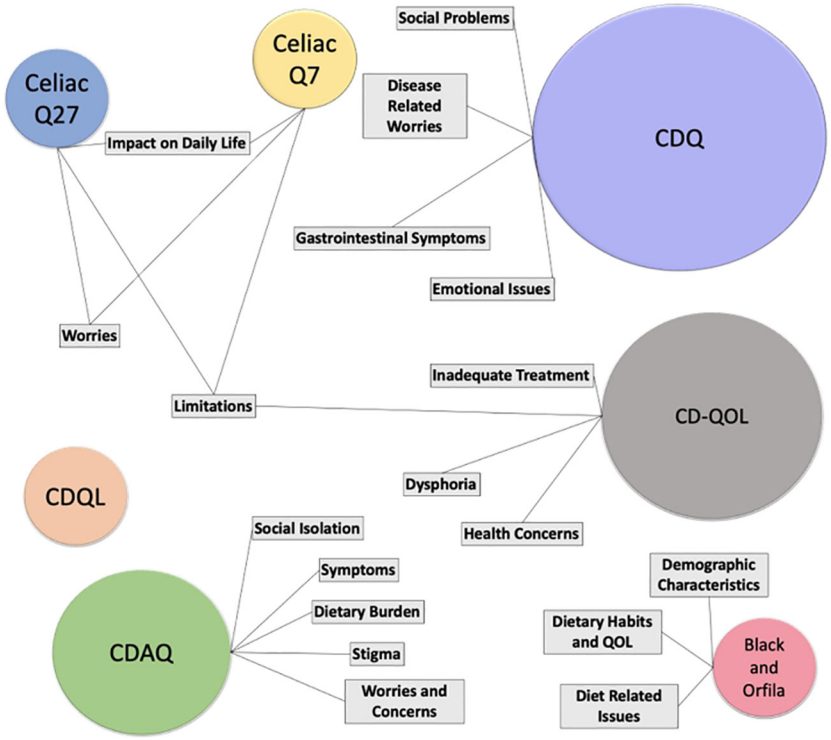


FIGURE 4
Graphical representation of the present domains in the included instruments and their respective intersections. Most utilized instruments are represented with proportional bigger circles.

populations. The adapted versions were applied to populations in Italy (25, 26, 46), Portugal (28, 53), France (29, 51), Turkey, Brazil (21, 45), Poland (9), Iran (38), Argentina (43), Morocco (52), and Spain (50). Furthermore, the original CDQ was employed in studies conducted in Germany (17), Italy (23), Poland (9), Australia (40), Hungary (47), UK (8). Overall, the CDQ has been applied across 21 different studies (48.4%) spanning 15 countries, corresponding to 71,42% of the nations that investigated the thematic and are contemplated in this review.

In the original paper, the total score of QOL indicated that in Germany, people with CD presented lower QOL (143.1) than people without CD-associated disease (157.6), suggesting that celiac does negatively impact patients' well-being (18). As the CDQ was developed for the German population, performing cultural adaptation as well as translation and validation of the tool to other countries is strongly recommended to minimize bias in QOL assessment and, consequently, data interpretation. In addition, since the original version of CQD is designed for on-paper applications, adapting to a web-based version is interesting for online applications.

The CD populations that presented the highest and lowest CDQ scores were Italian (159 score) (26) and Portuguese (103 score) (53). As the instrument has been applied only once in Portugal, it is not possible to compare the QOL scores over time. However, as the data was collected during COVID-19 pandemic, it could have negatively interfered with the score, especially over the social domain punctuation (53). However, as discussed by the Portuguese study, isolation can have a positive effect on GFD adherence, and it can be analyzed in Brazilian scores (53).

Brazil´s first assessment of celiac QOL was in 2018 and obtained a 119.79 (21) and the second CDQ application took place during COVID-19 and found a 125.26 score (45). Authors associate the improvement in celiac well-being in the pandemic period to the reduced social interactions involving gluten-containing food, which have negative repercussions in social and emotional domains, as well as the increase of home meal preparation that is a protective factor to GFD adherence, impacting positively in the emotional, social, worries and symptoms scales 383838.

Regarding the psychometric quality and quantitative parameters, the Cronbach's alpha for the CDQ domains ranged from 0.80 to 0.91, as instruments are viable when Cronbach's alpha is higher than 0.7 (18). All adaptations of the questionnaire presented over 0.7 values of Cronbach's alpha. Therefore, the CDQ is a valid instrument to measure QOL of celiac patients that contemplates HRQOL dimensions.

Celiac disease specific quality of life instrument

The CD-QOL is a quantitative, self-administered questionnaire of 20 items distributed across four subscales: limitations, dysphoria,

health concerns, and inadequate treatment (19). Researchers sought to capture in the instrument how patients perceive changes in their daily lives after diagnosis by consulting a celiac support group for input during the focus group stage (19). The answers to each item are allocated into a 5-point Likert Scale response, ranging from "not at all" to "a great deal".

Each subscale is associated with factors that can negatively impact the QOL of individuals with CD. The first factor is called limitations and refers to the feeling of facing difficulties in belonging to or being part of routine events, such as eating with coworkers, not being able to eat conventional foods on important occasions (e.g., birthday cake) or struggling to take long trips due to CD and GFD (19). The second factor is dysphoria and is associated with a feeling of emotional dissatisfaction or discomfort due to the CD, the items in this category question if the participant feels depressed, frightened, or overwhelmed about having CD; it also inquires If the person believes not to have enough knowledge about CD (19). The third subscale, health concerns, is based on items of concern of increased risk of stigma due to the disease (19). The last category is inadequate treatment and it´s items inquire if patients feel like the GFD is sufficient treatment for CD (19).

It is noticeable that the CD-QOL approach focuses more on the individuals' perceptions of external elements and challenges related to adhering to a gluten-free lifestyle rather than on gastrointestinal or extra gastrointestinal symptoms affecting the quality of life, from an urge to use the bathroom to sexual activities. The main difference between the CDQ and the CD-QOL instruments is that the first focuses on repercussions of the CD in physiological repercussions, psychological symptoms, and impact in daily activities (e.g., work, leisure, etc.); whereas the second targets attitudes and perceptions of the celiac population in routine events such as socializing. The CD-QOL does not include any item to measure the physical impact of CD since the individuals in its population, which included American celiac support group members, did not emphasize symptoms as a struggle, which could be a characteristic of the USA population or public policies. That highlights the need to perform a cultural adaptation and validation of the questionnaire when assessing the QOL of people with CD. The questionnaire was applied in 17 papers (39,53%) of 9 countries and applied in 8 nations (38.09% of all countries included in this review).

The original version of CD-QOL was utilized in the USA (19, 31, 33, 34), Canada (35), Brazil (5). Cultural adaptation and validation were conducted in Italy (25), Spain (20, 27, 32, 41, 42, 59), and Iran (49); the CD-QOL was also adapted to the Netherlands (39), but it was not applied to the population, it was used to develop new questionnaires. The Spanish version of the instrument (27) was applied to the Spanish-speaking countries of Paraguay (60) and Chile (48); however, it was not adapted to South American specificities. It´s possible to suggest that all four subscales of the CD-QOL are susceptible to changes due to regional influences in the exposome and public policies such as regulations for specialized health service support to people with CD, therefore the cultural adaptation is recommended even for countries with same mother language (55).

CeliacQ-27 and CeliacQ-7

The CeliacQ-27 and CeliacQ-7 aim to evaluate CD HRQOL. It was developed and validated in Dutch, considering the cross-cultural adaptation of the CD-QOL to the Netherlands (19, 39). These questionnaires offer a unique approach by comparing different phases of CD, including active/clinical remission periods as well as instances where individuals may deviate from their gluten-free diet.

The CeliacQ-27 consists of 27 questions categorized into three domains: limitations (11 items), worries (10 items), and impact on daily life (6 items) (39). The limitations domain is related to patients' perceptions of daily life restrictions because of the CD or GFD. The worries domain questions about mental and general challenges associated with CD (39). The third domain, impact on daily life, comprises questions about the social influences of the CD and GFD (39). Higher scores in de CeliacQ27 equal better QOL. The internal consistency of the questionnaire domains ranged from Chronbach´s α of 0.87 and 0.92, demonstrating good to excellent reliability (39).

Its shorter version contained only seven questions (CeliacQ-7) and was created by excluding all items with loads <0.70 Chronbach´s α in the Dutch version of the CD-QOL (39). The final version obtained a 0.88 Chronbach´s α and a high correlation with the CeliacQ-27 (39).

Since the CeliacQ-27 and the CeliacQ-7 are both derived from the Danish version of the CD-QOL, the two do not include questions to assess the repercussions of the physical symptoms associated with CD in patients' well-being (39). However, introducing a condensed questionnaire could increase participation rates in surveys and be an interesting tool for ambulatory assistance and follow-ups, especially for patients who have been following a GFD.

Coeliac disease assessment questionnaire

The Coeliac Disease Assessment Questionnaire (CDAQ) was developed in two stages in 2018 (36, 37). The premise for developing this instrument was that the questionnaires available at the time were constructed without considering patient-reported outcome measures (PROM), which consider patients' point of view, not necessarily measured by biological markers or associated with clinical outcomes (61).

In the first phase of development, qualitative interviews were conducted and analyzed through a data framework, which revealed six common themes reported by the participants: symptoms, gluten-free diet, emotional health, impact on activities, relationships, and financial issues (36). In the first phase, 64 items were present in the instrument (36). The subsequent phase was centered on the item reduction of the first version of the instrument; in this sense, items were refined through item appraisal, expert review, cognitive interviews and translatability assessment (37).

The resultant instrument comprises 32 items in five domains: stigma, dietary burden, symptoms, social isolation and worries, and

concerns (37). Concerning its psychometric quality and quantitative parameters, this instrument presented Cronbach's alpha between 0.82 and 0.88 for all domains. These values demonstrate the viability of the instrument, since an instrument of this type is considered viable when its Cronbach's alpha is higher than 0.7 (62). Another highlight regarding CDAQ's quantitative parameters is related to its strong intraclass correlation (0.86) with SF-36's domains, the Short Form Health Survey developed by the World Health Organization (WHO) (37).

Regarding its application, the questionnaire is structured on a five-point Likert scale (never, rarely, sometimes, often and always), which is later converted into a scale of 0-100, with 100 being the highest quality of life (QOL) (37). Two studies (4.65%) included in this review used this instrument, one being the pilot study developed by Crocker et al. (2018) (36) in the United Kingdom and another carried out through a telephone survey in the same region in 2020 (44).

In a study of 276 people (166; 61.9% women and 110; 38.1% men), the results revealed an average quality of life score of 53.6 on the instrument´s proposed 0-100 scale (37). Significant differences ($p < 0.05$) were found between the two groups, with male participants showing a higher overall quality of life (60.91) compared to women (49.18). However, potential reasons for this difference were not explored by the authors (37).

The other study that used the CDAQ as an instrument to assess the QOL with CD evaluated the effectiveness of telephone monitoring in improving the QOL of this population, evaluating, in addition to the quality of life, adherence to a GFD (44). The results demonstrated that although the intervention was effective in improving adherence to the gluten-free diet, quality of life parameters did not differ significantly between the treated and control groups, with emphasis on assessments in the "dietary burden" domain, which assesses the difficulty of diet be followed (44).

Based on its quantitative parameters, the CDAQ is an appropriate instrument for assessing the QOL in patients with CD. However, the low application of this questionnaire in studies outside the UK stands out, in addition to the fact that, to date, studies regarding the translation of this instrument into other languages have not been carried out. A possible hypothesis is that the instrument is relatively new (2018) compared to other instruments already developed and applied in different countries, such as the CDQ, which was developed in 2006 (15, 37).

Coeliac disease quality of life questionnaire

The CDQL consists of a 44-item questionnaire developed in 2017 for CD patients of all ages and applied in one of the studies contemplated in this review (n=1, 2,32%) (11). It was designed in Ireland and Denmark and occurred in three phases: focus groups to collect celiacs' insights and important aspects of QOL that should be in the final questionnaire; CD patients responded to the pilot version of the CDQL; refinement of the final version of the CDQL and application to Danish participants (11).

The final version of the instrument was web-based and estimated to take 5-10 minutes to complete (11). It included twelve items about background information, covering demographic characteristics and diagnosis data; two generic QOL questions; and 30 CD specific questions attributed to ten scales, the first two being general and followed by eight CD specific categories (11). The CDQL evaluates specific CD scales including: worries-about-symptoms (1 general item with thirteen alternatives); symptoms (1 general item with thirteen alternatives); contacting health care (3 items); having coeliac disease and following a gluten-free diet (7 items); communicating about coeliac disease and gluten-free diet (4 items); others´ handling my coeliac disease (3 items); confronting gluten-containing food (4 items); knowing about coeliac disease and gluten-free food (3 items); gluten-free food supply (3 items); evaluating having coeliac disease in overall (3 items) (11).

For scoring procedures, items are evaluated through a 5-point Likert scale, alternating among ´very unwell´, ´unwell´, ´neutral´, ´well´ or ´very well´. All response choices were followed by a smiley communicating the analogous emotion (11). The final score was calculated using the average score of each scale, higher results on the Likert scale indicate better QOL (11).

Though the CDQL was developed for children, adolescents and adults, the focus group step included only a few adults, which poses a limiting factor despite the items being formulated in a non-age-restricted way (11). On the other hand, this instrument presents the opportunity to evaluate patient reported HRQOL across different ages using a unique questionnaire (11). As far as we know, the CDQL has not been reapplied or translated/adapted culturally to any other country. Further studies are necessary to corroborate the pilot study results and their applicability to all age segments.

Self-developed questionnaire (Black and Orfila)

One of the studies included in this review (2.32%) aimed to analyze the quality of life of participants in an observational cohort conducted in 2011 with 146 CD patients who were members of the Coeliac UK Charity (24). However, in addition to the fact that this study did not use an instrument created and validated by other authors, it also included a food frequency questionnaire, seeking to relate the quality of life with the participants' dietary habits (24).

In this sense, the authors developed a questionnaire composed of 32 questions, of which 10 are related to demographic variables, 10 to dietary habits (including availability of gluten-free foods and accidental ingestion of gluten due to cross-contamination) and 12 questions related to quality of life (24). It is important to highlight that although the questionnaire developed by the authors is not validated, such questions were derived from previously validated questionnaires, such as the Canadian Celiac Health Survey and the EPIC-norfolk food frequency questionnaire (63, 64).

It´s structured on a Likert scale, with five points: all of the time, most of the time, some of the time and never (24). Furthermore, the results were interpreted based only on the frequencies of answers on

each point, with no corresponding scale to assess the quality of life (24).

As a main result, the cohort study demonstrated that CD impacts participants' daily habits; however, most participants reported good physical health (24). Also, 97% of the participants reported good dietary compliance, with results supported by the food frequency questionnaire (24). Regarding the critical situations that affect the quality of life of these people, anxiety and depression related to social isolation resulting from dietary restriction and pain resulting from both intentional and unintentional gluten ingestion have been reported (24).

As the main limitation of this instrument, it is important to highlight that given the fact that it has not been validated or culturally adapted, the results from this study cannot be extrapolated to other populations with celiac disease. Also, no statistical assessment of the psychometric constructs of the items and sections was performed.

Domains of the included instruments

As presented in Figure 4, CD symptoms are explicitly described only in the domains of the CDQ (17) and CDAQ (37). However, while the CDQ domain related to symptoms only presents questions regarding bowel movements, diarrhea, gas, bloating and abdominal cramps, CDAQ includes symptoms related to mental health, such as tiredness, exhaustion, limitation of daily activities and general pain (36, 37).

Yet, issues regarding the same symptoms are also assessed in the instrument developed by Black and Orfila (24); however, under both domains of Dietary Habits and QOL and Diet-related issues. In the CDQ (17) instrument, such constructs are assessed under the "emotional issues" domain and in Celiac Q27 and Celiac Q7 in the Impact of Daily Life domain (39).

The "worries" domain is present in the CDQ (17), Celiac Q7 and Q27, CD-QOL (19) and CDAQ (36, 37) instruments. However, different scales relate to which spheres of life such worries refer. While in Celiac Q7 and 27 (39) such worries include conditions that relate to problems such as food availability outside the household, social interactions, and unpredictable bowel movements, in other questionnaires such as the CDAQ (36, 37), CDQ (17), and CD-QOL (19) such conditions are better stratified into other specific domains such as "Dietary Burden", and "Social Problems". In the CDAQ instrument, the concern regarding the availability of safe gluten-free food is measured in questions from the "dietary burden" domain (36, 37).

The domain entitled "Limitations" is found in the Celiac Q27 575757, Celiac Q7 (39) and CD-QOL (19) instruments. In both CeliacQ27 and Celiac Q7, questions assigned under this domain regard quotidian challenges present in patients' lives, such as persistent symptoms, changes in the composition of foods previously labeled as "gluten-free" and situations regarding social acceptance while coexisting with celiac disease (39). In the context of CD-QOL (19), similar questions are present in the limitations´ domain, thus showing similarities between those three instruments.

However, questions regarding similar situations before addressed in the "Limitations" domain in CD-QOL (19), Celiac Q27 and Celiac Q7 (39) are also present in other instruments, for example, in "Dietary Related Issues" domain in the instrument created by Black and Orfila (24), "Disease related worries" (CDQ (17)) and "Social isolation" (CDAQ (36, 37),.

It is important to highlight that evaluating domains is challenging, given the semantic obstacles related to the proposed construct to be evaluated by different instruments (65). Furthermore, given the context that most instruments undergo translation and cultural validation, possible changes in the meaning of the constructs may occur (66, 67). Such differences make it difficult to compare the effectiveness of different instruments which, despite being individually statistically validated, may not be possible to be applied together given the differences between the literal meanings of the domains present (65–67).

The assessment of QOL in individuals with CD plays a crucial role in gaining insight into the well-being and impact of the disease. To effectively measure QoL, researchers have developed and validated various questionnaires that consider the unique experiences and management strategies associated with CD. Notably, two questionnaires stood out, the CDQ and the CD-QOL. Since the first focuses on the physical and mental symptoms related to the disease and the second focuses on the emotional repercussions of adhering to the GFD treatment for life (dysphoria), the CDQ application is an interesting option for countries that struggle with public policies for CD patients and patients with active CD; whereas the CD-QOL could be used for countries that have GF and CD regulations and populations in remission. When comparing results among different populations, it is preferable to utilize culturally validated instruments, which have been applied across multiple countries, providing greater comparability between study findings.

Author contributions

AF: Conceptualization, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. BdL: Formal Analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. PF: Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. SF: Investigation, Visualization, Writing – review & editing. RR: Investigation, Visualization, Writing – review & editing. AR: Funding acquisition, Resources, Supervision, Writing – review & editing. ET-L: Funding acquisition, Writing – review & editing. CC: Funding acquisition, Writing – review & editing. RZ: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The study was partially supported by FAPDF N° 539/2022 and the Brazilian National Council for Scientific and Technological Development (CNPq - N° 302602/2021-6). This work was also funded by National Funds through the FCT-Foundation for Science and Technology, I.P., within the scope of the projects Ref[a] UIDB/05507/2020 and UIDB/00681/202.

Acknowledgments

The authors acknowledge the University of Brasília, *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES), *Fundação de Apoio à Pesquisa do Distrito Federal* (FAP-DF) and the Brazilian National Council for Scientific and Technological Development (CNPq) for their scientific support. This work was supported by the FCT—Foundation for Science and Technology, Portugal. Furthermore, we would like to thank the Centre for Studies in Education and Innovation (CI&DEI), Centre for Natural Resources, Environment and Society (CERNAS) and the Institute Polytechnic of Viseu for their support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1396589/full#supplementary-material

References

1. Biagi F, Corazza GR. Mortality in celiac disease. *Nat Rev Gastroenterol Hepatol.* (2010) 7:158–62. doi: 10.1038/nrgastro.2010.2

2. Rodrigues M, Yonaminez GH, Satiro CA. Rate and determinants of non-adherence to a gluten-free diet and nutritional status assessment in children and adolescents with celiac disease in a tertiary Brazilian referral center: A cross-sectional and retrospective study. *BMC Gastroenterol.* (2018) 18:1–8. doi: 10.1186/s12876-018-0740-z

3. Queiroz MS, Nery M, Cançado EL, Gianella-Neto D, Liberman B. Prevalence of celiac disease in Brazilian children of short stature. *Braz J Med Biol Res.* (2004) 37:55–60. doi: 10.1590/S0100-879X2004000100008

4. Casellas F, Rodrigo L, López Vivancos J, Riestra S, Pantiga C, Baudet JS, et al. Factors that impact health-related quality of life in adults with celiac disease: A multicenter study. *World J Gastroenterol.* (2008) 14:46–52. doi: 10.3748/wjg.14.46

5. Castilhos AC, Gonçalves BC, Macedo e Silva M, Lanzoni LA, Metzger LR, Kotze LMS, et al. Quality of life evaluation in celiac patients from southern Brazil. *Arq Gastroenterol.* (2015) 52:171–5. doi: 10.1590/S0004-28032015000300003

6. Itzlinger A, Branchi F, Elli L, Schumann M, Itzlinger A, Branchi F, et al. Gluten-free diet in celiac disease—Forever and for all? *Nutrients.* (2018) 10:1796. doi: 10.3390/nu10111796

7. Aspasia S, Emmanuela-Kalliopi K, NikoLaos T, Eirini S, Ioannis S, Anastasia M. The gluten-free diet challenge in adults with coeliac disease: the hellenic survey. *PEC Innovation.* (2022) 1:1–5. doi: 10.1016/j.pecinn.2022.100037

8. Dimidi E, Kabir B, Singh J, Ageridou A, Foster C, Ciclitira P, et al. Predictors of adherence to a gluten-free diet in celiac disease: do knowledge, attitudes, experiences, symptoms, and quality of life play a role? *Nutrition.* (2021) 90:1–9. doi: 10.1016/j.nut.2021.111249

9. Zysk W, Głąbska D, Guzek D. Social and emotional fears and worries influencing the quality of life of female celiac disease patients following a gluten-free diet. *Nutrients.* (2018) 10:1414. doi: 10.3390/NU10101414

10. Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology.* (2001) 120:636–51. doi: 10.1053/gast.2001.22123

11. Skjerning H, Hourihane J, Husby S, DunnGalvin AA. Comprehensive questionnaire for the assessment of health-related quality of life in coeliac disease (CDQL). *Qual Life Res.* (2017) 26:2831–50. doi: 10.1007/s11136-017-1632-3

12. Farage P, Zandonadi RP, Austin J. Sci the gluten-free diet: difficulties celiac disease patients have to face daily. *Austin J Nutri Food Sci.* (2014) 2:1027–5.

13. World Health Organization (WHO). *WHO WHO | WHOQOL: measuring quality of life*. WHO (2014).

14. Abreu Paiva LM, Gandolfi L, Pratesi R, Harumi Uenishi R, Puppin Zandonadi R, Nakano EY, et al. Measuring quality of life in parents or caregivers of children and adolescents with celiac disease: development and content validation of the questionnaire. *Nutrients.* (2019) 11:2302. doi: 10.3390/nu11102302

15. Häuser W, Stallmach A, Caspary WF, Stein J. Predictors of reduced health-related quality of life in adults with coeliac disease. *Aliment Pharmacol Ther.* (2007) 25:569–78. doi: 10.1111/j.1365-2036.2006.03227.x

16. Paarlahti P, Kurppa K, Ukkola A, Collin P, Huhtala H, Mäki M, et al. Predictors of persistent symptoms and reduced quality of life in treated coeliac disease patients: A large cross-sectional study. *BMC Gastroenterol.* (2013) 13:1–8. doi: 10.1186/1471-230X-13-75

17. Häuser W, Gold J, Stein J, Caspary WF, Stallmach A. Health-related quality of life in adult coeliac disease in Germany: results of a national survey. *Eur J Gastroenterol Hepatol.* (2006) 18:747–53. doi: 10.1097/01.meg.0000221855.19201.e8

18. Hauser W, Gold J, Stallmach A, Caspary WF, Stein J. Development and validation of the celiac disease quality of life measure for adult patients with celiac disease. *Jornal Clin Gastroenterol.* (2007) 41:157–66. doi: 10.1097/01.mcg.0000225516.05666.4e

19. Dorn SD, Hernandez L, Minaya MT, Morris CB, Hu Y, Leserman J, et al. The development and validation of a new coeliac disease quality of life survey (CD-QOL). *Aliment Pharmacol Ther.* (2010) 31:666–75. doi: 10.1111/j.1365-2036.2009.04220.x

20. Casellas F, Rodrigo L, Lucendo AJ, Fernández-Bañares F, Molina-Infante J, Vivas S, et al. Benefit on health-related quality of life of adherence to gluten-free diet in adult patients with celiac disease. *Spanish J Gastroenterol.* (2015) 107:196–201.

21. Pratesi CB, Häuser W, Uenishi RH, Selleski N, Nakano EY, Gandolfi L, et al. Quality of life of celiac patients in Brazil: questionnaire translation, cultural adaptation and validation. *Nutrients.* (2018) 10:1167. doi: 10.3390/nu10091167

22. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* (2021) 372:1–9. doi: 10.1136/bmj.n71

23. Zampieron A, Daicampi C, Martin A, Buja A. Quality of life in adult celiac disease in a mountain area of northeast Italy. *Gastroenterol Nurs.* (2011) 34:313–9. doi: 10.1097/SGA.0b013e3182248a73

24. Black JL, Orfila C. Impact of coeliac disease on dietary habits and quality of life. *J Hum Nutr Dietetics*. (2011) 24:582–7. doi: 10.1111/j.1365-277X.2011.01170.x

25. Zingone F, Iavarone A, Tortora R, Imperatore N, Pellegrini L, Russo T, et al. The Italian translation of the celiac disease-specific quality of life scale in celiac patients on gluten free diet. *Digestive Liver Dis*. (2013) 45:115–8. doi: 10.1016/j.dld.2012.10.018

26. Marchese A, Klersy C, Biagi F, Balduzzi D, Bianchi PI, Trotta L, et al. Quality of life in coeliac patients: Italian validation of a coeliac questionnaire. *Eur J Intern Med*. (2013) 24:87–91. doi: 10.1016/j.ejim.2012.09.015

27. Casellas F, Rodrigo L, Molina-Infante J, Vivas S, Lucendo AJ, Rosinach M, et al. Transcultural adaptation and validation of the celiac disease quality of life transcultural adaptation and validation of the celiac disease quality of life (CD-QOL) survey, a specific questionnaire to measure quality of life in patients with celiac dis. *Rev Espanõla Enfermedades Digestivas*. (2013) 105:585–93. doi: 10.4321/S1130-01082013001000003

28. Lobão C, Gonçalves R, Baltazar RM. Desenvolvimento da versão portuguesa do celiac disease questionnaire. *Rev Internacional Cienc Sociales*. (2013) 2:1–8. doi: 10.37467/gka-revsocial.v2.1229

29. Pouchot J, Despujol C, Malamut G, Ecosse E, Coste J, Cellier C. Validation of a french version of the quality of life "Celiac disease questionnaire". *PloS One*. (2014) 9:1–9. doi: 10.1371/journal.pone.0096346

30. Aksan AAA, Mercanligil SM, Häuser W, Karaismailo E, Mercanlıgil SM, Häuser W, et al. Validation of the turkish version of the celiac disease questionnaire (CDQ). *Health Qual Life Outcomes*. (2015) 13:1–7. doi: 10.1186/s12955-015-0272-y

31. Lee AR, Wolf R, Contento I, Verdeli H, Green PHR. Coeliac disease: the association between quality of life and social support network participation. *J Hum Nutr Dietetics*. (2016) 29:383–90. doi: 10.1111/jhn.12319

32. Rodríguez-Almagro J, Hernández-Martínez A, Lucendo AJ, Casellas F, Solano-Ruiz MC, Siles-González J, et al. Health-related quality of life and determinant factors in celiac disease. A population-based analysis of adult patients in Spain. *Spanish J Gastroenterol*. (2016) 108:181–189. doi: 10.17235/reed.2016.4094/2015

33. Mahadev S, Gardner R, Lewis SK, Lebwohl B, Green PH. Quality of life in screen-detected celiac disease patients in the United States. *J Clin Gastroenterol*. (2016) 50:393–7. doi: 10.1097/MCG.0000000000000433

34. Lee J, Clarke K. Effect of clinical and laboratory parameters on quality of life in celiac patients using celiac disease-specific quality of life scores. *Scand J Gastroenterol*. (2017) 52:1235–9. doi: 10.1080/00365521.2017.1350283

35. Dowd AJ, Jung ME. Self-compassion directly and indirectly predicts dietary adherence and quality of life among adults with celiac disease. *Appetite*. (2017) 113:293–300. doi: 10.1016/j.appet.2017.02.023

36. Crocker H, Jenkinson C, Peters M. Quality of life in coeliac disease: qualitative interviews to develop candidate items for the coeliac disease assessment questionnaire. *Patient Relat Outcome Meas*. (2018) 9:211–20. doi: 10.2147/prom.s149238

37. Crocker H, Jenkinson C, Peters M. Quality of life in coeliac disease: item reduction, scale development and psychometric evaluation of the coeliac disease assessment questionnaire (CDAQ). *Aliment Pharmacol Ther*. (2018) 48:852–62. doi: 10.1111/apt.14942

38. Barzegar F, Pourhoseingholi MA, Rostami-nejad M, Gholizadeh S, Malekpour MR, Sadeghi A, et al. Transcultural adaptation and validation of Persian version of celiac disease questionnaire (CDQ); A specific questionnaire to measure quality of life of Iranian patients. *Galen Med J*. (2018) e1106:1–7. doi: 10.22086/gmj.v0i0.1106

39. Burger JPW, Van Middendorp H, Drenth JPH, Wahab PJ, Evers AWM. How to best measure quality of life in coeliac disease? A validation and comparison of disease-specific and generic quality of life measures. *Eur J Gastroenterol Hepatol*. (2019) 31:941–7. doi: 10.1097/MEG.0000000000001432

40. Harnett JE, Myers SP. Quality of life in people with ongoing symptoms of coeliac disease despite adherence to a strict gluten-free diet. *Sci Rep*. (2020) 10:7–11. doi: 10.1038/s41598-020-58236-8

41. Fueyo-Díaz R, Montoro M, Magallóon-Botaya R, Gáscon-Santos S, Asensio-Martínez Á., Palacios-Navarro G, et al. Influence of compliance to diet and self-efficacy expectation on quality of life in patients with celiac disease in Spain. *Nutrients*. (2020) 12:1–15. doi: 10.3390/nu12092672

42. Casellas F, Argüelles F, Burgos R, van der Hofstadt Rovira M. National survey on the experiences of people with celiac disease in Spain. The CELIAC-SPAIN project. *Rev Espanola Enfermedades Digestivas*. (2020) 112:343–54. doi: 10.17235/reed.2020.6929/2020

43. Selleski N, Zandonadi RP, Milde LB, Gandolfi L, Pratesi R, Häuser W, et al. Evaluation of quality of life of adult patients with celiac disease in Argentina: from questionnaire validation to assessment. *Int J Environ Res Public Health*. (2020) 17:7051. doi: 10.3390/ijerph17197051

44. Muhammad H, Reeves S, Ishaq S, Mayberry JF, Jeanes YM. Telephone clinic improves gluten-free dietary adherence in adults with coeliac disease: sustained at 6 months. *Frontline Gastroenterol*. (2021) 12:586–92. doi: 10.1136/flgastro-2020-101643

45. Falcomer AL, Farage P, Pratesi CB, Pratesi R, Gandolfi L, Nakano EY, et al. Health-related quality of life and experiences of Brazilian celiac individuals over the course of the sars-cov-2 pandemic. *Nutrients*. (2021) 13:1582. doi: 10.3390/NU13051582

46. Schiepatti A, Maimaris S, De Queiros C, Archela M, Rusca G, Costa S, et al. Long-Term adherence to a gluten-Free diet and quality of life of celiac patients after transition to an adult referral center. *Dig Dis Sci*. (2021) 67:1–9. doi: 10.1007/s10620-021-07231-8

47. Szőcs H, Horváth Z, Vizin G. The mediating role of shame in the relationship between stigma and quality of life in patients with celiac disease. *Orv Hetil*. (2021) 162:1968–76. doi: 10.1556/650.2021.32258

48. Parada A, Méndez C, Espino A, Reyes Á., Santibáñez H. Adherence to a gluten-free diet and quality of life in Chilean celiac patients. *Rev Espanola Enfermedades Digestivas*. (2021) 113:429–31. doi: 10.17235/reed.2020.7293/2020

49. Nikniaz Z, Asghari Jafarabadi M, Ghaffarifar S, Ravand Z, Akbari Namvar Z, Shirmohammadi M. The persian translation and validation of the celiac disease quality of life questionnaire (CDQOL). *Health Qual Life Outcomes*. (2021) 19:1–7. doi: 10.1186/s12955-021-01694-z

50. de Lourdes Moreno M, Sánchez-Muñoz D, Sousa C. Quality of life in teenagers and adults with coeliac disease: from newly Spanish coeliac disease questionnaire validation to assessment in a population-based study. *Front Nutr*. (2022) 9:887573. doi: 10.3389/fnut.2022.887573

51. Enaud R, Tetard C, Dupuis R, Laharie D, Lamireau T, Zerbib F, et al. Compliance with gluten free diet is associated with better quality of life in celiac disease. *Nutrients*. (2022) 14:1–10. doi: 10.3390/nu14061210

52. Guennouni M, Admou B, Elkhoudri N, Bouchrit S, Ait Rami A, Bourrahouat A, et al. Quality of life of moroccan patients with celiac disease: arabic translation, cross-cultural adaptation, and validation of the celiac disease questionnaire. *Arab J Gastroenterol*. (2022) 23:246–52. doi: 10.1016/J.AJG.2022.06.009

53. Chaves C, Raposo A, Zandonadi RP, Nakano EY, Ramos F, Teixeira-Lemos E. Quality of life perception among Portuguese celiac patients: A cross-sectional study using the celiac disease questionnaire (CDQ). *Nutrients*. (2023) 15:1–12. doi: 10.3390/nu15092051

54. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al. Global prevalence of celiac disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. (2018) 16:823–36. doi: 10.1016/j.cgh.2017.06.037

55. Falcomer AL, Luchine BA, Gadelha HR, Szelmenczi JR, Nakano EY, Farage P, et al. Worldwide public policies for celiac disease: are patients well assisted? *No prelo*. (2020) 65:937–45. doi: 10.1007/s00038-020-01451-x

56. Catassi C, Gatti S, Lionetti E. World perspective and celiac disease epidemiology. *Digestive Dis*. (2015) 33:141–6. doi: 10.1159/000369518

57. Jacoby A, Rannard A, Buck D, Bhala N, Newton JL, James OFW, et al. Development, validation, and evaluation of the PBC-40, a disease specific health related quality of life measure for primary biliary cirrhosis. *Gut*. (2005) 54:1622–9. doi: 10.1136/gut.2005.065862

58. Guyatt G, Mitchell A, Irvine EJ, Singer J, Williams N, Goodacre R, et al. New measure of health status for clinical trials in inflammatory bowel disease. *Gastroenterology*. (1989) 96:804–10. doi: 10.1016/S0016-5085(89)80080-0

59. Fueyo-díaz R, Magallón-botaya R, Gascón-santos S, Palacios-navarro G, Sebastián-domingo JJ, Fueyo-d R, et al. The effect of self-efficacy expectations in the adherence to a gluten free diet in celiac disease. *Psychol Health*. (2019) 0:1–16. doi: 10.1080/08870446.2019.1675658

60. Real-Delor RE, Centurión-Medina IC. Quality of life in Paraguayan adults with celiac disease. *Duazary: Rev Internacional Cienc la Salud*. (2018) 15:61–70. doi: 10.21676/2389783X.2026

61. Makhni EC, Meadows M, Hamamoto JT, Higgins JD, Romeo AA, Verma NN. Patient reported outcomes measurement information system (PROMIS) in the upper extremity: the future of outcomes reporting? *J Shoulder Elbow Surg*. (2017) 26:352–7. doi: 10.1016/J.JSE.2016.09.054

62. Pesudovs K, Burr JM, Harley C, Elliott DB. The development, assessment, and selection of questionnaires. *Optometry Vision Sci*. (2007) 84:663–74. doi: 10.1097/OPX.0B013E318141FE75

63. Welch AA, Luben R, Khaw KT, Bingham SA. The CAFE computer program for nutritional analysis of the EPIC-norfolk food frequency questionnaire and identification of extreme nutrient values. *J Hum Nutr Diet*. (2005) 18:99–116. doi: 10.1111/j.1365-277X.2005.00593.x

64. Cranney A, Zarkadas M, Graham ID, Butzner JD, Rashid M, Warren R, et al. The Canadian celiac health survey. *Dig Dis Sci*. (2007) 52:1087–95. doi: 10.1007/S10620-006-9258-2

65. Dean DL, Hender JM, Rodgers TL, Santanen EL. Identifying quality, novel, and creative ideas: constructs and scales for idea evaluation 1. *J Assoc Inf Syst*. (2006) 7:646. doi: 10.17705/1jais

66. Yu DSF, Lee DTF, Woo J. Issues and challenges of instrument translation. *Western J Nursing Res*. (2004) 26:307–20. doi: 10.1177/0193945903260554

67. Wang WL, Lee HL, Fetzer SJ. Challenges and strategies of instrument translation. *Western J Nursing Res* (2006) 28:310–21. doi: 10.1177/0193945905284712

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 05 July 2024
DOI 10.3389/fimmu.2024.1405344

OPEN ACCESS

EDITED BY
Ger Rijkers,
University College Roosevelt, Netherlands

REVIEWED BY
Ana Maria Calderon De La Barca,
National Council of Science and Technology (CONACYT), Mexico
Richard E. Goodman,
University of Nebraska-Lincoln, United States

*CORRESPONDENCE
Isabel Comino
icomino@us.es

†These authors have contributed equally to this work and share first authorship

RECEIVED 22 March 2024
ACCEPTED 10 June 2024
PUBLISHED 05 July 2024

CITATION
Ruiz-Carnicer Á, Segura V, Moreno MdL, Coronel-Rodríguez C, Sousa C and Comino I (2024) Transfer of celiac disease-associated immunogenic gluten peptides in breast milk: variability in kinetics of secretion. *Front. Immunol.* 15:1405344. doi: 10.3389/fimmu.2024.1405344

Transfer of celiac disease-associated immunogenic gluten peptides in breast milk: variability in kinetics of secretion

Ángela Ruiz-Carnicer[1†], Verónica Segura[1†], María de Lourdes Moreno[1], Cristóbal Coronel-Rodríguez[2], Carolina Sousa[1] and Isabel Comino[1*]

[1]Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain, [2]Health Center Amante Laffón, Seville, Spain

Background: Exposure to antigens is crucial for child immune system development, aiding disease prevention and promoting infant health. Some common food antigen proteins are found in human breast milk. However, it is unclear whether gluten antigens linked to celiac disease (CD) are transmitted through breast milk, potentially impacting the development of the infant's immune system.

Objective: This study aimed to analyze the passage of gluten immunogenic peptides (GIP) into human breast milk. We evaluated the dynamics of GIP secretion after lactating mothers adopted a controlled gluten-rich diet.

Methods: We prospectively enrolled 96 non-CD and 23 CD lactating mothers, assessing total proteins and casein in breast milk, and GIP levels in breast milk and urine. Subsequently, a longitudinal study was conducted in a subgroup of 12 non-CD lactating mothers who adopted a controlled gluten-rich diet. GIP levels in breast milk and urine samples were assayed by multiple sample collections over 96 hours.

Results: Analysis of a single sample revealed that 24% of non-CD lactating mothers on a regular unrestricted diet tested positive for GIP in breast milk, and 90% tested positive in urine, with significantly lower concentrations in breast milk. Nevertheless, on a controlled gluten-rich diet and the collection of multiple samples, GIP were detected in 75% and 100% of non-CD participants in breast milk and urine, respectively. The transfer dynamics in breast milk samples were long-enduring and GIP secretion persisted from 0 to 72 h. In contrast, GIP secretion in urine samples was limited to the first 24 h, with inter-individual variations. In the cohort of CD mothers, 82.6% and 87% tested negative for GIP in breast milk and urine, respectively.

Conclusions: This study definitively established the presence of GIP in breast milk, with substantial inter-individual variations in secretion dynamics. Our findings provide insights into distinct GIP kinetics observed in sequentially

collected breast milk and urine samples, suggesting differential gluten metabolism patterns depending on the organ or system involved. Future research is essential to understand whether GIP functions as sensitizing or tolerogenic agents in the immune system of breastfed infants.

KEYWORDS

antigens, breast milk, celiac disease, gluten exposure, gluten-free diet, gluten immunogenic peptides

1 Introduction

Celiac disease (CD) is a systemic disorders triggered by exposure to dietary gluten in genetically predisposed individuals (1, 2). It is a common disease that can affect individuals of all ages and is characterized by a wide spectrum of clinical manifestations. The symptoms include abdominal pain, bloating, nausea, vomiting, and/or diarrhea. Additionally, extra-intestinal manifestations can occur, such as blistering skin rashes, ataxia, bone disease, and issues in the reproductive and endocrine systems, among others (3).

The diagnostic rate for this pathology has increased over the last 10 years (4). Worldwide epidemiological data show that CD is ubiquitous, with a prevalence of 1.4% (5), and is higher in females than in males (4–8). The increase in this prevalence may, in part, be attributed to improved recognition and testing of the disease. However, there may also be a real increase in the incidence of this immune disorder, related to environmental factors that promote a loss of tolerance to dietary gluten (9). Breastfeeding and the timing of gluten introduction have been particularly considered influential factors (10). Nevertheless, the available evidence regarding the relationship between CD and breast milk remains inconclusive. Extended breastfeeding and, notably, breastfeeding at the time of gluten introduction seem to decrease the risk of developing CD or at least postpone its onset (11, 12). However, conflicting findings persist, as some studies have been unable to establish breastfeeding as protective against the development of CD (13–15).

Breastfeeding significantly influences the composition of the intestinal microbiota in the infant and offers substantial potential to prevent allergic diseases (16, 17). The study of breast milk composition is crucial not only for understanding the nutritional requirements of infants, but also for evaluating its impact on neurodevelopment, immune system maturation, and gut development, among others (18). Additionally, breastfeeding plays a dual role in promoting tolerance through the presence of immunomodulatory substances and facilitating antigen transfer. The presence of food antigens in breast milk is a significant source of early oral exposure that is critical for establishing and reinforcing oral tolerance (19).

Common food antigens found in human breast milk include proteins derived from egg (ovalbumin) (20), cow milk (casein and beta-lactoglobulin) (21), and peanuts (arah h1 and arah h2) (22, 23). The frequency and concentration of these dietary proteins in breast milk vary widely among women, with approximately 50% excreting them at concentrations ranging from 0.1 to over 1000 ng/ml (24–26). However, the presence of certain proteins/peptides including gluten remains unclear (27, 28). Gluten proteins have unique properties compared to other dietary proteins. In particular, they contain high proportions of glutamine and proline residues, making them resistant to degradation by gastrointestinal proteases. Consequently, relatively long gluten peptides with intact immunotoxic epitopes accumulate in the lumen of the small intestine and cross the epithelial barrier, ultimately entering the systemic circulation (29–31). This raises the possibility of their subsequent secretion through the mammary glands into breast milk. Therefore, the possible presence of gluten peptides in breast milk could promote oral tolerance or sensitivity to gluten in breastfed infants.

Our research group is a pioneer in detecting and analyzing gluten in human samples. In particular, monoclonal antibodies (moAbs) have been used to develop immunoassays for the sensitive and specific detection of the gluten immunogenic peptides (GIP). These assays have enabled to measure GIP concentrations in human samples, including feces and urine, confirming their absorption into the bloodstream (32–36). Given their resistance to gastrointestinal digestion, GIP play a key role in triggering immunogenic responses within T cells of individuals diagnosed with CD. However, to date, there have been no studies that confirm the existence of gluten peptides in breast milk and if they are immunogenic. On this basis, the aim of this study is to understand the transfer and secretion dynamics of GIP in breast milk. Initially, we carried out a cross-sectional study to analyze the GIP in breast milk samples within a cohort of mothers with and without CD and assessed total protein and caseins secretion. Secondly, we conducted an exploratory longitudinal clinical study to elucidate the differential kinetics of GIP secretion in human milk and urine by examining non-CD mothers after a controlled gluten-rich diet.

2 Materials and methods

2.1 Study design and participants

2.1.1 Cross-sectional study

This cross-sectional study was conducted between September 2020 and September 2023, and included lactating mothers with and

without CD. Potential volunteers were invited to participate via the following official notification: Centro de Salud Amante Laffón (Sevilla, Spain), Federación de Asociaciones de Celíacos de España (FACE), Asociación Provincial de Celíacos de Sevilla (Asprocese) and social networks (https://celiacos.org/la-universidad-de-sevilla-estudia-la-presencia-de-peptidos-del-gluten-en-la-leche-materna/).

A questionnaire was administered to assess the gestational age of the children at delivery and during sample collection process. Furthermore, mothers with CD were asked about the date of CD diagnosis, results of the last duodenal biopsy study, and serum CD antibodies. The inclusion criteria for the cohort of non-CD mothers were as follows: (1) over 18 years of age; (2) regular gluten consumption; and (3) no prior diagnosis of CD, non-CD gluten sensitivity, food allergies, food intolerances, or other gastrointestinal diseases. The inclusion criteria for the cohort of mothers with CD were as follows: (1) over 18 years of age; (2) breastfeeding mothers previously diagnosed with CD, and (3) all volunteers had followed a gluten-free diet (GFD) for at least 2 years prior to enrolling in the study. The exclusion criteria were as follows: (1) participants with associated pathologies or severe psychiatric diseases; and (2) participants who did not collect the samples properly.

This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the regional research ethics committee (ID/Numbers: 0364-N-20, Comunidad Autónoma de Andalucía, Spain), and written informed consent was obtained from all participants.

Detailed instructions were provided to all participants at the beginning of the study. Breast milk and urine samples were consecutively collected from each participant. The parameters analyzed were total protein, casein and GIP level in breast milk, and GIP level in urine.

2.1.2 Longitudinal study

A longitudinal study was conducted in a selected subgroup of non-CD mothers who were instructed to complete a two-phase study: (1) an initial 3-day phase with a controlled gluten-rich diet and (2) a second 5-day phase with a strict GFD. During this study, participants were provided with a menu planning with foods rich in gluten (pizza, sandwich, wheat pancakes, pasta, croutons, lasagna, etc.) for the first period, and menus with gluten-free (unprocessed or minimally processed) foods for the second phase (Supplementary Figure S1). All volunteers collected consecutive urine and milk samples at 0 h during the controlled gluten-rich diet and at 3, 6, 24, 48, 72, and 96 h after the start of the GFD (Figure 1).

2.2 Milk and urine collection

The participants were instructed to collect 10 mL of mature milk and 5 mL of urine in a sealed sterile container. Mothers expressed the milk samples manually or by pump and immediately froze the milk and urine sample at −20°C until collected by the researchers. Once in the laboratory, the samples were homogenized, and after aliquoting, they were stored at −20°C. To optimize the milk protocol, the following were used: 1) whole breast milk samples, and 2) whey samples obtained by centrifuging an aliquot of the milk sample for 5 min at 8000 x *g* (removal of fat content and cellular elements that could interfere with immunoassays). To ensure temporal coherence and minimize potential data variations, milk and urine samples were collected consecutively over time.

2.3 Total protein concentration

The protein concentration in the whole milk and whey was quantified using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) adapted to microplates. Briefly, 25 µL of milk/whey or standard diluted was added to 96-well plates in duplicate. After covering the plates and

A

Characteristics [a]	*Non-CD mothers*
	n = 12
Mothers	***Mean SE***
Age	34 (2.8)
Mode of delivery	
Vaginal	12 (100)
Cesarean	0 (0)
Infants	
Baby's age (months)	10,1 (7.7)
Sex	
Male	8 (67)
Female	4 (33)

B

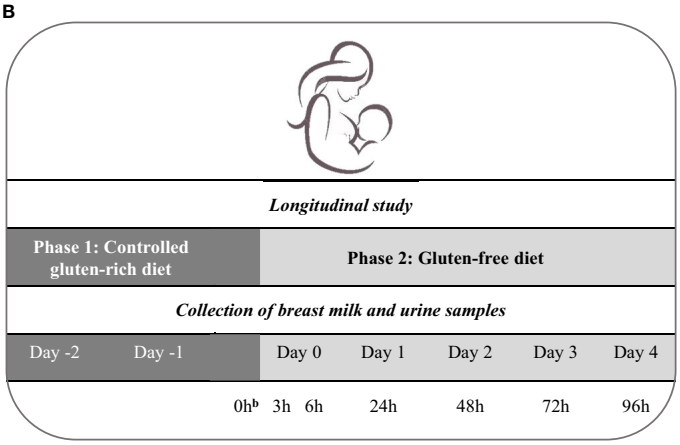


FIGURE 1
Characteristics of the participants and design of the longitudinal study. **(A)** Characteristics of the participants included **(B)** Study design for the determination of the kinetics of GIP secretion in breast milk and urine. [a]Data of continuous variables are expressed as mean and standard error (SE) (in brackets), and data of categorical variables are expressed as absolute numbers and percentage (in brackets). [b]Collection of breast milk and urine samples after breakfast with gluten. CD, celiac disease; GFD, gluten-free diet.

incubating at 37°C for 30 minutes, optical densities were read at 562 nm in the Multiskan Sky Reader (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA). Each sample was participants to duplicate analyses on different dates.

2.4 Casein concentration

The casein concentration in breast milk samples was measured by sandwich-type enzyme-linked immunosorbent assay (ELISA) using the AlerTox ELISA Casein Kits (AlerTox ELISA, Hygiena Diagnostic España S.L., Seville, Spain), following the manufacturer's guidelines with minor modifications. Briefly, breast milk samples were incubated for 15 min at 60°C with gentle agitation in extraction buffer and centrifuged. ELISA was performed using a microtiter plate and standards previously diluted 1:100. The samples were then incubated with a casein-conjugated antibody for 20 min. The TMB casein substrate solution was then added. Color development was stopped with a stop solution of casein, and absorbance at 450 nm was measured using a Multiskan Sky Reader microplate reader (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). A minimum of two separate aliquots from each sample were examined on different days.

2.5 Determination of GIP in milk and urine samples

GIP in milk and urine were measured using a lateral flow immunoassay (LFIA) (iVYCHECK GIP Urine kit based on G12 and A1 moAbs, Biomedal S.L., Seville, Spain) following the manufacturer's recommendations. Samples were homogenized and mixed with a conditioning solution. Thereafter, 100 µL of the mixture was added to the immunochromatographic cassette and visual interpretation of the results was carried out after 30 min. The limit of detection (LoD) was established through visual examination (2.25 ng GIP/mL), and the measuring range was 3.12–25 ng GIP/mL that was determined using the iVYCHECK Reader (Biomedal S.L.). Each sample was run in duplicate, and at least two different aliquots of each sample were tested on different days.

2.6 Statistical analysis

The results of the quantitative variables were expressed using the median and interquartile range (IQR), and those of the qualitative variables were expressed as percentages. The goodness-of-fit to normality was calculated using the Shapiro–Wilk test.

The Mann–Whitney U test was used to compare quantitative variables in independent groups, and the Wilcoxon signed-rank test was used to compare quantitative variables in dependent groups. The McNemar test was used to compare qualitative variables within dependent groups. For all the cases, $P < 0.05$ was considered statistically significant. The data analysis was performed using SPSS 26.0 software for Windows (SPSS Inc.).

3 Results

3.1 Participants

We recruited 96 non-CD lactating mothers taking a normal unrestricted diet with a median age of 35 (IQR: 32–37) and 23 CD lactating mothers under long-term GFD with a median age of 35 (IQR: 31–37). In non-CD mothers, the mode of delivery of the fetus was: 81% (78/96) vaginal and, 17% (16/96) cesarean. For CD mothers, the mode of delivery of the fetus was: 17% (4/23) vaginal and, 4% (1/23) cesarean. The gestational age, 40 (IQR: 39–40) weeks, was similar in both cohorts. However, the median of the baby´s age was 2 (IQR:1–5) months in non-CD mothers and 8 (IQR: 4–20) months in CD-mothers. The demographic and clinical characteristics are summarized in Table 1.

3.2 Cross-sectional study

As this was a non-interventional study, and neither the amount of fluid ingested, nor the feeding pattern of the lactating mothers was modified. Additionally, urine and breast milk samples were collected only once.

3.2.1 Determination of total protein and casein in breast milk

To evaluate whether differences in the diets of non-CD and CD mothers could affect the protein content of their breast milk, we analyzed protein concentrations before and after whey separation. The protein concentration was higher in whole milk than in whey in both cohorts (Wilcoxon test, $P<0.001$) (Figure 2A). In CD mothers, the median was 9.7 (IQR= 9.1–12.8) and 9 mg/mL (IQR =8–11) for whole breast milk and whey samples, respectively, while in non-CD mothers was 10.7 (IQR= 9.7–12) and 9.8 mg/mL (IQR=8.6–11.6). Protein levels values were similar between the study cohorts, with no significant differences (Mann–Whitney U test, $P=0.273$).

Bovine casein is one of the non-human proteins most frequently found in human breast milk (21, 37, 38). In this study, casein was detected in 78.15% of the samples (93/119) (Figure 2B), specifically 65% (16/23) in the cohort of CD mothers and 79% (76/96) in non-CD mothers (median 62.5 ng/mL; IQR 50–76 and median 60.7 ng/mL; IQR 52.2–73.4, respectively). As expected, the results showed no significant differences between the bovine casein values of the control and CD mothers (Mann–Whitney U test, $P=0.797$) (Figure 2C).

3.2.2 Determination of GIP in breast milk

We investigated the presence of gluten in breast milk by quantifying GIP using specific moAbs (G12/A1). In the cohort of non-CD mothers without gluten restriction in their diet, 24% (23/96) of breast milk samples contained detectable levels of GIP. There were no significant differences in the GIP levels of whole milk and whey for these mothers. Urine samples tested positive for GIP in 90% (86 out of 96) of volunteers within this cohort. The milk and urine samples were collected consecutively over time, and our findings revealed that the concentrations of GIP in the milk were

10.3389/fimmu.2024.1405344

TABLE 1 Characteristics of the lactating mothers included in the study.

Characteristics of the lactating mothers	
Total	119
Non-CD mothers	96 (81)
CD mothers	23 (19)
Non-CD mothers	
Age (years)[a]	35 (32–37)
Gestational age (weeks)[a]	40 (39–40)
Mode of delivery	
Vaginal	78 (81)
Cesarean	16 (17)
Nd	2 (2)
Early lactation[b]	74 (78)
Late lactation[c]	21 (22)
Baby's sex	
Female	40 (42)
Male	50 (52)
Nd	6 (6)
Baby's age (months)[a]	2 (1–5)
CD mothers	
Age (years)[a]	35 (31–37)
Gestational age (weeks)[a]	40 (39–40)
Mode of delivery	
Vaginal	4 (17)
Cesarean	1 (4)
Nd	18 (78)
Early lactation[b]	7 (30)
Late lactation[c]	16 (70)
Baby's sex	
Female	6 (26)
Male	5 (22)
Nd	12 (52)
Baby's age (months)[a]	8 (4–20)

Categorical variables are expressed as absolute numbers and percentage (in brackets). CD, celiac disease; Nd, no data available (Data were not self-reported by study participants). [a]Data are expressed as median (IQR), [b]Milk produced prior to 6 months after delivery, [c]Milk produced from 6 months after delivery onward.

significantly lower than those in the urine (average range according to the Mann–Whitney U test: 60.71 and 132.39, respectively) ($P < 0.001$) (Figure 3). However, 96% (22/23) of mothers who tested positive in breast milk also had a GIP positive result in urine.

In the cohort of CD mothers, 82.6% (19/23) tested negative for GIP in breast milk. GIP were detected in only four breast milk samples and three urine samples from 23 participants, and only one of them showed significant GIP levels (> limit of quantification, LoQ) for both samples. No significant differences were found between the GIP values of the milk and urine samples ($P > 0.05$).

These results suggest the presence of GIP across both biological fluids, albeit at varying levels, underscoring the importance of comprehensive assessment methods in elucidating GIP dynamics in lactating mothers with CD.

3.3 Longitudinal study

3.3.1 Kinetics of GIP secretion in breast milk

A parallel longitudinal descriptive study was conducted using a subgroup of 12 volunteers from the cohort of non-CD mothers. This study aimed to elucidate GIP kinetics in breast milk after maternal ingestion of gluten. For this purpose, all volunteers were instructed to complete a two-phase study: phase 1 on a controlled gluten-rich diet, and phase 2 on a strict GFD. GIP levels were quantified in breast milk and urine samples taken at various time points, from 0 to 96 h after gluten ingestion and at the onset of the GFD. The study design and the characteristics of mothers are summarized in Figure 1.

The results were GIP positive in 75% (9/12) of the participants in breast milk samples and 100% (12/12) of the urine samples (Figure 4), considering at least one positive sample per participant. After adopting a gluten-rich diet (0 h), 54.5% (6/11) of the volunteers tested positive for GIP in their breast milk. At 3 and 6 h from the beginning of the GFD, GIP was detected in 33.3% (4/12) and 16.7% (2/12) of the volunteers, respectively. In addition, we found positive GIP in breast milk on the following hours: at 24, 48 and 72 h in 16.7% (2/12), 25% (3/12) and 8.3% (1/12) of the participants, respectively. The means GIP values detected in breast milk were similar between 0 and 48 h, however, significant differences were found after 72 h ($P<0.05$) (Figure 5A). No GIP was detected in breast milk after 72 h in the volunteers studied. However, the period of GIP excretion in urine after the start of GFD is limited to the first 24 h (100% of urine samples at 0h, 92% at 3 and 6 h, and 17% at 24 h) (Figure 5B).

4 Discussion

To our knowledge, this study represents the first instance of detecting immunogenic gluten peptides in breast milk samples. The presence of GIP in breast milk indicates that gluten is absorbed through the intestinal mucosa, enters the bloodstream, and is subsequently secreted by the mammary gland. The detection of measurable amounts of GIP in breast milk suggests that infants are exposed to gluten antigens before their introduction into the diet, which could potentially impact CD development and other gluten-related disorders.

Our findings reveal that the secretion of GIP in breast milk exhibits dynamic and fluctuating changes over time, likely influenced by various factors such as maternal diet, hormonal fluctuations, and the feeding patterns of the infant, among others. This dynamic nature underscores the inherent complexity involved in studying GIP in breast milk. Following a gluten-containing diet

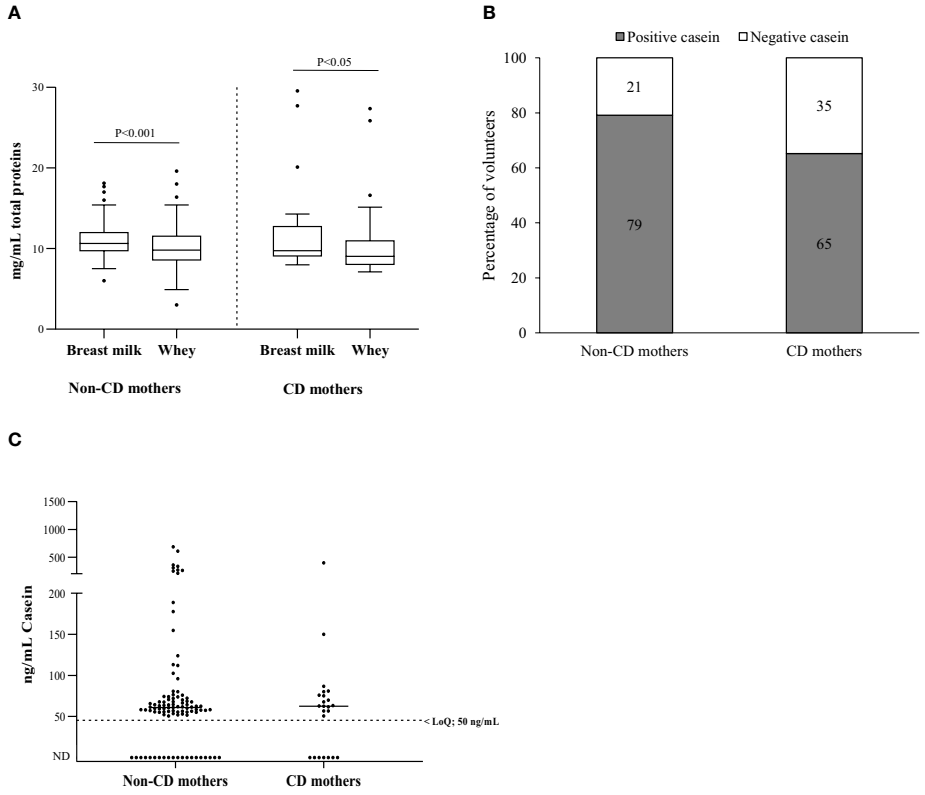


FIGURE 2
Total proteins and bovine caseins in breast milk. **(A)** Total protein content (mg/mL) in breast milk and whey (milk serum) of non-CD and CD mothers; **(B)** percentage of non-CD and CD mothers with casein in breast milk; **(C)** casein concentration in non-CD and CD mothers. Differences between groups were tested with a Wilcoxon signed-rank test in dependent groups and Mann–Whitney U test in independent groups. CD, celiac disease; LoQ, limit of quantification; ND, not detectable.

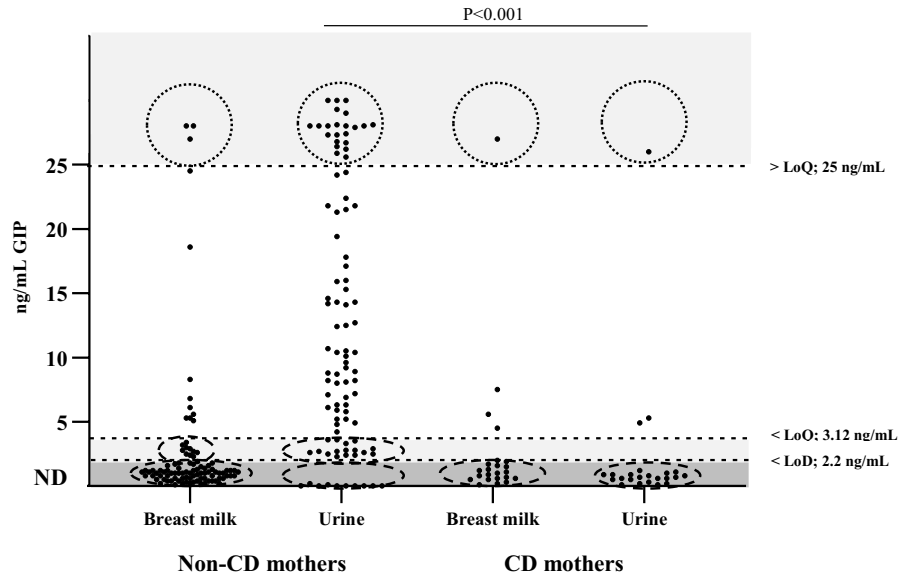


FIGURE 3
Detection and quantification of GIP in urine and breast milk of non-CD mothers and CD mothers. n = 96 (non-CD mothers), n = 23 (CD mothers). The data points include 2 values per volunteer. Differences in GIP levels in breast milk and urine between the 2 groups were tested with a Mann–Whitney U test. CD, celiac disease; GIP, gluten immunogenic peptides; LoD, limit of detection; LoQ, limit of quantification; ND, not detectable.

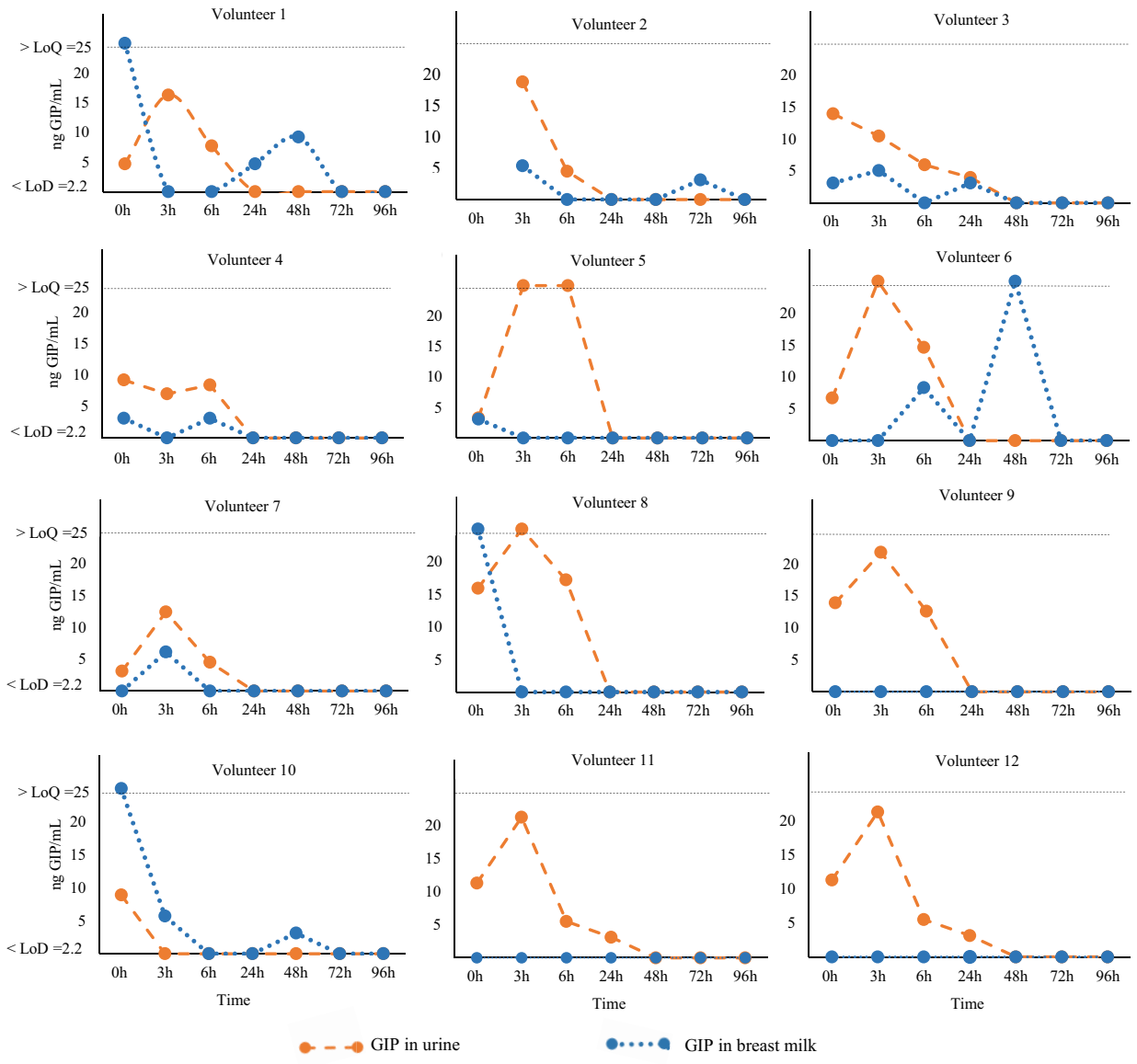


FIGURE 4
Kinetics of GIP secretion in breast milk and urine. Individual GIP excretion kinetics of 12 non-CD mothers on a GFD for over 96 h. The dashed orange lines correspond to breast milk samples, and blue lines represent urine samples. GFD, gluten-free diet; GIP, gluten immunogenic peptides; LoQ, limit of quantification; LoD, limit of detection.

challenge and the collection of multiple breast milk samples (ensuring at least one positive sample from each participant), the percentage of GIP-positive participants was to 75%. The secretion kinetics extended from 0 to 72 hours after the gluten-containing diet challenge and the commencement of the GFD. It is important to note that a considerable degree of intra- and interindividual variation was observed in the secretion of GIP, as previously reported for other food antigens (39–42).

Chirdo et al. (28) detected undegraded gliadins in all milk samples analyzed from lactating mothers following a normal diet, using a polyclonal antibody. The concentrations of gliadins observed exceeded those reported in our study to immunogenic gluten peptides. These variations may be attributed to differences in the study populations, the identified epitopes, and the specificity of the antibodies. The polyclonal antibodies are produced from a mixture of different immune cells, and they may bind to unrelated proteins, causing cross-reactivity. This cross-reactivity can lead to false signals or interference in assays. In contrast, our study has the advantage of detecting GIP using G12 and A1 moAbs, known for their reactivity with tandem epitopes contained in the major immunogenic peptides associated with CD (36, 43).

As expected, in the cohort of mothers with CD on a GFD, the majority of the breast milk samples (82.6%, 19/23) tested negative for GIP. Only four breast milk samples tested positive for GIP. Although the mothers with CD in our study followed a GFD, the possibility of dietary lapses cannot rule out. A GFD is difficult to maintain, and adult patients with CD consume unsafe amounts of gluten on average (44–46). Therefore, these results may be

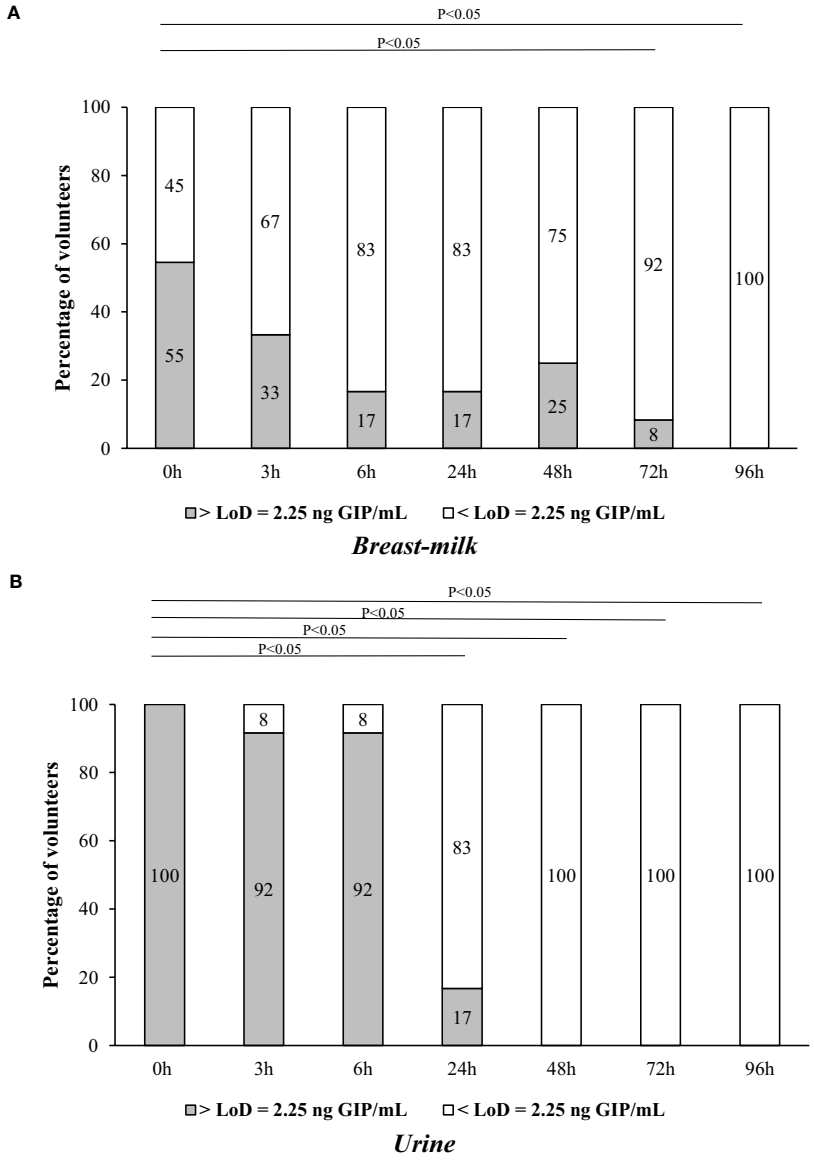


FIGURE 5
Comparison of GIP levels in breast milk and urine of 12 non-CD mothers on a GFD for over 96 h after gluten ingestion. **(A)** GIP secretion in breast milk; **(B)** GIP excretion in urine. Comparative analysis via the McNemar test of GIP levels before and after consumption of gluten. GIP, gluten immunogenic peptides; LoD, limit of detection.

attributed to possible transgressions in the GFD as no food intake control was implemented for mothers with CD.

Understanding how other proteins manifest in breast milk is fundamental it provides crucial insights into the composition of breast milk and its role in infant nutrition and development. In our study, no significant differences were detected in the total protein levels in breast milk between non-CD and CD mothers. Thus, our findings suggest that a GFD does not influence variations in the levels of this macronutrient in breast milk. These results agree with those of prior studies that have demonstrated a remarkable consistency in the macronutrient composition of breast milk across different populations despite potential variations in maternal nutritional status (47). With respect to food antigens, we also proved the transference of bovine casein into breast milk that represents one of the most commonly secreted non-human proteins (21). The levels of this protein in breast milk were in the ng/mL range, similar to those of GIP and other food antigens (48). No significant statistical differences in bovine casein levels were observed between the non-CD and CD cohorts. This result was expected, given that cow milk proteins are not only present in dairy products and their derivatives, but are also widely incorporated into a myriad of manufactured items such as bread, cold cuts, sausages, frozen fish, sweeteners, preserves, and medicines. Additionally, numerous additives used in the food industry are derived from milk (49).

The transfer of proteins from the maternal intestinal tract to the mammary gland is not yet well understood (24, 48, 50–52). Additionally, food proteins secreted into the breast milk appear at highly variable concentrations at different time points following

exposure and in different forms, either as free proteins or as components of immune complexes (24). This makes it impossible to precisely predict the concentration at which food antigens/allergens will appear in breast milk and their impact on the immune response of the breast-fed children. For example, ovalbumin was detected in 68% of breast milk samples following egg ingestion, while beta-lactoglobulin was found in 62.5% of lactating women after consuming cow milk, exhibiting variable appearance and disappearance over a 15-hour sampling period. Another study observed the presence of beta-lactoglobulin in breast milk up to 7 days after oral intake of cow's milk (37). Kinetic analysis of peanut protein transfer into breast milk revealed that 11 out of 23 mothers excreted peanut proteins, with 73% showing appearance within 1 hour of ingestion. In this work, in a cohort of non-CD mothers, taking a single sample of breast milk and urine consecutively over time, we have demonstrated that the percentage of GIP- positive milk samples was significantly lower as compared to urine samples. The detection of GIP in the urine of 90% of non-CD mothers implied that the vast majority of volunteers in this cohort consumed gluten; however, this gluten intake was not reflected in breast milk (with only 24% breast milk being GIP positive). To understand this differential excretion/secretion GIP pattern between urine and breast milk and the excretion kinetics of gluten peptides in breast milk we designed a two-phase longitudinal trial: phase 1 involved a gluten-rich diet by menu planning, and phase 2 enforced a strict GFD (Figure 1). We measured GIP levels in the breast milk at various time points, ranging from 0 to 96 h after gluten ingestion and at the commencement of a GFD. The period of GIP excretion in urine after initiating the GFD was confined to the initial 24 h, with 100% of the volunteers testing positive for GIP during this period. In contrast, the period of GIP secretion in breast milk was extended to 72 h, with 75% of volunteers showing positive GIP results. This result confirmed the differential kinetics of GIP in breast milk and urine samples collected sequentially.

Gluten proteins are initially digested by the enzymes in the mother's intestinal tract. However, this digestion is often incomplete, leaving behind resistant peptides that can cross the intestinal barrier. It is probable that these peptides reach the mammary glands throughout the entire lactation process. We hypothesize that these peptides may undergo partial degradation before being secreted into the mammary glands, potentially extending the time of secretion. Consequently, the mammary gland could serve as a reservoir for certain proteins, including gluten. This speculation arises from observations that mothers who followed the same diet, with menu planning, exhibited fluctuating gluten secretion and interindividual differences.

In this longitudinal trial, the children were breastfed during the milk sample collection period, and this may limit the scope of our results. It should be noted that not all breast milk samples could be collected within the designated 0 to 96 h timeframe, and GIP could have been secreted in some uncollected milk samples. Pooled 24h milk samples are generally considered gold standards for collection; however, they are not possible if the mother wants to maintain normal breastfeeding behaviors (42). Furthermore, it is likely that the variations in the rate and concentration at which gluten is secreted into breast milk can be attributed to the complexity and content of meals consumed before or after gluten intake within our study group, and this could also contribute to the variations observed.

Our findings provide a conclusive demonstration of the presence of GIP in breast milk. Notable aspects of this study include the analysis of breast milk samples and the sequential analysis of urine samples using a specific assay designed for detecting GIP, coupled with the collection of multiple samples over several days. We now have the molecular tool to further investigate whether and how GIP are processed and secreted into breast milk. This investigation has raised important questions about the role that these proteins may play in the development of the immune system of infants, particularly considering that exposure to gluten in children occurs before the introduction of complementary feeding, as was previously understood. Therefore, future studies are necessary to know whether GIP act as sensitizing or tolerogenic agents in the immune system of breastfed babies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Dictamen Único En La Comunidad Autónoma De Andalucía. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

ÁR-C: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Resources. VS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Resources. MLM: Writing – review & editing, Funding acquisition, Project administration, Investigation. CC: Writing – review & editing, Investigation. CS: Writing – original draft, Writing – review & editing, Investigation. IC: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing, Investigation, Methodology, Resources.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by grant from Consejería de Economía y Conocimiento, Junta de Andalucía (project P18-RT-3004) PAIDI 2018 Spain.

Acknowledgments

We would like to thank the Federation of Celiac Associations of Spain (FACE) and the Provincial Association of Celiacs of Seville (Asprocese) for their outreach to the population, as well as the volunteer mothers who enrolled in the study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1405344/full#supplementary-material

References

1. Al-Toma A, Volta U, Auricchio R, Castillejo G, Sanders DS, Cellier C, et al. European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. *United Eur Gastroenterol J*. (2019) 7:583–613. doi: 10.1177/2050640619844125

2. Besser HA, Khosla C. Celiac disease: mechanisms and emerging therapeutics. *Trends Pharmacol Sci*. (2023) 44:949–62. doi: 10.1016/j.tips.2023.09.006

3. Caio G, Volta U, Sapone A, Leffler DA, De Giorgio R, Catassi C, et al. Celiac disease: a comprehensive current review. *BMC Med*. (2019) 17:142. doi: 10.1186/s12916-019-1380-z

4. King JA, Jeong J, Underwood FE, Quan J, Panaccione N, Windsor JW, et al. Incidence of celiac disease is increasing over time: a systematic review and meta-analysis. *Am J Gastroenterol*. (2020) 115:507–25. doi: 10.14309/ajg.0000000000000523

5. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al. Global prevalence of celiac disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. (2018) 16:823–36.e2. doi: 10.1016/j.cgh.2017.06.037

6. Mardini HE, Westgate P, Grigorian AY. Racial differences in the prevalence of celiac disease in the us population: national health and nutrition examination survey (NHANES) 2009-2012. *Dig Dis Sci*. (2015) 60:1738–42. doi: 10.1007/s10620-014-3514-7

7. Lebwohl B, Green PHR, Söderling J, Roelstraete B, Ludvigsson JF. Association between celiac disease and mortality risk in a swedish population. *JAMA*. (2020) 323:1277–85. doi: 10.1001/jama.2020.1943

8. Ludvigsson JF, Murray JA. Epidemiology of celiac disease. *Gastroenterol Clin North Am*. (2019) 48:1–18. doi: 10.1016/j.gtc.2018.09.004

9. Lebwohl B, Rubio-Tapia A. Epidemiology, presentation, and diagnosis of celiac disease. *Gastroenterology*. (2021) 160:63–75. doi: 10.1053/j.gastro.2020.06.098

10. Villamil E, Rodríguez-Camejo C, Puyol A, Fazio L, Colistro V, Hernández A. Immune profiling of breast milk from mothers with treated celiac disease. *Pediatr Res*. (2021) 89:488–95. doi: 10.1038/s41390-020-0901-y

11. Akobeng AK, Ramanan AV, Buchan I, Heller RF. Effect of breast feeding on risk of coeliac disease: a systematic review and meta-analysis of observational studies. *Arch Dis Child*. (2006) 91:39–43. doi: 10.1136/adc.2005.082016

12. Aronsson CA, Agardh D. Intervention strategies in early childhood to prevent celiac disease-a mini-review. *Front Immunol*. (2023) 14:1106564. doi: 10.3389/fimmu.2023.1106564

13. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Tonutti E, Amarri S, et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. *N Engl J Med*. (2014) 371:1295–303. doi: 10.1056/NEJMoa1400697

14. Vriezinga SL, Auricchio R, Bravi E, Castillejo G, Chmielewska A, Crespo-Escobar P, et al. Randomized feeding intervention in infants at high risk for celiac disease. *N Engl J Med*. (2014) 371:1304–15. doi: 10.1056/NEJMoa1404172

15. Silano M, Agostoni C, Sanz Y, Guandalini S. Infant feeding and risk of developing celiac disease: a systematic review. *BMJ Open*. (2016) 6:e009163. doi: 10.1136/bmjopen-2015-009163

16. Borba V, Sharif K, Shoenfeld Y. Breastfeeding and autoimmunity: Programing health from the beginning. *Am J Reprod Immunol*. (2018) 79(1). doi: 10.1111/aji.12778

17. van den Elsen LWJ, Garssen J, Burcelin R, Verhasselt V. shaping the gut microbiota by breastfeeding: the gateway to allergy prevention? *Front Pediatr*. (2019) 7:47. doi: 10.3389/fped.2019.00047

18. Samuel TM, Zhou Q, Giuffrida F, Munblit D, Verhasselt V, Thakkar SK. Nutritional and non-nutritional composition of human milk is modulated by maternal, infant, and methodological factors. *Front Nutr*. (2020) 7:576133. doi: 10.3389/fnut.2020.576133

19. Nuzzi G, Di Cicco ME, Peroni DG. Breastfeeding and allergic diseases: What's new? *Children (Basel)*. (2021) 8:330. doi: 10.3390/children8050330

20. Kosmeri C, Rallis D, Kostara M, Siomou E, Tsabouri S. Characteristics of exogenous allergen in breast milk and their impact on oral tolerance induction. *Front Pediatr*. (2022) 10:830718. doi: 10.3389/fped.2022.830718

21. Franco C, Fente C, Sánchez C, Lamas A, Cepeda A, Leis R, et al. Cow's milk antigens content in human milk: a scoping review. *Foods*. (2022) 11:1783. doi: 10.3390/foods11121783

22. Bernard H, Ah-Leung S, Drumare MF, Feraudet-Tarisse C, Verhasselt V, Wal JM, et al. Peanut allergens are rapidly transferred in human breast milk and can prevent sensitization in mice. *Allergy*. (2014) 69:888–97. doi: 10.1111/all.12411

23. Vadas P, Wai Y, Burks W, Perelman B. Detection of peanut allergens in breast milk of lactating women. *JAMA*. (2001) 285:1746–8. doi: 10.1001/jama.285.13.1746

24. Macchiaverni P, Tulic MK, Verhasselt V. Antigens in breast milk: possible impact on immune system education. In: *Handbook of dietary and nutritional aspects of human breast milk*, vol. 5. Wageningen Academic, Leiden, The Netherlands (2013). p. 447–60. doi: 10.3920/978-90-8686-764-6_25

25. Palmer DJ, Gold MS, Makrides M. Effect of cooked and raw egg consumption on ovalbumin content of human milk: a randomized, double-blind, cross-over trial. *Clin Exp Allergy*. (2005) 35:173–8. doi: 10.1111/j.1365-2222.2005.02170.x

26. Fukushima Y, Kawata Y, Onda T, Kitagawa M. Consumption of cow milk and egg by lactating women and the presence of beta-lactoglobulin and ovalbumin in breast milk. *Am J Clin Nutr*. (1997) 65:30–5. doi: 10.1093/ajcn/65.1.30

27. Troncone R, Scarcella A, Donatiello A, Cannataro P, Tarabuso A, Auricchio S. Passage of gliadin into human breast milk. *Acta Paediatr Scand*. (1987) 76:453–6. doi: 10.1111/j.1651-2227.1987.tb10498.x

28. Chirdo FG, Rumbo M, Añón MC, Fossati CA. Presence of high levels of non-degraded gliadin in breast milk from healthy mothers. *Scand J Gastroenterol*. (1998) 33:1186–92. doi: 10.1080/00365529850172557

29. Shan L, Molberg Ø, Parrot I, Hausch F, Filiz F, Gray GM, et al. Structural basis for gluten intolerance in celiac sprue. *Science*. (2002) 297:2275–9. doi: 10.1126/science.1074129

30. Heyman M, Abed J, Lebreton C, Cerf-Bensussan N. Intestinal permeability in coeliac disease: insight into mechanisms and relevance to pathogenesis. *Gut*. (2012) 61:1355–64. doi: 10.1136/gutjnl-2011-300327

31. Palanski BA, Weng N, Zhang L, Hilmer AJ, Fall LA, Swaminathan K, et al. An efficient urine peptidomics workflow identifies chemically defined dietary gluten peptides from patients with celiac disease. *Nat Commun*. (2022) 13:888. doi: 10.1038/s41467-022-28353-1

32. Comino I, Real A, Vivas S, Síglez MÁ, Caminero A, Nistal E, et al. Monitoring of gluten-free diet compliance in celiac patients by assessment of gliadin 33-mer equivalent epitopes in feces. *Am J Clin Nutr*. (2012) 95:670–7. doi: 10.3945/ajcn.111.026708

33. Comino I, Fernández-Bañares F, Esteve M, Ortigosa L, Castillejo G, Fombuena B, et al. Fecal gluten peptides reveal limitations of serological tests and food questionnaires for monitoring gluten-free diet in celiac disease patients. *Am J Gastroenterol*. (2016) 111:1456–65. doi: 10.1038/ajg.2017.110

34. Comino I, Segura V, Ortigosa L, Espín B, Castillejo G, Garrote JA, et al. Prospective longitudinal study: Use of faecal gluten immunogenic peptides to

monitor children diagnosed with coeliac disease during transition to a gluten-free diet. *Aliment Pharmacol Ther*. (2019) 49:1484–92. doi: 10.1111/apt.15277

35. Moreno M, Cebolla Á, Muñoz-Suano A, Carrillo-Carrion C, Comino I, Pizarro Á, et al. Detection of gluten immunogenic peptides in the urine of patients with coeliac disease reveals transgressions in the gluten-free diet and incomplete mucosal healing. *Gut*. (2017) 66:250–7. doi: 10.1136/gutjnl-2015-310148

36. Ruiz-Carnicer Á, Garzón-Benavides M, Fombuena B, Segura V, García-Fernández F, Sobrino-Rodríguez S, et al. Negative predictive value of the repeated absence of gluten immunogenic peptides in the urine of treated celiac patients in predicting mucosal healing: new proposals for follow-up in celiac disease. *Am J Clin Nutr*. (2020) 112:1240–51. doi: 10.1093/ajcn/nqaa188

37. Picariello G, De Cicco M, Nocerino R, Paparo L, Mamone G, Addeo F, et al. Excretion of dietary cow's milk derived peptides into breast milk. *Front Nutr*. (2019) 6:25. doi: 10.3389/fnut.2019.00025

38. Dekker PM, Boeren S, Wijga AH, Koppelman GH, Vervoort JJM, Hettinga KA. Maternal allergy and the presence of nonhuman proteinaceous molecules in human milk. *Nutrients*. (2020) 12:1169. doi: 10.3390/nu12041169

39. Daly SE, Di Rosso A, Owens RA, Hartmann PE. Degree of breast emptying explains changes in the fat content, but not fatty acid composition, of human milk. *Exp Physiol*. (1993) 78:741–55. doi: 10.1113/expphysiol.1993.sp003722

40. Ruel MT, Dewey KG, Martínez C, Flores R, Brown KH. Validation of single daytime samples of human milk to estimate the 24-h concentration of lipids in urban Guatemalan mothers. *Am J Clin Nutr*. (1997) 65:439–44. doi: 10.1093/ajcn/65.2.439

41. Kent JC, Mitoulas LR, Cregan MD, Ramsay DT, Doherty DA, Hartmann PE. Volume and frequency of breastfeedings and fat content of breast milk throughout the day. *Pediatrics*. (2006) 117:e387–395. doi: 10.1542/peds.2005-1417

42. Keikha M, Bahreynian M, Saleki M, Kelishadi R. Macro- and micronutrients of human milk composition: are they related to maternal diet? A comprehensive systematic review. *Breastfeed Med*. (2017) 12:517–27. doi: 10.1089/bfm.2017.0048

43. Morón B, Bethune MT, Comino I, Manyani H, Ferragud M, López MC, et al. Toward the assessment of food toxicity for celiac patients: characterization of monoclonal antibodies to a main immunogenic gluten peptide. *PloS One*. (2008) 3: e2294. doi: 10.1371/journal.pone.0002294

44. Silvester JA, Comino I, Kelly CP, Sousa C, Duerksen DRDOGGIE BAG Study Group. Most patients with celiac disease on gluten-free diets consume measurable amounts of gluten. *Gastroenterology*. (2020) 158:1497–99.e1. doi: 10.1053/j.gastro.2019.12.016

45. Silvester JA, Comino I, Rigaux LN, Segura V, Green KH, Cebolla A, et al. Exposure sources, amounts and time course of gluten ingestion and excretion in patients with coeliac disease on a gluten-free diet. *Aliment Pharmacol Ther*. (2020) 52:1469–79. doi: 10.1111/apt.16075

46. Wieser H, Ruiz-Carnicer Á, Segura V, Comino I, Sousa C. Challenges of Monitoring the gluten-free diet adherence in the management and follow-up of patients with celiac disease. *Nutrients*. (2021) 13:2274. doi: 10.3390/nu13072274

47. Fields DA, Demerath EW. Human milk composition: nutrients and bioactive factors. *Pediatr Clin North Am*. (2013) 60:49–74. doi: 10.1016/j.pcl.2012.10.002

48. Schocker F, Jappe U. Breastfeeding: maternally transferred allergens in breast milk: protective or sensitizing? *Mol Nutr Food Res*. (2022) 66:e2200066. doi: 10.1002/mnfr.202200066

49. Al-Beltagi M, Saeed NK, Bediwy AS, Elbeltagi R. Cow's milk-induced gastrointestinal disorders: From infancy to adulthood. *World J Clin Pediatr*. (2022) 11:437–54. doi: 10.5409/wjcp.v11.i6.437

50. Benn CS, Böttcher MF, Pedersen BV, Filteau SM, Duchén K. Permeabilidad paracelular epitelial mamaria en madres atópicas y no atópicas versus atopia infantil. *Pediatra Inmunol alérgico*. (2004) 15:123–6. doi: 10.1046/j.1399-3038.2003.00138.x

51. Monks J, Neville MC. Albumin transcytosis across the epithelium of the lactating mouse mammary gland. *J Physiol*. (2004) 560(Pt 1):267–80. doi: 10.1113/jphysiol

52. Pastor-Vargas C, Maroto AS, Díaz-Perales A, Villaba M, Diaz NC, Vivanco F, et al. Sensitive detection of major food allergens in breast milk: first gateway for allergenic contact during breastfeeding. *Allergy*. (2015) 70:1024–7. doi: 10.1111/all.12646

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 05 August 2024
DOI 10.3389/fimmu.2024.1433304

OPEN ACCESS

EDITED BY
Kosuke Nishi,
Ehime University, Japan

REVIEWED BY
Björn Ragnar Weström,
Lund University, Sweden
Jocelyn Silvester,
Boston Children's Hospital and Harvard Medical School, United States

*CORRESPONDENCE
Carolina Sousa
csoumar@us.es

RECEIVED 15 May 2024
ACCEPTED 22 July 2024
PUBLISHED 05 August 2024

CITATION
Rodríguez-Ramírez R, Fernández Peralbo MA, Mendía I, Long JCD, Sousa C and Cebolla Á (2024) Urinary excretion of gluten immunoreactive peptides as an indicator of gastrointestinal function after fasting and dietary provocation in healthy volunteers. *Front. Immunol.* 15:1433304. doi: 10.3389/fimmu.2024.1433304

Urinary excretion of gluten immunoreactive peptides as an indicator of gastrointestinal function after fasting and dietary provocation in healthy volunteers

Raquel Rodríguez-Ramírez[1,2], María Auxiliadora Fernández Peralbo[1], Irati Mendía[1], Joshua C. D. Long[1], Carolina Sousa[3]* and Ángel Cebolla[1]

[1]Research and Development Department, Biomedal S.L., Seville, Spain, [2]Inorganic Chemistry Department, Faculty of Science, University of Granada, Granada, Spain, [3]Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain

Introduction: Understanding intestinal permeability is paramount for elucidating gastrointestinal health and pathology. The size and nature of the molecule traversing the intestinal barrier offer crucial insights into various acute and chronic diseases, as well as the evolution of some conditions. This study aims to assess the urinary excretion kinetics of gluten immunogenic peptides (u-GIP), a unique class of dietary peptides detectable in urine, in volunteers under controlled dietary conditions. This evaluation should be compared to established probes like lactulose, a non-digestible disaccharide indicative of paracellular permeability, and mannitol, reflecting transcellular permeability.

Methods: Fifteen participants underwent simultaneous ingestion of standardized doses of gluten (10 g), lactulose (10 g), and mannitol (1 g) under fasting conditions for at least 8 hours pre-ingestion and during 6 hours post-ingestion period. Urine samples were collected over specified time intervals. Excretion patterns were analyzed, and correlations between the lactulose-to-mannitol ratio (LMR) and u-GIP parameters were assessed.

Results: The majority of u-GIP were detected within the first 12 hours post-ingestion. Analysis of the variability in cumulative excretion across two sample collection ranges demonstrated that lactulose and u-GIP exhibited similar onset and excretion dynamics, although GIP reached its maximum peak earlier than either lactulose or mannitol. Additionally, a moderate correlation was observed between the LMR and u-GIP parameters within the longest urine collection interval, indicating potential shared characteristics among permeability pathways. These findings suggest that extending urine collection beyond 6 hours may enhance data reliability.

Discussion: This study sheds light on the temporal dynamics of u-GIP in comparison to lactulose and mannitol, established probes for assessing intestinal permeability. The resemblance between u-GIP and lactulose

excretion patterns aligns with the anticipated paracellular permeability pathway. The capacity to detect antigenic food protein fragments in urine opens novel avenues for studying protein metabolism and monitoring pathologies related to the digestive and intestinal systems.

KEYWORDS

intestinal permeability, urine, gluten immunogenic peptides, lactulose, mannitol

Introduction

The integrity of the epithelial barrier within the intestine is a crucial determinant of the pathogenesis of various gastrointestinal diseases. Assessing intestinal permeability is essential for elucidating the origin of symptom in undiagnosed patients, monitoring gastrointestinal disorders, and investigating the role of the intestine in multifarious diseases (1). Intestinal permeability enables a balanced exchange of fluids and solutes between the intestinal lumen and bloodstream, constituting a key characteristic of the protective barrier (2). Regulation of molecular passage occurs through transcellular absorption and paracellular absorption, mediated by tight junctions between intestinal epithelial cells (3). Gut barrier dysfunction has been associated not only with chronic gastrointestinal conditions like inflammatory bowel disease or irritable bowel syndrome, but also with metabolic disorders, alcoholic liver disease, chronic arthritis, and neuropsychiatric disorders (4). Generally, transport mechanisms in the intestinal mucosa allow the passage of amino acids, dipeptides, and tripeptides and limited quantities of larger peptides via transcytosis after binding to receptors on the intestinal membrane. These peptides pose a high risk of acting as antigens and consequently contributing to the development of food allergies and intolerance in the event of intestinal barrier dysfunction (5).

The investigation into gluten, a complex mixture of proteins called prolamins, present in wheat, barley, rye, oats, and their derivatives has been driven by its association with celiac disease. The incomplete digestion of gluten results in proline-rich, digestion-resistant immunogenic peptides. These peptides, hereafter referred to as GIP (gluten immunogenic peptides), can be detected in feces (6) and urine (7). The presence of GIP in the urine of healthy individuals after gluten consumption suggests they can cross the intestinal epithelium into the bloodstream, undergo filtration by the kidney, and ultimately be excreted in the urine (5). Given the size of detectable GIP, they are likely to traverse the paracellular pathway, which is compromised in individuals with increased intestinal permeability to large molecules. This renders them as a unique model for studying the absorption of biologically active macromolecules, that can be monitored at various metabolic stages using diverse methodologies (8).

The determination of intestinal permeability has long been a topic of debate in scientific circles. Established methodologies involve the oral administration of tracer molecules, such as labeled chromium-ethylenediaminetetraacetic acid (51Cr-labeled EDTA), or non-digestible sugars, such as lactulose (lac) and mannitol (man), followed by the analysis of urinary excretion. Although these tests have been utilized for decades, they suffer from drawbacks including time-intensive procedures, lack of standardization, inability for retrospective analysis, and limited validity due to uncertainties surrounding normal reference standards (9).

The lactulose/mannitol test, designed to discern paracellular and transcellular absorption dynamics, involves orally administering a solution containing lactulose, a disaccharide which cannot cross an intact epithelia, and mannitol, a monosaccharide capable of transcellular transport (10). After ingestion, the urinary excretion of lactulose and mannitol is quantified over a specified duration, with the resulting lactulose-to-mannitol ratio (LMR) serving as a surrogate marker of intestinal permeability (11). In conditions where intestinal permeability is increased, as is commonly observed in various gastrointestinal disorders, a higher LMR indicates increased transfer of substances across the intestinal suggesting compromised epithelial barrier integrity (12). Despite its widespread utilization, the lactulose/mannitol test has limitations. It measures the permeability of small sugar molecules that lack immunogenic activity, thus not allowing analysis of the ability of antigenic macromolecules to pass the epithelial barrier, which can cause and exacerbate underlying inflammatory conditions and autoimmune diseases. Additionally, lactulose has a low molecular weight, and the transfer of this substance through the intestinal barrier does not reflect the transfer of dietary proteins and the overall immune response (lactulose, 382 Da; mannitol, 182 Da; gliadin peptides, ~ 2000-4000 Da) (13). Moreover, establishing normative LMR thresholds for healthy and diseased states remains a subject of ongoing investigation, highlighting the need for further refinement and standardization within the realm of intestinal permeability assessment methodologies. Furthermore, researchers have explored the potential of various endogenous proteins such as lipopolysaccharide-binding protein or zonulin as biomarkers for

intestinal permeability. However, consensus regarding their clinical utility has yet to be reached (4).

Multiple observations indicate that in conditions in which intestinal function/health is compromised, GIP are able to cross the intestinal epithelium more readily, likely due to increased permeability. Firstly, it has been observed that in celiac patients consuming equivalent gluten challenges, there was a variation in the amount of urinary GIP detected and those patients with the highest level of uGIP showed a greater degree of villus atrophy progression (unpublished results). Additionally, utilizing a specific peptidomics workflow using LC-MS, urine samples from celiac patients at diagnosis have shown up to four times higher number of gluten peptides compared to those from healthy volunteers (14). These preliminary findings suggest that quantifying GIP excreted in urine could serve as an indicator of intestinal permeability to immunogenic macromolecules. In this study, we investigated the kinetic and dynamic patterns of u-GIP excretion after simultaneous consumption of gluten, lactulose, and mannitol to explore pathways of epithelial barrier translocation and preliminarily assess its potential as a standard food antigen probe in intestinal permeability analysis.

Materials and methods

Study population

Fifteen healthy volunteers were included using the criteria: (1) age >18 years; (2) absence of diagnoses for CD, non-celiac gluten sensitivity, food allergies, food intolerances, or other gastrointestinal diseases; (3) willingness to adhere to a strict diet regimen; and (4) capability to collect daily urine samples. Exclusion criteria included: (1) presence of concurrent pathologies. Participants failing to collect samples correctly on at least 70% of occasions were not excluded during participant recruitment but were excluded during the analysis phase. The study was conducted according to the guidelines of the Declaration of Helsinki. All participants provided written informed consent, and the study received approval from the local ethics committee (n. 1308-N-23).

Study design

The study comprised two stages: a washout period and an intake/collection period (Figure 1). The washout period lasted for 32 hours (including a final 8 hours fasting period) during which volunteers adhered to a gluten-free diet and abstained from dairy and foods high in sorbitol and/or mannitol. Before ingestion, volunteers provide a urine sample to confirm the absence of target compounds. Gluten (10 g), lactulose (10 g), and mannitol (1 g) were ingested after a period of eight hours fasting. Participants fasted for the initial 4 hours post-ingestion, after which they commenced scheduled liquid intake (250 mL every 2 hours). At the 6-hours mark, participants began the prescribed diet. The study concluded 15 hours post-ingestion of the compounds.

Throughout the study, diet adherence and fluid intake were assessed using a food recall questionnaire, and participants recorded daily food consumption details. A schematic of the study timeline is illustrated in Figure 1.

Compounds administration

The compounds comprised 10 g of gluten powder (Aurora Intelligent Nutrition, Sevilla, Spain), an oral solution sachet of lactulose (Duphalac™, Abbott Laboratories, S.A., Madrid, Spain), and 1 g of mannitol (Acofarma, Madrid, Spain). For mannitol intake, 50 mL of water was added to the tube and ingested after shaking.

To administer the gluten intake, a portion of the content was suspended in 125 mL of water, post-ingestion, an additional 125 mL of water was added to ensure complete suspension of the remaining powder.

Urine collection

Comprehensive instructions were provided to all participants at the study's commencement. Subjects were equipped with all necessary materials for urine collection, including plastic screw-

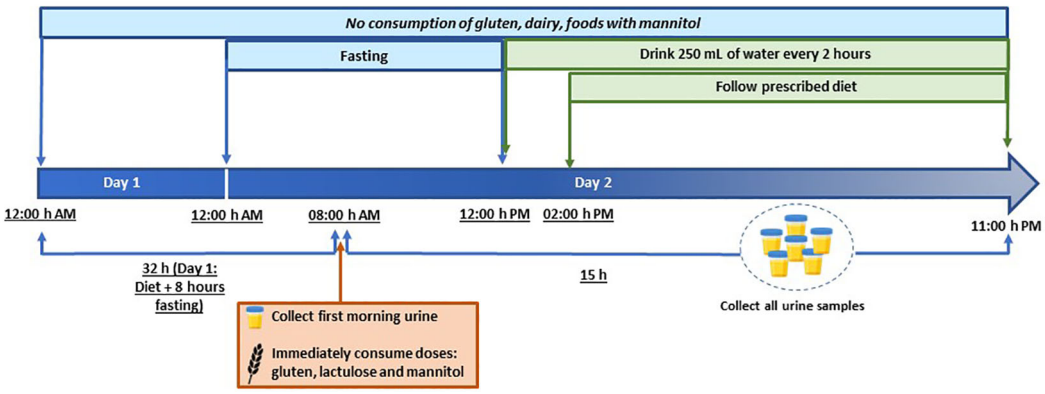


FIGURE 1
Study timeline showing the periods of fasting, gluten/lactulose/mannitol consumption, liquid consumption and sample collection.

capped containers, labels, cool bags, isothermal boxes, and cool packs. Participants were instructed to collect the entire urine sample from each micturition, noting the date and time of collection. All urine samples were preserved in isothermal boxes with cool packs at 4–8°C and deposited within 48 hours of collection. Samples were then frozen at -20°C until processing. GIP concentration in urine remained stable throughout the freeze-thaw process.

Urine analysis

The volume of each urine sample was recorded, and when multiple containers were required for the same urination, they were mixed and homogenized. Additionally, some mixed samples were analyzed at intervals of 0–6-hours and 2–15-hours. To create these mixtures, 10% of the volume of each container was utilized. To prevent bacterial growth, 100 µL of 1% chlorhexidine diacetate was added. Aliquots of 1 mL were done and stored at -20°C until analysis.

u-GIP analysis

Qualitative analysis of GIP in urine was conducted using a lateral flow immunoassay (LFIA) with iVYCHECK GIP Urine (Biomedal S.L., Seville, Spain), following the manufacturer's guidelines. Thawed urine samples were homogenized and mixed with a conditioning solution. Subsequently, 100 µL of the mixture was added to the immunochromatographic cassette, and visual interpretation of results occurred after 30 minutes. A positive outcome was determined if the test line exhibited a red color accompanied by a green color on the control line. A negative result was confirmed when only the control line displayed a green color. The Limit of Detection (LoD) determined by visual inspection was 2.50 ng GIP/mL urine. GIP concentration in urine was also assessed on the immunochromatographic strips using the iVYCHECK Reader (Biomedal S.L., Seville, Spain). Reader calibration was performed before urine analysis using the α-gliadin 33-mer peptide as a standard. The measuring range established for this method was 3.12–25 ng GIP/mL urine. Results were expressed as ng GIP per mL of urine. Each sample underwent duplicate runs, and at least two aliquots of each sample were tested.

Lactulose/Mannitol analysis

The determination of lactulose and mannitol analytes was conducted using a method developed and validated by LC-MS/MS, with a linear range from of 10–1200 mg/L. The LC-MS/MS system comprised an HPLC coupled a triple quadrupole mass spectrometer (QSight 220, Perkin Elmer™, Waltham, Massachusetts, USA), equipped with an electrospray ion source.

HPLC separation was performed using a 100 x 2.1 mm, Hypercarb™ column (Thermo Scientific, Waltham, Massachusetts, USA), operating at a flow rate of 0.25 mL/min. Elution was carried out with a 25-minute linear gradient from 1 to 4% acetonitrile in water containing 0.1% formic acid, with the oven temperature set at 11°C. The injection volume was 5 μL, and the total analysis time was 27 minutes.

Multiple Reaction Monitoring (MRM) was employed, with parent and fragments ions monitored at Q1 and Q3, respectively. Optimization of parent and daughter ions, along with collision voltages, was conducted through experiments where pure standards were infused into the mass spectrometer in the mobile phase. The ESI source operated in the negative mode with the following mass parameters: Drying Gas, 120 (arbitrary units); Hot-Surface Induced Desolvation (HSID) Temperature, 250°C; Nebulizer gas, 350 (arbitrary units); Electrospray voltage, -5500 V; and Source Temperature, 300°C.

Aliquots were thawed and agitated for one minute using a vortex mixer. Subsequently, they were centrifuged for 5 minutes at 5000 g to remove sediments. Fifty microliters of the supernatant were collected and brought to a final volume of 1 mL with water. After shaking, the same dilution was repeated, with an additional 50 µL of internal standard (2 mg/mL melezitose) added. The final dilution was 1:400 (v/v).

Statistical analysis

Quantitative variables results are presented using both the mean (SD) and median (IQR or range), while categorical variables are expressed as absolute (N) and cumulative (%) frequencies. The LMR values were then multiplied by 100. Interquartile tests were performed using RStudio (Version 2022.02.3 + 492, RStudio, Inc., Boston, MA, USA), and correlation tests were conducted using Microsoft Excel (Version 2401, Microsoft Corporation, Redmond, WA, USA).

Results

Participants and samples

Fifteen individuals, comprising 12 (80%) females and three (20%) males, completed the study. The median age of participants was 35 years (IQR 29–41). None of the participants had been diagnosed with any relevant diseases. Based on exhaustive food recall data, including specific brands and detailed consumption information, all participants adherent to the prescribed diet and consumed the provided compounds.

Urine analysis

Throughout the study, 107 urine samples were collected from all participants. To ensure data integrity, rigorous exclusion criteria were applied, in fact, five samples were excluded due to their duration exceeding the predefined study window.

It is essential to emphasize the meticulous attention paid to the mixing process, particularly regarding urine samples collected at 2–15-hours intervals. While most samples adhered strictly to the study protocol, exceptions were made for two volunteers urines samples during the 2–15-hours interval mixing due to their temporal

deviation beyond the stipulated range. This decision was justified by the time gap between the last urination before the temporal limitation and the subsequent excretion, warranting their inclusion for measurement purposes. Similar exceptions were noted during the 0–6-hour pooling process, totaling three instances.

Considering the aforementioned exceptions the statistical analyses provided valuable insights into the mixing procedures. For the 0–6-hours interval, the median number of urine samples utilized for mixing was 2.00 (IQR 2-3), with a mean pooled six-hourly urine volume of 348 mL ± 176 mL. In contrast, during the 2–15-hours interval, there was a median of six urine samples (IQR 4-6) employed for mixing, resulting in an average total volume of 983 mL ± 331 mL.

Excluding baseline urination, the average number of urinations per participant was determined to be 6 (IQR 5-7). Importantly, baseline urinations showed non-detectable levels of u-GIP and lactulose, indicating the successful adherence to the pre-study dietary requirements. However, an average of 25 ppm ± 17 ppm of mannitol was detected in these samples, suggesting the challenges associated with adhering to a mannitol-free diet, despite recommendations.

Excretion kinetics

The excretion kinetics of u-GIP were investigated following ingestion, revealing insightful patterns among participants. On average, the initial urine sample with detectable levels containing GIP was excreted 2 hours and 53 minutes after ingestion, with most volunteers producing this sample as their first urine output following ingestion. The average peak excretion time was 4 hours and 08 minutes. Notably, exceptions were observed in two volunteers, whose first urine occurred approximately one hour after ingestion. These samples tested negative for GIP, possibly suggesting incomplete bladder emptying before ingestion. The detectable excretion of GIP extended to 9 hours and 43 minutes post-ingestion, with the first undetectable urine excreted, on average, at 12 hours and 55 minutes post-ingestion. Intriguingly, a double excretion peak was identified in 40% of volunteers, suggesting potential variability in u-GIP excretion dynamics. Above the upper limit of quantification (values represented by red squares in Figure 2), approximate concentrations were used based on extrapolation from the standard curve.

Analysis of lactulose excretion kinetics (Figure 2) revealed distinct temporal dynamics among participants. The first positive lactulose urine sample was typically excreted approximately 2 hours and 44 minutes post-ingestion, closely aligning with the timing of u-GIP excretion. Similar to u-GIP excretion, the first urine after ingestion coincided with the first urine with detectable levels, except for one volunteer, whose first urine after ingestion occurred just one hour later and showed a negative result for lactulose, possibly indicating incomplete bladder emptying during lactulose ingestion. The average for the peak excretion time was 5 hours and 38 minutes. Lactulose excretion persisted for an average of 10 hours and 21 minutes post-ingestion, with the first urine with undetectable levels appearing, on average at 13 hours and 23 minutes post-ingestion. Intriguingly, lactulose did not reach negativity during the study for two volunteers, suggesting distal

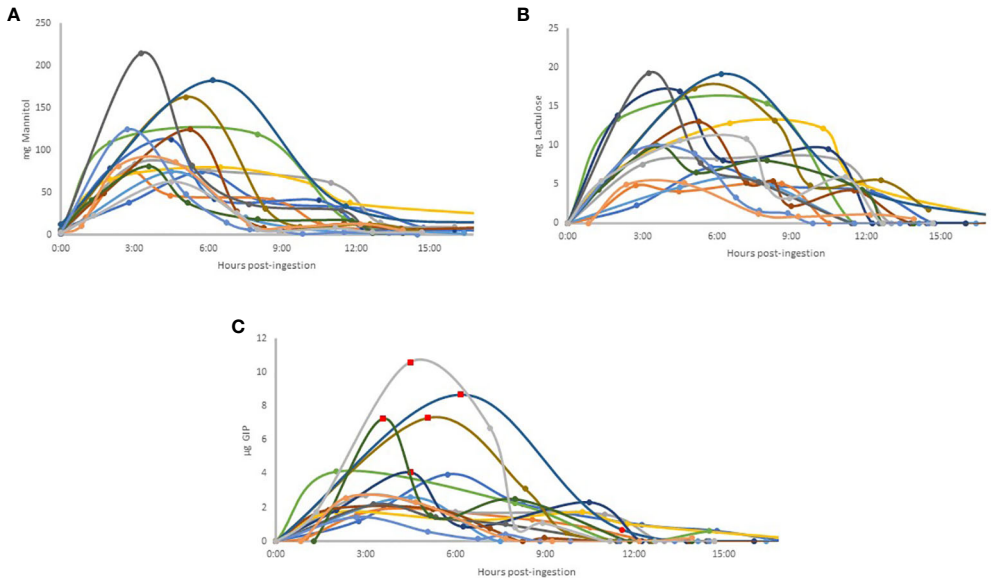


FIGURE 2
Individual excretion patterns in urine: Mannitol **(A)**; Lactulose **(B)**; GIP **(C)**. Each volunteer is represented by a color that is the same for all three compounds studied. In Figure C, each value extrapolated above the upper limit for accurate quantification of GIP is represented by a red square on the graph, based on the standard curve, for illustrative purposes.Abbreviations: GIP, Gluten Immunogenic Peptides.

absorption of the molecule. Moreover, a double excretion peak was evident in 46.67% of volunteers, indicating potential variability in lactulose absorption/excretion patterns.

As mentioned previously, all baseline urine samples exhibited quantifiable values for mannitol (Figure 2). The average peak excretion time was 4 hours and 52 minutes. Notably, a double excretion peak was identified in 40% of volunteers, with two volunteers exhibiting double excretion peaks for all three compounds studied. None of the volunteers reached negativity during the study (Figure 2).

The comparison of the time after ingestion at which the peak excretion occurred for each compound and each volunteer, as well as the corresponding urine number, was analyzed (Table I). For all three compounds, the peak excretion (on average for all participants) occurred in the third urine sample. In 50% of volunteers, the time and, consequently, the urine matched for all three compounds. In 81% of volunteers, the excretion peaks for lactulose and mannitol coincided in the same urine sample.

Analysis of the variability in cumulative excretion within two ranges of sample collection

The excretion percentages for lactulose and mannitol were calculated based on both the ingested and total determined excreted quantities. Subsequently, the percentages of both compounds excreted in the 0–6-hours and 2–15-hours interval mixtures were determined. The excretion percentage in the 2–15-hours interval mixture was considered as 100%, and the proportion of this mix excreted within the 0–6-hours window was then calculated. For lactulose, the mean excretion rate was found to be 56.54% ± 16.53%, while for mannitol, the mean excretion rate was observed to be 72.54% ± 16%.

Regarding the excretion of u-GIP, due to the unavailability of precise ingestion data and the uncertainty surrounding the exact percentage of GIP in the 10 g of gluten consumed, the u-GIP quantity is utilized for calculations by using α-gliadin 33-mer (a principal contributor to gluten immunotoxicity) (15) as a standard. Employing the same methodology as with the aforementioned compounds, a mean excretion rate of 55.97% ± 20.66% is derived for the 0–6-hours interval mixture, with 100% representing the excretion in the 2-15-hours interval mixture.

The Lactulose-to-Mannitol ratio (LMR) was calculated for each participant in the 0–6-hours and 2–15-hours interval mixtures, yielding means of 1.04 ± 0.36 and 1.43 ± 0.53, respectively. Individual coefficients of variation between the ratios of each time interval were calculated for each participant, resulting in a mean of 18.81% ± 14.33%. The correlation graph between the LMR of the two intervals demonstrated a moderate correlation with an R^2 value of 0.5977.

Outliers' analysis

A study on outliers was conducted with the volunteers to determine if any participant demonstrated outlier values in the assessed parameters (LMR and u-GIP) at different time intervals (Figure 3). Out of the four parameters analyzed, volunteer 15 consistently exhibited outlier values across all parameters, while three other volunteers showed outlier values specifically in the LMR parameter within the 0–6-hours interval. Outlier calculations were performed using the Interquartile Range (IQR) method.

Analysis of the correlation between the different parameters

Considering that LMR is the most commonly used methodology for assessing intestinal permeability, it served as the reference control for analyzing u-GIP metabolism, particularly the absorption/excretion kinetics. Scatterplots were generated to compare the data from all volunteers for the different compounds. In addition to comparing the quantities of LMR and u-GIP at the two-time intervals, the correlation was also studied using only the percentage of lactulose excreted. A moderate correlation was observed between the LMR and u-GIP quantity in the 2–15-hours interval (R^2 = 0.5225), inclusive of outliers. When the same representation but disregarding outlier results is done, only lactulose vs u-GIP comparison in the 2–15-hours interval shows a certain correlation (R^2 = 0.4159). There is no correlation observed in the 0–6-hours interval.

Discussion

In this study, we evaluated the excretion dynamics of lactulose and mannitol, widely used probes for assessing intestinal permeability, in urine samples collected from healthy volunteers who consumed controlled doses under regulated dietary conditions. Furthermore, simultaneous consumption of gluten in addition to lactulose and mannitol was conducted to explore the pathways of epithelial barrier translocation of gluten peptides and evaluate their potential as a biomarker in intestinal permeability analysis.

GIP represent a heterogeneous mixture of peptides of varying sizes and degrees of hydrolysis. The assay used can detect both small peptides and intact gliadin protein. Given the heterogeneous mix of gluten digestion products, the relative quantification of GIP concentration is provided in reference to a standard curve of the immunogenic 33-mer GIP peptide. It is important to note that the detected signal does not exclusively indicate the presence of this specific peptide.

One notable finding was the comparative ease of adhering to a short-term gluten-free diet over abstaining from mannitol-containing foods, supported by the analysis of baseline urine samples from all participants. The straightforward identification of gluten sources contrasted with the complexity of identifying and avoiding various mannitol-rich foods (16). Despite instructions to avoid mannitol consumption during the washout period, all participants exhibited detectable concentrations of mannitol in their urine samples before ingesting the pure compound. Taking into account the average mannitol value found in baseline urines (25 ppm ± 17 ppm) and the average values for all volunteers in the

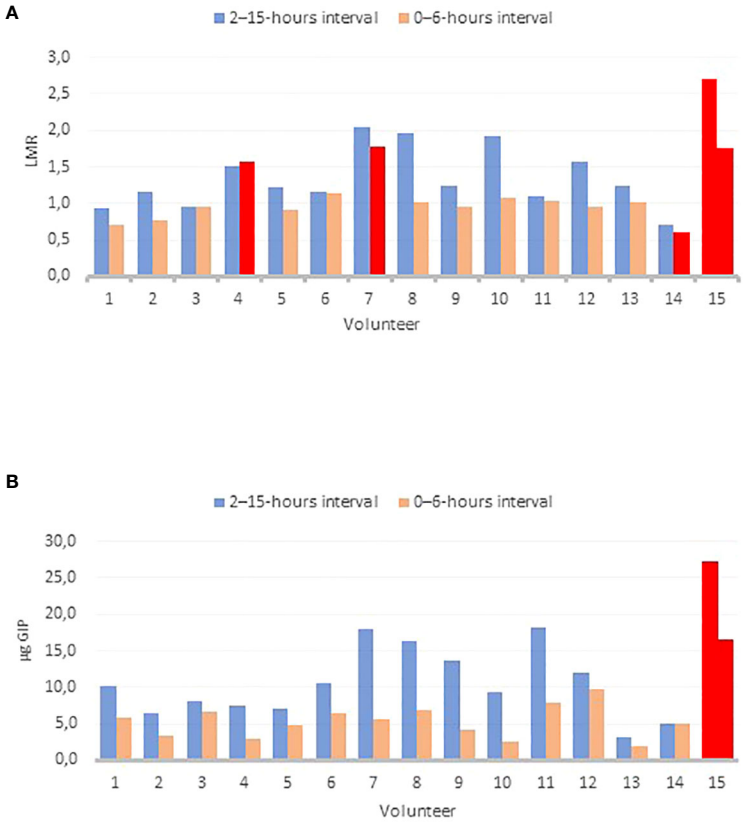


FIGURE 3
Outlier' plots for different parameters: LMR 0–6 hours and 2–15-hours intervals **(A)**; GIP 0–6-hours and 2–15-hours interval **(B)** The Interquartile Range (IQR) method was utilized for outlier detection. Outlier' results are shown in red. Abbreviations: LMR, lactulose-to-mannitol ratio; GIP, Gluten Immunogenic Peptides.

different intervals (638 ppm ± 366 ppm for the 0–6-hours interval and 291 ppm ± 42 ppm for the 2–15-hours interval), it is concluded that the contribution of baseline urine is insignificant."

Our results revealed a median duration of u-GIP excretion of approximately 10 hours, with the first urine with undetectable levels sample collected, on average, around 13 hours post-ingestion. These findings align with those of Coto et al. (7), who reported u-GIP detection within 1–15 hours after ingesting 2 grams of gluten in a homogeneous breakfast among their study participants. Notably, our study observed a mean peak in u-GIP excretion approximately 4 hours post-ingestion, contrasting with the findings of Coto et al. (7) of a peak between 6 and 9 hours. This discrepancy may stem from differences in the amount and the form of gluten ingested. Unlike the previous study, which did not incorporate a fasting period after gluten ingestion, our participants consumed gluten on an empty stomach in dispersed powder form. In contrast, Coto et al. (7) demonstrated that gluten was administered in capsules with breakfast, requiring metabolism alongside the meal. These results underscore the efficacy of administering gluten in suspension after a fasting period, enhancing metabolic efficiency.

In comparison, Moreno et al. (17) reported a narrower detection window for u-GIP, with detectable levels observed between 3- and 15-hours post-ingestion. Furthermore, they noted an extended time for u-GIP disappearance after a normal gluten-containing diet, ranging from 16 to 34 hours, compared to our study. These differences highlight the influence of gluten dosage and dietary context on u-GIP excretion dynamics and/or the higher sensitivity owing to the previous solid-phase extraction protocol of the samples.

Our study provides novel insights into the temporal patterns of u-GIP excretion following gluten ingestion under fasting conditions. The observed variations underscore the complex interplay between gluten exposure and GIP metabolism, with potential implications for gastrointestinal physiology and metabolic health. When the excretion patterns of specific volunteers were analyzed (Figure 4), significant heterogeneity was observed. The plots illustrate the variance between individuals with different excretion rates, including instances of double-peaked excretion profiles.

A comparison of the excretion dynamics between lactulose/mannitol and u-GIP revealed remarkable similarities, particularly in the temporal patterns observed. Both lactulose and u-GIP exhibited comparable times to the first urine with quantifiable levels sample post-ingestion, with lactulose detected at 2 hours and 44 minutes and u-GIP at 2 hours and 53 minutes, suggesting a similar excretion dynamic.

Similarly, the duration of excretion, as indicated by the time to the first urine with undetectable levels sample post-ingestion,

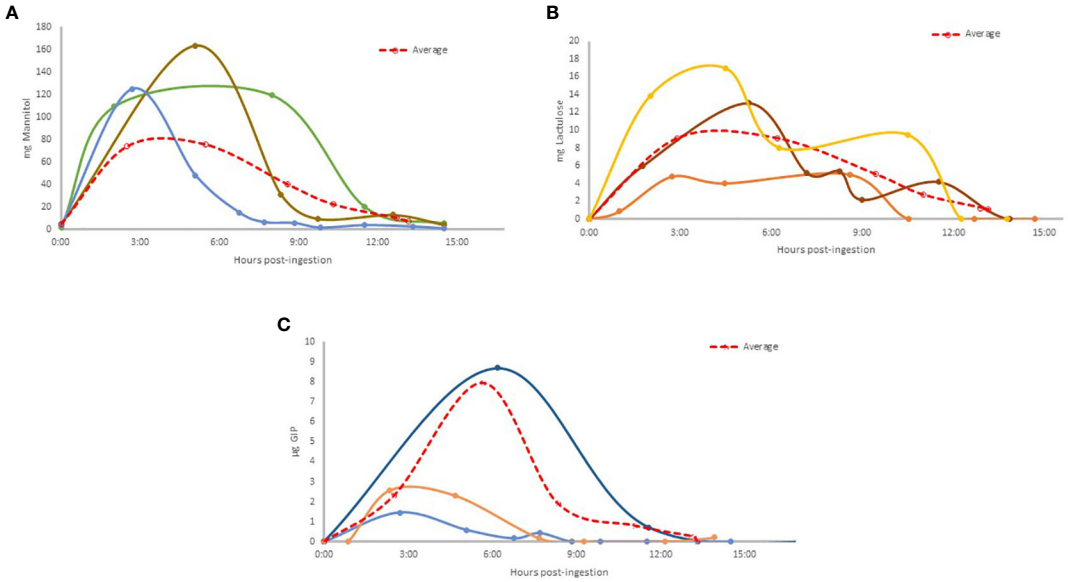


FIGURE 4
Excretion patterns of selected volunteers where: Mannitol excreted **(A)** (Light blue: Vol. 13; Brown: Vol.10; Green: Vol. 6); Lactulose excreted **(B)** (Brown: Vol. 8; Orange: Vol. 2; Yellow: Vol. 7); u-GIP excreted **(C)** (Light blue: Vol. 13; Orange: Vol. 14; Dark blue: Vol. 11). The average results are shown in a dashed red line.

remained consistent for lactulose (13 hours and 23 minutes) and u-GIP (12 hours and 55 minutes), emphasizing their parallel kinetics. As mentioned previously, all baseline urine samples tested positive for mannitol, indicating pre-existing exposure, and none reached undetectable levels during the study period. Notably, the peak excretion times differed slightly, with lactulose and mannitol peaking at approximately 5 hours, and u-GIP peaking at 4 hours and 8 minutes. These findings underscore the feasibility of the GIP-based test, as a reliable marker of intestinal permeability, with the advantage of utilizing a real food antigen that may be implicated in immunological disorders and without transgressions by the participants.

Analyzing the findings presented in Table 1, it can be concluded that while excretion patterns varied among individual volunteers—some exhibiting early excretion peaks at 2 hours post-ingestion and others at 8 hours post-ingestion—it is notable that the third urine sample corresponded to the time of maximum concentration for all three compounds. This finding could inform the development of more accurate protocols and interpretation of future studies related to the excretion kinetics of these compounds.

The presence of volunteers with multiple excretion peaks aligns with existing literature, where the temporal curves for the percentage excretion of mannitol and lactulose exhibited a bimodal pattern with early and later peak (11). In the previous study, the initial peak in mannitol excretion occurred between one- and two-hours post-dosage. However, in our data, the first peak for mannitol was observed at around 4.5 hours, which does not coincide with their findings. We did observe a similarity with the second peak for lactulose at 4 hours, which corresponds to the first peak in our study. Since the previous study only collected data up to

TABLE 1 Characteristics of peak excretion time for each compound and volunteer.

	Component	Peak time (h)	Urine number		Component	Peak time (h)	Urine number
Volunteer 1	**Mannitol**	5:45	3	**Volunteer 9**	**Mannitol**	3:15	2
	Lactulose	5:45	3		**Lactulose**	3:15	2
	GIP	5:45	3		**GIP**	3:15	2
Volunteer 2	**Mannitol**	2:43	3	**Volunteer 10**	**Mannitol**	5:05	2
	Lactulose	8:35	5		**Lactulose**	5:05	2
	GIP	4:28	2		**GIP**	5:05	2
Volunteer 3	**Mannitol**	6:00	3	**Volunteer 11**	**Mannitol**	6:10	2
	Lactulose	6:00	3		**Lactulose**	6:10	2

(Continued)

10.3389/fimmu.2024.1433304

TABLE 1 Continued

	Component	Peak time (h)	Urine number		Component	Peak time (h)	Urine number
	GIP	3:00	2		**GIP**	6:10	2
Volunteer 4	**Mannitol**	6:30	3	**Volunteer 12**	**Mannitol**	3:35	3
	Lactulose	6:30	3		**Lactulose**	3:35	3
	GIP	2:00	2		**GIP**	3:35	3
Volunteer 5	**Mannitol**	4:30	2	**Volunteer 13**	**Mannitol**	2:41	2
	Lactulose	7:30	3		**Lactulose**	2:41	2
	GIP	7:30	3		**GIP**	2:41	2
Volunteer 6	**Mannitol**	8:00	3	**Volunteer 14**	**Mannitol**	4:40	4
	Lactulose	8:00	3		**Lactulose**	4:40	4
	GIP	2:00	2		**GIP**	2:20	3
Volunteer 7	**Mannitol**	4:30	3	**Volunteer 15**	**Mannitol**	4:30	3
	Lactulose	4:30	3		**Lactulose**	7:10	4
	GIP	4:30	3		**GIP**	4:30	3
Volunteer 8	**Mannitol**	5:15	3	**Media**	**Mannitol**	4:52	2,7
	Lactulose	5:15	3		**Lactulose**	5:38	3,0
	GIP	5:15	3		**GIP**	4:08	2,6

GIP, Gluten Immunogenic Peptides.

6 hours, it is unknown whether a later peak would have appeared, as we found in our results at 10:36 for lactulose and 12:10 for mannitol. Additionally, we detected one case with up to three excretion peaks, indicating further interindividual variations in the absorption and excretion of these compounds. These findings underscore the intricate interplay between excretion dynamics and urine characteristics, highlighting the complexity of excretion dynamics and the need to consider individual variability in future studies on intestinal permeability.

The urinary levels of GIP were notably within the µg range, while those of lactulose were in the mg range, despite similar intake quantities. This discrepancy suggests that gluten peptides demonstrate lower permeability than lactulose and/or undergo significantly greater hydrolysis, rendering them more challenging to detect. However, the immunogenicity of gluten peptides holds greater biological relevance than saccharide molecules when analyzing potential systemic inflammatory responses.

Two different collection intervals were tested. Firstly, 2–15-hours interval was chosen because it is documented as the period during which the majority of GIP are excreted (7). Thus, this collection period should capture the vast majority of GIP excreted following the 10g gluten. However, it was determined, that collecting urine for 15 hours would be logistically challenging for patients necessitating the testing of a shorter collection time frame. Given that multiple studies collect urine for 6 hours to measure lactulose/mannitol (18), the 0–6-hour period was also selected.

Different time intervals for urine collection offer varied interpretations of the lactulose and mannitol test, depending on excretion time and gastrointestinal tract location. According to Odenwald et al. (19), the period from 0 to 2 hours after ingestion reflects small intestinal permeability. Between 2 to 8 hours after ingestion, markers are distributed in both the small and colonic intestines, indicating their combined presence in these regions. However, urine collected between 8 and 24 hours provides a more accurate reflection of colonic permeability. It is crucial to recognize that during this later period, colonic microbiota may degrade lactulose and mannitol, limiting their effectiveness in evaluating colonic permeability (20).

Our results, comparing the cumulative excretion within the 0–6-hours range against the 2–15-hours interval, revealed that lactulose and u-GIP did not reach the 60% excretion mark, whereas mannitol exceeded the 70% threshold. Regarding mannitol, it should be noted that there is a minimal dietary contribution (See Results). These findings are consistent with previous investigations, notably those by Camilleri et al. (21) estimating that over 70% of mannitol is absorbed before reaching the colon. Regarding the interpretation of our results, we observed a moderate correlation between the LMR calculated at the two intervals. This correlation has been observed in previous studies, such as that by Camilleri et al. (21) who noted an increase in LMRs in later urine collections, suggesting potentially higher permeability of the colon compared to the small intestine. They hypothesized that a significant portion of the mannitol may have been absorbed by the small intestine, reducing its availability for absorption in the colon, unlike lactulose, according to our data.

A review of 24 studies concluded that the mean LMR in healthy individuals was 1.4, which is consistent with our findings (1.04 for the 0–6-hours interval and 1.43 for the 2–15-hours interval) (22).

Controversy surrounds the establishment of cutoff value for LMR indicating altered intestinal permeability. Some studies set the normal value at 2.5 (23), while others used 3 (24), although this may depend on various factors such as the test procedure. Therefore, in this study, no cutoff value was considered for data evaluation. Instead, an outlier analysis was conducted to identify any anomalous results compared to the rest of the group, given that only healthy volunteers were involved. Volunteer 15 was exhibited unusual values for both the LMR and u-GIP in both intervals, as shown in Figure 3. The likelihood of this occurrence is 0.008%. Detecting an outlier with elevated values for both parameters is an intriguing finding that supports the feasibility of using u-GIP as a potential measure of intestinal permeability. However, further research and analyses involving a significant number of participants (both healthy and diseased) are necessary to confirm this correlation and determine the clinical value of u-GIP in assessing intestinal permeability.

Correlations between LMR and u-GIP parameters at both time intervals were evaluated, along with % Lac and u-GIP. Our analysis detected a moderate correlation between the LMR and u-GIP parameters during the 2–15-hours interval (R^2 = 0.5225) when considering the presence of an outlier. However, upon excluding this outlier, the correlation weakened, suggesting an insignificant relationship between the two variables. This observation is consistent with the expected outcomes in the healthy control group, where variations in intestinal permeability parameters were anticipated to be minimal. Interestingly, Ordiz et al. (25) mentioned in their study involving the L:M test in 1669 rural Malawian children, that the strong direct correlation between percentage lactulose and percentage mannitol excretion does not support the use of mannitol as a normalizing factor for lactulose. They suggested that the use of percentage lactulose excretion alone provides more information about gut integrity than the LMR. Conversely, when outliers were removed from the analysis of % Lac vs u-GIP for the 2–15-hours interval, a moderate correlation (R^2 = 0.4159) emerged. This finding suggests a potential association between lactulose and u-GIP excretion rates within this timeframe, indicating underlying physiological mechanisms that warrant further investigation.

In this study, significant similarities have been identified between lactulose and u-GIP. Both compounds exhibit comparable temporal patterns in urinary excretion, including similar times to first quantifiable urine sample and duration of excretion. Furthermore, comparing excretion across the studied intervals (0–6-hours and 2–15-hours) reveals slower absorption rates for both u-GIP and lactulose. These findings support the theory that gluten peptides are predominantly absorbed via the paracellular pathway, akin to lactulose, due to their substantially larger molecular size compared to molecules predominantly absorbed via the transcellular route in the small intestine.

One notable weakness of the study lies in the dietary conditions post the 6-hour mark, where participants resumed consuming varied foods. This variability in dietary intake among participants introduces uncertainty regarding its potential impact on the excretion dynamics of the substances tested. It remains unclear whether these dietary variations could have altered the results due to minor dietary transgressions. This factor collectively highlights the need for careful consideration and further exploration in future research to mitigate such potential biases.

While these results are promising and demonstrate the feasibility of an intestinal permeability test based on u-GIP, further studies involving individuals exhibiting gastrointestinal conditions that may suggest altered permeability are necessary to compare and validate u-GIP as a marker. One potential concern regarding the use of gluten in assessing intestinal damage, particularly in the context of celiac disease, should not pose a problem during the diagnosis, as a gluten challenge is essential for reliable diagnosis. Given that occasional gluten ingestion is common and often asymptomatic (8, 26), it can also be considered a valuable specialized tool for monitoring the progression of intestinal damage during the gluten-free diet. This can be achieved using urinary GIP after a single gluten intake and for characterizing clinical symptoms (presence or type of symptoms). Given the disparities observed in the dynamics of this cohort comprising 15 volunteers, despite standardized conditions minimizing other dietary factors, exploring the potential for intraindividual variability justifies further investigation. Further clinical studies on individuals with gastrointestinal conditions where changes in permeability are potentially present will ascertain the utility of this approach in estimating intestinal permeability to food antigens.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Virgen del Rocío and Virgen Macarena University Hospitals (n. 1308-N-23). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

RR: Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft. MP: Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Writing – review & editing. IM: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. JL: Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing. CS: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. ÁC: Conceptualization,

Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by Grant DIN2020-011104 funded by MCIN/AEI/10.13039/501100011033 and, by "European Union NextGenerationEU/PRTR, Grant funded by CTA (23/1107) and CDTI (IDI-20230350).

Acknowledgments

The authors would like to thank the volunteers who enrolled in the study.

Conflict of interest

ÁC is the founder and current CEO of Biomedal S.L. Seville, Spain; RR-R, MF, IM, and JL are employees at Biomedal S.L.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, et al. Intestinal permeability - a new target for disease prevention and therapy. *BMC Gastroenterol.* (2014) 14:1–25. doi: 10.1186/s12876-014-0189-7

2. Fasano A. Leaky gut and autoimmune diseases. *Clin Rev Allergy Immunol.* (2012) 42:71–8. doi: 10.1007/s12016-011-8291-x

3. Salvo-Romero E, Alonso-Cotoner C, Pardo-Camacho C, Casado-Bedmar M, Vicario M. The intestinal barrier function and its involvement in digestive disease. *Rev Espanola Enfermedades Digestivas*. (2015) 107:686–96. doi: 10.17235/reed.2015.3846/2015

4. Bischoff SC, Kaden-Volynets V, Filipe Rosa L, Guseva D, Seethaler B. Regulation of the gut barrier by carbohydrates from diet – Underlying mechanisms and possible clinical implications. *Int J Med Microbiol.* (2021) 311(4):151499. doi: 10.1016/j.ijmm.2021.151499

5. Caminero A, Nistal E, Herrán AR, Pérez-Andrés J, Vaquero L, Vivas S, et al. Gluten metabolism in humans. In: *Wheat and Rice in Disease Prevention and Health*. Academic Press (2014). p. 157–70.

6. Coto L, Sousa C, Cebolla A. Individual variability in patterns and dynamics of fecal gluten immunogenic peptides excretion after low gluten intake. *Eur J Nutr.* (2022) 61:2033–49. doi: 10.1007/s00394-021-02765-z

7. Coto L, Sousa C, Cebolla A. Dynamics and considerations in the determination of the excretion of gluten immunogenic peptides in urine: individual variability at low gluten intake. *Nutrients.* (2021) 13:2624. doi: 10.3390/nu13082624

8. Ruiz-Carnicer A, Garzon-Benavides M, Fombuena B, Segura V, Garcia-Fernandez F, Sobrino-Rodriguez S, et al. Negative predictive value of the repeated absence of gluten immunogenic peptides in the urine of treated celiac patients in predicting mucosal healing: New proposals for follow-up in celiac disease. *Am J Clin Nutr.* (2020) 112:1240–51. doi: 10.1093/ajcn/nqaa188

9. Seethaler B, Basrai M, Neyrinck AM, Nazare JA, Walter J, Delzenne NM, et al. Biomarkers for assessment of intestinal permeability in clinical practice. *Am J Physiol Gastrointest Liver Physiol.* (2021) 321:G11–7. doi: 10.1152/ajpgi.00113.2021

10. Drabińska N, Krupa-Kozak U, Jarocka-Cyrta E. Intestinal Permeability in Children with Celiac Disease after the Administration of Oligofructose-Enriched Inulin into a Gluten-Free Diet—Results of a Randomized, Placebo-Controlled, Pilot Trial. *Nutrients.* (2020) 12:1736. doi: 10.3390/nu12061736

11. Sequeira IR, Lentle RG, Kruger MC, Hurst RD. Standardising the lactulose mannitol test of gut permeability to minimise error and promote comparability. *PloS One.* (2014) 9(6):E99256. doi: 10.1371/journal.pone.0099256

12. Schoultz I, Keita ÅV. The intestinal barrier and current techniques for the assessment of gut permeability. *Cells.* (2020) 9:1–30. doi: 10.3390/cells9081909

13. Lebreton C, Ménard S, Abed J, Moura IC, Coppo R, Dugave C, et al. Interactions among secretory immunoglobulin A, CD71, and transglutaminase-2 affect permeability of intestinal epithelial cells to gliadin peptides. *Gastroenterology.* (2012) 143:698–707. doi: 10.1053/j.gastro.2012.05.051

14. Palanski BA, Weng N, Zhang L, Hilmer AJ, Fall LA, Swaminathan K, et al. An efficient urine peptidomics workflow identifies chemically defined dietary gluten peptides from patients with celiac disease. *Nat Commun.* (2022) 13:888. doi: 10.1038/s41467-022-28353-1

15. Morón B, Bethune MT, Comino I, Manyani H, Ferragud M, López MC, et al. Toward the assessment of food toxicity for celiac patients: characterization of monoclonal antibodies to a main immunogenic gluten peptide. *PloS One.* (2008) 3:e2294. doi: 10.1371/journal.pone.0002294

16. Bellini M, Tonarelli S, Mumolo MG, Bronzini F, Pancetti A, Bertani L, et al. Low fermentable oligo-di-and mono-saccharides and polyols (Fodmaps) or gluten free diet: What is best for irritable bowel syndrome? *Nutrients.* (2020) 12:1–13. doi: 10.3390/nu12113368

17. Moreno MDL, Cebolla Á, Munõz-Suano A, Carrillo-Carrion C, Comino I, Pizarro Á, et al. Detection of gluten immunogenic peptides in the urine of patients with coeliac disease reveals transgressions in the gluten-free diet and incomplete mucosal healing. *Gut.* (2017) 66:250–7. doi: 10.1136/gutjnl-2015-310148

18. Teixeira TFS, Boroni Moreira AP, Souza NCS, Frias R, Gouveia Peluzio M do C. Mediciones de permeabilidad intestinal: Aspectos generales y posibles riesgos. *Nutr Hosp.* (2014) 29:269–81. doi: 10.3305/nh.2014.29.2.7076

19. Odenwald MA, Turner JR. Intestinal permeability defects: is it time to treat? *Clin Gastroenterol Hepatol.* (2013) 11:1075–83. doi: 10.1016/j.cgh.2013.07.001

20. Vanuytsel T, Tack J, Farre R. The role of intestinal permeability in gastrointestinal disorders and current methods of evaluation. *Front Nutr.* (2021) 8. doi: 10.3389/fnut.2021.717925

21. Camilleri M, Nadeau A, Lamsam J, Linker Nord S, Ryks M, Burton D, et al. Understanding measurements of intestinal permeability in healthy humans with urine lactulose and mannitol excretion. *Neurogastroenterol Motility.* (2010) 22:15–26. doi: 10.1111/j.1365-2982.2009.01361.x

22. Gan J, Nazarian S, Teare J, Darzi A, Ashrafian H, Thompson AJ. A case for improved assessment of gut permeability: a meta-analysis quantifying the lactulose: mannitol ratio in coeliac and Crohn's disease. *BMC Gastroenterol.* (2022) 22:16. doi: 10.1186/s12876-021-02082-z

23. Marshall JK, Thabane M, Garg AX, Clark W, Meddings J, Collins SM. Intestinal permeability in patients with irritable bowel syndrome after a waterborne outbreak of acute gastroenteritis in Walkerton, Ontario. *Aliment Pharmacol Ther.* (2004) 20:1317–22. doi: 10.1111/j.1365-2036.2004.02284.x

24. Duerksen DR, Wilhelm-Boyles C, Parry DM. Intestinal permeability in long-term follow-up of patients with celiac disease on a gluten-free diet. *Dig Dis Sci.* (2005) 50:785–90. doi: 10.1007/s10620-005-2574-0

25. Ordiz MI, Davitt C, Stephenson K, Agapova S, Divala O, Shaikh N, et al. EB 2017 Article: Interpretation of the lactulose:mannitol test in rural Malawian children at risk for perturbations in intestinal permeability. *Exp Biol Med.* (2018) 243:677–83. doi: 10.1177/1535370218768508

26. Russell AK, Lucas EC, Henneken LM, Pizzey CJ, Clarke D, Myleus A, et al. Stool gluten peptide detection is superior to urinary analysis, coeliac serology, dietary adherence scores and symptoms in the detection of intermittent gluten exposure in coeliac disease: a randomised, placebo-controlled, low-dose gluten challenge study. *Nutrients.* (2024) 16(2):279. doi: 10.3390/nu16020279

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 16 October 2024
DOI 10.3389/fimmu.2024.1425982

OPEN ACCESS

EDITED BY
Simone Baldi,
Department of Experimental and Clinical Medicine, Italy

REVIEWED BY
Maria Georgina Herrera,
Ruhr University Bochum, Germany
Diego S. Vazquez,
National Scientific and Technical Research Council (CONICET), Argentina

*CORRESPONDENCE
Werner Tschollar
Werner.Tschollar@amyra.com
Stefan Engeli
Stefan.Engeli@med.uni-greifswald.de

†These authors share first authorship

‡These authors share last authorship

RECEIVED 30 April 2024
ACCEPTED 16 September 2024
PUBLISHED 16 October 2024

CITATION
Mourabit S, Römer S, Bonner ER, Winter F, Tschollar J, Tzvetkov MV, Weitschies W, Engeli S and Tschollar W (2024) Exopeptidase combination enhances the degradation of isotopically labelled gluten immunogenic peptides in humans.
Front. Immunol. 15:1425982.
doi: 10.3389/fimmu.2024.1425982

Exopeptidase combination enhances the degradation of isotopically labelled gluten immunogenic peptides in humans

Sulayman Mourabit[1†], Sarah Römer[2†], Erin R. Bonner[1], Fabian Winter[3], Julian Tschollar[1], Mladen V. Tzvetkov[2], Werner Weitschies[3], Stefan Engeli[4‡*] and Werner Tschollar[1‡*]

[1]AMYRA Biotech AG, Basel, Switzerland, [2]Department of General Pharmacology, Institute of Pharmacology Center of Drug Absorption and Transport (C_DAT), University Medicine Greifswald, Greifswald, Germany, [3]Department of Pharmaceutical Technology and Biopharmaceutics, Institute of Pharmacy, Center of Drug Absorption and Transport (C_DAT), University of Greifswald, Greifswald, Germany, [4]Department of Clinical Pharmacology, Institute of Pharmacology, Center of Drug Absorption and Transport (C_DAT), University Medicine Greifswald, Greifswald, Germany

Introduction: Celiac disease is a common autoimmune-like enteropathy caused by an aberrant response to incompletely digested dietary gluten. Gluten immunogenic peptides including the immunodominant 33-mer are thought to be resistant to proteolytic digestion by human gastrointestinal peptidases. We developed a novel enzyme therapy approach to support gluten peptide digestion using a combination of two tandem-acting exopeptidases, AMYNOPEP, that complement the intrinsic enzymatic activity of intestinal brush border enterocytes.

Methods: We evaluated the effects of AMYNOPEP supplementation on 33-mer degradation *in vitro* and *in vivo*. In a cross-over clinical study, healthy volunteers with no gastrointestinal disorders were given stable isotope (SI) labelled 33-mer peptides in the presence of varying peptide substrates and caloric loads, with and without AMYNOPEP. 33-mer degradation products (SI-labelled single amino acids) were measured in the blood plasma using LC-MS/MS.

Results: AMYNOPEP achieved rapid, complete amino-to-carboxyl terminal degradation of the 33-mer *in vitro*, generating single amino acids and dipeptides. In healthy volunteers, AMYNOPEP supplementation significantly increased 33-mer degradation and absorption of SI-labelled amino acids even in the presence of competing substrates. Specifically, we observed a 2.8-fold increase in the C_{max} of stable isotope-labelled amino acids in the presence of wheat gluten. The absorption kinetics of labelled amino acids derived from 33-mer digestion with AMYNOPEP closely resembled that of SI-labelled X-Proline dipeptides administered without enzyme supplementation, highlighting the rapid hydrolytic activity of AMYNOPEP on polypeptides.

Conclusions: AMYNOPEP achieved complete degradation of the 33-mer into single amino acids and dipeptides *in vitro* and significantly improved 33-mer degradation kinetics in healthy volunteers, as measured by labelled amino acid detection, warranting further investigation into the potential therapeutic benefits of exopeptidase combinations for patients with gluten-related health disorders including celiac disease.

KEYWORDS

gluten, gluten immunogenic peptide, celiac disease, exopeptidase, glutenase, 33-mer, enzyme therapy, enzyme supplementation

Introduction

Celiac disease (CeD) is a common autoimmune-like enteropathy affecting 1-1.4% of the global population (1, 2) with its incidence rising by 7.5% annually over the past several decades (3). CeD is caused by an aberrant response to specific peptide fragments released during dietary gluten digestion. A limited number of gluten immunogenic peptides (GIPs) are considered to be immunodominant, including the α_2-gliadin derived 33-mer peptide which carries six overlapping T cell epitopes (4). The 33-mer is thought to be stable to proteolytic digestion by human gastric, pancreatic, and intestinal peptidases due to its abundance of proline residues (5), though studies of duodenal biopsies have shown the 33-mer to be almost fully degraded during intestinal transport in healthy individuals (6). Interestingly, the 33-mer peptide has also been shown to spontaneously form peptide self-aggregates *in vitro* (7, 8), which may further interfere with its digestion. In patients with CeD, GIP exposure reactivates a CD4+ T cell-driven immunological response resulting in a broad range of gastrointestinal (GI) and systemic symptoms (as reviewed elsewhere (9, 10)). A lifelong gluten-free diet (GFD) is seen as the only effective approach to prevent gastrointestinal symptoms in CeD patients. However, most patients do not experience complete mucosal healing on a GFD even with well-controlled symptoms (11, 12) and up to 80% of GFD-adhering patients experience inadvertent gluten contamination (13), highlighting a need for more effective CeD therapeutics.

Enzyme therapies currently in development aim to support the body's natural digestion of gluten peptides using exogenous bacterial, fungal, or plant-derived peptidases. These therapies have almost exclusively focused on stomach-acting endopeptidases (ENPs), a class of enzyme that generates peptides of variable lengths by cleaving intra-chain residues (e.g., AN-PEP, Latiglutenase, TAK-062). While ENPs, such as pepsin, trypsin, and chymotrypsin, partially digest peptides into progressively smaller chains, exopeptidases (EXPs) complete digestion by systematically cleaving peptide bonds on either terminal end into absorbable lengths (*e.g.*, single amino acids, dipeptides). The majority of EXPs are anchored to enterocytes at the brush border membrane (BBM) or released by BBM vesicles into the lumen of the small intestine (14, 15). Patients with CeD experience damage to the intestinal BBM and reduced activity of certain brush border EXPs (16, 17). For instance, activity of the endogenous proline-specific dipeptidyl peptidase-IV (DPP-IV) was shown to be reduced by an average of 70% in CeD patients compared to healthy individuals without gastrointestinal diseases (17), likely aggravating the indigestibility of GIPs and their accumulation.

Here, we investigated a novel enzyme therapy approach for the digestion of GIPs using a combination of two exopeptidases, AMYNOPEP, that complements the intrinsic exopeptidase activity of enterocytes. AMYNOPEP consists of two tandem-acting aminopeptidases (a monoaminopeptidase and dipeptidyl peptidase) that digest peptides from the amino- to carboxy-terminal to generate absorbable single amino acids and dipeptides. We assessed the action of AMYNOPEP enzymes on stable isotope (SI) labelled 33-mer peptide digestion using a quantitative LC-MS/MS method for near-real-time detection of SI-labelled amino acids in the blood of healthy volunteers.

Results

AMYNOPEP rapidly and completely degrades the 33-mer peptide into dipeptides and single amino acids *in vitro*

The degradation activity and efficacy of AMYNOPEP was assessed on the 33-mer *in vitro* (1:10 enzyme ratio, see **Methods**). Since both enzymes are aminopeptidases, the 33-mer was expected to be degraded through stepwise cleaving events beginning at the amino-terminal end and resulting in the release of single amino acids (leucine, L; glutamine, Q; phenylalanine, F) and X-proline (XP) dipeptides (QP, FP, LP, and (tyrosine, Y) YP, Figure 1A). Within 30 minutes of incubation with AMYNOPEP, less than 0.05% of the full 33-mer sequence was detectable (Figure 1B), and all anticipated degradation products (XP dipeptides and single

10.3389/fimmu.2024.1425982

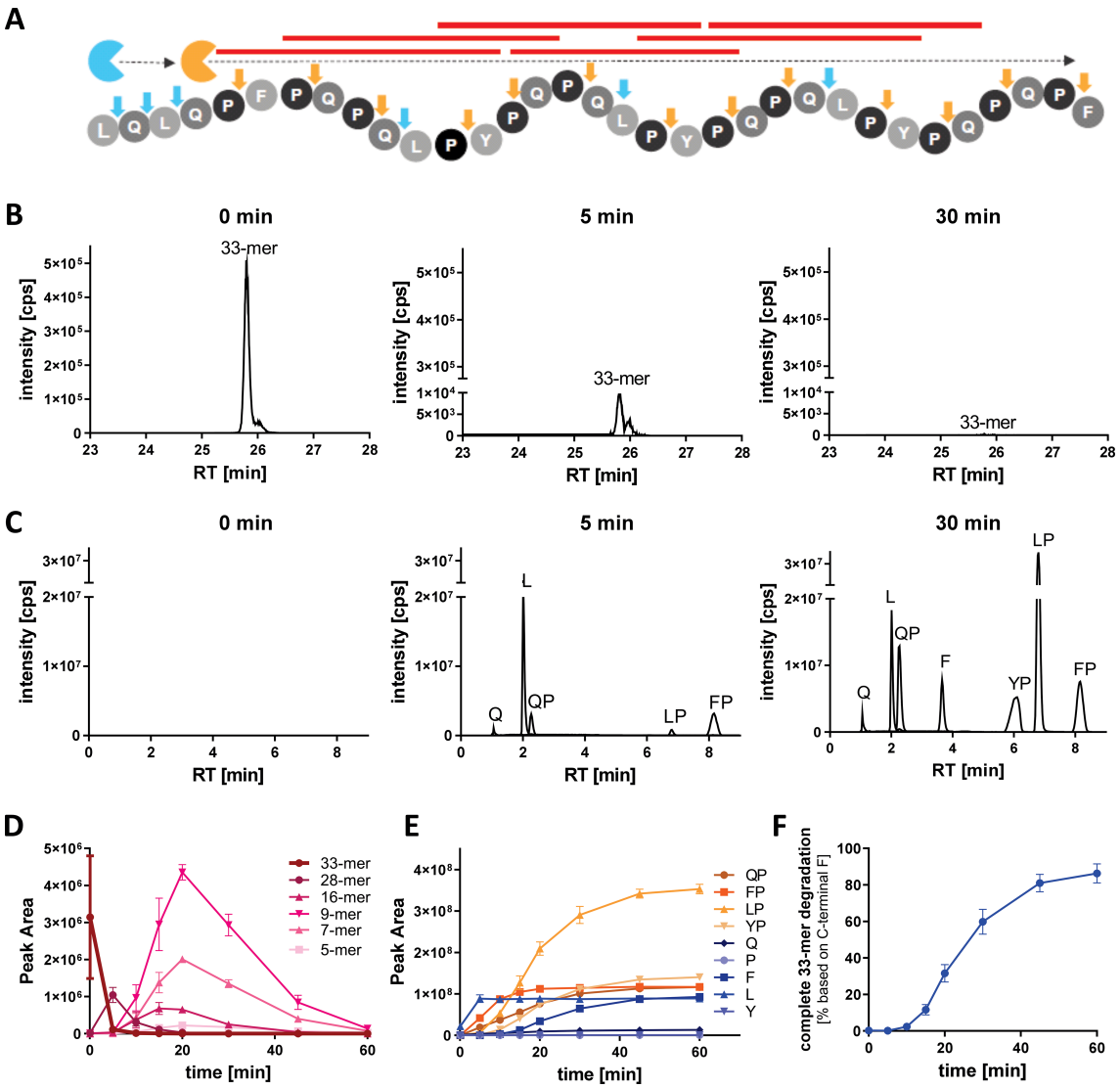


FIGURE 1
AMYNOPEP rapidly and completely degrades the 33-mer GIP into single amino acids and dipeptides *in vitro*. **(A)** Schematic representation of the AMYNOPEP mode-of-action. Tandem EXP activity ensures systematic degradation of the 33-mer through stepwise cleavage of single amino acids (L, Q, F), mediated by the aminopeptidase (blue), and cleavage of XP dipeptides (QP, FP, LP, YP) mediated by the dipeptidyl peptidase (orange). Cleaving sites for each enzyme are indicated by arrows. Red lines represent immunogenic HLA-DQ2/-DQ8 epitopes. **(B)** LC-MS/MS analysis of full length 33-mer degradation 0, 5, and 30 min after addition of AMYNOPEP. **(C)** Detection of XP dipeptides and amino acids during 33-mer degradation 0, 5, and 30 min after addition of AMYNOPEP. Peaks are labelled with their corresponding degradation product. **(D)** Time-dependent degradation of the 33-mer by AMYNOPEP and appearance of selected peptide intermediates, 28-mer (FPQPQLPYPQPQLPYPQPQLPYPQPQPF), 16-mer (LPYPQPQLPYPQPQPF), 9-mer (LPYPQPQPF), and 5-mer (QPQPF). **(E)** Corresponding time-dependent appearance of XP dipeptides and free amino acids. **(F)** Quantitative determination of complete 33-mer degradation based on the detection of F that is only cleaved once as single amino acid during the last cleavage step. **(D–F)** show means and SD of three technical replicates.

amino acids) were detected (Figure 1C). We further analyzed the extent of 33-mer digestion by monitoring the appearance of degradation intermediates: 28-mer, 16-mer, 9-mer, and 5-mer peptides (Figure 1D). The degradation intermediates appeared in a time-dependent manner, in line with progressive N-to-C terminal degradation of 33-mer peptides. Accordingly, XP dipeptides and free amino acids increased in a time-dependent manner (Figure 1E). XP dipeptides appeared to be stable to enzymatic cleavage, as indicated by the lack of detectable free P or Y residues. Given the lack of XP cleavage, detection of free F residues indicated complete degradation of the 33-mer down to the carboxy-terminal-most F residue. Thus, the presence of detectable F within 30 minutes of AMYNOPEP incubation proved complete N-to-C terminal degradation of the 33-mer peptide *in vitro*. Using the terminal F, it could be estimated that 81% to 91% of analyzed 33-mer was degraded completely down to single amino acids and XP dipeptides after 60 minutes of incubation (Figure 1F). To further assess the complete degradation of the 33-mer peptide by AMYNOPEP *in vitro*, we monitored all theoretically possible 33-mer degradation intermediates expected to result from AMYNOPEP's mode-of-

action, revealing complete degradation of the measured peptide products by 60 minutes (Supplementary Figure 1). Faster 33-mer degradation *in vitro* was observed with an enzyme ratio of 1:1 (Supplementary Figure 2), thus a 1:1 enzyme ratio was used for all subsequent studies in healthy volunteers.

SI-labelled amino acids are detectable in the plasma of healthy volunteers

To evaluate the *in vivo* detection efficacy of labelled 33-mer degradation products, we performed a pilot study wherein SI-labelled XP dipeptides were orally administered to healthy volunteers (n=9 total) and subsequently measured in plasma and urine using HPLC-MS/MS (Figure 2, top panel pilot cohort). Volunteers (n=3) were given 50 mg of each SI-labelled XP dipeptide (F*P*, LP*, and L*P*) diluted in water, and the appearance of SI-labelled products was assessed over 72 hours in plasma and pooled urine samples. Only trace amounts (<LOQ) of XP dipeptides were detected in plasma (Figure 3A) and urine. In contrast, all three SI-labelled amino acids (L*, F*, and P*) that would result from the cleavage of their respective XP dipeptides were detectable in plasma in a time-dependent manner following oral administration (Figure 3A). Since SI-labelled amino acids were only partially excreted in urine with 0.002% to 0.017% of the orally administered amount (data not shown), subsequent *in vivo* studies did not include urine analyses.

To assess differences in SI-labelled amino acid digestion and absorption in the presence of a caloric load and competing peptide substrate, we administered 50 mg of each XP dipeptide with 100 mL of Peptamen (n=3 volunteers, Figure 2 top panel). Peptamen did not influence the detection of XP dipeptides which were again only present in trace amounts in the plasma (data not shown). Peptamen resulted in decreased detection of F* and P* in plasma, as seen through a decrease in AUC of 2.1-fold and 2.4-fold, respectively

A

Pilot study (n=9)			
	Pilot 1 (n=3)	**Pilot 2 (n=3)**	**Pilot 3 (n=3)**
XP dipeptides (L*P*, LP*, F*P*)	50 mg / peptide	50 mg / peptide	5 mg / peptide
Drink	Water only	Water + 4 g whey protein drink	Water + 4 g whey protein drink

B

Proof-of-concept study (n=14)		
Comprehensively labelled 33-mer LQLQP*F*PQP*QL*P*YP*QP*QL*P*YP*QP*QL*P*YP*QP*QP*F		
	Week 1	**Week 2**
Cohort 1 (n=5)	50 mg 33-mer + apple juice	50 mg 33-mer + apple juice + 40 mg AMYNOPEP
Cohort 2 (n=5)	50 mg 33-mer + 4 g whey protein drink	50 mg 33-mer + 4 g whey protein drink + 40 mg AMYNOPEP
Strategically labelled 33-mer LQLQPFPQPQLPYPQPQL*PYPQPQLP*YPQPQPF		
Cohort 3 (n=4)	100 mg 33-mer + 5 g wheat gluten	100 mg 33-mer + 5 g wheat gluten + 40 mg AMYNOPEP

FIGURE 2
Overview of pilot and proof-of-concept study design assessing AMYNOPEP supplementation on 33-mer peptide digestion in healthy volunteers. *Pilot study:* To assess detection of orally administered amino acids in blood, a pilot study was conducted beforehand. **(A)** SI-labelled XP dipeptides were administered and resorption in the form of SI-labelled amino acids was measured in blood. **(B)** *Proof-of-concept study (Cohorts 1-3):* Two different isotope labelled peptides were used to evaluate 33-mer degradation: (1) a comprehensively labelled peptide with labels covering epitopes throughout the peptide, and (2) a strategically labelled peptide to evaluate end-to-end peptide digestion. A cross-over study design was utilized in which healthy volunteers followed a specified protocol on week one, followed by the same protocol with the addition of AMYNOPEP on week 2. In cohort 1, five volunteers were given 50 mg of comprehensively labelled peptide with apple juice. In cohort 2, five volunteers were given 50 mg of comprehensively labelled peptide with Peptamen. In cohort 3, four volunteers were given 100 mg of strategically labelled peptide with 5 g of wheat gluten. Blood was collected at regular intervals and 33-mer digestion products were measured as described in the Methods.

10.3389/fimmu.2024.1425982

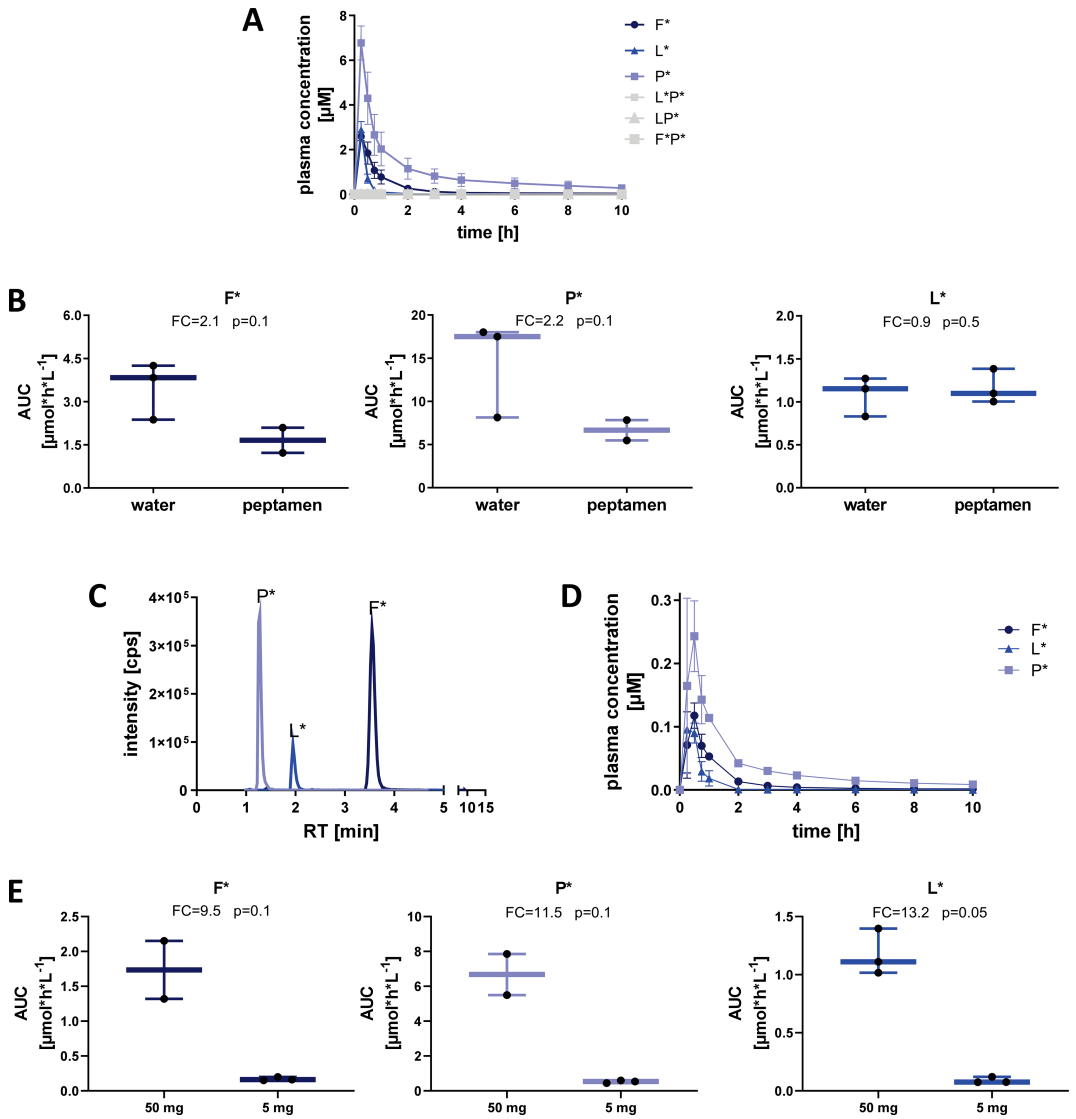


FIGURE 3
Detection of SI-labelled single amino acids in plasma after oral administration of XP dipeptides in healthy volunteers. **(A)** Plasma concentration [µM] of labelled dipeptides and amino acids after oral administration of L*P*, LP*, and F*P* (50 mg each) with water. Means and SD of three individuals are shown. **(B)** Influence of Peptamen (hydrolyzed protein) on absorption and detection of labelled amino acids after oral administration of dipeptides (50 mg each). Means and min/max of three individuals are shown. **(C)** LC-MS/MS detection intensity [cps] of labelled amino acids 1 hour after oral administration of dipeptides (5 mg each) taken with Peptamen. **(D)** Plasma concentration [µM] of labelled amino acids after administration of 5 mg of each labelled XP taken with Peptamen. **(E)** Influence of dose reduction from 50 mg to 5 mg of each labelled XP taken with Peptamen on plasma AUC of labelled amino acids. Box plots show the median, 25th to 75th percentiles and whisker show the min/max of three healthy individuals per cohort. FC, fold-change; AUC, area under the curve.

(Figure 3B). In contrast, detection of L* was not significantly altered in the presence of Peptamen relative to water.

To assess the detection limit of our system, we lowered the dipeptide dose to 5 mg per XP, in combination with 100 mL Peptamen (n=3 volunteers, Figure 2 top panel). The 10-fold dipeptide dose reduction resulted in clearly detectable F*, P*, and L* in plasma (Figures 3C, D), with a proportional reduction in plasma AUC of 9.5-fold, 11.5-fold, and 13.2-fold, respectively (Figure 3E). Based on the XP dipeptide absorption data, we proceeded to administer at least 50 mg of labelled 33-mer peptides in subsequent studies.

AMYNOPEP supplementation rapidly increases the degradation of 33-mer peptides in healthy volunteers

To assess enzyme efficacy in healthy volunteers, we analyzed degradation of two different SI-labelled 33-mer peptides with and without AMYNOPEP (Figure 2, middle and lower panels). The first was a comprehensively labelled peptide with SI labels distributed throughout the sequence, covering all immunogenic epitopes with the goal of ensuring sufficient detection of enzyme activity *in vivo* (Figure 4A). In the first cohort (n=5 volunteers, Figure 2 middle

10.3389/fimmu.2024.1425982

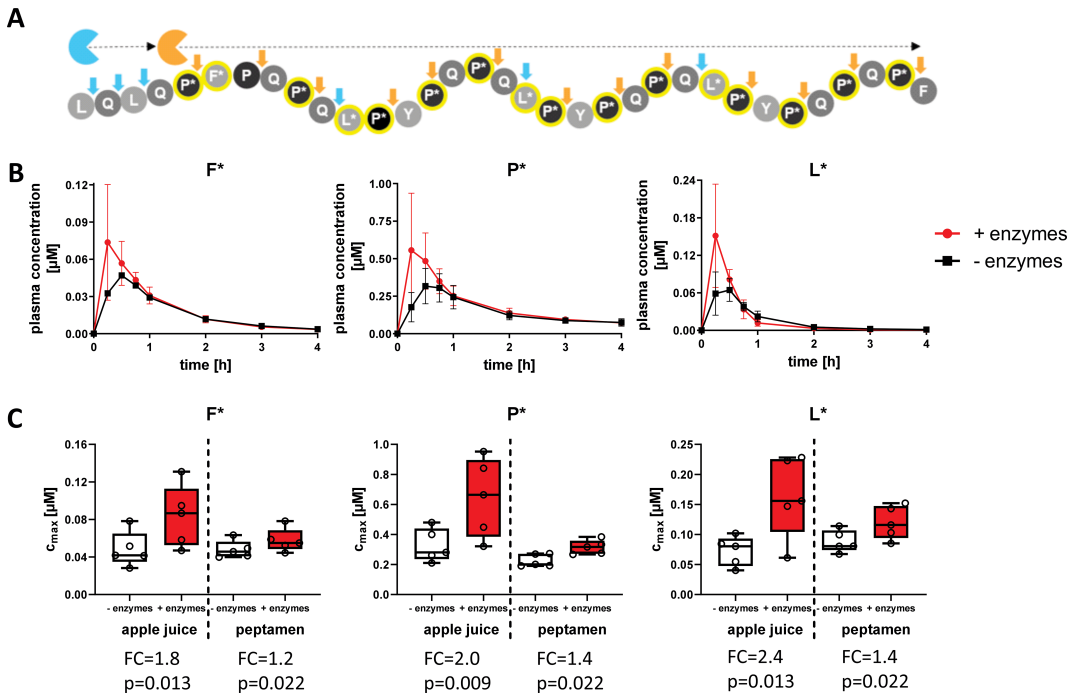


FIGURE 4
Rapid and enhanced degradation of the 33-mer peptide with AMYNOPEP supplementation. **(A)** Schematic overview of comprehensively labelled 33-mer peptide digestion by AMYNOPEP enzymes (represented by blue and orange sectors). Cleavage sites are indicated by arrows. **(B)** 50 mg of labelled 33-mer peptide was administered to five healthy volunteers with apple juice, preceded by administration of AMYNOPEP (visit 2) or plain water (visit 1). Plasma concentration of labelled amino acids (F*, P*, L*) was measured over the course of four hours. Means and SD of five healthy individuals are shown **(C)** C_{max} of labelled amino acids in plasma, with and without AMYNOPEP supplementation, and co-administration of apple juice (left side of graphs) or Peptamen (right side of graphs). Box plots show the median, 25th to 75th percentiles and whisker show the min/max of five healthy individuals. FC, fold-change; C_{max}, maximum concentration.

panel), 50 mg of comprehensively labelled 33-mer peptide were administered with apple juice, a low-caloric fluid to facilitate rapid gastric emptying, and to avoid the introduction of competing peptides that could affect enzyme activity. Interestingly, 33-mer degradation was also observed in volunteers without AMYNOPEP, though AMYNOPEP supplementation resulted in significantly higher plasma C_{max} of all labelled amino acids in the first 30 min post-administration compared to control (Figures 4B, C). Specifically, AMYNOPEP resulted in significant 1.8, 2.0, and 2.4-fold-change increases in maximum plasma concentrations (C_{max}) of F*, P*, and L*, respectively (Figure 4C). In addition, AMYNOPEP supplementation resulted in significantly increased AUCs of F* (FC=1.3, p=0.025), P* (FC=1.2, p=0.006) and L* (FC=1.3, p=0.018) compared to control (Supplementary Figure 3).

In the second cohort (n=5 volunteers, Figure 2 middle panel), we assessed the effect of competing peptide substrates in the form of 100 mL Peptamen on the degradation activity of 33-mer by AMYNOPEP. As expected, the addition of Peptamen decreased the efficacy of 33-mer digestion when compared to enzyme administration with apple juice, as shown by decreased C_{max} (Figure 4C). However, even with Peptamen, significant increases in C_{max} of F* (FC=1.2, p=0.022), P* (FC=1.4, p=0.022) and L* (FC=1.4, p=0.022) were observed with AMYNOPEP compared to control. Additionally, AMYNOPEP supplementation still resulted in significantly increased AUCs of F* (FC=1.1, p=0.022), P* (FC=1.2, p=0.022) and L* (FC=1.1, p=0.022) in the presence of Peptamen compared to controls.

AMYNOPEP supplementation significantly decreased time to C_{max} (t_{max}) for F* (t_{max}= 22.5 min with AMYNOPEP vs 31.5 min without, p=0.048) and P* (t_{max}= 24 min with AMYNOPEP vs. 33 min without, p=0.032) when administered with apple juice (Supplementary Figure 4). No significant differences in t_{max} were observed with Peptamen (Supplementary Figure 4).

We further assessed AMYNOPEP enzyme efficacy using a second SI-labelled 33-mer peptide with strategic labeling of single L* and P* residues, to assess the extent of N-to-C terminal peptide digestion (Figure 2 bottom panel, Figure 5A). Detection of L* would indicate degradation of the first half of the 33-mer and disruption of four out of six immunogenic epitopes, while the downstream P* shows disruption of all immunogenic epitopes. In a third cohort (n=4 volunteers), we administered 100 mg of the strategically labelled 33-mer with 5 g of wheat gluten as a competitive substrate (Figure 2). AMYNOPEP supplementation resulted in significantly increased C_{max} of P* (FC=2.8, p=0.034) and L* (FC=3.4, p=0.034) compared to control, even in the presence of gluten (Figures 5B, C). We observed a trend in which AMYNOPEP decreased t_{max}, which was reached at 22.5 min post-administration of AMYNOPEP for both P* and L*, compared to t_{max} of 45 min without enzyme (p=0.054 for both; Supplementary Figure 5).

10.3389/fimmu.2024.1425982

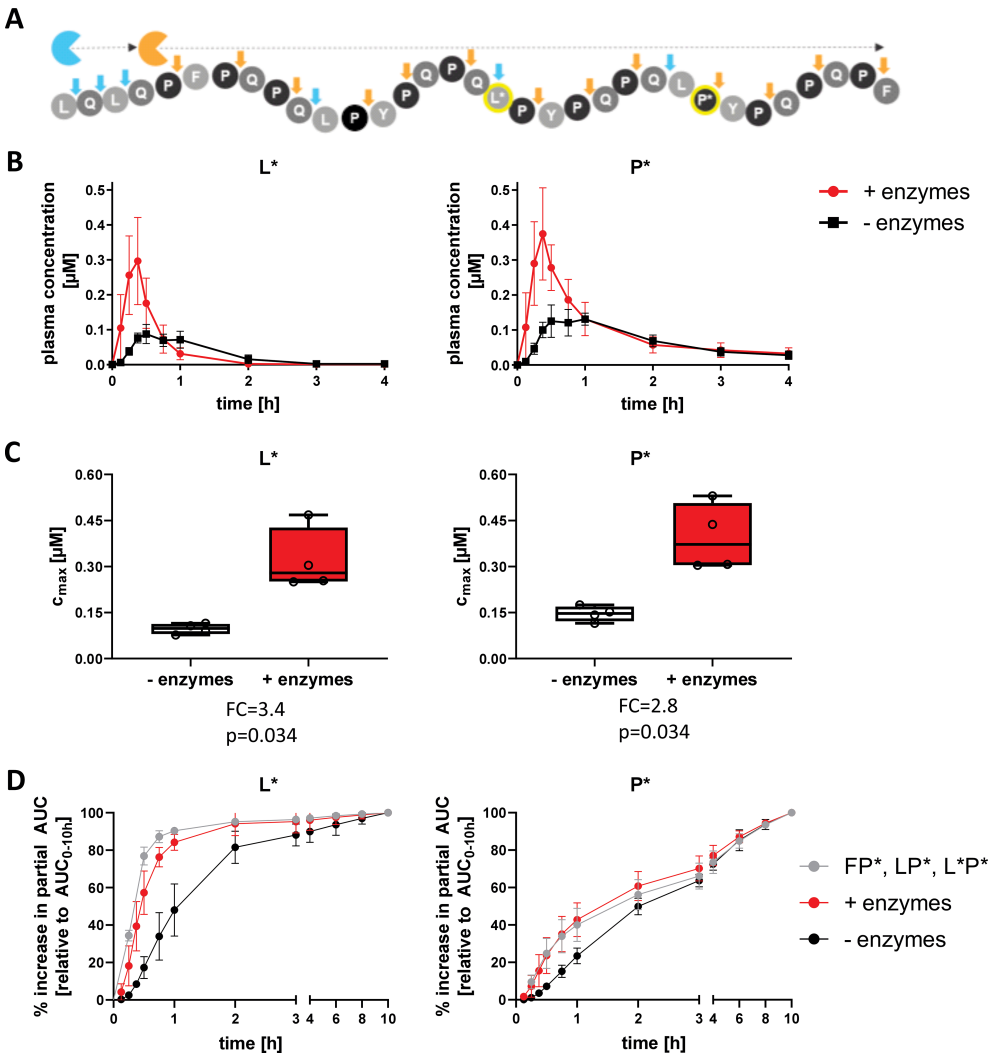


FIGURE 5
Comprehensive degradation of SI-labelled 33-mer by AMYNOPEP in the presence of gluten. **(A)** Schematic overview of strategically labelled 33-mer peptide digestion by AMYNOPEP enzymes (represented by blue and orange sectors). Cleavage sites are indicated by arrows. **(B, C)** 100 mg of strategically labelled 33-mer peptide was administered to four healthy volunteers with 5 g wheat gluten, with (red) or without (black) AMYNOPEP. Influence of enzyme addition on L* and P* plasma concentrations over time **(B)** and on maximum plasma concentrations (C_{max}, **C)** were determined. **(D)** Comparison of amino acid absorption after administration of XP dipeptides (F*P*, LP*, L*P*) with water or 33-mer strategically labelled peptide with and without addition of AMYNOPEP. Plasma concentration-time plots show means and SD of four healthy individuals. Box plots show the median, 25th to 75th percentiles and whiskers show and min/max of four healthy individuals. C_{max}, maximum concentration; AUC, area under the curve; FC, fold-change.

Most importantly, the absorption kinetics of single amino acids resolving from 33-mer degradation after AMYNOPEP addition closely resembled that of the amino acids when administered as XP dipeptides in the pilot study (Figure 5D). This finding reinforces the rapid action of AMYNOPEP, whereby the digestion and absorption of amino acids from a long (>30-amino acid) peptide was comparable to that of ingested dipeptides.

Discussion

We have demonstrated rapid, systematic N-to-C terminal degradation of 33-mer peptides by AMYNOPEP *in vitro*, with corresponding degradation of SI-labelled 33-mer peptides in healthy volunteers even in the presence of competing substrates (Peptamen, wheat gluten). While the study was limited by its focus on one representative SI-labelled GIP, the positive results described herein warrant further investigation into AMYNOPEP's efficacy at digesting other proteolytically-resistant and immunogenic peptides. Given the observed end-to-end degradation mode of action of AMYNOPEP *in vitro*, the same mechanism very likely underlies the improved degradation of 33-mer peptides *in vivo*, with AMYNOPEP supporting endogenous digestive enzyme machinery. Our strategy to use SI-labelled 33-mer combined with quantitative LC-MS/MS detection allowed us to distinguish between SI-labelled 33-mer degradation products and naturally occurring amino acids in plasma samples, including those from competing substrates co-administered with SI-labelled 33-mer. Considering the N-to-C

terminal degradation of peptides by AMYNOPEP, the two differently SI-labelled peptides provided complementary information. Whereas the comprehensively labelled peptide allowed for sensitive detection of even minor degradation, the strategically labelled peptide gave us an indication of the extent of 33-mer digestion. Following the mode-of-action demonstrated *in vitro*, detection of labelled L* would indicate degradation of half of the 33-mer and four out of six immunogenic epitopes, whereas detection of labelled P* would show further N-to-C terminal degradation and disruption of all six immunogenic epitopes.

We observed 33-mer degradation in healthy volunteers even without AMYNOPEP. These findings further support that the 33-mer is not indigestible in healthy humans, but that its digestion in the small intestine may be performed by rate-limited enzymes (*e.g.*, brush border EXPs (18)). In line with this, research on duodenal biopsies from active CeD, GFD-managed CeD, and healthy volunteers showed that enterocytes of healthy individuals and GFD-managed, but not active CeD patients, can fully digest certain digestion-resistant gliadin peptides (6), suggesting defects in BBM peptidase activity in active CeD. As noted, compromised enterocyte functionality and reduced activity of certain BBM peptidases in CeD reinforce the need to supplement or replace BBM deficiencies. To date, there have been limited research and clinical studies aimed at developing EXP-based enzyme therapies, perhaps due to a limited understanding of the human BBM proteome and lack of suitable *in vitro* models to study the human BBM (19, 20), the source of most EXPs (14). Some exogenous EXPs have been studied in human and commercial settings, including DPP-IV and separately, leucyl aminopeptidase, demonstrating favorable safety profiles of these enzymes. DPP-IV is already available as an over-the-counter dietary supplement in several products, and a food enzyme leucyl aminopeptidase recently underwent safety evaluation showing no safety concerns under conditions of use in eight food manufacturing processes (21). Regarding the safety of AMYNOPEP, we collected data on adverse events during the course of study participation, and no volunteers reported adverse events related to the ingestion of study materials (AMYNOPEP or SI-labelled peptides, see Appendix I for more details on safety data). We demonstrated here that EXP supplementation *via* AMYNOPEP greatly increased 33-mer degradation and SI-labelled amino acid absorption *in vivo*, even in healthy volunteers with presumably intact BBM. Our approach, with quantitative LC-MS/MS measurement of SI-labelled 33-mer degradation products, allowed for sensitive, non-invasive monitoring of GIP degradation in near-real-time. We did not detect SI-labelled XP dipeptides *in vivo*, likely due to endogenous processing of dipeptides into single amino acids by enterocytes (as demonstrated in the pilot study).

Contrary to many ENP-based enzyme therapies under development for the treatment of CeD, our enzyme combination targets peptide degradation in the small intestine. The focus of most enzyme therapies on peptide digestion in the stomach likely stems from concerns that GIP digestion must occur before gastric emptying to prevent immune activation in the small intestine. However, recent research into the immunological timeline of events following gluten ingestion revealed a one-to-four-hour window before peak detection of immune markers in the blood and associated gastrointestinal symptoms (22), suggesting that fast-acting enteric enzymes may still digest gluten peptides in time to prevent adverse effects. Furthermore, enzyme approaches relying solely on gluten digestion in the stomach are likely to be ineffective due to limited mixing in the stomach (23, 24) and rapid gastric emptying of low-caloric fluids and small particles (23, 25). Stomach-targeting enzyme supplements may therefore be rapidly emptied into the small intestine, where they might experience greatly reduced activity or inactivation due to changes in pH or vulnerability to trypsin, thereby missing their window of opportunity to efficiently digest gluten peptides. Our novel mode-of-action is based on combinatorial EXP action in the small intestine, hence addressing these limitations, complementing the body's EXP activity of luminal or brush border origin to achieve thorough digestion of GIPs into absorbable single amino acids and dipeptides. While our study was limited by a small sample size of healthy volunteers and did not include patients with CeD, our SI-labelled approach offers a highly sensitive and accurate detection method for assessing enzyme efficacy in near real-time. Future enzymatic approaches to gluten digestion should account for issues of motility and enzyme mode-of-action, with a particular focus on intestinal activity and EXP supplementation.

Materials and methods

Materials

All labelled peptides, XP dipeptides, and amino acids were synthesized using 13C and 15N stable isotopes (SI, labelling further indicates as *). Labelled 33-mer (LQLQ**P***F*PQ**P***Q**L***P***Y**P***QP*Q**L***P***Y**P***Q**P***QL*P*YP*QPQPF and XP dipeptides phenylalanine-proline (F*P*), leucine-proline (LP*), and leucine-proline (L*P*) were synthesized by Intavis Peptide Services GmbH (Tübingen, Germany). Labelled and non-labelled amino acids (F, P, L, Y, Q) were obtained from Sigma-Aldrich (Taufkirchen, Germany). Non-labelled 33-mer was obtained from Ontores biotechnologies (Shanghai, China). Non-labelled XP dipeptides FP, LP, YP and QP as well as 28-mer, 16-mer, 9-mer and 5-mer were obtained as SpikeTides™ for method development from JPT Peptide Technology GmbH (Berlin, Germany). LC-MS/MS grade acetonitrile, methanol, and formic acid were obtained from Merck (Darmstad, Germany). LoBind 1.5mL reaction tubes were obtained from Sarstedt (Nümbrecht, Germany). Peptamen™ (Neutral SmartFlex) was obtained from Nestlé Health Science Gmbh (Frankfurt, Germany). Wheat gluten was obtained from Veganz Group AG (Berlin, Germany). Hydrogencarbonate ($NaHCO_3$) was obtained from Arnold Holste Wwe. GmbH & Co. KG (Bielefeld, Germany).

In vitro digestion of 33-mer peptide by AMYNOPEP

In vitro digestion of 33-mer peptides was performed using a two-form enzyme combination, AMYNOPEP, which utilizes two tandem-acting aminopeptidases. The 33-mer digestion was done in Tris buffer at pH 7.0 using an enzyme mixture consisting of 0.86

mg/L dipeptidyl peptidase and 8.6 mg/L aminopeptidase (1:10 enzyme ratio). Enzyme mixture was incubated with 100 mg/L 33-mer in a final volume of 1.8 mL. Digestions were performed at 37°C and 350 rpm. Samples of 200 µL were collected at 0, 5, and 30 min and immediately transferred to new 1.5 mL reaction tubes prefilled with 1.6 mL 0.1% TFA to stop the reaction. Samples were stored at -80°C prior to LC-MS/MS measurement, for which samples were diluted 1:5 in 0.1% formic acid. 5 µL or 10 µL of samples were analyzed to detect the intact 33-mer, degradation intermediates (28-mer: FPQPQLPYPQPQLPYPQPQLPYPQPQPF; 16-mer: LPYPQPQLPYPQPQPF; 9-mer: LPYPQPQPF; 5-mer: QPQPF), and degradation products (dipeptides and amino acids). In order to determine the proportion of complete degradation, the amount of cleaved F, which is only generated in the last step of degradation, was quantified and set in relation to the theoretical maximum achievable concentration with complete degradation of all 33-mer molecules. Detailed description of LC-MS/MS method to detect *in vitro* degradation products can be found in Supplementary Tables 1, 2.

Pilot study demonstrating SI-labelled amino acid detection in plasma of healthy volunteers

Healthy volunteers were selected based on no previous history of CeD, NCGS, or wheat allergy. The study (German Register of Clinical Studies, DRKS00033099) was approved by the Ethics Committee of the University Medicine Greifswald, and was performed in adherence to the declaration of Helsinki (2013 Version), the Medical Association's Professional code of conduct for Mecklenburg-Vorpommern (BOÄ M-V) and the data protection regulation of the EU (EU-DSGVO) and of Mecklenburg-Vorpommern (DSG-MV). Written informed consent was obtained from all volunteers prior to enrollment in the study.

A mixture of all three SI labelled XP dipeptides (F*P*, LP*, and L*P*) were orally administered to volunteers (n=9 total), divided into three cohorts of three volunteers each (see Figure 2). Cohort 1 received 50 mg of each dipeptide in 300 mL water. Cohort 2 received 100 mL Peptamen first, then 50 mg of each dipeptide in 150 mL water, followed by another 50 mL water. All drinks were taken within a minute. Cohort 3 received 100 mL Peptamen first, then a reduced dose of 5 mg of each dipeptide in 150 mL water, followed by another 50 mL water. All drinks were taken within a minute. Blood samples were collected after 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 14 h, 48 h and 72 h after dosing. Urine was collected and pooled at 0-4 h, 4-6 h, and 6-10 h, and was collected as spot urine at 48 h and 72 h after dosing.

Proof-of-concept study evaluating 33-mer peptide degradation by AMYNOPEP in healthy volunteers

Healthy volunteers were selected based on no previous history of CeD, NCGS, or wheat allergy. The study (German Register of Clinical Studies, DRKS00033108) was approved by the Ethics Committee of the University Medicine Greifswald, and was performed in adherence to the declaration of Helsinki (2013 Version), the Medical Association's Professional code of conduct for Mecklenburg-Vorpommern (BOÄ M-V) and the data protection regulation of the EU (EU-DSGVO) and of Mecklenburg-Vorpommern (DSG-MV). Written informed consent was obtained from all volunteers prior to enrollment in the study.

Volunteers were divided into three cohorts, utilizing differently labelled 33-mer peptides and different food intake conditions. Two differently SI-labelled 33-mer peptides were used: (1) a comprehensively labelled 33-mer (LQLQ**P*F***PQ**P*QL*P***YP*QP***QL*P*YP*QP*QL*P*YP*QP*QP*F), and (2) a strategically labelled 33-mer (LQLQPFPQPQLPYPQPQ**L***PYPQPQL**P***YPQPQPF). Bold asterisked letters indicate SI-labelled amino acids. The study was designed as a cross-over study, with study visits one week apart. During visit 1, each volunteer received the labelled 33-mer with apple juice, Peptamen, or wheat gluten without AMYNOPEP. During visit 2, each volunteer received the labelled 33-mer with apple juice, Peptamen, or wheat gluten plus AMYNOPEP. Consistently across the study cohorts, AMYNOPEP was prepared in a 1:1 enzyme ratio (20 mg of each enzyme) dissolved in 40 mL water with 500 mg sodium bicarbonate to neutralize gastric acid.

In cohort 1, volunteers (n=5) received 40 mL water with 500 mg sodium bicarbonate, and 5 min later, 50 mg of comprehensively labelled 33-mer dissolved in 20 mL water and 180 mL apple juice (week 1). Apple juice was treated with 2 g sodium bicarbonate before administration to neutralize fruit acids. In week 2, volunteers first received 40 mg of AMYNOPEP in 40 mL water with 500 mg sodium bicarbonate, and 5 min later, received the same treatment conditions as in week 1. In cohort 2, volunteers (n=5) followed the same study protocol as in cohort 1, but apple juice was replaced with 100 mL of Peptamen (containing partially hydrolyzed whey protein, 100 kCal, 4 g peptides per 100 mL; caloric content approximately similar to the volume of apple juice in cohort 1) neutralized with 1 g sodium bicarbonate. In cohort 3, volunteers (n=4) received 100 mg of strategically labelled 33-mer together with 5 g of wheat gluten dissolved in 180 mL of water (week 1). Wheat gluten was previously neutralized by 2 g of sodium bicarbonate. In week 2, volunteers received 40 mg of AMYNOPEP, and after 5 min, received the same treatment conditions as in week 1.

Blood samples were collected after 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, and 24 h after treatments, and plasma amino acid levels were monitored at each time point by LC-MS/MS. In cohorts 2 and 3, additional blood samples were collected at 7.5 min and 22.5 min after treatments.

Sample preparation

Plasma samples were thawed on ice, mixed, and 100 µL of each sample was transferred to a 1.5 mL LoBind reaction tube. Plasma samples were precipitated after adding 200 µL of acetonitrile/methanol (10:1), and incubated on ice for 15 min. Samples were centrifuged at 16,000 *g* for 15 min at 4 °C. 100 µL

supernatant was dried under nitrogen at 40 °C. Samples were reconstituted in 100 µL 0.1% formic acid and 5 µL was applied to the LC-MS/MS instrument.

Analysis of LC-MS/MS data

Labelled XP dipeptides and single amino acids (P*, L*, F*) in plasma samples were quantified by LC-MS/MS using Nexera LC40 HPLC system (Shimadzu, Duisburg, Germany) coupled to an API 6500+ ™ tandem mass spectrometer (AB Sciex, Darmstadt, Germany). For chromatographic separation an ACQUITY UPLC HSS PFP Column (100 Å, 1.8 µm, 2.1 mm X 150 mm; Waters, Eschborn, Germany) protected by a SecurityGuard C18 column (4x2 mm; Phenomenex, Aschaffenburg, Germany) was used. Chromatographic separation was performed at an oven temperature of 40°C and the solvent flow rate was set to 400 µL/min. Elution was achieved by varying composition of solvent A (90% acetonitrile + methanol (6 + 1), 0.1% formic acid in H_2O) and solvent B (0.1% formic acid in H_2O) using following LC conditions: 0-1.4 min 0% solvent A; 1.4-10.4 min 0-50% solvent A; 10.5-12.9 min 80% solvent A; 13-15 min 0% solvent A. To decrease the introduction of debris into the MS, a valve was directed to waste at 0.1-1 min and 9.5-14.9 min.

MS detection of labelled amino acids and XP dipeptides was performed in positive mode with curtain gas set to 40, gas 1 of 50, gas 2 of 70, temperature of 500°C and an IS of 3500. Detection was achieved using MS parameters and mass transitions listed in Supplementary Table 3.

The LC-MS/MS method was verified to be specific for the quantitative determination of P*, F*, L*, F*P*, LP* and L*P* in human plasma and urine. No interferences of the analytical signals with the biological matrix were observed. The calibration curves for labelled amino acids were linear between 0.002 µM and 5 µM for F*, P* and L*. This correlates to 0.24 to 605 ng/ml P*, 0.35 to 875 ng/ml F and 0.28 to 690 ng/ml L*. The calibration curves for F*P*, LP* and L*P* were linear between 0.005 µM and 5 µM. This correlates to 1.39 to 1390 ng/ml for F*P*, 1.17 to 1170 ng/ml for LP* and 1.21 to 1205 ng/ml for L*P*. The correlation coefficients for all analytes ranged between 0.9986 and 1.

Analysis of *in vitro* samples was performed semi quantitatively. Non-labelled XP dipeptides and single amino acids from *in vitro* samples were measured applying the method described above using mass transitions and MS parameters listed in Supplementary Table 1. To estimate total degradation, measurement of carboxy-terminal F that is only cleaved in the last step of 33-mer digestion by exopeptidases, was performed quantitatively. Detection of 33-mer and its degradation intermediates (28-mer, 16-mer, 9-mer, 7-mer and 5-mer; see Supplementary Table 2) was achieved using an ACQUITY UPLC BEH C18 Column (130Å, 1.7 µm, 2.1 mm X 50 mm (Waters) protected by a SecurityGuard C18 column (4x2 mm; Phenomenex, Aschaffenburg, Germany) for chromatographic separation. Chromatographic separation was performed at an oven temperature of 40°C and the solvent flow rate was set to 400 µL/min. Elution was achieved using following LC conditions: 0-4 min 0% solvent A; 4-6 min 0-15% solvent A; 6-20 min 15-30% solvent A; 20-26 min 30-40% solvent A, 26.1-28 min 60% solvent A, 28.1-31.2 min 0% solvent A. MS detection was performed in positive mode with curtain gas set to 40, gas 1 of 50, gas 2 of 50, temperature of 450°C and IS of 5500.

Pharmacokinetics analyses

LC-MS/MS-generated chromatograms were analyzed using Analyst 1.7.2 (Sciex, Darmstadt, Germany). The pharmacokinetics parameter AUC was calculated using GraphPad Prism version 8.0 (GraphPad Prism Software Inc., La Jolla, CA, USA). Partial AUCs were calculated manually using the trapezoidal rule.

Statistical analyses

Statistical analyses were performed using SPSS Statistics Version 28 (SPSS Inc., IBM, Chicago, IL, USA). To compare the effects between cohorts in the dipeptide resorption study the one-tailed Mann-Whitney-Test was applied. To compare the effects between enzyme supplementation and controls during the proof-of-concept study the one tailed Wilcoxon matched-pairs signed rank test was applied.

Data availability statement

The data generated during this study are available upon request from the corresponding authors. Access to the data will be considered on a case-by-case basis, and any requests will be evaluated in accordance with ethical guidelines and data sharing policies. Please contact the corresponding authors for further information.

Ethics statement

The studies involving humans were approved by Ethics Committee of the University Medicine Greifswald. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SM: Conceptualization, Project administration, Writing – original draft, Writing – review & editing. SR: Methodology, Investigation, Formal analysis, Visualization, Writing – review & editing. ERB: Writing – original draft, Writing – review & editing, Visualization. FW: Writing – review & editing. JT: Funding acquisition, Project administration, Writing – review & editing. MVT: Conceptualization, Supervision, Formal analysis, Writing – review & editing. WW: Conceptualization, Supervision, Writing – review & editing. SE: Conceptualization, Supervision, Investigation, Writing – review & editing. WT: Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was sponsored by AMYRA Biotech AG.

Acknowledgments

The authors would like to thank all volunteers for their participation in the clinical study. The authors would also like to thank Intavis Peptide Services GmbH for synthesis of the stable isotope-labelled peptides used in the present study.

Conflict of interest

Authors SM and JT were employed by AMYRA Biotech AG. EB has a consulting agreement with AMYRA Biotech AG. SR, MT, WW, and SE have contract research agreements with AMYRA Biotech AG. WT is co-founder of and shareholder in AMYRA Biotech AG.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

The authors declare that this study received funding from AMYRA Biotech AG. The funder(s) had the following involvement in the study: study design, decision to publish, and preparation of the manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1425982/full#supplementary-material

References

1. Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, et al. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. *Ann Med*. (2010) 42:587–95. doi: 10.3109/07853890.2010.505931

2. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al. Global prevalence of celiac disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. (2018) 16:823–836.e822. doi: 10.1016/j.cgh.2017.06.037

3. King JA, Jeong J, Underwood FE, Quan J, Panaccione N, Windsor JW, et al. Incidence of celiac disease is increasing over time: A systematic review and meta-analysis. *Am J Gastroenterol*. (2020) 115:507–25. doi: 10.14309/ajg.0000000000000523

4. Sollid LM, Tye-Din JA, Qiao SW, Anderson RP, Gianfrani C, Koning F. Update 2020: nomenclature and listing of celiac disease-relevant gluten epitopes recognized by CD4. *Immunogenetics*. (2020) 72:85–8. doi: 10.1007/s00251-019-01141-w

5. Shan L, Molberg Ø, Parrot I, Hausch F, Filiz F, Gray GM, et al. Structural basis for gluten intolerance in celiac sprue. *Science*. (2002) 297:2275–9. doi: 10.1126/science.1074129

6. Matysiak-Budnik T, Candalh C, Dugave C, Namane A, Cellier C, Cerf-Bensussan N, et al. Alterations of the intestinal transport and processing of gliadin peptides in celiac disease. *Gastroenterology*. (2003) 125:696–707. doi: 10.1016/s0016-5085(03)01049-7

7. Amundarain MJ, Herrera MG, Zamarreño F, Viso JF, Costabel MD, Dodero VI. Molecular mechanisms of 33-mer gliadin peptide oligomerisation. *Phys Chem Chem Phys*. (2019) 21:22539–52. doi: 10.1039/c9cp02338k

8. Herrera MG, Dodero VI. Gliadin proteolytical resistant peptides: the interplay between structure and self-assembly in gluten-related disorders. *Biophys Rev*. (2021) 13:1147–54. doi: 10.1007/s12551-021-00856-z

9. Anderson RP. Review article: Diagnosis of coeliac disease: a perspective on current and future approaches. *Aliment Pharmacol Ther*. (2022) 56 Suppl:1, S18–S37. doi: 10.1111/apt.16840

10. Anderson RP. Innate and adaptive immunity in celiac disease. *Curr Opin Gastroenterol*. (2020) 36:470–8. doi: 10.1097/MOG.0000000000000672

11. Daveson AJM, Popp A, Taavela J, Goldstein KE, Isola J, Truitt KE, et al. Baseline quantitative histology in therapeutics trials reveals villus atrophy in most patients with coeliac disease who appear well-controlled on gluten-free diet. *GastroHep*. (2020) 2:22–30. doi: 10.1002/ygh2.380

12. Adelman DC, Murray J, Wu TT, Mäki M, Green PH, Kelly CP. Measuring change in small intestinal histology in patients with celiac disease. *Am J Gastroenterol*. (2018) 113(3):339–47. doi: 10.1038/ajg.2017.480

13. Hollon JR, Cureton PA, Martin ML, Puppa EL, Fasano A. Trace gluten contamination may play a role in mucosal and clinical recovery in a subgroup of diet-adherent non-responsive celiac disease patients. *BMC Gastroenterol*. (2013) 13:40. doi: 10.1186/1471-230X-13-40

14. Hooton D, Lentle R, Monro J, Wickham M, Simpson R. The secretion and action of brush border enzymes in the mammalian small intestine. *Rev Physiol Biochem Pharmacol*. (2015) 168:59–118. doi: 10.1007/112_2015_24

15. McConnell RE, Benesh AE, Mao S, Tabb DL, Tyska MJ. Proteomic analysis of the enterocyte brush border. *Am J Physiol Gastrointest Liver Physiol*. (2011) 300:G914–926. doi: 10.1152/ajpgi.00005.2011

16. Smith MW, Phillips AD. Abnormal expression of dipeptidylpeptidase IV activity in enterocyte brush-border membranes of children suffering from coeliac disease. *Exp Physiol*. (1990) 75:613–6. doi: 10.1113/expphysiol.1990.sp003439

17. Detel D, Persić M, Varljen J. Serum and intestinal dipeptidyl peptidase IV (DPP IV/CD26) activity in children with celiac disease. *J Pediatr Gastroenterol Nutr*. (2007) 45:65–70. doi: 10.1097/MPG.0b013e318054b085

18. Hausch F, Shan L, Santiago NA, Gray GM, Khosla C. Intestinal digestive resistance of immunodominant gliadin peptides. *Am J Physiol Gastrointest Liver Physiol*. (2002) 283:G996–G1003. doi: 10.1152/ajpgi.00136.2002

19. Mamone G, Picariello G. Optimized extraction and large-scale proteomics of pig jejunum brush border membranes for use in *in vitro* digestion models. *Food Res Int*. (2023) 164:112326. doi: 10.1016/j.foodres.2022.112326

20. Verhoeckx K, Bøgh KL, Dupont D, Egger L, Gadermaier G, Larre C, et al. The relevance of a digestibility evaluation in the allergenicity risk assessment of novel proteins. Opinion of a joint initiative of COST action ImpARAS and COST action INFOGEST. *Food Chem Toxicol*. (2019) 129:405–23. doi: 10.1016/j.fct.2019.04.052

21. Efsa Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Lambré C, Baviera JMB, Bolognesi C, Cocconcelli PS, Crebelli R, et al. Safety evaluation of the food enzyme leucyl aminopeptidase from non-genetically modified Aspergillus oryzae strain NZYM-EX. *EFSA J*. (2023) 21:e8507. doi: 10.2903/j.efsa.2023.8507

22. Goel G, Tye-Din JA, Qiao SW, Russell AK, Mayassi T, Ciszewski C, et al. Cytokine release and gastrointestinal symptoms after gluten challenge in celiac disease. *Sci Adv*. (2019) 5:eaaw7756. doi: 10.1126/sciadv.aaw7756

23. Koziolek M, Grimm M, Schneider F, Jedamzik P, Sager M, Kuhn P, et al. Navigating the human gastrointestinal tract for oral drug delivery: Uncharted waters and new frontiers. *Adv Drug Delivery Rev*. (2016) 101:75–88. doi: 10.1016/j.addr.2016.03.009

24. Schulze K. Imaging and modelling of digestion in the stomach and the duodenum. *Neurogastroenterol Motil*. (2006) 18:172–83. doi: 10.1111/j.1365-2982.2006.00759.x

25. Koziolek M, Garbacz G, Neumann M, Weitschies W. Simulating the postprandial stomach: physiological considerations for dissolution and release testing. *Mol Pharm*. (2013) 10:1610–22. doi: 10.1021/mp300604u

frontiers | Frontiers in Nutrition

TYPE Review
PUBLISHED 07 January 2025
DOI 10.3389/fnut.2024.1501817

OPEN ACCESS

EDITED BY
Simone Baldi,
Department of Experimental and Clinical Medicine, Italy

REVIEWED BY
Stefano Guandalini,
The University of Chicago, United States
Jernej Dolinsek,
Maribor University Medical Centre, Slovenia

*CORRESPONDENCE
Davide Scalvini
✉ davide.scalvini01@universitadipavia.it

RECEIVED 25 September 2024
ACCEPTED 06 December 2024
PUBLISHED 07 January 2025

CITATION
Scalvini D, Scarcella C, Mantica G, Bartolotta E, Maimaris S, Fazzino E, Biagi F and Schiepatti A (2025) Beyond gluten-free diet: a critical perspective on phase 2 trials on non-dietary pharmacological therapies for coeliac disease.
Front. Nutr. 11:1501817.
doi: 10.3389/fnut.2024.1501817

Beyond gluten-free diet: a critical perspective on phase 2 trials on non-dietary pharmacological therapies for coeliac disease

Davide Scalvini[1]*, Chiara Scarcella[2], Giulia Mantica[1], Erica Bartolotta[1], Stiliano Maimaris[1], Erica Fazzino[1], Federico Biagi[1,2] and Annalisa Schiepatti[1,2]

[1]Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy, [2]Istituti Clinici Scientifici Maugeri IRCCS, Gastroenterology Unit of Pavia Institute, Pavia, Italy

Coeliac disease is an immune-mediated chronic enteropathy, with a prevalence of around 1% in the general population and occurring in genetically susceptible individuals after the ingestion of gluten proteins present in wheat, rye and barley. Currently, a strict lifelong gluten-free diet is the cornerstone of treatment of coeliac disease. However, maintaining strict dietary adherence is challenging for many patients, due to the high costs, the highly restrictive nature of the diet and the impact on patients' quality of life. Moreover, a tiny minority of coeliac patients can develop pre-malignant/malignant complications of coeliac disease, a group of conditions, that despite being rare, are still burdened by a poor prognosis due to the lack of effective therapies. Therefore, the development of pharmacological treatments as an alternative to or supportive of a gluten-free diet is still an unmet need. The identification of new pathogenetic targets in the last years has enabled the development of several candidates molecules, many of which have been investigated in phase 2/3 clinical trials. In this narrative review we aim to summarise the investigational therapies that have been evaluated in phase 2/3 trials and provide a critical overview on the latest advances in this field.

KEYWORDS

coeliac disease, gluten-free diet, alternative therapies, pharmacological therapies, persistent symptom

1 Introduction

Coeliac disease (CeD) is a chronic immune-mediated enteropathy developing in genetically susceptible individuals after the ingestion of gluten (1–4). CeD is characterised by a prevalence of around 1% in the general population, a very heterogeneous clinical picture and an increased mortality compared to the general population, predominantly due to the development of pre-malignant and malignant complications such as refractory CeD, abdominal lymphomas and small-bowel adenocarcinoma (1–6). A strict lifelong gluten-free diet (GFD) is the cornerstone of treatment for CeD, leading to resolution of symptoms and small bowel lesions in the vast majority of patients (1–4). However, great interest has been devoted to alternative/supportive therapies for several reasons (Table 1).

Firstly, a GFD can be demanding for many patients to maintain due to psychological, economic and social barriers (7–10), and in addition to this, many patients also experience persistent symptoms despite a GFD (11–13). Persistence of symptoms despite a GFD is a common and relevant clinical scenario, that can affect up to 30–50% of coeliac patients and be due to many different underlying etiologies, either related or unrelated to CeD itself, and

TABLE 1 Reasons for needing alternative/supportive therapies to a gluten-free diet.

Persistence of symptoms/histological lesions despite a GFD
Lack of effective therapies for refractory and complicated CeD
Inadvertent gluten ingestion
Availability of GF foodstuffs and other barriers to long-term adherence (economical, social e.g.)
Palatability of GF foodstuffs
Individual super-sensitivity to gluten

GFD, gluten-free diet; CeD, coeliac disease; GF, gluten-free.

with significant variability in terms of clinical severity (11–13). In some cases, unsatisfactory response to a GFD can be due to development of malignant complications of CeD, which, although rare, are burdened by a very dismal prognosis and for which, currently, no standardised and curative treatments are available (14–16). Patients can also experience persistent symptoms due to voluntary or involuntary transgressions to a GFD, or because some of them have been reported to be supersensitive to gluten (11–13).

The dissatisfaction of many coeliac patients with a GFD (17) and their interest regarding the possibility of novel therapies (18), put together with recent developments into the underlying pathogenetic mechanisms of CeD (19) have provided ample fuel for research aiming to develop alternative or supportive non-dietary treatments for CeD.

This review aims to provide a state-of-the-art summary and a critical overview on the different types of non-dietary therapies for CeD that have been proposed and evaluated in phase 2/3 clinical trials so far.

2 Criteria for literature search

We performed a systematic search of the literature on experimental non-dietary therapies for CeD using the PubMed and Embase databases. The search was conducted on January 17, 2024 using search strings designed to identify relevant phase 2/3 trials focussing on CeD and its non-dietary treatments, including pharmacological and other experimental therapies. Only full-text papers were considered for inclusion. No temporal or language restrictions were applied to the search. The search terms encompassed various synonyms and keywords related to CeD and therapeutic approaches to ensure a broad coverage of the existing literature. The exact search strings used for each database are listed below:

- PubMed:

(celiac disease[mesh] OR coeliac disease[title] OR celiac disease[title] OR celiac disease[ot] OR coeliac disease[ot] OR gluten sensitive enteropathy[title]) AND (treatment[title/abstract] OR drug[title/abstract] OR pharmacological[title/abstract] OR trial[title/abstract])

- Embase:

('celiac disease':ti,kw OR 'coeliac disease':ti,kw OR 'gluten sensitive enteropathy':ti,kw) AND ('treatment':ti,ab,kw OR 'drug':ti,ab,kw OR 'pharmacological':ti,ab,kw OR 'trial':ti,ab,kw)

Search results from both databases were then merged, and after removing duplicates, we screened the titles and abstracts of the retrieved articles to identify relevant studies. Additionally, we reviewed the reference lists of selected articles and reviews to identify any additional relevant studies that may not have been captured by our initial search.

The flowchart in Figure 1 illustrates the results of our literature search and our screening process for identifying eligible articles. Overall, 7,286 records were retrieved by our literature search. After removing duplicates and screening titles and abstracts, 69 papers were considered for inclusion. After full-text review 26 of them were included. Finally, 1 additional paper published after our literature search was also included, so 27 papers were included overall.

3 From pathophysiology of coeliac disease to therapeutic targets

A thorough description of the pathophysiology of CeD is beyond the scope of this review. However, we would like to provide the readers with a description of the different molecules tested so far, which have been classified according to their mechanisms of action and their specific pathogenetic targets. This is illustrated and briefly described in Figure 2.

4 Peptidases to digest gluten

Gluten owes its immunogenicity to its high content of proline and glutamine, which are not efficiently degraded by the enzymes of the gastrointestinal tract. Consequently, these proteins are capable of triggering the immune response in individuals with CeD.

The degradation of gliadin peptides at the level of the stomach/intestinal lumen before they reach the *lamina propria* aims at preventing the activation of the immune cascade leading to the intestinal damage. This therapeutic approach is usually based on the use of peptidases able to degrade gluten through their proteolytic action, usually identified as to glutenases. Table 2 summarises the phase 2 trials on glutenases. Several trials have been conducted evaluating 2 different types of endopeptidases, namely ALV003 and AN-PEP (20–28), which are described below.

4.1 ALV003 (latiglutenase)

ALV003, also known as latiglutenase, is the most commonly studied glutenase. ALV003 is a glutenase composed of two gluten-specific proteases: ALV001 and ALV002. ALV001 is a genetically engineered form of cystine endoprotease B, isoform 2 sourced from barley (*Hordeum vulgare*) while ALV002 is a modified form of prolyl endopeptidase extracted from the bacterium *Sphingomonas capsulata*. As of today, ALV003 is being studied through phase 2 trials as the molecule has progressed beyond phase 1 trials, demonstrating tolerability and safety (20).

In a randomised, double-blind study, the efficacy of ALV003 was evaluated by pre-treating food with this glutenase and assessing the T-cell response in 20 patients (including 10 treated with a placebo). Unlike the food pre-treated with ALV003, 6 out of 10 patients in the placebo-treated gluten group exhibited gluten-specific T-cell response in peripheral blood. Both groups, however, experienced gastrointestinal symptoms after ingestion. It is worth noting that, in contrast to other studies

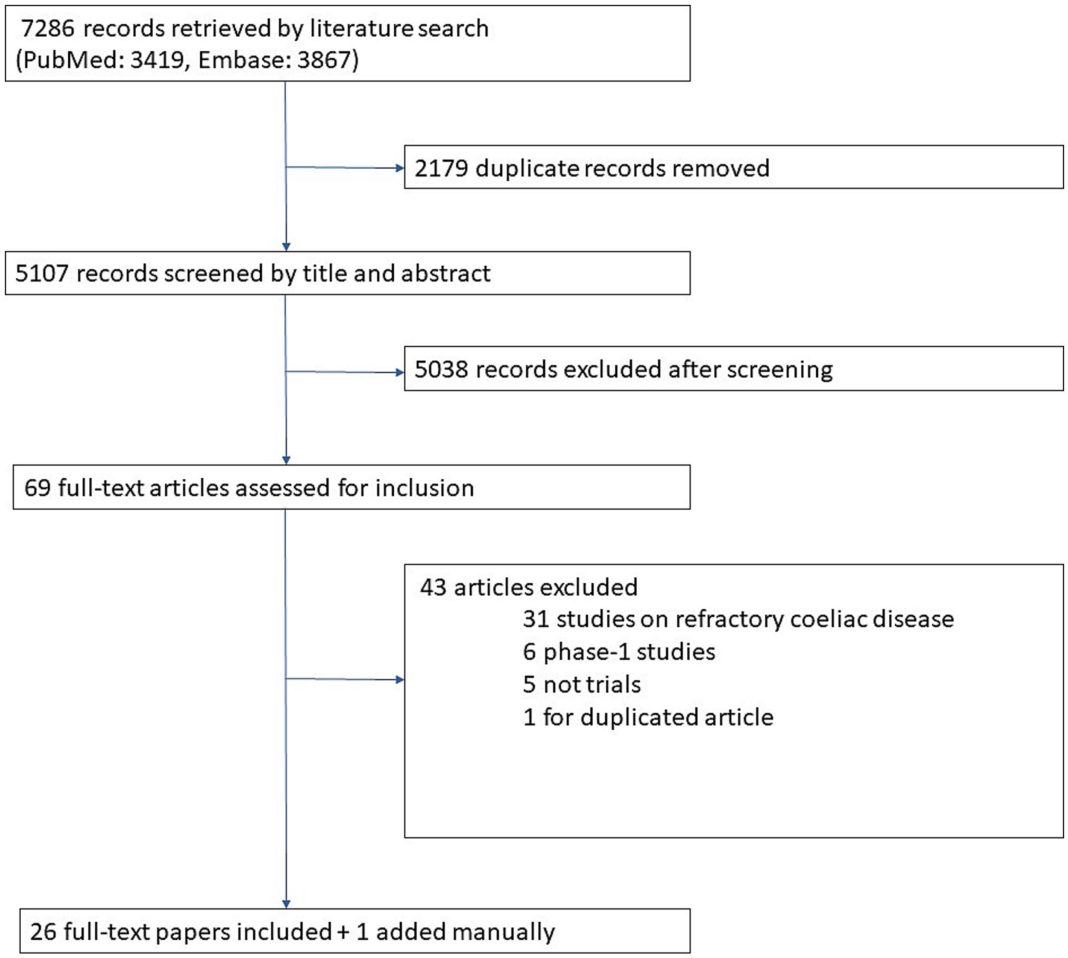


FIGURE 1
Flowchart of studies included the review.

investigating ALV003 where a dose of 2 g of gluten for 6 weeks was administered (22, 24), this one involved the administration of 16 g (21).

In a randomised, placebo-controlled, double-blind clinical phase 2 trial by Lähdeaho et al., ALV003 appeared to mitigate gluten-induced damage to the small intestinal mucosa in patients with CeD, within the context of a daily gluten-free diet containing up to 2 g of gluten for 6 weeks, although a statistically significant difference in the presence of any symptoms was not found (22). Conversely, Murray et al. in a multicenter, randomised, double-blind, placebo controlled, dose-ranging study enrolled 494 symptomatic coeliac patients on a GFD for at least 1 year with duodenal mucosal atrophy to assess the efficacy and safety of ALV003. The primary endpoint evaluated any histological changes in the mucosa, while the secondary endpoints included the number of intraepithelial lymphocytes (IELs), antibody positivity, symptom frequency, and drug safety. Although the drug was well tolerated by all participants, the study's endpoints were not achieved, as ALV003 failed to improve villous atrophy or reduce the severity and frequency of symptoms (23).

In a subsequent study including 50 patients receiving 2 g/day of gluten for 6 weeks and 1,200 mg of latiglutenase a reduction in both mucosal damage and symptom severity compared to placebo, was demonstrated (24).

In the ALV003–1221 clinical trial, a multi-center, randomised, double-blind, placebo-controlled study, although the primary endpoint to achieve histological improvement was not met, treated subjects experienced significant improvement in symptoms and quality of life (QOL). There was a statistically significant, dose-dependent reduction in the severity and frequency of symptoms (abdominal pain, bloating, tiredness, and constipation) in subjects treated with ALV003. Interestingly, Diarrhoea and nausea were the only symptoms which did not improve after receiving the glutenase (25).

Overall, ALV003 (latiglutenase) shows mixed prospects. While it demonstrated some efficacy in symptom reduction, inconsistent results across trials and failure to meet primary endpoints in larger studies suggest limited future development potential.

4.2 AN-PEP

An endoprotease derived from Aspergillus Niger named AN-PEP is able to degrade both whole gluten and gluten peptides into non-immunogenic residues within minutes (26). A total of 2 studies have evaluated AN-PEP so far (27, 28). Both these studies had

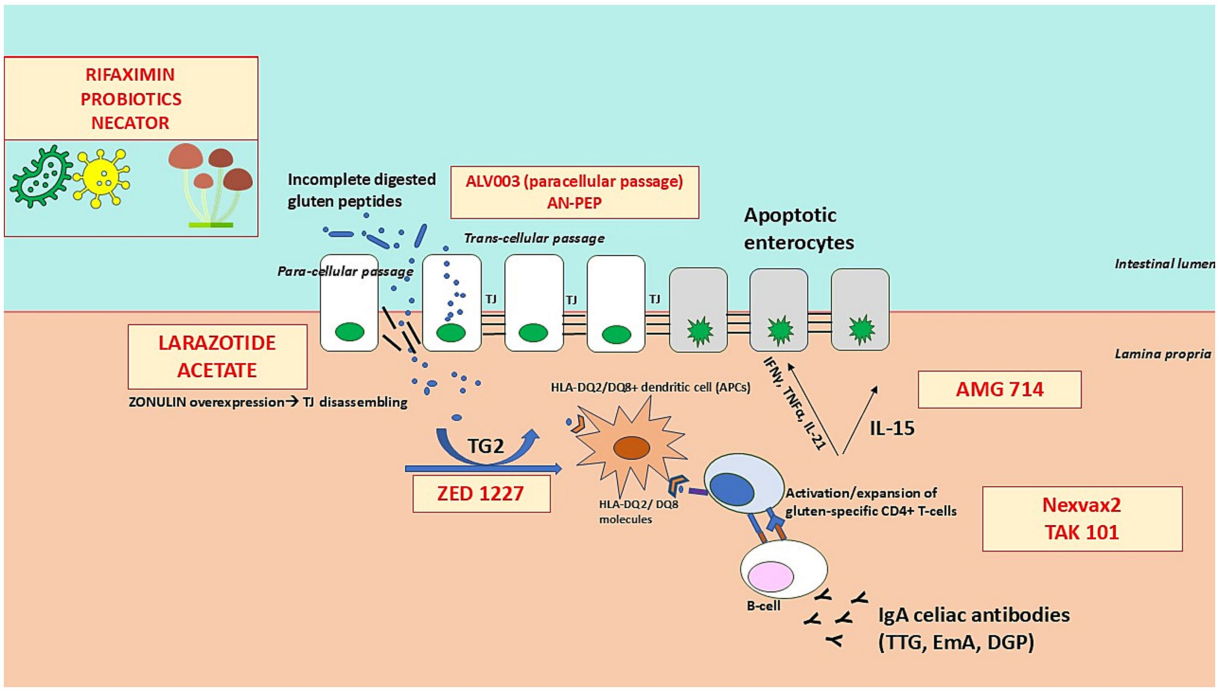


FIGURE 2
Therapeutic targets and mechanisms of action in coeliac disease. The figure illustrates the key steps in the pathogenesis of coeliac disease and highlights where current investigational therapies intervene. These therapies target different underlying mechanisms, including: (1) Glutenases (e.g., ALV003, AN-PEP) that enzymatically degrade immunogenic gluten peptides in the gastrointestinal lumen to prevent immune activation; (2) Intestinal barrier modulators (e.g., larazotide acetate) that enhance tight junction function to reduce intestinal permeability and prevent translocation of gluten peptides; (3) Tissue transglutaminase-2 (TG2) inhibitors (e.g., ZED1227) that block the deamidation of gluten peptides, reducing their immunogenicity; (4) Immunotherapies (e.g., Nexvax2, TAK-101) that aim to induce immune tolerance by modulating gluten-specific T-cell responses; and (5) Immunomodulators targeting pro-inflammatory cytokines (e.g., AMG 714, an anti-IL-15 antibody) to suppress immune-mediated intestinal inflammation. By disrupting various stages of the immune response to gluten, these therapies offer potential alternative or adjunctive treatments to the gluten-free diet in coeliac disease.

limitations due to the small sample size and the short duration of gluten intake (2 weeks). In a randomised double-blind placebo-controlled pilot study, the safety and efficacy of AN-PEP were evaluated. However, prevention of histological damage after receiving gluten and AN-PEP, i.e., the primary endpoint, was not met, despite the overall good tolerability by all participants (27).

A recent RCT investigated the role of AN-PEP in reducing stool gluten immunogenic peptides (GIP). While the use of AN-PEP has been associated with a lower incidence of severe GI symptoms, it failed to meet the primary endpoint, as a significant decrease of stool GIP was not found in patients receiving AN-PEP when compared with the placebo group (28).

The presence of nausea, bloating and abdominal pain were the most commonly reported adverse events (AEs) during the administration of gluten-digestive peptidases; however, their incidence rates did not statistically differ from the placebo group.

To summarise, future development for AN-PEP appears limited due to its failure to significantly reduce GIP or prevent histological damage without major modifications to improve its efficacy.

5 Intestinal barrier modulators

The intestinal barrier, including its epithelial integrity and tight junctions, is obviously crucial in CeD (29). Tight junctions appear to play a particularly important role in CeD by maintaining intestinal barrier integrity. The main components of tight junctions include occludins, claudins, junctional adhesion molecules (JAM), and zonulin. After gluten exposure, epithelial cell rearrangement and loss of barrier integrity are observed, causing an inappropriate immune response to environmental antigens like gluten (29, 30). These observations prompted many researchers to conduct studies evaluating barrier modulators as alternative therapies to GFD.

5.1 Larazotide acetate

Four phase-2 studies have examined larazotide acetate, also known as AT-1001, an 8-amino-acid synthetic peptide able to decrease the intestinal permeability, by acting as an antagonist of the zonulin, a key protein in regulation of the gut's tight junctions. Larazotide acetate is a paracellular permeability inhibitor derived from a protein produced by *Vibrio Cholerae* and it regulates tight junctions, preventing the passage of gluten into the mucosal *lamina propria* and the subsequent trigger of the inflammatory response. This drug has no effect on the transcellular passage of gluten (31, 32). Table 3 summarise the results of these studies.

A recent meta-analysis of RCTs on larazotide acetate including 626 CeD patients who underwent ingestion of gluten ranging from

TABLE 2 Phase-2 studies on gluten digestive endopeptidase.

Study	Type	Molecule	Population	Endpoints (primary; secondary)	Gluten challenge	Adverse events	Key results
Tye-Din et al. (21)	Double-blind RCT	ALV003	Adult CeD on GFD >8 weeks, HLA DQ2+	IFN-γ ELISpot responses; symptom response and antibody levels after GC	16 g/day for 3 days	Nausea, bloating, abdominal pain (the only one more frequent in intervention group)	ALV003 pre-treatment abolished immune responses but not symptoms
Lähdeaho et al. (22)	Double-blind RCT	ALV003	CeD on GFD >1 year, in remission, TG2-IgA negative	Vh:Cd ratio, IEL densities, serologic markers, symptoms, QOL	2 g/day for 6 weeks	Nausea, vomiting, abdominal pain (no differences between groups)	ALV003 prevented significant mucosal deterioration
Murray et al. (23)	Double-blind RCT, dose-ranging	ALV003	CeD on GFD >1 year, with GI symptoms	Change in Vh:Cd; IELs, serology, symptoms, safety	None	Bloating, nausea, abdominal pain (no differences between groups except for one moderate episode of fungal infection attributable to the treatment)	No improvement of histology and symptom scores compared with placebo; significant improvements in histology and symptom scores in all groups
Syage et al. (25)	Double-blind RCT, dose-ranging	ALV003	Seropositive and seronegative CeD, Vh:Cd $\leq$2.0	Symptoms, QOL, serology	None	No serious AEs reported	Dose-dependent improvement in symptoms and QOL for seropositive patients
Murray et al. (24)	Double-blind RCT	ALV003	CeD on GFD >1 year, Vh:Cd >2, TTG negative	Prevention of mucosal damage; Symptoms, IELs	2 g/day for 6 weeks	Nausea, bloating, Diarrhoea (no differences between groups)	Reduced gluten-induced intestinal mucosal damage and symptom severity
Tack et al. (27)	Double-blind RCT pilot study	AN-PEP	CeD on GFD >1 year, Marsh 0 or I, TTG and EMA negative	Safety and efficacy with gluten challenge	7 g/day for 2 weeks	No serious AEs; mild and transient gastrointestinal complaints	Well tolerated; primary endpoint not met due to lack of clinical deterioration upon placebo
Stefanolo et al. (28)	Double-blind RCT, exploratory	AN-PEP	CeD on GFD >2 years	Stool GIP; CSI, CeD-specific serology, QOL	Inadvertent gluten exposure	No major AEs reported.	AN-PEP did not reduce overall GIP stool excretion, but lowered prevalence of severe symptoms vs. placebo

RCT, Randomised Controlled Trial; CeD, Coeliac Disease; GFD, Gluten-Free Diet; Vh:Cd, Villus height:Crypt depth ratio; IEL, Intraepithelial Lymphocytes; QOL, Quality of Life; TTG, Tissue Transglutaminase; EMA, Endomysial Antibodies; GIP, Gluten Immunogenic Peptides; GC, gluten challenge; CSI, Coeliac Symptom Index; AEs, adverse events.

10.3389/fnut.2024.1501817

TABLE 3 Phase-II studies on Larazotide.

Study	Type	Molecule	Population	Endpoints (primary; secondary)	Gluten challenge	Adverse events	Key results
Paterson et al. (31)	Double-blind RCT	AT-1001	Adult CeD on GFD >6 months, anti-tTG ≤10 EU	Intestinal permeability (LAMA ratio); GI discomfort, AEs, global outcomes, urinary nitrites/ nitrates, PBMC markers, cytokine levels	2.5 g for 3 days	Diarrhoes, abdominal discomfort and flatulence. Gastrointestinal symptoms were more frequently detected in the placebo group.	No permeability increases in AT-1001 group; 70% increase in placebo. Fewer GI symptoms in AT-1001 group.
Leffler et al. (57)	Double-blind RCT, dose-ranging	Larazotide Acetate	Adult CeD on GFD ≥6 months, in remission	Intestinal permeability (LAMA ratio); Clinical symptoms (GSRS and CeD GSRS), QOL measures, TTG levels	2.4 g/day for 14 days	Common AEs included headache and UTI.	No difference in LAMA ratios. 0.25 and 4.0 mg doses prevented worsening of GI symptoms vs. placebo.
Kelly et al. (32)	Double-blind RCT, exploratory	Larazotide Acetate	Adult CeD on GFD >6 months, anti-tTG ≤10 EU	Intestinal permeability (LAMA ratio); Clinical symptoms (GSRS), TTG levels	2.7 g/day for 6 weeks	Common AEs included gastrointestinal disorders, fatigue, headache with similar rates between groups	No difference in LAMA ratios. Larazotide reduced symptoms and anti-TTG levels vs. placebo. Similar AEs.
Leffler et al. (56)	Double-blind RCT	Larazotide Acetate	Adult CeD on GFD ≥12 months, anti-tTG IgA <4	Clinical symptoms (weekly CeD-GSRS); Change from baseline in CeD-GSRS, CeD PRO GI and Abdominal domain scores	None	No drug-related serious AEs	0.5 mg dose reduced symptoms vs. placebo. 1 and 2 mg doses no different from placebo. Safety comparable to placebo.

RCT, Randomised Controlled Trial; CeD, Coeliac Disease; GFD, Gluten-Free Diet; LAMA, Lactulose-to-Mannitol; TTG, Tissue Transglutaminase; GI, Gastrointestinal; GSRS, Gastrointestinal Symptom Rating Scale; PBMC, Peripheral Blood Mononuclear Cell; QOL, Quality of Life; PRO, Patient-Reported Outcome; AEs, Adverse Events.

10.3389/fnut.2024.1501817

2.5 grams for 3 days up to 2.7 grams for 6 weeks, of which 456 receiving Larazotide acetate and 161 receiving a placebo, showed that the drug reduced the weekly number of symptomatic days and improved symptom severity scores compared to the placebo in patients undergoing gluten challenge. Unfortunately, it failed to demonstrate a reduction in intestinal permeability compared to placebo (33).

During treatment with larazotide acetate, no severe AEs were reported. As previously mentioned, it was able to significantly reduce the incidence of Diarrhoea, abdominal pain, and bloating, which were the most frequently AEs in both the intervention and control groups.

Despite Larazotide acetate's ability to alleviate gastrointestinal symptoms, it seems unlikely to be a definitive cure for coeliac patients. Instead, it may be considered a complementary option to a GFD in patients with persistent symptoms rather than a substitute of GFD itself.

So far, larazotide investigations have been discontinued despite showing some promise in symptom management.

6 Modulators of the immune response to gluten

The third possible strategy to achieve gluten tolerance is to apply drugs that modulate the immune response to gluten and gluten-related peptides (34).

Several molecules and mechanisms have been investigated trying to block different pathways in CeD pathogenesis, as illustrated in Figure 2 and summarised in Table 4.

6.1 Nexvax2

Nexvax2, was the first therapeutic vaccine created to treat CeD. It consists of synthetic peptides recognised by gluten specific CD4+ T-lymphocytes, leading to their non-reactivity to further gluten stimuli (35).

A phase 1 randomised placebo-controlled trial was initially conducted to evaluate the safety and tolerability of Nexvax2, highlighting that the vaccine did not cause changes in circulating lymphocyte subgroups and no significant changes in the villus-crypt ratio. A subsequent phase 2 study was conducted to evaluate the efficacy of Nexvax2, but it did not demonstrate any beneficial effect in lowering the levels of circulating coeliac antibodies (anti-tTG, anti-DGP), improving duodenal histology and reducing gastrointestinal symptoms (35–37). Regarding AEs, nausea and bloating were usually more frequent in Nexvax2 group (35, 36).

So far, trials on Nexvax2 have been discontinued after unsatisfactory results of phase 2 studies.

6.2 TAK-101

Recently, another drug used to induce immunotolerance was engineered, its name is TAK-101 and it consists of gliadin encapsulated in nanoparticles to induce tolerogenic effects (38).

By administering the drug TAK-101 intravenously instead of subcutaneously as Nexvax2, antigen-presenting cells (APCs) with tolerogenic properties in the liver and spleen are activated instead of APCs with immunogenic properties in the skin and lymph nodes. This different approach allows for the induction of an anergic state in gluten-specific T-lymphocytes, while simultaneously activating regulatory T-lymphocytes, which are crucial for achieving the desired tolerogenic effect.

In a phase 2a trial, 33 patients were randomised to TAK-101 and placebo. The number of circulating gliadin-specific IFN-gamma spot-forming T-cells in response to oral gluten challenge was reduced in TAK-101 group compared to placebo. Furthermore, this drug prevented the deterioration of villous-crypt ratio compared to placebo, even if this did not reach the statistical significance ($p = 0.1$). On the contrary, TAK-101 did not induce clinical changes and did not decrease the percentage of IELs (38).

TAK-101 was generally well tolerated, and no serious AEs occurred. Flushing, headache, back pain, were the most commonly reported AEs, with significant differences between treatment and placebo groups.

Regarding future perspectives, TAK-101 seems promising with its unique mechanism of action, but larger trials are needed to confirm preliminary results.

6.3 AMG 714

The monoclonal antibody AMG 714 administered by intravenous infusion exploits a different mechanism of action, namely the inhibition of IL-15 production by APCs and epithelial cells (39).

IL-15 plays a fundamental role in the activation and proliferation of lymphocytes, making CD4+ T-lymphocytes insensitive to the inhibition of regulatory T-lymphocytes and promoting the loss of tolerance to food antigens. AMG 714 did not induce statistically significant changes in the villous-crypt ratio compared with placebo, but only an improvement in lymphocyte density and clinical picture was observed. The authors therefore concluded that AMG 714 may be used beneficially in coeliac patients with persistent symptoms despite a GFD (39).

The effect of AMG 714 was also investigated for type 2 refractory coeliac disease (RCD) in a RCT, given its pathophysiological link with IL-15. After 10 weeks of AMG 714 or placebo, there was no difference between the groups in terms of histological endpoints; nevertheless, patients in the AMG 714 group showed improvement of symptoms compared to the placebo group (40). Serious AEs were reported in 5 patients (26.3%) in the AMG 714 group (pneumococcal infection, elevated transaminases, balance disorder, tuberculosis, and cerebellar syndrome). Safety profile was considered acceptable by the authors considering the severity of RCD type 2. Nasopharyngitis was also commonly reported in AMG 714 group compared to the placebo group (42%vs. 11%).

Overall, the results obtained for AMG714 were poorly satisfactory. Future developments are unlikely due to poor efficacy and concerns regarding its safety profile.

TABLE 4 Phase-2 studies on pharmacological modulators of the immune response to gluten and transglutaminase 2 inhibitor.

Study	Type	Molecule	Mechanism	Population	Endpoints (primary; secondary)	Gluten challenge	Adverse events	Key results
Goel et al. (35)	Double-blind RCT	Nexvax2	Gluten peptide-based antigen-specific immunotherapy	HLA-DQ2.5+ CeD, 18–70 years, on GFD	AEs; Safety, tolerability, duodenal histology, antibodies, IGRA	9 g/day on days 1–3 and on days 8–10 as cookies	Nausea, vomiting, abdominal pain were more frequent in Nexvax2 group	More AEs with Nexvax2. No significant differences in other endpoints.
Truitt et al. (36)	Double-blind RCT	Nexvax2	Gluten peptide-based antigen-specific immunotherapy	HLA-DQ2.5+ CeD, 18–70 years, GFD >12 months	Safety, tolerability, bioavailability; Pharmacokinetics	Yes	Headache, abdominal distension, nausea were more frequent in Nexvax2 group	Subcutaneous dosing safe, well-tolerated. Higher exposure than intradermal.
Tye-Din et al. (37)	Double-blind RCT	Nexvax2	Gluten peptide-based antigen-specific immunotherapy	HLA-DQ2.5+ CeD, 18–70 years, GFD >12 months	CeD symptoms post-gluten; Blood IL-2, individual symptoms	10 g bolus vital gluten	Nausea, Diarrhoea abdominal pain (no differences between groups)	Nexvax2 did not reduce gluten-induced symptoms or IL-2 elevation.
Kelly et al. (38)	Phase 1/2a RCT	TAK-101	Gliadin-nanoparticle tolerance induction	HLA-DQ2/8+ CeD, 18–75 years, GFD ≥6 months	PK, safety, tolerability, IFN-γ + cells; Enteropathy, IELs, gut-homing T cells	≥14 days	Flushing, headache, back pain. No serious AEs occurred (no differences between groups)	88% reduction in IFN-γ + cells. No Vh:Cd deterioration in TAK-101 group.
Lähdeaho et al. (39)	Double-blind RCT	AMG 714	Anti-IL-15 antibody	CeD, 18–80 years, GFD >12 months	Vh:Cd ratio change; IEL density, symptoms, antibodies	2-4 g daily for 10 weeks	Gastrointestinal symptoms (no differences between groups). Injection site reactions occurred more frequently in AMG group.	No significant Vh:Cd difference. 300 mg improved IEL density and symptoms.
Celier et al. (40)	Double-blind RCT	AMG 714	Anti-IL-15 antibody	Adults with confirmed refractory CeD type 2	Change in aberrant IEL from baseline to week 12; histological scores, patient-reported symptoms	None, GFD continued	Nasopharyngitis (42% AMG 714 vs. 11% placebo) Five serious AEs in AMG 714 group vs. one in placebo	No significant difference in primary endpoint but associated with symptom improvement
Schuppan et al. (41)	Double-blind RCT	ZED1227	Transglutaminase 2 inhibitor	HLA-DQ2/8+ CeD, 18–65 years, GFD >12 months	Vh:Cd change; IELs density, symptom scores, QoL	3 g/day for 6 weeks	Headache, nausea, vomiting, abdominal pain. No differences between groups, except for rash (3 patients, 8%) in the 100 mg group.	ZED1227 attenuated mucosal damage. 100 mg dose may improve symptoms and QoL.

RCT, Randomised Controlled Trial; CeD, Coeliac Disease; GFD, Gluten-Free Diet; Vh:Cd, Villus height to Crypt depth ratio; IEL, Intraepithelial Lymphocyte; QoL, Quality of Life; PK, Pharmacokinetics; IL, Interleukin; IFN, Interferon; AEs, Adverse Events.

10.3389/fnut.2024.1501817

7 Inhibitors of tissue transglutaminase 2

7.1 ZED1227

ZED1227 is an orally administered small molecule tissue transglutaminase (TG2) inhibitor that selectively binds to the active form of TG2, thus preventing the formation of deamidated gliadin, its antigenic presentation resulting in gluten-induced T-cell activation (41).

In a double-blind, placebo-controlled study, ZED1227 demonstrated efficacy compared to placebo in reducing mucosal injury and preserving the villous-crypt ratio (p-value <0.001) in CeD patients undergoing a moderate-dose gluten challenge (3 g/daily for 6 weeks), in all proposed dosage (10, 50, 100 mg). Moreover, the effectiveness of ZED1227 has been shown to be dose dependent, with doses ranging from 50 to 100 mg exhibiting greater efficacy in preventing intestinal villous atrophy compared to 10 mg. Furthermore, 100 mg of ZED1227, was effective in inhibiting the increase of IELs consequently to gluten ingestion.

Regarding AEs, headache, nausea, vomiting were the most commonly reported, but there were no differences between groups, except for rash, which occurred in 3 patients (8%) in the 100 mg treatment group.

ZED1227 appears to be the most promising candidate drug with demonstrated dose-dependent efficacy and a good safety profile. It is likely to progress to further development and there is ongoing recruitment for a phase II, double-blind, randomised, placebo-controlled trial in coeliac patients with persistent symptoms despite a GFD (EudraCT/CTIS number 2023–506150-21).

8 Miscellanea

8.1 Probiotics

There is significant evidence that gut microbiota can influence and alter the immune system, playing an important role in maintaining a healthy state. Consequently, it is plausible that in genetically susceptible host, imbalances between microbiota and immunity could lead to the onset of a major immune-mediated inflammatory disease, including CeD (42, 43). Three randomised placebo-controlled trials have investigated the role of probiotics as an alternative to a GFD.

In a three-month double-blind, placebo-controlled randomised study, *Bifidobacterium longum* CECT7347 was found to attenuate the inflammatory effects of dysbiotic intestinal microbiota, decreasing peripheral CD3+ T lymphocytes ($p = 0.004$), slightly reducing TNF-α concentration (even though it was not statistically significant, $p = 0.067$), reducing the numbers of the *Bacteroides fragilis* group ($p = 0.02$) and the content of fecal IgA ($p = 0.011$) (44).

Others examined the role of VSL#3™, a well-known probiotic mixture used in inflammatory bowel disease, on patients with CeD. Harnett et al. randomised 42 CeD patients with only partial symptom improvement despite strict adherence to a GFD, in a group treated with VSL#3™ and a placebo group for 12 weeks (45). Unfortunately, no significant differences were found between the two groups at the end of the treatment in bacteria, mycotoxins, or parasites composition, nor for blood urea levels or urinary organic acids.

8.2 Rifaximin

Rifaximin is a non-absorbable, broad spectrum antibiotic, which acts as an inhibitor of bacterial RNA synthesis and it is mainly used to treat travelers' Diarrhoea and irritable bowel syndrome. Chang et al. conducted a single-center, double-blind, randomised, placebo-controlled study involving 50 patients to evaluate the improvement of gastrointestinal symptoms in patients with non-responsive CeD with a dose of 1,200 mg per day for 10 days of rifaximin. After randomisation, authors concluded that rifaximin did not improve symptoms in CeD patients with persistent gastrointestinal symptoms following a GFD (46).

8.3 Budesonide

The efficacy of budesonide for RCD is well known, on the contrary fewer were the studies about its role in acute reactions to gluten or as alternative of GFD (47, 48).

The impact of budesonide was assessed, in an *in vivo* and *in vitro* pilot study, in 20 patients randomised to GFD with or without 6 mg/day of budesonide (49).

Individuals receiving both a GFD and budesonide reported higher well-being scores, increased body weight, reduced frequency of evacuations, and decreased stool weight compared to those on a gluten-free diet. Duodenal biopsies in CeD patients and non-CeD patients were exposed *in vitro* to gliadin (0.5 mg/mL) and budesonide (10–30 μg/mL) for 3 and 24 h. *In vitro* budesonide led to a decrease in epithelial tyrosine phosphorylation and histocompatibility leucocyte antigen complex DR (HLA-DR) expression induced by gliadin-derived peptides and in cyclo-oxygenase (COX)-2 and intercellular adhesion molecule (ICAM)-1 in the *lamina propria* compared to those treated with gliadin alone (49).

Budesonide was also assessed to evaluate its effect on histological response, but no statistically significant differences were observed regarding Marsh grading and villous-height in the studies (48, 50). No major AEs occurred during the therapy with budesonide.

8.4 *Necator americanus*

Parasitic helminths may potentially regulate gut microbiota and alter the progression of inflammatory disease.

A successful small trial (12 patients) was conducted by an Australian team by inoculating subcutaneously *Necator americanus* larvae in CeD patients undergoing gluten challenge (GC), which prevented the worsening of villous trophism and symptoms (51). However, a subsequent larger (54 patients) randomised, placebo-controlled trial failed to reproduce the previous results but confirmed the protective effects on symptoms (52). However, the inoculation of *Necator americanus* larvae appeared safe, with no severe AEs occurred.

9 Clinical trials pitfalls

A major problem of the trials conducted so far is the heterogeneity of aspects related to the populations recruited, the endpoints and the outcomes measures. Thus, this represents a barrier to compare and

generalise the results of these studies. We will briefly discuss the major pitfalls emerging from phase 2 trials conducted so far.

9.1 Concept of cross contamination

Cross-contamination and inadvertent gluten intake have always been a significant fear for coeliac patients to cope with. However, to define the concept of cross contamination to a GFD is very difficult, as currently no precise definition exists in the literature. It is well known that 50 mg of gluten/day for 90 days represents the minimal toxic dose for coeliac patients; on the other hand, 10 mg of gluten/day is the maximum non-toxic amount of gluten for coeliac patients (53, 54). With regard to these doses of gluten, it has been previously shown that 50 mg of gluten (equivalent to 0.05 grams of gluten) are contained in food samples that a well instructed and conscientious coeliac patient is not likely to eat by mistake. In practical terms, 50 mg of gluten are contained in a large breadcrumb, with a size of approximately 1–2 cm, if we consider that gluten is 75% of the whole protein content of wheat (55). In the trials conducted so far where gluten was administered to patients, the dose varied between 2 g (roughly equivalent to a slice of bread or a packet of crackers) and 16 g per day (roughly equivalent to a large serving of Italian pasta) (20–39, 56, 57), which is definitely a toxic dose of gluten that is very unlikely to be eaten by mistake. A recent international consensus on outcomes measures for CeD trials established that 9 g of gluten/die is the maximum amount tolerated for clinical trials to simulate normal ingestion (58), which is more or less the equivalent of 90 gr of common Italian pasta (a medium portion).

Another relevant aspect to consider is that, although inadvertent gluten intake has been repeatedly reported as a leading cause for persistent symptoms in CeD (11–13), particularly in those patients who may be supersensitive (59), it is very difficult to properly ascertain its causative role in clinical practice. In this regard, a recent study by our group showed that minimal and inadvertent ingestion of gluten in coeliac patients who had been correctly instructed on how to follow a GFD is likely to have no role on triggering intestinal symptoms (60).

10 Study population

Heterogeneity of coeliac patients enrolled in the trials is another point to critically consider. The vast majority of trials enrolled adult coeliac patients with confirmed diagnosis based on both serology and duodenal histology, who have been on a GFD for at least 6–12 months even without evidence of histological response to a GFD at time of enrolment. Additionally, the majority of them lacked a 'baseline biopsy' before recruitment into the trials, and only some of them performed a follow-up duodenal biopsy in the 6 months prior to enrolment due to clinical reasons (21, 32, 36, 57).

Adequate knowledge of the GFD is a crucial requirement for coeliac patients, and several reports highlight the association between a comprehensive knowledge of gluten-free living and a better adherence to a GFD (61–63). Consequently, knowledge about a GFD should be assessed before enrolment in a clinical trial, but unfortunately this has not been systematically done and was limited to self-reported adherence.

Furthermore, HLA DQ2.5 typing was also used as a diagnostic criterion for many trials (21, 35–37), which potentially represents a limit towards excluding other patients expressing HLA-DQ8 molecules or other rarer haplotypes such as HLA-DQ2.2 and HLA-DQ7.5.

Special subgroups such as CeD patients with persistent symptoms and refractory CeD were only rarely included and evaluated (23, 40), unfortunately with unsuccessful results.

Finally, all the trials conducted so far involved adult coeliac patients only and no data on pediatric populations are available. This is an important aspect to be considered in the future, also based on recent EU regulations.

10.1 Gluten-challenge (dose, duration, vehicle)

A major factor to consider in the evaluation of drug efficacy is the administration of a gluten challenge. In fact, dose, vehicle of gluten administration and duration of gluten-challenge in trials have not been standardised so far. As previously mentioned, GC dose varied between 2 g and 16 g per day (20–39, 56, 57). This aspect is even more challenging if we consider that also in clinical practice diagnostic gluten-challenge is complex to perform. A recent ESPGHAN position paper (64) provides guidance on how to perform GC in children, although this is mainly based on expert opinion, whereas in adults no guidelines provide guidance for gluten challenge (65–67). Table 5 summarises the main concerns related to GC in clinical trials.

Run-in periods are useful to reduce drop-outs from a trial (68); however, run-in periods were introduced only in 5 trials (24, 27, 32, 36, 37). Recently, run-in periods have been suggested for trials contemplating gluten-challenge in order to increase compliance and reduce confounding in the evaluation of symptomatic response (58).

The length of GC varied greatly among studies, ranging from a single bolus dose to up to 10 weeks in one case (31, 37, 39). Furthermore, the type of gluten vehicle was not standardised and several different methods of administration were used, such as capsules but also baked products such as cookies, bread and biscuits that may be rich in fermentable oligo-, di-, and monosaccharides and polyols (FODMAP) (32, 35, 39, 40, 57), which are known to trigger symptoms in IBS and also in coeliac patients on a GFD (69, 70).

Additionally, the adherence to GC in the trials was not extensively evaluated, neither with specific questionnaires nor with objective tools such as GIP (24, 28). Lastly, the influence of the so called 'trial effect' on patients enrolled in trials should also be considered, as this may lead patients to improve their adherence to the GFD, potentially confounding the beneficial effect of the drug compared to controls (23).

10.2 Histological outcomes measures

The precise definition of histological recovery is a mandatory outcome to establish before starting a trial. This concept is challenging also in clinical practice, as many parameters should be considered such as the patchiness of duodenal lesions, the amount of time required for healing, the histological criteria adopted. So far, histology has been the primary endpoint of 5 trials (22–24, 39, 41) and this was effectively met only in one (41). Moreover, different methods (villous height to crypt depth (Vh:Cd) ratio, lactulose-to-mannitol (LAMA)

TABLE 5 Phase-2 studies performing gluten challenge.

Study	GC dose	GC duration	GC vehicle	GFD duration before study	Run-in period	Baseline histology
Goel et al. (35)	9 g/day for days 1–3; then 9 g/day for days 8–10	3 + 3 days	Cookies (3 g gluten each)	>12 months	No	Yes
Truitt et al. (36)	6 g	Single bolus	10 g vital wheat gluten flour in water	>12 months	No	No
Tye-Din et al. (37)	10 g bolus	Single dose	10 g vital wheat	>12 months	No	Yes
Schuppan et al. (41)	3 g/day	6 weeks	1 biscuit/day	>12 months	No	Yes
Kelly et al. (38)	12 g/day for 3 days, then 6 g/day for 11 days	14 days	NA	>6 months	No	Yes
Lähdeaho et al. (39)	2-4 g/day	10 weeks	Two cookies/day (Finnish rusks or double-baked cake breads)	>12 months	No	Yes
Paterson et al. (31)	2.5 g bolus	Single dose	Pudding with 2.5 g amygluten 160 powder	>6 months	No	Yes
Leffler et al. (65)	2.4 g/day	14 days	Capsules (amgluten 160 powder)	>6 months	No	No
Kelly et al. (32)	2.7 g/day	6 weeks	Capsules (450 mg gluten each)	>6 months	Yes, 7-days placebo	No
Tye-Din et al. (21)	16 g/day	3 days	Flour slurry mixed with orange juice or soy milk	>8 weeks	No	No
Lähdeaho et al. (22)	2 g/day	6 weeks	Breadcrumb	>12 months	No	Yes
Murray et al. (24)	2 g/day	6 weeks	NA	>12 months	Yes, 14-days placebo	Yes
Tack et al. (27)	7 g/day	2 weeks	Toast	>12 months	Yes, 14-days placebo	Yes

GC, Gluten Challenge; GFD, Gluten-Free Diet; NA, Not AvailableLEGENDS.

10.3389/fnut.2024.1501817

ratio and densities of IELs) have been used to evaluate histological changes, which makes it difficult to compare the results and inevitably introduces an observer variability. Indeed, LAMA is not specific for CeD, but it only provides an indirect and less appropriate measure of histological damage by assessing intestinal permeability (31, 32, 56, 57). According to a recent consensus, a Vh:Cd ratio ≥ 2.5 or ≥ 3 or Marsh 1 lesions were considered necessary criteria to enter a trial where gluten challenge is performed in order to avoid the ethical concerns related to offering gluten to patients with persistent villous atrophy (58).

10.3 Inclusion of patient related outcomes

The use of PROs as trial endpoints has been gaining importance over the last decade, due to their extensive application in pharmacological trials, particular those related to inflammatory bowel disease and functional gastro-intestinal disorders (71–73).

PROs provide measures of patients' QOL and assess how objective clinical effects alter the subjective sphere and viceversa. Indeed, patients' clinical characteristics such as anxiety, resilience and hyper-vigilance could potentially skew the results, contaminate trial's endpoints and change symptoms perception (74–76). Furthermore, PROs promote a more patient-centered evaluation and regulatory agencies such as the European Medicine Agency and the Food and Drugs Administration have also recognised their significance.

Few CeD trials have investigated PRO (37, 56), but their inclusion is desirable in future trials as suggested by a recent international consensus (58).

11 Considerations on efficacy of alternative pharmacological drugs

This review has summarised the current evidence about molecules evaluated in phase II trials in the last two decades, which may potentially support/replace the GFD in coeliac patients. The pursuit of an alternative, non-pharmacological therapy to GFD is highly requested by patients and industry and could represent a significant improvement in all instances where conventional therapy alone is insufficient. Although the development of alternative therapies has spanned over two decades, with varying degrees of industry interest and investment, several factors have contributed to the slow progress, including the complexity of the disease mechanism, challenges in trials design, and the high bar set by the effectiveness of the GFD.

In fact, so far, none among the proposed molecules has yet demonstrated a significant efficacy, particularly in the prevention of gluten-induced histological damage. Indeed, promising preliminary phase-II results have been observed only with ZED1227, a transglutaminase-2 inhibitor, whose administration has reduced gluten-induced mucosal damage, demonstrating a good safety profile (41). However, the small sample size precludes to give definitive results. This molecule is currently undergoing a phase IIb trial, under the name rebranded in TAK-227 (EudraCT number 2020–004612-97) (77).

The remaining therapies aiming to induce immune tolerance to gluten have failed to meet the primary endpoint represented by the prevention of the histological damage, although a minimal positive effect on the prevention of gluten-induced damage has been shown for TAK-101 (38). For this reason, a new trial is currently ongoing (NCT04530123) (78).

Currently, phase 2 studies on glutenases are yielding disappointing results regarding their effectiveness, particularly in the prevention of mucosal damage after gluten challenge. Therefore, their potential target population may be represented by patients with ongoing symptoms despite a GFD and no histological damage.

Larazotide held high interest in the past, but now it is clear that it is unable to prevent mucosal damage (31, 32, 56, 57). Nevertheless, it may be still considered for symptoms control in the absence of mucosal damage/organic disorders.

In conclusion, the possibility to develop alternative or supportive therapies to a GFD still remains a priority in the research agenda in this field. Identification of specific subgroups of patients and meaningful endpoints together with uniformity in the trial methodology are major areas to implement in the future.

Author contributions

DS: Writing – original draft, Writing – review & editing, Data curation. CS: Data curation, Writing – original draft. GM: Data curation, Writing – original draft. EB: Data curation, Writing – original draft. SM: Data curation, Formal analysis, Writing – review & editing. EF: Data curation, Writing – review & editing. FB: Data curation, Writing – review & editing. AS: Conceptualization, Data curation, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was partially supported through the Ricerca Corrente funding of the Italian Ministry of Health.

Acknowledgments

DS is attending the PhD Course in Experimental Medicine, University of Pavia.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

References

1. Lebwohl B, Sanders DS, Green PHR. Coeliac disease. *Lancet*. (2018) 391:70–81. doi: 10.1016/S0140-6736(17)31796-8

2. Ludvigsson JF, Bai JC, Biagi F, Card TR, Ciacci C, Ciclitira PJ, et al. British Society of Gastroenterology. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. *Gut*. (2014) 63:1210–28. doi: 10.1136/gutjnl-2013-306578

3. Al-Toma A, Volta U, Auricchio R, et al. European Society for the Study of coeliac disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. *United European Gastroenterol J*. (2019) 7:583–613. doi: 10.1177/2050640619844125

4. Rubio-Tapia A, Hill ID, Semrad C, Kelly CP, Greer KB, Limketkai BN, et al. American College of Gastroenterology guidelines update: diagnosis and management of celiac disease. *Am J Gastroenterol*. (2023) 118:59–76. doi: 10.14309/ajg.0000000000002075

5. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al. Global prevalence of celiac disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. (2018) 16:823–836.e2. doi: 10.1016/j.cgh.2017.06.037

6. Maimaris S, Schiepatti A, Biagi F. Systematic review with meta-analysis: cause-specific and all-cause mortality trends across different coeliac disease phenotypes. *Aliment Pharmacol Ther*. (2024) 59:592–605. doi: 10.1111/apt.17867

7. Hall NJ, Rubin G, Charnock A. Systematic review: adherence to a gluten-free diet in adult patients with coeliac disease. *Aliment Pharmacol Ther*. (2009) 30:315–30. doi: 10.1111/j.1365-2036.2009.04053.x

8. Schiepatti A, Maimaris S, Nicolardi ML, Alimenti E, Vernero M, Costetti M, et al. Determinants and trends of adherence to a gluten-free diet in adult celiac patients on a long-term follow-up (2000–2020). *Clin Gastroenterol Hepatol*. (2022) 20:e741–9. doi: 10.1016/j.cgh.2020.12.015

9. See JA, Kaukinen K, Makharia GK, Gibson PR, Murray JA. Practical insights into gluten-free diets. *Nat Rev Gastroenterol Hepatol*. (2015) 12:580–91. doi: 10.1038/nrgastro.2015.156

10. Schiepatti A, Maimaris S, Randazzo S, Maniero D, Biti R, Caio G, et al. Resilience in adult coeliac patients on a gluten-free diet: a cross-sectional multicentre Italian study. *Nutrients*. (2024) 16:2595. doi: 10.3390/nu16162595

11. Penny HA, Baggus EMR, Rej A, Snowden JA, Sanders DS. Non-responsive coeliac disease: a comprehensive review from the NHS England National Centre for refractory coeliac disease. *Nutrients*. (2020) 12:216. doi: 10.3390/nu12010216

12. Schiepatti A, Maimaris S, Lusetti F, Scalvini D, Minerba P, Cincotta M, et al. High prevalence of functional gastrointestinal disorders in celiac patients with persistent symptoms on a gluten-free diet: a 20-year follow-up study. *Dig Dis Sci*. (2023) 68:3374–82. doi: 10.1007/s10620-022-07727-x

13. Leffler DA, Dennis M, Hyett B, Kelly E, Schuppan D, Kelly CP. Etiologies and predictors of diagnosis in nonresponsive celiac disease. *Clin Gastroenterol Hepatol*. (2007) 5:445–50. doi: 10.1016/j.cgh.2006.12.006

14. Biagi F, Gobbi P, Marchese A, Borsotti E, Zingone F, Ciacci C, et al. Low incidence but poor prognosis of complicated coeliac disease: a retrospective multicentre study. *Dig Liver Dis*. (2014) 46:227–30. doi: 10.1016/j.dld.2013.10.010

15. Biagi F, Schiepatti A, Maiorano G, Fraternale G, Agazzi S, Zingone F, et al. Risk of complications in coeliac patients depends on age at diagnosis and type of clinical presentation. *Dig Liver Dis*. (2018) 50:549–52. doi: 10.1016/j.dld.2017.12.001

16. Biagi F, Marchese A, Ferretti F, Ciccocioppo R, Schiepatti A, Volta U, et al. A multicentre case-control study on complicated coeliac disease: two different patterns of natural history, two different prognoses. *BMC Gastroenterol*. (2014) 14:139. doi: 10.1186/1471-230X-14-139

17. Aziz I, Evans KE, Papageorgiou V, Sanders DS. Are patients with coeliac disease seeking alternative therapies to a gluten-free diet? *J Gastrointestin Liver Dis*. (2011) 20:27–31.

18. Tomal J, McKiernan D, Guandalini S, Semrad CE, Kupfer S. Celiac patients' attitudes regarding novel therapies. *Minerva Gastroenterol Dietol*. (2016) 62:275–80.

19. Dahal-Koirala S, Fremgaard Risnes L, Sollid LM. Chapter 3 - pathogenesis of coeliac disease: a disorder driven by gluten-specific CD4+ T cells In: A Schieptti and D Sanders, editors. Coeliac disease and gluten-related disorders. London, UK: Academic Press (2022). 41–68.

20. Siegel M, Garber ME, Spencer AG, Botwick W, Kumar P, Williams RN, et al. Safety, tolerability, and activity of ALV003: results from two phase 1 single, escalating-dose clinical trials. *Dig Dis Sci*. (2012) 57:440–50. doi: 10.1007/s10620-011-1906-5

21. Tye-Din JA, Anderson RP, Ffrench RA, Brown GJ, Hodsman P, Siegel M, et al. The effects of ALV003 pre-digestion of gluten on immune response and symptoms in celiac disease in vivo. *Clin Immunol*. (2010) 134:289–95. doi: 10.1016/j.clim.2009.11.001

22. Lähdeaho ML, Kaukinen K, Laurila K, Vuotikka P, Koivurova OP, Kärjä-Lahdensuu T, et al. Glutenase ALV003 attenuates gluten-induced mucosal injury in patients with celiac disease. *Gastroenterology*. (2014) 146:1649–58. doi: 10.1053/j.gastro.2014.02.031

23. Murray JA, Kelly CP, Green PHR, Marcantonio A, Wu TT, Mäki M, et al. No difference between latiglutenase and placebo in reducing villous atrophy or improving symptoms in patients with symptomatic celiac disease. *Gastroenterology*. (2017) 152:787–798.e2. doi: 10.1053/j.gastro.2016.11.004

24. Murray JA, Syage JA, Wu TT, Dickason MA, Ramos AG, van Dyke C, et al. Latiglutenase protects the mucosa and attenuates symptom severity in patients with celiac disease exposed to a gluten challenge. *Gastroenterology*. (2022) 163:1510–1521.e6. doi: 10.1053/j.gastro.2022.07.071

25. Syage JA, Green PHR, Khosla C, Adelman DC, Sealey-Voyksner JA, Murray JA. Latiglutenase treatment for celiac disease: symptom and quality of life improvement for seropositive patients on a gluten-free diet. *GastroHep*. (2019) 1:293–301. doi: 10.1002/ygh2.371

26. Mitea C, Havenaar R, Drijfhout JW, Edens L, Dekking L, Koning F. Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for coeliac disease. *Gut*. (2008) 57:25–32. doi: 10.1136/gut.2006.111609

27. Tack GJ, van de Water JM, Bruins MJ, Kooy-Winkelaar EM, van Bergen J, Bonnet P, et al. Consumption of gluten with gluten-degrading enzyme by celiac patients: a pilot study. *World J Gastroenterol*. (2013) 19:5837–47. doi: 10.3748/wjg.v19.i35.5837

28. Stefanolo JP, Segura V, Grizzuti M, Heredia A, Comino I, Costa AF, et al. Effect of aspergillus Niger prolyl endopeptidase in patients with celiac disease on a long-term gluten-free diet. *World J Gastroenterol*. (2024) 30:1545–55. doi: 10.3748/wjg.v30.i11.1545

29. Cardoso-Silva D, Delbue D, Itzlinger A, Moerkens R, Withoff S, Branchi F, et al. Intestinal barrier function in gluten-related disorders. *Nutrients*. (2019) 11:2325. doi: 10.3390/nu11102325

30. Cukrowska B, Sowińska A, Bierła JB, Czarnowska E, Rybak A, Grzybowska-Chlebowczyk U. Intestinal epithelium, intraepithelial lymphocytes and the gut microbiota - key players in the pathogenesis of celiac disease. *World J Gastroenterol*. (2017) 23:7505–18. doi: 10.3748/wjg.v23.i42.7505

31. Paterson BM, Lammers KM, Arrieta MC, et al. The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: a proof of concept study. *Aliment Pharmacol Ther*. (2007) 26:757–66. doi: 10.1111/j.1365-2036.2007.03413.x

32. Kelly CP, Green PH, Murray J, DiMarino A, Colatrella A, Leffler D, et al. Larazotide acetate in patients with celiac disease undergoing a gluten challenge: a randomized placebo-controlled study. *Aliment Pharmacol Ther*. (2013) 37:252–62. doi: 10.1111/apt.12147

33. Hoilat GJ, Altowairqi AK, Ayas MF, Alhaddab NT, Alnujaidi RA, Alharbi HA, et al. Larazotide acetate for treatment of celiac disease: a systematic review and meta-analysis of randomized controlled trials. *Clin Res Hepatol Gastroenterol*. (2022) 46:101782. doi: 10.1016/j.clinre.2021.101782

34. Kenison JE, Stevens NA, Quintana FJ. Therapeutic induction of antigen-specific immune tolerance. *Nat Rev Immunol*. (2023) 24:338–57. doi: 10.1038/s41577-023-00970-x

35. Goel G, King T, Daveson AJ, Andrews JM, Krishnarajah J, Krause R, et al. Epitope-specific immunotherapy targeting CD4-positive T cells in celiac disease: two randomised, double-blind, placebo-controlled phase 1 studies. *Lancet Gastroenterol Hepatol*. (2017) 2:479–93. doi: 10.1016/S2468-1253(17)30110-3

36. Truitt KE, Daveson AJM, Ee HC, Goel G, MacDougall J, Neff K, et al. Randomised clinical trial: a placebo-controlled study of subcutaneous or intradermal NEXVAX2, an investigational immunomodulatory peptide therapy for celiac disease. *Aliment Pharmacol Ther*. (2019) 50:547–55. doi: 10.1111/apt.15435

37. Tye-Din JA, Daveson AJM, Goel G, Goldstein KE, Hand HL, Neff KM, et al. Efficacy and safety of gluten peptide-based antigen-specific immunotherapy (Nexvax2) in adults with celiac disease after bolus exposure to gluten (RESET CeD): an interim analysis of a terminated randomised, double-blind, placebo-controlled phase 2 study. *Lancet Gastroenterol Hepatol*. (2023) 8:446–57. doi: 10.1016/S2468-1253(22)00428-9

38. Kelly CP, Murray JA, Leffler DA, Getts DR, Bledsoe AC, Smithson G, et al. TAK-101 nanoparticles induce gluten-specific tolerance in celiac disease: a randomized,

double-blind, placebo-controlled study. *Gastroenterology*. (2021) 161:66–80.e8. doi: 10.1053/j.gastro.2021.03.014

39. Lähdeaho ML, Scheinin M, Vuotikka P, Taavela J, Popp A, Laukkarinen J, et al. Safety and efficacy of AMG 714 in adults with celiac disease exposed to gluten challenge: a phase 2a, randomised, double-blind, placebo-controlled study. *Lancet Gastroenterol Hepatol*. (2019) 4:948–59. doi: 10.1016/S2468-1253(19)30264-X

40. Cellier C, Bouma G, van Gils T, Khater S, Malamut G, Crespo L, et al. Safety and efficacy of AMG 714 in patients with type 2 refractory coeliac disease: a phase 2a, randomised, double-blind, placebo-controlled, parallel-group study. *Lancet Gastroenterol Hepatol*. (2019) 4:960–70. doi: 10.1016/S2468-1253(19)30265-1

41. Schuppan D, Mäki M, Lundin KEA, Isola J, Friesing-Sosnik T, Taavela J, et al. A randomized trial of a transglutaminase 2 inhibitor for celiac disease. *N Engl J Med*. (2021) 385:35–45. doi: 10.1056/NEJMoa2032441

42. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res*. (2020) 30:492–506. doi: 10.1038/s41422-020-0332-7

43. Pozo-Rubio T, Olivares M, Nova E, de Palma G, Mujico JR, Ferrer MD, et al. Immune development and intestinal microbiota in celiac disease. *Clin Dev Immunol*. (2012) 2012:654143:–654112. doi: 10.1155/2012/654143

44. Olivares M, Castillejo G, Varea V, Sanz Y. Double-blind, randomised, placebo-controlled intervention trial to evaluate the effects of *Bifidobacterium longum* CECT 7347 in children with newly diagnosed coeliac disease. *Br J Nutr*. (2014) 112:30–40. doi: 10.1017/S0007114514000609

45. Harnett J, Myers SP, Rolfe M. Probiotics and the microbiome in celiac disease: a randomised controlled trial. *Evid Based Complement Alternat Med*. (2016) 2016:9048574. doi: 10.1155/2016/9048574

46. Chang MS, Minaya MT, Cheng J, Connor BA, Lewis SK, Green PHR. Double-blind randomized controlled trial of rifaximin for persistent symptoms in patients with celiac disease. *Dig Dis Sci*. (2011) 56:2939–46. doi: 10.1007/s10620-011-1719-6

47. Mukewar SS, Sharma A, Rubio-Tapia A, Wu TT, Jabri B, Murray JA. Open-capsule budesonide for refractory celiac disease. *Am J Gastroenterol*. (2017) 112:959–67. doi: 10.1038/ajg.2017.71

48. Therrien A, Silvester JA, Leffler DA, Kelly CP. Efficacy of enteric-release oral budesonide in treatment of acute reactions to gluten in patients with celiac disease. *Clin Gastroenterol Hepatol*. (2020) 18:254–6. doi: 10.1016/j.cgh.2019.03.029

49. Ciacci C, Maiuri L, Russo I, Tortora R, Bucci C, Cappello C, et al. Efficacy of budesonide therapy in the early phase of treatment of adult coeliac disease patients with malabsorption: an in vivo/in vitro pilot study. *Clin Exp Pharmacol Physiol*. (2009) 36:1170–6. doi: 10.1111/j.1440-1681.2009.05211.x

50. Newnham ED, Clayton-Chubb D, Nagarethinam M, Hosking P, Gibson PR. Randomised clinical trial: adjunctive induction therapy with oral effervescent budesonide in newly diagnosed coeliac disease. *Aliment Pharmacol Ther*. (2021) 54:419–28. doi: 10.1111/apt.16446

51. Croese J, Giacomin P, Navarro A, et al. Experimental hookworm infection and gluten microchallenge promote tolerance in celiac disease. *J Allergy Clin Immunol*. (2015) 135:508–516.e5. doi: 10.1016/j.jaci.2014.07.022

52. Croese J, Miller GC, Marquart L, Llewellyn S, Gupta R, Becker L, et al. Randomized, placebo-controlled trial of experimental hookworm infection for improving gluten tolerance in celiac disease. *Clin Transl Gastroenterol*. (2020) 11:e00274. doi: 10.14309/ctg.0000000000000274

53. Catassi C, Fabiani E, Iacono G, D'Agate C, Francavilla R, Biagi F, et al. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. *Am J Clin Nutr*. (2007) 85:160–6. doi: 10.1093/ajcn/85.1.160

54. Akobeng AK, Thomas AG. Systematic review: tolerable amount of gluten for people with coeliac disease. *Aliment Pharmacol Ther*. (2008) 27:1044–52. doi: 10.1111/j.1365-2036.2008.03669.x

55. Shewry PR, Halford NG, Belton PS, Tatham AS. The structure and properties of gluten: an elastic protein from wheat grain. *Philos Trans R Soc Lond Ser B Biol Sci*. (2002) 357:133–42. doi: 10.1098/rstb.2001.1024

56. Leffler DA, Kelly CP, Green PH, Fedorak R, DiMarino A, Perrow W, et al. Larazotide acetate for persistent symptoms of celiac disease despite a gluten-free diet: a randomized controlled trial. *Gastroenterology*. (2015) 148:1311–9.e6. doi: 10.1053/j.gastro.2015.02.008

57. Leffler DA, Kelly CP, Abdallah HZ, Colatrella AM, Harris LA, Leon F, et al. A randomized, double-blind study of larazotide acetate to prevent the activation of celiac disease during gluten challenge. *Am J Gastroenterol*. (2012) 107:1554–62. doi: 10.1038/ajg.2012.211

58. Lebwohl B, Ma C, Lagana SM, Pai RK, Baker KA, Zayadi A, et al. Standardizing randomized controlled trials in celiac disease: an international multidisciplinary appropriateness study. *Gastroenterology*. (2024) 166:88–102. doi: 10.1053/j.gastro.2023.08.051. Epub 2023 Sep 11

59. Penny HA, Rej A, Baggus EMR, Coleman SH, Ward R, Wild G, et al. Non-responsive and refractory coeliac disease: experience from the NHS England National Centre. *Nutrients*. (2022) 14:2776. doi: 10.3390/nu14132776

60. Schiepatti A, Bellani V, Perlato M, Maimaris S, Klersy C, Biagi F. Inadvertent and minimal gluten intake has a negligible role in the onset of symptoms in patients with coeliac disease on a gluten-free diet. *Br J Nutr*. (2019) 121:576–81. doi: 10.1017/S0007114518003616

61. Silvester JA, Weiten D, Graff LA, Walker JR, Duerksen DR. Is it gluten-free? Relationship between self-reported gluten-free diet adherence and knowledge of gluten content of foods. *Nutrition*. (2016) 32:777–83. doi: 10.1016/j.nut.2016.01.021

62. Vernero M, Schiepatti A, Maimaris S, et al. The GLU-10: a validated ten-point score to identify poorly instructed celiac patients in need of dietary interventions. *Minerva Gastroenterol*. (2022) 68:91–7. doi: 10.23736/S2724-5985.21.03037-0

63. Halmos EP, Deng M, Knowles SR, Sainsbury K, Mullan B, Tye-Din JA. Food knowledge and psychological state predict adherence to a gluten-free diet in a survey of 5310 Australians and new Zealanders with coeliac disease. *Aliment Pharmacol Ther*. (2018) 48:78–86. doi: 10.1111/apt.14791

64. Mearin ML, Agardh D, Antunes H, al-toma A, Auricchio R, Castillejo G, et al. ESPGHAN special interest group on celiac disease. ESPGHAN position paper on management and follow-up of children and adolescents with celiac disease. *J Pediatr Gastroenterol Nutr*. (2022) 75:369–86. doi: 10.1097/MPG.0000000000003540

65. Leffler D, Schuppan D, Pallav K, Najarian R, Goldsmith JD, Hansen J, et al. Kinetics of the histological, serological, and symptomatic responses to gluten challenge in adults with coeliac disease. *Gut*. (2013) 62:996–1004. doi: 10.1136/gutjnl-2012-302196

66. Rispo A, Guarino AD, Siniscalchi M, et al. The crackers challenge": a reassuring low-dose gluten challenge in adults on gluten-free diet without proper diagnosis of coeliac disease. *Dig Liver Dis*. (2024):S1590-8658(24)00305-0. doi: 10.1016/j.dld.2024.03.004

67. Popp A, Laurikka P, Czika D, Kurppa K. The role of gluten challenge in the diagnosis of celiac disease: a review. *Expert Rev Gastroenterol Hepatol*. (2023) 17:691–700. doi: 10.1080/17474124.2023.2219893

68. Ulmer M, Robinaugh D, Friedberg JP, Lipsitz SR, Natarajan S. Usefulness of a run-in period to reduce drop-outs in a randomized controlled trial of a behavioral intervention. *Contemp Clin Trials*. (2008) 29:705–10. doi: 10.1016/j.cct.2008.04.005

69. Lusetti F, Schiepatti A, Scalvini D, Maimaris S, Biagi F. Efficacy of a low-FODMAP diet for coeliac patients with persistent IBS-like symptoms despite a gluten-free diet: a systematic review. *Nutrients*. (2024) 16:1094. doi: 10.3390/nu16071094

70. Varjú P, Farkas N, Hegyi P, Garami A, Szabó I, Illés A, et al. Low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet improves symptoms in adults suffering from irritable bowel syndrome (IBS) compared to standard IBS diet: a meta-analysis of clinical studies. *PLoS One*. (2017) 12:e0182942. doi: 10.1371/journal.pone.0182942

71. Spiegel BM. Patient-reported outcomes in gastroenterology: clinical and research applications. *J Neurogastroenterol Motil*. (2013) 19:137–48. doi: 10.5056/jnm.2013.19.2.137

72. Williet N, Sandborn WJ, Peyrin-Biroulet L. Patient-reported outcomes as primary endpoints in clinical trials of inflammatory bowel disease. *Clin Gastroenterol Hepatol*. (2014) 12:1246–56.e6. doi: 10.1016/j.cgh.2014.02.016

73. Weldring T, Smith SM. Patient-reported outcomes (PROs) and patient-reported outcome measures (PROMs). *Health Serv Insights*. (2013) 6:61–8. doi: 10.4137/HSI.S11093

74. Motta LS, Gosmann NP, Costa MA, et al. Placebo response in trials with patients with anxiety, obsessive-compulsive and stress disorders across the lifespan: a three-level meta-analysis. *BMJ Ment Health*. (2023) 26:e300630. doi: 10.1136/bmjment-2022-300630

75. Wong MW, Hsiao SH, Wang JH, Yi CH, Liu TT, Lei WY, et al. Esophageal hypervigilance and visceral anxiety contribute to symptom severity of laryngopharyngeal reflux. *Am J Gastroenterol*. (2023) 118:786–93. doi: 10.14309/ajg.0000000000002151

76. Söderquist F, Syk M, Just D, Kurbalija Novicic Z, Rasmusson AJ, Hellström PM, et al. A cross-sectional study of gastrointestinal symptoms, depressive symptoms, and trait anxiety in young adults. *BMC Psychiatry*. (2020) 20:535. doi: 10.1186/s12888-020-02940-2

77. Clinicaltrialsregister.eu. Clinical trials register: trial 2020-004612-97. Available at: https://www.clinicaltrialsregister.eu/ctr-search/trial/2020-004612-97/DE (Accessed September 18, 2024).

78. Clinicaltrials.gov. Study NCT04530123. Available at: https://clinicaltrials.gov/study/NCT04530123 (Accessed September 18, 2024).

frontiers | Frontiers in Nutrition

TYPE Brief Research Report
PUBLISHED 25 February 2025
DOI 10.3389/fnut.2025.1500632

OPEN ACCESS

EDITED BY
Carolina Sousa,
Sevilla University, Spain

REVIEWED BY
Israel Parra-Ortega,
Federico Gómez Children's Hospital, Mexico
Verónica Segura,
University of Sevilla, Spain

*CORRESPONDENCE
Fernando Gabriel Chirdo
✉ fchirdo@biol.unlp.edu.ar

†These authors have contributed equally to this work

RECEIVED 01 October 2024
ACCEPTED 27 January 2025
PUBLISHED 25 February 2025

CITATION
Ruera CN, Guzman L, Menendez L, Orellano L, Girard Bosch MC, Catassi C and Chirdo FG (2025) Typing of HLA susceptibility alleles as complementary tool in diagnosis of controversial cases of pediatric celiac disease. *Front. Nutr.* 12:1500632. doi: 10.3389/fnut.2025.1500632

Typing of HLA susceptibility alleles as complementary tool in diagnosis of controversial cases of pediatric celiac disease

Carolina Naymé Ruera[1†], Luciana Guzman[2†], Lorena Menendez[2], Laura Orellano[2], María Cecilia Girard Bosch[2], Carlo Catassi[3] and Fernando Gabriel Chirdo[1*]

[1]Departmento de Ciencias Biologicas, Facultad de Ciencias Exactas, UNLP, Instituto de Estudios Inmunologicos y Fisiopatologicos (IIFP) (UNLP-CONICET), La Plata, Argentina, [2]Hospital de Niños Superiora Sor María Ludovica, La Plata, Argentina, [3]Department of Pediatrics, Università Politecnica delle Marche, Ancona, Italy

Objectives: Diagnosis of celiac disease (CeD), an immune-mediated disorder, is based on clinical presentation, a panel of serological markers, and the histopathological findings in duodenal biopsies. Commonly, pediatric CeD patients fulfill these criteria for diagnosis. However, lack of correlation between serology tests and histology, or no accessible biopsies because of clinical conditions or during the COVID pandemic, are conditions that led to inconclusive diagnoses. Since the majority of CeD patients carry HLA-DQ2 and/or DQ8 alleles, HLA testing is used as a complementary tool in diagnosis though is costly and not broadly available for gastroenterology centers.

Methods: We performed a retrospective study to assess the performance of HLA testing when applied to selected groups of patients who could not be definitely diagnosed following the common algorithm. Eighty patients underwent testing for CeD-related HLA-DQ2 and DQ8 alleles.

Results: HLA typing contributed to diagnosis in 34 patients with positive serology but normal mucosa or those who presented negative serology or slightly positive serology (less than 3 times ULN) and duodenal histopathological changes. In patients with normal histology and negative or slightly positive serology, or those who did not undergo intestinal biopsy (39 in total), HLA typing contributed to CeD diagnosis in 23 cases, only 16 patients were admitted for a clinical follow-up program.

Conclusion: HLA-DQ typing supported the diagnosis in 57 of 80 children (71.2%) with previously inconclusive results, providing a beneficial approach for diagnosing celiac disease (CeD) in selected cases.

KEYWORDS

celiac disease, HLA, diagnosis, potential CeD, pediatric

1 Introduction

Celiac disease (CeD) is one of the most prevalent immune-mediated chronic gut disorders, that develops in genetically susceptible individuals, triggered by the intake of a group of proteins from wheat, barley, and rye, commonly named as gluten. Diagnosis of CeD is based on the clinical presentation, presence of a panel of specific antibodies in peripheral blood,

histological demonstration of mucosal damage in duodenal biopsies, and clinical improvement when patient adheres to a gluten-free diet (GFD). CeD may appear at any age with typical gastrointestinal symptoms, but also with minimal and variable intestinal and extraintestinal manifestations, or even with an asymptomatic presentation. CeD is also associated with type I diabetes, autoimmune thyroid disease or other conditions such as Down syndrome and IgA deficiency (1).

Genes encoding for alpha and beta chain of HLA class II molecules are the most strongly genetic factor associated to CeD. Four HLA class II alleles account for the highest relative risk for an HLA-disease association. These are commonly described as HLA-DQ2.5 (encoded by either a *cis* haplotype: HLA-DQA1*0501/HLA-DQB1*0201 or a *trans* haplotype configuration HLA-DQA1*0501/HLA-DQB1*0301 and HLA-DQA1*0201/HLA-DQB1*0202), HLA-DQ2.2 (encoded by HLA-DQA1*0201 and HLA-DQB1*0202), the HLA-DQ8 heterodimer (encoded by the HLA-DQA1*0301 and HLA-DQB1*0302), and rarely the DQ7.5 type (encoded by the HLA-DQA1*05 and HLA-DQB1*0301). Though almost all CeD patients carry one or a combination of these alleles, these are also frequent in non-CeD individuals. Very few CeD cases not carrying any of the HLA susceptibility alleles have been reported (2). Consequently, the absence of these alleles makes very unlikely the disease (high negative predictive value), while their presence is not confirmatory (low positive predictive value) (3, 4).

The prevalence of CeD is estimated at around 1% worldwide, however, this disorder is deeply underdiagnosed. Poor disease awareness, failures in the health system, particularly in developing regions, increasing findings of asymptomatic presentations, and drawbacks during the diagnostic protocol, result in a high rate of undiagnosed patients (5).

Reference centers have followed different algorithms for the diagnosis of CeD, according to their own experiences or performance of each step in the procedure (clinic, laboratory, and pathology evaluations). In the year 2012 the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) proposed that the diagnosis of CeD may definitely be established in patients with compatible symptoms having anti-transglutaminase 2 (TG2) IgA levels 10 times above the cut-off value, followed by positive IgA EmA in a second blood sample, and carrying HLA-DQ2 and/or DQ8, avoiding the requirement of the intestinal biopsy (6). More recently, this consensus was reviewed and the current ESPGHAN recommendation considers that positive serology (anti-TG2 IgA levels 10 times above the cut-off value) plus EmA positivity determines the diagnosis even in asymptomatic patients. As HLA typing does not provide a cost-effective outcome, it was not recommended (7).

CeD diagnosis can be reached in most of the cases following these guidelines, however, complex situations are sometimes observed in clinical practice. Asymptomatic presentation, lack of correlation between serology and histological findings, unavailable biopsies, and starting the gluten-free diet (GFD) before performing complete investigations may lead to difficulties in reaching a final diagnosis. Additional studies, such as the evaluation of intraepithelial lymphocytes subsets by flow cytometry (8), biomarkers as intestinal FABP in serum (9, 10), intestinal deposits of anti-TG2 IgA (11), may help to solve these cases, but these tools are still not broadly available.

Algorithms followed by different specialized gastroenterology units may include HLA-DQ typing but this technique is costly and is not easily available for most of the centers (3). Therefore, we aimed to assess the impact of performing HLA-DQ typing when applied to selected groups of patients who could not be diagnosed following the routine protocol as a consequence of normal histology or negative serology, or lack of histopathological assessment due to the lack of endoscopy procedures because of clinical conditions, parental denial, or COVID pandemia.

2 Materials and methods

A total of 360 pediatric patients between 1 and 16 years old were diagnosed as CeD in the period between January 2015 and July 2023, at the gastroenterology Unit of Sor María Ludovica Children's Hospital in La Plata (Argentina). This Hospital is a reference center for the diagnosis of CeD and the patients were referred from different Public Health Units of the Province of Buenos Aires. Patients followed a protocol for Celiac Disease diagnosis in the Gastroenterology Unit.

The diagnosis of CeD was based on the clinical presentation, serology, and histological analysis of intestinal biopsies. Patients with suspected CeD and/or positive serology underwent upper endoscopy, except in those for whom the procedure was not medically indicated. HLA-DQ typing was performed in all patients. After diagnosis, patients were evaluated by a nutritionist to start the GFD. Patients were followed up to monitor dietary compliance to the diet and for clinical examination.

This retrospective study included 80 pediatric patients with suspected CeD whose clinical evaluation, serology, and histopathological findings did not support a definite diagnosis of CeD or those in whom upper endoscopy procedure could not be performed.

Cases diagnosed as CeD on the basis of concordant clinical presentation, positive serology and histopathological findings in the duodenal biopsies, as well as type 1 diabetes mellitus patients, were excluded from this study.

2.1 Clinical presentation

Gastrointestinal symptoms included chronic diarrhea, abdominal pain, bloating, and weight loss. Extraintestinal symptoms were anemia, decreased bone mineralization, increased levels of liver enzymes, dermatitis herpetiformis, short stature, and delayed puberty.

2.2 Serology

Serum samples were kept frozen at −20°C until analysis in the Immunology Section of the Hospital de Niños Sor María Ludovica. IgA anti-TG2 antibodies were determined by an ELISA test (Quanta Lite R H-tTG ELISA, Inova Diagnostic). IgG anti-DGP were performed by an ELISA test [QUANTA Lite® Gliadin IgG II (DGP), Inova Diagnostics]. Total serum IgA concentration was determined by nephelometric technique (IMMAGE® 800, Beckman Coulter).

Samples presenting IgA anti- TG2 antibodies <20 UA/mL were considered normal. Positive samples were defined as presenting IgA anti-TG2 antibody levels higher than the upper level of normal (ULN). Serology values for IgA anti-TG2 antibodies of 200 UA/mL and 60 UA/mL correspond to 10× ULN and 3× ULN, respectively. All

positive samples for anti-TG2 antibodies were evaluated in a second separated blood sample for IgA anti-endomysial antibodies (EMA) (Inova Diagnostics).

2.3 Intestinal biopsy

The small intestine biopsy was performed by upper gastrointestinal videoendoscopy under general anesthesia. A Fujinon 530 video esophagoduodenoscope (Video-Gastroscope Fujinon EG-530D) was used. During the procedure, the second portion of the duodenum was reached, 4 samples of the second duodenal portion (D2) and 2 biopsies of the duodenal bulb were taken. The samples were placed in formalin for histological evaluation following Marsh–Oberhuber classification (12).

2.4 HLA-DQ typing

Genomic DNA extraction from white blood cells was performed using the QIAamp® DNA Blood Kit (QIAGEN® Inc., Valencia, CA). High resolution HLA genotyping was performed by multiplex polymerase chain reaction (PCR) with biotinylated primers, followed by reverse hybridation of the PCR products with arrays of sequence-specific DQA1 and DQB1 oligonucleotide probes. This is followed by a stringent wash step to remove any mismatched amplified material. Then, streptavidin conjugated with alkaline phosphatase is added and bound to any biotinylated hybrid previously formed; using INNO-LIPA HLA-DQ kits, according to the manufacter's instructions (Fujirebio, Gent, Belgium). Results were analyzed by using the sixth version of the LiRAS interpretation software for LiPA HLA. The nomenclature was based on "Nomenclature for Factors of the HLA System, 2010" (13).

2.5 Patient groups

The patients with suspected symptoms included in the study population were divided into 4 groups as follows:

Group 1: Patients with positive serology and small intestine with normal histology.

Group 2: Patients with negative serology and small intestine with normal histology.

Group 3: Patients with negative serology and small intestine with histopathological changes (Marsh 2 or 3).

Group 4: Patients with who did not undergo intestinal biopsy due to medical contraindication.

When a definite diagnosis could not be reached, patients with symptoms suggestive of celiac disease were included in a follow-up program. In these cases, clinical evaluation was performed every 6 months while serological testing on a normal gluten-containing diet was performed every 12 months.

In the period from 2015 to 2023, patients in Group 1, 2 and 3 underwent endoscopy and histological assessment of intestinal biopsy based on the guidelines from Celiac Disease Expert Committee approved by the National Health Ministry in Argentina (14).

2.6 Statistical analysis

Statistical analysis was performed with Graph-Pad Prism software (San Diego, United States). A chi-square test was applied to assess statistical significance.

3 Results

In this study, a total of 80 pediatric patients out of 360 with gastrointestinal symptoms did not reach a final diagnosis after the first work-up due to lack of histological evaluation or discrepancy between the serological and histopathological findings. To assess whether HLA-DQ typing can contribute to the diagnosis of CeD, these cases were distributed into the four groups described in section 2.5.

3.1 Group 1

Group 1 included 27 patients with compatible gastrointestinal symptoms and positive serology but a normal villous architecture at the small intestinal biopsy. According to current knowledge, these are cases of "Potential" CeD, a condition that may evolve into fully-expressed CeD or revert to normal over time (5). Fourteen of them (52%) presented both anti-TG2 antibodies at high titer (>10 times ULN) and EMA positivity. All these cases presented compatible HLA and were definitely considered as affected by CeD. Upon discussion with the family, patients started a GFD and all of them showed a positive clinical response (Figure 1). Thirteen other patients had anti-TG2 antibody low levels ranging from 1 to 3 ULN. Among these, six patients were excluded from having CeD as they tested negative for EMA and

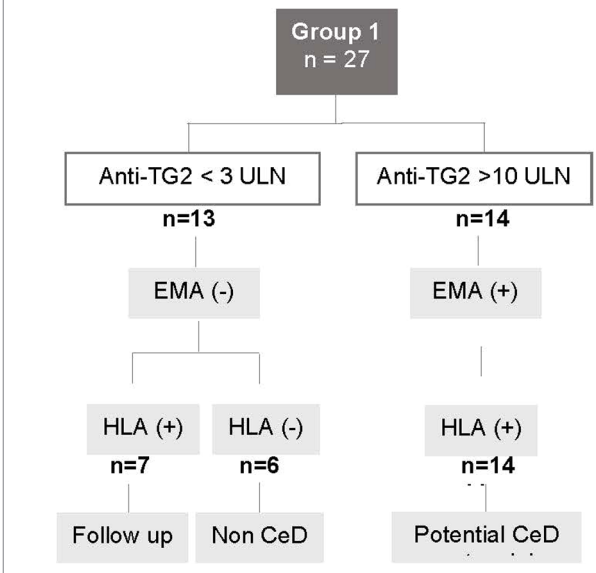


FIGURE 1
Flowchart for Group 1. Patients with positive serology and small intestine with normal histology. CeD, celiac disease; anti-TG2, anti-transglutaminase 2 antibody levels; ULN, upper limit of normal; EMA, anti-endomysial antibody; HLA, patients carrying the HLA CeD-compatible alleles.

lacked HLA susceptibility alleles (22%). The remaining seven patients (26%), who had HLA predisposing genes but were EMA negative, were enrolled in a follow-up program and left on a normal diet.

3.2 Group 2

Group 2 comprised 18 patients who had suggestive symptoms, negative serology, and normal histology. CeD was ruled out in seven patients because they lacked HLA predisposing genes. The remaining 11 patients, with persistence of symptoms and compatible HLA genes, were maintained on a normal diet and entered a follow-up program (Figure 2A).

3.3 Group 3

Group 3 included 14 patients with compatible symptoms, negative serology and histopathological changes in the small intestine (Marsh 2, 3 score). HLA predisposing alleles were absent in 7 cases, ruling out CeD. The remaining 7 patients carried HLA susceptibility alleles. Among them, four patients were under 3 years old, exhibited low levels of IgA, and had a Marsh 3 score. A GFD was introduced, leading to a positive clinical response (Figure 2B).

In the search for other causes (i.e., upper digestive bleeding and esophageal atresia), Marsh 3 scores were occasionally observed in the duodenum of two patients. After excluding other conditions such as inflammatory bowel disease, dietary allergies, HIV, and primary immunodeficiency, these two patients were placed on a GFD, resulting in a favorable clinical outcome. Endoscopy and duodenal biopsy 6 months after starting the GFD showed mucosal recovery, and patients were diagnosed with seronegative CeD. The remaining patient presented with severe malnutrition, negative serology, and a Marsh 3 score in the duodenal biopsies. After a positive response to the GFD, and the assessment of duodenal biopsy 6 months later, a diagnosis of CeD was confirmed. The frequency of seronegative CeD was 3/360 CeD patients.

3.4 Group 4

Group 4 included 21 symptomatic patients who, due to pre-existing clinical conditions such as heart disease, epilepsy, or severe malnutrition, could not undergo endoscopy. In addition, this group included children who suffered from gastrointestinal symptoms during the COVID-19 pandemic. According to the recommendations of the different Scientific Societies, endoscopy procedures were not performed during this period.

Seven patients presented a negative serology, 4 of them presented a not compatible HLA and CeD was ruled out. The remaining 3 presented compatible HLA and symptoms, therefore were admitted to the follow up program. Eleven cases presented anti-TG2 levels higher than 10 UNL. All of them were EMA positive, carried compatible HLA and were diagnosed as CeD. In these cases, HLA typing was requested following the local guidelines (13).

Finally, there were 3 patients with anti-TG2 levels between 1 and 10 UNL and compatible HLA. Two of them were EMA positive and were diagnosed as CeD. Then a good response to the GFD was observed. The remaining case presented anti-TG2 levels below 3 UNL, EMA negative and was admitted in a clinical and serological follow-up program (Figure 3).

4 Discussion

Cases showing a discrepancy between the results of serological markers and duodenal histology or those who do not undergo an endoscopy procedure to take duodenal biopsies are common causes for inconclusive CeD diagnosis. HLA typing is a costly technique and is not easily available in many gastroenterology centers. However, this is a valuable tool to exclude the disease when patients do not carry the CeD-compatible alleles and could be beneficial when applied in selected groups of patients.

As the Caucasian central European is the main ethnic contribution to the population studied, DQ2.5 was the most frequent allele found (23 out of 32 diagnosed patients, 16 of them homozygous) (Table 1).

Studies from European populations have shown the dominance of the DQ2 allele in celiac patients (15). However, other ethnic groups

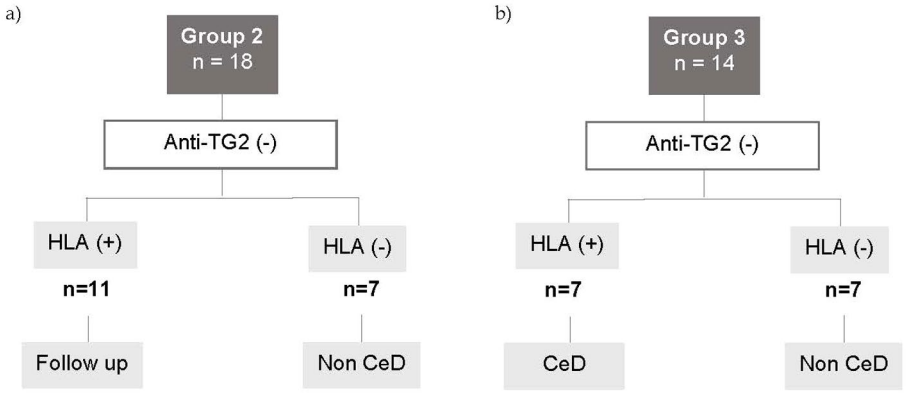


FIGURE 2
Flowchart for Groups 2 and 3. **(A)** Patients with negative serology and small intestine with normal histology. **(B)** Patients with negative serology and small intestine with histopathological changes. CED, celiac disease; anti-TG2 (–), negative anti-transglutaminase 2 antibody levels; HLA+, patients carrying the HLA CED- compatible alleles.

10.3389/fnut.2025.1500632

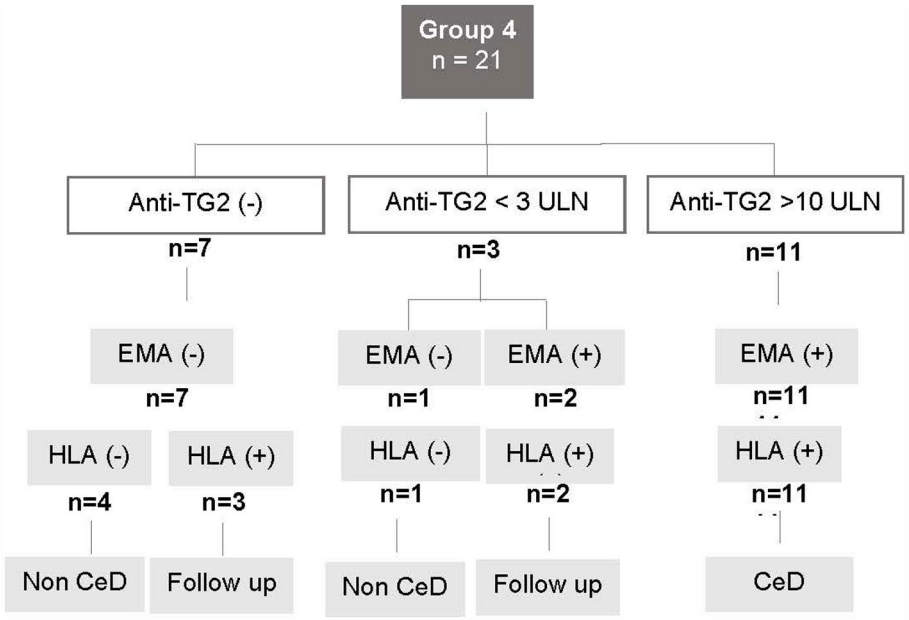


FIGURE 3
Flowchart for Group 4. Patients with who did not undergo intestinal biopsy due to medical contraindication. CeD, celiac disease; anti-TG2, anti-transglutaminase 2 antibody levels; ULN, upper limit of normal; EMA, anti-endomysial antibody; HLA+, patients carrying the HLA CeD-compatible alleles.

TABLE 1 HLA alleles distribution.

	Group 1 *n* = 27	Group 2 *n* = 18	Group 3 *n* = 14	Group 4 *n* = 21	Total *n* = 80	CeD *n* = 32
DQ2.5/DQ2.5	10	5	1	10	26	16
DQ2.5/x	4	3	2	4	13	7
DQ2.5/DQ8	1	1	—	—	2	1
DQ8/x	3	2	—	2	7	4
DQ2.2/x	—	—	1	—	1	1
DQ7.5	1	—	3	—	4	3
Negative	6	7	7	5	25	—

may exhibit different patterns. For instance, research on Indian populations (16) and Native Amerindians in Argentina (17), Chile (18), and Mexico (19) has identified DQ8 as the most frequent allele contributing to CeD. Additionally, the prevalence of the DQ7 allele varies among celiac patients across different communities (20).

Altogether, this study shows that HLA typing may contribute as a useful complementary tool in the diagnosis of CeD (Figure 4A). Group 1 included patients with so-called potential CeD (5), a situation that may be difficult to ascertain in clinical practice due to the discrepancy between the results of CeD serology and intestinal histology. HLA determination was a useful confirmatory test in 52% of these cases, but at the same time helped to exclude CeD in further 22%, therefore leaving only 26% of patients in need of follow-up to reach a final diagnosis. In Group 2, cases presented the typical condition in which absence of HLA susceptibility alleles ruled out the pathology, while the low level of antibodies or negative serology determined that patient entry in a follow-up program. In Group 3, all cases received a conclusive diagnosis either because they were CeD or disease was excluded. Noteworthy in this group, two patients younger than 3 years old presented with severe malnutrition, abdominal distention, and chronic diarrhea. Serology was negative, likely secondary to their poor nutritional state. Histopathological assessment revealed severe villous atrophy. These patients followed a GFD with good response. In these cases, HLA evaluation supported the diagnosis. HLA typing was found helpful to select a group of seronegative or presenting low anti-TG2 antibody levels individuals at risk (17), or to exclude the disease when HLA was not compatible. Group 4 included patients evaluated during the COVID pandemia. As a referral center in the Buenos Aires Province, 400 endoscopies are performed in average, with 40–50 diagnosis of CeD per year. However, during the pandemia, endoscopy procedures were solely performed in extreme emergency cases. As a result, HLA evaluation was useful in supporting the CeD diagnosis in these cases. Considering the results across all study groups, it is evident that HLA typing contributed in making a diagnostic decision in a significant number of cases ($p = 0.0016$) (Figure 4B). As mentioned before, the highest contributions were observed in Groups 1, 3, and 4, where HLA typing contributed to the diagnose in 74, 100, and 76% of cases, respectively. In Group 2, HLA typing contributed to the diagnosis in only 39% of cases.

As indicated, patients with persistent and isolated low level of anti-TG2 antibodies were included in a clinical and serological follow up protocol. Some of these patients will be eventually diagnosed as CeD, while others may normalize the serology. In a prospective study, Auricchio et al. (21) reported that 32% of 280 potential CeD patients

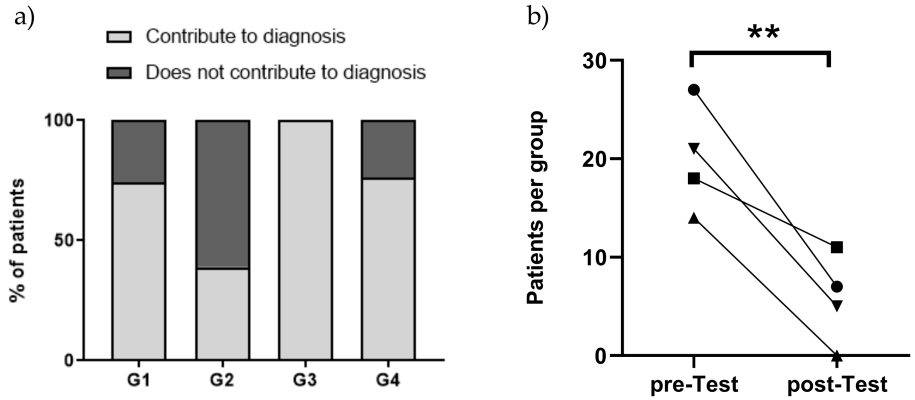


FIGURE 4
(A) Performance of HLA typing to CeD diagnosis per group. **(B)** Impact of HLA typing on diagnosis in study groups. The comparison of diagnosed cases before (pre-test) and after (post-test) HLA typing revealed a significant overall difference ($p = 0.0016$). Statistical analysis for each group is as follows: ● Group 1 ($p = 0.019$), ■ Group 2 ($p = 0.481$), ▲ Group 3 ($p = 0.0001$), ▼ Group 4 ($p = 0.027$). A chi-square test was applied to assess statistical significance.

enrolled in the study have presented negative serology in successive blood samples and none of them developed villous atrophy over 60 months of follow up. This exemplifies the complex scenario for CeD diagnosis for some of the cases. Although not tested in our study due to the limited number of cases, DQ typing may also be beneficial for individuals already on a gluten-free diet (GFD) (22). This aspect is highly relevant and warrants further evaluation in referral centers that could enroll large number of patients.

HLA typing has typically been used as an exclusion criterion in the absence of susceptibility HLA. However, various diagnostic algorithms have highlighted the importance of positive results as well. The study by Lionetti et al. (23) demonstrated the value of HLA typing as a mass screening tool in the pediatric population.

5 Conclusion

In summary, though HLA-DQ typing is not required for CeD diagnosis in all cases, it is a valuable complementary tool in evaluating cases with suspected CeD, emphasizing its crucial role in ruling out the disease when negative and providing diagnostic support in challenging clinical situations. Since it is expensive and not broadly available, here we show that its use in selected groups of patients such as in the context of serology-histology discrepancy, lack of upper endoscopy and histological assessment, contribute to the diagnosis of celiac disease.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. In adherence to ethical guidelines, all patient data were anonymized to protect confidentiality.

Ethics statement

The studies involving humans were approved by Instituto de Desarrollo e Investigaciones Pediátricas-IDIP, La Plata. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

CR: Writing – original draft, Writing – review & editing. LG: Writing – original draft, Writing – review & editing. LM: Writing – original draft, Writing – review & editing. LO: Writing – original draft. MB: Writing – original draft. CC: Writing – review & editing. FC: Writing – original draft, Writing – review & editing, Conceptualization, Supervision.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Lindfors K, Ciacci C, Kurppa K, KEA L, Makharia GK, Mearin ML, et al. Coeliac disease. *Nat Rev Dis Primers*. (2019) 5:3. doi: 10.1038/s41572-018-0054-z

2. Karell K, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L, et al. HLA types in celiac disease patients not carrying the DQA1 *05-DQB1 *02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. *Hum Immunol*. (2003) 64:469–77. doi: 10.1016/S0198-8859(03)00027-2

3. Pallav K, Kabbani T, Tariq S, Vanga R, Kelly CP, Leffler DA. Clinical utility of celiac disease-associated HLA testing. *Dig Dis Sci*. (2014) 59:2199–206. doi: 10.1007/s10620-014-3143-1

4. Iversen R, Sollid LM. The immunobiology and pathogenesis of celiac disease. *Annu Rev Pathol Mech Dis*. (2023) 18:47–70. doi: 10.1146/annurev-pathmechdis-031521-032634

5. Catassi C, Verdu EF, Bai JC, Lionetti E. Coeliac disease. *Lancet*. (2022) 399:2413–26. doi: 10.1016/S0140-6736(22)00794-2

6. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr*. (2012) 54:136–60. doi: 10.1097/MPG.0b013e31821a23d0

7. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition guidelines for diagnosing coeliac disease 2020. *J Pediatr Gastroenterol Nutr*. (2020) 70:141–56. doi: 10.1097/MPG.0000000000002497

8. Núñez C, Carrasco A, Corzo M, Pariente R, Esteve M, Roy G. Flow cytometric analysis of duodenal intraepithelial lymphocytes (celiac lymphogram): a diagnostic test for celiac disease. *Methods Cell Biol*. (2023) 179:143–55. doi: 10.1016/bs.mcb.2022.11.004

9. Adriaanse MPM, Tack GJ, Passos VL, Damoiseaux JGMC, Schreurs MWJ, van Wijck K, et al. Serum I-FABP as marker for enterocyte damage in coeliac disease and its relation to villous atrophy and circulating autoantibodies. *Aliment Pharmacol Ther*. (2013) 37:482–90. doi: 10.1111/apt.12194

10. Bottasso Arias NM, García M, Bondar C, Guzman L, Redondo A, Chopita N, et al. Expression pattern of fatty acid binding proteins in celiac disease enteropathy. *Mediators Inflamm*. (2015). doi: 10.1155/2015/738563

11. Koskinen O, Collin P, Lindfors K, Laurila K, Mäki M, Kaukinen K. Usefulness of small-bowel mucosal transglutaminase-2 specific autoantibody deposits in the diagnosis and follow-up of celiac disease. *J Clin Gastroenterol*. (2010) 44:483–8. doi: 10.1097/MCG.0b013e3181b64557

12. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol*. (1999) 11:1185–94. doi: 10.1097/00042737-199910000-00019

13. Espino L, Núñez C. The HLA complex and coeliac disease. *Int Rev Cell Mol Biol*. (2021) 358:47–83. doi: 10.1016/bs.ircmb.2020.09.009

14. Ortiz G, Toca MDC, Mora M, Orsi M, Furnes R, Litwin N, et al. Enfermedad celíaca en pediatría: características clínicas y metodología diagnóstica [Pediatric coeliac disease: Clinical features and diagnostic process]. *Arch Argent Pediatr*. (2024) 28:e202410461. doi: 10.5546/aap.2024-10461

15. Sánchez-Valverde F, Martínez-Ojinaga E, Donat E, Bodas A, Bandrés E, Torres R, et al. Geographical distribution of risk genotypes in pediatric patients with celiac disease in Spain. *Hum Immunol*. (2023) 84:290–5. doi: 10.1016/j.humimm.2023.01.010

16. Verma AK, Mechenro J, Monachesi C, Venugopal G, Catassi GN, Lionetti E, et al. Distribution of celiac disease predisposing genes HLA-DQ2 and HLA-DQ8 in the native population of southern India. *Indian J Gastroenterol*. (2022) 41:240–6. doi: 10.1007/s12664-022-01251-6

17. López Vázquez A. Influencia de las moléculas de histocompatibilidad no clásicas mica y micb en la heterogeneidad cl í nica de la enfermedad cel í aca In: Tesis Doctoral. Oviedo: University of Oviedo (2015)

18. Araya M, Oyarzun A, Lucero Y, Espinosa N, Pérez-Bravo F. DQ2, DQ7 and DQ8 distribution and clinical manifestations in celiac cases and their first-degree relatives. *Nutrients*. (2015) 7:4955–65. doi: 10.3390/nu7064955

19. Cerda-Contreras E, Ramírez-Cervantes KL, Granados J, Mena L, Núñez-Álvarez C, Uscanga L. Is celiac disease better identified through HLA-DQ8 than through HLA-DQ2 in Mexican subjects? *Rev Gastroenterol Mex*. (2018) 83:410–3. doi: 10.1016/j.rgmxen.2018.06.003

20. Rouvroye MD, Roos A, Bergkamp F, Haagen IA, van der Pol P, Neefjes-Borst EA, et al. The frequency of HLA-DQ7 in patients at risk of coeliac disease: a haplotype to be reckoned with for screening? *Hum Immunol*. (2024) 85:111158. doi: 10.1016/j.humimm.2024.111158

21. Auricchio R, Mandile R, del Vecchio MR, Scapaticci S, Galatola M, Maglio M, et al. Progression of celiac disease in children with antibodies against tissue transglutaminase and normal duodenal architecture. *Gastroenterology*. (2019) 157:413–420.e3. doi: 10.1053/j.gastro.2019.04.004

22. Rubio-Tapia A, Hill ID, Semrad C, Kelly CP, Lebwohl B. American College of Gastroenterology guidelines update: diagnosis and management of celiac disease. *Am J Gastroenterol*. (2023) 118:59–76. doi: 10.14309/ajg.0000000000002075

23. Lionetti E, Pjetraj D, Gatti S, Catassi G, Bellantoni A, Boffardi M, et al. Prevalence and detection rate of celiac disease in Italy: results of a SIGENP multicenter screening in school-age children. *Dig Liver Dis*. (2023) 55:608–13. doi: 10.1016/j.dld.2022.12.023

Frontiers in Immunology

Explores novel approaches and diagnoses to treat immune disorders.

The official journal of the International Union of Immunological Societies (IUIS) and the most cited in its field, leading the way for research across basic, translational and clinical immunology.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact