

Online Supplementary Appendix

ACKNOWLEDGEMENTS

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Supplementary Appendix Table S-1. Adverse Events Leading to Withdrawal From the Study

Dosing Group	Patient no.	TEAE?	Preferred Term	Drug Related?
Placebo	1	Yes	Diarrhea Nausea Abdominal pain - upper	Probably Probably Probably
	2	Yes	Colitis – microscopic	No
	3	Yes	Headache	Probably
0.5 mg TID	1	Yes	Basedow's Disease	Unlikely
	2	Yes	Migraine	Possibly
	3	No	Myalgia Musculoskeletal stiffness Asthenia Arthritis	No No No No
	4	Yes	Eructation Flatulence Abdominal pain – upper	Probably Probably Probably
	5	Yes	Gastroesophageal reflux disease	No
1.0 mg TID	1	Yes	Diarrhea	No
	2	Yes	Vertigo	Probably
	3	Yes	Abdominal distension Fatigue Fecal incontinence	Unlikely Unlikely Unlikely
	4	Yes	Celiac disease Gastroenteritis viral	Unlikely No
	5	Yes	Diarrhea	Possibly
2.0 mg TID	1	Yes	Anxiety	No
	2	Yes	Headache	Yes
	3	No	Arthralgia Myalgia Headache Asthenia Nausea	No No No No No
	4	No	Headache	No
	5	Yes	Memory impairment Disturbance in attention Constipation Nausea	Unlikely Unlikely Possibly Possibly
TID=three times daily; TEAE=treatment-emergent adverse event MedDRA coding system				

Supplementary Appendix Table S-2. Treatment-Emergent Adverse Events Occurring in ≥3% of Patients in any Treatment Group

	Larazotide Acetate (n=258)			
	Placebo (n=84), n (%)	0.5 mg TID (n=85), n (%)	1.0 mg TID (n=84), n (%)	2.0 mg TID (n=87), n (%)
Subjects with at least one AE	53 (63.1)	54 (63.5)	52 (61.9)	54 (62.1)
System organ class Preferred term				
Gastrointestinal disorders	21 (25.0)	20 (23.5)	28 (33.3)	27 (31.0)
Diarrhea	7 (8.3)	7 (8.2)	5 (6.0)	8 (9.2)
Nausea	9 (10.7)	4 (4.7)	7 (8.3)	7 (8.0)
Constipation	2 (2.4)	3 (3.5)	7 (8.3)	5 (5.7)
Abdominal pain	4 (4.8)	1 (1.2)	5 (6.0)	6 (6.9)
Vomiting	6 (7.1)	2 (2.4)	2 (2.4)	3 (3.4)
Abdominal pain – upper	2 (2.4)	3 (3.5)	4 (4.8)	2 (2.3)
Abdominal distention	1 (1.2)	2 (2.4)	5 (6.0)	1 (1.1)
Flatulence	0	2 (2.4)	4 (4.8)	3 (3.4)
Gastroesophageal reflux disease (GERD)	0	2 (2.4)	1 (1.2)	6 (6.9)
Dyspepsia	3 (3.6)	1 (1.2)	2 (2.4)	2 (2.3)
Infections and Infestations	25 (29.8)	23 (27.1)	23 (27.4)	18 (20.7)
Sinusitis	5 (6.0)	5 (5.9)	5 (6.0)	4 (4.6)
Nasopharyngitis	4 (4.8)	4 (4.7)	5 (6.0)	3 (3.4)
Influenza	4 (4.8)	3 (3.5)	4 (4.8)	2 (2.3)
Urinary tract infection	2 (2.4)	1 (1.2)	1 (1.2)	5 (5.7)
Gastroenteritis, viral	2 (2.4)	1 (1.2)	3 (3.6)	2 (2.3)
Upper respiratory tract infection	0	2 (2.4)	1 (1.2)	3 (3.4)
Pharyngitis streptococcal	0	3 (3.5)	1 (1.2)	0
Nervous System Disorders	10 (11.9)	8 (9.4)	6 (7.1)	12 (13.8)
Headache	8 (9.5)	2 (2.4)	5 (6.0)	6 (6.9)

General disorders and administration site conditions	2 (2.4)	4 (4.7)	5 (6.0)	8 (9.2)
Fatigue	0	1 (1.2)	3 (3.6)	6 (6.9)
Musculoskeletal and connective tissue disorders	5 (6.0)	6 (7.1)	3 (3.6)	5 (5.7)
Fatigue	0	1 (1.2)	3 (3.6)	6 (6.9)
Back pain	3 (3.6)	1 (1.2)	1 (1.2)	2 (2.3)
Skin and subcutaneous tissue disorders	5 (6.0)	5 (5.9)	3 (3.6)	3 (3.4)
Respiratory, thoracic and mediastinal disorders	4 (4.8)	4 (4.7)	2 (2.4)	5 (5.7)
Cough	3 (3.6)	0	0	0
Investigations	3 (3.6)	6 (7.1)	1 (1.2)	4 (4.6)
Low serum ferritin	1 (1.2)	3 (3.5)	0	0
Psychiatric Disorders	1 (1.2)	3 (3.5)	1 (1.2)	2 (2.3)
Patients are counted only once per category. A treatment-emergent adverse event (TEAE) was defined as an AE that occurred during double-blind treatment phase any time between the first dose and 7 days after the last dose of study drug. TEAEs coded using MedDRA version v.14.1				

Supplementary Appendix Table S-3. Adverse Events Relating to Iron Status in the Placebo and 0.5mg groups

Subject	Treatment	Baseline ferritin	week 12/end of treatment ferritin
1	0.5mg Larazotide acetate	8.5	25.3
2	0.5mg Larazotide acetate	7.0	6.8
3	0.5mg Larazotide acetate	10.7	7.3*
4	0.5mg Larazotide acetate	5.9	9.8
5	Placebo	10.2	40.6
6	Placebo	11.7	8.0

*early termination

Supplementary Appendix Figure S-1. Tight Junction Structure and Mechanism of Action of Larazotide Acetate

Larazotide acetate is a Tight Junction (TJ) regulator octapeptide inhibitor of *Vibrio Cholera* zonula occludens toxin. TJs comprise over 50 proteins, including the transmembrane proteins occluding claudin, junctional adhesion molecule (JAM), and cytoplasmic scaffolding proteins zonula occludens (ZO)-1, ZO-2, and ZO-3. Larazotide acetate inhibits the cytoskeletal rearrangement and ZO-1 redistribution caused by gliadin in epithelial cells, regulates actin rearrangement, and stabilises TJ *in vitro* in response to various stimuli, including inflammatory cytokines, bacterial products, and gliadin. Additionally, larazotide acetate inhibits transepithelial gliadin transport *in vitro* and decreases intestinal permeability and preserves barrier function *in vitro* and *in vivo*. From Martinez et al. Cellular and Molecular Basis of Intestinal Barrier Dysfunction in the Irritable Bowel Syndrome. *Gut and Liver* 2012;6(3):305-315. © Korean Association of Medical Journal Editors. <http://gutnliver.org>

Supplementary Appendix Figure S-2. No Increase or Worsening of Serum Antibody Levels

Supplementary Appendix A. The Gastrointestinal Symptom Rating Scale (CeD-GSRS)

(*Asterisks represent the 10 items on the Celiac Disease domains of the GSRS [CeD-GSRS])

This survey contains questions about how you have been feeling and what it has been like during the past week. Mark the choice that best applies to you and your situation with an "X" in the box

1.* Have you been bothered by PAIN OR DISCOMFORT IN YOUR UPPER ABDOMEN OR THE PIT OF YOUR STOMACH during the past week?

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

2. Have you been bothered by HEARTBURN during the past week? (By heartburn we mean an unpleasant stinging or burning sensation in the chest.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

3. Have you been bothered by ACID REFLUX during the past week? (By acid reflux we mean the sensation of regurgitating small quantities of acid or flow of sour or bitter fluid from the stomach up to the throat.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

4.* Have you been bothered by HUNGER PAINS in the stomach during the past week? (This hollow feeling in the stomach is associated with the need to eat between meals.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

5.* Have you been bothered by NAUSEA during the past week? (By nausea we mean a feeling of wanting to throw up or vomit.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort

- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

6.* Have you been bothered by RUMBLING in your stomach during the past week?
(Rumbling refers to vibrations or noise in the stomach.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

7.* Has your stomach felt BLOATED during the past week? (Feeling bloated refers to swelling often associated with a sensation of gas or air in the stomach.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

8.* Have you been bothered by BURPING during the past week? (Burping refers to bringing up air or gas from the stomach via the mouth, often associated with easing a bloated feeling.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

9.* Have you been bothered by PASSING GAS OR FLATUS during the past week?
(Passing gas or flatus refers to the need to release air or gas from the bowel,
often associated with easing a bloated feeling.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

10. Have you been bothered by CONSTIPATION during the past week?
(Constipation refers to a reduced ability to empty the bowels.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

11.* Have you been bothered by DIARRHEA during the past week? (Diarrhea refers to a too-frequent emptying of the bowels.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

12.* Have you been bothered by LOOSE STOOLS during the past week? (If your stools (motions) have been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being loose.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

13. Have you been bothered by HARD STOOLS during the past week? (If your stools (motions) have been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being hard.)

- No discomfort at all
- Minor discomfort
- Mild discomfort

- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

14.* Have you been bothered by an URGENT NEED TO HAVE A BOWEL MOVEMENT during the past week? (This urgent need to go to the toilet is often associated with a feeling that you are not in full control.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

15. When going to the toilet during the past week, have you had the SENSATION OF NOT COMPLETELY EMPTYING THE BOWELS? (This feeling of incomplete emptying means that you still feel a need to pass more stool despite having exerted yourself to do so.)

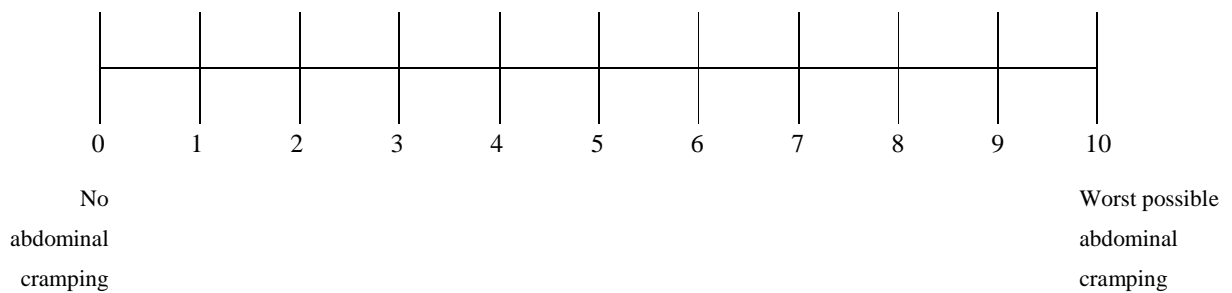
- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

Appendix B. Celiac Disease Patient Reported Outcome (CeD PRO)®

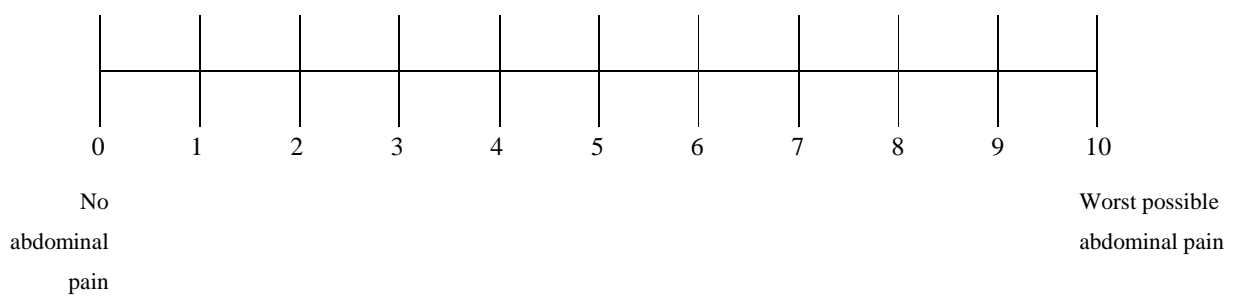
Instructions: These questions ask about how you feel each day. Please complete the daily diary every evening, at approximately the same time.

1. Thinking about your worst experience in the past 24 hours, how severe was each of the following symptoms? On the following screens, please tap a number to indicate how you felt.

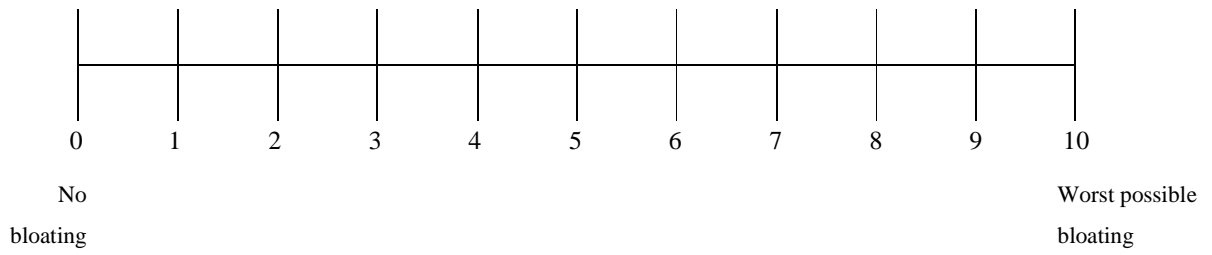
a. How severe was your abdominal cramping?



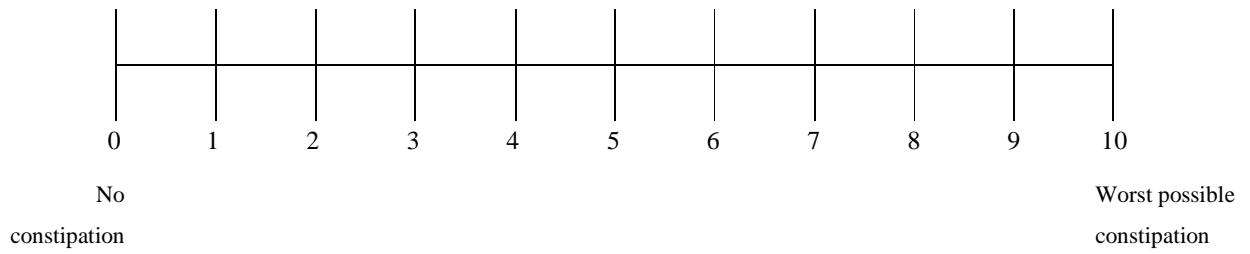
b. How severe was your abdominal pain?



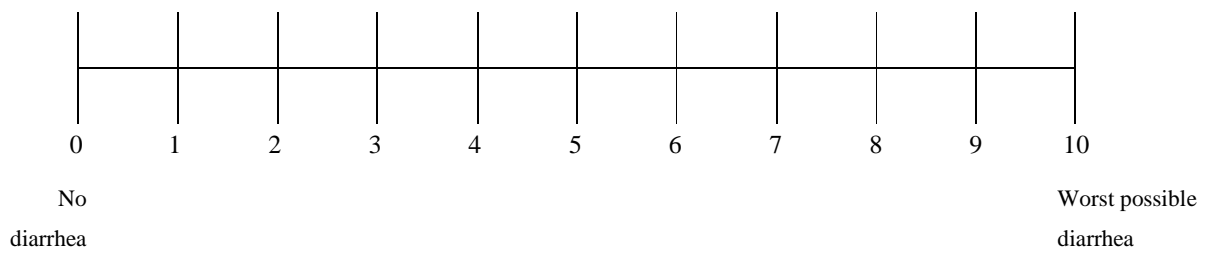
c. How severe was your bloating?



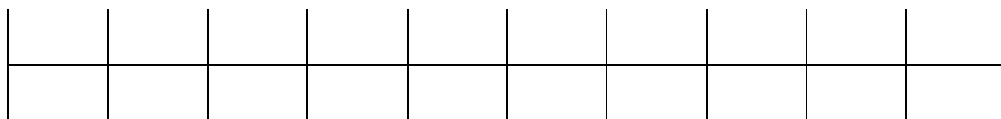
d. How severe was your constipation?



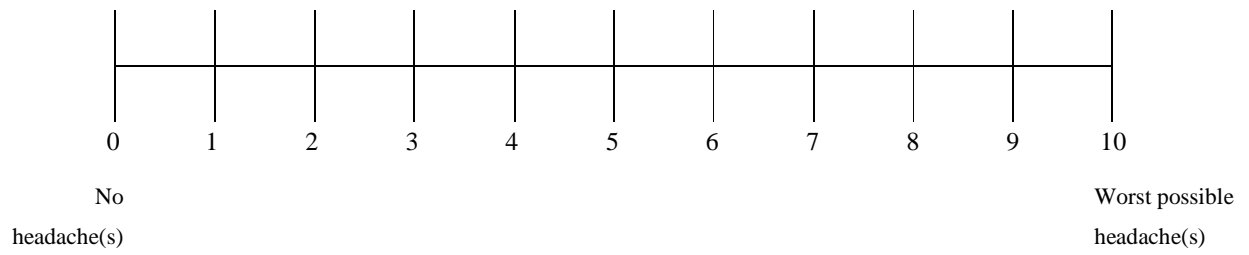
e. How severe was your diarrhea?



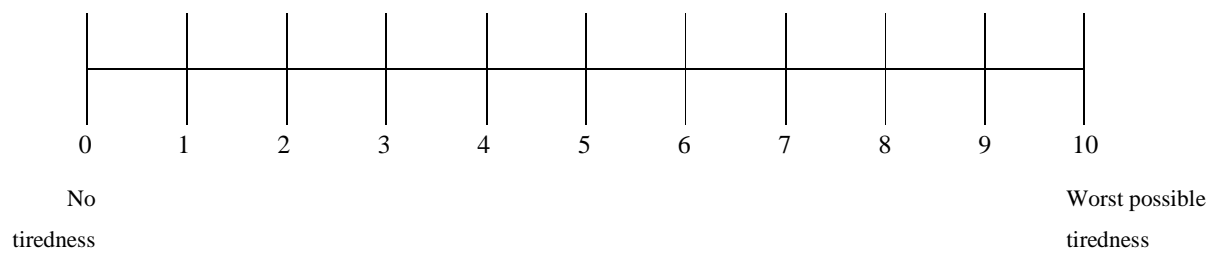
f. How severe was your gas (flatulence)?



j. How severe was your headache(s)?



k. How severe was your tiredness?



APPENDIX C. GLUTEN-FREE DIET COMPLIANCE QUESTIONNAIRE (GFDCQ)

Participant ID: _____ Date of Interview: _____

Name of Interviewer: _____

NOTE: This interview should be conducted by the study staff. Study subjects **SHOULD NOT** complete the interview directly on the interview form.

1. Are you currently following a gluten free diet?

1 Yes

0 No If no, please explain: _____

2. How confident are you that you fully understand the gluten free diet?

0 Not at all confident

1 Somewhat confident

2 Very confident

3. During the time that you were taking your study medication, did you ever knowingly consume a food item or beverage that contained gluten?

0 No

1 Yes If yes, what food? _____

Quantity of food consumed? _____

When food was consumed? _____

4. During the time that you were taking your study medication, did you eat a food or beverage that you thought was gluten free but found out later it contained gluten or was contaminated with gluten?

0 No

1 Yes If yes, what food? _____

Quantity of food consumed? _____

When food was consumed? _____

5. During the time that you were taking your study medication, were you reading ingredient labels to detect gluten in foods?

1 Yes

0 No If no, was it because you

2 Called manufacturers

3 Used only naturally gluten-free, unprocessed foods

4 Used only foods labeled gluten free

5 Bought only foods on lists of GF foods (shopper's guide from stores, support groups, websites)

6 Did not shop for food since the time you started taking your study medication

7 Other: _____

6. Since you started taking your study medication, have you been reading labels or otherwise verifying the gluten free status on any of the following items:

6a. Prescription medications

0 No If no, was this because:

88 You did not need a prescription since the you started taking your study medication

77 Other: _____

1 Yes If yes, how did you verify the gluten free status?

- 5 Called the manufacturer yourself
- 4 Asked the pharmacist to call the manufacturer
- 3 Used a website to verify
- 2 Checked with prescribing medical provider
- 77 Other _____

6b. Over the counter (OTC) medications

0 No If no, was this because:

88 You did not need OTC medications since you started taking your study medication

77 Other: _____

1 Yes If yes, how did you verify the gluten free status?

- 5 Called the manufacturer yourself
- 4 Asked the pharmacist to call the manufacturer
- 3 Used a website to verify
- 2 Checked with a medical provider
- 77 Other _____

7. During the time that you were taking your study medication, have you been reading labels...

- 3 Every time item was purchased
- 2 First time item was purchased
- 1 Intermittently when purchasing items
- 0 Never

88 Have not shopped since you started taking your study medication

77 Other: _____

8. During the time that you were taking your study medication, how did you manage cross contamination at home (check all that apply)

3 Entire household is gluten free, so I didn't worry about cross contamination (SKIP TO QUESTION 9))

8a. Cooking

2 Dedicated items for gluten free cooking (toaster/toaster oven)

1 Shared prep items (e.g., toaster/toaster oven) but cleaned between gluten free and non-gluten free foods

0 I did not manage cross contamination

8b. Food items

3 Dedicated items for gluten free foods (condiments, butter, peanut butter)

2 Shared food items, but items are in squeeze bottles (condiments, butter, peanut butter)

1 "Single dip rule" followed (clean utensil is inserted into bottle but not reinserted after touching gluten containing food item)

0 I did not manage cross contamination for food items

9. Since you started taking your study medication, have you taken Holy Communion?

0 No

1 Yes If yes, do you?

2 Take the regular communion host

3 Provide church with a gluten free communion host

4 Provide church with a low gluten communion host

Do you?

5 Drink wine from communal cup

6 Drink wine from separate cup

7 Avoid wine if bread is dipped in wine

10. During the time that you were taking your study medication, did you eat away from home (someone else handles the food prep i.e.: at a restaurant, friend's or relative's house)?

0 No (SKIP TO QUESTION 12)

Yes If yes, how many times? _____

11. When you ate away from home did you

(CHECK ALL THAT APPLY)

11a. At Restaurants

1 Identify yourself as someone with special dietary needs

2 Depend on the menu description for gluten free foods

3 Eat only in restaurants with a gluten free menu

4 Ask to speak to the Chef or manager regarding ingredients used

5 Ask that your foods be prepared on clean, uncontaminated surfaces

6 Request special prep of foods order (i.e.: no croutons, no sauces, and no bun)

7 Ask if naturally gluten-free foods (like French fries) are made in dedicated fryer/on dedicated/clean surfaces

11b. At a friend/relative's house

1 Identify yourself as someone with special dietary needs (i.e., remind friends/relatives of your diet)

2 Look at food/ask for descriptions to determine the likelihood that they are gluten free

3 Ask to speak to the person who prepared the meal regarding ingredients used

4 Ask that your foods be prepared on clean, uncontaminated surfaces

5 Bring own food

6 Do not eat

11c. At other social events

1 Identify yourself as someone with special dietary needs

2 Look at food/ask for descriptions to determine the likelihood that they are gluten free

3 Ask to speak to the host/hostess, manager or person who prepared the food regarding the ingredients used

4 Bring own food

5 Do not eat

12. During the time that you were taking study medication, did you contact a celiac support group, either local or national, for information on Gluten Free Foods?

0 No

1 Yes If yes, name of support group _____

13. Since you started taking your study medication, did you take any trips requiring you to stay away from home overnight?

0 No

1 Yes

13a. If yes, were you able to follow the gluten free diet while traveling?

1 Yes

0 No

If no was it because:

2 Could not find gluten free foods at restaurants/airport/etc

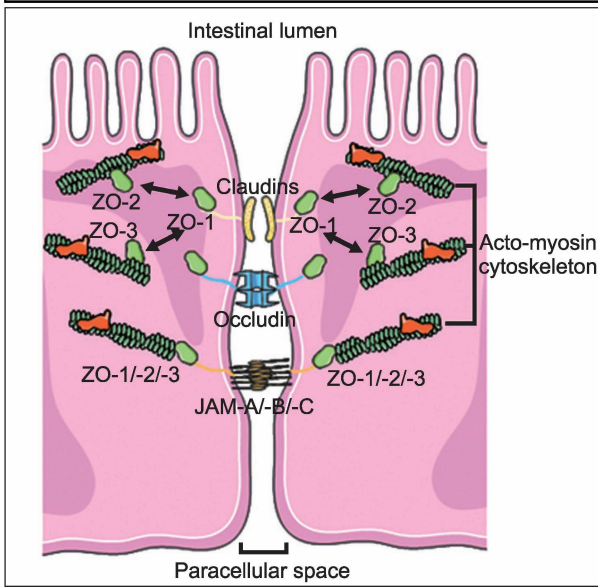
3 Could not afford gluten free alternatives

4 Gluten free foods not available during travel

5 Too much hassle/embarrassment to get gluten free foods

6 Other

External (Gliadin Peptides)

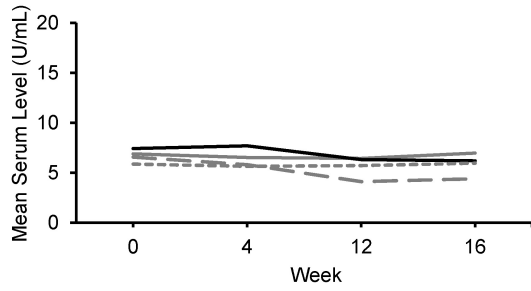


Internal (inflammatory cytokines)

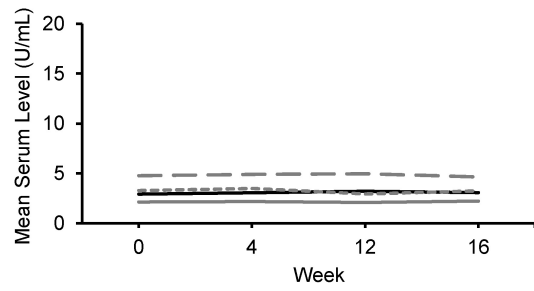
CLIN1001-012, Serum Antibody Levels

— Placebo — 0.5 mg - - - 1.0 mg - - - 2.0 mg

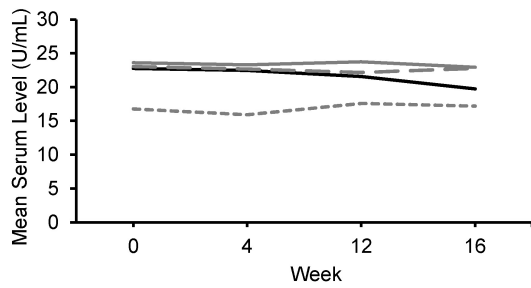
A. Serum Anti-tTG (IgA)



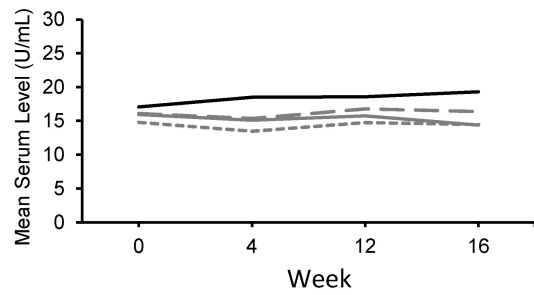
B. Serum Anti-tTG (IgG)



C. Serum Anti-DGP (IgA)



D. Serum Anti-DGP (IgG)



MITT population, n=339