Author's response to reviews

Title: IgG and IgG4 antibodies in subjects with irritable bowel syndrome: a case control study in the general population

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Author's response to reviews: see over
Dear Sir,

We thank you for the kind possibility to address the comments on our paper “IgG and IgG4 antibodies in subjects with irritable bowel syndrome: a case control study in the general population”. The responses to the referees are listed below point-by-point, and a revised manuscript has been uploaded.

We would also like to thank the reviewers for their useful comments regarding our paper.

Referee 1: Lena Öhman

General comments

1.1 We agree that the information of how gastrointestinal symptoms are described can be misinterpreted. Therefore, the text where the assessment of gastrointestinal symptoms were described was changed to: “The following information was gathered from the questionnaires: demographics, smoking habits, activity habits, diet, common diseases (asthma, bronchitis, diabetes, osteoporosis, fibromyalgia, mood disorders, heart attack, angina, cerebral stroke) (number of diseases, score 0-9), allergic rhinitis, mood disorders as measured by the Hopkins Symptom Checklist 10 (HSCL10) (score 1.0-4.0, mental distress ≥ 1.85), musculoskeletal complaints (score 0-12), and gastrointestinal symptoms of which IBS was defined according to a translation of the Rome II criteria"
“We considered that in a journal like BMC gastroenterology the Rome II criteria are well known. The text were food intake was described is extended with “The food groups cereals and meat were not included.”

The text where the analysis of IgG and IgG4 antibodies are described is changed to “The IgG antibodies against food panel (FP)5 (milk, egg, cod, wheat, soybean and peanut) and FP73 (beef, chicken, lamb and pork), as well as IgG antibodies against milk, wheat, cod, egg, chicken, beef, pork, brewers’ yeast and Candida albicans and IgG4 antibodies against milk, wheat, egg and Candida albicans were measured using the ELISA technique (Siemens Healthcare Diagnostics AS). Lower and upper detection limits for IgG antibodies were 2.0 mg/L and 200 mg/L, respectively and for IgG4 antibodies 0.2 mg/L and 50 mg/L, respectively.”

The discussion of choice of dietary assessment is included under the “Discussion section” with: “In general a FFQ can never catch the complete diet, as there necessarily will be limitations on how many food items the questionnaire can contain [2]. Additionally, as the FFQ is retrospective the method is hampered by recall bias. The subjects with IBS might recall better than subjects without IBS, as subjects with IBS might consider what kind of food intake causes pain/discomfort. Food diaries are more exact. However, in surveys with many participants it is mostly too demanding to use food diaries.”

A discussion of our way to determine food intolerance is included in the manuscript: “As we found no reason to use serum levels of food- and yeast-specific IgG and IgG4 antibodies to determine food and yeast hypersensitivity, this may not be the best way to determine food hypersensitivity. There may be other components than proteins of the offending food that causes the hypersensitivity. Or there might be other material than sera where the antibodies can be detected with higher sensitivity, such as faecal samples or mucosal biopsies. In this study there was however no access to faecal samples or mucosal biopsies.”
The “Background section” is changed to “About two-thirds of subjects with irritable bowel syndrome (IBS) relate their symptoms to food intake [3,4]. Most such subjects modify their diet, and a portion of these subjects have an inadequate diet [3]. IgE-mediated food allergy is uncommon and explains the symptoms in only a minority of subjects with IBS [5]. IgG antibodies are purposed to give a more delayed response to specific antigens than IgE antibodies [6], and the subclass IgG4 might induce histamine release, like IgE antibodies, and is found to be synthesized under influence of T-helper 2 cytokines like IgE antibodies [7]. One study found higher titres for some food-specific IgG antibodies in subjects with IBS compared to controls, but no significant correlation between symptom severity and IgG antibody titres [8]. Other studies have shown reduced IBS symptoms when excluding foods for which IgG and IgG4 antibodies were raised [9-11]. The role of IgG-mediated food hypersensitivity is inconclusive [12].

Intestinal colonization of Candida albicans has been described as one cause of IBS symptoms by the popular press [13]. Refined carbohydrates are proposed to facilitate Candida growth in the gastrointestinal tract [14]. One study showed a higher intake of carbohydrates in subjects with IBS compared to controls [15]. The role of Candida albicans in the aetiology of IBS remains unclear.

We hypothesised that subjects with IBS would have IgG- and IgG4-mediated food and yeast hypersensitivity. The primary aim of this case-control study was to compare food and yeast IgG and IgG4 antibodies in subjects with and without IBS in an unselected general population and to relate these values to food intake. Additionally, we wanted to assess associations between the severity of symptoms and levels of IgG and IgG4 antibodies within the IBS population.”
1.6 The multiple imputation description is changed with “. For the regression analyses, missing values were handled by multiple imputations. All variables to be included in the regression analyses were included in the imputation model, which consisted of 62 variables including demographics, dietary intake, common diseases, common blood tests and food and yeast IgG and IgG4 antibodies. Right skewed variables were log-transformed before use in the imputation model, and 20 datasets were created.”

Referee 2: Thierry Piche

Major points

1.1 We agree that the stratification between IBS subgroups is interesting. We have now included the following text in the “Results section”: Multivariate regression analyses on IBS subgroups versus subjects without IBS showed significant higher values of IgG antibodies against Candida albicans in diarrhoea predominant and alternating IBS. Significant lower values of IgG antibodies against beef and significant higher values against fish were found in constipation predominant IBS. IgG4 antibodies and IgG antibodies against food panels were not associated with subgroups of IBS.

1.2 See answer referee 1, 1.1.

1.3 The patients had no colonoscopy with histological analysis to exclude other diseases with symptoms similar to those seen in subjects with IBS.

1.4 We agree that stratification in IBS subjects with and without allergic rhinitis is of interest and this was also performed before the first upload of the manuscript. As the number of subjects with allergic rhinitis was just 59 and therefore the number of variables to be included in the logistic regression model is just six when following the rule of thumb of ten cases per variables, we did not include these data in the manuscript. There was however no principal differences with respect to results of the analysis between IBS
subjects with and without allergic rhinitis, but the results should be interpreted with caution as the sample size is too small for the use in a logistic regression analyses.

Minor points

2.1 The “Discussion section” is extended with the following text: “In addition to the antigens tested in this study, there are many other antigens that could be associated with IBS and/or severity of symptoms. The antigens used in our study were selected to cover the most common staple foods which are described as offending to subjects with IBS. Additionally, possible inhalants could have been included.”

2.2 The “Background section” is extended with an explanation on why IgG4 was an interesting IgG subclass to analyse.

2.3 We had no information on IBS duration, origin of IBS and we did not exclude celiac disease. Regards medication, the information on histamine antagonist and mast cell stabilisers was inadequate.

References


I thank you in advance.

On behalf of the authors
Yours sincerely

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