

# Linköping University Post Print

## Screening-detected and symptomatic untreated celiac children show similar gut microflora-associated characteristics

Bo Tjellstrom, Lars Stenhammar, Lotta Högberg, Karin Fälth-Magnusson, Karl-Eric Magnusson, Tore Midtvedt, Tommy Sundqvist and Elisabeth Norin

N.B.: When citing this work, cite the original article.

### Original Publication:

Bo Tjellstrom, Lars Stenhammar, Lotta Högberg, Karin Fälth-Magnusson, Karl-Eric Magnusson, Tore Midtvedt, Tommy Sundqvist and Elisabeth Norin, Screening-detected and symptomatic untreated celiac children show similar gut microflora-associated characteristics, 2010, SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY, (45), 9, 1059-1062.

<http://dx.doi.org/10.3109/00365521.2010.483738>

Copyright: Informa Healthcare

<http://informahealthcare.com/>

Postprint available at: Linköping University Electronic Press

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-60499>

November 5<sup>th</sup>, 2009

## **Screening-detected and symptomatic untreated celiac children show similar gut microflora-associated characteristics**

Tjellström Bo<sup>\*§</sup>, Stenhammar Lars<sup>§□</sup>, Högberg Lotta<sup>§□</sup>, Fälth-Magnusson Karin<sup>□</sup>, Magnusson Karl-Eric<sup>#</sup>, Midtvedt Tore<sup>\*</sup>, Sundqvist Tommy<sup>#</sup>, Norin Elisabeth<sup>\*</sup>

*\*Department of Microbiology, Tumor and Cell biology,, Karolinska Institute, Stockholm; §Pediatric Clinic, Norrköping Hospital, Norrköping; □Division of Pediatrics and #Division of Medical Microbiology, Department of Clinical and Molecular Medicine, Linköping University, Linköping, Sweden*

No conflicts of interest exist.

*Running title:* Gut microflora-associated characteristics in celiac disease

*Corresponding author:* Bo Tjellström, MD, PhD, Department of Microbiology, Tumor and Cell biology, Karolinska Institute, Nobels väg 16, SE-171 77 Stockholm, Sweden.

Email address: [bo.tjellstrom@ki.se](mailto:bo.tjellstrom@ki.se)

## ABSTRACT

*Aim:* The aim of this study was to investigate the metabolic function of intestinal microflora in children with screening-detected celiac disease (CD) to see if there is an aberrant gut flora in screening-detected CD similar to symptomatic CD and contrary to healthy controls.

*Methods:* As part of a Swedish multicenter screening for CD, 912 12 year-old children were screened with serum anti-human tissue transglutaminase-IgA. Small bowel biopsy specimens from children with positive serology revealed 17 individuals with CD. The functional status of the intestinal microflora was evaluated by gas-liquid chromatography of short chain fatty acids (SCFAs) in fecal samples. Our previously published findings in children with symptomatic CD and healthy controls were used as comparison.

*Results:* The children with screening-detected CD had a similar fecal SCFA profile to children with symptomatic CD, and significantly different from that in healthy children.

*Conclusions:* This is the first study on SCFA patterns in fecal samples from children with screening-detected CD. The similarity of the fecal SCFA profile in screening-detected and symptomatic CD indicates common pathogenic mechanisms. This could open the way for new therapeutic or prophylactic measures based on novel biological principles.

*Key words:* Celiac disease; children; screening; short chain fatty acids

## INTRODUCTION

The diagnosis of celiac disease (CD) in individuals with symptoms suggestive of malabsorption is based on the finding of characteristic abnormalities of the small bowel mucosa (1). These intestinal changes and clinical symptoms should improve on a gluten-free diet (GFD) according to criteria formulated by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (2), North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) (3) and American Gastroenterological Association (AGA) (4). Since the advent of serological celiac markers in the 1970s, numerous screening studies have shown the existence of more or less symptom-free cases of CD, so called silent CD. “The celiac iceberg” has been used as a metaphor for the diagnostic situation with clinically overt CD cases being above the waterline and silent cases forming part of the submerged iceberg (5).

We have previously reported the finding of a significantly different short chain fatty acid (SCFA) profile in fecal samples from children with symptomatic CD compared to healthy controls, reflecting an aberrant gut microflora (6). The aim of the present study was to study the metabolic function of intestinal microflora in children with screening-detected CD compared to symptomatic CD and healthy controls. We found that the fecal SCFA profile in screening-identified,

non-symptomatic individuals with CD was similar to children with symptomatic CD but differed significantly from that in healthy controls.

## PATIENTS AND METHODS

### *Patients*

The study was performed in the town of Norrköping, Sweden, between September 2005 and June 2006 as part of a Swedish multicenter CD screening study on 12 year-old schoolchildren (ETICS, Exploring the Iceberg of Celiacs in Sweden) (7). A total of 1,350 children in Norrköping were invited to take part in the study, of whom 912 delivered serum samples. Eight children had previously diagnosed CD and were excluded from the study. Serum samples were analyzed for anti-human tissue transglutaminase of isotypes IgA and IgG (Celikey<sup>®</sup>, Phadia GmbH, Freiburg, Germany) and total serum IgA. All children with increased antibody levels (n=24) had small bowel biopsy performed by capsule or endoscopically. Mucosal specimens were light-microscopically graded according to Marsh (8). Seventeen children (8 boys, 9 girls; median age 12 years; all born in 1993) had small bowel enteropathy consistent with CD. Further details on case ascertainment have recently been published (7).

None of the children in this study had been treated with antibiotics within 3 months prior to fecal sampling. The children delivered fecal samples when they were on a normal gluten-containing diet. The fecal samples were frozen

immediately or at least within 20 min of passage, and stored at  $-20^{\circ}\text{C}$  pending analysis.

For comparison we used our previously reported results from SCFA analysis of fecal samples from 36 children (12 boys, 24 girls; median age 4.7 yr, range 0.7 – 10 yr) with symptomatic CD at presentation and 42 healthy children (23 boys, 19 girls; median age 3.0 yr, range 0.25 – 5.75 yr) (6).

#### *Microflora-associated characteristics*

A microflora-associated characteristic (MAC) is defined as the recording of any anatomical structure, physiological, biochemical or immunological function in an organism that has been influenced by the microflora (9). In the present study we used the fecal SCFA pattern as MAC.

SCFA analyses were performed on fecal samples at the Karolinska Institute, Stockholm, Sweden. The fecal material was homogenized after addition of distilled water containing 3 mmol/L of 2-ethylbutyric acid (=internal standard) and  $\text{H}_2\text{SO}_4$  (0.5 mmol/L). A 2 mL sample of the homogenate was vacuum distilled according to the method of Zijlstra *et al.* (10), modified by Höverstad *et al.* (11). The distillate was analyzed using gas-liquid chromatography and quantitated using internal standardization. Flame ionization detection was employed. The results were expressed in mmol/kg wet weight. The following

SCFAs were analyzed: acetic acid; propionic acid; *i*-butyric acid; *n*-butyric acid; *i*-valeric acid; *n*-valeric acid; *i*-caproic acid; and *n*-caproic acid.

### *Statistical analysis*

Statistical analysis was performed using Student's *t*-test with Bonferroni correction. Caproic acid results showed a skewed distribution and were thus tested by the Wilcoxon rank sum test. A *p* value less than 0.05 was considered significant.

### *Ethical considerations*

The study was approved by the Research Ethics Committees of Umeå University and Linköping University, Sweden.

## RESULTS

A fecal sample was obtained from 16 of 17 screening-detected celiac children. Fecal material was too little to permit analysis in one case. Table 1 presents the results of SCFA analysis of fecal samples from the remaining 15 screening-detected celiac cases compared with our previously reported results from symptomatic celiac children with untreated disease and healthy controls (6). The SCFA pattern was similar in screening-detected CD cases to children with untreated, symptomatic disease apart from acetic acid and *i*-caproic acid, which were significantly higher in the screening-detected children ( $p < 0.05$ ).

## DISCUSSION

In the present study we found that the fecal SCFA pattern in children with untreated, screening-detected, asymptomatic CD differed significantly from healthy reference children. This finding corresponds to our previously published results with significant differences between children with untreated, symptomatic CD and healthy controls (6). These results could indicate that children with asymptomatic and symptomatic CD have a common “celiacogenic” gut microflora, which differs from that in healthy children. Furthermore, celiac children on gluten-free diet still have the same SCFA pattern as do children with untreated CD (6). An aberrant gut flora in patients with CD might thus reflect a genuine phenomenon of CD that is not affected by diet, gut inflammation or the autoimmune status of the patient.

We recently reported striking differences in some microflora-associated characteristics seen in fecal samples from non-celiac, first-degree relatives of celiac children compared to healthy adults (12). The pattern of microflora-associated characteristics in non-celiac relatives also differed from that in the celiacs. One hypothesis is that non-celiac relatives have a different gut microflora compared to their celiac family members, a “celiacoprotective” gut microflora, thereby protecting them from developing CD.

The mean age of the untreated, screening-detected celiac children in the present study was 12 years. The untreated symptomatic celiac children and the children in the control group were younger. However, we consider the age difference between the groups studied to be of minor importance since it has been shown that children have already established their permanent gut microflora by 2 years-of-age (13). Furthermore, we have reported that the SCFA pattern does not differ significantly in celiac children less than 6 years-of-age compared to those between 6 and 10 (6).

Most children with screening-detected CD consider themselves to be symptom-free, at least until they experience improvement after introduction of GFD (14). Symptom-free, screening-detected celiac patients, who do not experience improvement on GFD, cannot be classified according to the ESPGHAN, NASPGHAN or AGA criteria, which require clinical improvement on GFD (2-4). Thus, the definition of screening-identified CD remains a problem (15). Moreover, celiac individuals identified at screening cannot be expected to comply with a life-long GFD as well as symptomatic cases (16). We do not know for sure that screening-identified cases of CD run the same risk for long-term complications as do symptomatic cases (1,17). It might even be that the pathogenesis of clinically identified CD differs from that of screening-detected disease. However, the results of the present study indicate that screening-

detected CD and symptomatic CD share a common pathogenic feature regarding the metabolic function of the gut flora.

CD is still an under-diagnosed chronic disease (18) and many undiagnosed cases can only be detected by screening. The present study suggests that a common pathogenic mechanism may operate in symptomatic and silent CD. If this is confirmed in other studies, individuals with untreated silent CD may be exposed to the same risk for long-term complications as celiacs with symptomatic disease (1,17,18). This is an argument in favour of a mass-screening for CD in children (19-21), although many aspects of widespread screening must be considered (15,22).

In conclusion, this is the first study of the fecal SCFA pattern in children with screening-detected CD. Our findings indicate that screening-detected CD and CD diagnosed on clinical grounds have common pathogenic mechanisms at the microbial level. Further research on the intestinal bacterial flora in CD is needed to unravel this pathogenic mechanism. A better understanding of the impact of the gut microflora may hopefully open the possibility of treatment of CD, based on novel biological principles, or even prophylaxis against the disease (23).

## ACKNOWLEDGEMENTS

We express our sincere gratitude to research nurses Gudrun Hellgren and Ann-Catrin Andersson and the staff at the Pediatric Clinic in Norrköping for their help in collecting the fecal samples, and to Anna-Karin Persson at Karolinska Institute for excellent analytical work with the fecal samples. The study was financially supported by the Swedish Research Council (Medicine), FORSS (the Health Research Council in the South-east of Sweden), FORMAS (the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning) and the Bengt E Gustafsson Fund.

## REFERENCES

1. Di Sabatino A, Corazza GR. Coeliac disease. *Lancet* 2009;373:1480-93.
2. Walker-Smith JA, Guandalini S, Schmitz J, Shmerling DH, Visakorpi JK. Revised criteria for diagnosis of coeliac disease. *Arch Dis Child* 1990;65:909-11.
3. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S et al. Guideline for the diagnosis of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2005;40:1-19.
4. Rostom A, Murray JA, Kagnoff MF. American Gastroenterological Association (AGA) institute technical review on the diagnosis and management of celiac disease. *Gastroenterology* 2006;131:1981-2002.
5. Logan RFA. Problems and pitfalls in epidemiological studies of coeliac disease. In: Auricchio S, Visakorpi JK, eds. *Common food intolerances 1: Epidemiology of coeliac disease*. Basel: Karger, 1992, p 14-24.
6. Tjellström B, Stenhammar L, Högberg L, Fälth-Magnusson K, Magnusson K-E, Midtvedt T et al. Gut microflora associated characteristics in children with celiac disease. *Am J Gastroenterol* 2005;100:2784-8.
7. Myléus A, Ivarsson A, Webb C, Danielsson L, Hernell O, Högberg L et al. Celiac disease revealed in 3 % of Swedish 12-year-olds born during an epidemic. *J Pediatr Gastroenterol Nutr* 2009;49:170-6.
8. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('coeliac sprue'). *Gastroenterology* 1992;102:330-54.
9. Midtvedt T, Björneklett A, Carlstedt-Duke B, et al. The influence of antibiotics upon microflora associated characteristics in man and animals. In: Wostman BS editor. *Germfree research; microflora control and its application to the biochemical sciences*. Progress in clinical and biological Research, vol. 181. New York: Alan R. Liss Corporation Inc., 1985:241-4.

10. Zijlstra JB, Beukema J, Wothers BG, Byrne BM, Groen A, Dankert J. Pretreatment methods prior to gas chromatographic analysis of volatile fatty acids from fecal samples. *Clin Chim Acta* 1977;78:243-50.
11. Höverstad T, Björneklett A. Short-chain fatty acids and bowel functions in man. *Scand J Gastroenterol* 1984;19:1059-65.
12. Tjellström B, Stenhammar L, Högberg L, Fälth-Magnusson K, Magnusson K-E, Midtvedt T et al. Gut microflora associated characteristics in first-degree relatives of children with celiac disease. *Scand J Gastroenterol* 2007;42:1204-8.
13. Midtvedt A-C. The establishment and development of some metabolic activities associated with the intestinal microflora in healthy children. Medical dissertation. Karolinska Institute, Stockholm, Sweden, 1994.
14. Fasano A. Clinical presentation of celiac disease in the pediatric population. *Gastroenterology* 2005;128:S68-S73.
15. Hoffenberg EJ. Should all children be screened for celiac disease? *Gastroenterology* 2005;128:S98-S103.
16. Fabiani E, Taccari LM, Räscht I-M, Di Giuseppe S, Coppa GV, Catassi C. Compliance with gluten-free diet in adolescents with screening-detected celiac disease: a 5-year follow-up study. *J Pediatr* 2000; 136:841-3.
17. Green PHR, Cellier C. Celiac disease. *N Engl J Med* 2007;357:1731-43.
18. Rubio-Tapia A, Murray JA. Celiac disease beyond the gut. *Clin Gastroenterol Hepatol* 2008;6:722-3.
19. Ravikumara M, Nootigattu VKT, Sandhu BK. Ninety percent of celiac disease is being missed. *J Pediatr Gastroenterol Nutr* 2007;45:497-9.
20. Fasano A. Should we screen for coeliac disease? Yes. *BMJ* 2009;339:b3592.
21. Evans KE, McAllister R, Sanders DS. Should we screen for coeliac disease? No. *BMJ* 2009;339:b3674.
22. Mariné M, Fernández-Banares F, Alsina M, Farré C, Cortijo M, Santaolalla R et al. Impact of mass screening for gluten-sensitive enteropathy in working population. *World J Gastroenterol* 2009;15:1331-8.
23. Neish AS. Microbes in gastrointestinal health and disease. *Gastroenterology* 2009;136:65-80.

Table 1. Short chain fatty acid levels of children with untreated screening-detected celiac disease, celiac children with untreated symptomatic disease and healthy controls.

Short chain fatty acids			
Type of acid §	Untreated screening-detected CD	Untreated symptomatic CD	Healthy controls
Acetic acid	76*** 15 (27)	50*** 36 (22)	25 114 (6.7)
Propionic acid	11 15 (4.8)	14 36 (6.2)	11 114 (5.9)
<i>i</i> -butyric acid	2.2 15 (1.7)	2.3*** 36 (1.0)	1.6 114 (1.1)
<i>n</i> -butyric acid	16 15 (8.2)	15 36 (8.2)	15 114 (11)
<i>i</i> -valeric acid	2.9 15 (3.1)	3.0** 36 (1.4)	2.1 114 (1.6)
<i>n</i> -valeric acid	1.8 15 (1.4)	1.7 36 (1.0)	1.4 114 (1.2)
<i>i</i> -caproic acid	0.8* 15 (1.1)	0.3 36 (0.5)	0.2 114 (0.4)
<i>n</i> -caproic acid	0.7 15 (1.2)	0.2 36 (0.3)	0.2 114 (0.3)
Total SCFA	111*** 15 (42)	86*** 36 (31)	57 114 (19)

There were 15 children with untreated, screening-detected CD, 36 children with untreated, symptomatic CD and 42 healthy control children. Untreated children delivered fecal samples on 1 occasion, healthy controls on 1 - 4 occasions.

SCFA = short chain fatty acid; CD = celiac disease; n = number of fecal samples analyzed.

§ Results are presented as Mean (mmol/kg feces), No. of samples, (SD).

Significant difference *versus* healthy controls: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

