

Mucin Genes Expression in the Intestine of Crohn's Disease Patients: a Systematic Review and Meta-analysis

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ABSTRACT

Background & Aims: The mucus layer of the intestinal tract is the main barrier between luminal microbes and the mucosa, and has an essential role in the body defense mechanisms. Previous research could not establish consistent results for mucin genes expression in Crohn's disease (CD) patients. In this meta-analysis we looked at the mucin expression in CD patients and compared it with healthy controls.

Method. English medical literature searches were conducted for mucin expression in the mucosa of the ileum and colon of CD patients and compared it with normal mucosa. Case-control studies were included. Meta-analysis was performed by using Comprehensive meta-analysis software. Pooled odds ratios and 95% confidence intervals were calculated.

Results. We found 160 eligible studies. Twenty studies were rejected because they have been performed in animals or did not have full text, and 134 studies were excluded because of language, being editorials, review articles, or because of duplications. We were left with 6 case-control studies from 4 countries that fulfilled the inclusion criteria, published till 31.12.2015. No significant heterogeneity was demonstrated: $Q = 149.256$, $df(Q) = 40.00$, $I^2 = 73.2\%$ (less than 75%). We found a decrease of 34% in the total mucin expression in CD patients (Odds Ratio 0.660, 95% CI 0.486-0.897, $P = 0.008$). We also found a significantly decreased expression in CD patients for MUC5AC, MUC5B and MUC7.

Conclusion. We demonstrated a global decrease in mucin expression in CD patients when compared with healthy controls.

Key words: Mucin – MUC – gene expression – Crohn's disease.

Abbreviations: CD: Crohn's disease; IMH: Immunohistochemistry; ISH: In situ hybridization; MUC: mucin; SNP: single nucleotide polymorphism; UACL: ulcer-associated cell lineage.

INTRODUCTION

The mucus layer of the intestinal tract is the main barrier between luminal microbes and the mucosa, and has an essential role in body defense mechanisms [1]. Mucins, high-molecular-weight glycoproteins, are heavily glycosylated, and are responsible for the viscosity of the mucus layer. Change in the mucin secretion may be a primary event in Crohn's disease (CD) or secondary to inflammation. Several studies looked at mucins expression, comparing involved

mucosa of CD patients with normal mucosa of patients or healthy controls.

There are 21 mucin (*MUC*) genes in the human genome that encode two types of mucins, secreted and membrane-bound mucins [1]. The main mucin secreted in the intestine is MUC2, but most of the membrane-bound mucins are also expressed in the small and large bowel [2]. Membrane-bound mucins are involved in cell signalling and important cellular processes such as immune modulation, growth, adhesion and motility. The more glycosylated side chains and the more monosaccharides per chain, the higher is the viscosity and protection ability of mucins.

Previous research could not establish consistent results for mucin genes expression in CD patients [1]. Most of the mucin genes had decreased expression of RNA and some also for the protein; some trials compared involved ileal mucosa with normal mucosa of the same patients, and some with healthy

controls. Thus, we performed a meta-analysis of these studies, to establish the knowledge as a basis for further investigation.

There is a normal ongoing process of mucin degradation in equilibrium with synthesis. Thus, bacteria are usually absent from the mucin inner layer [3]. Disruption of the mucus layer exposes the mucosa and the immune system to bacteria, fungi, viruses and toxins, and may have a significant role in the pathogenesis of CD.

In this meta-analysis we looked at studies which described mucin expression in CD patients and compared it with healthy controls.

METHODS

Search strategy

English medical literature searches were conducted for mucin expression in the mucosa of the involved ileum and colon of CD patients, as compared with normal mucosa of CD patients or healthy controls. Searches were performed through December 31, 2015, using MEDLINE, PubMed, Scopus, EMBASE, and CENTRAL. Search terms were: mucin and Crohn's disease. Hand search of articles references was also performed. Only fully published human studies in English were included (Fig. 1).

Study selection

Case-control studies comparing mucin expression in the ileal or colonic mucosa in patients with CD were included. We selected only studies that used standard immunohistochemistry (IMH) with antibodies against mucin proteins or methods for measuring RNA encoded by mucins axons. We looked at all kinds of mucins that have been studied, in most cases more than one in a single study.

Data extraction

Mucin gene expression in the ileal or colonic mucosa was compared quantitatively between involved (inflamed) or normal mucosa. In the first run we considered every study where more than one mucin was compared as a composite of several studies, and calculated all the sub-studies together.

Then, in nested calculations, we isolated comparisons of different mucins, combined the sub-studies of different papers.

When studies reported immunohistochemical or in situ hybridization (ISH) data in qualitative measures (such as means or averages) we looked at tables and figures where we could count the positive cases.

Statistical analysis

The meta-analysis was performed by using the Comprehensive meta-analysis software (Version 3, Biostat Inc., Englewood, NJ, United States). Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to compare mucin expression in individual studies.

Heterogeneity between studies was evaluated using the Cochran Q-test, and it was considered to be present if the Q-test P value was less than 0.10. I^2 statistic was used to measure the proportion of inconsistency in individual studies, with $I^2 > 75\%$ representing substantial heterogeneity. We also calculated a potential publication bias.

RESULTS

Altogether we found 160 eligible studies. Twenty studies were rejected because they were performed in animals or did not have full text, and 134 studies were excluded because of language, being editorials, review articles, or because of duplications. We were left with six case-control studies from four countries, comparing mucin expression in CD patients and healthy controls that fulfilled the inclusion criteria, published till 31.12.2015 [4-9] (Fig. 1, Table I). There were 49 sub-studies (stratifying data according to the kind of MUC expressed). Funnel plot denied a significant publication bias (Fig. 2).

Weiss et al. could not demonstrate any change in MUC2 and MUC3 expression between patients with active CD and controls normal mucosa [4]. Hanski et al. demonstrated an increased expression of MUC2 protein by IMH in 16 mucosal biopsies of CD patients in comparison with normal controls, but no increase in RNA expression by ISH [5]. This observation was due to the alteration of the post-transcriptional

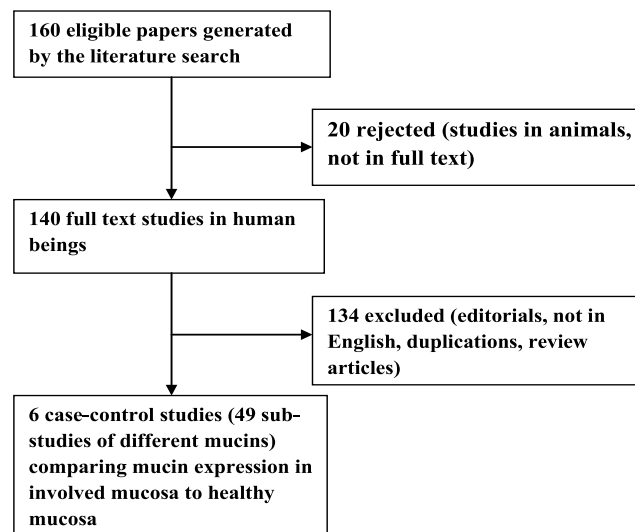


Fig. 1. Flow chart of the articles identified for the meta-analysis.

Table I. Studies comparing mucin expression in the involved ileal and colonic mucosa of CD patients with healthy mucosa

Author	Country	Year	Subgroup - substudies	CD N	Mucin expression N	Control N	Mucin expression N
Weiss AA [4]	USA	1996	MUC2 RNA involved ileal and colonic CD	5	5	7	7
Weiss AA [4]	USA	1996	MUC3 RNA involved ileal and colonic CD	5	5	7	7
Hanski C [5]	Germany	1999	MUC2 ISH>+ involved area in colonic CD	16	16	13	13
Hanski C [5]	Germany	1999	MUC2 IMH>x9 involved area in colonic CD	16	10	13	0
Buisine MP [6]	France	1999	MUC1 RNA>0.2 healthy area in CD	16	7	13	6
Buisine MP [6]	France	1999	MUC1 RNA>0.2 involved area in CD	15	6	13	6
Buisine MP [6]	France	1999	MUC2 RNA>0.3 healthy area in CD	15	4	14	6
Buisine MP [6]	France	1999	MUC2 RNA>0.3 involved area in CD	15	3	14	6
Buisine MP [6]	France	1999	MUC3 RNA>0.5 healthy area in CD	16	3	14	7
Buisine MP [6]	France	1999	MUC3 RNA>0.5 involved area in CD	15	1	14	7
Buisine MP [6]	France	1999	MUC4 RNA>0.4 healthy area in CD	16	2	14	7
Buisine MP [6]	France	1999	MUC4 RNA>0.4 involved area in CD	15	2	14	7
Buisine MP [6]	France	1999	MUC5AC RNA>0.06 healthy area in CD	15	3	14	7
Buisine MP [6]	France	1999	MUC5AC RNA>0.06 involved area in CD	15	1	14	7
Buisine MP [6]	France	1999	MUC5B RNA>0.1 healthy area in CD	14	2	14	11
Buisine MP [6]	France	1999	MUC5B RNA>0.1 involved area in CD	15	4	14	11
Buisine MP [6]	France	1999	MUC6 RNA>0.1 healthy area in CD	15	5	14	5
Buisine MP [6]	France	1999	MUC6 RNA>0.1 involved area in CD	15	5	14	5
Buisine MP [7]	France	2001	MUC1 ISH>+ healthy area in CD	19	3	14	6
Buisine MP [7]	France	2001	MUC1 ISH>+ involved area in CD	19	14	14	6
Buisine MP [7]	France	2001	MUC2 ISH>+ healthy area in CD	19	11	14	6
Buisine MP [7]	France	2001	MUC2 ISH>+involved area in CD	19	18	14	6
Buisine MP [7]	France	2001	MUC 2 IMH>+ healthy area in CD	2	2	2	2
Buisine MP [7]	France	2001	MUC 2 IMH>+ involved area in CD	9	9	2	2
Buisine MP [7]	France	2001	MUC3 ISH>+ healthy area in CD	19	11	14	6
Buisine MP [7]	France	2001	MUC3 ISH>+ involved area in CD	19	18	14	6
Buisine MP [7]	France	2001	MUC4 ISH>+ healthy area in CD	19	11	14	7
Buisine MP [7]	France	2001	MUC 4 ISH>+ involved area in CD	19	18	14	7
Buisine MP [7]	France	2001	MUC5AC ISH>+ healthy area in CD	19	0	14	7
Buisine MP [7]	France	2001	MUC5AC ISH>+ involved area in CD	19	6	14	7
Buisine MP [7]	France	2001	MUC5AC IMH>+ healthy area in CD	2	0	2	0
Buisine MP [7]	France	2001	MUC5AC IMH>+ involved area in CD	9	6	2	0
Buisine MP [7]	France	2001	MUC5B ISH>+ healthy area in CD	19	0	14	7
Buisine MP [7]	France	2001	MUC5B ISH>+ involved area in CD	19	4	14	7
Buisine MP [7]	France	2001	MUC5B IMH>+ healthy area in CD	2	0	2	0
Buisine MP [7]	France	2001	MUC5B IMH>+ involved area in CD	9	6	2	0
Buisine MP [7]	France	2001	MUC6 ISH>+ healthy area in CD	19	0	14	7
Buisine MP [7]	France	2001	MUC6 ISH>+involved area in CD	19	4	14	7
Buisine MP [7]	France	2001	MUC6 IMH>+ healthy area in CD	2	0	2	0
Buisine MP [7]	France	2001	MUC6 IMH>+ involved area in CD	9	6	2	0
Buisine MP [7]	France	2001	MUC7 ISH>+ healthy area in CD	10	0	14	7
Buisine MP [7]	France	2001	MUC7 ISH>+involved area in CD	10	0	14	7
Kaneko Y [8]	Japan	2008	MUC5AC IMH>1 involved area in ileal CD	19	19	5	0
Kaneko Y [8]	Japan	2008	MUC6 IMH>1 involved area in ileal CD	19	19	5	0
Kaneko Y [8]	Japan	2008	MUC2 IMH>1 involved area in ileal CD	19	11	5	5
Hensel KO [9]	Germany	2014	MUC1 RNA>x3 involved area in CD	5	5	5	0
Hensel KO [9]	Germany	2014	MUC1 RNA>x3 healthy area in CD	5	5	5	0
Hensel KO [9]	Germany	2014	NUC2 RNA>x3 involved area in CD	5	0	5	5
Hensel KO [9]	Germany	2014	MUC2 RNA>x3 healthy area in CD	5	5	5	0

IMH: immunohistochemistry; ISH: in situ hybridization; CD: Crohn's disease; N: number of patients

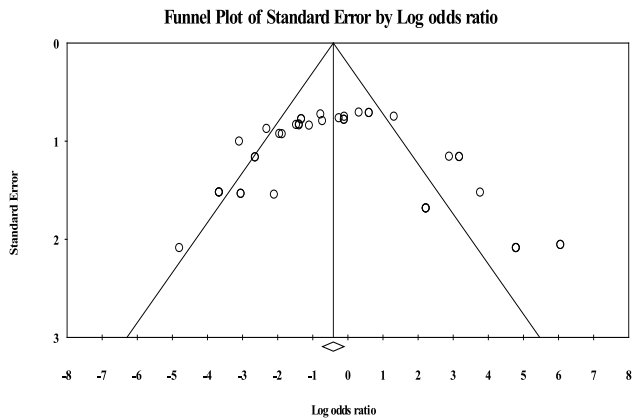


Fig. 2. Funnel plot for publication bias.

modification on MUC2, with low sulphation. Buisine and colleagues looked at alterations in mucin genes expression, measuring RNA, in the ileal mucosa of 16 patients with active CD inflammation, in normal looking mucosa of these patients and in 14 controls with irritable bowel syndrome. The expression of MUC1, MUC4, MUC5B was lower in CD patients than in controls [6]. The investigators suggested a

primary mucosal defect of these genes in CD. In a second study, two years later, the same group looked at mucin gene expression in the intestinal epithelial cells in 19 CD patients and 14 controls, probably the same irritable bowel disease controls as in their previous study [7]. They used ISH and IMH and found lesser expression of MUC1, MUC4, MUC5AC, MUC5B, MUC6 and MUC7. Kaneko and colleagues looked at the ulcer-associated cell lineage (UACL) induced in the small intestine of CD patients [8]. They performed IMH staining with monoclonal antibodies against MUC5AC, MUC6 and MUC2 in ileal biopsies taken from 19 patients and 5 controls. Positive staining for MUC5AC and MUC6 were found in all the patients and in none of the controls. Staining for MUC2 was positive in 11 of the 19 patients versus all the controls. The authors concluded that the UACL is associated with the decreased expression of MUC2 and increased expression of MUC5AC and MUC6. They also found increased expression of PDX-1 transcription factor, which regulates the development of gastric mucosa. Hensel et al. demonstrated decreased mRNA levels of MUC2 in the inflamed mucosa of the terminal ileum of 5 CD patients when compared with 5 non-inflamed mucosa or 5 healthy controls [9]. MUC1 mRNA levels were higher in inflamed as well as in non-inflamed mucosa.

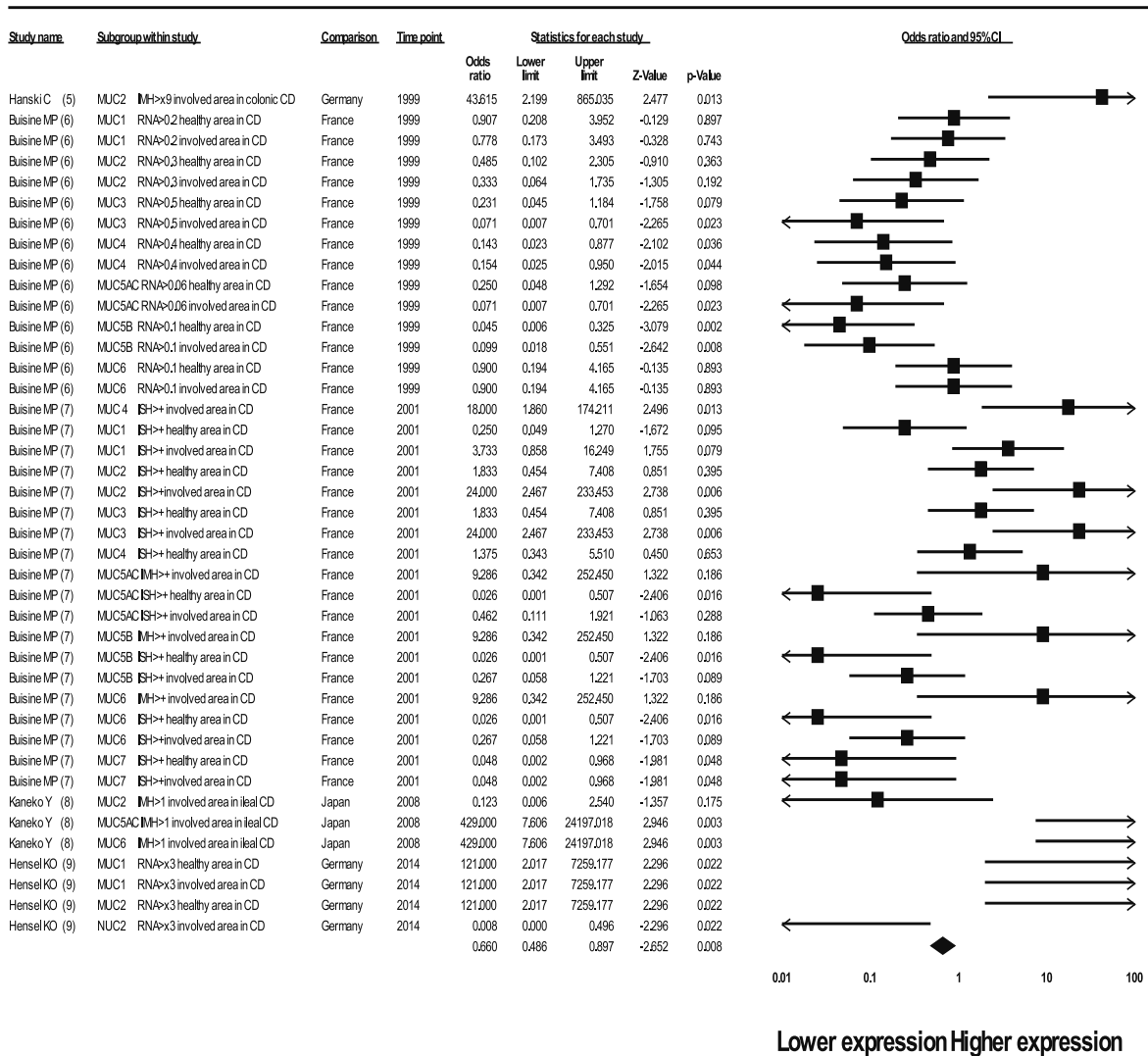


Fig. 3. Meta-analysis of mucin expression in Crohn's disease patients versus controls (6 studies, 49 sub-studies).

Out of the 49 sub-studies, the results of 21 reached significance (Fig. 3) [4-9]. In 9 sub-studies mucin expression was higher in CD patients than in controls (Fig. 3) [5, 7-9]. In 12 sub-studies mucin expression was lower than in controls (Fig. 3) [6, 7]. No significant heterogeneity was demonstrated in the included studies: $Q = 149.256$, $df(Q) = 40.00$, $I^2 = 73.2\%$ (less than 75%) [10]. Odds ratio for mucin expression in CD patients versus healthy controls was 0.660 with 95%CI 0.486-0.897, $P = 0.008$ (Fig. 3). Thus, we could demonstrate a

significant decrease of 34% in mucin expression in CD patients. When we looked at the specific mucins expression we found a significant decreased expression in CD patients for MUC5AC, MUC5B and MUC7 (Fig. 4). The odds ratio for MUC5AC was 0.392 with 95%CI 0.164 – 0.937, $P = 0.035$, for MUC5B was 0.147 with 95%CI 0.060 – 0.363, $P < 0.001$, and for MUC7 0.048 with 95%CI 0.006 – 0.401, $P = 0.005$. Decreased expression was found also for MUC3 and MUC4, and increased expression for MUC1 and MUC2, but these did not reach significance.

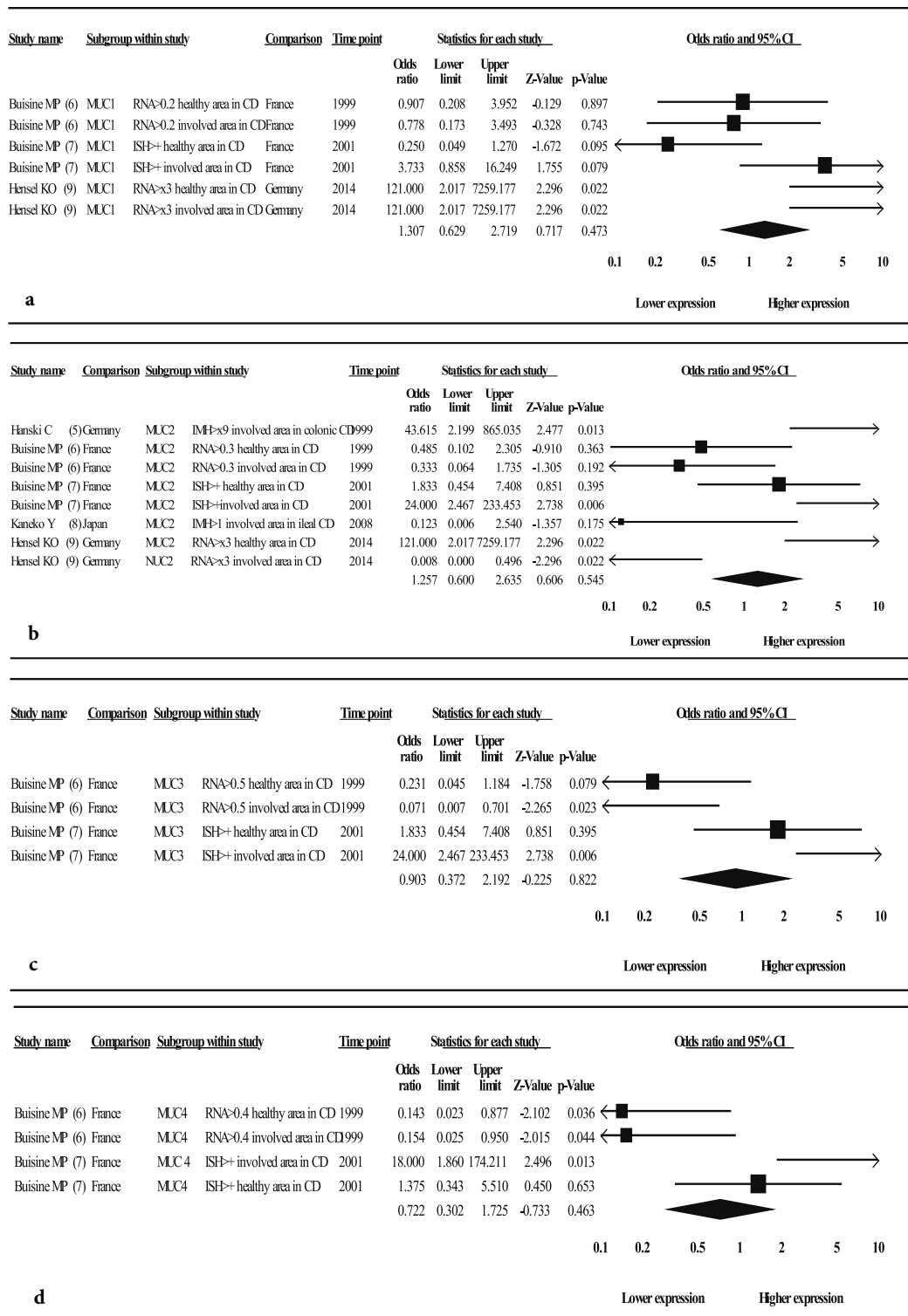


Fig. 4 (continued)

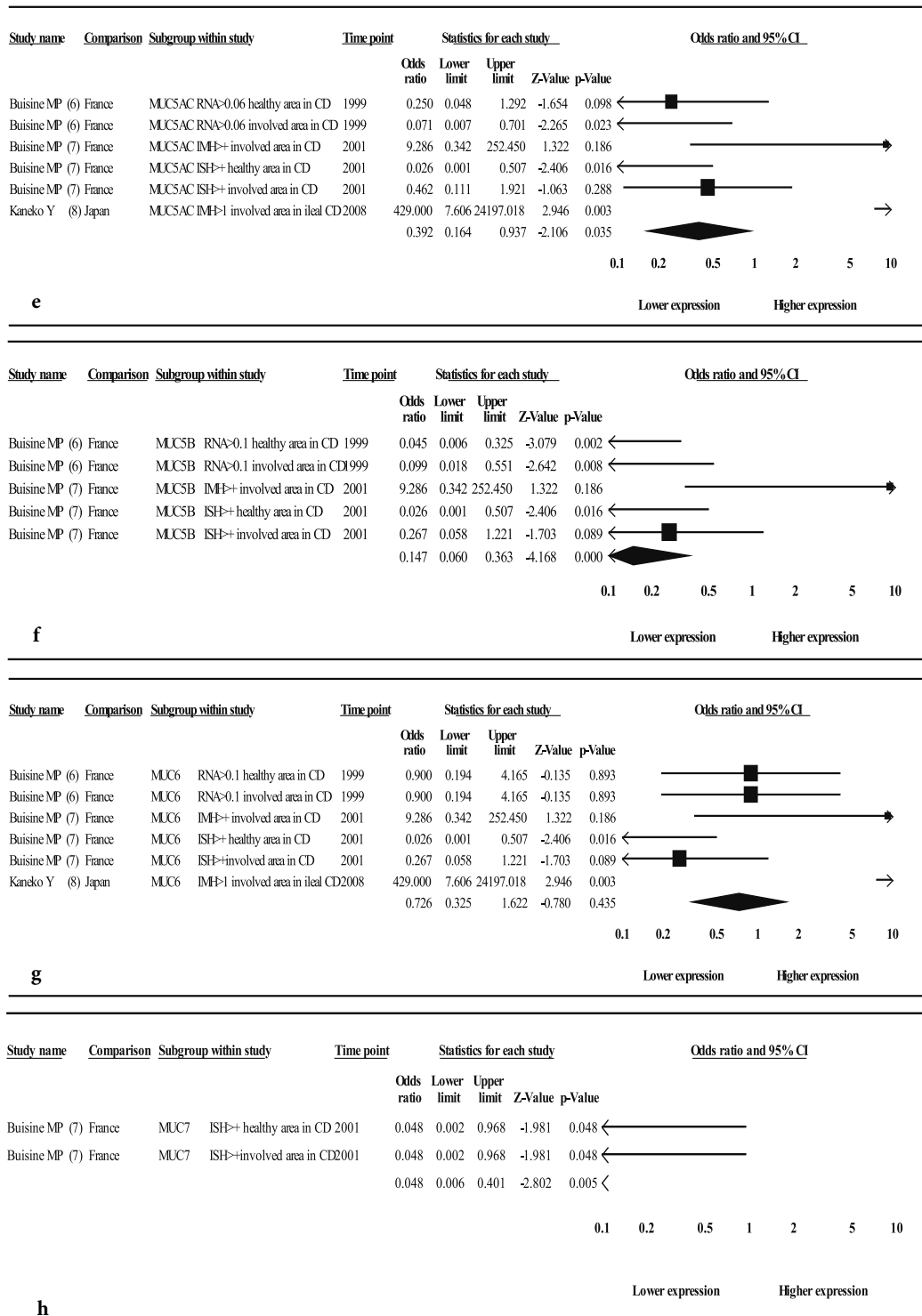


Fig. 4. Meta-analysis of mucin expression in CD patients versus controls, sub-studies of specific MUCs: a. MUC1; b. MUC2; c. MUC3; d. MUC4; e. MUC5AC; f. MUC5B; g. MUC6; h. MUC7

DISCUSSION

We could demonstrate in the meta-analysis calculation a decrease of 34% in the total mucin expression in CD patients. However, we need to be mindful of the fact that, although 12 sub-studies showed lower mucin gene expression, 9 studies showed higher expression. The main influence on the result

was due to a significant decrease in the expression of MUC5AC, MUC5B and MUC7.

We cannot be sure that the decrease in mucin expression is specific for CD, or a result of a non-specific inflammation. Even though, the inconsistent findings in CD in comparison with ulcerative colitis support the specific effect of CD [1]. Several differences were described, comparing mucin expression in CD

versus UC: mucus thickness increased in CD but decreased in UC; goblet cell numbers were unchanged in CD but decreased in UC; mucin sulfation was unchanged in CD but decreased in UC. MUC1 and MUC4 expression were decreased in CD but increased in UC [1].

Gastric metaplasia has been described in active ileal CD, and mucins that are typical for gastric mucosa such as MUC5AC and MUC6 were expressed in the inflamed mucosa, with a marked absence of MUC2 [7, 8]. This „UACL” was expressed in addition to epidermal growth factor and trefoil peptides, which are involved in mucin secretion, mucosal repair and ulcer healing. In addition, Kaneko et al. demonstrated a decrease in CDX2 (intestine-specific transcription factor) and an increase in the expression of PDX-1 (protein that regulates development of gastric mucosa) [8].

In our meta-analysis, we demonstrated a global decrease in mucin expression in CD patients when compared with healthy controls. In an interim analysis we found a significant decrease in MUC5AC, MUC5B and MUC7 expression. No significant increase or decrease in expression was found for MUC1, MUC2, MUC3, MUC4 and MUC6.

Two papers were not included in our meta-analysis. Moehle et al. found no expression of MUC6, MUC7, MUC8, MUC9, MUC11, MUC15, MUC16, and MUC18 in the colonic and ileal mucosa of 9 CD patients, 7 in active disease and 2 in remission [11]. Strong downregulation of mRNA levels was also found for MUC1, MUC2, MUC4, MUC5B, MUC12, MUC13, MUC17, and MUC20 in CD ileal and colonic mucosa. We could not add this study to our meta-analysis since RNA results were present only in fold change for the group but not for individual cases. Kyo et al. reported evidence that “MUC3” consists of two genes, MUC3A and MUC3B, which encode membrane-bound mucins. They also reported that SNPs of MUC3A, involving a tyrosine residue with a proposed role in cell signalling, might confer genetic predisposition to CD [12].

It is not clear to us whether the decrease in mucin expression in CD patients is a primary or a secondary event, and if this kind of observation has anything to do with the etiology, pathology or prognosis of the disease. In addition, the effect of treatment regimens, such as 5-amino-salicylic acid, immunomodulators, steroids or biologic therapies on mucin expression, could not be excluded. These issues should be further explored in direct studies.

CONCLUSION

We demonstrated a global decrease in mucin expression in CD patients when compared with healthy controls.

Conflicts of interest: No conflict to declare.

STUDY HIGHLIGHTS

What is current knowledge?

- The mucus layer of the intestinal tract is the main barrier between luminal microbes and the mucosa.
- The mucus layer is composed of secreted and membrane-bound mucins, and has an essential role in the body defense mechanisms.

- Previous research could not establish consistent results for mucin genes expression in Crohn's disease (CD) patients.

What is new here?

- In this meta-analysis a decrease of 34% in the total mucin expression in CD patients was demonstrated.
- A significant decreased expression in CD patients for MUC5AC, MUC5B and MUC7 was also found.
- A global decrease in mucin expression in CD patients may be a significant factor in the disease etiology.

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Expresia genelor mucinei în mucoasa intestinului la pacienții cu boală Crohn: referat sistematic și meta-analiză

ABSTRACT / REZUMAT

Premize: Stratul de mucus al tractului intestinal reprezintă principala barieră între microbii din lumen și mucoasa intestinală, și are un rol esențial între mecanismele de apărare ale organismului. Cercetările anterioare nu au adus rezultate concrete privind expresia genelor mucinei la pacienții cu boală Crohn (BC). **Scop:** în această meta-analiză am evaluat expresia mucinei la pacienții cu BC și am comparat-o cu cea constatată la martorii sănătoși.

Metodă. Cercetarea literaturii medicale în limba engleză a urmărit expresia mucinei în mucoasa ileală și colonică la pacienții cu BC comparativ cu cea din mucoasa martorilor. Au fost incluse toate studiile tip caz-control. Meta-analiza a fost efectuată cu ajutorul softului Comprehensive meta-analysis. Au fost calculate șansele de risc (odds ratio) și intervalele de confidență 95% (95% CI).

Rezultate. Am identificat 160 studii eligibile: 20 au fost eliminate deoarece au fost efectuate pe animale sau nu aveau textul întreg, iar 134 fiindcă erau scrise în altă limbă decât engleza, erau editoriale, referate, sau duplicate. Au rămas 6 studii caz-control din 4 țări care au îndeplinit criteriile de includere și au fost publicate până în 31.12.2016. Nu a existat o heterogenitate semnificativă: $Q=149.256$, $df(Q)=40.00$, $I^2=73.2\%$ (sub 75%). Odds ratio calculată a fost 0.660, cu 95%CI 0.486-0.897, $P=0.008$, respective indicând o reducere cu 34% în expresia totală a mucinei la pacienții cu BC. Am constatat de asemenea o reducere semnificativă a expresiei pentru MUC5AC, MUC5B și MUC7 la pacienții cu BC.

Concluzie. Am demonstrat o reducere globală a expresiei mucinei în mucoasa intestinală la pacienții cu BC comparativ cu martorii sănătoși.