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TOPIC HIGHLIGHT

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Campylobacter concisus and inflammatory bowel disease

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Abstract

Investigation of the possible role of *Campylobacter concisus* (*C. concisus*) in inflammatory bowel disease (IBD) is an emerging research area. Despite the association found between *C. concisus* and IBD, it has been difficult to explain how *C. concisus*, a bacterium that is commonly present in the human oral cavity, may contribute to the development of enteric diseases. The evidence presented in this review shows that some *C. concisus* strains in the oral cavity acquired zonula occludens toxin (*zot*) gene from a virus (prophage) and that *C. concisus* Zot shares conserved motifs with both *Vibrio cholerae* Zot receptor binding domain and human zonulin receptor binding domain. Both *Vibrio cholerae* Zot and human zonulin are known to increase intestinal permeability by affecting the tight junctions. Increased intestinal permeability is a feature of IBD. Based on these data, we propose that a primary barrier function defect caused by *C. concisus* Zot is a mechanism by which *zot*-positive *C. concisus* strains may trigger the onset and relapse of IBD.

Key words: *Campylobacter concisus*; Inflammatory bowel disease; Zonula occludens toxin; Tight junctions; Intestinal permeability

Core tip: *Campylobacter concisus* (*C. concisus*) is an oral bacterium that was previously shown to be associated with inflammatory bowel disease (IBD). Evidence presented in this review shows that some strains of *C. concisus* acquired zonula occludens toxin (*zot*) gene from a virus (prophage), suggesting that a primary barrier function defect caused by *C. concisus* Zot is a mechanism by which *zot*-positive *C. concisus* strains may trigger the onset and relapse of IBD.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract[1]. The two major clinical forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). The etiology of IBD is not fully understood. Multiple contributors including genetic

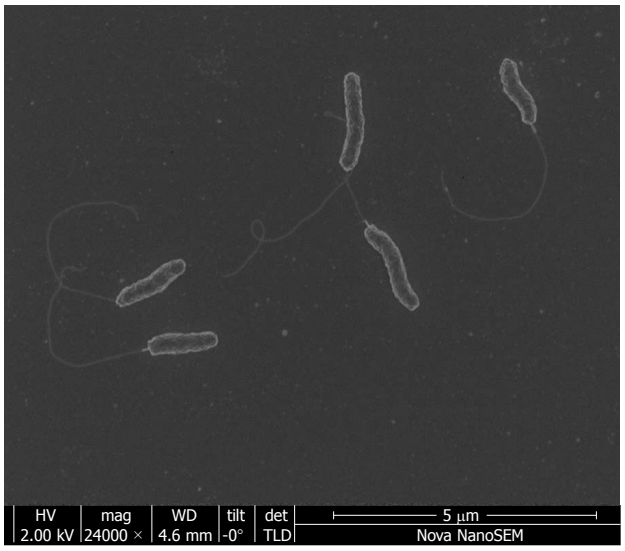

Figure 1 Electron microscopic image of *Campylobacter concisus*.

factors, environmental factors and intestinal microbiota have been suggested to play a role in the development of IBD[1]. The pathogenesis of IBD is thought to result from a dysregulated response of the intestinal mucosal immune system to luminal commensal microbes[1].

The highest incidence of IBD, both CD and UC, is in young adults[2]. This implies that in most of the patients with IBD, the intestinal mucosal immune system has maintained a non-hostile relationship with the intestinal commensal microbes for decades prior to the onset of the disease. Given this, the uncontrolled attack of the intestinal mucosal immune system to luminal commensal bacteria would have been initiated by a trigger. Such a trigger may not necessarily be a dominant intestinal bacterial species or a long-term intestinal resident microbe. Evidence presented in this review suggests that zonula occludens toxin gene (*zot*)-positive *Campylobacter concisus* (*C. concisus*) strains, a bacterium colonizing the human oral cavity, may be a trigger of IBD.

C. concisus

C. concisus is a Gram negative bacterium that requires microaerobic or anaerobic conditions enriched with H_2 for growth[3]. Cells of *C. concisus* are curved, with a size of (0.5 - 1) × (2 - 6) μm. *C. concisus* is motile, driven by a single polarized flagellum (Figure 1).

HUMANS ARE THE NATURAL HOST OF *C. CONCISUS* AND THE ORAL CAVITY IS THE PRIMARY COLONIZATION SITE

Tanner *et al*[4] first reported the isolation of *C. concisus* from patients with gingivitis in 1981. Zhang *et al*[5] examined the presence of *C. concisus* in saliva samples obtained from healthy individuals of different age groups and found that *C. concisus* is commonly present in the human oral cavity. In that study, *C. concisus* was detected in 97% (57/59) of saliva samples collected from healthy individuals aged 3-60 years old by polymerase chain reaction (PCR) targeting 16S rRNA gene and cultured from 75% (44/59) of these saliva samples using a filtration method. A study from Petersen *et al*[6] also detected a high prevalence of *C. concisus* in the human oral cavity: in this study *C. concisus* was detected in 100% of saliva samples (11/11) collected from healthy individuals by a PCR targeting 16S rRNA gene. Despite its high prevalence in the human oral cavity, *C. concisus* is not a dominant oral bacterial species[7].

Table 1 Detection of *Campylobacter concisus* in samples obtained from healthy individuals

Samples	Cultivation of *C. concisus*	Detection of *C. concisus* DNA by PCR
Human saliva	75%	97%-100%
Human feces	0%-2.8%	33%
Human intestinal biopsies	0%	2%-38%

Data shown in this table were obtained from references 5-10 and 17-21. To date, no studies have detected *Campylobacter concisus* (*C. concisus*) in samples obtained from healthy animals. The collective data suggest that humans are the natural host of *C. concisus* and the oral cavity is the primary colonization site. PCR: Polymerase chain reaction.

In comparison to the high isolation of *C. concisus* from saliva samples, the isolation rates of *C. concisus* from fecal samples collected from healthy individuals were much lower. Using the filtration method, Engberg *et al*[8] isolated *C. concisus* from 2.8% (3/107) of fecal samples and Nielsen *et al*[9] did not isolate *C. concisus* from any of 108 fecal samples collected from healthy individuals. The low isolation rates of *C. concisus* from fecal samples suggest that the human intestinal tract of healthy individuals is a less optimal site for *C. concisus* colonization compared to the oral cavity.

To date, *C. concisus* has not been detected in any healthy animals. In a study examining the presence of *Campylobacter* species in fecal samples collected from 70 healthy pet dogs using a quantitative PCR targeting the 60 kDa chaperonin gene, Chaban *et al*[10] detected seven different campylobacter species but not *C. concisus*. Various campylobacter species have been detected in fecal samples collected from animals or birds, but *C. concisus* was not detected[3,10,11]. Lynch *et al*[12] isolated *C. concisus* from 10% (18/185) of chicken meat and 3% of beef meat (6/186) samples. However, whether chicken and cattle are natural hosts of *C. concisus* cannot be determined from these data.

C. concisus has been detected in some animals with gastrointestinal disorders. Petersen *et al*[6] detected *C. concisus* in 12.5% (1/8) of saliva samples from pet cats with dental diseases by PCR targeting the 16S rRNA gene[6]. In addition, Chaban *et al*[10] detected *C. concisus* in fecal samples of 9% of dogs with diarrhea (6/65).

The collective data suggest that humans are the natural host of *C. concisus,* with the human oral cavity being the primary colonization site (Table 1).

Table 2 Genetic relatedness of enteric and oral *Campylobacter concisus* strains

Enteric *C. concisus* strains from patients with IBD	Genetically related *C. concisus* strains
A strain isolated from intestinal biopsies of patient No. 1	An oral strain from patient No. 6
A strain isolated from intestinal biopsies of patient No. 3	An oral strain from patient No. 3 (identical)
A strain isolated from luminal fluid of patient No. 3	An oral strain from healthy No. 1

Data shown in this table were obtained from reference 13. The genetic relatedness was assessed based on the sequences of six housekeeping genes. These data provide evidence showing that *Campylobacter concisus* (*C. concisus*) strains colonizing the intestinal tract of patients with IBD originating from either the patient's own oral *C. concisus* strains or oral *C. concisus* strains from other individuals. IBD: Inflammatory bowel disease.

DIVERSITY OF *C. CONCISUS* STRAINS COLONIZING THE HUMAN ORAL CAVITY

C. concisus strains colonizing the human oral cavity are greatly diverse. On examination of oral *C. concisus* strains isolated from individual patients with IBD and healthy controls, it was found that *C. concisus* strains isolated from each individual had unique protein patterns on sodium dodecyl sulphate polyacrylamide gel electrophoresis[5,13,14]. Furthermore, some individuals were colonized with multiple *C. concisus* strains in the oral cavity, with as many as three different *C. concisus* strains having been isolated from individual patients with IBD or healthy controls[5,13,14]. A significantly higher number of patients with active IBD were colonized with multiple *C. concisus* strains in the oral cavity compared to healthy controls[14].

COLONIZATION OF ORAL *C. CONCISUS* STRAINS IN THE INTESTINAL TRACT

It is estimated that 1-1.5 L of saliva is produced daily in humans, most of which is swallowed[15]. Thus, the human oral cavity is a constantly available source, transporting *C. concisus* from the oral cavity along with saliva to lower parts of the gastrointestinal tract. However, as mentioned previously, both the detection of *C. concisus* by PCR and the cultivation of *C. concisus* from fecal samples were much lower than that from saliva samples, indicating that *C. concisus* routinely transported from the oral cavity to the intestines does not commonly establish colonization there.

Nevertheless, intestinal colonization of oral *C. concisus* strains does occur in some individuals. For example, in a study comparing the housekeeping genes of *C. concisus* strains isolated from intestinal biopsies of patients with IBD and controls, Ismail *et al*[13] found that a *C. concisus* strain isolated from intestinal biopsies of a patient with UC had housekeeping genes identical to that of an oral *C. concisus* strain isolated from the same patient, providing evidence that the oral *C. concisus* strains are in fact able to colonize the human intestinal tract (Table 2).

In addition to an individual's own oral *C. concisus*, *C. concisus* detected in the intestinal tract may also come from a different source, most likely through materials contaminated with saliva from others. For example, in the above patient with UC, while the *C. concisus* strain isolated from intestinal biopsies had housekeeping genes identical to that of the patient's own oral *C. concisus* strain, the *C. concisus* strain isolated from the luminal fluid of this patient had a genetic relationship closely related to an oral *C. concisus* strain from a healthy control, rather than to the patient's own oral *C. concisus* (Table 2)[13].

The human intestinal environment is not optimal for *C. concisus* colonization in general, as suggested by the low isolation rate of *C. concisus* from fecal samples of healthy individuals (Table 1). Given this, *C. concisus* intestinal colonization is most likely a short-term event in most individuals. However, with the human oral cavity as a constantly available source of *C. concisus,* repeated intestinal colonization of *C. concisus* may occur.

Whether the characteristics of a given oral *C. concisus* strain or the intestinal environment of an individual, or both, plays the major role in determining whether or not intestinal colonization of oral *C. concisus* strains will occur is currently unknown. A previous study by Haag *et al*[16] showed that intestinal microbiota shifts towards elevated commensal *Escherichia coli* loads abrogated colonization resistance against *Campylobacter jejuni* in mice. Currently, it is not clear whether the dysbiosis associated with IBD plays a role in *C. concisus* intestinal colonization.

PREVALENCE OF *C. CONCISUS* IN THE INTESTINAL TRACT OF PATIENTS WITH IBD AND HEALTHY CONTROLS

A number of studies have examined the prevalence of *C. concisus* in the intestinal tract of patients with IBD using PCR methods. Most of these studies detected a significantly higher prevalence of *C. concisus* DNA in patients with IBD and controls[17-21]. The reported detection rates of *C. concisus* by PCR in enteric samples (biopsies and fecal samples) were 33%-69% in patients with IBD and 2%-38% in controls[17-21]. Analysis of the data in these studies revealed a number of interesting findings.

Firstly, different PCR strategies affect the detection of *C. concisus* in enteric samples. This was best seen in the study conducted by Man *et al*[18], who compared the prevalence of *C. concisus* in fecal samples collected from 54 children with CD, 33 healthy controls and 27 non-IBD controls using two different PCR methods. The first PCR method employed campylobacter genus specific PCR (primers C412F/C1288R) and sequencing the PCR products to determine campylobacter species. The second PCR method was a nested PCR, using campylobacter genus specific PCR (primers C412F/C1288R) followed by *C. concisus* specific PCR (primers Concisus F/Concisus R). These two PCR methods yielded very different results in detection of *C. concisus* in the same samples. The prevalence of *C. concisus* in children with CD, healthy controls and non-IBD controls detected by *C. concisus* genus specific PCR

Table 3 Detection rates of *Campylobacter concisus* in fecal samples by two polymerase chain reaction methods

	Campylobacter genus PCR	Nested PCR
CD (n = 54)	19%	65%
Healthy controls (n = 33)	12%	33%
Non-IBD controls (n = 27)	0	37%

An example showing that different polymerase chain reaction (PCR) methods affect the detection of *Campylobacter concisus* (*C. concisus*) in intestinal samples. Data included in this table were from reference 18. Primers C412F and C1288R were used in campylobacter genus PCR. In nested PCR, PCR products amplified by primers C412F and C1288R were amplified again using *C. concisus* specific primers Concisus F and Concisus R. The nest PCR detected a significantly higher intestinal prevalence of *C. concisus* in patients with Crohn's disease (CD) compared to both healthy controls and non-inflammatory bowel disease (IBD) controls. The genus PCR detected a significantly higher intestinal prevalence of *C. concisus* in patients with CD compared to non-IBD controls, but not healthy controls.

was 19% (10/54), 12% (4/33) and zero (0/27) respectively. The nested PCR greatly increased the detection of *C. concisus* in the same cohort of samples, with the prevalence of *C. concisus* being 65% (35/54) in children with CD, 33% (11/33) in healthy controls and 37% (10/27) in non-IBD controls. The nested PCR, but not the genus specific PCR, detected a significantly higher prevalence of *C. concisus* in children with CD as compared to healthy controls[18] (Table 3). Indeed, in studies revealing a significant difference in intestinal prevalence of *C. concisus* between patients with IBD and controls, nested PCR was used to examine all of the samples or part of the samples[17-20].

Secondly, collection of multiple intestinal biopsies increases the detection of intestinal prevalence of *C. concisus*. A study by Mahendran *et al*[20] showed that in comparison to the collection of one biopsy, the collection of four biopsies from each individual greatly increased the detection of *C. concisus* (Figure 2).

Thirdly, despite the increased prevalence of *C. concisus* detected by PCR in the intestinal tract of patients with IBD, the isolation rates of *C. concisus* from intestinal biopsies of patients with IBD were low (3%-7.7%)[17,20,21]. This suggests that *C. concisus* detected in most of the enteric samples were at a low number or in a nonculturable state.

ADVERSE EFFECTS OF *C. CONCISUS* ON INTESTINAL EPITHELIAL CELLS

A number of adverse effects of *C. concisus* on intestinal epithelial cells have been described. Using *in vitro* cell culture models (Caco2 cells or HT-29 cells), epithelial adhesion and invasion, damage of the barrier function and up-regulation of Toll-like receptors-4 expression by *C. concisus* have been reported. Different strains showed varying degrees of ability to induce such adverse effects[13,22-25]. The underlying molecular mechanisms responsible for these effects have not yet been investigated.

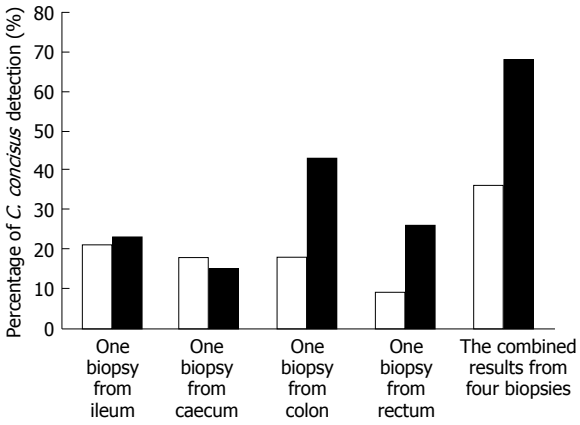

Figure 2 An example showing that collection of multiple intestinal biopsies increases the detection of intestinal prevalence of *Campylobacter concisus*. Data included in Figure 2 were from reference 20. In both patients with inflammatory bowel disease (IBD) (right column) and healthy controls (left column), the detection of intestinal prevalence of *Campylobacter concisus* (*C. concisus*) was greatly increased using four biopsies as compared to using one biopsy. In healthy controls, *C. concisus* detection rates in a single biopsy collected from ileum, caecum, colon and rectum were 21%, 18%, 18% and 9% respectively. If results from the four biopsies were used in determining the intestinal prevalence of *C. concisus*, the intestinal prevalence of *C. concisus* in healthy controls was 36%. Similarly, in patients with IBD, *C. concisus* detection rates in a single biopsy collected from ileum, caecum, colon and rectum were 23%, 15%, 43% and 26% respectively, and the intestinal prevalence of *C. concisus* in patients with IBD was 68% when four biopsies were taken into consideration.

C. CONCISUS ZOT GENE AND IBD

Mahendran *et al*[14] examined the prevalence of *zot* gene in 56 oral *C. concisus* strains isolated from saliva of 19 patients with IBD and 20 healthy controls. This study showed that 30% (17/56) of the oral *C. concisus* strains carried the *zot* gene. The *zot*-positive *C. concisus* strain was present in 55% (6/11) of patients with active IBD and 40% (8/20) of healthy controls. Some IBD patients with active disease 18% (2/11) were colonized with multiple *zot*-positive *C. concisus* strains in the oral cavity. Interestingly, polymorphic forms of the *C. concisus zot* gene resulting in the substitution of valine at amino acid position 270 were found to be associated with active IBD.

The *zot* gene was first discovered in *Vibrio cholerae* where it is carried by a filamentous prophage[26,27]. The *zot* gene in *V. cholerae* is required for phage production; a *V. cholerae* strain with *zot* gene mutation did not produce phage particles into the culture supernatant[28]. The *V. cholerae* Zot toxin was shown to increase intestinal permeability and to be associated with mild to moderate diarrhea[26,29,30].

Previous studies have found a human intestinal Zot analogue, namely zonulin, which is a physiological regulator that increases the intestinal permeability[31,32].

C. CONCISUS ZOT GENE IS A COMPONENT OF A CHROMOSOMALLY INTEGRATED PUTATIVE PROPHAGE

Stothard *et al*[33] established a visual database of bacterial

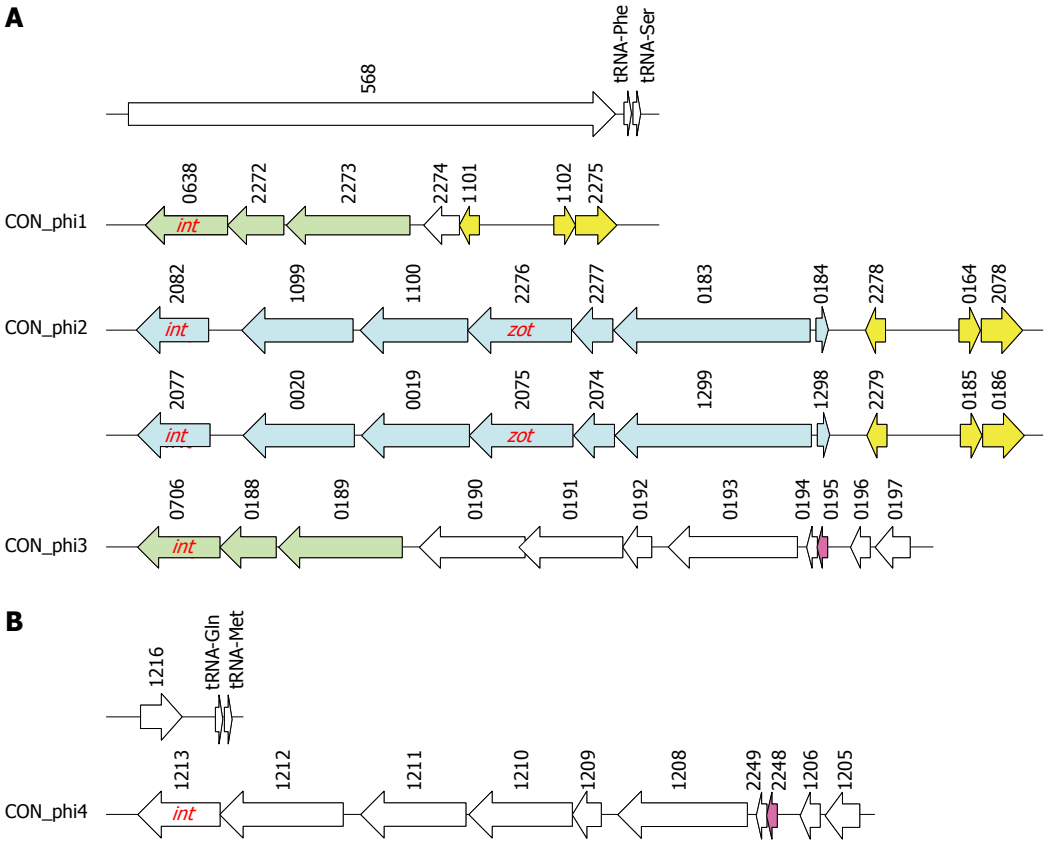

Figure 3 Genetic structures of putative prophage identified in *Campylobacter concisus* strain 13826. A: Multiple prophages at nucleotide position between 1576686 and 1614075; B: One putative prophage at nucleotide position between 939158 and 946901. Identical genes were indicated with the same color. *int*: A gene encodes phage integrase; *zot*: Zonula occludens toxin gene. The numbers are the locus tags.

chromosome maps in which *C. concisus* strain 13826 (Accession No. CP000792. 1) was included. Stothard *et al*[33] identified a region from nucleotide position 1576683 to 1615449 (38767 bp) in the genome of *C. concisus* strain 13826 as an "incomplete prophage". In this region, 39 genes with open reading frames were identified, with four of these genes encoding integrases and 10 genes encoding phage-like proteins[33].

The genetic structures of this "incomplete prophage" region are shown in Figure 3A and the proteins encoded by genes in this region are shown in Table 4. We compared the genes within this region using publicly available softwares[34,35]. A number of genes that have identical nucleotide sequences, including CCC13826_1099, CCC13826_0020, CCC13826_1102, CCC13826_0164, CCC13826_0185, CCC13826_2082 and CCC13826_2077 were annotated with identical protein names.

Interestingly, the region that was considered as an "incomplete prophage" by Stothard *et al*[33] turned out to be four putative prophages, each beginning with a phage integrase (Figure 3 and Table 4). The first prophage had a genome size of 5.2 kb, which contained seven protein-encoding genes. The second prophage and third prophage were identical, each having a genome size of 9.6 kb consisting of 10 protein-encoding genes. The fourth prophage contained 11 protein-encoding genes with a genome size of 8.6 kb. We named the first prophage CON_phi1, the second prophage and third prophage CON_phi2 and the fourth prophage CON_phi3. The *zot* gene is a component of CON_phi2. Comparison of the proteins encoded by genes in CON_phi2 with that encoded by genes in CTX phage, the phage that carries the *zot* gene in *V. cholera,* did not show high similarities except for the Zot protein (data not shown), suggesting the CON_phi2 is a previously uncharacterised prophage.

A study by Kaakoush *et al*[36] found that two hypothetical proteins encoded by CCC13826_0191 and CCC13826_1210 have 47% and 46% similarity respectively to *C. concisus* Zot. Here we found that CCC13826_0191 is a gene of CON_phi3 and CCC13826_1210 is a gene of an additional putative prophage, which was named CON_phi4. A number of genes in CON_phi3 and CON_phi4 had high similarities, however, CON_phi4 did not contain the gene that corresponding to CCC13826_0188 in CON_phi3 (Table 5).

IDENTIFICATION OF CONSERVED MOTIFS SHARED BY *C. CONCISUS* ZOT AND ZONULIN/ZOT RECEPTOR BINDING DOMAINS

Kaakoush *et al*[36] compared Zot sequences in *C. concisus* strain 13826 and *V. cholerae* strain 86015 and reported that

Table 4 Proteins encoded by putative prophages in *Campylobacter concisus* strain 13826

Nucleotide position	Locus tag	Encoded proteins	Size (aa)
1576686..1581986	CCC13826_0568	Hypothetical protein	1766
1582376..1583269	CCC13826_0638	Phage integrase	297
1583269..1583880	CCC13826_2272	Glutathionylspermidine synthase family	203
1583895..1585235	CCC13826_2273	Bacteriophage replication gene A protein	446
1585467..1585853	CCC13826_2274	Protein yitk	128
1585894..1586115	CCC13826_1101	Phosphonate uptake transporter	73
1586807..1587043	CCC13826_1102	Sensory box protein	78
1587044..1587496	CCC13826_2275	Hypothetical protein	150
1587598..1588506	CCC13826_2082	Phage integrase	302
1588755..1589966	CCC13826_1099	Putative phage replication protein A	403
1590015..1591184	CCC13826_1100	ATP binding protein (ABC) transporter	389
1591228..1592352	CCC13826_2276	Zonula occludens toxin (Zot) family protein	374
1592354..1592800	CCC13826_2277	Hypothetical protein	148
1592797..1594923	CCC13826_0183	Hypothetical protein	708
1594955..1595095	CCC13826_0184	Hypothetical protein	46
1595477..1595698	CCC13826_2278	Phosphonate uptake transporter	73
1596412..1596648	CCC13826_0164	Sensory box protein	78
1596649..1597101	CCC13826_2078	Hypothetical protein	150
1597203..1598111	CCC13826_2077	Phage integrase	302
1598360..1599571	CCC13826_0020	Putative phage replication protein A	403
1599620..1600789	CCC13826_0019	ABC transporter	389
1600833..1601957	CCC13826_2075	Zot family protein	374
1601959..1602405	CCC13826_2074	Hypothetical protein	148
1602402..1604528	CCC13826_1299	Hypothetical protein	708
1604560..1604700	CCC13826_1298	Hypothetical protein	46
1605082..1605303	CCC13826_2279	Phosphonate uptake transporter	73
1606017..1606253	CCC13826_0185	Sensory box protein	78
1606254..1606706	CCC13826_0186	Hypothetical protein	150
1606808..1607701	CCC13826_0706	Phage integrase	297
1607701..1608312	CCC13826_0188	Glutathionylspermidine synthase family	203
1608327..1609667	CCC13826_0189	Bacteriophage replication gene A protein	446
1609899..1611041	CCC13826_0190	Type Ⅱ and Ⅲ secretion system protein	380
1610992..1612116	CCC13826_0191	Hypothetical protein	374
1612118..1612438	CCC13826_0192	Hypothetical protein	106
1612537..1613946	CCC13826_0193	Hypothetical protein	469
1613956..1614075	CCC13826_0194	Alkyl hydroperoxide reductase	39
1614090..1614209	CCC13826_0195	Hypothetical protein	39
1614489..1614707	CCC13826_0196	Hypothetical protein	72
1614801..1615178	CCC13826_0197	Hypothetical protein	125

aa: Amino acids.

the biological active domain (FCIGRL) previously found in *V. cholerae* Zot was not found in *C. concisus* Zot. In this review, we compared the sequence of *C. concisus* Zot with human zonulin receptor binding domain and *V. cholerae* Zot receptor binding domain previously reported[32,37]. Interestingly, we found that *C. concisus* Zot shares conserved motifs with both the human zonulin receptor binding domain and the *V. cholerae* Zot receptor binding domain (Table 6). These data suggest that *C. concisus* Zot may increase intestinal permeability using a mechanism that is similar to the human zonulin and *V. cholerae* Zot, affecting the tight junctions through proteinase activated receptor 2 activation[37,38]. The motif "GRFLSYHG" is located at amino acid position 123-130 in *C. concisus* Zot, which was found in all oral *zot*-positive *C. concisus* strains that we previously isolated as well as in the *C. concisus* strain 13826[14]. The polymorphisms of *C. concisus zot* gene that Mahendran *et al*[14] previously detected were not in the receptor binding domain, suggesting that these polymorphisms may impact on the function of *C. concisus* Zot, if there is any, using a different mechanism rather than affecting the binding of *C. concicsus* Zot to the receptor.

INCREASED INTESTINAL PERMEABILITY IN PATIENTS WITH IBD

Increased intestinal permeability is a feature of both CD and UC[39-43]. While epithelial cell death and proinflammatory cytokines may damage the intestinal epithelial barrier during active disease, evidence shows that increased intestinal permeability may precede the initial onset or relapse of IBD. An early study from Hollander *et al*[39] reported that increased intestinal permeability was detected not only in patients with CD but also in their healthy relatives. A family history of IBD is a known risk factor for IBD[44]. Irvine *et al*[41] reported that an individual with a family history of CD had elevated intestinal permeability eight years prior to the onset of clinical symptoms and diagnosis of CD. Wyatt *et al*[43] measured the intestinal

Table 5 The similarity of proteins in CON_phi3 and CON_phi4

CON_phi3	CON_phi4	Similarity[1]
CCC13826_0706	CCC13826_1213	23.23%
CCC13826_0188		
CCC13826_0189	CCC13826_1212	93.72%
CCC13826_0190	CCC13826_1211	98.95%
CCC13826_0191	CCC13826_1210	92.25%
CCC13826_0192	CCC13826_1209	92.03%
CCC13826_0193	CCC13826_1208	58.90%
CCC13826_0194	CCC13826_2249	100%
CCC13826_0195	CCC13826_2248	97.44%
CCC13826_0196	CCC13826_1206	61.11%
CCC13826_0197	CCC13826_1205	95.24%

[1]Similarity is the percentage of identical amino acids.

Table 6 Motifs shared by *Campylobacter concisus* zonula occludens toxin and zonulin/zonula occludens toxin receptor binding domains

C. concisus Zot[1]	**GRF LSYHG**
Human adult intestine zonulin	**GGXL**
Human fetal intestine zonulin	**GGVLVQPG**
C. concisus Zot[2]	**GRF LSYHG**
V. cholerae Zot	FC**IGRLCVQDG**

[1]*Campylobacter concisus* zonula occludens toxin (Zot) and zonulin binding domain shares a motif (bold letters): Non-polar G, variable, non-polar, non-polar L, variable, polar, variable and non-polar G; [2]*C. concisus* Zot and *Vibrio cholerae* Zot shares a motif (bold letters): non-polar G, basic polar R, non-polar, non-polar, variable, polar, variable and non-polar G. Comparison of *C. concisus* Zot and zonulin/Zot receptor was performed in this review. The amino acid sequences of human intestinal zonulin and *V. cholerae* Zot were obtained from reference 32.

permeability in patients with quiescent CD and found that those with increased intestinal permeability were at a significantly higher risk of clinical relapse. These data suggest that increased intestinal permeability occurred prior to the onset and relapse of the disease may be a possible aetiological factor of IBD.

C. CONCISUS ZOT: A POTENTIAL TRIGGER OF IBD THROUGH CAUSING PRIMARY BARRIER DEFECT

The human zonulin and *V. cholerae* Zot toxin are known to increase intestinal permeability through affecting the tight junctions[31,32,37]. In this review, we found that *C. concisus* Zot has conserved motifs shared by the zonulin/Zot binding receptor domains. Given this, it is very likely that *C. concisus* Zot also affects the tight junctions.

Based on the information obtained from previous publications and the analysis that we have performed in this review, we propose a mechanism by which *C. concisus*, an oral bacterium, may trigger the onset or relapse of IBD: that some oral *C. concisus* strains acquire *zot* gene from a virus (prophage). With the human oral cavity as the reservoir of *C. concisus*, repeated intestinal colonization of *C. concisus* and release of *C. concisus* Zot due to prophage induction may occur, which is likely to result in a prolonged primary epithelial barrier defect and translocation of macromolecule such as luminal microbes and their products. In genetically susceptible individuals, this may trigger the development of IBD.

Damage to the intestinal epithelial tight junctions may also lead to the development of diarrhea. Indeed, in addition to its association with IBD, *C. concisus* has been frequently isolated from non-IBD-related diarrheal stool samples[9,45,46].

If some oral *C. concisus* strains are indeed involved in the development of human IBD, the question as to why the lesions of IBD occur more often in the intestinal tract rather than in the oral cavity arises. One explanation is that the virulence factors that are associated with IBD are more often expressed in the intestinal tract rather than in the oral cavity. For example, the expression of *C. concisus* Zot may require induction of prophage from the *C. concisus* genome. As prophage induction usually occurs when bacterial cells are under stressful conditions[47], the fact that *C. concisus* uses the human oral cavity as its primary colonization site suggests that the oral cavity is not a stressful site for *C. concisus*. However, as the *C. concisus* travels to the more hostile lower parts of the gastrointestinal tract, the stressful environment may trigger the induction of *C. concisus* prophage.

Another possible factor that may reduce the pathogenic effect of *C. concisus* Zot in the oral cavity is that the epithelium in the oral cavity is a stratified squamous epithelium, either keratinized or non-keratinized[48]. In contrast, the intestinal epithelium is a simple columnar epithelium[48]. The impact on permeability caused by Zot, even it is expressed in the oral cavity, in multiple layers of squamous epithelium may not be as evident as that in the single layered columnar epithelium.

C. CONCISUS ZOT: A POTENTIAL ENVIRONMENTAL FACTOR CONTRIBUTING TO THE INCREASED RISK OF IBD IN INDIVIDUALS WITH A FAMILY HISTORY OF IBD

A family history of IBD is a risk factor for developing IBD[44]. In addition to genetic factors, environmental factors have been shown to be involved in the increased incidence of IBD in members with a family history of this disease[49,50]. We suggest that *C. concisus* Zot is one such factor. This suggestion is based on the findings that the higher numbers of the relatives of patients with IBD have increased intestinal permeability and that some oral *C. concisus* strains carry the *zot* gene that encodes a toxin known to promote this[14,39,41]. This hypothesis remains to be further assessed by examining the correlation between colonization of *zot*-positive *C. concisus* strains and the increased intestinal permeability in family members of patients with IBD.

CONCLUSION

The evidence presented in this review shows that some *C. concisus* strains colonizing the human oral cavity acquired *zot* gene from a virus (prophage). We are currently examining the biologic activities of *C. concisus* Zot, the expression of Zot in *zot*-positive *C. concisus* strains isolated from patients with IBD and controls as well as the presence of *C. concisus* Zot in the oral cavity and intestinal tract of patients with IBD and controls, which will provide further information in understanding the role of *C. concisus* Zot in IBD and other human diseases.

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