IMPACTS OF EARLY LIFE ADVERSITY ON
MICROBIOTA AND IMMUNE FUNCTIONING IN
INDIVIDUALS WITH MAJOR DEPRESSIVE DISORDER

by

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Abstract

Early life stress may contribute to depression vulnerability, possibly through immune system development and gut microbial colonization patterns. Increased pro-inflammatory cytokines and differences in gut microbiota composition have been seen in depressed individuals. The current study investigated inflammatory and bacterial profiles of 39 individuals with a current major depressive episode and 43 healthy volunteers with varying severity of childhood trauma. Plasma interleukin (IL)-6/IL-10 ratios was positively correlated with depression severity, and childhood trauma explained variance in severity of symptoms above and beyond that accounted for by the IL-6/IL-10 ratios. Expression of *Faecalibacterium prausnitzii* (*F. prausnitzii*) in stool was decreased in depressed participants compared to healthy controls. Importantly, this relationship was significant only in participants who had experienced moderate-to-severe physical neglect. These findings suggest a relationship between particular gut bacteria and depressive symptoms that is dependent on the presence of specific types of early life trauma.

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Abbreviations

ANOVA: Analysis of variance, **BAI:** Beck anxiety inventory, **BDI-II:** Beck depression inventory-II, **BMI:** Body mass index, **CRP:**C-reactive protein, **CTE:** Childhood trauma exposure, **CTQ-SF:** Childhood trauma questionnaire- short form, **DSM-5:** Diagnostic and statistical manual of mental disorders fifth edition, **EDTA:** ethylenediaminetetraacetic acid, **HAMDS:** Hamilton's depression scale, **Ig:** Immunoglobulin, **IL:** Interleukin, **IMHR:** Institute of Mental Health Research, **INF γ :** Interferon-gamma, **LPS:** lipopolysaccharide, **MADRS:** Montgomery–Åsberg Depression Rating Scale, **MDD:** Major Depressive Disorder, **MINI:** Mini-international neuropsychiatric interview, **PDD:** Persistent depressive disorder, **SCID:** Structured Clinical Interview for the DSM-5, **SSRI:** Selective serotonin reuptake inhibitor, **SNRI:** Serotonin-norepinephrine reuptake inhibitor, **TNF- α :** Tumor necrosis factor alpha,

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Introduction

Major depression is the leading cause of disability worldwide (WHO, 2017). Depression negatively impacts daily functioning in multiple ways, including fatigue, sleep disturbances, weight loss, inability to concentrate, inability to feel pleasure, lack of motivation, and thoughts of suicide (American Psychiatric Association, 2013). Unfortunately, treatments for depression are not optimal: in one of the largest antidepressant trials, the “Sequenced Treatment Alternatives to Relieve Depression” (STAR*D), which tested the efficacy of a variety of antidepressant interventions, including selective serotonin reuptake inhibitors (SSRIs) and cognitive-behavioural therapy, only a quarter of the participants achieved remission after a first intervention, and half of the participants failed to respond to a second intervention (Fava et al., 2003, Rush et al., 2004). In a more recent review of 15 clinical trials of standard antidepressant treatments, less than 40% of treated participants achieved remission (Dodd et al., 2013). Adverse side effects associated with use of antidepressant medications are also problematic as they represent the leading reason for treatment discontinuation (Samples & Mojtabai, 2015). The devastating impacts of depression, combined with limited treatment efficacy and adverse side effects, make finding new treatments for depressive illnesses a critical topic for research. To develop informed and effective treatments, more knowledge about the processes influencing depression development and severity is required. Increasing evidence suggests that inflammatory activation and changes in the composition of bacteria residing in the digestive tract (gut microbiota) may alter central nervous system functioning, increasing risk to develop depression (Kelly et al., 2016b; Kiecolt-Glaser et al., 2015). Early life stress, which is a risk factor for depression, has also been shown to promote changes in the gut microbiota and inflammation (Baumeister et al., 2016; O’Mahony et al., 2009). Therefore, the current study investigated

whether specific bacteria and inflammatory factors in depressed and healthy individuals are different in those with a history of early life trauma, and whether these changes are linked to the presence and severity of depressive symptoms.

Early Life Trauma and Depression

Stress is one of the main contributing factors to depression. High interpersonal chronic stress (troubles with best friend relationships, peer social circles, romantic relationships, family relationships, etc.) has been related to increased risk of developing a major depressive episode in young adults (Vrshek-Schallhorn et al., 2015). It is also well supported that cortisol (the “stress hormone”) is altered (usually increased) in the blood, saliva and urine of depressed individuals (Bearden et al., 2009; Booij et al. 2015). In fact, several animal models of depression rely on forms of stress (e.g., restraint stress, chronic social stress) to elicit depressive-like behaviors in rodents (Czéh et al., 2016). Stress appears to be especially detrimental for mental health when experienced in early life. For example, childhood adversity is more common in depressed than in non-depressed individuals (Danese et al., 2008; Kelly et al., 2016a). Also, early life trauma, such as neglect or physical/sexual abuse, increases the risk for developing depression and correlates with symptom severity (Chu et al., 2013). In addition, maternal separation (an animal model of early life stress) elicits depressive-like behaviour in rodents (De Palma et al., 2015; O’Mahony et al., 2009), supporting the possibility that childhood trauma is a large contributor to depression vulnerability and severity. Although the relationship between early life stress/trauma and depression has been supported by many animal and clinical studies, the mechanisms behind this connection remain unclear.

Multiple mechanisms have been proposed to underlie depression, with a dysregulation of monoamine pathways being the most popular theory (Chaudhury, Liu, & Han 2015). Standard

antidepressant treatments (e.g., SSRIs and serotonin-norepinephrine reuptake inhibitors [SNRIs]) are targeting these pathways and as previously mentioned, lack in efficacy for a significant proportion of depressed individuals (Fava et al., 2003, Rush et al., 2004). This lack of treatment success has led researchers to investigate alternative depression pathways. Studies have shown that depressed individuals have increased circulating levels of pro-inflammatory cytokines (signaling molecules between immune cells) (Dowlati et al., 2010; Kelly et al., 2016a; Valkanova et al., 2013; Xia et al., 2018) and changes in the composition of the gut microbiota (Aizawa et al., 2016; Jiang et al., 2015; Kelly et al., 2016a; Lin et al., 2017; Liu et al., 2016; Naseribafrouei et al., 2014). Interestingly, pro-inflammatory cytokines and gut bacteria are also influenced by early life stress. For example, levels of interleukin (IL)-6 (a pro-inflammatory cytokine) were more pronounced in healthy adult individuals with childhood trauma compared to those without (Carpenter et al., 2010). As well, increased abundance of Proteobacteria and decreased abundance of lactic acid bacteria and Actinobacteria were seen in stool of children whose mothers reported high distress during pregnancy (Zijlmans et al., 2015). Similarly, offspring of stressed pregnant monkeys had reduced overall numbers of *Bifidobacteria* and *Lactobacilli* species (Bailey, Lubach, & Coe, 2004). Evidence suggests that stress during early life is common in depressed individuals and can have an effect on inflammatory and microbiota profiles, two systems also shown to be related to depression. Taken together, it is possible that early life stress could be influencing depression through bacterial and/or inflammatory changes, although this has yet to be thoroughly investigated.

Inflammatory Factors Implicated in Depression Vulnerability and Severity

The inflammatory profiles of depressed individuals have been the topic of interest for many studies: particularly the pro-inflammatory cytokines IL-6 and tumor necrosis factor (TNF)-

α which have been shown to be increased in the circulatory system of individuals with depression (Dowlati et al., 2010; Kelly et al., 2016a; Valkanova et al., 2013; Xia et al., 2018). Increased serum IL-6 and TNF- α levels also correlated with Hamilton Depression Scale scores, suggesting that these cytokines may influence depression severity (Fan et al., 2017). Interestingly, Walsh et al., (2016) found that the association between plasma IL-6 levels and depression severity in pregnant adolescents was only displayed in those who had experienced high levels of childhood trauma, suggesting a link between childhood adversity and depression-related inflammation. Increased serum IL-6 levels were also found in depressed adult individuals with a history of childhood trauma (especially physical and emotional abuse) compared to depressed individuals without (Moreira et al., 2018). In a longitudinal study, increased serum IL-6 levels in women predicted depression 6 months prior to a depressive episode, and the cytokine elevations persisted throughout the episode in women with a history of childhood trauma only (Miller and Cole, 2012). Together, these findings suggest that stress experienced early in life could possibly influence the connections between peripheral inflammatory activation (particularly in relation to IL-6) and depression.

In addition to IL-6, other inflammatory markers in the blood have been related to depression, one of which is the acute phase protein C-reactive protein (CRP). CRP is involved in immune signalling: it is produced by the liver and increases following secretions of pro-inflammatory cytokines, especially IL-6 (Thompson et al., 1999). CRP then binds to lysophosphatidylcholine (a phospholipid expressed on the surface of dead/dying cells and some bacteria), which activates further inflammatory processes (specifically the complement system, which enhances the ability of antibodies to clear microbes from an organism, among other functions) (Thompson et al., 1999). Increased plasma CRP levels have been reported in

individuals with major depression compared to healthy controls (Kelly et al., 2016a; Xia et al., 2018). Reductions of depressive symptoms over time (measured 1 year from baseline) were associated with reductions in serum CRP in individuals with type-2 diabetes (Herder et al., 2018). As well, serum CRP levels were elevated in treatment-resistant depressed individuals and importantly, were strongly associated with childhood adversity, specifically among individuals who mentioned “feeling unloved as a child or wishing for a different childhood” (Chamberlain et al., 2018), suggesting a link between depression-related changes in CRP and early childhood adversity, similar to what was observed with IL-6. Miller and Cole (2012) also reported that in women with a history of early life trauma, the transition to depression was accompanied with increases in serum CRP levels, which remained elevated for 6 months after depressive symptoms subsided. Together, this evidence suggests that CRP is increased in depressed individuals and that this relationship may be influenced by the presence of early life trauma.

In addition to pro-inflammatory cytokines, anti-inflammatory cytokines have also been implicated in depression. For instance, IL-10 was decreased in the serum of unmedicated depressed individuals compared to healthy controls (Dhabhar et al., 2009). Interestingly, serum IL-10 was increased in depressed individuals who had experienced childhood trauma (especially physical abuse) compared to depressed individuals who had not (Moreira et al., 2018), suggesting that early life adversity could be a mediator of the connection between anti-inflammatory cytokines and depression. Likewise, Pan et al., (2013) reported that administering IL-10 via intracerebroventricular injections reduced stress-induced depressive-like behaviors in rats. As well, IL-10 deficient female mice (IL-10^{-/-}) on a Balb/c background) showed increased depressive-like behaviours compared to control mice (wild-type Balb/c), which were reversed by administration of IL-10, and female mice overexpressing serum IL-10 (PMT10) showed a

decrease in depressive-like behaviour (Mesquita et al., 2008). Taken together, it seems like IL-10 could have protective effects against depression. The increase of circulating IL-10 levels seen in depressed individuals and those with early life trauma could be due to a compensatory effort by the immune system to reduce stress-related inflammation (Moreira et al., 2018).

Microbiota Alterations Implicated in Depression Vulnerability and Severity

Within the digestive system, there live approximately 100 trillion microbes (including bacteria, archaea, protists, fungi and viruses), collectively referred to as the gut microbiota (Frank and Pace, 2008). Traditional function of gut bacteria are to help maintain immune functioning, supplement the host's diet (eg. producing vitamins), and aid in digestion (eg. metabolizing non-digestible carbohydrates) (Frank and Pace, 2008). The digestive tract is also innervated with over 100 million neurons throughout (Blackshaw et al., 2007; Furness, 2006). This expansive community of gut microbes may influence the central nervous system (and vice-versa), and this possible bidirectional relationship has been rapidly gaining interest among researchers. The gut microbiota can be manipulated by many factors, including diet, antibiotics, pathogens, and most importantly stress (Sherwin et al., 2016). For example, socially stressed adult mice showed decreased relative abundance of *Bacteroides* spp. and increased relative abundance of *Clostridium* spp. compared to non-stressed mice (Bailey et al., 2011). The gut microbiota is also altered in individuals with depression. Decreased overall species richness (number of species) and diversity (number of species and how evenly distributed they are), as well as altered relative abundance of specific bacterial taxa have been reported in individuals with depression compared to healthy controls (Aizawa et al., 2016; Jiang et al., 2015; Kelly et al., 2016a; Liu et al., 2016; Lin et al., 2017; Naseribafrouei et al., 2014). As previously mentioned, stress can affect the gut microbiota when experienced in adulthood such as in

socially stressed adult mice (Bailey et al., 2011), as well as in early life such as in the children of mothers who reported high distress during pregnancy (Zijlmans et al., 2015) and in the offspring of stressed pregnant monkeys (Bailey, Lubach, & Coe, 2004).

Currently, there is not a clear consensus on the particular taxa potentially involved in the development of depression, vulnerability to the illness, and/or symptom severity, likely due to heterogeneity of sample populations (e.g., gender, medication, duration of depression, onset of depression). For example, the genus *Prevotella* spp. was reported as both increased (Liu et al., 2016; Lin et al., 2017) and decreased (Jiang et al., 2015; Kelly et al., 2016a) in depressed individuals compared to healthy controls. Nevertheless, decreased abundance of the genus *Faecalibacterium* spp. (Chen et al., 2018; Jiang et al., 2015), and increased abundance of the genus *Alistipes* spp. (Jiang et al., 2015; Naseribafrouei et al., 2014) and the Enterobacteriaceae family (Chen et al., 2018; Jiang et al., 2015) have been consistently reported in MDD individuals compared to healthy controls. *Prevotella* spp. is a genus of Gram-negative bacteria associated with production of butyrate (a short-chain fatty acid known as an important energy source for intestinal cells) and inflammatory regulation (Kelly et al., 2016a). As previously mentioned, studies have shown contrasting results when determining *Prevotella* spp. abundance in depressed versus healthy individuals (Jiang et al., 2015; Kelly et al., 2016a; Liu et al., 2016; Lin et al., 2017). Yet evidence suggests that *Prevotella* spp. could be protective against depression, as increased *Prevotella* spp. levels have been associated with the Mediterranean diet, a preventive strategy in depression (De Filippis et al., 2015; Opie et al., 2015). It is possible that the increase of *Prevotella* spp. seen by Lin and collaborators (2017) was due to the escitalopram treatment given to all participants with depression, as *Prevotella* spp. increases in treatment-responsive depressed patients have also been reported (Gerritsen et al., 2011; Jiang et al., 2015). Stress has

been shown to affect *Prevotella* spp. abundance as well, as decreased abundance of *Prevotella* spp. was seen in both fecal and cecal contents of rats stressed by tail shocks compared to non-stressed rats (Maslanik et al., 2012). Contrarily, increased *Prevotella* spp. was seen in piglets in response to weaning (a stressful time in development) (Guevarra et al., 2018), indicating that *Prevotella* spp. could be responsive to other early life stressors as well. Together, evidence suggests that *Prevotella* spp. likely responds differently to various stressors, perhaps explaining the contrasting results in depression studies.

A member of the Gram-positive *Faecalibacterium* spp. genus, *Faecalibacterium prausnitzii* (*F. prausnitzii*, which also produces butyrate), is one of the most abundant bacterium species in healthy human adults (Miquel et al., 2013). The *Faecalibacterium* spp. genus has been related to anti-inflammatory activity within the gut (Sokol et al., 2008). Low levels of *F. prausnitzii* in particular have been reported in inflammatory diseases conditions such as ulcerative colitis, irritable bowel disorder and Crohn's disease (Miquel et al., 2013), suggesting that *Faecalibacterium* species may be involved in anti-inflammatory processes. Importantly, *Faecalibacterium* spp. was decreased in participants with depression compared to healthy controls (Chen et al., 2018; Jiang et al., 2015) and a negative correlation between the genus and depression severity has been observed (Jiang et al., 2015), indicating that *Faecalibacterium* spp. could be protective against depression, possibly through its anti-inflammatory effects. Interestingly, *F. prausnitzii* was decreased in rats that had been subjected to neonatal maternal separation compared to non-separated rats, and administration of *F. prausnitzii* reduced colonic hypersensitivity induced by neonatal maternal separation or restraint stress (Miquel et al., 2016), suggesting that this species can be protective against stress-induced intestinal dysfunction.

Enterobacteriaceae is a family of inflammation-related lipopolysaccharide (LPS) (a bacterial endotoxin found on the membrane of Gram-negative bacteria) -producing, Gram-negative bacteria, which includes many bacterial species naturally found in the human digestive system, as well as pathogenic genera such as *Salmonella* spp., *Escherichia* spp., and *Enterobacter* spp. An overgrowth of the Enterobacteriaceae family has been seen in depressed individuals compared to healthy controls (Chen et al., 2018; Jiang et al., 2015), suggesting a potential involvement of these bacteria in depression. It has been suggested that increased intestinal permeability seen in depressed individuals (Maes, Kubera, & Leunis, 2008) may allow Gram-negative bacteria (including Enterobacteriaceae) to enter into lymph nodes and/or systemic circulation (Berg and Garlington, 1979; O'Malley et al., 2010) and thus contribute to the increased inflammation commonly seen in depressed individuals. Immunoglobulin (Ig)A and/or IgM (antibodies used by the immune system to neutralize pathogens) are directed against pathogenic bacteria (such as those from the Enterobacteriaceae family), and have been seen to be increased in individuals with depression (Maes et al., 2012), further suggesting that an increase in pathogenic bacteria could promote the inflammatory activation seen in depressed individuals. Interestingly, studies have shown that infection with pathogenic bacteria from the Enterobacteriaceae family (e.g., shiga toxin-producing *Escherichia coli*) was related to higher instances of depression compared to the overall population (Lowe et al., 2014). Piglets that were stressed by early weaning showed a more rapid and severe reaction (diarrhea and reductions in weight gain) to orally administered F18 *E. coli* (a member of the Enterobacteriaceae family) compared to late-weaned piglets (McLamb et al., 2013), suggesting that early life stress could promote vulnerability to certain pathogenic bacteria in the Enterobacteriaceae family later in life.

In turn, this could possibly increase depression vulnerability via intestinal and circulating inflammation.

Importantly, in addition to associations established between specific bacterial taxa and depression, some evidence has pointed towards a potential causal link between gut bacteria, circulating inflammatory factors, and depressive symptoms. Transferring human fecal samples into germ-free rats (born and raised in sterile environments and thus devoid of any microbial colonization) increased anhedonia (which is considered a feature of depression) and plasma levels of IL-6, TNF- α , and CRP in rats that received samples from depressed participants, but not in those rats that received samples from healthy controls (Kelly et al., 2016a). The gut microbiota is sensitive to stress, particularly in early life. While the literature currently lacks human studies on the effects of childhood adversity on the gut microbiota, animal models of early life stress such as maternal separation have been shown to elicit gut microbiota perturbations in mammals (De Palma et al., 2015; O'Mahony et al., 2009). Circulating elevations of TNF- α and depressive-like behaviours were also apparent in maternally separated rodents (O'Mahony et al., 2009). Interestingly, maternal separation induced anxiety- and depression-like behavior (symptoms of anxiety are highly co-morbid with depression (Gorman, 1996)) only in specific-pathogen-free mice, but not in germ-free mice, suggesting that the presence of bacteria was necessary for behavioural effects of maternal separation to take place (De Palma et al., 2015). Evidence suggests that commensal bacteria (especially LPS-producing bacteria such as the Enterobacteriaceae family and *Prevotella* spp.) are also necessary for stress-induced inflammation. For example an increase in plasma IL-1 β (a pro-inflammatory cytokine) was seen in rats stressed by tail-shocks, however this increase was attenuated in rats treated with antibiotics or LPS blockers (Maslanik et al., 2012). This same relationship was not seen for IL-6

and IL-10, suggesting that there are specific cytokines involved in stress-activated microbiota pathways.

In summary, early life stress has been associated with depression vulnerability and severity (Chu et al., 2013; Danese et al., 2008; Kelly et al., 2016a) and has been shown to elicit gut dysbiosis (De Palma et al., 2015; O'Mahony et al., 2009; Zijlmans et al., 2015) and immune activation (Carpenter et al., 2010; O'Mahony et al., 2009; Zijlmans et al., 2015), features that are apparent in individuals with depression (Jiang et al., 2015; Kelly et al., 2016a). It is not yet clear, however, whether these features are a result of depression, or contributing factors to depression vulnerability and severity. While some animal studies have hinted at a causal relationship between gut microbiota and depressive symptoms (Kelly et al., 2016a), there have yet to be any human studies to attempt to determine whether microbiota and/or inflammation perturbations are factors that precede depression. Since it is difficult to examine individuals before and after depression onset, examining the influence of childhood adversity on these factors in depressed and healthy populations could help determine whether microbiota/inflammation perturbations differ in individuals with early childhood trauma, and potentially lead to depression. This would not only provide an alternative to the monoamine hypothesis of depression, but would offer possible pathways to examine to investigate how childhood trauma is contributing to depression later in life. To date, however, whether a history of childhood adversity influences inflammatory and gut microbiota changes in relation to depressive symptoms has yet to be investigated in clinical populations.

Goals and Hypotheses of the Current Study

The goal of the current study is to investigate targeted bacterial species and inflammatory factors in depressed and healthy individuals with varying childhood trauma severity and to link

these bacterial/inflammatory profiles to depression severity. It was hypothesized that participants with depression would show increased plasma levels of IL-6, CRP, TNF- α , and IL-10 (Lin et al., 2017), decreased abundance of *Prevotella* spp. (Jiang et al., 2015; Kelly et al., 2016a) and of *Faecalibacterium* spp. (Chen et al., 2018; Jiang et al., 2015), and increased abundance of the Enterobacteriaceae family (Chen et al., 2018; Jiang et al., 2015) compared to healthy control participants. It was also expected that changes in gut bacteria and increases in IL-6, CRP, TNF- α , and IL-10 would be more pronounced in participants with more severe early life trauma. Finally, it was hypothesized that childhood trauma would moderate the relationships between inflammatory/bacterial measures and depression severity. Understanding the relationships between early life trauma, microbiota/inflammatory changes and depression incidence and severity will provide valuable information for treatment and diagnosis research.

Materials and Methods

Participants:

The population consisted of 82 participants between the ages of 22 and 55, with 39 experiencing a current depressive episode and 43 sex- and age-matched healthy control participants. All participants were recruited from the community via posters and online advertisements and screened via a phone interview for psychiatric conditions (e.g., bipolar disorder, schizophrenia, substance abuse disorders) using a modified, study-specific version of the Mini-International Neuropsychiatric Interview (MINI) Screen 7.0.2 (Sheehan et al., 1998; Appendix A). Exclusion criteria (Appendix B), also assessed during the phone interview, included the following: chronic inflammatory diseases (e.g., inflammatory bowel disease, lupus, multiple sclerosis, rheumatoid arthritis); irritable bowel syndrome; obesity (BMI > 30); cardiovascular diseases; diabetes; liver cirrhosis or fatty liver disease; a current bacterial, fungal,

or viral infection; use of antibiotics, probiotics, or prebiotics in the last month; changes in antidepressant, anxiolytic, antipsychotic medication in the last month; change in smoking habits (past 6 months); and illicit drug use (past 6 months – not including marijuana).

Experimental design:

Once eligibility was confirmed, a description of the study protocol, approved by the Research Ethics Board of The Royal's *Institute of Mental Health Research* (IMHR, protocol #2016012) was provided and informed consent was obtained during an in-person visit at the Royal Ottawa Mental Health Centre. Eligible participants with suspected depression were then interviewed by a clinical psychologist or a psychiatrist using the Structured Clinical Interview for the DSM-5 (SCID-V, First et al., 1996) to confirm the presence of a current major depressive episode as the primary diagnosis. This interview also served to classify participants by depression type: Major Depressive Disorder (MDD, episodic-symptoms of depression for at least two weeks that are contrasting to participants "norm") or Persistent Depressive Disorder (PDD, chronic- symptoms of depression for at least two years, no contrast to "norm") and to detect any confounding co-morbid psychiatric disorders (e.g., schizophrenia, bipolar disorder) or substance abuse, which would deem the participant ineligible. If eligible, depressed participants were then scheduled for a second appointment for the administration of the remaining study procedures. Healthy control participants were not scheduled for an appointment with a psychologist or psychiatrist, and instead proceeded directly to the study procedures after consent was obtained. The study procedures involved providing a blood sample (drawn at the Diagnostic Services of the Royal Ottawa Mental Health Centre by a medical laboratory technician with required qualifications) and completing a socio-demographics form (which included questions related to diet, birth method, and supplement use; Appendix C) and three study questionnaires (Childhood

Trauma Questionnaire – Short Form, Beck Depression Inventory-II, and Beck Anxiety Inventory; Appendix D). At the end of the visit, participants were provided with a stool self-collection kit (Norgen Biotek Corp, Stool Nucleic Acid Collection and Preservation System, Cat.# 63710) to take home and were asked to collect a stool sample within 2 days following the appointment according to the manufacturer's instructions (which were described in details during the appointment), record the date of stool collection on the collection tube, and return it within two weeks. Participants received a monetary compensation upon reception of the stool sample, and the researcher recorded the date of sample receipt. The stool sample was stored at room temperature in a locked cabinet until subsequent analyses.

Experimental Procedures and Materials

Study questionnaires:

The Childhood Trauma Questionnaire-Short Form (CTQ-SF) is a 25-item self-report measure of childhood trauma exposure (Bernstein et al., 2003). The questionnaire is divided into the following subscales: physical abuse, sexual abuse, emotional abuse, emotional neglect, and physical neglect. Questions are scored on a 5-point scale as 1 = never true, 2 = rarely true, 3 = sometimes true, 4 = often true, 5 = very often true, based on how often each event happened to the participant from birth to age 17. Seven of the questions are reverse-scored. The score of each subscale range from 5 to 25, with a total CTQ score between 25 and 125. In general, the total CTQ score is associated with severity of childhood trauma exposure (CTE). The following cut-off scores of each CTQ subscale determine the presence of moderate-to-severe CTE: 1) emotional abuse ≥ 13 , 2) emotional neglect ≥ 15 , 3) sexual abuse ≥ 8 , 4) physical abuse ≥ 10 , and 5) physical neglect ≥ 10 . The CTQ-SF has demonstrated good criterion-related validity (Bernstein et al., 2003).

The Beck Depression Inventory-II (BDI-II) is a 21-item self-report questionnaire which assesses presence and severity of depressive symptoms (Beck et al., 1996). Each item consists of four statements in increasing severity about a particular symptom of depression (assigned increasing numerical values), of which the participant chooses the one that best represents their symptoms over the past two weeks. Higher total scores represent more severe depressive symptoms, and the following cut-offs provided by the inventory are used to categorize depression severity: 0-13 minimal; 14-19 mild; 20-28 moderate; 29-30 severe.

The Beck Anxiety Inventory (BAI) is a 22-item self-report questionnaire which assesses presence and severity of symptoms of anxiety over the past week (Beck et al., 1988). The inventory uses a 4-point Likert scale ranging from 0-not at all, to 3-severely. Participants are presented with a list of anxiety symptoms and asked to use the scale to rate how severely each symptom has bothered them over the past week. Higher total scores correspond to more severe anxiety, and the following cut-offs provided by the inventory are used to categorize anxiety severity: 0-7 minimal level of anxiety; 8-15 mild anxiety; 16-25 moderate anxiety; 26-63 severe anxiety. The inventory notes that women with anxiety disorders tend to score 4 points higher than men with anxiety disorders. The BAI correlates with the revised Hamilton Anxiety Rating Scale (Beck et al., 1988).

Fecal Microbiota Analysis:

Bacterial DNA from the stool samples was extracted and purified using the Stool DNA Isolation Kit according to the manufacturer's instructions (Norgen Biotek Corp, Catalog #27600). Briefly, preserved samples were aliquoted into bead tubes and vortexed with a buffer solution to promote cell lysis, centrifuged to separate debris, attached to a binding column, washed and centrifuged twice and eluted from the column to collect DNA. DNA yields and

purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). DNA was stored at -20°C until subsequent analysis.

Isolated DNA was amplified, in triplicates, in simultaneous quantitative polymerase chain reactions (qPCR). SsoAdvanced™ Universal SYBR® Green Supermix detection was used according to the manufacturer's protocol (Bio-Rad, Catalog #1725274) and a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Canada) collected data. Cycle thresholds for each bacteria of interest within the stool sample (target) were determined using the CFX Manager™ Software Version 3.0 (Bio-Rad, Canada) and data was expressed as a percentage of total bacteria determined with the use of Total bacteria as the reference gene. Primer sequences used were as follows: Total bacteria, F: 5'- ACT CCT ACG GGA GGC AGC AG-3', R: 5'-ATT ACC GCG GCT GCT GG-3'; *Prevotella* spp., F: 5'-GAA GGT CCC CCA CAT TG-3', R: 5'-CGC KAC TTG GCT GGT TCA G-3'; *F. Prauzini*, F: 5'-GAG CCT CAG CGT CAG TTG GT-3', R: 5'-CCA TGA ATT GCC TTC AAA ACT-3'; Enterobacteriaceae, F: 5'-CAT TGA CGT TAC CCG CAG AAG AAG C-3', R: 5'-CTC TAC GAG ACT CAA GCT TGC-3'. The thermal cycling protocol consisted of 1 cycle of 3 minutes at 98°C and 40 cycles of 15 seconds at 98°C followed by 30 seconds at 60°C. The specificity of amplified products (amplicons) was examined for each gene using their melt curve to ensure only the DNA sequence of interest had been amplified. Amplification curves and Cq values across biological and technical replicates were examined and technical replicates with a standard deviation higher than 0.2 had one replicate excluded from the subsequent analysis if it significantly deviated from the others and contributed to the high standard deviation.

Plasma levels of pro- and anti-inflammatory cytokines and CRP:

Blood was collected in pre-chilled ethylenediaminetetraacetic acid (EDTA)-coated tubes that had been stored at 4°C, immediately placed on wet ice for transport, and then centrifuged at 1000 x g for 15 minutes at 4°C. For each participant, plasma was aliquoted in volumes of 500-750uL into 1.5ml nuclease-free microfuge tubes, which were then stored at -80°C until subsequent analysis.

Plasma levels of IFN γ , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-17A, TNF- α , and CRP were determined, in duplicates, via a Multiplex immunoassay using a High Sensitivity 9-Plex Human ProcartaPlex™ Panel (Thermo Fisher Scientific; Cat# EPXS090-12199-901) and a CRP Human ProcartaPlex™ Simplex Kit (Thermo Fisher Scientific; Cat# EPX01A-10288-901).

Briefly, plasma samples were thawed on ice and the antigen standard was prepared. Magnetic beads were diluted and added to the plate, along with Universal Assay Buffer, plasma samples, standards and blanks. The plate was then fixed to a magnetic plate and washed once as per the wash procedure. The plate was sealed, shaken for 30 minutes at room temperature and incubated overnight at 4°C (for CRP, the plate was incubated for 2 hours at room temperature on a shaking table set to 500rpm). The plate was then washed twice and the detection antibody was added. The plate was sealed and let incubate for 30 minutes on a shaker table set to 500rpm at room temperature. The plate was washed twice and Streptavidin, R-Phycoerythrin Conjugate (SAPE) solution was added, and the plate was incubated again for 30 minutes on a shaker table at 500rpm at room temperature. For the 9-plex only, an amplification reagent was added to the plate, and the plate was incubated at room temperature on a shake plate set to 500rpm for 30 minutes, and then a second amplification reagent was added and the plate was again incubated. For the 9-plex and CRP, the plate was again washed twice and then reading buffer was added. The plate was sealed and incubated at room temperature for 5 minutes on a shaker table set to

500rpm, and then was run on the Bio-Plex 200 System. A Bio-plex Manager software (Bio-Rad) analysed the data according to the manufacturer's instructions. Values above the minimal detectable concentration but outside the standard curve range were extrapolated from the standard range provided by the Bio-Plex Manager™ software.

Statistical Analyses

Statistical analyses were conducted with SPSS Statistics 20 for Windows 10.0 (SPSS Science, Chicago, IL, USA). Statistical significance was determined at $p < 0.05$ for between-groups comparisons and at $p < 0.01$ for correlations. Data was cleaned for outliers by converting to z-scores and bringing outliers just within range (Z score of ± 3.29), or by removing the participant and all associated data (this was done only for one participant).

Demographic data in Depressed and Healthy Control participants were compared using chi-squared tests. Questionnaire scores (MINI, BDI-II, BAI, CTQ-SF and CTQ-SF subscales) as well as plasma levels of inflammatory factors (CRP, INF-g, IL-1 β , IL-10, IL-12p70, IL-17, IL-2, IL-4, IL-6, TNF- α and IL-6/IL-10 ratio) and percentages of expression of bacteria (*Prevotella* spp., Enterobacteriaceae, *F. prausnitzii*) were analyzed using a series of one-way analysis of variance (ANOVA) with Diagnostic group as the between-groups factor (Depressed vs. Healthy Controls participants as well as MDD, PDD, and Healthy Control participants). Follow-up comparisons comprised of *t* tests with a Bonferroni correction to maintain the alpha level at .05 were conducted when the overall ANOVA was significant. In addition to between-groups comparisons, Pearson correlations between questionnaire scores, plasma levels of inflammatory factors and percentages of expression of bacteria were conducted to determine the presence, strength and direction of any relationships.

To determine whether childhood trauma moderated relationships between inflammatory/bacterial measures and severity of depressive symptoms, plasma inflammatory factors and stool bacteria that differed between groups or that correlated with BDI-II scores were included in a series of moderation analyses, with the selected inflammatory factor or stool bacteria as the predictor, BDI-II score as the outcome, and CTQ-SF total score or CTQ-SF subscales as a moderator.

Finally, to determine if childhood trauma could account for any variance in depression severity above and beyond that accounted for by a selected inflammatory/bacterial measure, a hierarchical linear regression was conducted with any bacterial or inflammatory measure that showed a significant relationship with BDI-II or CTQ-SF scores (either between group difference or correlation - as determined above) entered on the first step, CTQ-SF scores entered on the second step, and BDI-II scores as the outcome measure.

Results

Demographic data and dietary intake among depressed and healthy control participants

Eighty-two participants (39 depressed and 43 healthy controls) met eligibility criteria and were initially included in the study. Among these, one healthy control participant did not return the fecal sample but completed the study questionnaires and provided a blood sample. One healthy control participant was excluded from the final analyses due to abnormally elevated plasma cytokine levels, which could have potentially been related to a recent infection, as this participant reported antibiotic use one month prior to participation (just at the limit of the antibiotic-free timeframe outlined in the eligibility criteria). Therefore, the final analyses included 39 depressed and 42 healthy control participants (41 controls for stool bacteria analyses), for a total of 81 participants. According to the SCID-5 assessment, 21 of the 39

depressed participants fulfilled the criteria for MDD (episodic symptoms over a 2 week period), and 18 fulfilled the criteria for PDD (chronic symptoms for 2 years or longer). Twenty-one of the depressed participants also fulfilled criteria for comorbid Generalized Anxiety Disorder (GAD), although this was not considered the primary diagnosis. As depicted in Tables 1 and 2, age, gender, smoking status, alcohol use, cannabis use, language, ethnicity, education, employment, marital status, birth method, main diet, and whether participants took daily vitamins/supplements did not differ between depressed and healthy control participants (p 's > 0.05). Importantly, depressed participants reported consuming fermented food products significantly less often than healthy control participants, $X^2(3, N = 81) = 17.608, p = 0.001$ (Figure 1).

Psychological measures in depressed and healthy control participants

Characteristics of depressed and healthy control participants in terms of psychological measures as well as SCID-5 diagnoses are summarized in Table 3. As expected, depressed participants had higher scores on the MINI ($t(55) = -14.03, p < 0.001$), BDI-II ($t(62) = -17.00, p < 0.001$) and BAI ($t(54) = -9.35, p < 0.001$) compared to healthy control participants (Figure 2). The depressed group also had higher total CTQ-SF scores than healthy controls ($t(79) = -3.23, p = 0.002$), as well as on Subscale 1 (Emotional Abuse) ($t(64) = -3.37, p = 0.001$), Subscale 2 (Physical Abuse) ($t(63) = -2.44, p = 0.018$), Subscale 4 (Emotional Neglect) ($t(79) = -3.15, p = 0.002$) and Subscale 5 (Physical Neglect) ($t(62) = -2.85, p = 0.006$) of the questionnaire (Figure 2). Subscale 3 scores (Sexual Abuse) of the CTQ-SF did not significantly differ between groups ($p > 0.05$).

To determine whether psychological, inflammatory and microbiota data would differ among depression subtypes, the depressed group was further divided into two subgroups based on participants' SCID-5 diagnosis. Twenty-one participants met the criteria for Major Depressive Disorder (MDD, $n = 21$) whereas eighteen met the criteria for Persistent Depressive Disorder

(PDD, n=18). Descriptive data on psychological measures for the PDD and MDD groups is summarized in Table 4. Scores for the MINI, BDI-II and BAI were all higher in the MDD and PDD groups compared to healthy controls ($p<0.001$), but no difference was seen between MDD and PDD groups ($p>0.05$) (Figure 3A,B,C). In contrast, the CTQ-SF scores were higher in the PDD subgroup compared to healthy controls ($p<0.001$) and to the MDD subgroup ($p=0.026$) (Figure 3D). Analysis of the CTQ-SF subscales revealed that scores for Subscales 1 (Emotional Abuse), 4 (Emotional Neglect), and 5 (Physical Neglect) were higher in PDD participants but not in MDD participants compared to healthy controls ($p=0.001$). In Subscales 4 and 5, scores were higher in the PDD subgroup compared to the MDD subgroup (p 's=0.009 and 0.004, respectively). No group differences were seen in the mean scores of Subscales 2 (Physical Abuse) or 3 (Sexual Abuse) ($p>0.05$).

Inflammatory and bacterial profiles among depressed and healthy control participants

The inflammatory and bacterial profiles of depressed and healthy control participants are outlined in Table 5. Plasma levels of CRP, INF γ , IL-1 β , IL-10, IL-12p70, IL-2, IL-4, IL-6, TNF- α , as well as the IL-6/IL-10 ratios (a measure of proper immune functioning) were comparable among depressed and healthy control participants ($p>0.05$). As seen in Figure 3, expression of *F. prausnitzii* was lower in depressed participants compared to healthy controls ($t(66)=2.248$, $p=0.028$), whereas *Prevotella* spp. and Enterobacteriaceae did not differ between the two groups.

The distinction between MDD and PDD inflammatory and bacterial profiles is summarized in Table 6. There were no significant differences between MDD and PDD subgroups, or between these subgroups and healthy control participants ($p>0.05$).

Relationships between psychological characteristics, plasma levels of inflammatory factors and stool expression of bacterial populations

Correlations between psychological measures are summarized in Tables S1-S3 (supplementary material). Because of the large number of correlations assessed, only those that were significant beyond the 0.01 alpha level were considered to be significant. Correlational analyses including the two groups combined show that BDI-II scores positively correlated with BAI scores ($r(79)=.734, p<0.001$), CTQ-SF scores ($r(79)=.412, p<0.001$) and Subscales 1 ($r(79)=.437, p<0.001$), 4 ($r(79)=.391, p<0.001$), and 5 ($r(79)=.380, p<0.001$) of the CTQ-SF. Likewise, BAI scores were positively correlated with CTQ-SF total scores ($r(79)=.370, p<0.001$), as well as CTQ-SF Subscales 1 ($r(79)=.398, p<0.001$), 2 ($r(79)=.337, p=0.002$), and 4 ($r(79)=.296, p=0.007$). When healthy controls were assessed separately, BDI-II scores stayed positively correlated with BAI scores ($r(40)=.691, p<0.001$) and both BDI-II scores ($r(40)=.471, p=0.002$) and BAI scores ($r(40)=.471, p=0.002$) remained positively correlated with CTQ-SF Subscale 2 (Physical Abuse). In contrast, none of the correlations within the depressed group reached significance at $p<0.01$. In both the MDD and PDD subgroups, CTQ-SF total scores positively correlated with each of the CTQ-SF subscales ($p<0.001$).

The correlations between plasma levels of inflammatory factors, stool expression of bacterial populations and psychological characteristics are summarized in Tables S4-S9 (supplementary material). As depicted on Figure 5, the IL-6/IL-10 ratios were positively correlated ($p<0.05$) with BDI-II scores when all participants were considered ($r(65)=0.277, p=0.023$). No other relationships between plasma inflammatory factors, stool bacteria, and psychological characteristics were significant at $p<0.01$. The positive association between IL-6/IL-10 ratios and BDI-II scores approached significance in depressed ($r(33)=.385, p=.029$) but not healthy control ($p=.519$) participants when considered separately. Among depressed participants, however, Subscale 2 of the CTQ-SF (Physical Abuse) was positively correlated

with plasma levels of IL-6 ($r(37)=.412$, $p=0.009$) (Figure 6). In healthy controls, the association between this subscale and IL-6/IL-10 ratios was just short significance at $p<0.01$ ($r(33)=.345$, $p=0.043$). There was also a positive relationship between plasma levels of IL-6 and of IL-10 in healthy controls ($r(40)=.879$, $p<0.001$) and in depressed participants ($r(37)=.567$, $p<0.001$) (Figure 7). Additional relationships were identified when the depressed group was further divided into MDD and PDD subgroups. In the MDD subgroup, BDI-II scores negatively correlated with plasma levels of IL-1 β ($r(19)=-.595$, $p=0.004$) (Figure 8). Alternatively, in the PDD subgroup BAI scores positively correlated with plasma levels of IL-1 β ($r(16)=.579$, $p=0.012$) (Figure 9). As well, the positive relationship between CTQ-SF Subscale 4 (Emotional Neglect) and *F. prausnitzii* expression in the MDD subgroup almost reaches significance ($r(16)=.546$, $p=0.019$) (Figure 10).

Contribution of childhood trauma to relationships between inflammatory factors or bacterial expression and depression severity

Comparisons of inflammation and bacterial measures between participants with moderate-to-severe trauma (scores above cut-offs) and low-to-no-trauma (scores below cut-offs) regardless of depression status were conducted using the cut-off criteria for each CTQ-SF subscale as follows: 1) Emotional Abuse ≥ 13 , 2) Physical Abuse ≥ 10 , 3) Sexual Abuse ≥ 8 , 4) Emotional Neglect ≥ 15 , and 5) Physical Neglect ≥ 10 (Bernstein et al., 2003). Participants with moderate-to-severe sexual abuse ($n=11$) had increased expression of *Prevotella* spp. compared to those with low-to-no sexual abuse ($n=60$) ($t(69)=-2.794$, $p=0.007$). As well, participants with moderate-to-severe physical neglect ($n=14$) had decreased expression of *F. prausnitzii* compared to those with low to no physical neglect ($n=54$) ($t(66)=2.121$, $p=0.038$). No other differences between moderate-to-severe and low-to-no trauma were seen for bacterial expression on the

remaining CTQ-SF subscales. Plasma levels of inflammatory factors were comparable between participants with and without a history of moderate-to-severe childhood trauma (for each subscale).

Based on the significant association between depression severity and the IL-6/IL-10 ratios (all groups considered), a moderation analysis was conducted to examine whether there was any moderating effect of childhood trauma on this relationship. The moderation analysis showed that childhood trauma did not moderate the relationship between IL-6/IL-10 ratios and depression severity ($\Delta R^2 = 0.0000$, $F(1,77) = 0.001$, $p > 0.05$). To determine whether childhood trauma could account for any variance in depression severity above and beyond the variance accounted for by the IL-6/IL-10 ratios, a hierarchical linear regression was conducted with IL-6/IL-10 ratios on the first step and the CTQ-SF total scores on the second step. The overall model was found to be significant ($R = 0.455$, $R^2 = 0.183$, $F(2,64) = 8.37$, $p = 0.001$) and the addition of childhood trauma in the model significantly accounted for additional variance in depression severity ($R^2 \text{ change} = 0.131$, $\beta = 0.362$, $t = 3.25$, $p = 0.002$).

Based on the observation that percentages of *F. prausnitzii* expression were reduced in depressed participants as well as in participants with a history of moderate-to-severe childhood physical neglect, a moderation analysis was conducted to determine whether childhood trauma and more specifically the presence of physical neglect would moderate the relationship between *F. prausnitzii* and depression severity. The moderation analysis showed that childhood trauma did not moderate the association between *F. prausnitzii* and depression severity ($\Delta R^2 = 0.0049$, $F(1,64) = 0.3856$, $p > 0.05$). Importantly, however, when only early life physical neglect was used as a moderator (rather than total scores of childhood trauma), this specific category of trauma did moderate the relationship between *F. prausnitzii* and depression severity, $\Delta R^2 = .053$, $F(1, 64) =$

4.01, $p=0.0494$. Specifically, lower stool expression of *F. prausnitzii* was associated with increased depression severity (higher BDI-II scores), but only in participants with a history of early-life physical neglect ($B = -392.48$, $t = -2.18$, $p<0.0327$) and not among those without a history of physical neglect ($B = -24.111$, $t = -0.627$, $p>0.05$ (Figure 11).

Discussion

The goal of the current study was to examine targeted bacterial species and inflammatory factors in depressed and healthy individuals with and without childhood trauma, and to link these bacterial/inflammatory profiles to depression severity. Consistent with previous findings (Jiang et al., 2015; Chen et al., 2018), expression of *F. prausnitzii* was decreased in depressed individuals compared to healthy volunteers. More importantly, less *F. prausnitzii* was seen in individuals with more severe symptoms of depression, but only in participants with moderate-to-severe physical neglect during childhood and not those with low-to-no physical neglect. Also, higher IL-6/IL-10 ratios were seen in those with greater depression severity, and childhood trauma added strength to this relationship above and beyond the capacity of the IL-6/IL-10 ratios to predict severity of depressive symptoms. Together, these findings support our hypothesis that childhood trauma would influence gut microbiota and inflammation in relation to symptom severity in depressed individuals.

The decreased expression of *F. prausnitzii* (percentages of total bacteria) seen in depressed participants is consistent with a previous study by Jiang et al., (2015). In contrast with this study, however, we did not find that reductions of this species corresponded to increased depression severity. This may be due to a difference in the study questionnaires used to determine depression severity (HAMDS and MADRS versus the BDI-II in the present study), which may focus on different symptoms of depression. Decreased *F. prausnitzii* expression was

also seen in participants with moderate-to-severe physical neglect compared to those with low-to-no physical neglect, irrespective of whether participants were depressed or mentally healthy. As well, increased *Prevotella* spp. was seen in participants with moderate-to-severe sexual abuse compared to those with low-to-no sexual abuse. Although an altered gut microbiota has been observed in toddlers whose mothers were stressed during their pregnancy (Zijlmans et al., 2015) and in maternally separated rodents (O'Mahony et al., 2009), no studies have investigated the bacterial profiles of individuals who experienced differing subsets of childhood trauma. The current results thus present a novel finding that specific categories of childhood trauma may be associated with specific microbial changes in the intestinal system. Importantly, in line with our initial hypothesis, decreased *F. prausnitzii* corresponded to increased depression severity but only in participants with moderate-to-severe physical neglect during childhood compared to those with low-to-no physical neglect. It should be noted that the CTQ-SF questions in the Physical Neglect subscale ask about whether basic human needs (e.g., supplying food, clean clothing, and medical care) were met by parental figures during childhood, as well as whether parents were often intoxicated and were generally “taking care of them”. Of the five subscales in the CTQ-SF, physical neglect might relate the most to the maternal separation model of early life stress used in animals. Therefore, it is interesting that this subscale was found to moderate a relationship between gut bacteria and depression severity, considering that *F. prausnitzii* has been reported to be decreased in rats that had been subjected to maternal separation compared to control rats (Miquel et al., 2016), and that maternal separation has been shown to increase depressive-like symptoms in mice (De Palma et al., 2015).

Furthermore, physical abuse was positively associated with plasma IL-6 levels among depressed participants but not among healthy volunteers. This result is consistent with a meta-

analysis demonstrating that different types of trauma were related to different inflammatory measures, with physical abuse being associated with increases in IL-6 (Baumeister et al., 2016). The current study did not replicate the other associations found in this meta-analysis, including sexual abuse and IL-6, physical/sexual abuse and TNF- α , and CRP and neglect. It is possible that the small sample size and heterogeneity of our sample (differing depression symptomology, medication, BMI, etc) could have contributed to increased variance and thus prevented relationships from reaching statistical significance.

The ratio of IL-6/IL-10 levels in circulation (serum or plasma) has been used as a measure of proper immune functioning (such that a higher ratio indicates a lack of an anti-inflammatory response of IL-10 to an increase of inflammatory IL-6), and has previously been seen to be increased in depressed individuals compared to healthy volunteers (Firdaus et al., 2009), indicating that depression could be related to a dysregulation of inflammatory and anti-inflammatory cytokines. The current study found a slight but non-significant increase in the IL-6/IL-10 ratio in the depressed group. Most importantly, the IL-6/IL-10 ratio was the only inflammatory measure to correlate with severity of depressive symptoms (such that higher IL-6/IL-10 ratios corresponded with greater depression severity) across both groups. This relationship was not seen in healthy controls when groups were considered separately, but a trend towards increased IL-6/IL-10 ratios in individuals with more severe symptoms was seen in the depressed group, suggesting that an imbalance in pro- and anti-inflammatory cytokines may contribute to depression symptoms. As well, plasma concentrations of IL-6 and IL-10 were positively correlated with each other within both the depressed and healthy control groups, and this correlation was stronger in healthy controls. Previous studies have shown a complete dissociation between serum IL-6 and IL-10 concentrations in depressed participants, but highly

correlated IL-6 and IL-10 in healthy controls (Firdaus et al., 2009), further suggesting that IL-6 and IL-10 signaling might be dysregulated in our depressed participants. Interestingly, childhood trauma had an additive effect on the impact of IL-6/IL-10 dysregulation on depression severity, suggesting that childhood trauma and a flawed IL-6/IL-10 ratio can both increase depression severity, and that the presence of both is more detrimental than one alone. What is still unclear based on our results is whether IL-6/IL-10 functioning is influenced by childhood trauma. Our findings suggest that childhood trauma did not moderate the relationship between IL-6/IL-10 ratios and depression severity, but future studies with larger sample sizes need to be conducted to confirm this finding.

Contrary to previous literature, circulating inflammatory factors were comparable among depressed individuals and healthy controls. Plasma levels of CRP, IL-6 and TNF- α were slightly increased in the depressed group, which is consistent with previous studies (Kelly et al., 2016; Dowlati et al., 2010; Valkanova et al., 2013; Xia et al., 2018), but these changes did not reach significance. In a rat model of depression (induced by repeated central administration of LPS), treatment with fluoxetine (a commonly used SSRI medication for depression) increased levels of IL-10 in the habenula of depressed and control rats (Zhao et al., 2018), suggesting IL-10 may be reactive to SSRI medication. TNF- α has also shown sensitivity to antidepressant treatment: plasma levels became lower after a 12-week treatment with SSRI/SNRI in MDD patients (Mao et al., 2018). Therefore it is possible that the inclusion of depressed participants taking medication in our study has washed out any cytokine changes in depressed participants.

In contrast with previous findings (Jiang et al., 2015; Kelly et al., 2016; Liu et al., 2016; Lin et al., 2017), other than decreased *F. prausnitzii* changes in abundance of *Prevotella* spp. or increased abundance of the Enterobacteriaceae family were not observed in depressed

participants compared to healthy controls. It was found, however, that depressed participants ingested less fermented foods than healthy controls. *Lactobacillus* spp., a bacterial genus found naturally in fermented foods (Bernardeau, Guguen, & Vernoux, 2006), is decreased in depressed participants (Aizawa et al., 2016). Importantly, children who ingested milk fermented with *Lactobacillus paracasei* showed an increase in *Faecalibacterium* spp. compared to those given a placebo (Berni Canani et al., 2017). It is possible that the reduction in fermented food intake in depressed participants could have resulted in reduced *Lactobacillus* spp., which then may have induced the observed decrease of *F. prausnitzii*. The interactions between bacterial taxa and downstream behavioural effects are complex, and more research is needed to determine whether there may be a specific pathway involving diet and microbiota in relation to depression severity.

As expected, depressed individuals had increased depression and anxiety symptoms, as well as more instances of early life trauma than healthy volunteers. Scores for the CTQ subscales were higher in depressed individuals than controls for all subscales except for sexual abuse. A meta-analysis has shown that childhood sexual abuse is associated with depression in adulthood (Lindert et al., 2014), however most of these studies used questionnaires other than the CTQ-SF to measure sexual (and sometimes physical) abuse (e.g., Conflict Tactics Scales, Adverse Childhood Experience (ACE) Questionnaires, Childhood Trauma Screener (CTS)). It is thus possible that different screening measures may be more accurate in measuring sexual abuse, and inclusion of these different measures may have revealed a relationship between sexual abuse and depression symptoms.

When looking at the depressed and healthy control groups separately, anxiety symptoms, depression symptoms and childhood trauma were all highly correlated in healthy controls, but surprisingly not in individuals with depression. The absence of expected relationships between

psychological measures in the depressed group is possibly due to the increased variance of symptoms (especially depression severity) in the depressed group, which could have hidden an otherwise significant relationship. It is interesting to note that childhood trauma and severity of depressive symptoms were associated with each other in the healthy control population, suggesting that this relationship may be present even at sub-depression-diagnosis thresholds.

In most, if not all, of the available published reports on depression and the gut microbiota, it was unclear whether MDD participants had a strict diagnosis of MDD (episodic in nature) or included individuals with PDD (previously known as chronic MDD or dysthymia). Because PDD shares many diagnostic criteria and symptoms with MDD, individuals with PDD may also meet the criteria for a major depressive episode based on the DSM-5 (American Psychiatric Association, 2013). In fact, PDD may be more prevalent than MDD, as many individuals experiencing depressive symptoms fall into the PDD category with one study reporting 61% of their depressed cohort fulfilling the criteria for PDD and only 39% for episodic MDD (Ildirli, Şair, & Dereboy, 2015). Therefore, it is likely that many human studies on depression are including people with PDD in their MDD group, but to our knowledge no studies have compared the microbial or inflammatory profiles of these two diagnostic groups.

The current study included diagnostic information from the SCID-5 that allowed for a distinction between PDD and MDD diagnoses. Based on this diagnostic separation, important differences in trauma, condition co-morbidity and inflammatory and bacterial measures were identified. Importantly, PDD participants experienced higher amounts of childhood trauma than MDD participants, especially emotional and physical neglect. The bacterial and inflammatory profiles of PDD and MDD groups were comparable, although a slight, but non-significant decrease in *F. prausnitzii* was observed in PDD but not in MDD participants ($p > 0.05$),

suggesting that neither subgroup was driving the bacterial change among depressed individuals described earlier. Physical abuse in the PDD subgroup was also positively correlated with anxiety severity, a relationship that was not apparent in the MDD subgroup. Although not significant, there was a trend for higher anxiety severity in the PDD group compared to the MDD group, which is consistent with the findings of Ildirli, Şair, & Dereboy, (2015), in that PDD participants were more likely to have a comorbid anxiety disorder. Within the MDD subgroup, IL-1 β negatively correlated with depression severity, which contrasts with a meta-analysis showing that IL-1 was positively associated with depression, in both clinical and community-based samples (Howren, Lamkin, & Suls, 2009), that administration of IL-1 β can induce depressive-like symptoms (Maes, Song & Yirmiya, 2012), and another study that has reported no difference in serum IL-1 β levels in depressed participants compared to healthy controls (Jiang et al., 2015). However, in the meta-analysis by Maes, Song & Yirmiya (2012), some studies (not all) showed a decrease in IL-1 β in response to antidepressant medication, which, considering our sample includes individuals on medication, could explain the negative relationship we found. Interestingly, in the PDD group, IL-1 β showed a trend towards a positive relationship with anxiety severity, but not with depression severity. It is unclear as to why we are seeing this discordant result but it could be that previous studies did not account for the relative amount of PDD and MDD diagnoses, since we have shown that the IL-1 β relationship with depression/anxiety symptoms differs between these two groups.

It is possible that the inclusion of individuals with a PDD diagnosis in samples of depressed participants (which may account for up to 46% of the depressed sample based on the current study) could be causing the large amount of discrepancy in the current literature investigating the gut microbiota and inflammatory profiles of depressed individuals, and thus

future studies should investigate further these distinct, although related, diagnoses. The current results in relation to PDD and MDD diagnoses also point towards individualized medicine when treating depression, as the expression of different symptoms may be related to the expression of different physical biomarkers (here inflammatory or microbial), which could potentially be targeted for treatment.

One of the major findings of the current study, the observation that decreased *F. prausnitzii* corresponded to more severe depression symptoms specifically in those with a history of moderate-to-severe childhood physical neglect, opens up a new perspective on the potential evolutionary advantages gut microbiota changes could have on the host. It is possible that the decreased *F. prausnitzii* seen in depressed individuals could be an adaptive response to early life stress (in the case of the current study childhood physical neglect). In previous research, presence of gut microbiota was required for maternal separation to induce depressive- and anxiety-like behaviour (in contrast, these behavioural changes were not seen in germ-free mice) (De Palma et al., 2015). It is possible that changes in microbial composition elicited by early life stress are promoting behaviours which may have at one point been adaptive for the host. For example, the increased immobility in the tail suspension test seen in maternally separated mice (De Palma et al., 2015) could act to conserve energy for future stressful situations, which in a natural mouse environment would be an adaptive behaviour since mice that were naturally maternally separated were likely born in an environment rich with other stressors, all of which would require energy to endure. This extra-cautious host behaviour would be beneficial to the bacterial colonies as well if it resulted in greater host survival. In humans, the lethargy and social withdrawal often seen in depressed individuals, referred to as “sickness behaviour” (Stieglitz et al., 2015) possibly induced through a similar cascade of early life stress and microbial manipulation of immune

functioning (for example, reduced *F. prausnitzii* could result in reduced anti-inflammatory cytokine IL-10), may have once also been advantageous, preserving energy after a highly stressful event in order to recuperate and avoid additional stressors. However, in modern society where physical threats are less prominent, these behaviours may become maladaptive. Avoiding stressors may be beneficial if an organism is hiding from a predator, however this strategy does not work as well if one is hiding from an academic deadline. More research is needed on this topic, however our results indicating that early life physical neglect is required for a negative relationship between *F. prausnitzii* and depressive symptoms to exist may support the hypothesis of an adaptive nature of bacterial manipulation of host behaviour.

Conclusion

The current findings confirm that differences exist in the bacterial profiles of individuals with depression compared to healthy controls (i.e., decreased *F. prausnitzii*). Importantly, the relationship between *F. prausnitzii* and depression severity was apparent only in individuals with higher instances of childhood physical neglect, suggesting that bacterial variations in depressed individuals may be influenced by specific types of childhood trauma. As well, inflammatory and gut microbial profiles may vary according to depression type or symptom subsets (i.e., plasma IL-1 β levels and *Prevotella* spp. relating to anxiety rather than depression in PDD individuals but not in MDD individuals), and depression severity (particularly IL-6/IL-10 ratio). It was also seen that childhood trauma combined with the IL-6/IL-10 ratios are stronger predictors of depression severity. This study also suggests that the specific type of childhood trauma and the subtype of depression are important to account for when examining the relationships between microbiota and depression. Importantly, our results suggest that the current variability of results within this field may be due to the lack of control for depression and trauma subtypes. Future studies

investigating depression and gut microbiota should control for both depression type and childhood trauma in an effort to reduce sample heterogeneity and strengthen results. In conclusion, this study is the first to present evidence that the relationship between *F. prausnitzii* and depressive symptoms is dependent on the presence of physical neglect in childhood. Since the composition of the gut microbiota can be easily measured via a non-invasive fecal sample, there exists potential for the use of fecal microbiota as a biomarker for depression (precision medicine), and thus a tool for depression diagnosis. The possibility of treating depression by targeting the gut microbiota (e.g., prebiotics, probiotics) is currently of interest (Akkasheh, et al. 2016), and future studies will benefit greatly from the information provided by this study.

Limitations

Although every effort was made to reduce the limitations involved in this study, the following must be acknowledged. Body Mass Index (BMI- a calculation involving weight and height) is known to affect the relationship between IL-6, CRP and depressive symptoms (Howren, Lamkin, & Suls, 2009). This study did not control for BMI (other than excluding those with a BMI over 30), and therefore the relationships noted in this study (or lack thereof) involving IL-6 and CRP may change when BMI is properly accounted for. Medication was also not controlled for in this study, which could have influenced the relationships between certain cytokines (e.g., IL-6, IL-1 β , TNF α) with depression severity (Maes, Song & Yirmiya, 2012; Mao et al., 2018). Importantly, healthy control participants were not administered the SCID-5 (nor did they meet with a clinical psychologist or psychiatrist like MDD participants), and therefore it is possible that a few control participants could have fulfilled criteria for MDD or PDD, but were missed when screened by the MINI (which is limited in its ability to screen for depressive symptoms as only three questions related to depression were included and we noted afterward

that participants in the depression group frequently answered these negatively). Analysis of inflammatory factors in plasma yielded very low concentrations (sometimes extrapolated from the standard curve) and it is possible that a floor effect hid potential relationships involving inflammatory measures. Finally, individual factors of depression (i.e., age of onset, duration of episode, first episode/recurring, treatment resistance) were also not controlled for, and seeing as we found important differences between PDD and MDD groups, it is possible that more differences could be highlighted if participants were further separated by other individual factors. Indeed, such differences have already been seen, for example IL-10 was increased in those with late onset depression but not early-onset depression (Zou et al., 2018). Future studies should thus be careful to control for BMI, administer all study procedures/evaluations to both depressed and healthy control groups, and control for individual factors of depression to reduce limitations.

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Table 1. Demographic data for depressed and healthy control groups.

Demographics	Healthy Control (n=42)	Depressed (n=39)	
Age Mean (+/-SD)	34.29 (+/-9.051)	32.87 (+/-8.55)	$p = 0.472$
Sex (Male/Female)	18/24	13/26	$p = 0.378$
Smoking Status			$p = 0.585$
Never	35	29	
Current	6	8	
Past	1	2	
Alcohol Use			$p = 0.916$
Never	11	9	
Light	23	24	
Moderate	6	4	
Heavey	2	2	
Cannabis Use			$p = 0.252$
Never	35	30	
Occasionally	3	4	
Moderate	4	2	
Heavey	0	3	
Antidepressant Medication	N/A	24	
Language			$p = 0.539$
English	39	38	
French	1	0	
Bilingual	2	1	
Ethnicity			
African-American	1	3	$p = 0.640$
Asian	6	2	
Caucasian	29	27	
First Nations/ Metis	0	1	
Hispanic	0	1	
Other	6	5	
Education (highest reached)			$p = 0.234$
Grade 7 to 12 without graduating high school	0	2	
Some University/College	3	6	
Completed University/College	26	23	
Completed Graduate School	13	8	
Employment Status			$p = 0.192$
Student	8	4	
Part-Time	6	5	
Full-Time	23	21	
Homemaker	1	0	
Unemployed - Seeking	4	4	
Unemployed - Disability	0	5	
Marital Status			$p = 0.559$
Single	23	19	
Married/Cohabiting	17	15	
Divorced/Separated	2	4	
Widowed	0	1	
Birth Method			$p = 0.441$
Vagnial	33	31	
Cesarian	4	6	
Unknown	5	2	

Table 2. Dietary data for depressed and healthy control groups.

Diet	Healthy Control (n=42)	Depressed (n=39)	
Fermented Food Intake			$p = 0.001^{**}$
Never	1	8	
Less than once/week	11	20	
More than once/week	19	5	
Daily	11	6	
Diet			$p = 0.167$
Primarily Vegetarian	2	7	
Primarily Meat	7	6	
Both	33	26	
Vitamins/Supplements	20	21	$p = 0.507$
$^{**}p < 0.01$			

Table 3. Clinical measures of depressed and healthy control participants.

Clinical Measures	Healthy Control (n=42)	Depressed (n=39)	
Mini Score Mean (+/- SD)	1.93 (+/-1.61)	9.03 (+/-2.61)*** (n=35)	$p < 0.001$
BDI Score Mean (+/-SD)	5.49 (+/-5.97)	30.46 (+/-8.05)***	$p < 0.001$
BAI Score Mean (+/-SD)	5.21 (+/- 4.48)	20.54 (+/-9.27)***	$p < 0.001$
CTQ-SF Score Mean (+/-SD)	13.33 (+/-11.34)	23.00 (+/-15.07)**	$p = 0.002$
CTQ Section 1	3.40 (+/-2.98)	6.36 (+/-4.67)**	$p = 0.001$
CTQ Section 2	1.40 (+/-2.18)	3.00 (+/-3.51)*	$p = 0.018$
CTQ Section 3	0.55 (+/- 1.43)	1.10 (+/-2.36)	$p = 0.211$
CTQ Section 4	5.07 (+/-4.21)	8.36 (+/-5.17)**	$p = 0.002$
CTQ Section 5	2.29 (+/-2.09)	4.13 (+/-3.50)**	$p = 0.006$
Diagnosis MDD/PDD	N/A	21/18	
GAD Comorbidity	N/A	21	
<i>*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to healthy controls</i>			

Table 4. Clinical measures for MDD and PDD subgroups and the healthy control group.

Clinical Measures	Healthy Control (n=42)	MDD (n=21)	PDD (n=18)
Mini Score Mean (+/- SD)	1.93 (+/-1.61)	9.06(+/-3.30, n=18)***	9.00(+/-1.70, n=17)***
BDI Score Mean (+/-SD)	5.49 (+/-5.97)	28.62(+/-6.95)***	32.61(+/-8.88)***
BAI Score Mean (+/-SD)	5.21 (+/- 4.48)	18.52(+/-8.91)***	22.89(+/-9.38)***
CTQ-SF Score Mean (+/-SD)	13.33 (+/-11.34)	17.90(+/-12.10)	28.94(+/-16.32)***†
CTQ Section 1	3.40 (+/-2.98)	5.33(+/-3.50)	7.56(+/-5.61)**
CTQ Section 2	1.40 (+/-2.18)	2.76(+/-3.10)	3.39(+/-4.34)
CTQ Section 3	0.55 (+/- 1.43)	0.67(+/-1.43)	1.61(+/-3.09)
CTQ Section 4	5.07 (+/-4.21)	6.33(+/-4.73)	10.72(+/-4.74)***‡
CTQ Section 5	2.29 (+/-2.09)	2.81(+/-2.64)	5.67(+/-3.80)***‡
GAD Comorbidity (no GAD/GAD)	N/A	9/12	9/9
p<0.01, *p<0.001, compared to healthy controls			
†p<0.05, ‡p<0.01, compared to MDD group			

Table 5. Inflammatory and bacterial profiles of depressed and healthy control groups (mean $\pm$ SD).

Inflammatory profile	Healthy Control (n=42)	Depressed (n=39)	
CRP	1279.31 ($\pm$ 1675.88)	1882.61 ($\pm$ 2220.74)	$p=0.170$
INF γ	0.41($\pm$ 0.48)	0.41 ($\pm$ 0.46)	$p=0.955$
IL-1 β	0.11($\pm$ 0.17)	0.11($\pm$ 0.19)	$p=0.931$
IL-10	0.05($\pm$ 0.08)	0.06($\pm$ 0.07)	$p=0.787$
IL-12	0.28($\pm$ 0.37)	0.30($\pm$ 0.32)	$p=0.805$
IL-17	0.23($\pm$ 0.35)	0.23($\pm$ 0.24)	$p=0.996$
IL-2	0.53($\pm$ 0.58)	0.60($\pm$ 0.47)	$p=0.546$
IL-4	0.45($\pm$ 0.79)	0.47($\pm$ 0.82)	$p=0.899$
IL-6	1.54($\pm$ 2.10)	2.03($\pm$ 2.13)	$p=0.296$
TNF α	0.59($\pm$ 0.66)	0.67($\pm$ 0.51)	$p=0.557$
IL-6/IL-10 ratio	31.80($\pm$ 18.73, n=35)	40.51($\pm$ 35.63, n=32)	$p=0.223$
Bacterial profile	Healthy Control	Depressed	
<i>Prevotella</i> spp.	0.90($\pm$ 0.16, n=39)	0.89($\pm$ 0.21, n=32)	$p=0.686$
Enterobacteriaceae	0.45($\pm$ 0.12, n=37)	0.42($\pm$ 0.11, n=30)	$p=0.328$
<i>F. prausnitzii</i>	0.36($\pm$ 0.04, n=37)	0.33($\pm$ 0.04, n=29)*	$p=0.028$
<i>*p<0.05 compared to healthy controls</i>			

Table 6. Inflammatory and bacterial profiles of MDD, PDD and healthy control participants (mean $\pm$ SD).

Inflammatory profile	Healthy Control (n=42)	MDD (n=21)	PDD (n=18)
CRP	1279.31 (+/-1675.88)	1895.85(+/-2291.14)	1867.16(+/-2201.72)
INF γ	0.41(+/- 0.48)	0.47(+/-0.51)	0.33(+/-0.39)
IL-1 β	0.11(+/-0.17)	0.13(+/-0.22)	0.09(+/-0.16)
IL-10	0.05(+/- 0.08)	0.06(+/-0.09)	0.05(+/-0.05)
IL-12	0.28(+/-0.37)	0.35(+/-0.38)	0.24(+/-0.22)
IL-17	0.23(+/-0.35)	0.26(+/-0.29)	0.19(+/-0.17)
IL-2	0.53(+/-0.58)	0.63(+/-0.52)	0.57(+/-0.40)
IL-4	0.45(+/-0.79)	0.53(+/-0.90)	0.40(+/-0.73)
IL-6	1.54(+/-2.10)	2.17(+/-2.07)	1.87(+/-2.24)
TNF α	0.59(+/-0.66)	0.72(+/-0.57)	0.60(+/-0.45)
IL-6/IL-10 ratio	31.80(+/-18.73, n=35)	39.87(+/-38.19)	41.31(+/-33.43)
Bacterial profile	Healthy Control	MDD	PDD
<i>Prevotella</i> spp.	0.90(+/-0.16, n=39)	0.88(+/-0.20, n=18)	0.88(+/-0.24, n=14)
Enterobacteriaceae	0.45(+/-0.12, n=37)	0.40(+/-0.10, n=17)	0.43(+/-0.13, n=13)
<i>F. prausnitzii</i>	0.36(+/-0.04, n=37)	0.33(+/-0.04, n=18)	0.34(+/-0.05, n=11)

Figure 1. Daily fermented food intake (% of group reporting each frequency). Depressed: n=39; Healthy control: n=42. Depressed participants consumed significantly less fermented foods than healthy control participants ($p = .001$).

*Figure 2. Clinical measures among depressed and healthy control participants. For each scale, high scores are indicative of increased severity. (A) MINI scores were higher in depressed participants compared to healthy control participants. (B) Beck Depression Inventory scores were higher in depressed participants compared to healthy control participants. (C) Beck Anxiety Inventory scores were higher in depressed compared to healthy control participants. (D) Childhood Trauma Questionnaire-Short Form total scores were higher in depressed compared to healthy control participants, as well as in Subscales 1, 2, 4, and 5. CTQ-SF subscales correspond to the following measures: 1- Emotional Abuse, 2- Physical Abuse, 3- Sexual Abuse, 4- Emotional Neglect, 5- Physical Neglect. Data represents means $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ relative to healthy control participants.*

*Figure 3. Clinical measures for the MDD, PDD and healthy control groups. For all scales, increasing scores are indicative of increasing severity. (A) MINI scores were higher in both MDD and PDD subgroups compared to healthy control participants. MINI scores did not differ between MDD and PDD subgroups. (B) Beck Depression Inventory-II scores were higher in both MDD and PDD subgroups compared to healthy control participants, BDI-II scores did not differ between MDD and PDD subgroups. (C) Beck Anxiety Inventory scores were higher both MDD and PDD subgroups compared to healthy control participants. BAI scores did not differ between MDD and PDD subgroups. (D) Childhood Trauma Questionnaire-Short Form scores were higher in PDD subgroups compared to healthy control and MDD participants. PDD scores for Subscale 1 were higher than healthy control scores. Subscales 2 and 3 did not show any group differences. Subscale 4 scores were higher in PDD participants compared to healthy control and MDD participants. Subscale 5 scores were higher in PDD participants compared to healthy control and MDD participants. CTQ subscales correspond to the following measures: 1- Emotional Abuse, 2- Physical Abuse, 3- Sexual Abuse, 4- Emotional Neglect, 5- Physical Neglect. Data represents means $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ relative to healthy control participants, and † $p < 0.05$ and ‡ $p < 0.01$ relative to MDD participants.*

Figure 4. Distributions of bacterial taxa (% of total bacteria present) in depressed and healthy control participants. *F. prausnitzii* was significantly reduced in depressed participants compared to healthy controls. No differences were detected between groups for *Prevotella* spp. or the *Enterobacteriaceae* family. Data represents means $\pm$ S.E.M. * $p < 0.05$ relative to healthy control participants.

Figure 5. Correlations between IL-6/IL-10 ratios and BDI-II scores across all participants. An increase in IL-6/IL-10 ratio corresponds to an increase in BDI-II scores, $p < 0.05$.

Figure 6. Relationship between CTQ-2 (Physical Abuse) and plasma IL-6 concentration in depressed participants only. Correlation is significant at $p < 0.01$.

Figure 7. Relationship between plasma levels of IL-6 and IL-10 for depressed and healthy control participants separately. Both correlations are significant at $p < 0.01$.

Figure 8. Relationship between BDI-II scores and plasma levels of IL-1 β for MDD and PDD participants separately. **indicates significant correlation at $p < 0.01$.

Figure 9. Relationship between BAI scores and plasma levels of IL-1 β for MDD and PDD participants separately. *indicates significant correlation at $p < 0.05$.

Figure 10. Relationship between CTQ-SF subscale 4 (Emotional Neglect) scores and *F. prausnitzii* expression for MDD and PDD participants separately. *indicates significant correlation at $p < 0.05$.

Figure 11. Moderating effect of physical neglect (CTQ subscale 5) on the relationship between F. praunitzii and BDI-II scores. The negative relationship between F. praunitzii and BDI-II scores was significant only in participants who experienced moderate-to-severe physical neglect in their childhood ($p < 0.05$).

Supplementary Material

Table S1. Pearson correlations for clinical measures for all participants

	MINI	BDI	BAI	CTQ	CTQ-1	CTQ-2	CTQ-3	CTQ-4	CTQ-5
MINI	-								
BDI	.787**	-							
BAI	.742**	.734**	-						
CTQ	.458**	.412**	.370**	-					
CTQ-1	.481**	.437**	.398**	.883**	-				
CTQ-2	.249*	.254*	.337**	.714**	.624**	-			
CTQ-3	.113	.008	.039	.438**	.288**	.097	-		
CTQ-4	.424**	.391**	.296**	.874**	.685**	.528**	.201	-	
CTQ-5	.404**	.380**	.269*	.798**	.641**	.367**	.292**	.704**	-

* correlation significant at the $p < 0.05$ level (two tailed)

** correlation significant at the $p < 0.01$ level (two tailed)

Table S2. Pearson correlations for clinical measures within depressed and healthy control groups

Healthy Control									
	MINI	BDI	BAI	CTQ	CTQ-1	CTQ-2	CTQ-3	CTQ-4	CTQ-5
MINI	-								
BDI	.038	-							
BAI	.232	.691**	-						
CTQ	.144	.338*	.341*	-					
CTQ-1	.197	.315*	.318*	.849**	-				
CTQ-2	-.082	.444**	.471**	.674**	.594**	-			
CTQ-3	.053	-.057	-.052	.529**	.256	.141	-		
CTQ-4	.245	.332*	.321*	.854**	.692**	.407**	.199	-	
CTQ-5	.079	.126	.111	.725**	.503**	.120	.395**	.717**	-
Depressed									
	MINI	BDI	BAI	CTQ	CTQ-1	CTQ-2	CTQ-3	CTQ-4	CTQ-5
MINI	-								
BDI	.146	-							
BAI	.319	.133	-						
CTQ	.392*	.198	.125	-					
CTQ-1	.482**	.260	.180	.879**	-				
CTQ-2	.227	-.002	.206	.739**	.616**	-			
CTQ-3	.237	-.023	.090	.419**	.374**	.039	-		
CTQ-4	.222	.163	-.019	.861**	.621**	.566**	.223	-	
CTQ-5	.321	.284	.051	.804**	.636**	.447**	.260	.657**	-
* correlation significant at the p<0.05 level (two tailed)									
** correlation significant at the p<0.01 level (two tailed)									

Table S3. Pearson Correlations for clinical measures within PDD and MDD subgroups

MDD									
	MINI	BDI	BAI	CTQ	CTQ-1	CTQ-2	CTQ-3	CTQ-4	CTQ-5
MINI	-								
BDI	.060	-							
BAI	.338	.329	-						
CTQ	.360	-.312	-.311	-					
CTQ-1	.597**	.086	-.081	.790**	-				
CTQ-2	.245	-.418	-.233	.876**	.562**	-			
CTQ-3	.250	-.296	-.123	.369	.284	.083	-		
CTQ-4	.114	-.363	-.443*	.854**	.528*	.682**	.314	-	
CTQ-5	.263	-.244	-.187	.778**	.538*	.832**	.115	.454*	-
PDD									
	MINI	BDI	BAI	CTQ	CTQ-1	CTQ-2	CTQ-3	CTQ-4	CTQ-5
MINI	-								
BDI	.351	-							
BAI	.388	-.140	-						
CTQ	.648**	.410	.334	-					
CTQ-1	.561*	.287	.282	.928**	-				
CTQ-2	.269	.235	.534*	.694**	.646**	-			
CTQ-3	.362	.011	.134	.394	.366	-.001	-		
CTQ-4	.586*	.456	.202	.838**	.664**	.523*	.085	-	
CTQ-5	.611**	.492*	.048	.766**	.664**	.233	.234	.706**	-

* correlation significant at the $p < 0.05$ level (two tailed)

** correlation significant at the $p < 0.01$ level (two tailed)

Table S4. Pearson correlations between inflammatory/bacterial and clinical measures

	MINI	BDI	BAI	CTQ	CTQ-1	CTQ-2	CTQ-3	CTQ-4	CTQ-5
CRP	.165	.079	.109	-0.004	.031	-.029	.049	-.017	-.043
INF γ	.105	-.035	.074	-.034	-.008	0.074	-.091	-.076	-.025
IL-1 β	.105	-.079	.057	.001	-.002	.085	-.018	-.009	-.058
IL-10	.153	-.023	-.010	.025	.061	.062	-.081	.007	.027
IL-12	.099	-.030	-.033	-.015	.031	.053	-.089	-.055	-.003
IL-17	.041	-.039	-.056	-.006	.015	.051	-.073	-.023	-.001
IL-2	.061	.042	-.020	.050	.019	.079	-.122	.097	.069
IL-4	.134	-.055	.041	-.010	.023	.045	-.014	-.040	-.050
IL-6	.130	.150	.088	.069	.106	.171	-.104	.040	.008
TNF α	.096	.015	.073	.010	.030	.095	-.092	.010	-.036
IL-6/IL-10 ratio	.125	.277*	.203	.051	.015	.212	-.024	.046	-.082
<i>Prevotella</i> spp.	.085	.009	.154	.150	.200	.183	.186	.032	-.006
Enterobacteriaceae	-0.159	-0.022	-0.081	-0.018	0.068	0.066	-0.002	-0.111	-0.07
<i>F. prausnitzii</i>	-0.185	-0.193	-0.138	-0.067	0.02	0.021	-0.111	-0.022	-0.233
* correlation significant at the p<0.05 level (two tailed)									
** correlation significant at the p<0.01 level (two tailed)									

Table S5. Pearson correlations between bacterial and inflammatory measures

Pearson Correlations			
	<i>Prevotella</i> spp.	Enterobacteriaceae	<i>F. prausnitzii</i>
CRP	.107	.141	-.081
INF γ	-.022	-.014	.099
IL-1 β	.075	-.038	.116
IL-10	-.025	-.085	.059
IL-12	-.004	-.091	.061
IL-17	-.001	-.067	.080
IL-2	.025	-.048	.064
IL-4	.074	-.046	.077
IL-6	-.064	-.026	-.025
TNF α	.082	.011	.096
IL-6/IL-10 ratio	.016	.015	-.087
* correlation significant at the p<0.05 level (two tailed)			
** correlation significant at the p<0.01 level (two tailed)			

Table S6. Pearson correlations between inflammatory/bacterial measures and clinical measures within depressed and healthy control groups

Healthy Control									
	MINI	BDI	BAI	CTQ	CTQ-1	CTQ-2	CTQ-3	CTQ-4	CTQ-5
CRP	.202	.081	.204	2.46	.216	.120	.218	.131	.255
INF γ	.056	.165	-.033	-.211	-.109	-.179	-.109	-.127	-.185
IL-1 β	.074	.129	.020	-.142	-.120	-.111	-.068	-.089	-.152
IL-10	.062	.123	-.091	-.107	-.027	-.119	-.112	-.054	-.086
IL-12	.005	.146	-.086	-.088	-.028	-.082	-.073	-.074	-.053
IL-17	-.053	.189	-.088	-.053	.004	-.049	-.078	-.038	-.024
IL-2	-.139	.172	-.099	.086	.058	-.003	-.058	.163	.147
IL-4	.066	.116	-.036	-.167	-.123	-.142	-.068	-.138	-.139
IL-6	.017	.176	-.061	-.163	-.150	-.134	-.076	-.098	-.157
TNF α	-.090	.206	-.095	-.013	.029	-.025	-.081	.011	.025
IL-6/IL-10 ratio	.036	.113	.005	.078	-.121	.345*	.144	.111	-.152
<i>Prevotella</i> spp.	-.066	.201	.121	.168	.239	.154	.133	.078	-.009
Enterobacteriaceae	-0.192	0.145	-0.007	-0.031	0.125	0.034	-0.012	-0.119	-0.153
<i>F. prausnitzii</i>	0.05	0.229	0.06	-0.087	0.076	0.012	-0.028	-0.024	-0.24
Depressed									
	MINI	BDI	BAI	CTQ	CTQ-1	CTQ-2	CTQ-3	CTQ-4	CTQ-5
CRP	-.042	-.231	-.088	-.249	-.153	-.192	-.137	-.208	-.269
INF γ	.267	-.255	.212	.117	.129	.330*	-.066	-.035	.083
IL-1 β	.198	-.386	.103	.101	.065	.249	.046	.047	-.015
IL-10	.334	-.276	-.028	.121	.114	.229	-.039	.044	.090
IL-12	.253	-.332*	-.086	.029	.064	.189	-.117	-.064	.017
IL-17	.222	-.346*	-.090	.053	.034	.198	-.064	-.004	.024
IL-2	.133	-.219	-.123	-.029	-.062	.150	-.243	-.009	-.022
IL-4	.288	-.318*	.089	.102	.114	.207	.059	.028	-.010
IL-6	.078	.056	.035	.186	.220	.412**	-.155	.088	.049
TNF α	.218	-.360*	.130	-.016	-.013	.216	-.118	-.014	-.137
IL-6/IL-10 ratio	-.034	.385*	.159	-.044	-.024	.132	-.129	-.061	-.133
<i>Prevotella</i> spp.	.438*	0.071	.362*	.189	.236	.234	.365	.038	.023
Enterobacteriaceae	0.097	0.236	0.025	0.097	0.138	0.175	0.022	-0.018	0.058
<i>F. prausnitzii</i>	0.201	.000	0.121	0.146	0.187	0.167	0.058	0.18	-0.112
* correlation significant at the p<0.05 level (two tailed)									
** correlation significant at the p<0.01 level (two tailed)									

Table S7. Pearson correlations between inflammatory and bacterial measures within groups

Healthy Control			
	<i>Prevotella</i> spp.	Enterobacteriaceae	<i>F. prausnitzii</i>
CRP	-.153	.044	-.066
INF γ	-.282	-.091	.061
IL-1 β	-.120	-.035	.034
IL-10	-.091	-.018	.055
IL-12	.005	-.085	.088
IL-17	.028	-.054	.098
IL-2	.082	-.024	.098
IL-4	-.053	-.044	.032
IL-6	-.157	-.002	.061
TNF α	-.017	.015	.093
IL-6/IL-10 ratio	-.193	-.226	.024
Depressed			
	<i>Prevotella</i> spp.	Enterobacteriaceae	<i>F. prausnitzii</i>
CRP	.341	.278	-.105
INF γ	.219	.102	.183
IL-1 β	.234	-.020	.273
IL-10	.046	-.170	.143
IL-12	-.008	-.098	.094
IL-17	-.036	-.103	.095
IL-2	-.022	-.069	.093
IL-4	.187	-.037	.210
IL-6	.030	-.016	-.035
TNF α	.211	.027	.198
IL-6/IL-10 ratio	.132	.247	-.079
* correlation significant at the p<0.05 level (two tailed)			
** correlation significant at the p<0.01 level (two tailed)			

Table S8. Pearson correlations between inflammatory/bacterial measures and clinical measures within MDD and PDD subgroups

MDD									
	MINI	BDI	BAI	CTQ	CTQ-1	CTQ-2	CTQ-3	CTQ-4	CTQ-5
CRP	.154	-.005	.169	-.291	-.046	-.223	-.125	-.276	-.449*
INF γ	.278	-.385	.097	.246	.195	.450*	-.119	.043	.329
IL-1 β	.177	-.595**	-.162	.126	.016	.161	.204	.069	.135
IL-10	.422	-.411	-.112	.375	.296	.346	.120	.240	.427
IL-12	.383	-.378	-.069	.278	.211	.297	.035	.120	.411
IL-17	.348	-.439*	-.103	.304	.179	.310	.104	.168	.435*
IL-2	.288	-.229	-.173	.175	.069	.129	.015	.100	.371
IL-4	.352	-.505*	-.079	.103	.070	.100	.217	-.008	.157
IL-6	.148	-.021	-.116	.268	.247	.350	-.118	.033	.495*
TNF α	.294	-.357	-.078	.076	.016	.066	.096	-.066	.208
IL-6/IL-10 ratio	.002	.291	.255	-.164	-.083	-.050	-.147	-.259	-.053
<i>Prevotella</i> spp.	.468	.170	.225	.062	.234	-.079	.276	.014	-.076
Enterobacteriaceae	.192	.219	.105	.05	.109	-.013	.230	.059	-.110
<i>F. prausnitzii</i>	.222	.020	-.064	.352	.406	.010	.412	.546*	-.111
PDD									
	MINI	BDI	BAI	CTQ	CTQ-1	CTQ-2	CTQ-3	CTQ-4	CTQ-5
CRP	-.464	-.465	-.388	-.251	-.249	-.170	-.164	-.169	-.170
INF γ	.273	.023	.506*	.149	.179	.247	.017	.032	.011
IL-1 β	.267	-.134	.579*	.186	.180	.401	-.018	.145	-.091
IL-10	.018	-.050	.227	-.069	.010	.152	-.147	-.145	-.185
IL-12	-.222	-.233	.005	-.125	.026	.123	-.232	-.188	-.305
IL-17	-.228	-.212	.008	-.152	-.050	.119	-.185	-.155	-.371
IL-2	-.305	-.197	-.021	-.203	-.162	.201	-.459	-.092	-.373
IL-4	.146	-.102	.378	.198	.215	.350	.001	.186	-.100
IL-6	-.048	.159	.229	.201	.251	.483*	-.167	.239	-.220
TNF α	.027	-.349	.512*	-.016	.019	.421	-.250	.038	-.420
IL-6/IL-10 ratio	-.129	.521	-.034	-.078	-.022	-.332	-.169	.212	-.283
<i>Prevotella</i> spp.	.459	-.031	.533*	.339	.268	.480	.301	.061	.093
Enterobacteriaceae	-.120	.181	-.145	.003	.067	.269	-.129	-.291	.049
<i>F. prausnitzii</i>	.191	-.103	.285	-.108	-.011	.274	-.139	-.324	-.229
* correlation significant at the p<0.05 level (two tailed)									
** correlation significant at the p<0.01 level (two tailed)									

Table S9. Pearson correlations between inflammatory and bacterial measures within MDD and PDD subgroups

MDD			
	<i>Prevotella</i> spp.	Enterobacteriaceae	<i>F. prausnitzii</i>
CRP	.429	.280	-.187
INF γ	.099	-.138	-.116
IL-1 β	.167	-.212	.086
IL-10	.142	-.254	.134
IL-12	.112	-.161	0.28
IL-17	.097	-.096	0.99
IL-2	.022	-.095	.148
IL-4	.220	-.221	.032
IL-6	.082	-.081	-.179
TNF α	.266	.024	.072
IL-6/IL-10 ratio	.108	.253	-.176
PDD			
	<i>Prevotella</i> spp.	Enterobacteriaceae	<i>F. prausnitzii</i>
CRP	.270	.489	.271
INF γ	.401	.458	.721*
IL-1 β	.347	.257	.589
IL-10	-.126	.022	.214
IL-12	-.226	.076	.299
IL-17	-.264	-.071	.126
IL-2	-.077	.010	.065
IL-4	.155	.184	.468
IL-6	-.021	.062	.124
TNF α	.156	.062	.399
IL-6/IL-10 ratio	.215	.322	.132
* correlation significant at the p<0.05 level (two tailed)			
** correlation significant at the p<0.01 level (two tailed)			

Appendix A – MINI questionnaire

M.I.N.I. Screen 6.0 © 1998 by David V. Sheenan et al.

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Appendix B – Exclusion Criteria

Medical Conditions Checklist for Exclusion Criteria

To be completed by the study investigator during the screening process (telephone interview for healthy control participants and appointment with clinical psychologist for MDD participants)

- ☐ History of inflammatory bowel disease or of irritable bowel syndrome
Specify condition: _____
- ☐ Obesity (BMI<30)
- ☐ History of cardiovascular diseases
- ☐ History of diabetes
- ☐ History of liver cirrhosis or fatty liver disease (please specify)
- ☐ Known current bacterial, fungal, or viral infection (please specify)
- ☐ Use of antibiotics, probiotics, or prebiotics in the last month (please specify)
- ☐ Do you take antidepressant, anxiolytic, or antipsychotic medication? _____
Changes in dose in the last month?
- ☐ Do you smoke? (or have you quit?) Approximate frequency? _____
Change in smoking habits (check if smoked in past 6 months)
- ☐ Illicit drug use (ex. marijuana, cocaine, prescription drugs without prescription; check if used in past 6 months and approximate frequency) _____
- ☐ Approximate number of alcoholic drinks per week: _____
- ☐ Changes in diet over last month (ex. became vegetarian, began fasting, body cleansing/detoxification, etc)

Other Notes: _____

Appendix C – Socio-demographic questionnaire

Demographics Form

The following is used for demographic purposes only, and will not be used to identify you.

Date of Birth (MM/DD/YYYY): _____

Sex: ☐ Male ☐ Female ☐ Other

Age:

Preferred Language: ☐ English ☐ French ☐ Other

Ethnicity: ☐ Asian ☐ Caucasian ☐ African-American ☐ First Nations
☐ Hispanic ☐ Other _____

Education: ☐ Grade 6 or less
☐ Grade 7 to 12 without graduating high school
☐ Graduated high school or equivalent
☐ Partial college education
☐ Graduated college program
☐ Completed graduate/professional school (e.g., Masters/PhD)

Employment status: ☐ Student
☐ Part-time employed
☐ Full-time employed
☐ Full-time homemaker
☐ Unemployed, seeking work
☐ Unemployed, disability
☐ Retired

Marital Status: ☐ Single
☐ Married or cohabiting
☐ Separated or divorced
☐ Widowed

Birth Method: ☐ Caesarian
☐ Vaginal
☐ Unknown

Diet:

Fermented foods: How often do you eat fermented foods (ex. yogurt, kefir, kombucha, kimchi, tempeh, sauerkraut)?

- ☐ Daily
☐ More than once a week
☐ Less than once a week
☐ Never

General Diet Habits: Please check food items that you consume regularly/are typical to your diet or cooking habits (check all that apply).

- ☐ Fresh fruits and vegetables
☐ Beans, lentils, rice, seeds
☐ Meat (ex. beef, chicken, pork, salami, burgers)
☐ Foods marketed as “high fiber” (ex. Quaker Oats Plus fiber, high fiber cereals)
☐ Ginger, garlic, or turmeric
☐ Olive oil
☐ Green or black tea
☐ Cheese-based dishes (ex. pizza, cheesy pasta)
☐ Pastries, cookies, or doughnuts
☐ Snack foods (ex. chips, crackers, popcorn)
☐ Breakfast sandwiches, to-go wraps/sandwiches (ex. pre-made or fast-food)

Vitamins and Supplements: Do you take any over-the-counter vitamins or supplements (ex. iron, calcium, multivitamin, B12)?

If so, please indicate what you take:

Appendix D – Study Questionnaires

Childhood Trauma Questionnaire © 1997 by David P. Bernstein and Laura Fink

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Beck Depression Inventory®-II © 1996 by Aaron T. Beck, Gregory K. Brown, and Robert A. Steer

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Beck Anxiety Inventory © 1988 by Aaron T. Beck, Gregory K. Brown, and Robert A. Steer

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