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Full Length Article

Gut bacterial taxonomic abundances vary with cognition, personality, and mood in the Wisconsin Longitudinal Study

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ABSTRACT

Animal studies have shown that the gut microbiome can influence memory, social behavior, and anxiety-like behavior. Several human studies show similar results where variation in the gut microbiome is associated with dementia, depression, and personality traits, though most of these studies are limited by small sample size and other biases. Here, we analyzed fecal samples from 313 participants in the Wisconsin Longitudinal Study, a randomly selected population-based cohort of older adults, with measured psycho-cognitive dimensions (cognition, mood, and personality) and key confounders. 16s V4 sequencing showed that *Megamonas* is associated with all measured psycho-cognitive traits, *Fusobacterium* is associated with cognitive and personality traits, *Pseudoramibacter_Eubacterium* is associated with mood and personality traits, *Butyvibrio* is associated with cognitive traits, and *Cloacibacillus* is associated with mood traits. These findings are robust to sensitivity analyses and provide novel evidence of shared relationships between the gut microbiome and multiple psycho-cognitive traits in older adults, confirming some of the animal literature, while also providing new insights. While we addressed some of the weaknesses in prior studies, further studies are necessary to elucidate temporal and causal relationships between the gut microbiome and multiple psycho-cognitive traits in well-phenotyped, randomly-selected population-based samples.

1. Introduction

The gut microbiome is hypothesized to be part of a complex and bi-directional communication between the gastrointestinal tract and the central nervous system, known as the gut-brain axis. The existing empirical research, largely based on animal models, documents links between disturbances in the gut microbiome and impaired memory, impaired social behavior, reduced exploratory behavior, changes in anxiety-like behavior and the startle reflex (Buffington et al., 2016; Gareau et al., 2011; MacFabe, 2015; Sanguinetti et al., 2019; Zhan et al., 2018). To date, very few human studies, especially among older adults specifically, have explored this relationship, and while these studies have been critical to building our understanding of the gut-brain axis, significant gaps in our knowledge remain.

A central knowledge gap in the existing research is whether there are *shared* relationships, across multiple domains, between gut microbial composition and psycho-cognitive traits. Existing studies have examined psycho-cognitive domains, including personality, mood, and cognition, separately from each other (for a review see (Dinan and Cryan, 2019). Comparisons across studies are difficult to draw because each domain has been explored in separate populations, which vary by age or development, geography, and underlying health conditions, all of which highly correlate with microbial composition (Herd et al., 2019; McFarland et al., 2019). Moreover, basic measurement differences in phenotypes across studies make it difficult to draw comparisons.

Even within specific domains, including cognition, mood and personality, there are large gaps in our knowledge, including limited replication of findings from human samples. For example, while a few studies

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have found relationships between cognition and microbial composition for infants and toddlers, and those with dementia, we cannot necessarily generalize these findings to adults and older adults (Carlson et al., 2018; Cattaneo et al., 2017; Christian et al., 2015; Gao et al., 2019; Vogt et al., 2017). One study focused on older adults found particular phyla associated with below versus above average cognitive performance, but it included only 43 adults deemed healthy, limiting the generalizability of the results to other older adult samples (Manderino et al., 2017a).

Human studies on personality and mood have similar limitations. Studies on personality find relationships to temperament and fear reactivity for infants (Aatsinki et al., 2019; Christian et al., 2015) and an abundance of certain commensal bacteria with neuroticism and conscientiousness in one sample of Korean adults (Kim et al., 2018), but there are no studies of older adults or any adults beyond the Korean sample. Studies on mood have shown relationships to bacterial alpha diversity, but they have been largely focused on major depressive disorder rather than general mood, like depressive symptoms, anxiety or anger (Jiang et al., 2015; Kelly et al., 2016; Valles-Colomer et al., 2019) or studied indirect links to mood, such as probiotic use (Huang et al., 2016; Jiang et al., 2015; Slykerman et al., 2017; Steenbergen et al., 2015; Tillisch et al., 2013).

Finally, there are some basic data limitations with the population studies employed in prior empirical work. First, they are typically not conducted on randomly-selected populations, which are required to ensure the reliability of findings in a particular population (Emerson, 2015). Another limitation is that most of these studies have more limited phenotypic measures that limit their ability to adjust for confounding.

Consequently, we address some key limitations in the existing research. First, we examine the association between diversity, composition and relative abundances in the gut microbiome across multiple psychocognitive measures (cognition, mood and personality) using the same sample. Using the same sample, rather than comparing the results from different outcomes across different studies, better allows for testing a shared relationship between multiple psycho-cognitive traits and taxonomic groups. Second, unlike prior studies, we employ a randomly selected population-based cohort of older adults, which increases the reliability of the findings from this study. Third, our focus on older adults is important given that we might expect that these relationships vary across age groups, but most research has not focused on older adults. Fourth, our study includes more extensive phenotypic measures for both outcomes (e.g. detailed measures of cognition, mood, and personality), as well as hypothesized confounders (e.g. net worth, educational attainment, diet, and medications) in the association between diversity, composition and relative abundances in the gut microbiome and multidimensional measures of cognition, mood, and personality.

2. Materials and methods

2.1. Participants

Data in this study come from the Wisconsin Longitudinal Study (WLS), a population-based cohort study with over 60 years of follow up (Herd et al., 2014). WLS is a one-third sample of all 1957 Wisconsin high school graduates and a randomly selected sibling, with spouses of graduates and siblings added in 2004. The first in-person questionnaire was administered at age 18 in 1957, followed by data collection in 1964, 1975, 1992, 2004, and 2011. Response rates range from 60 to 86% in the graduate and sibling samples in recent follow-up years, with higher response rates among women and those with higher educational attainment. One major limitation is that the sample is nearly all non-Hispanic White, reflecting the composition of Wisconsin high schools in 1957.

Following the most recent wave of WLS, a pilot study was conducted to collect fecal microbiota samples from WLS participants. 500 participants were randomly sampled conditional on (1) having participated in 2011, (2) living in one of 10 Wisconsin counties, and (3) being part of a sibling pair (Herd et al., 2017). Of these, n = 329 completed sampling for a 68.7% response rate, and another 100 spouses who were present at the time of the collection visit also participated. Of the 329, 134 are siblings. The present sample excludes spouses because psycho-cognitive dimensions were not measured for spouses in 2011. Those who refused had slightly lower IQs, lower educational levels, slightly higher BMI, and were more likely to be women (Herd et al., 2017). A survey administered concurrently with the fecal sample collection kit collected information on typical dietary behavior in the past year, as well as antibiotics, probiotics, and prebiotics taken in the past 6 months.

2.2. Variables

2.2.1. Measurement of mood, cognition, and personality

Cognitive function is a multidimensional construct referring to multiple mental faculties such as reasoning, problem solving, memory, and decision making. We aimed to capture associations with quantitative reasoning, verbal fluency, and memory/attention. We captured quantitative reasoning using a Rasch score derived from performance on the McArdle and Woodcock number series task. To capture verbal fluency and memory/attention, we used gender-specific factor scores from a confirmatory factor analysis validated using WLS data (Yonker et al., 2007). Verbal fluency is a weighted average of category and letter fluency scores, and memory/attention is a weighted average of delayed recall, immediate recall, and digit ordering. The survey instrument is available at https://www.ssc.wisc.edu/wlsresearch/documentation/flowcharts/.

Depression was measured by the Center for Epidemiological Studies – Depression Scale (CES-D,(Radloff, 1977)), using the sum of the category ratings for the 20 items capturing depressive symptoms over the past week. Anxiety was measured using the Spielberger Anxiety Index (Spielberger, 2010), which captures feelings of calm, worry, tension, relaxation, ease, and jitteriness over the past week. Anger was measured using the Spielberger Anger Index (del Barrio et al., 2004), which asks the number of times in the past week a respondent felt furious, like banging on the table, yelling at someone, or breaking things.

Personality traits were assessed using a modified version of the Five-Factor model inventory (John et al., 1991), which factors personality into Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness, each measured using a six-level Likert scale.

2.3. Confounders

Our aim in adjusting for covariates is not to isolate any causal effects per se, but instead to reduce, to the extent possible, the role of obvious alternative explanations for microbiome-outcome associations other than a causal effect of the microbiome on these domains. Covariates adjusted for in fully-adjusted models included age, sex, educational attainment (coded as high school or less, some college or associate's degree, bachelor's degree or higher), net worth (self-reported in 2011, log transformed), self-reported average number of days per week meat was consumed in the past year, number of vegetables consumed per week in the past year, medications in the past 6 months (antibiotics, proton pump inhibitors, other digestive system medications, nervous system medications, and psychiatric medications - each of these are separate binary covariates), current smoking in 2011 (binary), body mass index (calculated from self-reported weight and height in 2011), probiotic or prebiotic use in the previous 6 months, and self-reported diagnosis of diabetes, hypertension, and cardiovascular disease (separate binary covariates) as of 2011. While fecal samples, dietary information, and medications and probiotics were measured in participants at the same time (in 2014), the other measures were collected approximately 3 years prior (between 2011 and 2012). Table S1 provides descriptive statistics on these variables.

2.4. Microbiome sample processing

A full stool specimen was collected in the home, and kept at -80 °C for

up to 8 h before being flash frozen and stored at -80 °C. Full details of the stool collection protocol are published in (Dill-McFarland et al., 2019; Herd et al., 2017). The full DNA extraction, PCR, sequencing, and sequence processing protocol used in this study is published in (Dill-McFarland et al., 2019), with the exception that, in this analysis, we retained the raw number of reads and did not perform the rarefaction step. Briefly, to extract genomic DNA from fecal aliquots, samples were bead-beaten and subjected to centrifugation, followed by recovery of the aqueous phase, isopropanol precipitation, and PCR purification. The variable 4 (V4) region of the 16S rRNA gene was amplified using PCR with universal primers, and amplicons were sequenced using the MiSeq 2 × 250 v2 kit (Illumina, San Diego, CA, USA) using custom primers (Kozich et al., 2013). Following demultiplexing on the Illumina Miseq, sequences were processed in mothur v.1.36.1(Schloss et al., 2009), aligned to SILVA (Pruesse et al., 2007) and ultimately classified using GreenGenes(DeSantis et al., 2006). and grouped using 98% similarity. This is because SILVA contains only full-length 16S, which is better for alignment) while GreenGenes contains both full and partial 16S, which is better for taxonomic assignment.

2.5. Statistical analysis

We examined associations between abundances of 16S-based taxonomic groups and psycho-cognitive dimensions using weighted generalized linear models with log-2 link and negative binomial likelihood, implemented using the R packages 'edgeR' and 'ipw'. The response variable is the raw, unnormalized count of 16S reads in a given sample, with separate models for each taxon. Despite conceptualizing the microbiome as exposure, we modeled the taxonomic counts as a function of the psycho-cognitive dimensions, as no method to our knowledge has been developed to treat compositional counts as regressors without considerable loss of information.

We accounted for differences in library size by using an offset in our models, rather than rarefaction, as the latter unnecessarily discards information and induces spurious correlation(Aitchison, 1986; McMurdie and Holmes, 2014). As offset, we did not use edgeR's default normalization factor, but instead used the geometric mean of pairwise ratios (Chen et al., 2018), a measure developed specifically to account for the compositional and zero-inflated properties of 16S data. The sole regressor in each model was the psycho-cognitive score in question, scaled to unit variance. To adjust for potential confounders, we estimated a separate set of stabilized inverse probability of treatment weights for each psycho-cognitive dimension. The "treatment" here is the psycho-cognitive dimension, and the probability is the conditional density function of each psycho-cognitive dimension evaluated at an individual's observed value, conditional on that individual's covariate values. We used a Gaussian generalized linear model to estimate all these density functions, since all such variables were approximately normally distributed (Naimi et al., 2014).

The measure of association is therefore the log-2 ratio change in expected normalized abundance of a taxon for a one-standard deviation change in a given psycho-cognitive score, for the population defined by the distribution of confounders in the sample. The variance of this measure was estimated first using profile likelihood, then subsequently shrunk using empirical Bayes weighted likelihood, where the prior mean was a spline function of the mean normalized abundance, an approach to "borrowing strength" across taxa which is standard in gene expression analysis (McCarthy et al., 2012).

We calculated these models not at the raw OTU level, which may be classified at the species, genus, or more course taxonomic level (44% are classified most specifically at the genus level), but after collapsing the OTU table to the species, genus and family level using the command 'tax_glom' in the R package 'phyloseq', and removing any taxa unclassified at the level in question. In all cases, before model fitting but after collapsing, we performed filtering to retain only taxa with a count of 3 or more in 10 or more samples. For each model we included only samples with complete data on all covariates and the psycho-cognitive variable in question (i.e., "complete case analysis"), with anywhere from 28 to 64% of participants missing data, depending on the outcome.

We also conducted two simple sensitivity analysis: (1) because chronic disease could represent mediators rather than confounders (psycho-cognitive traits may affect health), we re-estimated all models without chronic disease covariates, and (2) because graduates and siblings may have correlated responses, for which our modelling approach does not allow straightforward correction of standard errors, we re-estimated all models restricting to graduates.

Throughout, we rely on p-values as a descriptive, continuous measure of contradiction between our data and a null hypothesis of no association. In some cases we filter results using p-value thresholds. These filters are purely heuristic and selected to maximize readability and interpretability of figures and tables given the impossibility of interpreting the 7056 tests conducted in this study; as such, they should be interpreted as descriptive rather than conclusive. Results of all statistical tests are presented in a supplemental table. In addition to the raw p-value, the supplemental table also provides Benjamini-Hochberg false discovery rates that take into account all 7056 tests.

3. Results

3.1. Descriptive statistics

Of n = 329 graduates and siblings agreeing to participate, n = 16 had insufficient material for sequencing, resulting in n = 313 for analysis. Due to item non-response in outcomes and covariates (Table S1), the final analytic sample contained n = 241 for quantitative reasoning, n = 192 for memory/attention, n = 116 for verbal fluency, n = 236 for depression, anxiety, and hostility, n = 235 for anger, n = 241 for all Big Five traits. Fig. 1 is a matrix of Pearson's correlation estimates between outcomes; there are strong correlations between anger and hostility (r = 0.7), anxiety and depression (r = 0.7), and moderate correlations between hostility and depression (r = 0.5), hostility and anxiety (r = 0.5), anger and anxiety (r = 0.4), neuroticism and anxiety (r = 0.4) and conscientiousness and agreeableness (r = 0.4). The remaining pairwise correlations between outcomes had $|r| \leq 0.3$. Psycho-cognitive dimensions did not correlate strongly with Shannon alpha diversity - absolute value of Pearson's correlation (adjusted for all potential confounders) was less than 0.1 for all except neuroticism (r = −0.21, 95% confidence interval: -.33, -0.07). Each trait explained less than 1% of variance in Aitchison's distance using distance-based redundancy analysis, partialling out all potential confounders.

Of 83 detected genera, *Bacteroides*, *Blautia*, *Faecalibacterium*, and *Ruminococcus* had the highest relative abundance in the majority of samples; most samples also had an appreciable proportion unclassified at the genus level (6.2 ± 3.5%). Most sequence reads in most samples were classified at the family level (98.4 ± 1.8%), with Lachnospiraceae, Ruminococcaceae, and Bacteroidaceae in the top 3 most abundant families for 95, 91, and 73% of samples, respectively. At the phylum level, a smooth gradient was evident in Firmicutes vs. Bacteroidetes abundance with overall Firmicutes dominance, such that 90% of samples had greater than 53% Firmicutes abundance and less than 38% Bacteroidetes abundance.

3.2. Abundances of multiple taxonomic groups are associated with psycho-cognitive traits

Full results of all differential abundance analyses are in Table S1, and summarized in Figs. 2 and 3. Table S1 also provides prevalence and mean relative abundance among positive samples for each taxon. Because of strong correlations between anxiety and depression, and between anger and hostility, readers should bear in mind that a sample association with one may equally reflect a population association with the other. To summarize Figs. 2 and 3, members of *Megamonas, Megasphaera,*

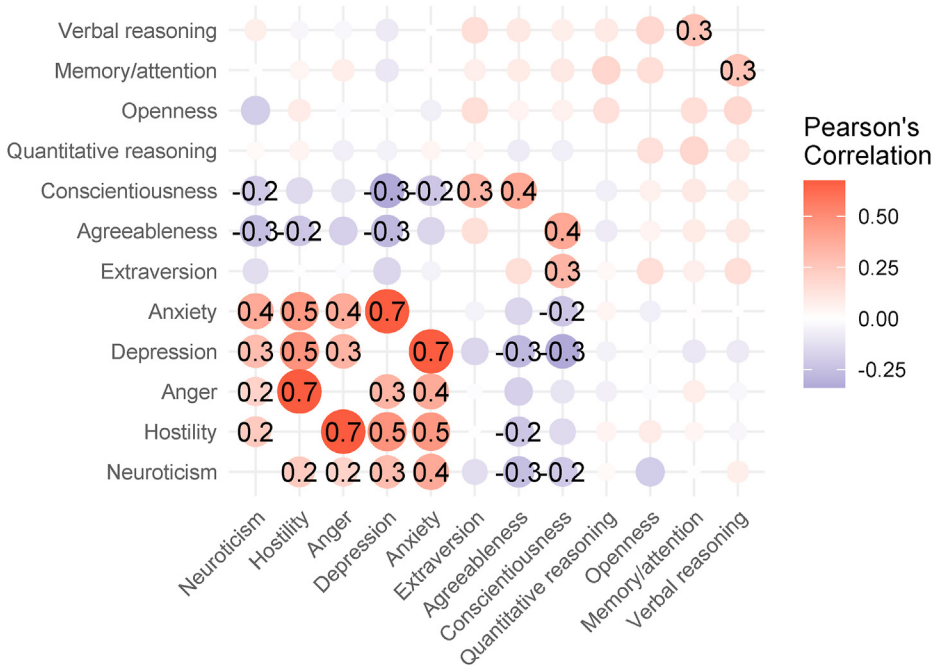

Fig. 1. [1 column]. Pairwise Pearson's correlation coefficient estimates between outcomes.

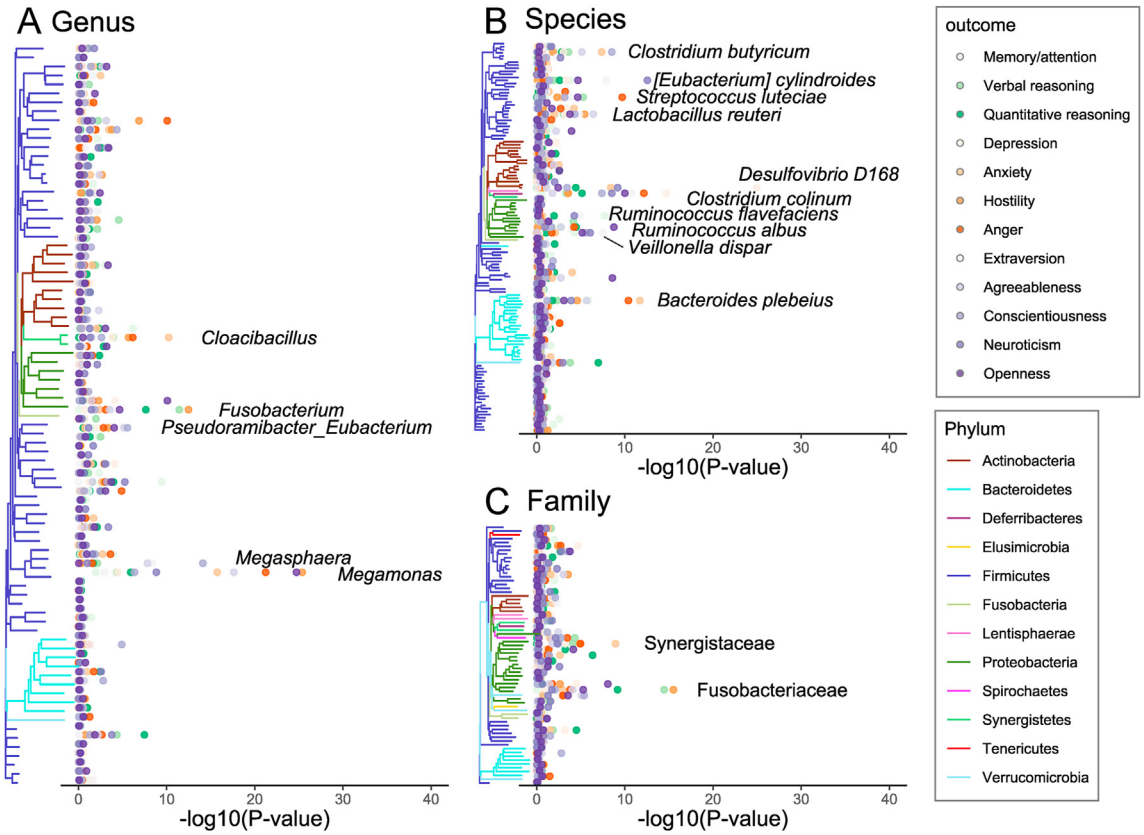

Fig. 2. [2column]. Phylogenetic distribution of P-values testing that linear model coefficients for each psycho-cognitive dimension equal zero. Tests are based on regressing taxonomic counts collapsed at the (A) genera-, (B) species-, and (C) family-level, on each psycho-cognitive dimension, adjusted for all confounders. Taxa are labelled if 3 or more traits had $p < 1e\text{-}4$.

Fusobacterium, Cloacibacillus, Pseudoramibacter_Eubacterium, Ruminococcus, Clostridium, Lactobacillus, Streptococcus, Bacteroides, and Desulfovibrio all robustly associate with at least two of mood, personality, and/or cognition. Fig. 2 shows (A) genera-, (B) species-, and (C) family-level associations with psycho-cognitive traits, all adjusted for confounders. Dots represent negative log-10 p-values, which do not indicate the strength of the association but the degree of contradiction between the data and the null hypothesis, which is influenced by taxon prevalence

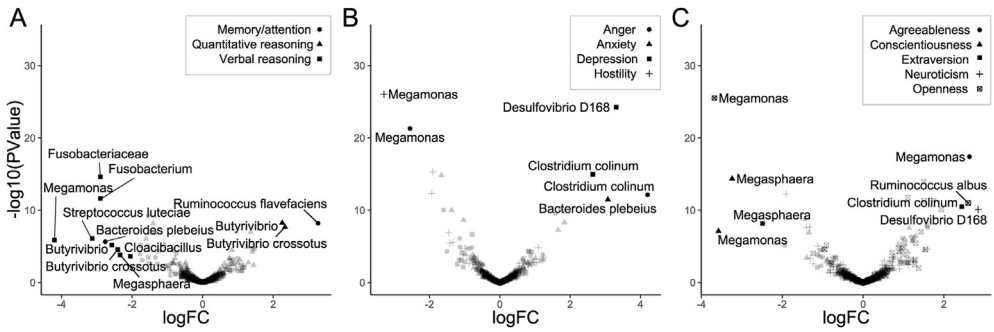

Fig. 3. [2-column]. Volcano plots highlighting the strongest associations with each phenotype. Log-fold-change (logFC) estimates are displayed for taxonomic counts collapsed at the family, genus, and species level, regressed on (A) cognition, (B) mood, and (C) personality variables, adjusted for all confounders. Taxa are labelled if the logFC had absolute value greater than 2.

and abundance distribution in addition to the effect measure estimate. We focus attention on "spikes" – locations in the phylogenetic tree where multiple traits show consistently elevated null hypothesis contradiction relative to other taxa, which we define roughly here as 3 or more traits with p-values less than 10^{-4} (corresponding to Benjamini-Hochberg false discovery rate <0.002). Taxa meeting this criterion are labelled in Fig. 2.

For instance, focusing on genera in Fig. 2A, low p-values are observed for the associations between the genus *Megamonas* and all traits. No species-level classifications are available for the detected *Megamonas* in our data, so this genus is not represented in Fig. 2B, and it does not appear to drive an association with its family, *Veillonellaceae*, in Fig. 2C. *Megamonas* is a relatively rare genus in this sample (11% prevalence, 1.6% mean abundance positive). *Megasphaera* is a more common, (25% prevalence) low abundance (mean = 1%) genus that is not further classified, with a spike primarily representing personality variables. *Fusobacterium* is common (prevalence 27%) but very low abundance (mean = 0.3%), and produces a spike in Fig. 2A reflecting its negative associations with quantitative reasoning, verbal fluency, openness, extraversion, and hostility; these also drive a spike in family *Fusobacteriaceae* (Fig. 2C). *Cloacibacillus* is a relatively common (prevalence 18%) low abundance (0.2%) genus that associates negatively with all mood traits (Fig. 2A), and appears to drive a spike in its family Synergistaceae (Fig. 2C). The spike for *Pseudoramibacter_Eubacterium* is driven largely by positive associations with agreeableness, conscientiousness, and openness.

Focusing instead on species in Fig. 2B reveals associations with several rarer members (prevalence ~7%) of common genera not appearing as genus associations in Fig. 1A. Two members of *Clostridium* contain spikes populated by positive associations with depression and anxiety, and varying personality traits, and two members of *Ruminococcus* appear associated with lower anger, greater quantitative reasoning (*R. albus),* greater memory and varying personality traits (*R. albus* and *R. flavefaviens*). The spike at *Desulfovibrio* D168 reflects strong positive associations with depression, anxiety, quantitative reasoning, and neuroticism. Other taxa associating with at least two psycho-cognitive domains include *L. reuteri* (mood and personality), *[Eubacterium] cylindroides* (mood, personality, and cognition), *S. luteciae* (mood, personality, and cognition), and *B. plebeius* (mood and personality).

Fig. 3 uses volcano plots to highlight the strongest associations with each phenotype; dots are labelled when the absolute logFC estimate is greater than 2. These strongly associated taxa largely corroborate the spikes seen in Fig. 2 and include *Megamonas, Megasphaera, Fusobacterium,* and *Cloacibacillus.* The one exception is *Butyrivibrio*, a relatively rare (12.5% prevalence, <1% relative abundance), butyrate-producing genus. *Butyrivibrio* and *B. crossotus* are both positively associated with quantitative reasoning and negatively associated with verbal reasoning.

3.3. Sensitivity analyses

The results of analyses dropping chronic disease covariates are in Table S2 and summarized in Fig. S1 (the equivalent of Fig. 2), and the results of analyses restricted to graduates are in Table S3 and summarized in Fig. S2. To summarize these, the key result that members of the genera *Megamonas, Megasphaera, Fusobacterium, Cloacibacillus, Pseudoramibacter_Eubacterium*, *Ruminococcus, Clostridium, Lactobacillus, Streptococcus, Bacteroides, and Desulfovibrio* all robustly associate with at least two of mood, personality, and/or cognition holds with or without adjustment for chronic disease adjustment and with or without inclusion of siblings.

Specifically, all "spikes" present in Fig. 2 are also visible and labelled in Fig. S1 (dropping chronic disease covariates), meaning at least 3 traits had p < 1e-4. In Fig. S2, all the same spikes are visible and labelled except *L. reuteri, S. luteciae, and R. flavefaciens*, although each still associate robustly with multiple domains - *R. flavefaciens* with greater memory/attention and greater neuroticism, *S. luteciae* with greater anger and greater agreeableness, and *L. reuteri* with greater neuroticism and lower memory.

3.4. Replication of previous findings

Faecalibacterium, Coprococcus, Butyricicoccus, Fusicatenibacter, and *Parabacteroides* were previously associated either with self-reported mental and emotional quality of life scores, or clinical depression (Valles-Colomer et al., 2019). We did not detect *Parabacteroides* or *Fusicatenibacter* in any of our samples, and for *Faecalibacterium, Coprococcus, Butyricicoccus,* and all psycho-cognitive traits, our data were not inconsistent with a null association, with log fold changes close to zero (range, −0.4 to 0.2) and large p-values (range, 0.2-0.9). On the other hand, *Butyrivibrio*, which was previously associated with greater mental and social functioning scores (Valles-Colomer et al., 2019), showed suggestive associations in our data with lower depression (logFC = -1, p = 0.007), anxiety (logFC = -0.5, p = 0.06), and anger (logFC = -0.4, p = 0.03). We also found *Butyrivibrio* to be strongly associated with greater quantitative (logFC 2.3, p = 6e-9) reasoning and lower verbal reasoning (logFC = -2.4, p = 6e-6). *Dialister*, previously negatively associated with depression (Valles-Colomer et al., 2019), was weakly positively associated with depression in our data (logFC = 0.4, p = 0.1).

4. Discussion

Although robust evidence links the gut microbiome to brain function, much of the evidence is based on animal models. Human studies on the gut-brain axis show promise in replicating animal findings but much of this research is hampered by bias resulting from design limitations such as having limited phenotypic data, non-randomly selected populations, and few studies of older adults. Importantly, to our knowledge, our study is the first randomly selected population-based gut microbiome cohort study on older adults looking at multiple psycho-cognitive traits, which allows for testing shared relationships between these multiple traits and

microbial composition.

Our findings point to shared associations between specific genera and a wide array of psycho-cognitive traits. The strongest findings in our study were associations between *Megamonas* and all measured psycho-cognitive traits, associations between *Butyvibrio* and cognitive traits, associations between *Fusobacterium* and cognitive and personality traits, associations between *Cloacibacillus* and mood traits, and finally an association between *Pseudoramibacter_Eubacterium* and personality. We do note, however, that *Megamonus* is present in just 11 percent of the sample. Finally, we were not able to replicate previous findings that *Faecalibacterium, Coprococcus,* and *Butyricicoccus* were associated with mental health; relative to another study, *Dialister* was weakly associated with depression in the reverse direction in our study (Valles-Colomer et al., 2019). However, *Butyrivibrio* was positively associated with mental health in both our and a previous study (Valles-Colomer et al., 2019).

In terms of cognition, we examined three factors: memory/attention, quantitative reasoning, and verbal fluency. *Megamonas* was negatively associated with verbal fluency and positively associated with memory/attention. *Butyrivibrio* was negatively associated with verbal fluency and positively associated with quantitative reasoning. *Fusobacterium* was negatively associated with verbal fluency and quantitative reasoning. Our results partially confirm differing levels of gut microbiota, including Proteobacteria and Firmicutes, by cognitive function, even after adjusting for confounders, though our associations are in the opposite direction than those found in one small and one large study on cognitively healthy adults (Manderino et al., 2017b; Verdi et al., 2018).

Within mood, we studied the following traits: anger, anxiety, hostility, and depressive symptoms. *Megamonas* was negatively associated with all four traits and *Butyrivibrio* was negatively associated with depression. These results are different from those found in another population-level study which found lower levels of *Coprococcus* and *Dialister* species in depressed Flemish participants (Valles-Colomer et al., 2019). In a meta-analysis on the gut microbiome and major depression, *Megamonas* had conflicting results across studies, being both positively and negatively associated with major depression and *Butyrivibrio* did not appear to be associated with major depression in any of the studies (Cheung et al., 2019). Similarly, in another small study, *Megamonas* was did not appear associated with generalized anxiety disorder (GAD), though other taxa were identified as less abundant in GAD such as *Roseburia* and *Lachnospira* (Jiang et al., 2018). Interestingly, one study of 34 individuals showed that several taxa, including members of *Ruminococcus* and *Faecalibacterium,* were associated with both major depression and with neuroactive substances such as isovaleric acid (Szczesniak et al., 2016). For recent reviews of gut microbiota and depression and of gut microbiota and mental health more generally see (Dinan and Cryan, 2019) and (Du Toit, 2019), respectively.

For personality, we examined the 'Big Five': agreeableness, conscientiousness, extraversion, neuroticism, and openness. *Megamonas* was negatively associated with conscientiousness, neuroticism, and openness, and positively associated with agreeableness. *Fusobacterium* was negatively associated with openness and extraversion. These results partially support the animal literature showing that differences in gut composition were associated with reduced exploratory behavior and social interaction (Buffington et al., 2016; Sanguinetti et al., 2019). Additionally, these findings are similar to those seen in one mother-toddler paired study with findings related to extraversion (Christian et al., 2015) and one large non-randomly selected adult Korean study with findings on neuroticism and conscientiousness (Kim et al., 2018), but not another study focused on temperament traits in infants (Aatsinki et al., 2019).

4.1. Public health implications

Our results indicate that several gut bacterial taxa are linked to multiple psycho-cognitive traits. Considered alongside previous research, this contributes some credibility to the growing hypothesis that treatments targeting the gut microbiome could be promising in treating or preventing disorders such as depression or cognitive impairment, which can significantly impact quality of life. Specifically, fecal transplantation has been shown to alter plasma levels of neuroactive substances (Kootte et al., 2017) as well as improve depression ratings (Kurokawa et al., 2018). The consumption of probiotics has been shown to reduce a risk factor for depression (Steenbergen et al., 2015) and is associated with brain activity in areas related to emotional processing (Tillisch et al., 2013).Two meta-analyses showed that probiotics reduced depressive symptoms in participants with major depressive disorder (Huang et al., 2016), though not in healthy participants (Ng et al., 2018). However, interventions of this type should also be tried at the population level, to determine if they are applicable on a wider scale, and potentially able to target multiple traits simultaneously.

4.2. Strengths and limitations

The major strength of this study is the fact that the sample is a randomly-selected population-based cohort of older adults with extensive phenotypic measures of psycho-cognitive traits, as well as possible confounders. This is the first US population-based study that addresses the microbiome and multiple dimensions of personality, highlighting a novel area for microbiome research. Additionally, the relationships found here between gut microbes and psycho-cognitive traits were robust to different covariate adjustment sets. Another strength of this study is the use of inverse probability treatment weights which allows our estimates to apply to a known population (Stürmer et al., 2006), although this is limited by missing data.

There are some study limitations. First, classifying the data to a different database (e.g. SILVA) may yield additional relationships, but given evidence that Greengenes maps well onto NCBI, it is unlikely that the patterns found here are false (Balvočiūtė and Huson, 2017). Second, the finding regarding *Megamonas* should be contextualized in the fact it was present in just 11 percent of the sample. Third, as in all human studies to varying degrees, there is risk of survivorship bias such that the factors leading to survival could be related to gut composition and/or psycho-cognitive traits; by age 75 approximately 20 percent of the sample was deceased. Finally, there was a ~3 year lag in timing between the phenotypic measures (particularly the cognition and psychological measures) as compared to the fecal sample collection, diet, and medication measures, leading to limiting our ability to address temporality in these relationships. The latter three measures were collected in 2014, whereas the remaining measures were collected between 2011–12. The simultaneous collection of the medication, diet, and fecal samples, however, was critical because medications and diet are the clearest possible confounder of the analyses. There are a few reasons why we are less concerned about a lag between the psycho-cognitive measures and the microbiome data. First, the gut microbiome is relatively stable over shorter periods in later life—there is little evidence of dramatic change among studies that have examined intra-individual change (Claesson et al., 2011; Mehta et al., 2018). Second, most of these measures (cognition, personality, well-being) are also relatively stable at this age (~age 70) over a 3 year period (Anusic and Schimmack, 2016; Clarke et al., 2011; Costa et al., 2019; Reas et al., 2017; Roberts et al., 2006;Yang and Yang, 2007).

Finally, while we were able to use this sample to explore shared taxonomic associations between multiple psycho-cognitive measures, the general pattern of relationships across multiple domains found in our study needs to be replicated in other studies with multiple measures of psycho-cognitive traits. The inclusion of studies with longitudinal data, capturing change over time in phenotypes and the gut microbiome, can also improve inferences about these relationships.

5. Conclusion

In conclusion, we have described reasonably robust associations between several taxa in the gut microbiome and multiple psycho-cognitive

traits in a randomly-selected population-based sample. This lends further support for the interconnectivity between the gut and the brain, and provides potential targets for prevention or intervention around the significant public health burden of depression and cognitive dysfunction.

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Declaration of competing interest

None to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2020.100155.

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