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What We Know, Think We Know, or Are Starting to Know

If ever a condition has evaded the reductionist research paradigm, it is arguably depression. Indeed, the impetus for the evolution of the "bio-psycho-social model" in the late 1970's came from the relative lack of fruition which the reductionist model had brought to the field of psychiatry [(1)].

Risk of developing depression is higher in individuals with family history of depression, individual history of depression, lower reward-responsiveness, sex [prevalence 1.5 times greater in women compared to men], and socio-economic factors like poverty, unemployment, life stressors, and physical illness [(2,3)].

Although the father of the bio-psycho-social model, George L. Engel, was rejecting the reductionist and dualist model [i.e., the separation of mind from body in research and understanding] those ~40yrs ago, the reality is that an understanding of the exact origins of depression remains elusive. This may, as some have argued, reflect a lack of conceptual clarity with psychiatric research regarding the nature of depression [i.e., is it more "bio", more "psycho", more "social", and/or other factors] [(4)]. More importantly, if the causal pathway(s) for any given individual are unique, why would there be an expectation of finding a unifying "cause" for depression [(4)]?

Nevertheless, we march on with paradigms lost, and where better to look when answers are elusive than the human gut microbiome. This search is based on the increasing understanding of the "gut-brain axis", which includes the central nervous system, the autonomic nervous system, and the enteric nervous system; bi-directional communication allows for the brain to influence functions in the gut, and the gut to influence brain function [(5)].

At the core of the gut-brain bi-directional axis of communication is the microbiota* [see ***Geek Box** below for further details], i.e., the compositions of bacteria in the human gut [(5)]. Could the microbiota be associated with depression?

*Geek Box: The Microbiota and 'Bacterial Core'

The "microbiome" is the term for the 'extended genome' provided by the bacteria in the human gut, i.e., what genes are expressed and functions they exert. The "microbiota" is the term for the different bacteria in the gut, i.e., what bacteria are present, and in what proportions.

During our evolution, human beings have colonised every corner of the planet, adopting diverse diets in radically different natural environments and climates. Our gastrointestinal tract is one of the largest interfaces with our external environment [other than the skin], providing for both the digestion and absorption of essential nutrients and the first line of immune defence.

Within our GI system, particularly the colon, is a dynamic ecosystem of bacteria. At the broadest level, there are 4 main divisions, known as 'phyla': Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria. These phyla are considered our "bacterial core", with the majority of bacterial types belonging to two major phyla, the Firmicutes and Bacteroidetes, and significant contributions from Actinobacteria and Proteobacteria.

Within each phylum, there are a multitude of different genus, and within the genus are individual species. It is within the composition of each phyla – at the genus and species level – that significant inter-individual variability is observed. Diversity in the human gut thus reflects the depth and breadth of variability within each major phylum.

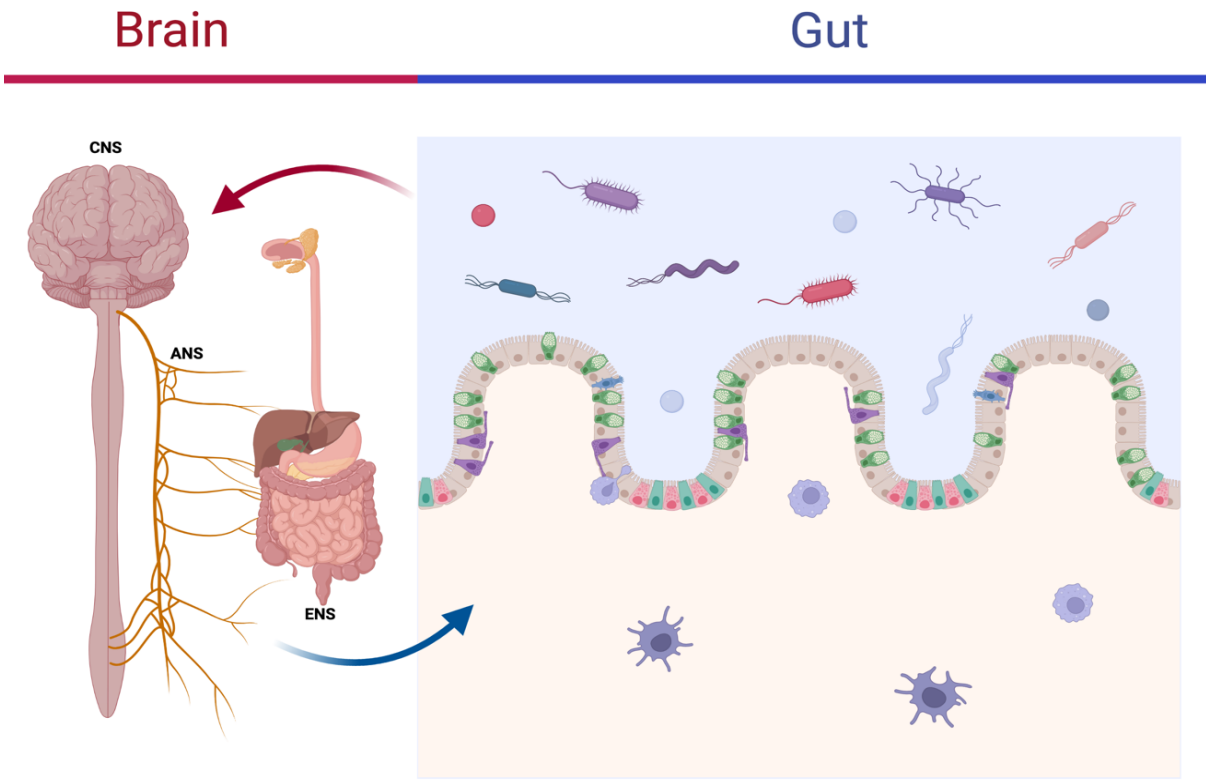

***Figure** illustrating the gut-brain axis. "**CNS**" = central nervous system; "**ANS**" = autonomic nervous system; "**ENS**" = enteric nervous system. The purple figurines against the beige background are dendritic cells, immune cells that are expressed in tissues exposed to the external environment, such as the gastrointestinal lining. The figurines against the purple background represent bacteria within the gastrointestinal tract. These areas are separated by the intestinal lining, at which layer interactions occur between host [i.e., you] and host immunity and the external world, in particular bacteria. These interactions may influence the CNS from the ENS, mediated by the ANS [the vagus nerve in particular], and feedback from the brain to the gut.*

The Study

The present study undertook two analyses; one analysis of Dutch participants from two cohorts, followed by a genetic analysis of associations between microbiota and major depressive disorder [MDD].

The first analysis investigated bacterial diversity and associations with depression in the following cohorts:

- **The Rotterdam Study [RS]**: 1,054 participants from RS that were not using anti-depressants were included in the analysis. Participants were those with completed faecal analysis samples, who had also completed an assessment of depressive symptoms [Center for Epidemiological Studies-Depression (CES-D) scale].
- **The Healthy Life in an Urban Setting [HELIUS] Cohort**: 1,539 participants from HELIUS were included in the analysis. Participants also had provided faecal samples, and depression was also assessed [Patient Health Questionnaire (PHQ-9)].

This first analysis investigated the associations between bacterial diversity and depression in each cohort, then combined the results from both in a meta-analysis. These outcomes were presented using two measures common in microbiome research: "alpha diversity" and "beta diversity".

Alpha diversity is a representation of within-person diversity, i.e., how many different bacterial species are present in an individual's microbiota. Beta diversity is a representation of the between-person diversity, i.e., the bacterial composition of an individual's microbiota compared to another.

The investigators then used genetic data to conduct a Mendelian Randomisation [MR] analysis of the potential causal links between genetic variants representing the microbiota identified from the cohort analyses and MDD.

Results: Average age of participants was 56yrs and 51yrs in the RS and HELIUS cohorts, respectively. 56% and 49% of the RS and HELIUS cohorts, respectively, were female. Mean BMI was 27kg/m2 and 26kg/m2 in the RS and HELIUS cohorts, respectively. Baseline depression scores were 4.7 [out of 49] and 3 [out of 21] in the RS and HELIUS cohorts, respectively.

***Associations Between Bacterial Diversity and Depression*:** Alpha diversity was negatively associated with depressive symptoms in both the RS and HELIUS cohorts, i.e., less diverse gut microbiota was associated with higher depressive symptom scores. Beta diversity was associated with depressive symptoms in the RS cohort but was not replicated in the HELIUS cohort.

24 genera of bacteria were identified as associated with depressive symptoms in the RS cohort, of which 13 were replicated in the HELIUS cohort. Of these replicated bacterial genera, 9 were negatively associated with depressive symptoms, i.e., lower abundance of these bacterial genera were associated with higher depressive symptom scores.

***MR of Microbiome Variants Associated with MDD*:** This analysis used genetic variants associated with the 13 bacterial genera that were significantly associated with depressive symptoms in the cohort's analysis. A genetic variant for only 1 bacterial genera, *Eggerthella*, was significantly associated with MDD.

The Critical Breakdown

Pros: For a complex study design involving faecal sample collection and analysis, the sample size including both cohorts of 2,593 participants was large. The analysis of the cohorts included adjustment for covariates which could be associated with both the microbiota and depressive symptoms, e.g., proton-pump inhibitor use, smoking, alcohol, sex, and age. The analysis was confined to individuals not using anti-depressant medications. The combination of "discovery" and "validation" cohorts reduced the possibility that the associations with the microbiota in one cohort reflected the particular characteristics of that cohort [more under ***Key Characteristic***, below]. The addition of a genetic MR analysis provided an additional insight into the potential direction of effect associated with identified bacterial genera, i.e., higher or lower bacterial abundance associated with higher depression risk.

Cons: Each cohort used different scales to assess depressive symptoms, which could influence classification of depressive symptoms [although the two instruments used appear to have moderate levels of agreement [(6)]]. Further, depressive symptoms were at the very low end of each scale in both cohorts, which may mean a lack of power to detect more robust associations. HELIUS is a multi-ethnic cohort, but the present analysis was confined to European ancestry participants, which limits replication value. The MR analysis was based on weak genetic variants and no genetic variants were strongly associated with the identified microbial genera [more under ***Interesting Finding***, below].

Key Characteristic

The use of "discovery" and "validation" cohorts is often used in genetic analyses, and provides a simple research design method to ensure that an observation found in one analysis is not a chance finding or finding due to uncontrolled residual factors influencing that outcome. For the present study, having faecal samples from two cohorts allowed for a similar approach to be taken for the microbiota.

Thus, in the present study, the RS cohort was analysed as the "discovery cohort" to identify gut microbiota associated with depression. The HELIUS cohort served as the "replication" or "validation" cohort, to provide support that the findings from the discovery cohort were not simply a reflection of the characteristics of that cohort.

The merits of this approach were evident in the fact that in the RS cohort, 24 bacterial genera were associated with depressive symptoms; were the study to have been confined to this cohort alone, it may have overestimated the microbial associations with depressive symptoms. By using the HELIUS cohort to replicate those findings, it resulted in 13 genera that were associated with the same direction of effect as in the RS cohort.

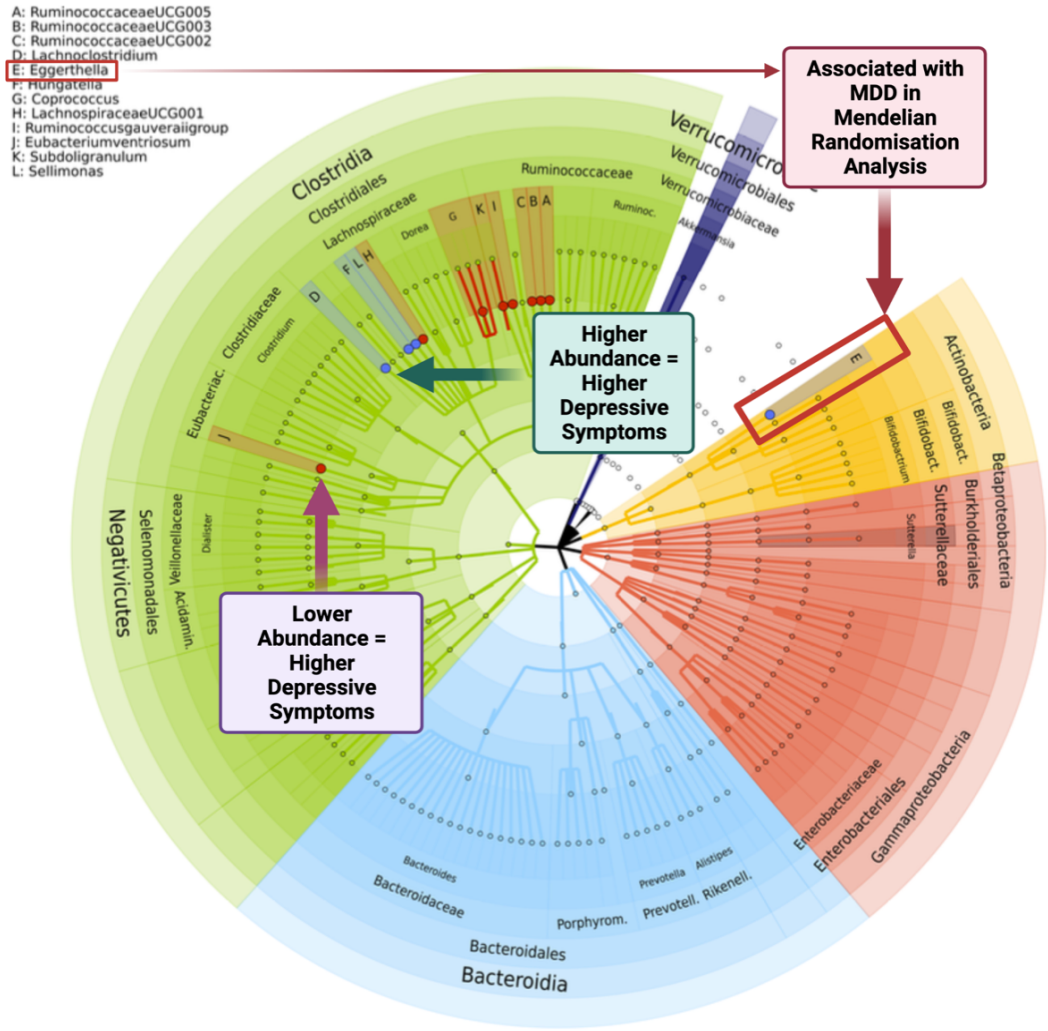

Figure *from paper illustrating the 13 bacterial genera associated with depression. The names at different layers of the circle represent the different level of organisation within each bacterial phylum [the outermost layer]. For ease of your interpretation, I've added a text box to illustrate that the red circled dots represent bacterial genera that were associated with depression when depleted in their abundance, while the blue circled dots represent bacterial genera that were associated with depression when greater in abundance.*

Interesting Finding

The MR analysis in the present study is an example of the limits of genetic analysis. It is common for MR analyses to begin with genetic data from "genome-wide association studies" [GWAS] to identify genetic variants that are associated with the exposure-outcome, which in this case were the microbiome and MDD.

However, for the microbiome there were either none, or very few, genetic variants that were significantly associated in GWAS with the microbiota identified in the cohort studies. As a compromise, the analysis used weak genetic variants, however, this limits the power and precision of any MR analysis [(7)]. One potential way to overcome this is to combine genetic variants that are associated with the exposure into one composite genetic risk score. In the present study, there was no association between the combined genetic risk score and MDD.

Of the 13 bacterial genera identified in the analysis of the cohorts, however, there was one genera [*Eggertella*] associated with MDD in the MR analysis. Importantly, the finding in the MR analysis was consistent with the direction of effect shown in the cohort analysis, i.e., that greater abundance of this bacterial genera was associated with higher depression scores.

While this suggests a potential causal link between higher abundance of this bacterial genera and MDD, it is important to recall that this is based off weak genetic associations. Thus, this finding is best considered an association, rather than cause-effect relationship, until further replication.

Relevance

When you have two poorly understood areas and combine them together, it can be awfully tempting to over-read and over-extrapolate from the research produced out of them. So let's start with a reminder that as an exposure we are still scratching the surface of the microbiome and microbiota, and as an outcome we are still scratching our heads about the aetiology of depression.

This means that interpreting a study like this requires staying within the bounds of "known knowns", rather than stray into "unknown unknowns". The first is that the overall dominance of the four bacterial phyla [*Firmicutes, Bacterioidetes, Actinobacteria, and Proteobacteria*] was observed in both cohorts, and is consistent with the taxonomy of the human gut microbiota [(8)].

At this juncture, however, it is difficult to say precisely whether there is any signature composition of the microbiota with regard to depression [(9)]. There is evidence that individuals with MDD have depleted levels of *Firmicutes* and increased *Bacteroidetes* [(10)]. But with the complex taxonomy of the gut microbiota, from phylum>class>order>family>genus>species, different studies produce a range of varying associations for depression [(9)].

In the present study, beta-diversity [i.e., differences in bacterial composition between individuals] was not associated with depressive symptoms in the HELIUS cohort. However, beta-diversity has been associated with depression in other research from

this cohort, including all ethnic groups in the HELIUS cohort [Dutch citizens of European, Surinamese, Ghanaian, Turkish and Moroccan origin] [11]. This analysis showed that beta-diversity explained 18% of the ethnic differences in depressive symptoms, i.e., the majority of the microbiota associations with depression were relatively consistent across ethnic groups.

Interestingly, in the analysis of all ethnicities in the HELIUS cohort, the association with alpha diversity [i.e., diversity of microbiota composition in an individual] and depressive symptoms was no longer evident after adjusting for the personality trait of neuroticism [11].

This highlights a crucial caveat; is a certain microbial composition merely a fellow traveller of other depression-related factors, or a causal contributor to those factors? This is what we do not know, and anyone pretending that we have any definitive answers in the area of the microbiota and mood is out over their skis on the evidence.

However, there is one broad conclusion that may be helpful: that alpha-diversity appears to be consistently associated with positive health outcomes, including depressive symptoms [11]. But is also more important to bear in mind that the effect sizes for the impact of diet on depression are small and confounded by other behavioural correlates of improved mood [12]. The most effective non-pharmacological intervention for depression is therapy [13].

Application to Practice

In this complex area, it is helpful to have generic markers of health status, and it appears that for all the complexity of the microbiota, the composite marker of alpha diversity is a positive. In practical terms, this means that greater the diversity in the composition of bacterial populations of the microbiota is a generic "good thing".

And it is also a good thing that we know that the composition of the microbiota is responsive to diet, in particular complex, non-digestible carbohydrates – fibres and non-starch polysaccharides – that result in bacterial fermentation and a diverse bacterial community [14,15]. To summarise in broad simple terms, the diversity in the composition of the microbiota corresponds to the diversity of complex carbohydrate structures in the diet, and microbial diversity is broadly associated with host health [14,15].

To what extent this may have meaningful impacts on depression is difficult to say but based on wider diet research it may not be much. Recommend a good high fibre diet, sure, but for depression let's major in the majors and recommend therapy.

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