Gut Microbiome alterations in Major Depressive Disorder: Relevance to Pathophysiology

Email: johnkelly@ucc.ie
johnkelly@johnrkelly
UCCpsychiatry@UCCpsychiatry

John R. Kelly[1,2], Yuliya Borre[1], Ciaran O' Brien[3], Elaine Patterson[3] , Sahar El Aidy[1,4], Jennifer Deane[3], Paul J. Kennedy[1], Sasja Beers[1], Karen Scott[1], Gerry Moloney[1], Lucinda Scott[2], Paul Ross[3], Catherine Stanton[3], John F. Cryan[1,5], Timothy G. Dinan[1,2], Gerard Clarke [1,2]

[1] APC Microbiome Institute, University College Cork, Cork, Ireland. [2] Department of Psychiatry and Neurobehavioural Science, University College Cork, Cork, Ireland. [3] Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland. [4] Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands. [5] Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland

1. Introduction

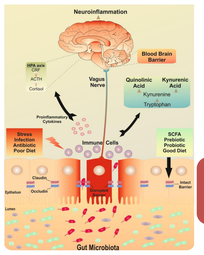


Figure 1: Signaling pathways involved in The Brain Gut Microbiota Axis [4].

The gut microbiota is a complex metabolic ecosystem, which interacts with the host via neuroimmune, neuroendocrine and neural pathways. These pathways are integral components of the brain-gut-microbiota axis and pre-clinical evidence suggests that the microbiota can recruit this bidirectional communication system to modulate brain development, function and behaviour [1]. Although it is well acknowledged that the pathophysiology of depression involves neuroimmune-endocrine dysregulation [2][3] the extent to which changes in the gut microbiota composition and function mediate dysregulation of these pathways in depression is currently unknown.

2. Aims

1. To determine the composition, richness and diversity of the gut microbiota in Depressed patients compared to healthy control participants and its relationship to: Short Chain Fatty acids (SCFAs), Immune activity (plasma cytokines), Hypothalamic-Pituitary-Adrenal axis (HPA-axis) function and Tryptophan metabolism
2. To determine the behavioural & physiological effects of a Fecal Microbiota Transplantation from Depressed patients & health controls to a microbiota depleted antibiotic rat model

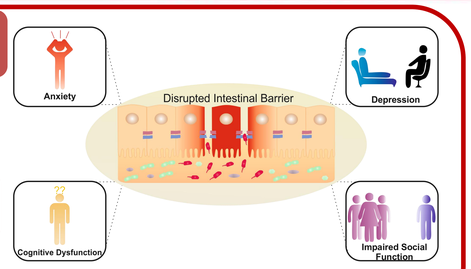


Figure 2:Activation of brain-gut-microbiota Axis signalling pathways via a compromised intestinal barrier with potential effects on mood, anxiety, cognition and social interaction [4].

3. Methods

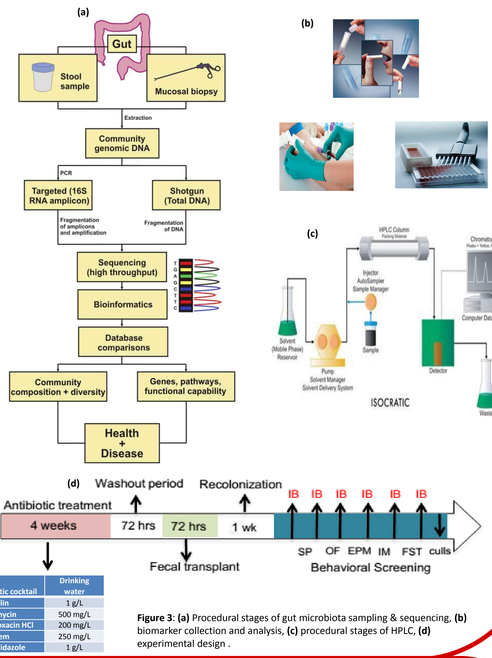
Study Population:
34 patients with DSM IV MDD & 33 healthy subjects matched for gender, age & ethnicity (see **Table 1** for demographics & clinical characteristics)

Measures:
Gut Microbiota Structure & Diversity: 16s rRNA gene sequencing
Microbial Metabolites: Short chain fatty acids (Gas Chromatography)
Hypothalamic-Pituitary-Adrenal (HPA) Axis: Salivary Cortisol (ELISA)
Inflammatory: plasma Cytokines & CRP (Meso Scale Discovery)
Tryptophan Metabolites: Plasma tryptophan & kynurenine (HPLC)
Intestinal Permeability: plasma Lipopolysaccharide Binding Protein
Subjective Mood & Stress: Hamilton Depression rating scale (HAMD 17) , Beck Depression & Anxiety scales (BDI & BAI), Perceived Stress scale (PSS), Pittsburgh Sleep Quality Index (PSQI)
Diet & Exercise: Food Frequency Questionnaire (FFQ), International Physical Activity Questionnaire (IPAQ)

Rats:
28 Male Sprague-Dawley rats

Behavioural tests:
Sucrose preference (SP), Open field (OF), Elevated plus maze (EPM), Intestinal motility (IM), Forced swim test (FST), Inoculation boost (IB) (twice a week)

Physiological outputs:
HPA Axis: Corticosterone 15 mins post FST
Inflammatory: plasma Cytokines & CRP
Tryptophan Metabolites: plasma tryptophan & kynurenine (HPLC)
Intestinal Permeability: plasma Lipopolysaccharide Binding Protein
Intestinal Motility: Transit Time



Antibiotic cocktail	Drinking water
Ampicillin	1 g/L
Vancomycin	500 mg/L
Ciprofloxacin HCl	200 mg/L
Imipenem	250 mg/L
Metronidazole	1 g/L

Figure 3: **(a)** Procedural stages of gut microbiota sampling & sequencing, **(b)** biomarker collection and analysis, **(c)** procedural stages of HPLC, **(d)** experimental design .

4. Subject Demographics

Demographics & Health Measures	Healthy Controls (n=33)	Depression (n=34)	p-value
Age mean (s.d.)	45.8 (11.9)	45.8 (11.5)	0.98
Sex Male (%)	19 (57.6)	21 (61.8)	0.73
BMI mean (s.d.)	24.58 (2.7)	26.2 (4.5)	0.07
Relationship status (% yes)	26 (78.8)	8 (23.5)	<0.001***
Employed (% yes)	31 (93.9)	16 (47.1)	<0.001***
Smoker (% current)	3 (9.1)	13 (38.2)	0.003
Dyslipidaemia (%)	4 (12.1)	7 (20.6)	0.51
Hypertension (%)	3 (9.1)	3 (8.8)	0.97
IPAQ Low (%)	7 (21.2)	13 (39.4)	0.18
IPAQ Moderate (%)	16 (48.5)	14 (42.4)	0.62
IPAQ High (%)	10 (30.3)	6 (18.2)	0.26
Metabolic Equivalent Task Units (MET) median, range	1386 (7287)	693 (7722)	0.10
HAMD 17 median (range)	NA	19.5 (14)	NA
Beck Depression mean, (s.d)	NA	32.4 (9.92)	NA
Beck Anxiety median, (range)	NA	25.5 (45)	NA
Duration of Depressive sx (months) median, (range)	NA	3.0 (72)	NA
Number of Depressive episodes median (range)	NA	1.0 (8)	NA
Depression in 1st degree relative (%)	2 (6.1)	21 (61.8)	<0.001***
Pittsburgh Sleep Quality Index (PSQI) mean, (s.d)	2.8 (1.8)	11.7 (4.3)	<0 .001***

5. Results

Altered Inflammatory & Tryptophan Metabolite Profile in Depression

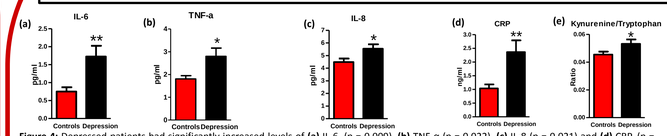


Figure 4: Depressed patients had significantly increased levels of **(a)** IL-6, (p = 0.009), **(b)** TNF-α (p = 0.022), **(c)** IL-8 (p = 0.021) and **(d)** CRP, (p = 0.001) compared to the healthy controls and had a significantly higher **(e)** kynurenine/tryptophan ratio (p = 0.049).

Elevated HPA Axis Activity & Perceived Stress in Depression

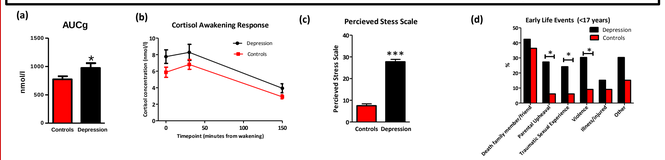


Figure 5: The depressed group had an increased cortisol output as measured by the **(a)** Area under the Curve with respect to ground (AUCg) (p = 0.045), increased **(b)** Cortisol Awakening Response (CAR) (p=0.026), higher **(c)** Perceived stress (p<0.0001), and more **(d)** life events before the age of 17 years compared to healthy controls.

Altered Gut Microbiota Composition, Diversity & Richness in Depression

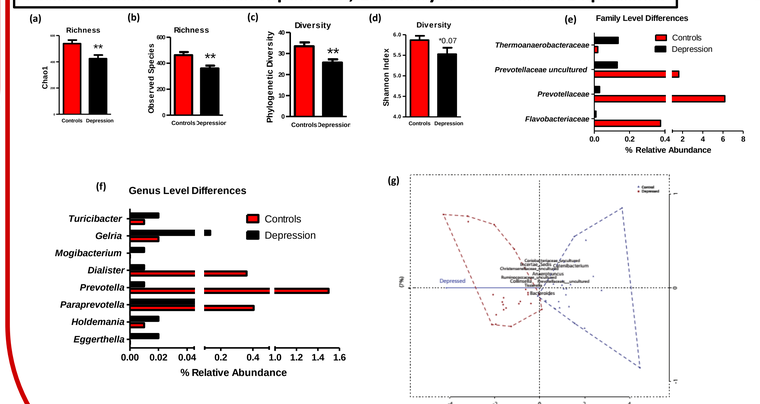


Figure 6: There was a significant reduction in richness as measured by **(a)** Chao1, (p = 0.004), **(b)** Total observed species, (p = 0.002)and **(c)** Phylogenetic Diversity, (p =0.0025), and a trend towards reduction in **(d)** Shannon diversity, (p = 0.072). **(e)** Significant Family level differences in % relative abundances & **(f)** significant Genus level differences in % relative abundances. **(g)** The significant differences at genus level between the depressed group and the controls which cluster by group in a Redundancy analysis plot.

Fibre, Short Chain Fatty Acids & Intestinal Permeability in Depression

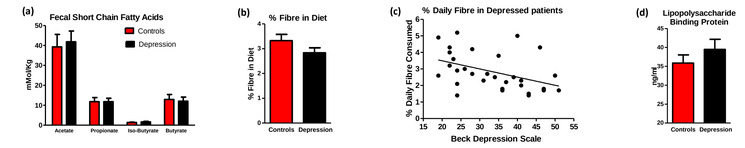


Figure 7: Functional implications of an altered Gut Microbiota: **(a)** There were no significant differences in fecal SCFAs. **(b)** There were no significant differences in the daily % fibre consumed between the groups (p=0.134) **(c)** though within the depressed group the daily % fibre in the depressed group negatively correlated with the Beck Depression Scale (r = -0.43, n = 31, p = 0.015). **(d)** There were no significant differences in LBP.

Fecal Microbiota Transfer (FMT) from Depressed patients Induces Depressive like behaviour & physiology in Rats

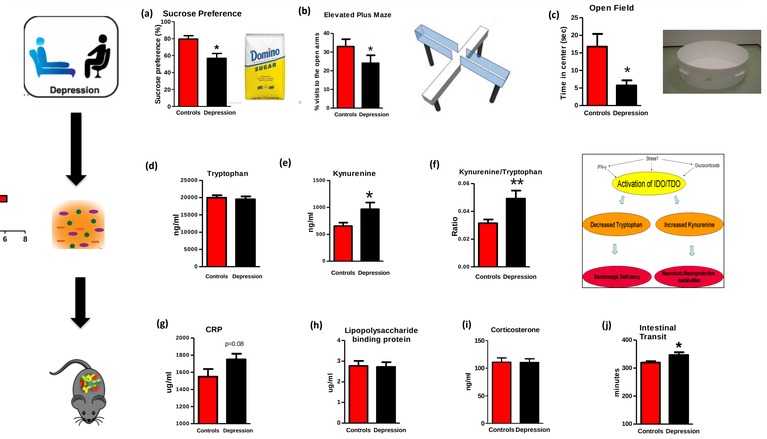


Figure 8: The rats that received the depressed FMT exhibited **(a)** anhedonia like behaviour, indicated by a decrease in the 24 hour 1% sucrose preference test (p = 0.022) and **(b)** an increase in anxiety like behaviour, measured by a decrease open arm visits in the elevated plus maze (EPM) (p = 0.029) and **(c)** a decrease in time spent in the open field (p = 0.013), compared to rats that received the healthy FMT. There were no differences in **(d)** plasma tryptophan levels (p = 0.686), but **(e)** plasma kynurenine levels (p = 0.029) and the **(f)** plasma Kynurenine/Tryptophan ratio (p = 0.008) were increased in rats that received the depressed FMT. **(g)** There was a trend for increased plasma CRP levels in the depressed FMT rats (p = 0.083), but no significant differences in **(h)** plasma LBP Lipopolysaccharide binding protein (LBP) levels or **(i)** plasma corticosterone levels. **(j)** Rats that received the depressed FMT demonstrated a significant decrease in intestinal transit time (p = 0.013).

6. Conclusions

Our results confirm that depression is associated with a distinct microbial signature which is capable of inducing alterations in behaviour and physiology when transferred to microbiota-depleted animals. This suggests that the gut microbiota may play a causal role in the development of core behavioural and neurobiological features of depression and may provide a tractable target in the treatment and prevention of depression.

Scan QR Code for link to UCC Psychiatry website poster and publication section

Acknowledgements & Disclosure

The authors are funded by Science Foundation Ireland (SFI), through the Irish Governments National Development Plan in the form of a centre grant (Alimentary Pharmabiotic Centre Grant Number SFI/12/RC/2273). The Alimentary Pharmabiotic Centre has conducted studies in collaboration with several companies including GSK, Pfizer, Cremo, Suntory, Wyeth and Mead Johnson. TGD has until recently been on the Board of Alimentary Health. Funding is also provided by the Health Research Board (HRB) through Health Research Awards (HRA_POR/2012/32; JFC, TGD and HRA-POR-2-14-647: GC, TGD). GC is supported by a NARSAD Young Investigator Grant from the Brain and Behaviour Research Foundation (Grant Number 20771).

References

[1] Cryan, J.F. and T.G. Dinan, Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci, 2012. 13(22968153): p. 701-712.
[2] Dowlati, Y., et al., A meta-analysis of cytokines in major depression. Biol Psychiatry, 2010. 67(5): p. 446-57.
[3] Lupien, S.J., et al., Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci, 2009. 10(6): p. 434-45.
[4] Kelly, Kennedy et al., Frontiers in Cellular Neuroscience. 2015. 10.3389/fncel.2015.00392