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Column: Editorial

Dear Editor,

We would like to thank for the positive and helpful comments on our manuscript “The impact of gut microbiota on neuropsychiatric disorders” previously submitted as an invited review in World Journal of Gastroenterology (ID00158194).

We submit now the revised version. The title has been changed according the editorial suggestion to "Influence of gut microbiota on neuropsychiatric disorders" and there is a point-by-point reply to reviewers ś comments.

We hope that this version has accomplished your expectatives. Please do not hesitate to contact me if you require further information.

We look forward to hearing from you,

Yours sincerely,

Pilar Codoñer-Franch, MD, PhD

Response to Reviewers

We thank both reviewers for their positive and useful comments which we consider very much improved our manuscript.

Reviewer #1:

COMMENTS TO AUTHORS

This manuscript is well organized and I enjoy reading it. The topic of gut microbiota and brain dysfunction is indeed a very interesting one. 1# The gut microbiota may be more dynamic than the human genome. This may pose a challenge to decipher the role of gut microbiota in the etiology and progress of neuropsychiatric diseases. What have we learned about the time scale on the changes of microbial community and development of the diseases? Does the disease development require a constant presence of particular microbial community or is the latter just a trigger? 2 # Regarding depression, alpha diversity seems to correlate either positively or negatively with depression. This needs to be clarified or there is no link between the two. 3# There is lack of information about the changes in gut microbiota in AD patients. I am interested in seeing more information and discussion about how the alteration in gut microbiota in diabetes or obesity may link to the risk for the development of AD. 4# Microglia appears to be a crucial cell type in these neuropsychiatric disorders, but only the effect of probiotic VSL#3 on microglia activation has been described in this manuscript. I would be cautious on the role of microglia unless more information can be provided. 5# Minor errors in grammar: Page 5, last paragraph, an extra "we" need to be deleted: "Thus, it is tempting to speculate that modulating the microbiota and its metabolic products we will enable us to modulate the epigenome and, thereby, prevent or treat mental illness." Page 19, last paragraph, a rearrangement of the sentence will make it clearer: "Another study showed a reduction in butyrate-producing bacteria (Blautia, Coprococcus and Roseburia) in feces and Faecalibacterium spp. in the mucosa of PD patients, together with an increase in Ralstonia in mucosal samples compared with the abundance of those microbiota members in controls[80]."

Reply:

1# We fully agree with the reviewer in this point. As already indicated on the introduction of the manuscript the gut microbiota is well known as a highly dynamic ecosystem. Now we have introduced a sentence highlighting that this indeed hampers the understanding of the role of gut microbiota in the etiology and progress of neuropsychiatric diseases. Regarding what we have learned about the time scale on the changes of microbial community and development of the diseases is that the onset of most of the neuropsychiatric disease usually are close to a period where the gut microbiota is more unstable and, therefore, at risk of suffering microbiota alterations. We have also now indicated on the introduction that most of the neuropsychiatric conditions are multifactorial disorders prompted by certain

environmental factors in genetically susceptible individuals, being suggested gut microbiota as a key trigger factor for many of them. We have also now specified that there is a need of further work to elucidate the exact complex gene–environment interactions and gut microbiota alterations that precede the onset of the different neuropsychiatric diseases and their manifestations in order to decipher the etiology of the neuropsychiatric disorders.

2# We apologize since there was a mistake on the text that now has been corrected. Indeed, in both indicated studies the authors observed a positive correlation between the alpha microbiota diversity and depression and now it has been indicated.

3# According to the reviewer´s suggestion we provide more information and discuss on the mechanisms by which the alterations in the gut microbiota composition associated with obesity and diabetes may be related with the higher risk of developing AD. We highly appreciate the reviewer´s suggestion since we believe the added discussion about how the alterations in gut microbiota in diabetes or obesity may be linked to the risk for the development of AD really improved a lot our manuscript.

We added the following paragraph according to the reviewer´s suggestion:

Different mechanisms may explain the link between gut microbiota alterations in obesity and T2D and the development of AD. For example, different studies have indicated that an altered gut microbiota linked to obesity increases intestinal permeability and contributes to systemic inflammation leading to insulin resistance and T2DM In turn, insulin resistance and T2DM is a risk factor for development of AD. Furthermore, the vascular effects of obesity and T2D, related to changes on the gut microbiota, also appear to play an important role in the development of AD.
A leading hypothesis on the pathophysiology of AD is the mis-metabolism of amyloid precursor protein. The Aβ peptide is derived from amyloid precursor protein (APP) by sequential cleavages of different proteases. The activity of these proteases involved in the generation of Aβ peptide is highly regulated by the inflammation, being the latter modulate as already mentioned by the gut microbiota. In fact, BACE1 enzyme is essential for the generation of β-amyloid and Interleukin 1β, considered as a risk factor for AD development, has been observed to aggravate plaque formation by induction of BACE1 expression.
However, although it has been suggested that alterations on gut microbiota observed in diabetes and obesity may be linked to the risk of developing AD, there is a need of further work to elucidate the specific gut´s microbes and the mechanisms involved in the link between obesity, T2D and AD.

We have now also added the following few references to the new paragraph:

1 **Everard A**, Cani PD. Diabetes, obesity and gut microbiota. *Best Pract Res Clin Gastroenterol* 2013; **27**: 73-83 [PMID: 23768554 DOI: 10.1016/j.bpg.2013.03.007]

2 **Ochoa-Repáraz J**, Kasper LH. The Second Brain: Is the Gut Microbiota a Link Between Obesity and Central Nervous System Disorders? *Curr Obes Rep* 2016; **5:** 51-64 [PMID: 26865085 DOI: 10.1007/s13679-016-0191-1]

3 **Cole SR** and Vassar R. The Alzheimer's disease β-secretase enzyme, BACE1. *Mol Neurodegener* 2007; **2:** 22 [PMID: 18005427 DOI: 10.1186/1750-1326-2-22]

4# Although we agree that only the effect of probiotic VSL#3 on microglia activation has been described on the manuscript we also added evidence supporting a role of the gut microbiota on microglia maturation and function. Indeed, we described that GF mice have displayed an impaired microglial function and that the oral treatment with SCFAs can rescue this impaired function. However, we agree with the reviewer´s suggestion and we added a sentence on the manuscript indicating that we need still to be cautious at the moment and to investigate further the different probiotics that could be used to modulate the microglial maturation and function.

5# Minor error in grammar has been corrected and we rearranged the indicated sentence to make it clearer which now reads as follows: "

"Another study showed that at the taxonomic level of genus, putative "anti-inflammatory "butyrate-producing bacteria from the genera *Blautia, Coprococcus*, and *Roseburia* were significantly more abundant in feces of controls than PD patients. On the other side, in this study it was also reported that bacteria from the genus *Faecalibacterium* were significantly found more abundant in the mucosa of controls than PD patients, whereas putative "pro-inflammatory" Proteobacteria of the genus *Ralstonia* were significantly more abundant in mucosa of PD patients than controls[80]."

Reviewer #2:

COMMENTS TO AUTHORS

This is a well written and informative article. It would be nice to add a couple of tables summarising the evidence on the role of gut microbiota. This may also help to reduce the size of the manuscript which appears a bit lengthy currently.

Reply:

We have now included a new couple of tables, as suggested. Those tables will allow the readers to have at a glance the described evidences that have supported the role of gut microbiota on behavior and neuropsychiatric disorders. The introduction of these tables allowed us to reduce the size of the manuscript as suggested.

Table 1. Preclinical evidences of the role of gut microbiota on behavior.
• Germ-free (GF) mice have shown impaired social behavior[41] • GF mice have displayed exaggerated stress response[22] and differences in anxiety-like behavior[23,24]. • GF mice have showed crucial changes in multiple neurotransmitters and their receptors in different brain regions[24]. • GF animals have exhibited an impaired neurogenesis[26] and structural and functional changes in the amygdala[27]. • GF mice have shown prefrontal cortical hypermyelination[28]. • Microglial function impaired in GF animals is rescued by the oral treatment with short chain fatty acids[12]. • Gut microbiota has been shown to modulate brain-derived neurotrophic factor, oxytocin and vasopressin brain levels[21]. • Different probiotic administrations to rats and mice have shown to achieve a reduction in anxiety-like and depressive-like behaviors[29,60].

Table 2. Current evidences linking gut microbiota to neuropsychiatric disorders.	
Autism	• Increase in microbiota diversity is associated with autism[45]. • Abundance of Bacteroidetes has found to be linked with severe autistic cases[45]. • Increase in short chain fatty acids has found in fecal samples from autistic children[46]. • A specific strain of the species *Lactobacillus reuteri* has shown to modulate oxytocin levels and reverse autism-related behavior[43].
Schizophrenia	• Dopamine, the key neurotransmitter associated with schizophrenia pathophysiology, is produced by components of the microbiota[55]. • Increased gastrointestinal inflammation is associated with schizophrenia[55]. • Intake of antibiotics is associated with the risk of schizophrenia[56].
Attention deficit hyperactivity disorder (ADHD)	• The risk of developing ADHD has been suggested to be associated with many perinatal risk factors, including delivery mode, gestational age, type of feeding, maternal health and early life stressors, all of them linked to gut microbiota alterations[58]. • Dietary components modulating gut microbiota may influence ADHD development or symptoms[58].
Depression	• Increase in gut microbiota alpha diversity is associated with depression[61,65]. • Lower numbers of Bifidobacterium and Lactobacillus have been found in individuals with depression[62]. • Increases in the genus *Eggerthella, Holdemania, Gelria, Turicibacter, Paraprevotella and Anaerofilm*, and reductions in *Prevotella* and *Dialister* have been found in individuals with depression[63]. • A negative correlation between *Faecalibacterium* spp. and severity of depressive symptoms has been reported[63]. • Role of diet on depression onset is suggested (Mediterranean diet seems to protect, whereas Western diet seems to be associated with

	an increased risk)[66]. • Different strains of *Lactobacillus rhamnosus, Lactobacillus helveticus Bifidobacterium longum, Bifidobacterium breve and Bifidobacterium infantis* have been shown to attenuate depression and anxiety-related behavior in rodents[60]. • A probiotic combination (*Lactobacillus helveticus R0052 and Bifidobacterium longum R0175*) has proven effective in increasing the subject's resilience to stress in humans[59].
Parkinson's disease (PD)	• Alterations in bowel function, mainly constipation, often precede the onset of motor symptoms associated with PD[78]. • Reduction in the levels of Prevotellaceae has been found in PD patients[82]. • Positive correlation between levels of Enterobacteriaceae and the severity of postural instability and gait difficulty was proven in PD patients[82]. • Reduction in short chain fatty acids[80] and butyrate-producing bacteria (*Blautia, Coprococcus, Faecalibacteriumspp and Roseburia*)[81] were found in fecal samples from PD patients. • GF mice overexpressing human α-synuclein (αSyn) display reduced microglia activation, αSyn aggregates and motor deficits (treatment with short chain fatty acids restored all major features of PD in GF mice)[79]. • Gut microbiota transfer from PD patients into GF mice overexpressing human α-synuclein (αSyn) enhances physical impairments whereas gut microbiota transfer from healthy human donor does not enhances those deficiencies[79].
Alzheimer's disease (AD)	• Risk factors for AD such as metabolic syndrome, type 2 diabetes and obesity are associated with gut microbiota alterations[88,89]. • Gut microbiota seems to be involved in the accumulation of amyloid plaques according to the results of a study using a mouse model of AD[90].

Reviewer #3:

COMMENTS TO AUTHORS

This is a very interesting and comprehensive paper presenting up-to-date knowledge regarding to interactions between gut microbiota and specific neuropsychiatric disorders. Please replace "germ-free" on "GF" on page 13, line 15 and on page 20 line 4.

Reply:

We highly appreciate the positive comments of the reviewer and made the suggested replacements.