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Bridging the gap between gut microbiota and social life: beyond autism, the case of alcohol use disorder

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Abstract

Background and Objective

Gut microbiota and social functioning are both substantially altered in individuals with severe alcohol use disorder (sAUD). Based on previous investigations of their relationship in autism spectrum disorder (ASD) and other psychiatric disorders, we investigated the interactions between gut-related measures and social functioning among sAUD patients. The social dimension has a crucial association with the risk of relapse after detoxification.

Design

Forty-six sAUD patients undergoing detoxification were categorized as dysbiotic or non-dysbiotic based on their gut microbiota composition, and compared with healthy controls in a cross-sectional study. Metabolomic profiles, inflammatory markers levels, dietary habits, psychological symptoms, and social functioning were compared. We applied a comprehensive assessment of social functioning combining sociodemographic data, an emotional intelligence questionnaire, a social cognition task and personal social networks (evaluated through mapping techniques).

Results

One third of the sAUD patients exhibited microbial alterations. The dysbiotic patients were younger, leaner, and reported higher alcohol craving compared to the non-dysbiotic patients. The dysbiotic subgroup also displayed altered metabolomic and nutritional profiles and a higher plasma IL-8 level. Interestingly, we observed a coherent profile of severe impairments across social functioning indexes in the dysbiotic group: they had lower sociability scores, displayed impairment in social cognition (with greater difficulty in spontaneously considering another's perspective), were more often divorced/separated and unemployed, and had a smaller, less cohesive, and less diverse personal social network compared to the non-dysbiotic group.

Conclusion

As previously shown in ASD, we found a significant relationship between gut dysbiosis and various aspects of social functioning in sAUD. Targeting the gut microbiota could offer a novel approach to address social impairments that mediate the risk of relapse in psychiatric disorders.

Introduction

Social isolation and loneliness are recognized as strong factors increasing morbidity and mortality [1–3], and both bear a strong relation to the expression and severity of mental health disorders [4–7]. More specifically, psychiatric patients with low availability and quality of their social network tend to experience more severe symptoms [8, 9], worse quality of life [10], less life satisfaction [11], and greater risk of hospitalization [12]. Alcohol use disorder (AUD) is a complex disorder manifesting in behavioural, neurobiological, and psychosocial impairments [13]. Indeed, social and emotional impairments are central aspects of the expression of the disorder [14]. Individuals with AUD typically exhibit difficulties in decoding the emotions expressed by others [15, 16], a reduced ability to understand the perspective of others [17], impairments of emotional regulation [18, 19], and vulnerability to social exclusion [20]. The social dimension is crucial for the outcome of AUD, as social difficulties strongly predict a high risk of post-detoxification relapse [21, 22] insofar as the quality of social support greatly influences the maintenance of abstinence and drinking rates after relapse [23]. Although social resources are of cardinal importance for coping with mental health disorders [24], the individual factors that determine such social resources are still largely unknown.

Intriguingly, emerging research has uncovered a link between gut microbiota and social behaviour, suggesting that the populations of microorganisms in our digestive system may play a crucial role in shaping social interactions and mental health [25]. The gut microbiota is a dynamic ecosystem that can be influenced by several factors, including stress, diet, or medication [26]. Recent studies in patients presenting with autistic spectrum disorders (ASD) raised the possibility of a link between altered gut microbiota composition and impaired social functioning)[27–29], as subsequently confirmed in preclinical models [25, 30, 31]. For example, germ-free mice displayed deficits in social recognition and social cognition [32]. Furthermore *Lactobacillus reuteri* supplementation restored the social behaviour in a mouse model of autism [33], and *Bifidobacterium longum* and specific *Lactobacillus* strain supplementation improved social functioning and decreased antisocial behaviour in children with ASD [34]. Several correlational studies have indicated a disturbance in the composition of the gut microbiota (i.e., reduced microbial diversity and abundance of beneficial bacteria) in neuropsychiatric populations exhibiting deficits in social functioning, including ASD [35], schizophrenia [36], social anxiety disorder [37], and AUD [38–40].

We have previously reported that mice transplanted with the gut microbiota of sAUD patients displayed a reduction in social behaviour [41], as likewise reported in mice receiving fecal transplant from ASD patients [42]. Such findings support a causal role for the gut microbiota in regulating social behaviour. Indeed, we have found that leaky gut and dysbiosis were associated with the severity of depression, anxiety, and alcohol craving in sAUD patients [43]. However, there has been no exploration of the link between gut microbiota and social functioning in a sAUD.

Given this background, we set about to examine the relationship between gut microbiota (with respect to its composition and function) and social functioning in sAUD patients. To obtain a broader evaluation of social functioning, we used a multimodal and comprehensive approach combining different tests and neuropsychological tasks. First, we administered a questionnaire for emotional intelligence and computed a “sociability sub-score”. We next assessed social cognition abilities, more particularly theory of mind (ToM), using a visual perspective task [44], aiming to investigate the psychological processes that support an individual’s integration into their social network. We also collected basic sociodemographic data regarding marital status and employment history. Finally, we applied the personal network sociogram approach to measure the composition, structure, importance and complexity of the patients’ social networks [45]. In our approach, we consider that emotional intelligence and social cognition influence social interactions that consequently influence the personal social network and marital status.

We also evaluated whether gut microbiota composition and function differences among our sAUD patients were related to environmental factors such as medication status [46] or diet, which previously showed associations with behavioural alterations in individuals with ASD [47] and AUD [48]. Our approach also included assessment of plasma inflammatory markers and metabolomic statuses, constituting key aspects of the gut-brain axis [49]. Overall, we thus tested the hypothesis that gut microbiome status in sAUD patients would predict key aspects of impairments in social interactions in patients undergoing detoxification.

Results

Identification and description of dysbiotic and non-dysbiotic sAUD patients

Based on previous findings of altered gut microbiota composition in only a subset of sAUD patients [43, 50], we segregated our population patients into non-dysbiotic and dysbiotic subgroups (Fig 1A). Here, we performed a Principal Coordinate Analysis (PCoA) with the Bray Curtis index and extracted the component scores for each of the healthy subjects (HS) and sAUD patients. We then identified the dysbiotic group sAUD patients according to a deviance criterion threshold of 1.65 SD of the mean score for the first component in the HS group. By this criterion, 16 patients (35%) were classified as dysbiotic and 30 (65%) were non-dysbiotic.

Regarding alpha-diversity, dysbiotic sAUD patients displayed a lower number of observed species, as well as lower Chao-1, Shannon, and Simpson indices compared to HS and non-dysbiotic patients (Fig. 1B). The dysbiotic patients had a mean of 157 bacterial species, representing a 25% reduction compared to non-dysbiotic patients (203 species) and HS (211 species). The LEfSe analysis revealed that 9 genera differed between the three groups of subjects. *Ruminococcus* as well as *Christensenellaceae R7* group, *Oscillospiraceae NK4A214* group, *Oscillospiraceae UCG 003* and an unknown genus from the family *Eggerthellaceae* were lower in dysbiotic patients than non-dysbiotic patients and HS (Fig. 1C-E). Three genera, namely *Parabacteroides*, *Lachnoclostridium*, *Erysipelatoclostridium* and *Flavonifractor*, were higher in dysbiotic patients compared to non-dysbiotic patients and HS (Fig. 1C-E).

Sociodemographic and clinical features of the participants

The sAUD patients and HS were matched for age, gender, and BMI. Family status did not differ significantly between groups (Table 1). The corresponding demographics after segregation of the sAUD patients according to gut dysbiosis (Table 2) showed that the dysbiotic sAUD patients were younger and had a lower BMI than non-dysbiotic patients. However, the two sAUD groups did not differ with respect to gender, educational level, or any variables related to alcohol consumption history, i.e., DSM-5 score, age of loss of control, duration of drinking habits, and quantity of ethanol consumed.

Table 1: Socio-demographic characteristics and clinical features of sAUD patients and healthy subjects

	HS n=14	sAUD n=46	p
Sociodemographic characteristics			
Age (y)	47.3 ± 11.5	48.2 ± 9.1	0.75
Gender (woman, n, %)	6 (43%)	16 (35%)	0.75
Educational level,n (%)			0.01
Primary	0 (0%)	5 (11%)	
Secondary	0 (0%)	15 (33%)	
Superior	14 (100%)	26 (56%)	
Family status, n (%)			0.30
In couple/married	9 (64%)	19 (41%)	
Single	3 (21%)	19 (41%)	
Separated/divorced	2 (14%)	8 (18%)	
Employment, yes, n (%)	12 (86%)	25 (54%)	0.07
Clinical examination			
Weight, kg	71.4 ± 10.7	72.2 ± 12.8	0.84
BMI, kg/m^2	23.7 ± 3.1	23.9 ± 3.3	0.83
MMSE score	29.4 ± 0.6	27.9 ± 2.4	< 0.01
Alcohol consumption (g/day)	7.5 ± 10.0	145.6 ± 51.1	< 0.01

Values are means ± SD. p values were calculated using a T-test and Chi 2 test or Fisher's test for categorical variables.

sAUD: severe alcohol use disorder; BMI: body mass index; MMSE; Mini-Mental State Examination.

Table 2: Socio-demographic characteristics and clinical features of sAUD patients according to gut dysbiosis

	Dysbiotic n=16	Non-dysbiotic n=30	p
Sociodemographic characteristics			
Age (y)	43.6 ± 7.3	50.7 ± 9.2	0.01
Gender (woman, n, %)	7 (44%)	9 (30%)	0.35
Educational level,n (%)			0.43
Primary	2 (12%)	3 (10%)	
Secondary	7 (44%)	8 (27%)	
Superior	7 (44%)	19 (63%)	
Clinical examination			
Weight, kg	66.7 ± 12.9	75.1 ± 11.8	0.03
BMI, kg/m^2	22.3 ± 3.3	24.8 ± 3.0	0.01
Cushman score	4.4 ± 2.1	4.7 ± 2.8	0.68
MMSE score	28.1 ± 2.1	27.8 ± 2.5	0.68
Alcohol history			
DSM-5 score	8.9 ± 1.7	8.2 ± 1.7	0.23
Age of loss of control (y)	29.4 ± 7.4	32.9 ± 11.9	0.26
Duration of drinking habit (y)	14.2 ± 7.6	17.7 ± 11.9	0.56
Alcohol consumption (g/d)	148.9 ± 58.5	144.1 ± 48.2	0.77
Morning alcohol use (yes, n, %)	8 (62%)	6 (35%)	0.27

Values are means ± SD. n=46. p values were calculated using a T-test or Mann Whitney Wilcoxon's test and Chi 2 test or Fisher's test for categorical variables

sAUD: severe alcohol use disorder; BMI: body mass index; DSM-5, Diagnostic and Statistical Manual of Mental Disorders fifth edition; MMSE; Mini-Mental State Examination.

Dysbiotic sAUD patients had a higher alcohol craving and a lower sociability score

Comparison of mood, alcohol craving and emotional intelligence showed similar scores for anxiety and depression in the dysbiotic and non-dysbiotic patient groups (Table 3). However, dysbiotic patients had higher mean craving score of the compulsive subscale, even after adjustment for potential confounders (Table 3).

Among the subscores for well-being, self-control, sociability, motivation, and emotionality in a questionnaire of emotional intelligence, only the sociability sub-score was associated with gut dysbiosis, persisting after adjustment for age, gender, BMI, and nutritional intake (Model 2 OR= 0.34, p=0.04, Table 3). The lower score in dysbiotic sAUD patients would predict relatively impaired social functioning.

Table 3: Psychological parameters of sAUD patients according to gut dysbiosis

	Dysbiotic n=16	Non Dysbiotic n=30		Model 1[b]		Model 2[c]		Model 3[d]	
	Mean± SD	Mean± SD	p[a]	OR	p	OR	p	OR	p
Mood									
Anxiety	48.2 ± 16.7	46.7 ± 13.8	0.67	0.99 [0.94 ; 1.04]	0.75	0.99 [0.93 ; 1.04]	0.59	0.96 [0.90 ; 1.03]	0.30
Depression	27.4 ± 14.1	25.1 ± 11.0	0.63	1.00 [0.94 ; 1.06]	0.95	1.01 [0.94 ; 1.06]	0.97	0.97 [0.89 ; 1.04]	0.36
Craving									
Total score	27.3 ± 4.6	23.2 ± 5.7	0.03	1.13 [0.97 ; 1.33]	0.10	1.14 [0.97 ; 1.34]	0.12	1.16 [0.94 ; 1.44]	0.17
Obsessive score	11.7 ± 3.4	10.0 ± 3.7	0.18	1.07 [0.88 ;1.32]	0.55	1.06 [0.85 ; 1.32]	0.59	1.01 [0.77 ; 1.33]	0.93
Compulsive score	15.6 ± 2.2	13.2 ± 2.8	**0.007**	1.64 [1.03 ; 2.62]	**0.037**	1.69 [1.05 ;2.71]	**0.03**	2.40 [1.01 ; 5.72]	**0.048**
Emotionnal intelligence									
Well-being	4.6 ± 1.0	4.7 ± 0.9	0.87	1.41 [0.53 ; 3.72]	0.49	1.63 [0.48 ; 3.34]	0.63	1.57 [0.51 ; 4.85]	0.43
Self-control	4.2 ± 0.9	4.3 ± 0.8	0.77	1.43 [0.49 ; 4.12]	0.51	1.27 [0.41 ; 3.90]	0.67	2.56 [0.52 ; 12.57]	0.25
Sociability	3.5 ± 1.0	4.3 ± 1.1	**0.03**	0.44 [0.17 ; 1.15]	0.09	0.34 [0.12; 0.97]	**0.04**	0.19 [0.04 ; 0.90]	**0.036**
Motivation	4.1 ± 1.4	4.6 ± 1.1	0.22	0.94 [0.41 ; 2.12]		0.87 [0.37 ; 2.05]	0.75	0.87 [0.37 ; 2.05]	0.99
Emotionality	4.6 ± 0.7	4.9 ± 0.9	0.38	0.81 [0.25 ; 2.60]	0.72	0.90 [0.39; 2.02]	0.79	0.94 [0.38; 2.31]	0.89

[a] p values were calculated using a T-test or Mann Whitney Wilcoxon's test.

[b] Logistic regression adjusted for age, gender and body mass index

[c] Logistic regression adjusted for age, gender, body mass index and nutritional intakes

[d] Logistic regression adjusted for age, gender, body mass index, nutritional intakes, family status and number of children.

sAUD: severe alcohol use disorder; OR: Odd ratio.

Dysbiotic sAUD group tended not to consider spontaneously another person's viewpoint

To investigate social cognition abilities that are considered as psychological processes supporting an individual's integration into a social network, we used a visual perspective-taking task (VPT) (see Fig 2A and methods section for explanation of the task). Results did not indicate any impairment in this task among sAUD patients (SI Appendix: Supplemental behavioural results).

In a previous VPT study conducted in healthy adults, presentation of an avatar in the image lead to a drop in the speed and accuracy of counting the number of disks seen by "You (the participant)" [44], due to interference from the natural tendency to capture the perspective of the avatar (Fig 2A). This phenomenon represents the degree of altercentric bias. We did report a trend towards a significant interaction between Congruency x Perspective x Group ($F(1, 43)= 3.07$, $p=0.087$), where the latter interaction is driven by the lesser altercentric bias concerning reaction time (RT) and accuracy in dysbiotic patients (Fig 2B and C).

Indeed, as in healthy adults, the non-dysbiotic patients also presented with altercentric bias in reaction time and accuracy that is marked by a significant increase in delay ($p=0.026$, Fig 2B) and a marginally significant drop in accuracy ($p=0.090$, Fig 2C). However, the dysbiotic group did not show these biases for time and accuracy ($p = 0.781$ and $p= 0.792$ respectively, Fig 2B and 2C), meaning that they failed to be influenced by the perspective of the other. Hence, combining RT and accuracy rates into Balanced Integration Scores (BIS;[51]) revealed that the dysbiotic patients had significantly less altercentric bias than the non-dysbiotic group ($t(42)= 2.522$, $p=0.016$; see Fig. 2E).

These converging trends and findings strongly suggest that the dysbiotic sAUD patients did not spontaneously consider the perspective of others (Fig. 2).

Dysbiotic sAUD patients displayed a smaller and less cohesive social network

Interestingly, family status differed according to the dysbiosis category (Table 4). Dysbiotic patients were more likely to live alone, with 80% being single, separated, or divorced, and less than 20% living as a couple or married. In the non-dysbiotic patients, only 47% lived alone, while 53% were in a couple or married. Also, the dysbiotic sAUD patients had a lower rate of employment (Table 4). Thus, microbiome status was associated with social structure as recorded in basic sociodemographic data.

Table 4. Social characteristics of sAUD patients according to gut dysbiosis

	Dysbiotic n=16	Non-Dysbiotic n=30	p[a]
Social characteristics			
Family status, n (%)			**0.023**
In couple / married	3 (18.8)	16 (53.3)	
Single/Separated/divorced	13 (81.2)	14 (46.6)	
Number of children	2 (1.2)	1 (1.5)	0.46
Employment, yes, n (%)	5 (31.3)	20 (66.7)	**0.047**

[a]p values were calculated using a Fisher's test or Mann Whitney Wilcoxon's test.

sAUD: severe alcohol use disorder

We next used the sociogram approach to analyse in more detail the personal social network of the sAUD patients (SI Appendix Fig S1, Table S1). Results showed that the regarding the size of the network, dysbiotic patients had smaller social networks, with lower numbers of alters and communities compared to non-dysbiotic sAUD group, when adjusted for potential confounders (Table 5). The dysbiotic group's network consisted of a mean of seven alters, compared to ten in the non-dysbiotic group. Among these alters, three were isolated in each group, meaning that they did not exchange information about "ego/the patient" with any other alter in the network. Structurally, the networks were less cohesive in the dysbiotic group, who had an almost two-fold higher transitivity index than the non-dysbiotic group (p=0.020 in models 1, 2 and 3; Table 5). Modularity was also associated with gut dysbiosis as dysbiotic patients showed a lower number of communities (p=0.030 in models 1, 2 and 3; Table 5 and Table S1).

There was no significant difference in the social network composition between dysbiotic and non-dysbiotic groups (SI Appendix, Fig. S2). About 50% of the network members were from the family sphere in the non-dysbiotic group versus 42% in the dysbiotic group (n.s.). Friends represented 21 and 26% of the network for non-dysbiotics and dysbiotics, respectively. Twenty-two percent of the alters belonged to the health care community in the non-dysbiotic group versus 13% in dysbiotic group, but this difference was not significant after adjustment for potential confounders (Table 5). Mental health care personnel represented approximately 10 and 7% of the social network of non-dysbiotic and dysbiotic patients, respectively (SI Appendix, Fig. S2).

In conclusion, gut dysbiosis in sAUD patients was associated with differences in personal network structure and lower size, but not in terms of composition.

Table 5: Structure of the social network of sAUD patients according to gut dysbiosis

	Dysbiotic n=15	Non-Dysbiotic n=25		Model 1[b]		Model 2[c]		Model 3[d]	
			p[a]	OR	p	OR	p	OR	P
Social network									
Density (%)	19.9 ± 16.3	20.0 ± 16.3	0.99	0.99 [0.95 ; 1.04]	0.79	0.99 [0.94 ; 1.05]	0.82	1.02 [0.95 ; 1.09]	0.66
Network size (no.)	7.53 ± 4.24	9.72 ± 3.69	0.09	0.76 [0.59 ;0.98]	**0.03**	0.73 [0.55 ;0.98]	**0.03**	0.60 [0.39 ; 0.91]	**0.016**
Number of dyads(no.)	5.93 ± 7.06	7.32 ± 5.22	0.17	0.92 [0.80 ; 1.05]	0.22	0.92 [0.80 ; 1.06]	0.25	0.89 [0.77 ; 1.04]	0.16
Number of triads (no.)	4.60 ± 9.70	3.68 ± 4.38	0.25	0.99 [0.89 ; 1.11]	0.94	1.01 [0.90 ; 1.13]	0.89	0.98 [0.87 ; 1.11]	0.75
Number of communities (no.)	3.60 ± 3.11	5.40 ± 2.06	**0.01**	0.65 [0.43 ; 0.98]	**0.04**	0.63 [0.41 ; 0.98]	**0.04**	0.45 [0.22 ; 0.91]	**0.02**
Number of community with more than 3 alters (no.)	0.67 ± 0.72	1.16 ± 0.69	**0.037**	0.10 [0.01 ; 0.63]	**0.01**	0.06 [0.01 ; 0.60]	**0.01**	0.04 [0.01 ; 1.06]	0.05
Cliques (no.)	0.73 ± 0.70	1.44 ± 1.29	0.11	0.41 [0.16 ; 0.99]	**0.03**	0.41 [0.16 ; 0.99]	**0.04**	0.32 [0.09 ; 1.13]	0.07
Degree (%)	5.7 ± 10.2	6.5 ± 8.3	0.91	1.01 [0.94 ; 1.09]	0.79	1.02 [0.94 ; 1.09]	0.70	1.07 [0.97 ; 1.17]	0.16
Betweenness (%)	8.1 ± 13.9	11.3 ± 23.2	0.67	0.99 [0.95 ; 1.03]	0.58	0.99 [0.94 ; 1.03]	0.51	1.01 [0.95 ; 1.06]	0.91
Closeness (%)	42.2 ± 28.1	43.6 ± 19.5	1.00	1.01 [0.97 ; 1.04]	0.74	1.01 [0.98 ; 1.05]	0.49	1.03 [0.98 ; 1.08]	0.23
Modularity (ranges from −1 to 1)	0.14 ± 0.20	0.28 ± 0.26	0.13	0.01 [0.01 ; 0.68]	**0.03**	0.01 [0.01 ; 0.61]	**0.03**	0.01 [0.01 ; 0.49]	**0.03**

Diameter (ranges from 0 to 6)	1.40 ± 1.06	1.64 ± 0.76	0.40	0.63 [0.27 ; 1.49]	0.29	0.62 [0.25 ; 1.55]	0.31	0.56 [0.18 ; 1.70]	0.30
Transitivity (%)	33.0 ± 36.6	58.9 ± 41.5	0.057	0.97 [0.95 ; 0.99]	**0.02**	0.97 [0.95 ; 0.99]	**0.02**	0.95 [0.92 ; 0.99]	**0.02**
Professional proportion (%)	13.1 ± 26.7	22.2 ± 17.2	**0.02**	0.98 [0.95 ; 1.03]	0.54	0.99 [0.96; 1.04]	0.83	0.99 [0.94 ; 1.04]	0.67
Gender homophily (%)	30.3 ± 30.0	49.4± 25.2	0.06	0.97 [0.94 ; 1.00]	0.08	0.97 [0.93 ; 1.01]	0.11	0.97 [0.93 ; 1.01]	0.17
Ring homophily (%)	46.1 ± 40.7	68.4 ± 29.9	0.09	0.99 [0.96 ; 1.01]	0.22	0.99 [0.96 ; 1.01]	0.33	0.99 [0.97 ; 1.02]	0.48
Largest full mesh (no.)	1.20 ± 1.93	1.64 ± 1.89	0.39	0.86 [0.57 ; 1.29]	0.45	0.88 [0.59 ; 1.33]	0.55	0.82 [0.50 ; 1.33]	0.42
Isolated (no.)	3.13 ± 2.90	3.16 ± 1.80	0.40	0.98 [0.69 ; 1.40]	0.92	1.01 [0.67 ; 1.43]	0.90	0.89 [0.56 ; 1.40]	0.61
Isolated dyads(no.)	0.47 ± 0.64	0.80 ± 0.96	0.33	0.57 [0.18 ; 1.83]	0.35	0.62 [0.19 ; 2.05]	0.43	0.60 [0.16 ; 2.24]	0.45

[a]p values were calculated using a T-test or Mann Whitney Wilcoxon's test.

[b] Logistic regression adjusted for age, gender and body mass index

[c] Logistic regression adjusted for age, gender, body mass index and nutritional intakes

[d] Logistic regression adjusted for age, gender, body mass index, familial status, the number of children and nutritional intakes

sAUD: salcohol use disorder; OR: Odd ratio.

Dysbiotic patients presented altered faecal and blood metabolomes

As potential mediators of the gut-brain axis, we made an exploratory comparison of fecal and blood metabolites in the two sAUD groups. This faecal metabolomic analysis highlighted 15 annotated metabolites that discriminated dysbiotic from non dysbiotic patients, where higher *L*-carnitine and 4-trimethylammoniobutanoic acid (also known as gamma-butyrobetaine, the precursor of L-carnitine) were the two most discriminating metabolites (Fig. 3A and B) in the dysbiotic group (VIP score=2.54 and p<0.0001). Dysbiotic patients also had higher fecal levels of the primary bile acids chenodeoxycholic acid and cholic acid (VIP=2.05, p=0.0002 and VIP=1.90, p=0.001), whereas they had lower

levels of the conjugated bile acid (BA) hydroxy-ketodeoxycholic acid (VIP=1.95, p=0.009; Fig. 3A and B). The dysbiotic group exhibited lower stercobilin and higher urobilin levels, these being bilirubin-derived metabolites. Tryptophan, choline and N8-acetylspermidine levels were higher and the histamine metabolite methylimidazoleacetic acid lower in the dysbiotic group (Fig. 3A and B). The SCFA levels did not differ between the two groups (SI Appendix, Fig. S3).

Plasma metabolite analysis revealed differences of 45 annotated metabolites in the sAUD subgroup comparison (VIP score ≥1.5; p-value <0.05). The top ten discriminant metabolites according to VIP score were kynurenine, phenylacetylglutamine (PAG), glycohyodeoxycholic acid (GHDCA), p-cresol sulfate, glycochenodeoxycholic acid (GCDCA), 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), pantoprazole, LPC 18:1, LPE 18:1 and 3-carboxy-4-methyl-5-pentyl-2-furanpropanoic acid (3-CMPFP) (Fig. 4A and B). Plasma kynurenine, PAG, p-cresol sulfate, CMPF, 3-CMPFP and LPC 18:1 were lower in the dysbiotic group compared to non-dysbiotic patients, while the other compounds were significantly higher (Fig. 4A and B). Dysbiotic patients also exhibited significantly higher plasma levels of conjugated BA (GHDCA, GCDCA, ursodeoxycholic acid [C24H40O4; UDCA], tauroursodeoxycholic (TUDCA) acid, glycochenodeoxycholic acid 7-sulfate, and glycocholic acid; Fig. 4A and B).

Dysbiotic sAUD patients had higher plasma IL-8 levels compared to non-dysbiotic patients

Inflammation being one of the main communication pathways between the gut and the brain, we measured several inflammatory parameters, including TNFα, IL-6, IL-8, IL-10, IL-18, MCP-1, IFNγ. Among these, the chemokine IL-8 was higher in the dysbiotic compared to non-dysbiotic patients, after adjustment for age, gender and BMI (model 1 OR=1.21, p=0.048, SI Appendix, Table S2); other inflammation markers did not differ between groups.

As gut microbiota alterations have been associated with liver disease in AUD, we also compared liver enzymes and markers of liver damage, which did not differ between the two groups (SI Appendix, Table S2). BDNF, a key regulator of synaptic plasticity, which is altered in depression and anxiety disorders and is modulated by gut microbiota, did not differ between dysbiotic and non-dysbiotic patients (SI Appendix, Table S2).

The subgroup differences in gut microbiota composition could be explicable by nutritional intake, but not medication use

Nutrition is one of the main factors influencing the composition of the gut microbiota [52]. We compared the nutritional profile of dysbiotic *versus* non-dysbiotic patients by PCA, including all important macro- and micronutrients. The diets of the two groups of sAUD patients were similar, as indicated by the overlapping PCA ellipses (SI Appendix, Fig. S4A). We then compared the intake of each nutrient individually (SI Appendix, Table S3). The dysbiotic and non-dysbiotic groups did not differ in terms of energy intake, but dysbiotic patients consumed less protein (p=0.03) and less dietary fiber (p=0.05). The consumption of beer, wine or spirits was similar between both groups of sAUD patients (SI Appendix, Fig. S4B).

Commonly used non-antibiotic drugs could also alter the composition and function of the gut microbiota [46, 53]; medication intake did not differ between dysbiotic and non-dysbiotic patients (SI Appendix, Table S4).

Social network position is linked to gut microbiota diversity, composition and function

After identifying alterations in the gut microbiota composition and function in sAUD patients, we tested whether specific bacterial genera or metabolites correlated with altered social functioning. To this analysis, the size and structure of the personal social network was significantly associated with microbial richness (SI Appendix, Fig. S5A). Indeed, the number of observed species and the Chao-1 index were both positively correlated with the number of

alters and the number of communities in the social network (SI Appendix, Fig. S5A). There was also a positive correlation between the alpha-diversity indices (Shannon and Simpson) and the modularity (SI Appendix, Fig. S5A).

We then tested for correlations with the bacterial genera that significantly differed between the dysbiotic and non-dysbiotic patients. Among these. *Erysipelatoclostridium* negatively correlated with the sociability subscore of the emotional intelligence questionnaire. We also found that *Lachnoclostridium*, *Flavonifractor* and *Erysipelatoclostridium*, three bacterial genera that were more abundant in dysbiotic patients, negatively correlated with the network size (number of alters and number of communities), while *Oscillospiraceae NK4A214* group and *Oscillospiraceae UCG_003* positively correlated (SI Appendix, Fig. S5B). Concerning the visual perspective task, *Oscillospiraceae UCG_003*, *Oscillospiraceae NK4A214 group* and *Christensenellaceae R7 group* were associated with an increased bias toward taking the perspective of others, while *Lachnoclostridium* and *Flavonifractor* were associated with lesser bias (SI Appendix, Fig. S5B).

Three of the faecal metabolites were significantly and negatively associated with sociability indexes (measured via psychological questionnaires, personal network, or the social cognition task), namely the bile acid chenodeoxycholic acid (CDCA), N-methyl-2-pyrrolidone, and tryptophan (SI Appendix, Table S5). Several blood metabolites were positively associated with the social network size (number of communities and of alters), namely PC 36:5, PC 38:6, CMPF and 3-CMPFP. Altercentric bias, which represents to tendency to consider the perspective of others, was negatively associated with PC 34:1, LPC 16:1, LPE 22:5. (SI Appendix, Table S5).

Discussion

Prior investigation of the relationship between abnormal gut microbiota and difficulties in social functioning in ASD patients [54] showed that diet is an important factor to consider [47]. Based on previous studies from our research group [41, 43, 55], we proposed that patients with sAUD would likewise show relationships between gut microbiota and social functioning. As observed previously [43, 50], only a subgroup (35%) of the present sAUD patients showed altered gut microbiota composition compared to HS. Overall, the dysbiotic group was younger, thinner, and had a higher alcohol craving score, a trait that is associated with higher relapse rate in detoxified sAUD patients [21].

To test the link to sociability, we applied a multimodal and comprehensive approach capturing a broad spectrum of social functioning by combining sociodemographic data, an emotional intelligence questionnaire, a social cognition task (visual perspective-taking task) and personal social networks (evaluated through mapping techniques). The dysbiotic group displayed a lower sociability score on the emotional intelligence scale, a decreased tendency to spontaneously adopt the others' viewpoint in the visual perspective task, and a smaller and less cohesive social network with fewer communities, as depicted in the sociogram. They also exhibited alterations in the metabolomic profile, specifically regarding carnitine, BA, lipids, kynurenine, urobilinoids, and gut-derived metabolites. Regarding inflammation markers, only IL-8 differed, with dysbiotic patients showing significantly higher levels after adjustment for age, gender, and BMI. There were no subgroup differences in terms of medication use. However, the dysbiotic sAUD patients consumed less protein and presented a trend towards lesser dietary fiber intake.

Decreased α-diversity has been considered an indicator of an unhealthy microbiota in association with chronic diseases such as obesity and diabetes, and likewise with unhealthy dietary habits [56]. In this study, lesser α-diversity was associated with lower protein intake, consistent with the role of dietary protein in determining gut microbiota composition and function [57]. Patients with impaired gut microbiota also tended to consume less dietary fiber, which is known to affect the growth of specific bacteria that can influence host metabolism and brain function [57, 58]. We found that the dysbiotic sAUD patients exhibited a higher relative faecal abundance of *Parabacteroides*, *Lachnoclostridium*, *Flavonifractor*, and *Erysipelatoclostridium* compared to the non-dysbiotic and HS groups. Increased

Parabacteroides, *Flavonifractor*, and *Lachnoclostridium* was previously reported in ASD, bipolar disorders, schizophrenia, and major depressive disorders compared to HS [27, 59–64], although not consistently across all studies. Conversely, the present dysbiotic patients displayed a gut microbiota poor in *Ruminococcus*, *Christensenellaceae R7 group*, *Oscillospiraceae NK4A214 group,* and *Oscillospiraceae UCG-003* compared to the non-dysbiotic and HS groups. Lower *Ruminococcus* abundance was already observed in AUD patients with high intestinal permeability [43]. Present findings of lower *Ruminococcus* negatively correlated with BA levels, which corroborates studies showing that certain BAs may inhibit the growth of bacteria, in particular *Ruminococcus bromii* [65]. Butyrate production by *Ruminococcus* is known to promote the intestinal barrier function, exert anti-inflammatory effects, and promote neurotrophic factors through its histone deacetylase activity [66, 67]. Furthermore, butyrate exerted beneficial effects on social and repetitive behaviour in an mouse model of ASD [68]. However, we did not observe altered levels of fecal butyrate or other SCFAs in the sAUD patients with altered gut microbiota. We observed an almost complete depletion of *Christensenellaceae* in the dysbiotic subjects, which is particularly interesting given the association of this bacterium family with better metabolic health [69], healthy aging [69, 70], and the bacterial population decreases in affective disorders, which is associated with increased oxidative stress and low-grade systemic inflammation [71, 72].

Interestingly, we demonstrated that sAUD patients with altered gut microbiota displayed a lower sociability score (emotional intelligence questionnaire), meaning that they subjectively perceived themselves as having more difficulties in being socially assertive, coping with others' emotions, and being effective in communication. To evaluate the nature of the social cognitive deficits explaining these social differences, we tested their visual perspective taking. Dysbiotic and non-dysbiotic patients both presented with a normal egocentric bias (i.e., the patient's viewpoint interferes in judgment about what the avatar sees). Interestingly, however, altercentric bias (i.e., the avatar's viewpoint interferes in judgment of what the patient him/herself sees) was absent only in the dysbiotic group, in contrast to our previous study involving healthy adults [44]. This highlights the loss of the spontaneous tendency of dysbiotic patients sAUD to consider another person's visual perspective, plausibly contributing to their reduced social network. Indeed, the sociogram approach revealed objectively that dysbiotic patients had a smaller and less cohesive social network than non-dysbiotic sAUD subjects, this in association with lower employment level and greater frequency of single living.

There is a prior report of social network of sAUD patients according to the Social Network Index, which showed lesser social network size and diversity among individuals with alcohol dependence compared to HS [73]. However, these previous findings did not capture the effective social structure of these individuals, whereas our sociogram methodology depicts a much deeper description of the personal network size, composition, and structure. AUD patients exhibited alterations in social cognition, particularly in the recognition of emotions, which persisted after three months of abstinence [74–76]. AUD patients also display reduced ability for taking the perspective of others [77]. These deficits may lie at the origin of social integration problems or difficulties in maintaining satisfactory interpersonal relationships, thus promoting social isolation [78]. One study reported that AUD patients have increased sensitivity to social rejection [20]. These aspects play a prominent role in the management of AUD, as 60% of relapses after detoxification can be directly attributed to emotional or interpersonal difficulties [21, 79].

The link between gut microbiota and sociability was recently highlighted in preclinical studies showing transfer of behavioural phenotype from donor to recipient mice after fecal material transplant [80, 81]. In our study, dybiotic sAUD patients with lower microbial diversity have deficits in sociability as well as a smaller social network. That observation is consistent the relation in a large non-clinical adult population between microbiome diversity and decreased indexes of sociability as measured by questionnaires [82]. We observed that microbial diversity correlated to different indexes of sociability, as well as certain bacterial genera, namely members of *Oscillospiracae* family,

Lachnoclostridium, *Flavonifractor*, *Erysipelatoclostridium* or *Christensenellaceae R7* group. Certain bacterial genera may influence the brain through neural, immune, or endocrine pathways [26]. In a murine model of autism, the ability of *Lactobacillus reuteri* administration to restore social behaviour was mediated by the vagus nerve [33]. Some neuroactive metabolites produced by gut bacteria could also modulate social behaviour. For example p-cresol, a by-product of bacterial fermentation of tyrosine, induced social behaviour deficits and microbial changes in mice [83], and is reportedly increased in urine or feces of autistic children [84, 85]. In our study, dysbiotic subjects had *lower* plasma levels of p-cresol sulfate, whithout showing any correlation with sociability indices. Metabolomic analysis revealed alterations in bile acids, lipids and CMPF in the dysbiotic sAUD patients; these metabolites could plausibly induce neurobiological changes and affect behaviour [86–89]. For example, CMPF, a gut-derived metabolite produced upon fatty fish uptake, was lower in the present dysbiotic patients, and correlated positively with the social network size and the number of communities. Treatment with CMPF reversed hepatic lipid accumulation and improved insulin sensitivity in obese mice [90]. This metabolite was also associated with a slower decrease in cognitive function in a cohort of middle-aged adults [91].

Serotonin modulates a wide range of cognitive functions linked to alcohol-related disorders, extending from reinforcement learning to social cognition [92, 93]. We observed elevated levels of the serotonin precursor tryptophan in stool samples from the dysbiotic patients, which might predict alterations in serotonin synthesis in brain. Interestingly, Luna *et al.* reported a microbial signature in children with ASD similar to present findings in dysbiotic patients, and showed that higher tryptophan levels in the gastrointestinal tract were associated with more severe behavioural symptoms, as well as reduced serotonin synthesis in brain [94]. In our study higher tryptophan levels were negatively correlated with the altercentric bias.

Studies in non-human primates and humans have shown that social contact impacts the composition of the gut microbiota, and that family members have a shared gut microbiota composition [95–98]. Therefore, we cannot exclude the possibility that social behaviour and the size of the social network themselves influence the gut microbiota composition in sAUD patients.

We note several limitations of our study. As previously mentioned, the cross-sectional data do not support inferences of causality. Indeed, gut microbiota and behaviour could have reciprocal effects. Our limited sample size calls for confirmation in a larger group. However, our study is the first to investigate the link between social functioning and gut microbiota in sAUD patients. A main strength of our approach lies in the use of different complementary tools to measure social functioning and structure, including mapping of the personal social network. Furthermore, we evaluated the influences of medication and diet, two factors known to strongly impact composition of the gut microbiota [46, 52].

In conclusion, we showed that impaired social functioning in sAUD patients was associated with gut dysbiosis, thus suggesting that promising preclinical results linking gut microbiome and sociability [41, 42] may also apply to humans, in particular to patients with sAUD. We show that lesser gut microbiota diversity is associated with reduced social functioning in AUD, as previously reported in ASD. This suggests that the microbiome could constitute a transdiagnostic process mediating deterioration of social abilities across a range of psychiatric disorders. Present results could contribute to the development of strategies to modulate gut microbiota or improve social cognition in AUD patients, and possibly in other disorders.

Materials and Methods

Subjects

The data of this cross-sectional study were obtained at the first time-point of a randomized, double-blind, placebo-controlled study evaluating the impact of prebiotic supplementation on the gut-liver-brain axis in patients with AUD [55]. Fifty AUD patients hospitalized for a 3-week highly standardized alcohol-detoxification program in the Cliniques Universitaires St Luc (Brussels, Belgium) enrolled on a voluntary basis in the study, with testing at the beginning of alcohol-withdrawal, before any intervention. A psychiatrist assessed the severity of AUD according to criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5). Inclusion criteria were the following: male or female, 18 to 65 years old, French speaking, and active alcohol consumption until at least 48 hours prior to admission. Patients suffering from another addiction (except tobacco), inflammatory bowel or other chronic inflammatory diseases (such as rheumatoid arthritis), cancer, metabolic diseases such as obesity (BMI≥ 30 kg/m^2), diabetes, or severe cognitive impairment (MMSE < 24), or having undergone bariatric surgery were excluded from the study, as were patients with known cirrhosis or significant hepatic fibrosis (≥F2) detected by Fibroscan (> 7.6 kPa) at admission. Other exclusion criteria were any use of antibiotics, probiotics, or prebiotics within two months prior to enrolment, and use of non-steroidal anti-inflammatory drugs or glucocorticoids within one month prior to enrolment.

AUD patients were matched for age, gender, and BMI with 14 healthy subjects (HS) with no AUD (Alcohol use disorders test [AUDIT] score <8 in males and <7 in females), who were recruited using flyers posted around Brussels. The study was approved by the institutional ethics committee (2017/04JUL/354 and 2014/14AOU/438), and all participants have signed informed consent prior to inclusion.

Outcomes

Gut microbiota analyses

Stool samples collected on day 2 of the detoxification program in 46 of the AUD patients were immediately stored -20°C and then transferred to a -80 °C freezer within 5 -10 hours. Genomic DNA was extracted from the feces using a QIAamp DNA Stool Mini Kit (Qiagen, Germany), including a bead-beating step, and following the protocol Q [99]. The composition of the gut microbiota was analysed by Illumina sequencing of the 16S rRNA gene, as previously described [55].

Metabolomics profiling

Plasma and fecal samples were kept in -80 °C until metabolite extraction. Samples were then thawed on ice, followed by acetonitrile extraction of plasma metabolites, and 80% methanol extraction of faecal metabolites. Pooled QC samples were used for instrument equilibration, including data-dependent MS/MS and MS drift correction. All samples were analyzed in the HILIC and RPLC separations both in ESI+ and ESI- ionization modes. Plasma RPLC data were acquired using a Thermo QExactive Classic coupled to a Vanquish Flex UHPLC (Thermo Fischer Scientific, Bremen, Germany), while HILIC data were acquired on an Agilent 6540 UHD QTOF coupled to a 1290 UHPLC (Agilent Technologies, Waldbronn, Karlsruhe, Germany). MS-Dial v. 4.80 was used for peak detection and alignment following data preprocessing with R (version 4.0.3) in the *notame* package [100]. Metabolites were annotated by manually inspecting *m/z* values, retention times, and fragmentation spectra against in-house library and public databases. The detailed protocol has been published earlier [101].

Semi-targeted analysis of fecal SCFAs

Fecal SCFAs were measured using a solid-phase microextraction coupled to gas chromatography mass spectrometry (SPME-GC-MS). Fecal samples were suspended in deionized H_2O and desalted by NaH_2PO_4. A fused silica 75 µm CAR/PDMS fiber was used for absorption of SCFAs, and the separation was obtained on an SPB-624 (60 m × 0.25

mm × 1.4 µm) fused silica column. The GC-MS instrument consisted of a Thermo Trace 1310 – TSQ 8000 Evo fitted with a TriPlus RSH autosampler (Thermo Scientific, Wilmington, DE, USA). EI voltage was set at 70 eV. Blank samples and analytical standard mixtures of acetic, propanoic and butyric acids were injected before and after each set of 26 biological samples. Identification of the SCFAs was based on comparison of the retention times and mass spectra against the analytical standards, as well as the NIST library.

Psychological symptoms assessment

Depression, anxiety, and alcohol craving

Self-reported questionnaires were used on days 1 and 2 to assess anxiety (State-Trait Anxiety Inventory [STAI form YA]), depression (the Beck Depression Inventory [BDI]), and alcohol craving (the Obsessive-Compulsive Drinking Scale modified version [OCDS]), all as described previously [102].

Social functioning: emotional intelligence, personal network and social cognition

The emotional intelligence (EI) was measured using the French version of the TEIque, which is a self-reported questionnaire consisting of 75 items, each rated on a 7-point scale ranging from 1 (strongly disagree) to 7 (strongly agree) [103]. TEIque assesses a global trait EI score, as well as scores in four specific factors: well-being, self-control, emotionality, motivation and sociability.

To measure instructed and spontaneous capacities to consider the perspective of others, AUD patients performed the visual perspective task (VPT) on day 1, as detailed elsewhere [44]. In brief, during the task the patient viewed a computer screen showing an adult avatar standing in the middle of a 3-walled room, along with 0 to 3 red discs hanging on the walls. The patient had to decide if a prompted number (ranging from 0 to 3) matched the number of discs visible on a screen from a prompted target perspective, which could be either the participant's perspective (self-perspective condition) or the perspective of the avatar (other-perspective condition) (Fig. 2A). The number of discs visible could be the same (congruent perspective condition) or different (incongruent perspective condition) for the two perspectives. Reaction times (RTs) and accuracy were recorded for all conditions [2 (perspective: self vs. other) × 2 (congruency: congruent vs. incongruent)]. As in previous studies [44, 104], only matching trials and accurate RTs were analysed. Performance is expected to weaken in incongruent perspective trials because either the self-perspective is spontaneously computed in other-perspective trials and interferes in the judgement of what the avatar sees, causing an *egocentric* effect (or bias or intrusion), or because the avatar's perspective is spontaneously computed in self-perspective trials and interferes in judging what the patient him/herself sees, causing an *altercentric* effect (or bias or intrusion).

Patients next completed the personal network (PN) survey, using the concentric circles or bullseye map format [105] during a face-to-face interview, as described in detail elsewhere [106]. Participants were interviewed using a simple question: "Who are the people who support you in your daily life?". and were then asked to list those supporters (hereafter designated as alters), and to define the type of support received in four specific areas: finances, housing, activities, and health. Alters were then placed on Hogan's bullseye map, and the participant was asked to link those whom they believed to exchange information ("who shares information about you?") (SI Appendix, Fig. S1). We then computed indices describing the social structure of the respondent's personal network: size (number of alters), cohesion of the PN (transitivity), and availability of different social circles (modularity). The composition of the PN was also computed as the percentages of health professionals, family members, and friends. Details of the different indicators obtained with the sociogram are described in SI Appendix, Table S1.

Diet anamnesis

On Day 2 of alcohol withdrawal, a trained dietician interviewed all participants regarding three non-consecutive 24-h dietary recalls (related to the week before hospitalization: week 0), as previously described [48]. Energy and nutrient intakes were evaluated using the Nubel Pro program (Nubel asbl, Belgium) and the French food composition database (CIQUAL 2017). Dietary fiber (DF), including soluble fiber, insoluble fiber, fructans, fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS) were evaluated using a specific database from the FiberTAG project [107].

Blood parameters

Fasting blood samples were collected on day 2. Blood samples were centrifuged at 1000 g for 15 min at 4°C, and the plasma was frozen at -80 °C in a biobank. Plasma concentrations of growth factors (brain derived neurotrophic factor [BDNF]) and inflammatory markers (IL-18, MCP-1, IFN-γ, IL-8, IL-10, TNF-a, IL-6) were determined using the Meso Scale Discovery (MSD) U-PLEX assay (Rockville, MD, USA).

Statistical analysis

Based on the calculated β-diversity index, we segregated our population of sAUD patients into two groups: non-dysbiotic and dysbiotic. For this, we performed a principal coordinate analysis (PCoA) with the Bray Curtis index and then extracted the component scores for each individual (HS and AUD). We then identified the dysbiotic group among sAUD patients according to a deviance criterion at a threshold of 1.65 SD of the mean score of the first component from the HS group.

To identify more precisely the differences in gut microbiota composition between HS, non-dysbiotic, and dysbiotic AUD patients, we used linear discriminant analysis effect size (LEfSE) [108]. The selected genera were then compared between the three groups of subjects using Kruskal–Wallis tests followed by a Dunn's test. P-values of Kruskal–Wallis tests were adjusted to control for the false discovery rate for multiple testing according to the Benjamini and Hochberg procedure. In this study, the HS group was used exclusively as a reference for the dysbiotic group in AUD patients and for microbial comparisons.

To compare the non-dysbiotic and the dysbiotic groups on other dimensions, we used the Mann-Whitney U-test or T-test, according to the data distribution. We then estimated multivariate associations between psychological symptoms, biological outcomes, the measures used to describe the social network, and the dysbiosis (yes vs no). Finally, we applied a logistic regression model adjusted for age, gender and body mass index [BMI] (model 1), subsequently adding the nutritional intake (model 2), and family status and number of children (model 3). To accommodate the subjects' entire nutritional patterns for the adjustment, we used PCA to construct a summary variable with all the nutrients assessed using the 24 h recall.

For the metabolomic analysis, we conducted partial least square discriminant analysis (PLS-DA) and the Mann-Whitney test. Based on the variable importance in projection (VIP) scores of the PLS-DA (VIP>1.5) and the p-value of the Mann-Whitney tests ($p<0.05$), we selected the metabolites best discriminating the two groups of AUD patients. Partial correlations adjusted for age, gender, and BMI were performed to study the relationships between social functioning and gut microbiota composition and function.

Statistical analyses were performed using SAS version 9.4, R studio version 3.5.1 and Graphpad Prism 8.0. A p-value or q-value < 0.05 was considered statistically significant. For the correlations between metabolome and other parameters, a p-value <0.01 was considered statistically significant.

We used the STROBE reporting guideline to draft this manuscript, and the STROBE reporting checklist is included in supplemental material.

Patient and public involvement

We have collaborations with representatives of the local “Alcoholicus Anonymus” group based in St Luc academic hospital in Brussels. We present the clinical studies and get their feedback on the acceptability and the way to present it to enrol patients. After the completion of the study and publication of the results, a focus group is organized to disseminate the results. However, this patient ‘organization is not involved in the design of the study.

Declarations

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Data availability statement

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request. The accession number for the raw data generated with the 16S rRNA gene sequencing reported in this paper is BioProject PRJNA745947 (SRA) (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA745947/).

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Figures

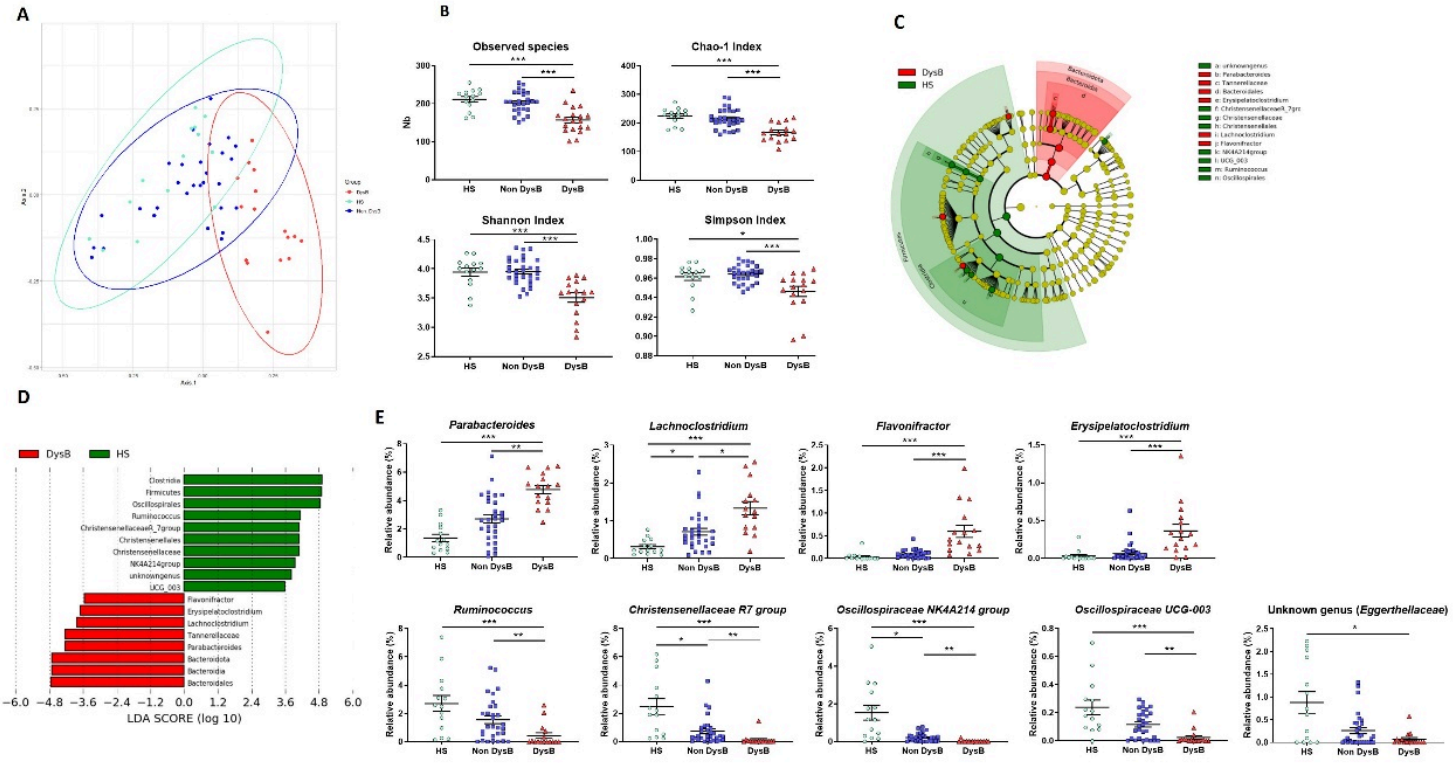

Figure 1

Gut microbiota composition in sAUD patients and in healthy subjects

A. Principal coordinate analysis (PCoA) with the Bray Curtis index for segregation of sAUD patients into dysbiotic (n = 16) and non-dysbiotic subgroups (n = 30). **B**. Indices of alpha-diversity. **C**. Cladogram of the LEfSe analysis showing differentially abundant bacterial taxa between dysbiotic sAUD patients and healthy subjects (HS). **D**. LDA scores obtained from the LEfSe analysis in dysbiotic sAUD patients and HS. **E**. Relative abundance of bacteria taxa in sAUD patients (dysbiotic (n = 16) and non-dysbiotics (n = 30)) and in HS (n = 14). For figures B and E, results are means ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001. HS: healthy subject; sAUD: severe alcohol use disorder.

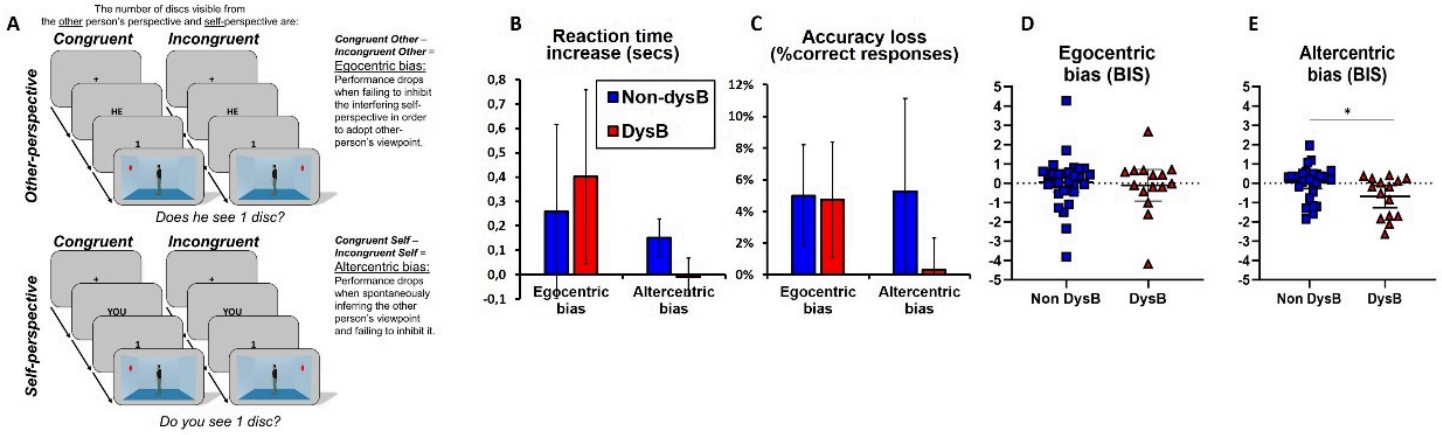

Figure 2

Visual perspective-taking task assessing social cognition in sAUD patients

A. Illustration of a trial from each of the four experimental conditions in the visual perspective-taking task. In the first trial, participants had to judge if the avatar in the room could see 1 disc. In the second trial, participants had to judge if they could see 1 disc. All conditions and the correct "yes" or "not" responses were equally distributed across 4 blocks of 52 trials, with random ordering. Each trial depicted 0 to 3 discs on the lateral walls, with response required withing 2 seconds . B-C. Increases in reaction time and drops in accuracy related to the egocentric and altercentric biases. D-E.Combination of reaction time and accuracy rates into the balanced integration score (BIS) related to the egocentric and altercentric biases. Results are means ± SD. Non-dysB: Non-dysbiotic; DysB: dysbiotic. * p < 0.05.

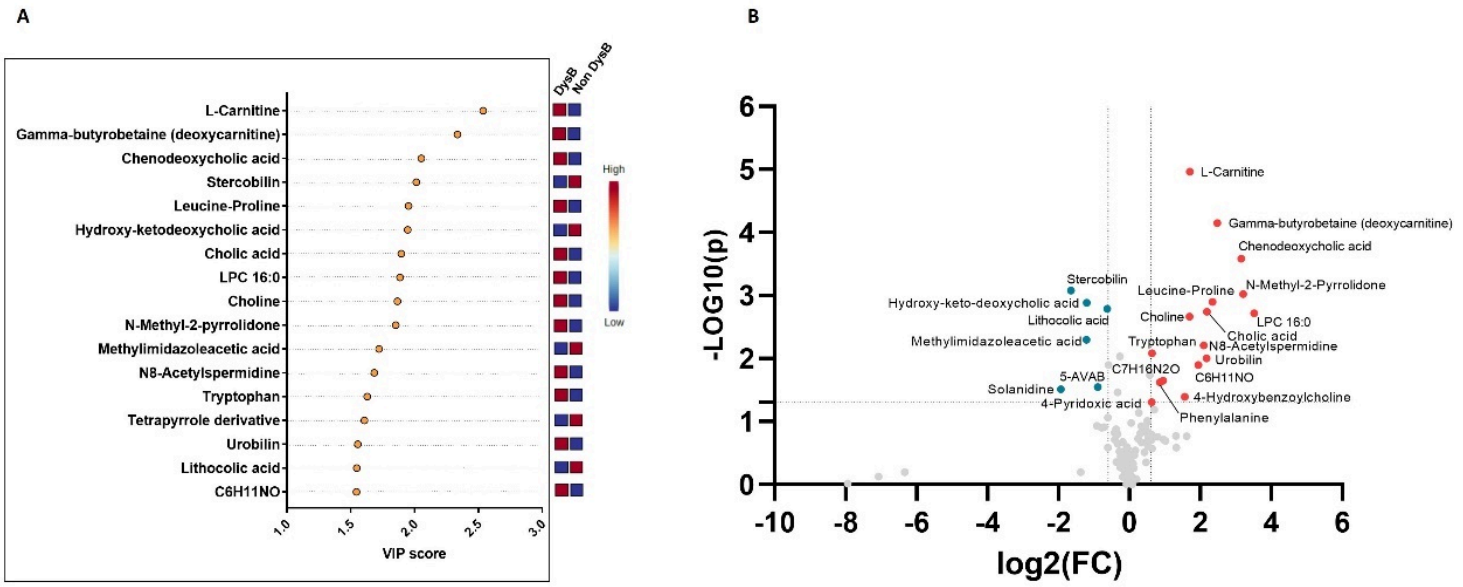

Figure 3

Faecal metabolome in sAUD patients

A. Variable importance in projection (VIP) score plot of the faecal metabolites that differed in dysbiotic and non-dysbiotic sAUD patients. **B** Volcano plot depicting the $\log_2$ fold change (FC) and the $\log_{10}$ p-value derived from Welch's t-test analysis for the contrast of dysbiotic and non-dysbiotic sAUD patients. Red dots represent metabolites that were increased in dysbiotic patients, while blue dots represent metabolites that were decreased in dysbiotic compared to non-dysbiotic patients.

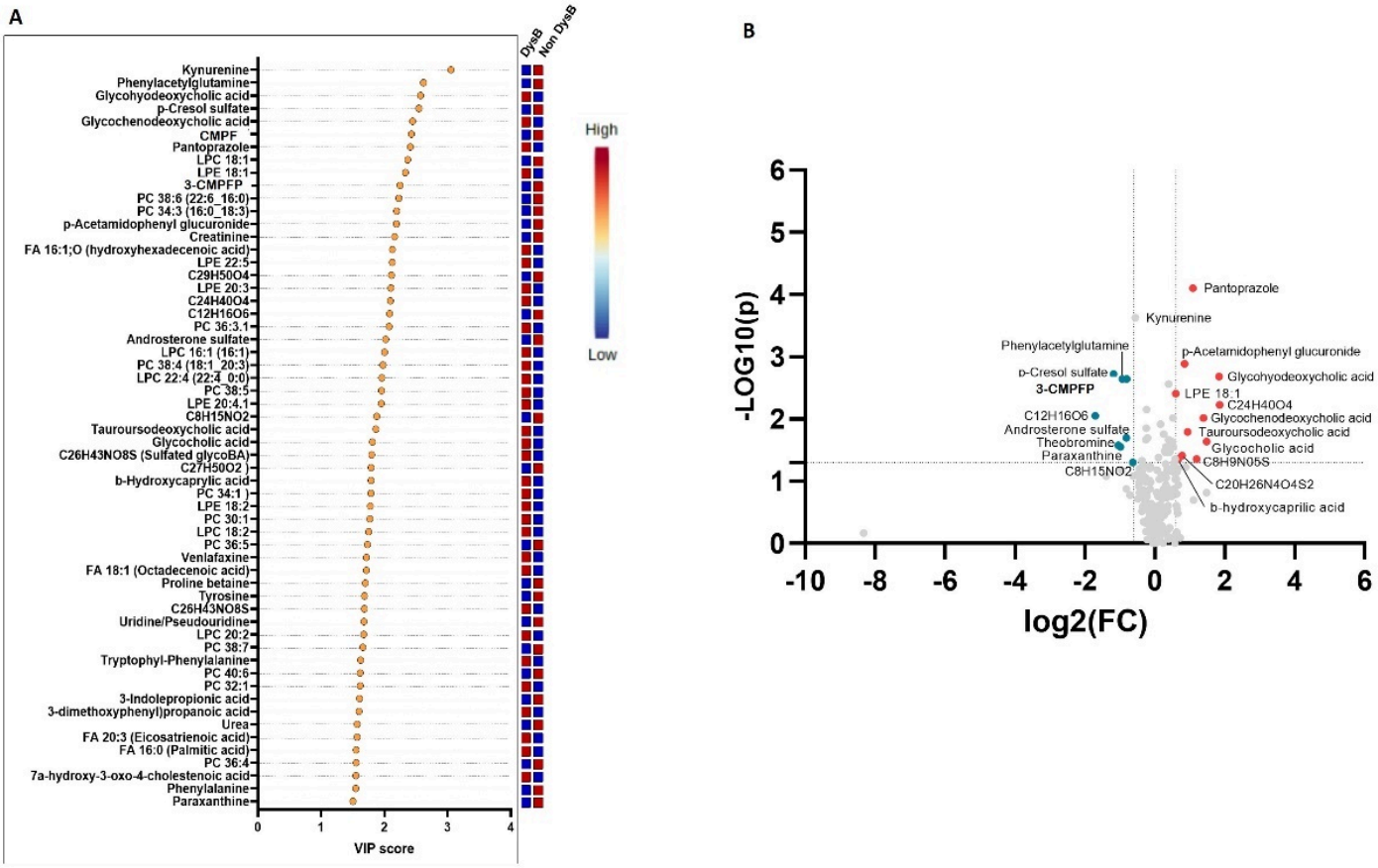

Figure 4

Blood metabolome in sAUD patients

A. Variable importance in projection (VIP) score plot of the metabolites that differed in dysbiotic and non-dysbiotic sAUD patients. **B** Volcano plot depicting the $\log_2$-fold change (FC) and the $-\log_{10}$ p-value derived from Welch's t-test analysis of the contrast between dysbiotic and non-dysbiotic sAUD patients. Red dots represent metabolites that were increased in dysbiotic patients while blue dots represent metabolites that were decreased in dysbiotic compared to non-dysbiotic patients.

Supplementary Files

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