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Faecal microbiota composition and impulsivity in a cohort of older adults with metabolic syndrome

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Impulsivity is an important determinant of human behaviour, affecting self-control, reasonable thinking and food choices. Recent evidence suggests a role for gut microbiota in human behaviour, but the relationship between gut microbiota and impulsive behaviours remains largely unexplored. To address this knowledge gap, the present study aims to explore the associations between faecal microbiota composition with trait and behavioural impulsivity, in a subcohort of the PREDIMED-Plus trial, including older adults presenting overweight/obesity. Fecal samples ($n = 231$) were profiled for their microbiota composition using 16 S rRNA amplicon sequencing and impulsivity was determined through four different assessments. Adherence to different dietary patterns was estimated through questionnaires. Beta diversity analyses showed a significant association with the Conner's Performance Test (CPT) in multivariate-adjusted models, and, in total, 13 bacterial genera associated with CPT. *Erysipelotrichaceae* UCG 003 showed the highest association with CPT and known butyrate producers such as *Butyricicoccus* spp., *Roseburia* spp., and *Eubacterium hallii* were among the identified bacteria. The bacteria *Lachnospiraceae* UCG 001, *Anaerostipes* and *Blautia* were associated with CPT and also the adherence to healthy and unhealthy plant-based diets. In addition, functional analysis showed a significant negative association between the CPT and the glucuronate and galacturonate metabolic pathways. From the other impulsivity assessments, two more associations were identified, for the genus *Phascolarctobacterium* with the Stroop test, and the genus *Lachnospiraceae* GAG 54 with the positive urgency subscore of UPPS-P Impulsive Behaviour Scale. Overall, our findings suggest potential links between the faecal microbiota composition and function with behavioural impulsive inattention as determined by the CPT.

Impulsivity is defined as "a predisposition toward rapid, unplanned reactions to internal or external stimuli without regard to the negative consequences of these reactions to the impulsive individuals or others"[1], and can be conceptualized as a psychological trait and behaviour. Trait impulsivity is the predisposition to act immediately and without forethought, whereas behavioural impulsivity is the observable action guided by a necessity to respond urgently to their emotions and without planning[2]. Excessive impulsivity has been associated

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with several psychiatric conditions[3], substance abuse[4], gambling disorders but also with metabolic disorders such as obesity[5], eating disorders[6,7], and cardiovascular problems[8].

Advancements in neuroscience have unveiled new insights for impulsive behaviours, identifying various factors involved with impulsivity such as gender influences[9] and neurotransmitters[10], and the role of genetics on impulsivity has been identified[11]. Although these findings improved the understanding of impulsive behaviour, still are not enough to define new treatments or prevention strategies. Hence it is necessary to seek other unknown factors that have been neglected and might provide clinicians/society with valuable insights to understand better impulsive behaviors.

Recently, there has been growing interest in the role of gut microbiota in human behavior[12]. Gut microbiota is the collective term that describes the microbial communities that colonize our gastrointestinal tract. The gut bacteria form a symbiotic relationship with the human body, aiding among others, in digestion, producing essential vitamins, and supporting the immune system[13]. Interestingly, gut microbes have been also linked with mental health and have been recognized as an important component of the gut-brain axis, the bidirectional communication between the central nervous system (the brain and spinal cord) and the enteric nervous system[14]. Alterations in gut microbiota composition have been reported in psychological conditions like depression[15,16], anxiety[17], and autism[18], further supporting gut microbiota as a potential regulator of behaviour through the gut-brain axis. Moreover, a systematic review showed interplays between gut microbiota composition and impulsivity-related behaviours and disorders[19], but the literature remains limited.

Diet is one of the main recognized factors shaping the gut microbiota composition and function[20], and certain dietary patterns, such as high-fat diets[21] or diets low in fiber[22], have been associated with alterations in the gut microbiota composition, which in turn may have implications for mental health and behaviour. Since impulsive individuals have been reported to follow unhealthy food choices[23] compared to non-impulsive individuals, it is possible due to their unhealthy dietary patterns their gut microbiota composition to be altered. Hence, the role of diet is also important to understand interactions between gut microbes and impulsive behaviours. Furthermore, a meta-analysis indicated that increased impulsivity was associated with elevated BMI values in a cardiometabolically healthy adult population[5]. In older adults with hypertension, a higher tendency towards impulsive decision-making was found to be associated with an increased likelihood of developing obesity[24]. Similar results were observed in both adult and older populations with and without obesity, indicating that increased reward sensitivity may be a contributing factor in the development of higher BMI during the aging process[25].

Given the limited knowledge of a potential relation between gut microbiota and impulsivity, here, in a cohort of Spanish older adults with overweight/obesity and metabolic syndrome, we study the association between gut microbiota and impulsivity using measurements of trait and behavioural impulsivity. Moreover, we examined whether adherence to different dietary patterns of the participants were associated with the potential relation between impulsivity and gut microbiota. We hypothesize that there is an association between gut microbiota composition and impulsivity and that adherence to different dietary patterns might influence this potential relationship.

Materials and methods

Study design

The PREDIMED-Plus study is an ongoing multicentre, randomized, parallel-group, primary prevention clinical trial conducted in Spain. The study design and methods have been published previously[26], and the study was registered at the International Standard Randomized Controlled Trial (ISRCT; http://www.isrctn.com/ISRCTN89898870) on the 24th of July 2014. The present study includes a subsample of PREDIMED-Plus participants for which data on impulsivity, faecal microbiota composition and dietary information was collected at baseline ($n=231$), from three recruiting centres in Spain (Supplementary Fig. 1). All participants provided written informed consent, and the study protocol and procedures were approved according to the ethical standards of the Declaration of Helsinki by the Research Ethics Committees from all the participating institutions: CEIC Hospital Universitari Sant Joan de Reus (13-7-25/7proj2), CEIm-PSMAR (2019/8612/I), CEIC Hospital Universitari de Bellvitge (PR240/13), Institutional Review Board of Valencia University (H1373255532771).

The present study showed a cross-sectional observational design using baseline data (before participants were randomized). Participants were men and women in the age group of 55–75 years and 60–75 years, respectively. Participants were free from cardiovascular disease at baseline but had obesity/overweight and met at least three criteria for metabolic syndrome. Faecal samples were collected at home using a sterilized airtight flask and delivered to the laboratory within 12 h of excretion under refrigerated conditions (i.e., to be kept frozen at -20°C at home until delivery to the laboratory).

Impulsivity assessment

Trait and behavioural impulsivity were assessed. Trait impulsivity was measured with the Impulsive Behaviour Scale (UPPS-P) questionnaire[2], validated in Spanish populations[27]. This questionnaire was composed of 59 items on a 4-point Likert scale, ranging from 1 "Agree strongly" to 5 "Disagree strongly". A total score of trait impulsivity was obtained by adding all the items, while impulsivity subfactors (lack of premeditation, lack of perseverance, sensation seeking, negative urgency, and positive urgency) added their respective items. Higher scores indicate higher trait impulsivity.

Behavioural impulsivity was assessed by the cognitive test Stroop Color Word Test (SCWT)[28], as well as by the computerized cognitive tests Conner's Performance Test Third Edition (CPT)[29] and Iowa Gambling Task (IGT)[30]. The SCWT consists of three tasks in which discrepancies between the written names of colours and the colours printed in these words were presented. An interference score was obtained, following specified methods[28], and higher scores indicate higher inhibitory control and consequently, lower behavioural impulsivity.

The CPT is a task in which participants have to press the crossbar in a computer when a specific stimulus appears. The commission score reports failed targets, and higher scores indicate higher behavioural impulsivity through lower attention ability[31]. The IGT is a task in which participants have to win as much money as they can by choosing from 4 desks, two being more advantageous and two being more disadvantageous options. Higher scores indicate better decision-making under risky decisions, and then lower behavioral impulsivity.

Covariates

Covariates were assessed at baseline through auto-reported questionnaires with the supervision of the PREDIMED-Plus staff. Sociodemographics: age (in years), sex, recruitment centre, and smoking status (never smoker, former smoker, current smoker); lifestyle: alcohol intake (g/day) measured using the validated 143 Frequency Food Questionnaire (FFQ) for the Spanish population[33], physical activity (MET min/week)[32] estimated by the validated Minnesota-REGICOR Short Physical Activity questionnaire, and body mass index (BMI) (kg/m^2); and medical history of disease: type 2 diabetes prevalence (no/yes). No antibiotic use was found for the current studied population.

Dietary assessment

The adherence to several dietary patterns was obtained using validated questionnaires or estimating the total score of the dietary patterns assessed using the 143-Food Frequency Questionnaire[33]. The dietary patterns evaluated are: (1) adherence to Mediterranean diet[34] and energy-reduced Mediterranean diet[35] determined based on a Mediterranean diet Adherence Screener (MEDAS) score, ranging from 0 to 14 points, or based on an energy-reduced Mediterranean diet (erMedDiet) score, ranging from 0 to 17 points, respectively; (2) adherence to a healthy and unhealthy plant-based diet[36], both ranging from 18 to 90 points; (3) adherence to the Western dietary style[23], with a possible score ranging from 12 to 60 points; and (4) adherence to Mediterranean-DASH diet Intervention for Neurodegenerative Delay (MIND), a diet tailored to protect against cognitive decline, with scores ranging from 0 to 15 points[37]. For all aforementioned dietary patterns evaluated, higher scores indicate higher adherence to their respective dietary pattern.

Microbiota composition analyses

Total DNA was isolated from the faecal samples using the QIAmp PowerFecal DNA kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. To profile the microbiota composition of the participants, the V4 region (515 F-806R) of the prokaryotic 16 S ribosomal RNA (rRNA) gene was amplified in triplicate PCR reactions using barcoded primers as described previously[38]. Artificial mock communities of known composition were included in each library as positive controls, and a subset of faecal samples were sequenced in duplicates as technical controls. Finally, to control for potential contaminant sequences, negative control samples were included in each library, using as DNA template, nuclease-free water, or material from DNA extraction blanks.

Raw sequence data were processed using the NG-Tax pipeline[39], with a read length of 100nt. Paired-end libraries were demultiplexed and only read pairs with perfectly matching barcodes were used for downstream steps. Amplicon sequence variants (ASV), were determined with the default settings and taxonomy was assigned to each ASV, using the USEARCH algorithm[40] and the Silva database (v138.1)[41].

Data analysis

To represent the general characteristics of the population we used the median and interquartile range for continuous variables, and percentages for categorical variables.

Alpha diversity indexes, Shannon and Simpson and observed genera were calculated using the publicly available package microbiome in R. For beta diversity analyses, Aitchison distances were calculated using the centered-log ratio (CLR) transformed data, using the package MicroViz[42]. Univariate analyses were conducted testing each variable individually while multivariate analyses were adjusted for age (in years), sex, body mass index (BMI) (kg/m^2), type 2 diabetes prevalence (no/yes), and recruitment centre. The variation explained by the impulsivity-related variables on the gut microbiota composition was calculated with a Permutational multivariate analysis of variance (PERMANOVA) test from the Vegan package in R, adjusting by age (in years), sex, BMI (kg/m^2), type 2 diabetes prevalence (no/yes), and recruitment centre.

To test the association of impulsivity-related scores (SCWT, IGT, CPT, and UPPS-P total and subfactor scores) with individual taxa the R package Linear models for differential abundance analysis of microbiome compositional data (LinDa)[43] was used, adjusting by age, sex, BMI, presence of type 2 diabetes (no/yes), and recruitment centre. The recruitment centre was included in the models as a random effect. A threshold of 25% prevalence (Supplementary Fig. 2) was applied for this analysis. FDR-corrected p values < 0.15 were considered to be statistically significant.

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2)[44] was used to perform analysis of the functional potential of the microbiome. Demultiplexed sequences were parsed and used as input for PICRUSt2 to generate a table of inferred per-sample MetaCyc pathways, and counts data was used as input in the LinDa package using the same settings with the compositional data.

Results

Baseline characteristics

A total of 231 faecal samples were available from the participants of the PREDIMED-Plus clinical trial, with data on impulsivity and dietary patterns. The characteristics of the study population are described in Table 1. Our cohort was balanced regarding sex, with females being 51%, with a median age of 65 years and a median BMI of 32 (kg/m^2). Subjects with diagnosed type-2 diabetes represented 17% of our study population. Data on dietary

Variable	*N*	Median (IQR) or *n* (%)
Age (years)	231	65 (62, 69)
Sex	231	
Female		117 (51%)
Male		114 (49%)
Civil status	231	
Married		172 (74%)
Single, divorced, or separated		35 (15%)
Widower		24 (10%)
Smoking status	231	
Never smoker		30 (13%)
Former smoker		76 (33%)
Smoker		125 (54%)
Educational level	231	
Primary school or less		123 (53%)
High school		77 (33%)
College		31 (13%)
Type 2 diabetes prevalence	231	41 (18%)
Obesity prevalence (BMI > 30)	231	176 (76%)
Body mass index (kg/m^2)	231	32.3 (30.1, 35.6)

Table 1. Characteristics of the study population.

Measurements	Univariate		Multivariate	
	R^2	p-value	R^2	p-value
SCWT	0.01404	**0.001*****	0.0039	0.538
CPT	0.00827	**0.031***	0.0077	**0.036***
IGT	0.00391	0.886	0.0047	0.603
UPPS-P total	0.00603	0.618	0.0034	0.855
UPPS-P negative urgency	0.00365	0.883	0.0030	0.971
UPPS-P positive urgency	0.00667	0.098#	0.0051	0.335
UPPS-P premeditation	0.0047	0.604	0.0049	0.453
UPPS-P perseverance	0.00351	0.887	0.0043	0.639
UPPS-P sensation seeking	0.00628	0.129	0.0040	0.683

Table 2. Results from the univariate and multivariate PERMANOVA analyses for each impulsivity measurement. Multivariate analyses were adjusted for diabetes prevalence, BMI, recruitment centre, sex and age.

patterns (Supplementary Table 1) was available from all the participants ($n = 231$). From the impulsivity data, only data from SCWT was collected from all the participants (Supplementary Table 2).

Association of alpha and beta diversity with impulsivity

First, the associations of alpha and beta diversity were tested with the impulsivity measurements. Alpha diversity -within the sample diversity- indexes, as determined by Shannon, observed genera and Simpson indexes, were not significantly associated with trait or behavioural impulsivity (Supplementary Tables 3–5).

Results from univariate beta diversity –between samples diversity- analyses showed significant associations between the bacterial composition with CPT and SCWT scores, while no associations were detected for the rest of the tests (Table 2). Results from multivariate models showed that only CPT remained significantly associated with the overall microbiota composition (p-value = 0.03, $R^2 = 0.007$). No association was shown in the case of the SCWT (Table 2). UPPS-P and its subfactors, along with the IGT, did not explain any part of the variation either in the univariate or the multivariate analyses (Table 2). From the rest of the covariates, age, presence of diabetes, and recruitment centre explained significantly part of the variation (Supplementary Table 6).

SCWT, Stroop Color Word Test; CPT, Conner's Performance Test; IGT, Iowa Gambling Test; UPPS-P, Urgency, Perseverance, Premeditation, Sensation Seeking, Positive Urgency Impulsive Behavior Scale. Significance levels are indicated by asterisks: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ and # indicates trend $p < 0.01$. All tests were performed with 999 permutations.

In total, twelve bacterial genera were identified to be significantly associated with CPT (Fig. 1A). The genera *Erysipelotrichaceae* UCG 003, *Butyricicoccus* and *Lachnospiraceae UCG 001*, *Blautia*, *Lachnospiraceae* NK4A136

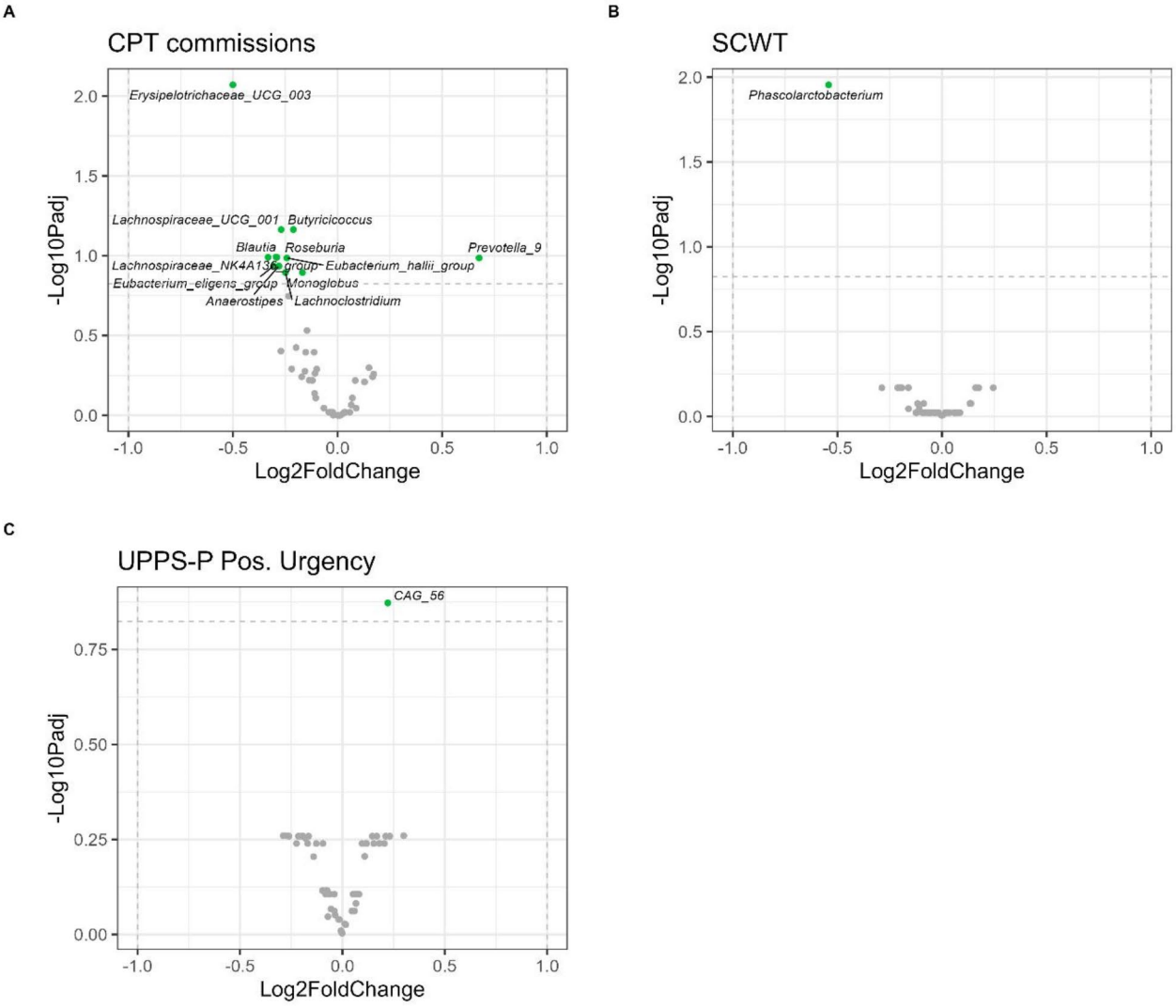

Fig. 1. Bacterial genera significantly associated with Conner's Performance Test (CPT) commissions as determined with linear mixed models, adjusting for age (in years), sex, BMI (kg/m^2), type 2 diabetes prevalence (no/yes), and recruitment centre as a random effect. The degree and direction of association of a specific taxon is indicated through the log2FoldChange, which represents the bias-corrected coefficients.

group, *Roseburia*, *Eubacterium hallii* group, *Anaerostipes*, *Eubacterium eligens*, *Lachnoclostridium*, *Monoglobus* were negatively associated with the CPT (Fig. 1A) while only the genus *Prevotella 9* was identified to be positively associated with the CPT (Fig. 1A). Moreover, the genus *Phascolarcobacterium* was negatively associated with the SCWT (Fig. 1B) and the uncultured taxa *Lachnospiraceae* CAG 56 was positively associated with the positive urgency facet from the UPPS-P questionnaire. No significant associations were shown between the bacterial genera tested with the IGT and any other facet from the UPPS-P questionnaire (Supplementary File 1).

Associations between bacterial taxa and adherence to dietary patterns

Next, the associations between bacteria and several dietary patterns were examined. Results showed that adherence to a healthy plant-based diet was associated with the microbiota composition and in total six genera, *Anaerostipes*, *Ruminococcus torques*, *Blautia* and *Ruminococcus gauvreauii*, were negatively associated with hPBD (Fig. 2A). The bacteria *Christensenellaceae* R7 group and *Oscillospiraceae* UCG 005 were positively associated with a hPBD. In addition a *Lachnospiraceae* UCG 001 was associated with unhPBD (Fig. 2B) and *Ruminococcus torques* was also negatively associated with adherence to the Med (Fig. 2C). Interestingly, the genera *Blautia*, *Anaerostipes and Lachnospiraceae UCG 001* were also detected to be associated with the CPT in the previous analysis (Fig. 1B). No associations were detected between gut microbiota composition and the other three dietary patterns (Supplementary File 2).

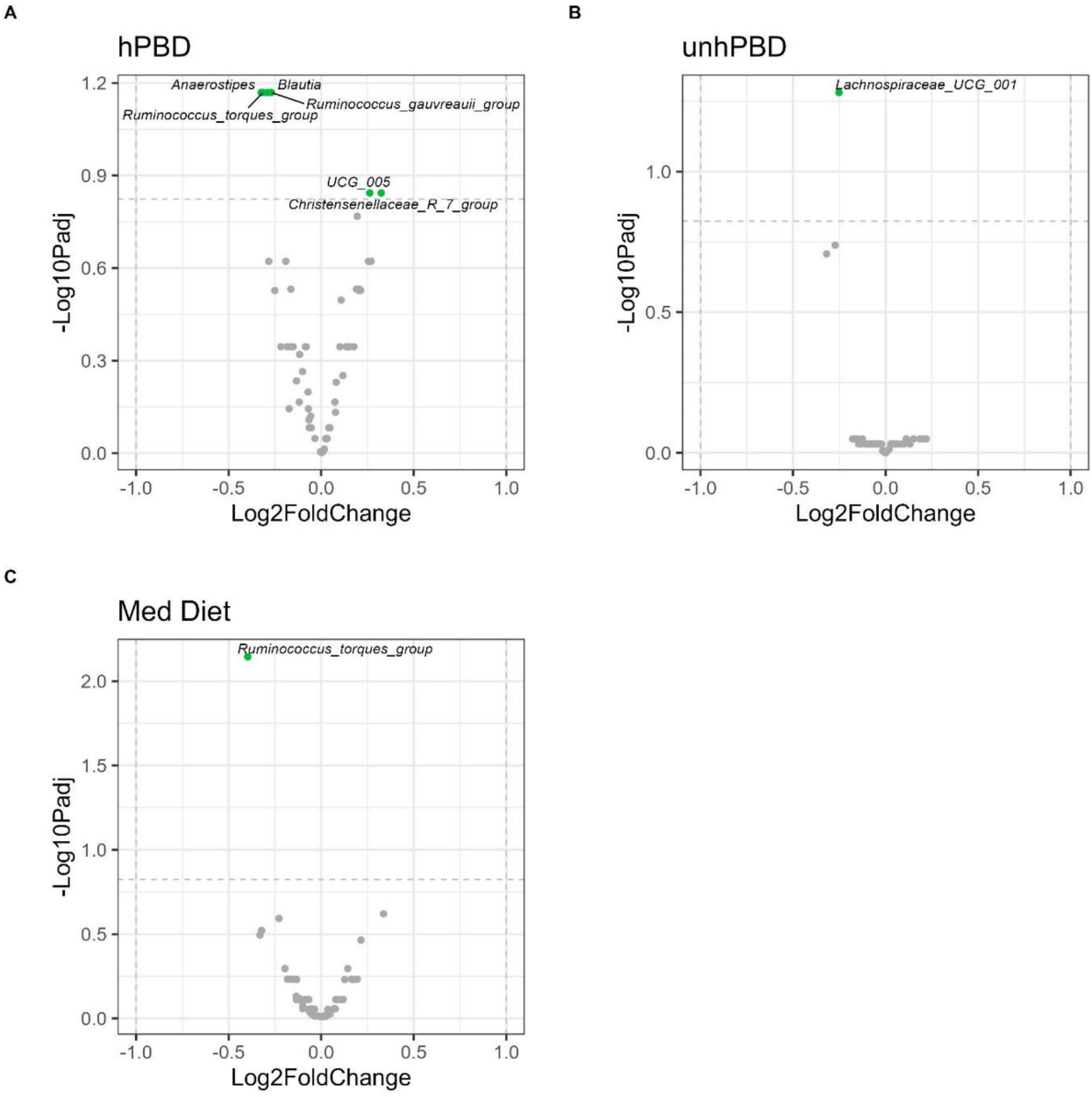

Fig. 2. Associations between bacterial genera with (A) healthy plant-based diet index (hBPD) and (B) unhealthy plant-based diet index (unhPBD), and (C) Mediterranean diet, as determined with linear mixed models, adjusting for age (in years), sex, BMI (kg/m^2), type 2 diabetes prevalence (no/yes), and recruitment centre as s random effect. The degree and direction of association in the abundance of a specific taxon is indicated through the log2FoldChange, which represents the bias-corrected coefficients.

Predicted microbial metabolic pathways associated with impulsivity

Except from compositional analyses, associations between metabolic pathways and impulsivity assessments were conducted. Results showed associations between CPT scores and the pathways for glucuronate and galacturonate metabolism (Fig. 3). Associations between metabolic pathways and the other impulsivity assessments did not produce any significant results (Supplementary File 3).

Discussion

In the present study, we assessed cross-sectionally the relation between measurements of trait and behavioural impulsivity and gut microbiota composition, in a cohort study of older adults presenting overweight/obesity and metabolic syndrome. Our results show that the cognitive inattention capacity, reflecting higher behavioural impulsivity and measured by the CPT score, was the strongest factor related to gut microbiota composition and

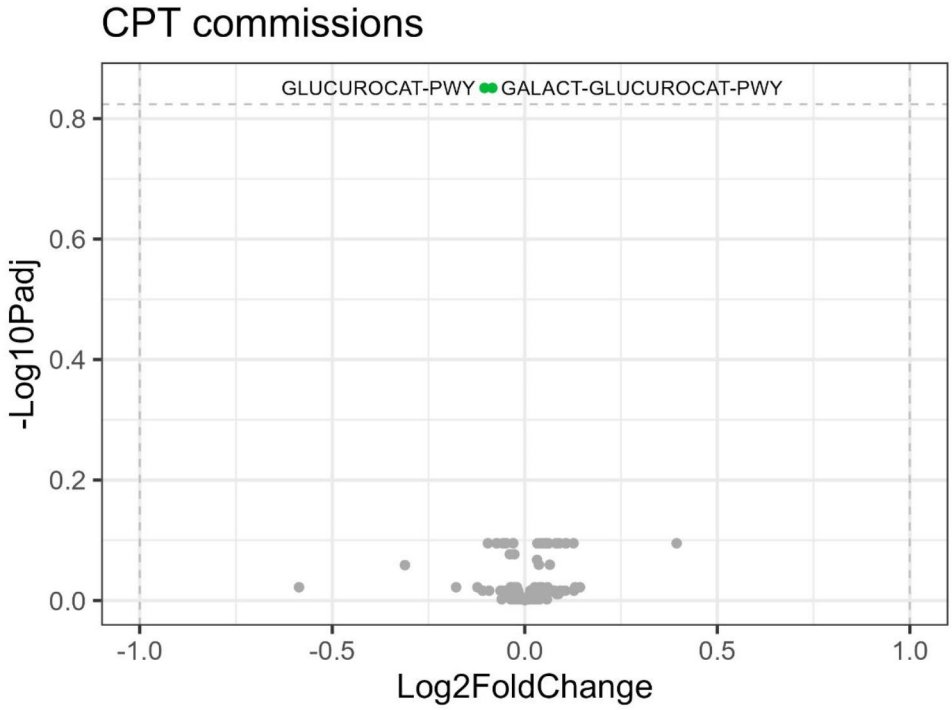

Fig. 3. Associations between microbial pathway abundances with Conner's Performance Test (CPT) commissions, as determined with linear mixed models, adjusting for age (in years), sex, BMI (kg/m^2), type 2 diabetes prevalence (no/yes), and recruitment centre as s random effect. The degree and direction of association in the abundance of a specific microbial pathway is indicated through the log2FoldChange, which represents the bias-corrected coefficients.

function. Moreover, SCWT was associated with the genus *Phascolarctobacterium* and the positive urgency facet from the UPPS-P with the bacterium *Lachnospiraceae* CAG 56. Furthermore, no associations were identified between the other four facets of the UPPS-P or the IGT with the gut microbiota composition. Overall, our results suggest a consistent association between behavioural impulsive inattention, measured by CPT, and the microbiota composition in this specific cohort.

The analysis of the CPT commissions assesses the individual's impulsivity, as higher commission errors indicate a difficulty in inhibiting impulsive responses exhibiting a lack of sustained attention capacities[31]. Our results indicate an inverse relation between CPT and the gut bacteria belonging to the genus *Erysipelotrichaceae*, *Butyricicoccus*, and *Lachnospiraceae*. The identified bacteria have been associated previously with the gut-brain axis. In a study comparing neurotypical and autistic young males, the presence of *Erysipelotrichaceae* UCG 003 was found to be decreased in the autistic participants[45]. Moreover, in animal models, phenotypes of both impulsive and non-impulsive rats showed correlations between the *Lachnospiraceae* family and behavioural measures of impulsivity[46]. Regarding *Butyricicoccus* spp., decreased abundances of the bacterium were detected in mice models after stress exposure[47,48]. Overall, the identified bacteria to be negatively associated with CPT might play a prominent role in the communication between the GBA and further research is necessary to validate and elucidate their role.

Members of *Butyricicoccus* spp. are known for their ability to produce butyrate[49], an important molecule for gut health that has been also associated with the gut-brain axis[50], for example, it has been found to stimulate the production of serotonin in the human gut[51]. Recently *Butyricicoccus* spp.has been reported as a key bacteria in venlafaxine treatment for depression, and its abundances were associated with serotonin levels in the brain[52]. Serotonin has been identified as an important hormone in impulsivity[53,54]. Interestingly, in a study of 1,180 children, it was found that serotonergic mechanisms have implications with behavioural impulsivity assessed by the CPT measurement[54]. Therefore, the relationships found in our study between behavioural inattention impulsivity and the specified gut microbiota genera might be related to butyrate production and its interplay with serotonin[54], although further research is warranted to validate this statement and other mechanisms could be also present.

Functional analysis showed associations between predicted bacterial metabolic pathways and impulsivity. Our results showed that participants with CPT presented lower abundances of glucuronidation and galacturonate pathways. Glucuronidation is an important metabolic pathway for the detoxification process of the human body[55]. Glucuronidation primarily takes place in the liver, where endogenous and exogenous compounds

are conjugated with a glucuronate that leads to their inactivation and excretion from the human body. In the gastrointestinal tract, the bacteria enzymes glucuronidases can metabolise glucoronate and unconjugate these substances. Notably, hormones, neurotransmitters, bile acids, and fatty acids are among the substances subjected to glucuronidation[56]. Of particular significance are neuroactive compounds like dopamine, norepinephrine, and serotonin[56]. Those neuroactive compounds are present in significant amounts in the gastrointestinal tract and play important roles such as gut motility and water absorption. Studies in germ-free mice showed that microbial glucuronidases are primarily responsible for dopamine and norepinephrine glucuronide hydrolysis[57]. Similarly, serotonin is also subjected to glucuronidation, and studies in germ-free mice showed that in the absence of bacterial glucuronidases, serotonin concentrations were depleted in mice faeces[58]. Consistently, selective inhibition of gut microbial β-glucuronidases in mice lead to a decreased abundance of serotonin in the colonic lumen of mice[59]. Our results suggest that a reduced abundance of the glucuronidation metabolic pathway is associated with higher impulsivity. Interestingly, higher impulsivity has been associated with lower serotonin levels, and based on our results this relationship might be mediated by microbial glucuronidation, however, actual quantification of serotonin or other compounds is essential to support such notion. To the best of our knowledge, this is the first study that reports such an association between impulsivity and microbial glucuronidation in humans, and it is important to be verified in future studies along with the levels of intestinal neurotransmitters and hormones.

The SCWT test is a widely used neuropsychological test that measures cognitive flexibility and inhibitory control[28]. Associations with the Stroop test and bacterial abundances have been previously reported, however, the identified genera are different[60], regarding the present work. Our results showed a negative association between the bacterial genus *Phascolarcobacterium* and SCWT. Higher abundances of *Phascolarctobacterium* were associated with lower SCWT scores, hence the presence of this bacterium is not considered beneficial. *Phascolarctobacterium faecium* has been reported to be prevalent in the human gut[61] and previous studies linked its abundance with mental health. An older study reported *Phascolarctobacterium* to be positively correlated with positive mood[62], but the rest of the studies reported negative associations with *Phascolarctobacterium* and measurements of human behaviour in line with our results. For example, enrichments in the genus *Phascolarctobacterium* were reported in individuals with schizophrenia[63], major depressive disorder[64], Alzheimer's disease[65], increased externalizing behaviour in adolescents, a characteristic of behavioural problems[66], and other neurological disorders[67,68]. Collectively, our results are in line with published literature to the genus *Phascolarcobacterium* and highlight the importance of further understanding the functional aspects of this bacterium and how it can potentially influence human behaviour.

Diet, an important regulator of the composition and function of intestinal microbiota, was also linked with impulsivity[69]. In the present study, we examined whether adherence to different dietary patterns was associated with the composition of the gut microbiota. Our results showed that adherence to the healthy plant-based diet was negatively associated with the abundance of several gut bacteria such as the genera *Blautia* and *Anaerostipes* even though these bacteria were negatively associated with CPT. Interestingly, the relative abundances of the uncultured genus *Lachnospiraceae UCG-001* were negatively associated with adherence to an unhPBD, and the same genus was also negatively associated with CPT, suggesting a potential interplay between diet, impulsivity and *Lachnospiraceae UCG-001*. Previous studies in humans implicated *Lachnospiraceae UCG-001* with reduced abundances reported in rodents with anhedonia[70] and in humans, where it was found to be decreased in depression in a cohort of Dutch population[16]. Moreover, a positive association between the potentially probiotic bacterium *Christensellaceae* spp. and healthy plant based diet, however no associations were detected with the impulsivity assessments. Overall, our results emphasise the effect of dietary on the gut microbiota and shows potential associations with bacteria that were identified to be associated with impulsivity.

Limitations of the present work include the specific type of population studied which is not representative of the general population but rather to this specific study group, older adults with overweight/obesity and metabolic syndrome. Moreover, we use 16 S rRNA amplicon sequencing which limits our analysis to genus-level identification of bacteria, the most refined level of analysis for this kind of technique. Information on metabolites of the community is also missing in our data, and it is an important component that can shed light on the relationship between gut microbiota and human behaviour. Some strengths should be also stated. The analyses performed included one of the largest populations studying associations between impulsivity and gut microbiota. Furthermore, impulsivity was assessed broadly, including both trait and behavioural impulsivity measurements and then, covering the comprehensive nature of the impulsivity construct. Finally, a potential and novel exploratory analysis between impulsivity and intestinal microbiomes through adherence to multiple dietary patterns was evaluated.

To conclude, the results of the present observational study conducted within the frame of the PREDIMED-Plus study identified a link between gut microbiota composition and behavioural impulsivity, as measured by CPT. Higher levels of CPT were associated with lower abundances of specific bacterial genera that have been associated with the gut-brain axis, and lower abundances of metabolic pathways related to glucuronidation. Moreover, the genus *Lachnospiraceae* UCG 001, was also related to adherence to an unhPBD suggesting a potential role for diet. Overall, our results pave the way to explore the relationship between gut microbiota and impulsivity.

Data availability

Due to signed consent agreements regarding data sharing, there are restrictions on data availability for the PREDIMED-Plus trial. These only allow access to external researchers for studies following the project purposes. Requestors wishing to access the PREDIMED-Plus trial data used in this study can make a request to the PREDIMED-Plus trial Steering Committee chair: predimed_plus_scommittee@googlegroups.comjordi.salas@urv.cat. The request will then be passed to members of the PREDIMED-Plus Steering Committee for deliberation.

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Author contributions

PK, CGM, JSS and CB, designed the current study; PK, JM and CGM provided essential materials; PK analysed the data; PK and CGM: wrote the draft manuscript; PK: wrote and edited the final manuscript; CB and JSS supervision; all authors participated in data interpretation, provided critical review and commentary on the draft of the manuscript, and read and approved the final manuscript.

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Declarations

Competing interests

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Additional information

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