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Original article

An association between chronic widespread pain and the gut microbiome

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Abstract

Objectives. Chronic widespread musculoskeletal pain (CWP) is a characteristic symptom of fibromyalgia, which has been shown to be associated with an altered gut microbiome. Microbiome studies to date have not examined the milder CWP phenotype specifically nor have they explored the role of raised BMI. The aim of this study was to investigate whether the microbiome is abnormal in CWP.

Methods. CWP was assessed using a standardized screening questionnaire in female volunteers from the TwinsUK cohort including 113 CWP cases and 1623 controls. The stool microbiome was characterized using 16S rRNA amplicon sequencing and amplicon sequence variants, and associations with CWP examined using linear mixed-effects models adjusting for BMI, age, diet, family relatedness and technical factors.

Results. Alpha diversity was significantly lower in CWP cases than controls (Mann–Whitney test, *P*-values 2.3e-04 and 1.2e-02, for Shannon and Simpson indices respectively). The species *Coprococcus comes* was significantly depleted in CWP cases ($P_{adj} = 3.04e\text{-}03$). A genome-wide association study (GWAS) performed for *C. comes* in TwinsUK followed by meta-analysis with three Dutch cohorts (total $n = 3521$) resulted in nine suggestive regions, with the most convincing on chromosome 4 near the *TRAM1L1* gene (rs76957229, $P = 7.4e\text{-}8$). A Mendelian randomization study based on the results of the GWAS did not support a causal role for *C. comes* on the development of CWP.

Conclusions. We have demonstrated reduced diversity in the microbiome in CWP, indicating an involvement of the gut microbiota in CWP; prospectively the microbiome may offer therapeutic opportunities for this condition.

Key words: body mass index, chronic widespread pain, gut microbiome, healthy eating index

CLINICAL SCIENCE

Rheumatology key messages

- Chronic widespread pain (CWP) is characterized by decreased alpha diversity of the gut microbiome.
- *Coprococcus comes* is the most significantly reduced in CWP.
- No evidence for causal relationship between the gut microbiome and CWP is seen.

Introduction

Chronic widespread musculoskeletal pain (CWP) is a common disorder, affecting some 5–15% of the general population, and presents a sizeable economic burden in terms of disability, work absence and healthcare costs [1]. Risk factors for CWP include increasing age, female sex, increasing BMI and lower socioeconomic status [2, 3]. CWP has a complex aetiology, and forms part of the fibromyalgia syndrome that includes fatigue and sleep disturbance in addition to CWP. CWP also co-occurs with other functional somatic syndromes such as chronic fatigue syndrome (CFS), irritable bowel syndrome, and mood disorders such as depression and anxiety [4].

Previous work has shown that genetic and environmental influences are important in the development of CWP [5]. Genetic factors that have shown associations with CWP in various studies have been summarized in

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several reviews [6, 7]. Overall results indicate that the nociceptive signalling system is rewired, and possibly disrupted by an excessive stress response [7]. We have shown that CWP shares common genetic determinants with low back pain [8] and with frailty [9]. A genome-wide study of DNA methylation also showed that epigenetic modification of neurological pathways is implicated in CWP [10] .

Overweight, obesity and raised BMI are the well-established risk factors for CWP [11, 12]. Longitudinal studies have provided evidence that BMI is raised prior to the onset of pain, rather than being a result of pain-induced immobility [13, 14]. We have shown that the influence of BMI on CWP risk is mediated through increased fat mass [15].

The human gut microbiome plays an important role in human health and disease. The composition of the human gut microbiome is affected by host genetics as well as diet, age, sex, weight, medication use and other environmental factors [16, 17], and the gut microbiome can in turn affect host metabolism [18]. Various studies have shown that a certain proportion of the gut microbiome is heritable [19, 20]; however, a recent study concluded that environmental factors have a greater effect than host genetics on the gut microbiome [21] .

Obesity has been associated with changes in the gut microbiome both in human and in mouse studies [22, 23], and there is considerable interest in modulating the gut microbiota for the prevention of obesity-related disease [24]. Short-chain fatty acids such as acetate, propionate and butyrate produced by the gut microbiome act as signalling molecules [25–27]. Acetate, in particular, influences insulin secretion and may promote obesity [28].

Thus, hypothetically, there is a link between gut microbiome, obesity and CWP. Accordingly, the aim of this study was to investigate the association of the gut microbiome with CWP in a large population sample, taking into account diet and BMI, and to address causality in the relationship using Mendelian randomization.

Methods

Study sample

Participants were individuals from the UK Adult Twin Registry (TwinsUK) [29]. The TwinsUK registry comprises volunteers from the general population recruited through national media campaigns. The cohort is predominantly female (83%), middle-aged, mainly of Northern European descent, and nearly equal in numbers of monozygotic and dizygotic same-sex twins. Participants have been characterized for a variety of clinical and behavioural traits including CWP. Twins from this registry have been shown to be similar to age-matched singletons for a range of health and lifestyle factors [30]. Twins have not been specifically recruited for the purpose of the current study; instead, participants were selected based on the availability of gut microbiome and phenotype of CWP (see below) data with the TwinsUK database. BMI was calculated from height and weight measurements taken during clinical visits. Zygosity was ascertained with the use of a questionnaire and confirmed by genotyping. All subjects provided written informed consent in accordance with the St Thomas' Hospital Research Ethics Committee, and were unaware of the precise hypotheses being tested. Ethical approval for microbiome studies within TwinsUK was provided by the NRES Committee London—Westminster (REC Reference No.: EC04/015).

CWP phenotype

A modified version of the London Fibromyalgia Epidemiology Study Screening Questionnaire (LFESSQ) [31] was used to screen for the presence of CWP. Volunteers from TwinsUK first completed a web-based or postal screening questionnaire. Those who answered yes to the question 'In the past 3 months, have you had pain in your muscles, bones and joints lasting at least 1 week?' were sent a more detailed paper questionnaire, with questions adapted from the LFESSQ. The questionnaire contained questions about musculoskeletal pain lasting >1 week in the right or left shoulders, arms or hands, in the right or left legs or feet, and in the neck, chest or back. Participants were also asked whether the pain had lasted >3 months. Each twin completed the questionnaires without reference to the co-twin. CWP cases were defined as those reporting pain on both sides of the body, above and below the waist, and in the axial skeleton, present for at least 3 months. Controls were individuals reporting pain but not meeting the criteria for CWP. Co-twins of the CWP cases were excluded from the controls. This was done to reduce the possible impact of shared genetic factors underlying both CWP and microbiome. We also carried out a discordant twin analysis, in which the healthy co-twins of CWP cases were compared. This analysis did not produce significant results likely because the sample size was too small, so the results are not reported here.

Dietary intake

The Healthy Eating Index (HEI) is a measure of diet quality developed by the United States Department of Agriculture (USDA) (https://www.fns.usda.gov/resource/healthy-eating-index-hei) to assess the extent to which food intake aligns with key dietary recommendations for Americans. The HEI uses a scoring system from 0 to 100, with a higher score indicating greater alignment with the dietary recommendations. Food intake for the TwinsUK cohort was determined from validated Food Frequency Questionnaires (FFQ) converted to HEI-2010 scores [32].

Faecal microbiome sample collection and 16S rRNA sequencing

The collection and processing of the faecal samples have been described previously [19]. Briefly, the

Table 1 Summary statistics of TwinsUK sample

Covariates	Controls ($n = 1623$)	CWP ($n = 113$)	Student's t-test P-value
Age, years	61.4 (11.3)	63.5 (9.3)	0.028
BMI, kg/m^2	25.7 (4.7)	27.9 (5.7)	6.1e-05
Healthy eating index	60.8 (10.0)	57.8 (9.6)	1.6e-03

Mean (S.D.) values are shown. CWP: chronic widespread pain.

participants collected faecal samples at home and the samples were either posted to King's College London on ice or refrigerated for up to 2 days prior to the twin pair annual clinical visit. Samples were stored at –80°C before being shipped on dry ice to Cornell University, and stored at –80°C until they were processed.

Genomic DNA was extracted from the faecal samples, and the V4 section of the 16S rRNA genes was amplified by PCR using the 515F and 806R primers [33]. The resulting amplicons were multiplexed using the Caporaso 12-base Golay barcodes [34] and sequenced as 250 bp paired-end reads using the Illumina MiSeq platform. Sequencing data for the microbiome samples used in this study are a subset of a larger study, and have been deposited in the European Nucleotide Archive (ENA) as part of previously published work [19, 35] under accession numbers ERP006339, ERP006342 and ERP015317.

Sequencing reads were demultiplexed and split per sample using QIIME 1.9.0 [36] to produce fastq sequencing data files. Amplicon sequence variants (ASVs) were obtained by denoising the raw sequencing data (fastq files) using the R/Bioconductor package DADA2 [37], which was applied separately per sequencing run ($n = 35$). The data were merged across sequencing runs, and chimeras removed, before finally assigning taxonomy of ASVs using the phyloseq R/Bioconductor package [38] and the SILVA rRNA gene database version 1.3.9 [39]. Final quality control steps were undertaken: sequences that were unassigned at the kingdom/phylum level or that were assigned to Eukaryota were removed. Samples with a sequencing depth of $<$10 000 were removed from further analysis.

Statistical analysis

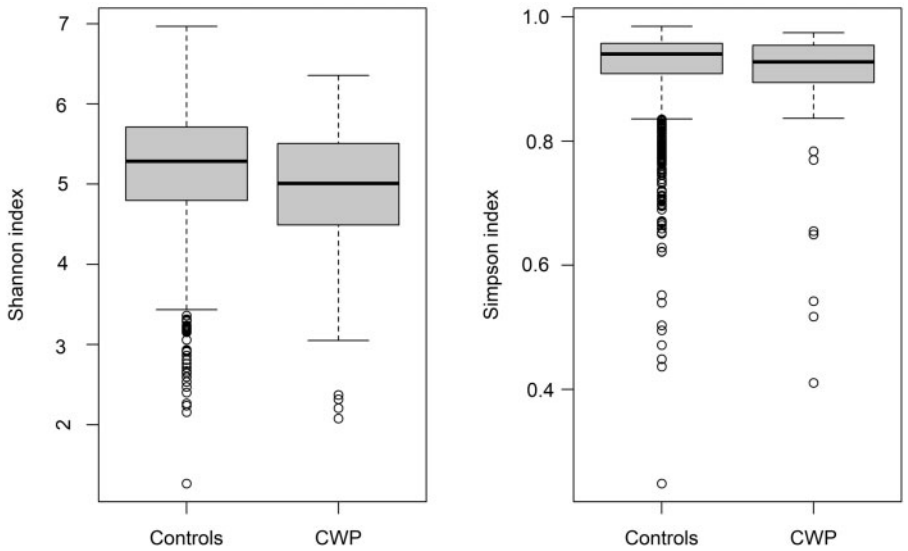
Association analysis of CWP and the microbiome

Association analysis of the gut microbiome with CWP was carried out using linear mixed-effects models with the *lme4* package (version 1.1.12) in R-3.3.0 (R Foundation for Statistical Computing, Vienna, Austria). ASV counts were transformed using the variance stabilization method in the R/Bioconductor package *DESeq2* (version 1.22.1 in R-3.5.2) [40]. ASVs were filtered to remove those not present in at least 5% of the samples, leaving 859 ASVs. Samples were filtered to remove those with fewer than 10 000 counts. Biological covariates included age, HEI, BMI and family relatedness. Technical covariates included sample sequencing depth, sequencing run, technician who extracted the DNA, technician who loaded the PCR plate, sample collection method, and FFQ date. Family relatedness and all technical covariates except sample sequencing depth were modelled as random effects. The variance stabilized ASV counts were used as the response variable in a linear model accounting for biological and technical factors. The Benjamini–Hochberg false discovery rate was used to control for multiple testing.

Causal inference

Causal inference was assessed using a combination of Mendelian randomization (MR) and polygenic risk score (PRS). Genome-wide association studies (GWAS) were carried out for bacteria of interest (see Results) and CWP. The bacteria counts were subjected to variance stabilizing transformation followed by an adjustment for technical covariates via residuals. The residuals have been further normalized using the *qqnorm* function in R statistical software to achieve a normal distribution. GWAS both for the bacterium and CWP was done using GEMMA software [41] adjusting for age, sex, BMI and family structure (via genomic relatedness matrix). The following filters were applied: minor allele frequency 0.03, Hardy–Weinberg equilibrium P-value 1e-6, imputation quality 0.7, and genotype missingness rate 0.95. To increase the precision of the bacterium GWAS, its results have been meta-analyzed with the results of GWAS of metagenomic data carried out in three Dutch cohorts—LifeLines-DEEP ($n = 916$), 500FG ($n = 410$), and MIBS-CO ($n = 93$)—as described elsewhere [42]. Meta-analysis was carried out for $n = 4\ 841\ 651$ overlapping single nucleotide polymorphisms (SNPs) using GWAMA software [43]. Following the completion of the GWAS, conditional and joint (COJO) analysis [44] was carried out to identify LD-independent association signals over the range of P-values: 2.5e-7, 5e-7, 2.5e-6, 5e-6, 2.5e-5, 5e-5, 2.5e-4, 5e-4, 2.5e-3, 5e-3, 2.5e-2 and 5e-2. For each set of SNPs resulting from the COJO analysis, we calculated PRSs as a weighted sum of genotype counts using regression coefficients from the GWAS as the weights. Predictive potential of the PRSs was assessed by regressing them on the phenotype of interest (the bacterium or CWP). The PRS with the highest coefficient of determination (R^2) was then used to predict the exposure phenotype followed by regression of the predicted exposure on the outcome phenotype, adjusting for age, sex and BMI. Power of the MR analysis was

Fig. 1 Alpha diversity values for Shannon and Simpson indices in CWP cases and controls



Alpha diversity was significantly lower in CWP cases (Mann–Whitney test, *P*-values 2.3e-04 and 1.2e-02, respectively). CWP: chronic widespread pain.

calculated using the online tool https://shiny.cnsgenomics.com/mRnd/ [45].

Results

The characteristics of the study population are presented in Table 1. Briefly, there were 1736 women in the TwinsUK dataset having pain questionnaire, dietary intake and gut microbiome data available. CWP cases were statistically significantly older, had higher BMI and lower HEI scores than controls.

Alpha diversity was significantly lower in CWP cases than controls, as measured by Shannon and Simpson metrics (Fig. 1).

Using linear mixed-effects models, we performed the case–control analysis of association between CWP and the microbiome using 859 ASVs present in at least 5% of samples; adjustments were done for age, BMI, HEI, family structure and technical covariates. Sixty ASVs had nominal *P*-values <0.05 (Table 2), with one remaining statistically significant after correcting for multiple testing: $\beta = -1.7227$ (0.3703) ($P_{adj} = 3.04e\text{-}03$).

The majority (44/60) of the ASVs with nominal *P*-values <0.05 were decreased in CWP cases compared with controls. The ASV that reached statistical significance after correction for multiple testing was assigned to *Coprococcus comes*, and it had a lower abundance in CWP cases than in controls.

The majority (38/44) of the ASVs nominally decreased in the CWP cases compared with the controls were assigned to Firmicutes of the order Clostridiales, with half assigned to the Lachnospiraceae family (19/38), and the rest mainly to the family Ruminococcaceae (16/38) (Table 2). Of the 60 ASVs with nominal *P*-values <0.05, the majority (48) were assigned at the genus level, but only 11 were assigned at the species level.

The majority (11/16) of the ASVs increased in the CWP cases compared with the controls with nominal *P*-values <0.05 were assigned to Firmicutes of the order Clostridiales, of which more than half were assigned to the Lachnospiraceae family (6/11) (Table 2).

An association between *C. comes* and CWP raises a question of causality: in the absence of confounders, either CWP causes the decrease of the bacterium abundance or the bacterium depletion causes the increased risk of CWP. We addressed this using Mendelian randomization and polygenic risk score. Neither meta-GWAS for the bacterium nor GWAS for CWP produced genome-wide significant associations (Fig. 2). However, nine loci achieved a suggestive significance threshold of $P < 1e\text{-}5$ for *C. comes*, with the most convincing one on chromosome 4 near *TRAM1L1* gene (Table 3; Fig. 2). Fifteen loci achieved suggestive significance for CWP, with the most convincing one on chromosome 11 near *SRSF8* gene (Table 4; Fig. 2). The best PRS in terms of predictive capacity of the bacterium comprised 895 SNPs having *P*-value $<5e\text{-}03$ ($R^2 = 0.157$, $P = 6.06e\text{-}58$). The levels of bacterium predicted using this PRS were not a statistically significant predictor for CWP ($\beta = -0.293$ (0.229); $P = 0.201$). The best PRS for CWP comprised 39 SNPs having *P*-value $<2.5e\text{-}05$ ($R^2 = 0.143$, $P = 9.49e\text{-}30$). The case–control classes of CWP predicted using this PRS were not predictive of the levels of *C. comes* ($\beta = -0.268$ (0.181); $P = 0.139$). The results suggest that neither *C. comes* nor CWP is likely a causal factor for each other. However, power estimates showed that our study has 22% power to detect significant causal effect of *C. comes* on CWP, while the power to detect causal effect of CWP on *C. comes* was 33%.

Table 2 Association between CWP and stool ASVs in TwinsUK

Internal ASV ID	Effect (β)	s.e.	Nominal *P*-value	ASV taxonomy
ASVs decreased in CWP				
85	−1.7227	0.3703	3.54e-06	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Coprococcus_3; s_comes
291	−0.4056	0.1426	4.49e-03	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_; s_
367	−0.2925	0.1051	5.44e-03	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Coprococcus_3; s_comes
782	−0.3320	0.1238	7.42e-03	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_GCA-900066575; s_
162	−1.1984	0.4472	7.44e-03	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminococcaceae_UCG-003; s_
255	−0.3490	0.1315	8.02e-03	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Roseburia; s_inulinivorans
332	−1.1333	0.4368	9.56e-03	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Lachnospiraceae_NK4A136_group; s_
118	−1.2962	0.5061	1.05e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Faecalibacterium; s_
695	−0.7161	0.2869	1.26e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminococcaceae_UCG-005; s_
631	−0.3418	0.1385	1.37e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_; s_
658	−0.6843	0.2836	1.59e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Howardella; s_ureilytica
238	−1.1532	0.4840	1.73e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Lachnospiraceae_UCG-010; s_
374	−1.0080	0.4261	1.81e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Family_XIII; g_Family_XIII_AD3011_group; s_
29	−0.8482	0.3597	1.85e-02	k_Bacteria; p_Firmicutes; c_Erysipelotrichia; o_Erysipelotrichales; f_Erysipelotrichaceae; g_Erysipelotrichaceae_UCG-003; s_
42	−1.2176	0.5202	1.94e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Lachnospiraceae_NK4A136_group; s_
482	−0.6015	0.2577	1.97e-02	k_Bacteria; p_Tenericutes; c_Mollicutes; o_Izimaplasmatales; f_; g_; s_
210	−0.3135	0.1349	2.03e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Faecalibacterium; s_
166	−0.7406	0.3201	2.08e-02	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Marinifilaceae; g_Odoribacter; s_splanchnicus
403	−0.7985	0.3509	2.30e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminococcaceae_NK4A214_group; s_
379	−0.4238	0.1864	2.31e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_; s_
748	−0.3009	0.1333	2.42e-05	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Family_XIII; g_Family_XIII_AD3011_group; s_
841	−0.2326	0.1069	2.98e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_; s_
284	−0.7409	0.3423	3.05e-02	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhodospirillales; f_; g_; s_
609	−0.0938	0.0439	3.27e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Dorea; s_formicigenerans
343	−0.9268	0.4344	3.30e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Lachnospiraceae_UCG-004; s_
169	−0.9253	0.4341	3.32e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminococcus_1; s_
708	−0.2969	0.1398	3.38e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminiclostridium_9; s_
587	−0.7418	0.3511	3.48e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Oscillibacter; s_
310	−0.1867	0.0886	3.52e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Blautia; s_obeum
626	−0.2869	0.1381	3.79e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Lachnospiraceae_NK4A136_group; s_
650	−0.2989	0.1439	3.79e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Marvinbryantia; s_
429	−0.3145	0.1518	3.85e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_; s_
464	−0.6522	0.3149	3.85e-02	k_Bacteria; p_Actinobacteria; c_Coriobacteriia; o_Coriobacteriales; f_Coriobacteriales_Incertae_Sedis; g_; s_
54	−1.1981	0.5792	3.88e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Agathobacter; s_
409	−0.8235	0.4020	4.07e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Lachnospiraceae_UCG-004; s_
474	−0.3302	0.1621	4.18e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Faecalibacterium; s_
328	−0.6931	0.3416	4.26e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminococcaceae_UCG-010; s_
143	−0.9961	0.4934	4.37e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_; g_; s_

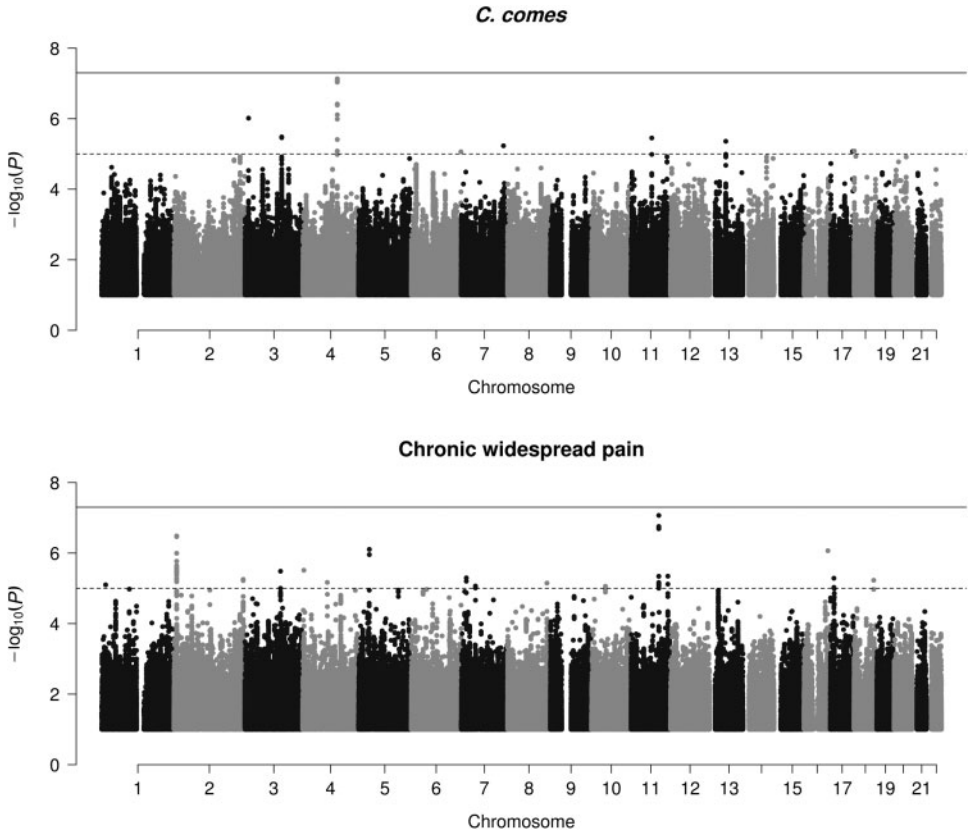
(continued)

Table 2 Continued

Internal ASV ID	Effect (β)	S.E.	Nominal *P*-value	ASV taxonomy
596	−0.2854	0.1430	4.61e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminococcaceae_UCG-005; s_
110	−0.9524	0.4781	4.65e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_; s_
594	−0.5822	0.2928	4.69e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminococcaceae_UCG-010; s_
355	−0.3037	0.1529	4.72e-02	k_Bacteria; p_Proteobacteria; c_Gammaproteobateria; o_Betaproteobacteriales; f_Burkholderiaceae; g_Parasutterella; s_excrementihominis
124	−0.3410	0.1720	4.76e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminococcaceae_UCG-002; s_
123	−0.2369	0.1208	5.00e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Subdoligranulum; s_
ASVs increased in CWP				
668	0.8838	0.2836	1.86e-03	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Shuttleworthia; s_
119	1.0217	0.3724	6.14e-03	k_Bacteria; p_Firmicutes; c_Bacilli; o_Lactobacillales; f_Streptococcaceae; g_Streptococcus; s_
844	0.5121	0.1880	6.53e-03	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Sellimonas; s_
220	1.1548	0.4573	1.17e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminiclostridium_5; s_
746	0.5170	0.2056	1.20e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Eisenbergiella; s_massiliensis
552	0.8018	0.3370	1.75e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminiclostridium_5; s_
338	0.3517	0.1516	2.05e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_; s_
20	1.2372	0.5413	2.24e-02	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Rikenellaceae; g_Alistipes; s_
461	0.6294	0.2791	2.43e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Lachnospira; s_
719	0.6488	0.2973	2.93e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_; g_; s_
441	0.7369	0.3461	3.34e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae; g_Ruminococcaceae_UCG-005; s_
173	0.7111	0.3375	3.52e-02	k_Bacteria; p_Firmicutes; c_Negativicutes; o_Selenomonadales; f_Veillonellaceae; g_Dialister; s_
724	0.5483	0.2626	3.70e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Christensenellaceae; g_Christensenellaceae_R-7_group; s_
710	0.6325	0.3058	3.87e-02	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Actinomycetaceae; g_Actinomyces; s_odontolyticus
589	0.5998	0.2908	3.93e-02	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Tyzzerella_3; s_
601	0.5768	0.2824	4.12e-02	k_Bacteria; p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Marinifilaceae; g_Butyricimonas; s_

Linear mixed-effects models were applied to test for association between ASVs and CWP in TwinsUK. Adjustment was done for age, BMI, HEI, family relatedness and technical covariates as detailed in the main text. ASV: amplicon sequence variant; CWP: chronic widespread pain.

FIG. 2 Manhattan plots for genome-wide association study results of *Coprococcus comes* (**A**) and chronic widespread pain (CWP) (**B**)



Discussion

The aim of this study was to examine the association between CWP and the gut microbiome while adjusting for the expected differences in BMI between cases and controls. Measures of species diversity were found to be significantly lower in CWP and we identified an ASV assigned to the species *Coprococcus comes* to be significantly decreased in CWP. Our Mendelian randomization study did not provide evidence for possible causal relationships between CWP and *C. comes*. However, this study was underpowered to show possible causal relationships (power achieved 22% and 33%).

C. comes is one of the most important butyrate-producing bacteria. Butyric acid produced by *C. comes* and other gut commensals exhibits remarkable anti-inflammatory effects in the gut. *C. comes* abundance was shown to decrease in inflammatory and immune-mediated disorders, such as Crohn's disease [46] and type 1 diabetes [47]. Even though our MR analysis was not significant, it still does not fully rule out a causal relationship between *C. comes* and CWP, given the low statistical power. While CWP as a causal factor for reduced *C. comes* can be explained by an overall drop of microbial abundance, the alternative route whereby the bacterium influences disease risk is more difficult to fathom. One possible explanation rests on the fact that a high-fat diet causes a decrease of *Coprococcus* species abundance in the gut [48] and people with CWP are known to have on average a diet higher in fat [49]. This is consistent with our finding of a statistically significantly lower HEI and higher BMI in CWP cases compared with controls (Table 1). These data reinforce the hypothesis that a high-fat diet promotes the decrease of *Coprococcus* and other anti-inflammatory species that in turn results in a low-grade inflammation, supporting the development of CWP.

Higher diversity of the gut microbiome has generally been associated with good health [50, 51], and the accumulation of health deficits (frailty index) has been linked with lower alpha diversity of the gut microbiota [52]. There is an established relationship between CWP and frailty in our cohort and others [9, 53, 54], and studies suggest that CWP may precede the accumulation of ill health, and potentially contribute to it [55]. This makes understanding the role of the microbiome critical as it offers a potential therapeutic mediator in the prevention of both CWP and frailty. While lower diversity in itself may not represent a deficit of the gut microbiota in CWP, the significantly lower gut microbiota diversity

Table 3 Suggestive results of meta-GWAS for *Coprococcus comes*

rs_number	Chr: position	Overlapped or nearest gene	Effect allele	Other allele	Effect size	s.e.	*P*-value	*Q*-statistic	*P*-value for *Q*-statistic	I^2	Total sample	Direction of effects
rs11551661	3:11871215	*TAMM41*	C	T	−0.1119	0.0228	9.6e-07	1.14	0.768	0.000	3521	−−−−
rs2735247	3:125477762	*GS1-388B5.8 / RP11-379B18.6*	G	A	−0.1123	0.0241	3.3e-06	6.89	0.076	0.564	3521	−−−−
rs76957229	4:118043418	*TRAM1L1 / AC107399.1*	T	C	0.1257	0.0233	7.4e-08	3.77	0.287	0.205	3521	++++
rs4716409	6:170234376	*RP1-182D15.2 / RP11-302L19.1*	A	T	−0.0934	0.0210	8.7e-06	1.39	0.708	0.000	3521	−−−−
rs59725556	7:145084002	*AC004911.2 / AC073055.2*	T	C	−0.1074	0.0237	5.9e-06	5.91	0.116	0.492	3521	−−−−
rs72958391	11:71265701	*KRTAP5-9 / KRTAP5-10*	C	G	−0.1141	0.0246	3.5e-06	6.55	0.088	0.542	3521	−−−−
rs4378518	13:56068607	*MIR5007 / HNF4GP1*	C	T	−0.0945	0.0206	4.4e-06	5.54	0.136	0.458	3521	−−−−
rs56396930	17:75743839	*RP11-316M20.1 / FLJ45079*	T	G	0.1079	0.0242	8.5e-06	1.92	0.589	0.000	3521	++++
rs62082666	18:923474	*ADCYAP1 / RP11-672L10.1*	A	G	−0.0935	0.0210	8.3e-06	1.67	0.643	0.000	3521	−−−−

Genome-wide association study was carried out for *C. comes* in TwinsUK ($n = 2118$) using GEMMA software. Adjustments were made for age, sex, BMI and kinship via genetic relatedness matrix. Subsequent meta-analysis was performed including three Dutch cohorts described elsewhere [42]. Top SNPs from regions of suggestive associations are provided ($P < 1e\text{-}5$). Overlapped and nearest genes are identified using SNPnexus (https://www.snp-nexus.org/v4/). GWAS: genome-wide association study; SNP: single nucleotide polymorphism. s.e: standard error.

Table 4 Suggestive results for GWAS for CWP

SNPID	Chr: position	Overlapped or nearest gene	Effect allele	Other allele	Effect size	s.e.	*P*-value	EAF	Sample size
rs61777763	1:14477359	*RNU6-1265P / RP11-344F13.1*	T	C	0.0935	0.0209	7.96e-06	0.062	3114
rs13429284	2:237102161	*ASB18*	C	A	0.0639	0.0141	5.57e-06	0.143	3114
rs34057310	2:9423678	*ASAP2*	T	C	0.0624	0.0122	3.27e-07	0.219	3114
rs9821958	3:122009508	*CASR / HNRNPA1P23*	G	A	0.0675	0.0145	3.27e-06	0.135	3114
rs35020435	4:4076195	*AC116562.2 / RP11-489M13.1*	C	T	0.0577	0.0123	3.07e-06	0.31	3114
rs34698918	5:37421837	*WDR70*	A	G	0.1084	0.0219	7.85e-07	0.061	3114
rs55728567	7:17792341	*AC006482.1 / SNX13*	T	C	0.0658	0.0144	5.05e-06	0.141	3114
rs142024481	8:134637071	*RP11-629O1.2 / SNORA40*	G	A	0.0948	0.0211	7.10e-06	0.061	3114
rs11259826	10:47600049	*AHCYP1 / ANTXRLP1*	T	C	−0.1310	0.0294	8.76e-06	0.038	3114
rs10893460	11:125867896	*CDON*	A	G	−0.0512	0.0111	4.53e-06	0.269	3114
rs78164056	11:94795234	*RP11-735A19.2 / SRSF8*	C	T	0.0787	0.0147	8.56e-08	0.136	3114
rs149091311	12:50885180	*LARP4 / DIP2B*	T	C	0.1805	0.0343	1.56e-07	0.036	3114
rs56117891	16:81861570	*PLCG2*	T	C	0.1296	0.0263	8.58e-07	0.039	3114
rs4792257	17:12251758	*RP11-471L13.2 / LINC00670*	A	G	−0.0928	0.0203	5.17e-06	0.937	3114
rs2849474	18:67569079	*CD226*	T	C	−0.0804	0.0177	5.90e-06	0.907	3114

GWAS for CWP was carried out in TwinsUK ($n = 3273$; 531 cases of CWP) using GEMMA software [41]. Adjustments were done for age, sex, BMI and kinship via genetic relatedness matrix. Top SNPs from regions of suggestive associations are provided ($P < 1e\text{-}5$). Overlapped and nearest genes are identified using SNPnexus (https://www.snp-nexus.org/v4/). CWP: chronic widespread pain; GWAS: genome-wide association study; SNP: single nucleotide polymorphism. s.e: standard error.

demonstrated here is indicative of a diseased state. As the gut microbiota represents a therapeutic target—via interventions such as diet, pro-biotics, drugs to target the microbiota and faecal transfer—the results of our study are in accordance with the gut microbiome representing a future therapeutic target in CWP.

A study of the gut microbiome in myalgic encephalomyelitis (ME)/CFS [56] found that bacteria of the genus *Coprococcus* were among the taxa decreased in ME/CFS patients compared with controls. CWP is common in chronic fatigue and both conditions are considered to lie on a spectrum of non-specific pain and fatiguing illnesses [57]. Another study of the gut microbiome in ME/CFS found decreased microbial diversity, in particular a decrease in relative abundance and diversity of members of the Lachnospiraceae family, including *Coprococcus* species [58].

Two recent studies demonstrated variation of gut microbiome in individuals with fibromyalgia (FM), a condition characterized by CWP as a major symptom [59, 60]. The study by Minerbi *et al.* [59] compared stool microbiome in 77 FM patients and 79 controls and identified no difference in alpha-diversity between cases and controls and mildly reduced beta-diversity in patients. They also found differential abundance for 72 operational taxonomic units belonging to phyla Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. The study by Clos-Garcia *et al.* [60] looked at 105 FM cases and 54 controls and identified reduced abundance of the *Bifidobacterium* and *Eubacterium* genera consistent with altered levels of neurotransmitters in the serum of the patients. Both studies provided evidence for potential of the gut microbiome as a biomarker of FM. However, the question of causality was not addressed.

Our study has limitations. For the case–control study, we looked at female twin volunteers only, so the results cannot be extrapolated to men. However, the prevalence of CWP is higher in women than in men so this middle-aged cohort is particularly relevant to the condition. The pain questionnaire used to diagnose CWP, the FFQ, and the faecal samples for gut microbiome analysis were obtained at different times, which in some cases may be several years apart. However, longitudinal studies have shown that the gut microbiota remain relatively stable in adults [61]. Also, longitudinal reporting of CWP in TwinsUK is reasonably consistent (Supplementary Table, available at *Rheumatology* online). Thus, we believe that even though the time difference between the stool sample acquisition and twins assessment might have played some role, our results still reflect true relationships between microbiome and CWP. Still, we cannot fully rule out the possibility of residual confounding due to longitudinal change of the diet, physical activity and other factors that may affect microbiota composition. Another potential limitation is the difference between cases and controls by age and BMI, so potentially the observed differences in microbiome can be attributable to these factors. However, the absolute differences in age is rather small (2 years, Table 1), so it is unlikely a significant factor in our study, also taking into account above-mentioned stability of microbiome with age. Concerning BMI, we cannot fully rule out its impact on the results; however, increased BMI is a characteristic feature of CWP, so it is inherently difficult to distinguish the impact of the two. As we statistically adjusted for BMI and age, the resultant differences in microbiome in CWP are likely attributable to CWP; however, collider bias cannot be excluded at this stage and this possibility needs to be explored further. Our analysis of the microbiome was limited by the use of 16S rRNA sequencing data, which did not enable assignment of genus or species to many of the ASVs. The use of shotgun metagenomics and metatranscriptomic data would provide more accurate species level assignment, as well as functional information about which genes and pathways are present and being expressed [62]. Finally, our Mendelian randomization study was underpowered (22% and 33%), so larger studies are warranted to look at possible causality between CWP and *C. comes*. Our estimates show that one needs a sample of at least 5500 individuals to achieve 80% to demonstrate causal impact of CWP on *C. comes* and 8700 to achieve 80% power to demonstrate causal impact of *C. comes* on the risk of CWP. While large GWAS for CWP using UK Biobank data are under way (Rahman *et al.* [unpublished results] Genome-wide association study identifies three loci associated with chronic widespread musculoskeletal pain; presented at the American Society of Human Genetics 69th Annual Meeting, October 15–19, 2019), GWAS of comparable size for *C. comes* are much more challenging.

In summary, our study establishes a suggestive connection between the gut microbiome and CWP. Lack of diversity of gut microbiome appears, in common with many conditions, and may potentially be addressed by dietary and other intervention. The role of *Coprococcus* merits further investigation and it may lie in a network of microbes which are indicative of poorer diet.

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Disclosure statement: The authors declare no conflict of interest.

Data availability statement

TwinsUK: Sequencing data for the microbiome samples used in this study have been deposited in the European

Nucleotide Archive (ENA) under accession numbers ERP006339, ERP006342, and ERP015317. Dutch cohorts: Sequencing data for the microbiome samples of the The LifeLines-DEEP and MIBS metagenomics sequencing data are available at the European Genome phenome Archive (EGA); LifeLines-DEEP, EGAS00001001704; MIBS, EGAS00001001924. The 500FG data are available at the Sequence Read Archive (SRA): PRJNA319574.

Other data are available on request from the TwinsUK Resource Executive Committee (TREC) (https://twinsuk.ac.uk/resources-for-researchers/access-our-data/).

Supplementary data

Supplementary data are available at *Rheumatology* online.

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