



OPEN ACCESS

ORIGINAL RESEARCH

Gut microbiota of obese subjects with Prader-Willi syndrome is linked to metabolic health

Lisa M Olsson ,¹ Christine Poitou,^{2,3} Valentina Tremaroli,¹ Muriel Coupaye,³ Judith Aron-Wisnewsky,^{2,3} Fredrik Bäckhed,^{1,4} Karine Clément,^{2,3} Robert Caesar ¹

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/gutjnl-2019-319322>).

For numbered affiliations see end of article.

Correspondence to

Dr Robert Caesar, The Wallenberg Laboratory, Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg 41345, Sweden; Robert.Caesar@wlab.gu.se and Professor Karine Clément, INSERM, Nutrition and obesity; systemic approaches (NutriOmics) research Unit, Sorbonne Université, Paris, France; karine.clement@inserm.fr

Received 20 June 2019

Revised 20 September 2019

Accepted 23 September 2019

Published Online First

14 October 2019

ABSTRACT

Objective The gut microbiota has been implicated in the aetiology of obesity and associated comorbidities. Patients with Prader-Willi syndrome (PWS) are obese but partly protected against insulin resistance. We hypothesised that the gut microbiota of PWS patients differs from that of non-genetically obese controls and correlate to metabolic health. Therefore, here we used PWS as a model to study the role of gut microbiota in the prevention of metabolic complications linked to obesity.

Design We conducted a case-control study with 17 adult PWS patients and 17 obese subjects matched for body fat mass index, gender and age. The subjects were metabolically characterised and faecal microbiota was profiled by 16S ribosomal RNA gene sequencing. The patients' parents were used as a non-obese control group. Stool samples from two PWS patients and two obese controls were used for faecal microbiota transplantations in germ-free mice to examine the impact of the microbiota on glucose metabolism.

Results The composition of the faecal microbiota in patients with PWS differed from that of obese controls, and was characterised by higher phylogenetic diversity and increased abundance of several taxa such as *Akkermansia*, *Desulfovibrio* and Archaea, and decreased abundance of *Dorea*. Microbial taxa prevalent in the PWS microbiota were associated with markers of insulin sensitivity. Improved insulin resistance of PWS was partly transmitted by faecal microbiota transplantations into germ-free mice.

Conclusion The gut microbiota of PWS patients is similar to that of their non-obese parents and might play a role for the protection of PWS patients from metabolic complications.

INTRODUCTION

Obesity is a heterogeneous disease associated with increased risk of metabolic perturbations such as insulin resistance, diabetes and cardiovascular disorders. Subpopulations of obese subjects may also be protected from metabolic abnormalities, at least for a period of their disease history.¹ Many different factors contribute to the metabolic consequences of obesity, including complex interactions between genetics, environment and lifestyle aspects, in which environmental factors may play critical roles as shown in large populations.² Recent research indicates that the gut microbiota can be considered an environmental factor and/or an integrator of

Significance of this study

What is already known on this subject?

- Obesity is associated with insulin resistance.
- The gut microbiota of patients with insulin resistance differs from that of healthy controls, and an altered microbiota has been suggested to be part of disease aetiology.
- Prader-Willi syndrome patients are morbidly obese but relatively protected against insulin resistance.

What are the new findings?

- The faecal microbiota of patients with Prader-Willi syndrome differs from that of obese subjects matched for body fat mass index, and is not different from that of non-obese subjects.
- The faecal microbiota of Prader-Willi syndrome patients is linked to markers of insulin sensitivity.
- Transplantation of faecal microbiota from Prader-Willi syndrome patients to germ-free mice transmits the insulin sensitivity of the donor.

How might it impact on clinical practice in the foreseeable future?

- The study supports the hypothesis that dysbiosis can worsen insulin tolerance and that the gut microbiota may be a suitable therapy target.
- Microbial taxa linked to butyrate production and intestinal mucus metabolism are identified as putative mediators of metabolic regulation.

environmental triggers that contribute to fat mass and obesity development.^{3,4} Several human studies have now investigated the relationship between the human gut microbiota and obesity phenotypes⁵⁻⁷ and a meta-analysis has shown significant associations between decreased alpha diversity, obesity and obesity severity.⁸ Due mainly to small group sizes, the different studies have revealed only few microbial taxa consistently associated with obesity.⁸ However, high alpha diversity in the faecal microbiota has generally been linked to a decreased relative risk of being obese.⁸

Whereas genome wide scans have shown moderate effect of genetic variants in body mass index (BMI) and related traits variance in large scale populations,⁹ it is established that



© Author(s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Olsson LM, Poitou C, Tremaroli V, et al. *Gut* 2020;**69**:1229–1238.

in some obese patients, genetic mutations are causal. This includes monogenic genetic disruptions of the leptin signaling pathway,¹⁰ pleiotropic syndromes and chromosomal rearrangements.¹¹

Among syndromic obesities, Prader-Willi syndrome (PWS) is the most common one.¹² PWS patients lack expression of paternal alleles on chromosome 15, related to several genetic mechanisms including paternal deletion, uniparental disomy and more rarely sporadic mutations and chromosome translocations. Whereas after birth, PWS patients experience difficulties in eating, during early infancy there is a switch toward hyperphagia and rapid weight gain as well as metabolic alterations. However, metabolic alteration in PWS patients is debated and it has been reported that despite major adiposity, PWS patients exhibit relative improved insulin sensitivity compared with subjects with common obesity.^{13–16} Many features in PWS patients could affect glucose homeostasis, including decreased baseline insulin levels,^{16–17} increased levels of the insulin sensitising hormone adiponectin,^{16–17} different body distribution of adipose tissue and an increased subcutaneous adipose tissue expandability with decreased inflammation and fibrosis,¹⁶ but the mechanism underlying the improved glucose metabolism of PWS patients remains elusive. We here used PWS as a model of genetically induced severe obesity with improved insulin resistance to investigate how the interaction between genetics and gut microbiota can affect individual metabolic status.

MATERIAL AND METHODS

Study population

To conduct a case-control study we examined two distinct populations matched for age, fat mass and gender. Subjects were recruited at the Pitié-Salpêtrière University Hospital, Paris, and exposed to the same clinical examinations. Stool samples were collected by similar procedures.

Between July 2007 and September 2015, 80 adults (age ≥ 16 years) with PWS were examined in the Nutrition Department (French Reference Centre for PWS, Nutrition department, Pitié-Salpêtrière Hospital, Paris). We obtained all authorisations of the Ministry of Research and the French Institutional Review Board (Comité de Protection des Personnes Ile de France 1, reference number 2014-mai-13577) to use the clinical data from our register for research purpose, including those performed with international collaborations as well as the analysis of faecal microbiota profiles. During the phase of recruitment (2014 to 2015), we proposed participation in the study to all patients coming for clinical follow-up in standard of care and to their parents. To be included in the study the subjects should have genetically confirmed PWS, be coming in standard of care visit in our centre, be living in their family home, have two living parents, have available measures of body composition evaluated by dual-energy X-ray absorptiometry (DXA), have agreed to participate in this research and have agreed to give stool samples. The exclusion criterion was presence of severe behavioural disorders. Seventeen patients with PWS (12 with a paternal deletion and 5 with uniparental disomy) fulfilled these criteria and were enrolled in the study. Three PWS patients had type 2 diabetes. The parents of the PWS patients were recruited as a control group (PWS parents). They had an average BMI of 25.5 kg/m² (BMI < 25, n = 10, 42%; 30 > BMI > 25, n = 11, 46%; BMI > 30, n = 3, 12%). All patients and parents gave informed consent. If the patient did not fully understand the informed consent, the consent of the patient's legally authorised representative was required for participation.

Table 1 Clinical characteristics of OC subjects, PWS patients and PWS parents

	OC (n=17)	PWS (n=17)	PWS (n=24)	P value
Female gender (%)	59	59	63	
T2D (%)	18	24	0	
BFMI	19.8±6	18.2±7.2		0.487
Fat mass (% DXA)	47.2±7	47.6±6.7		0.8759
Age (year)	31.4±8.9	29.4±7.8	59.1±7.3	0.4902
Weight (kg)	119.7±29.4	87.8±22.5	69.3±9.8	0.0013
Height (cm)	167.8±9	154.9±8.5	164.9±7.2	<0.001
BMI (kg/m ²)	42.3±8.7	37.4±10.1	25.5±3.7	0.1373
Fat mass (kg)	55.8±18	42.8±15.1		0.0299
Androgen fat (%)	55.6±7.5**	59.4±4.8		0.119
Fasting glycaemia (mmol/l)	5.1±0.5	4.6±1		0.0914
Fasting insulin (mU/l)	19.5±15.2**	8.8±5**		0.0236
HOMA-IR	4.5±3.5**	1.7±1**		0.0133
HbA1c (%)	5.6±0.6	5.7±0.5***		0.623
HDL-c (mmol/l)	1.2±0.4	1.2±0.3		0.557
LDL-c (mmol/l)	3.2±2.3	3±0.6		0.764
Triglycerides (mmol/l)	1.7±1.8	1±0.4		0.1298
Leptin (ng/ml)	51.5±29.1**	38.6±24.3**		0.2149
C-reactive protein (mg/l)	6.8±3.9**	12.4±12.5*		0.1948

The values in Table indicates average value \pm SD for continuous variables and percentage for factorial. P indicate significant differences in variable between OC and PWS using t-test, significant p values are in bold ($\alpha=0.05$). Body fat mass index is calculated as body fat (kg)/height (m²). * n=10; ** n=14; *** n=16.

BFMI, body fat mass index; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; HbA1c, haemoglobin A1c; HDL-c, high density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-c, low density lipoprotein cholesterol; OC, obese control; OWC, Over weight control; PWS, Prader-Willi syndrome; T2D, type 2 diabetes.

We also obtained faecal samples from subjects with common obesity that served as controls matched with the PWS patients for body fat mass index (BFMI) and percent fat mass (obese controls, OC). The OC group was selected among obese French subjects involved in the European project MetaCardis.¹⁸ Subjects provided written informed consent. The MetaCardis study was conducted in accordance with the Helsinki Declaration and is registered in clinical trial <https://clinicaltrials.gov/show/NCT02059538>

Gut microbiota composition as well as metabolic and inflammatory host phenotype are related to obesity. Since PWS patients have reduced height¹⁶ (table 1), the PWS patients and obese controls were matched for two height-independent parameters of obesity: per cent body fat mass and BFMI. BFMI is associated with features of the metabolic syndrome,¹⁹ provides information about body compartments and allows height-independent interpretation of nutrition status,²⁰ which is important since PWS have a body composition with excess body fat mass and lower lean body mass.¹⁶

Biochemical analyses and anthropometrics

Subjects underwent systematic explorations that included a thorough medical interview recording obesity comorbidities such as type 2 diabetes, a routine physical examination and fasting biological measurements as previously described.^{18–21} Blood samples were collected after an overnight fast. Fasting glucose, high density lipoprotein cholesterol (HDL-c), triglycerides and haemoglobin A1c (HbA1c) were measured using enzymatic methods. Fasting serum insulin was measured using a chemiluminescence assay (Insulin Architect, Abbott). Serum leptin was measured using the Human Leptin Quantikine ELISA Kit (R&D Systems, Inc). High-sensitivity c-reactive protein was

measured using an IMMAGE automatic immunoassay system (Beckman-Coulter).

Weight and height were assessed during the clinical inclusion visit according to standardised procedures using the same scale for all subjects. Body composition was assessed using the same device; a whole-body fan-beam DXA scan (Hologic Discovery W, software V.12.6, 2; Hologic, Bedford, Massachusetts) which evaluated per cent body fat mass. BFMI was calculated as body fat (kg)/height (m²).

Extraction of faecal genomic DNA

Human faecal samples were collected and stored at -80°C . Total genomic DNA was isolated from 100 to 150 mg of faeces or 100 mg of mouse caecum using a repeated bead-beating method.²² Briefly, samples were placed in Lysing Matrix E tubes (MP Biomedicals) and extracted twice in lysis buffer (4% w/v sodium dodecyl sulfate, 500 mmol/L NaCl, 50 mmol/L ethylenediaminetetraacetic acid, 50 mmol/L Tris-hydrochloride, pH 8) with bead beating at 5.0 m/s for 60 s in a FastPrep-24 instrument (MP Biomedicals). After each bead-beating cycle, samples were incubated at 90°C for 15 min and then centrifuged at full speed for 5 min at 4°C . Supernatants from the two extractions were pooled, and the DNA was recovered by isopropanol purification and then purified using the QIAamp DNA Mini kit (QIAGEN).

Profiling of faecal microbiota composition by sequencing of the 16S rRNA gene

The faecal microbiota was profiled by sequencing the V4 region of the 16S ribosomal RNA (rRNA) gene as previously been described.²³ Singletons and low abundant operational taxonomic units (OTUs) with a relative abundance $<0.002\%$ were excluded. We obtained an average of $61\,997 \pm 17\,134$ sequences/sample (mean \pm SD; range 48 603 to 123 343 sequences/sample); a total of 4 632 821 sequences and 1483 OTUs were included in the analyses. To correct for differences in sequencing depth between samples, 48 000 sequences were randomly subsampled from each sample and included in the analyses for the estimation of α -diversity and β -diversity. For the analysis of differential abundance we included OTUs with abundance of at least 0.1% and present in 20% of the samples ($n=166$), and genera (L6-features) with abundance of at least 0.1% and present in 10% of the samples ($n=66$). Representative sequences for OTUs that showed significant differential abundance or correlation with metadata were blasted against the NCBI 16S ribosomal RNA sequences (Bacteria and Archaea) database to obtain a more specific taxonomic annotation. 16S data are available at European Nucleotide Archive (accession number: PRJEB33908; <http://www.ebi.ac.uk/ena/data/view/PRJEB33908>).

Transfer of gut microbiota to germ-free mice

We selected two female donor pairs, each consisting of one individual with PWS and one OC subject. Swiss Webster male and female mice aged 9 to 10 weeks and fed regular chow diet were transplanted with faeces from each donor. Mice were kept in individually ventilated cages (ISOcage N System, Tecniplast) with a maximum of five mice per cage under a 12 hours light cycle and a room temperature of 21°C . Food and water was provided ad libitum. Frozen stools (500 mg) obtained from each human donor were resuspended in 5 mL reduced PBS. The mice were randomised into two groups based on body weight and colonised by oral gavage with 200 μL of faecal slurry from each donor. An insulin tolerance test (ITT) was performed 2 weeks after

colonisation when mice were fasted for 4 hour and injected with insulin (0.75 U/kg body weight). Blood glucose was measured in tail vein blood at 0, 30, 60, 90 and 120 min with a Contour Next EZ glucometer (Bayer). For the first colonisation, an intraperitoneal glucose tolerance test was also performed 3 weeks after colonisation. Mice were fasted for 4 hour and injected with d-glucose (2 g/kg body weight). Blood glucose was measured in tail vein blood at 0, 15, 30, 60, 90 and 120 min. Additional blood samples were collected at 0 and 15 min to analyse plasma insulin levels by insulin ELISA (Crystal Chem). Interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF α) were measured by ELISA in plasma collected from *vena cava* according to the manufacturers' protocols (R&D Systems). Colonisation of the recipient mice by the human gut microbiota was examined in caecal samples collected at the end of the experiment. Caecal samples were processed to profile the 16S rRNA gene as described above.

Statistical analyses

Statistical analyses were performed in R²⁴ and GraphPad Prism 7. Tests between groups were performed using Wilcoxon rank sum test, with adjustment for false discovery rate using Benjamini-Hochberg procedure.²⁵ Significance was defined for features with adjusted $p < 0.05$. Differences in composition of 16S rRNA gene profiles, using permuted multivariate analysis of variance (ANOVA), were tested using the *adonis* function in *vegan* (V2.4 to 5), while for distance-based redundancy analysis (dbRDA) we used the *capscale* function also in *vegan*.²⁶ Correlations between microbial taxa and clinical parameters were tested using Spearman's test with adjustment for false discovery rate using the Benjamini-Hochberg procedure.²⁵ Significance was defined for $p < 0.05$, while trends for $p < 0.1$. For mouse experiments the two-sided Student's t-tests was used. For tests between groups with repeated measurements a two-way ANOVA test for repeated measurement was used, which included a Sidak's multiple comparison.

RESULTS

Clinical profiles in subjects with PWS, their parents and patients with common obesity

Clinical characteristics of the subjects participating in the study are shown in [table 1](#). Severely obese subjects with PWS and common obesity (OC) were matched for BFMI, DXA-body fat percentage, age, gender and presence of type 2 diabetes. In line with the general characteristics of PWS, including dysmorphism and abnormal body fat distribution, the PWS patients were shorter, lighter, had lower total fat mass and lower BMI than OC subjects. Fasting serum insulin and homeostatic model assessment of insulin resistance (HOMA-IR) were lower in PWS patients compared with OC subjects despite similar fat mass index, in agreement with previous studies.¹⁶ Blood glucose levels and HbA1c did not differ between the groups, nor did levels of HDL-c, low density lipoprotein cholesterol, triglycerides, leptin or C-reactive protein. Parents of PWS patients (mean BMI 25.5 kg/m²) were used as an additional slightly overweight but non-obese control group sharing environmental and genetic conditions with PWS subjects.

The biological characteristics were similar in PWS patients with deletion and in those with uniparental disomy, with the exception of blood triglycerides that were moderately higher in the deletion group (Online Supplementary Table 1).

Differences in fecal microbiota composition between PWS patients and people with common obesity

The analysis of microbiota profiles by sequencing of the 16S rRNA gene showed that the microbiota of PWS patients was

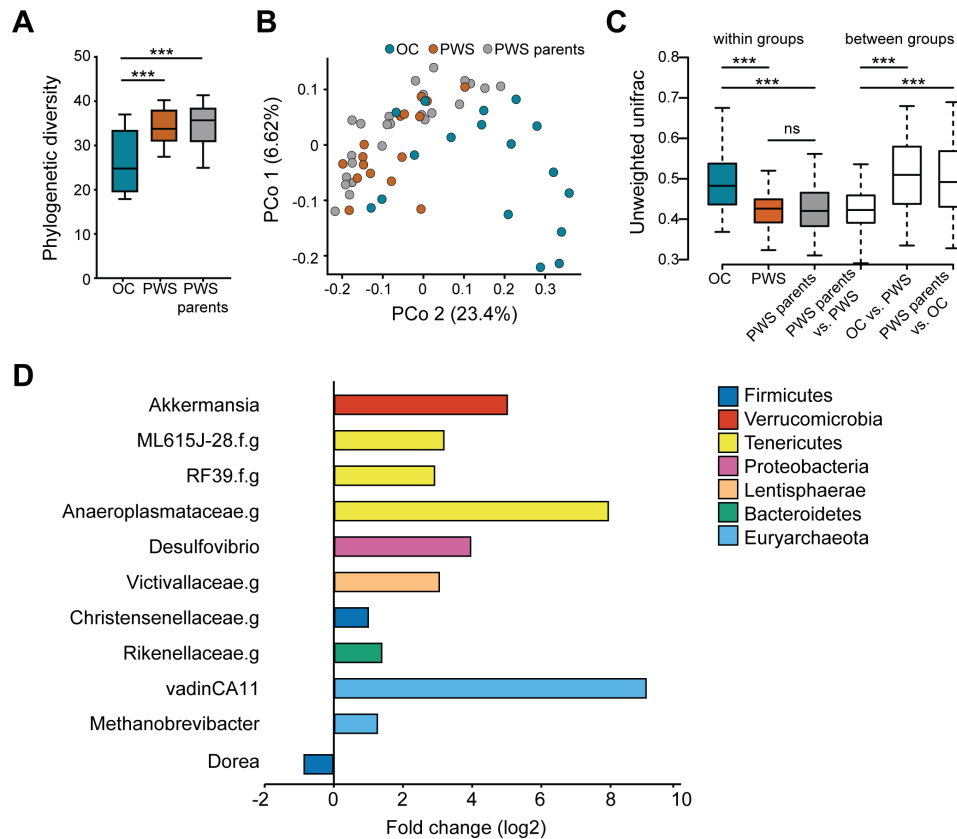


Figure 1 Composition of the gut microbiota in patients with Prader-Willi syndrome and in control subjects. (A) α -diversity expressed as phylogenetic diversity for obese controls (OC), patients with Prader-Willi syndrome (PWS) and PWS parents. (B) Principal coordinates analysis based on unweighted UniFrac showing the distribution along principal component (PCo) 1 and 2 of OC, PWS and PWS parents samples. The numbers in brackets next to the axis indicate the amount of compositional variation explained by each PCo. (C) Within-group and between-group β -diversity for the OC, PWS and PWS parents samples based on unweighted UniFrac (Wilcoxon rank sum test, *** $p < 0.001$). (D) Fold change for microbial genera with significantly different abundance in OC versus PWS samples (Wilcoxon rank sum test, significant level of 0.05 after adjustment for multiple comparisons). PWS, $n = 17$; OC, $n = 17$; PWS parents, $n = 24$. Boxes in plots indicate median and IQR. Whiskers specify $\pm 1.5 \times$ IQR from box's quartile.

strikingly different from that of OC individuals, and characterised by higher phylogenetic diversity similar to that of the PWS parents (phylogenetic diversity, PD, [figure 1A](#)). Ordination analysis showed different overall gut microbiota composition for PWS, OC and PWS parents ([Figure 1B](#) and Online Supplementary Figure 1), and in particular for unweighted UniFrac, the grouping of samples as OC, PWS and PWS parents explained about 12% of the compositional variation (adonis, 9999 permutations, $p = 0.001$). This analysis indicated that low abundant microbial taxa were important for the compositional variability between samples. Our analyses also showed that the overall differences in gut microbiota composition were due to differences in composition between OC and PWS, as well as between OC and PWS parents, while no significant difference was observed for the microbiota of PWS patients and that of their parents ($p = 0.15$, Online Supplementary Table 2). These results were confirmed by the analysis of between-group unweighted UniFrac, while analysis of within-group unweighted UniFrac showed a more heterogeneous gut microbiota composition for OC subjects compared with both PWS and PWS parents ([figure 1C](#)).

In agreement with the findings regarding overall microbiota composition, we found differential abundance of dominant genera in the faecal microbiota between OC and PWS samples as well as between OC versus PWS parents, but no difference for PWS versus PWS parents. Eleven genera were significantly

differentially abundant between PWS and OC. Ten genera increased in PWS, including *Akkermansia*, *Desulfovibrio* and genera classified in the Rikenellaceae, Victivallaceae and Christensenellaceae families, as well as three genera from the Tenericutes phylum and two genera from the Archaea domain (*Methanobrevibacter* and *vadinCA11*). Only one genus, here identified as *Dorea*, was significantly decreased in PWS compared with OC ([Figure 1D](#) and Online Supplementary Table 3). When comparing samples from OC and PWS parents we observed differential abundance of nine genera, seven of which were different for OC in comparison to PWS (Online Supplementary Table 3). However, the increase in Archaea (both *Methanobrevibacter* and *vadinCA11*) and the decrease in *Dorea* were not observed when comparing samples from OC and PWS parents, indicating that these genera could be specific for the PWS microbiota.

PWS faecal microbiota signature associates with insulin sensitivity markers independently of body fat mass

We used dbRDA to explore the relationships between gut microbiota composition and phylogenetic diversity as well as clinical and biological metabolic variables in PWS patients ($n = 12$) and OC subjects ($n = 14$) for whom all clinical parameters were available (Online Supplementary Table 4).

First, serum triglycerides (TG), HOMA-IR and phylogenetic diversity significantly contributed to compositional variation, and together constrained 25% of the total compositional variance (figure 2A and Online Supplementary Table 5). Importantly, parameters related to corpulence and adiposity were not significantly correlated to the variation of gut microbiota composition ($p=0.23$ and $p=0.19$ for fat mass (kg) and weight (kg), respectively; Online Supplementary Table 5). The phylogenetic diversity contributed mostly to the compositional variability and alone constrained 16% of the total variance (Online Supplementary Table 5). Moreover, in the dbRDA analysis the biplot vectors for serum triglycerides and HOMA-IR show opposite directions compared with phylogenetic diversity, indicating a possible negative correlation of both insulin resistance and serum lipid levels with phylogenetic diversity.

Second, microbial OTUs contributed significantly to the compositional variation of the faecal microbiota between PWS and OC in the constrained model. Interestingly, we found that OTU 3600504 corresponding to *Bacteroides vulgatus*, OTU 195937 corresponding to *Blautia luti* and OTU 185763 corresponding to *Faecalibacterium prausnitzii* had the strongest contributions to compositional variation in the microbiota in OC and PWS patients (Online Supplementary Table 7).

Third, we found significant correlations between 56 OTUs ($p<0.1$), phylogenetic diversity and metabolic variables (Figure 2B and Online Supplementary Table 7). The majority of these correlations were observed for phylogenetic diversity ($n=46$, $p<0.05$), in line with the dbRDA analysis. The abundance of several OTUs was nevertheless associated with the levels of serum insulin, HOMA-IR and triglycerides (Figure 2B and Online Supplementary Table 7). OTU 4438983 belonging to the Archaeon *Methanobrevibacter* showed the strongest significant negative correlations with these surrogates of insulin-resistance. In contrast, OTU 2724175 belonging to *Ruminococcus gnavus*, OTU 4483337 related to *Merdimonas faecis* and OTUs 3600504 and 3588390 belonging to *B. vulgatus* were strongly positively correlated to these markers (Figure 2B and Online Supplementary Table 7).

Finally, we found 18 OTUs with significant differential abundance in PWS versus OC samples (Figure 2C and Online Supplementary Table 8). Among these 18 OTUs, 8 OTUs belonging to Clostridiales ($n=7$) and Bacteroidales ($n=1$) were significantly more abundant in PWS and correlated negatively with serum insulin, HOMA-IR and TG (OTUs: 176269, 176318, 260414, 307238, 316732, 819353, 3327894, 4412540; Online Supplementary Table 7). The Clostridiales OTUs were highly prevalent in PWS samples (94% vs 52% for PWS vs OC, respectively) and showed a 8 to 400 times enrichment compared with OC (\log_2 fold change between 3.04 and 8.76; figure 2C and Online Supplementary Table 8). Only one OTU (2724175, matching to *R. gnavus*) showed decreased abundance in PWS samples and was more prevalent in OC samples (47% vs 88% for PWS vs OC) (Figure 2C, Online Supplementary Table 7,8). As indicated above, OTU 2724175 correlated negatively to phylogenetic diversity and positively to serum triglycerides and HOMA-IR (Figure 2B, Online Supplementary Table 7), and in addition was identified as a contributing feature for the compositional variation of the microbiota between PWS and OC samples in the dbRDA analysis (Figure 2A, Online Supplementary Table 6). Overall, these results indicate that gut microbiota features linked to PWS associate with metabolic markers eventually suggesting a contribution of microbiota to insulin sensitivity.

Influence of genetic subtypes of PWS on the faecal microbiota

Faecal microbiota phylogenetic diversity was not different in PWS patients with deletion ($n=12$) compared with those with disomy ($n=5$). However, Principal Coordinates Analysis (PCoA) of unweighted UniFrac in conjunction with non-parametric multivariate ANOVA showed a significant difference in overall microbiota composition between patients with deletion and those with disomy (Online Supplementary Table 9). Moreover, phylogenetic diversity, triglycerides and serum insulin were important variables explaining the compositional variation in the microbiota of PWS patients with deletion and disomy, each of these factors explaining more than 10% of the total variation (Online Supplementary Table 9). We also observed a trend for negative correlation between TG and phylogenetic diversity (Spearman's $Rho=-0.52$, $p=0.084$).

As we observed increased triglyceride levels in patients with deletion (Online Supplementary Table 1), we tested the relationship between microbiota compositional variation, genotype and triglyceride levels. Our analyses showed that the difference in overall microbiota composition between patients with deletion and those with disomy could be attributed primarily to the difference in serum triglycerides. Serum triglyceride was a stronger factor for microbiota compositional variation than genotype (Online Supplementary Table 9). These analyses point to a possible link between composition and richness of the gut microbiota and serum lipids in PWS subjects.

The improved insulin resistance of PWS compared to OC patients is transferred by gut microbiota transplantation into germ-free mice

Since we observed significant links between metabolic variables and the faecal microbiota of the PWS and OC subjects, we examined the impact of the gut microbiota on host metabolism by faecal microbiota transplantation (FMT) in germ-free mice. We selected stools from BFMI-matched PWS and OC donor pairs based on clinical parameters (Table 1, Online Supplementary Table 10), and faecal microbiota profile (Online Supplementary Table 10). The OC donors were specifically lacking, or had low abundance of, Archaea, Tenericutes, *Akkermansia*, Christensenellaceae and microbial taxa significantly contributing to the specific structures of the PWS and OC microbiota (Online Supplementary Table 10 and Supplementary Figure 2). Faecal microbiota from a PWS patient with deletion was used in the first donor pair. Fourteen days after colonisation recipient mice colonised with OC and PWS faecal microbiota did not differ in body weight, gonadal fat weight, adipocyte size distribution, fat mass/lean mass ratio or liver weight (figure 3A–E). However, mice transplanted with PWS microbiota had improved insulin tolerance compared with mice transplanted with OC microbiota (figure 3F–G). Fasting glucose levels, glucose tolerance, fasting insulin levels and insulin secretion in response to glucose were similar between the groups (figure 3H–K). Systemic levels of IL-6 did not differ between the groups (figure 3L) while the levels of $TNF\alpha$ were below the detection limit.

To confirm the observed difference in insulin tolerance, a second FMT experiment was conducted with a new donor pair (this time with a PWS patient with disomy) and ITT was performed 14 days after colonisation. In agreement with the first experiment, we confirmed that the recipient groups had similar body weight and fasting glucose levels (Online Supplementary Figure 3A–B) but mice transplanted with PWS faecal microbiota

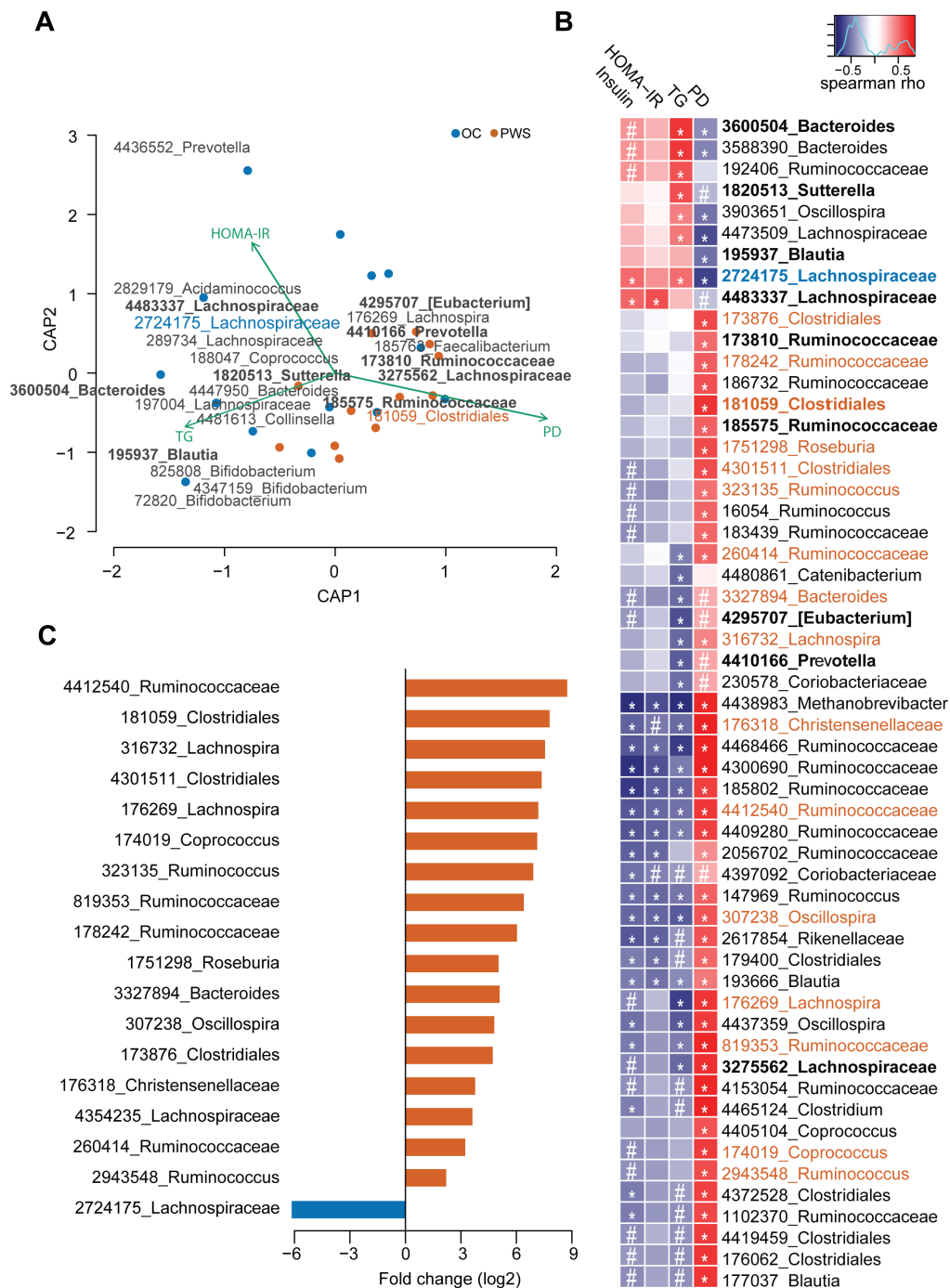


Figure 2 Features of the gut microbiota in Prader-Willi syndrome (PWS) patients. (A) Distance-based redundancy analysis for β -diversity dissimilarity distances and clinical as well as microbiota related factors that best explain the variation of gut microbiota composition between PWS and obese control (OC) samples (ie, HOMA-IR, serum triglycerides (TG) and phylogenetic diversity (PD)). The statistical model with these variables constrains 25.4% of the compositional variation ($p < 0.001$), with significance only for the first constrained principal coordinate (CAP1) ($p < 0.001$). Blue and red dots represent variance-reduced microbiota composition by the first two constrained principal coordinates (CAP1 and CAP2) for OC and PWS samples, respectively. Green arrows indicate the clinical and microbiota related variables correlated to faecal microbiota compositional variation. Operational taxonomic units (OTUs) numbers indicate OTUs with the strongest correlations to CAP1; OTUs in bold indicate features significantly correlated to clinical and microbiota related variables by the Spearman's test. OTUs 2724175 and 181059, indicated in blue and red colour, respectively, show OTUs with significant Spearman's correlations as well as significant differential abundance in OC (2724175) versus PWS (181059) samples, as shown in panels (B) and (C). (B) Significant Spearman's correlations between clinical parameters, PD and microbial OTUs. The red-blue colour scale indicates the direction and intensity of Spearman's correlations, with red and blue colours showing positive and negative correlations, respectively. # $p < 0.1$; * $p < 0.05$; exact values are indicated in online supplementary table 7. (C) \log_2 fold change of the abundance of the significantly enriched or depleted OTUs in OC and PWS samples (Wilcoxon rank sum test with significant level of 0.05 after adjustment for multiple testing). Red and blue colours indicate OTUs with significantly different abundance increased or decreased in PWS and OC samples, respectively. The analyses were performed on a subset of 12 PWS and 140C samples for which all clinical parameters were available. Samples with missing values in the clinical data were excluded from the analyses. HOMA-IR, homeostatic model assessment of insulin resistance.

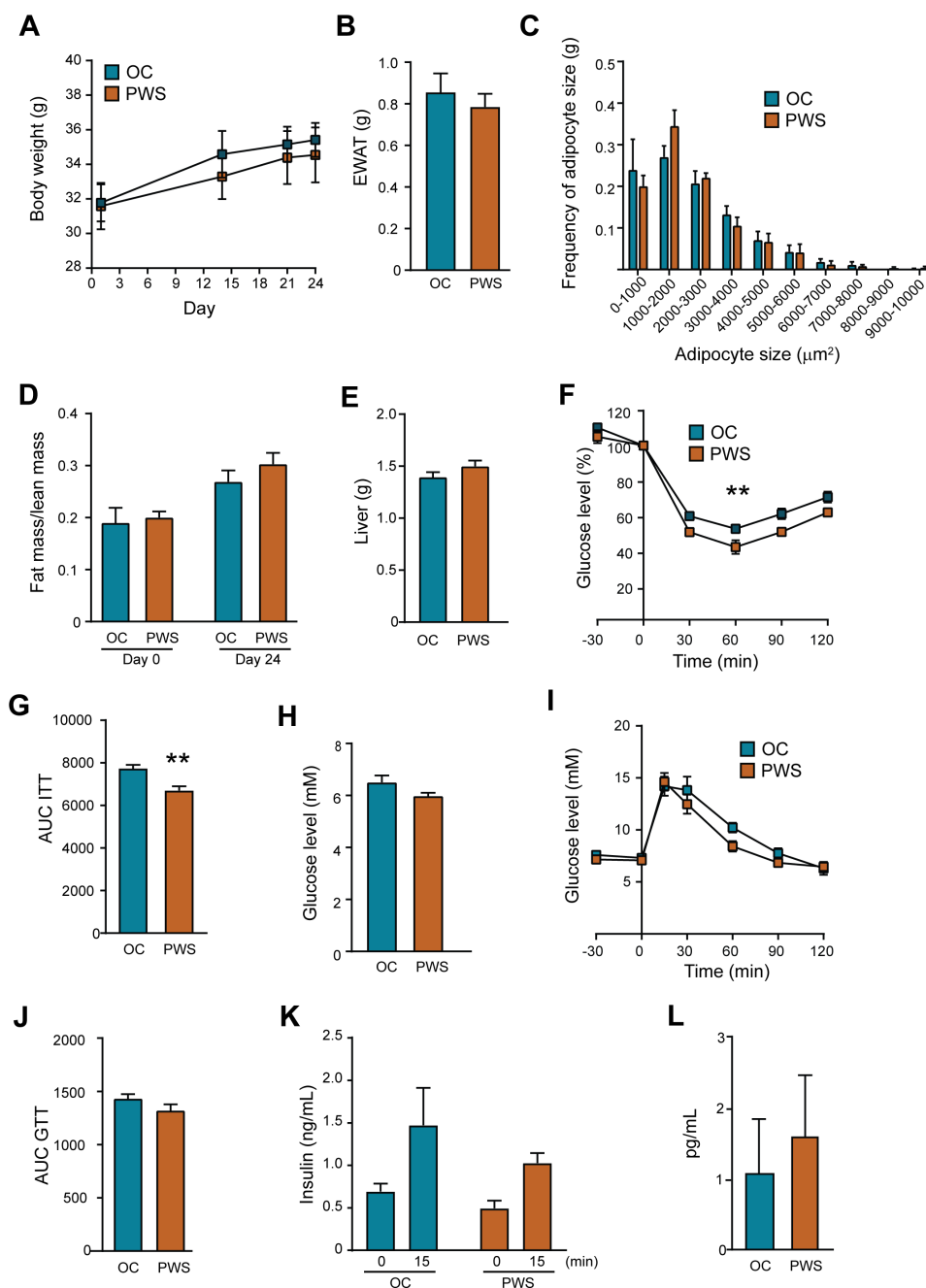


Figure 3 The microbiota of Prader-Willi syndrome (PWS) patients promotes improved insulin tolerance after transfer to germ-free mice. Phenotype of mice transplanted with microbiota from an obese control (OC) and a PWS donor (donor-pair 1, see online supplementary table 10). (A) Body weight gain after colonisation, (B) epididymal white adipose (EWAT) weight, (C) distribution of adipocyte cell sizes, (D) fat/lean mass ratio, (E) liver weight, (F and G) insulin tolerance, (H) fasting glucose, (I and J) glucose tolerance, (K) fasting insulin and insulin 15 min after glucose injection, (L) interleukin 6 levels. Mean±SEM are plotted. $n=7$ to 8 mice per group. ** $p<0.01$ according to two-way analysis of variance for repeated measurement (panel F) and t-test (panel G). AUC, area under the curve; GTT, glucose tolerance test.

had improved insulin tolerance (Online Supplementary Figure 3B-C).

The transmission of faecal microbiota from human donors to recipient mice was evaluated by 16S rRNA gene sequencing. Recipients of PWS microbiota had a tendency towards higher phylogenetic diversity than mice transplanted with OC microbiota (and figure 4A and online Supplementary Figure 4A), in agreement with the higher microbiota species diversity observed in PWS donors. PCoA analysis based on unweighted UniFrac showed similar overall composition for the microbiota of recipient mice with their human donors (figure 4B,C and Online

Supplementary Figure 4B, C), indicating that the variance in microbiota composition between the mice was due to the difference in microbiota composition between PWS and OC donors. Interestingly, we observed that five OTUs that were shown as important for the characterisation of the PWS and OC microbiota were captured as differentially abundant in the recipient mice (Online Supplementary Table 10). In particular, in the first FMT experiment we observed a significant increased abundance of OTU 2724175 (*R. gnavus*) in OC recipients, and of OTU 3327894 (*B. uniformis*) as well as OTU 819353 (identified as *Flintibacter butyricus* by Basic Local Alignment Search

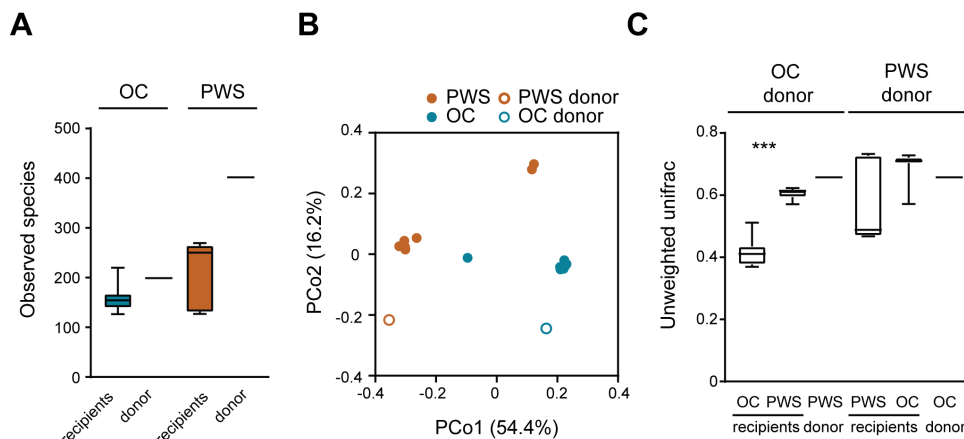


Figure 4 Microbiota composition in recipient mice after faecal microbiota transplantation. Data refers to donor-pair 1 (see online supplementary table 10). (A) α -diversity expressed as number of observed species in obese control (OC) and Prader-Willi syndrome (PWS) donors and in recipient mice. (B) Principalcoordinates (Pco) analysis of OC and PWS donors and mouse recipients. (C) Unweighted UniFrac distance between human donors and mouse recipients and between OC and PWS human donors. Mean \pm SEM are plotted. $n=7$ to 8 mice per group. *** $p<0.001$.

Tool (BLAST) analysis; Online Supplementary Table 7-8) in PWS recipients. Interestingly, OTU 3327894 (*B. uniformis*) was detected in all PWS mice but not in OC recipients (Online Supplementary Table 10). In the second FMT experiment we observed significantly increased abundance of OTU 195937 (*B. luti*) in OC recipients while OTU 2724175 (*R. gnavus*) was poorly transmitted from the donors and was only recovered in the OC recipients. These results indicate that the specific configurations of the PWS and OC microbiota could play important roles for insulin tolerance in the recipient mice, although further explorations are required to understand the specific role of these microbial taxa.

DISCUSSION

Here, we demonstrate that the faecal microbiota of adults with Prader-Willi syndrome significantly differs from that of obese subjects with common obesity matched for BFMI and per cent fat mass. Overall, our analyses on the PWS and OC microbiota matched for BFMI indicate an important link between intestinal microbes and metabolic markers such as serum triglycerides, insulin and HOMA-IR. Moreover, based on gut microbiota transfer experiments, we hypothesise that the specific configuration of the PWS gut microbiota may be a factor contributing to better insulin tolerance in the host, independently of fat mass.

To perform this study, we exploited a cohort of well-phenotyped PWS subjects and confirmed that after matching to BFMI, PWS patients had improved insulin resistance compared with OC subjects.^{13–16,27} PWS has been associated with decreased proportion of android fat,^{16,27–29} a phenotype linked to a healthier metabolic profile.³⁰ In our cohort the PWS patients had a tendency towards increased truncal fat, indicating that fat distribution may not be the only driving factor of improved insulin resistance in PWS.

In a previous study, it was shown that PWS subjects with deletion had increased BMI and fat mass when compared with subjects with the disomy.²¹ In our patient group the differences in gut microbiota composition between patients with deletion and patients with disomy was correlated by difference in serum triglycerides, although the number of patients was low in the disomy group ($n=5$). This observation needs further examination in an extended group of subjects.

Main results of our study are that the faecal microbiota of PWS patients had higher phylogenetic diversity and showed

different overall composition compared with the microbiota of OC subjects, with a profile in PWS that was not different from that of their non-obese parents. The only study that has previously investigated the gut microbiota of PWS patients comprises a cohort of Chinese children.³¹ In this study neither the gut microbiota composition, nor metabolic parameters, did differ between PWS patients and age-matched OC children. The discrepancy between this study and ours may be due to differences in age and ethnicity of the patients included, as well as the fact that we carefully matched our patient group for BFMI. It is particularly interesting to observe that the richness and composition of the PWS microbiota in our cohort was similar to that of their non-obese parents, possibly showing that PWS patients have a gut microbiota structure comparable to that of a non-obese population and/or that environmental conditions are important in shaping the gut microbiota.

Several studies have shown that microbiota richness, both in terms of species and microbial genes, is positively associated with metabolic health, such as improved glucose regulation and decreased obesity and inflammation.^{7,23,32} These associations are observed not only in obese/overweight subjects³³ but also in subjects with severe forms of obesities, as we previously have described.⁵

Many of the microbial genera with increased abundance in the PWS microbiota have consistently been associated with healthy metabolic profiles by several independent studies. This is the case for *Akkermansia*, and in particular *Akkermansia muciniphila*, which is decreased in obesity and diabetes,^{34,35} and which supplementation improves metabolism in experimental models.^{36,37} Similarly, *Christensenella*, *Methanobrevibacter smithii* and the Tenericutes phylum that were increased in PWS (ML615J-28 and RF39) can co-occur and be enriched in individuals with low body mass index, as described in a large twin study.³⁸ In addition, the abundance of *M. smithii* has been recently found to correlate negatively with the percentage of visceral fat.³⁹

Another large population study reported that *Akkermansia*, Christensenellaceae, the Tenericutes RF39 and Rikenellaceae are associated with lower serum triglycerides levels in addition to low BMI.⁴⁰ Interestingly, we found that the increase of the Archaea *Methanobrevibacter* and *vadinCA11*, and the decrease of the genus *Dorea*, are specifically associated with PWS, and not dependent on BFMI. *VadinCA11* has previously been found to be the second most prevalent methanogen in the human gut

and not to be mutually exclusive with the most prevalent methanogen *M. smithii*.³⁸ As we observed strong negative correlations between OTUs related to *M. smithii* and serum triglycerides and insulin levels, we could propose a protective role for this specific methanogenic Archaea in insulin tolerance and more broadly metabolic health even in case of severe obesity.

In contrast, *Dorea* has been associated with obesity⁴¹ and abundance of BMI-predictive plasma metabolites, including glutamate and branched-chain amino acids.⁴² In particular, *D. longicatena* has been found to be positively correlated with circulating leptin and negatively correlated with circulating adiponectin levels, indicating possible important roles in adipose tissue physiology.⁴¹ Whereas we did not explore adipose tissue samples in this study we have previously shown that subcutaneous adipose tissue from PWS was less proinflammatory and profibrotic although with higher adipocyte size than that of commonly obese subjects suggesting an improved adipose tissue expandability in PWS subjects.¹⁶

Diet-induced obesity results in the degradation of the outer mucus layer of the epithelium.³⁷ Several intestinal microbes distributed in diverse bacterial phyla have been characterised as mucin-degraders.⁴³ However, while the activity of mucin-degraders such as *A. muciniphila* appears beneficial for intestinal health and host metabolism,⁴⁴ mucus degradation by other bacteria such as *R. gnavus*⁴⁵ and *B. vulgatus* has been associated with opposite outcomes, including intestinal inflammation, inflammatory bowel diseases, obesity, metabolic disorders and prediabetes.⁴⁶ In addition, *B. vulgatus* is an opportunistic pathogen, present in many anaerobic infections and associated Crohn's disease.⁴⁷ OTUs 3600504 (*B. vulgatus*) and 2724175 (*R. gnavus*) were important features of the OC microbiota in our study. This observation is also in agreement with our previous report showing negative association between *B. vulgatus* with gut microbial richness and markers of insulin resistance in severe obesity.⁵ In line with these previous findings, we here show that these OTUs strongly contributed to overall community compositional variability, negatively correlated to microbiota phylogenetic diversity and positively correlated to serum triglycerides and insulin levels.

In addition to the possible negative impact of bacteria such as *B. vulgatus* and *R. gnavus*, we observed several microbial taxa beneficially associated with serum triglycerides, insulin and HOMA-IR. This includes *B. uniformis*, which has been previously identified as an important feature of the microbiota of lean subjects in a Chinese population.⁴¹ We observed beneficial associations also for several taxa with a poor taxonomic affiliation in the Clostridiales family, known to contain important butyrate producers. Depletion of butyrate-producing bacteria is a consistent feature of type 2 diabetes, prediabetes as well as severe obesity, as indicated by several independent studies.^{5 22 23 32} Here, we observed an important role for *F. prausnitzii* in the compositional variation between PWS and BFMI-matched OC microbiota, and significant enrichment of butyrate producers such as *E. eligens* and *Flintibacter butyricus*,⁴⁸ which displayed consistent negative correlations with serum triglycerides and insulin levels. Therefore, our results confirm the major importance of bacterial butyrate producers for metabolic regulation and a healthy gut, in line with observations that microbial butyrate production is an important modulator of intestinal inflammation.⁴⁹

We acknowledge some limitations of the study. The recruitment strategy applied may prevent us from extrapolate the results to all patients with PWS, which is a phenotypically heterogeneous group. Moreover, we are lacking assessment of food consumption, which is very difficult to obtain in patients

with PWS. This would have been very informative and a possible link to the differences in the microbiota identified.

Faecal microbiota transplantation to germ-free mice resulted in improved insulin tolerance in recipients of PWS microbiota, which demonstrated that the microbiota of PWS patients and OC subjects not only differs in composition, but also has the functional potential to impact host metabolism. The ability of the gut microbiota to directly modulate insulin sensitivity has been demonstrated through faecal microbiota transfers in humans, where transfer of microbiota from healthy subjects to patients with metabolic syndrome improves insulin sensitivity.⁵⁰ In our study, donors were not randomly selected but chosen based on the abundance of key microbial taxa that characterised the PWS faecal microbiota. Differences between the donors in key features of the microbiota such as diversity and overall composition were preserved in the recipient mice, and we observed successful transfer of both OC-associated and PWS-associated microbial taxa. Importantly, PWS recipient mice displaying insulin tolerance were colonised by *Flintibacter* as well as *B. uniformis*, but not with *R. gnavus* and *B. luti*, which were more abundant and prevalent in the OC recipient mice. Therefore, our results indicate that structural and functional features of the microbiota, including phylogenetic diversity but also mucus degradation properties and butyrate production might be important for the transmission of the host phenotype. However, markers of systemic inflammation did not differ between the recipient groups and the mechanisms underlying the difference in insulin tolerance cannot be determined from the performed experiments.

In conclusion, we show that despite severe obesity, PWS gut microbiota profiles differ from common obesity, but are not different from those of their non-obese parents. PWS gut microbiota strongly associate with indexes of metabolic health, a phenotype that could partly be transmitted in axenic animals. Future studies should explore whether the presence of methanogenic Archaea, butyrate production and intestinal mucus metabolism that characterise the PWS gut microbiota, causally explain the improved lipid profiles and insulin tolerance, and whether they play a role in the protection from metabolic consequences of obesity.

Author affiliations

¹The Wallenberg Laboratory, Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

²INSERM, Nutrition and obesity; systemic approaches (NutriOmics) research Unit, Sorbonne Université, Paris, Île-de-France, France

³Assistance Publique-Hôpitaux de Paris, Reference Center for Rare Diseases (Prader-Willi Syndrome), Nutrition Department, University Hospital Pitié Salpêtrière, Paris, Île-de-France, France

⁴Novo Nordisk Foundation Center for Basic Metabolic Research, Section for Metabolic Receptology and Enteroendocrinology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Acknowledgements We thank Louise Helldén, Carina Arvidsson, Antonio Molinaro, Manuela Krämer, Robert Jakubowicz and Anna Hallén for technical assistance. We also thank Dr Hichem Gahouti (obese control phenotyping) and Valentine Lemoine for their help in the clinical investigation of PWS and their parents (Pitié-Salpêtrière hospital, ICAN and nutrition department). The clinical investigations were performed at the Reference Center of Rare Diseases (Nutrition Department) and at the Human Nutrition Research Center (CRNH Ile de France), Pitié-Salpêtrière Hospital. The computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under Project SNIC 2018-3-350.

Contributors KC, RC, FB, VT and CP designed and directed the project; RC, CP, MC, LO, JAW, LH, CA, AM, MK, RJ, AH, HG and VL performed the experiments; LO, VT and RC analysed data; RC, LO, VT, KC and CP wrote the article with inputs from FB and MC.

Funding This study was supported by several sources: European Union, FP7 Health (METACARDIS) (HEALTH-F4-2012-30531), Svenska Forskningsrådet Formas (2017-01996_3 and 2017-02001), Foundation Leducq (17CVD01), Agence Nationale de la Recherche (ANR-11-DPBS-0001 and ANR-10-IAHU-05), the Swedish Research Council (Vetenskapsrådet) and grants from the Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (ALFGBG- 718101). FB is Torsten Söderberg Professor in Medicine and recipient of an ERC Consolidator Grant (European Research Council, Consolidator grant 615362-METABASE).

Competing interests FB is in the Scientific Advisory Board of MetaboGen, Sweden.

Patient consent for publication Not required.

Ethics approval All animal procedures were approved by the Gothenburg Animal Ethics Committee (152–2015).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement 16S data are available at European Nucleotide Archive (accession number: PRJEB33908; <http://www.ebi.ac.uk/ena/data/view/PRJEB33908>)

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Lisa M Olsson <http://orcid.org/0000-0001-9730-1915>

Robert Caesar <http://orcid.org/0000-0003-1017-2933>

REFERENCES

- Jacobini C, Pugliese G, Blasetti Fantauzzi C, et al. Metabolically healthy versus metabolically unhealthy obesity. *Metabolism* 2019;92:51–60.
- Rohde K, Keller M, la Cour Poulsen L, et al. Genetics and epigenetics in obesity. *Metabolism: clinical and experimental* 2018.
- Bäckhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 2004;101:15718–23.
- Rabot S, Membrez M, Bruneau A, et al. Germ-free c57bl/6j mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. *The FASEB Journal* 2010;24:4948–59.
- Aron-Wisniewsky J, Prifti E, Belda E, et al. Major microbiota dysbiosis in severe obesity: fate after bariatric surgery. *Gut* 2019;68:70–82.
- Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature* 2009;457:480–4.
- Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013;500:541–6.
- Sze MA, Schloss PD. Looking for a signal in the noise: revisiting obesity and the microbiome. *MBio* 2016;7:e01018–16.
- Ghosh S, Bouchard C. Convergence between biological, behavioural and genetic determinants of obesity. *Nat Rev Genet* 2017;18:731–48.
- Montague CT, Farooqi IS, Whitehead JP, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 1997;387:903–8.
- Farooqi IS, O'Rahilly S. Monogenic obesity in humans. *Annu Rev Med* 2005;56:443–58.
- Butler MG. Prader-will syndrome: obesity due to genomic imprinting. *Current genomics* 2011;12:204–15.
- Talebizadeh Z, Butler MG. Insulin resistance and obesity-related factors in Prader-Willi syndrome: comparison with obese subjects. *Clin Genet* 2005;67:230–9.
- Faienza MF, Ventura A, Lauciello R, et al. Analysis of endothelial protein C receptor gene and metabolic profile in prader-will syndrome and obese subjects. *Obesity* 2012;20:1866–70.
- Haqq AM, Muehlbauer MJ, Newgard CB, et al. The metabolic phenotype of Prader-Willi syndrome (PWS) in childhood: heightened insulin sensitivity relative to body mass index. *J Clin Endocrinol Metab* 2011;96:E225–E232.
- Lacroix D, Moutel S, Coupaye M, et al. Metabolic and adipose tissue signatures in adults with Prader-Willi syndrome: a model of extreme adiposity. *J Clin Endocrinol Metab* 2015;100:850–9.
- Haqq AM, Muehlbauer M, Svetkey LP, et al. Altered distribution of adiponectin isoforms in children with Prader-Willi syndrome (PWS): association with insulin sensitivity and circulating satiety peptide hormones. *Clin Endocrinol* 2007;67:944–51.
- Touch S, Assmann KE, Aron-Wisniewsky J, et al. Mucosal-associated invariant T (MAIT) cells are depleted and prone to apoptosis in cardiometabolic disorders. *Faseb j*;2018.
- Liu P, Ma F, Lou H, et al. The utility of fat mass index vs. Body mass index and percentage of body fat in the screening of metabolic syndrome. *BMC Public Health* 2013;13:629.
- Kyle UG, Schutz Y, Dupertuis YM, et al. Body composition interpretation. Contributions of the fat-free mass index and the body fat mass index. *Nutrition* 2003;19:597–604.
- Coupaye M, Tauber M, Cuisset L, et al. Effect of genotype and previous GH treatment on adiposity in adults with Prader-Willi syndrome. *J Clin Endocrinol Metab* 2016;101:4895–903.
- Karlsson FH, Tremaroli V, Nookaew I, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 2013;498:99–103.
- Allin KH, Tremaroli V, Caesar R, et al. Aberrant intestinal microbiota in individuals with prediabetes. *Diabetologia* 2018;61:810–20.
- R Core Team. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, 2018.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B* 1995;57:289–300.
- Oksanen J, Blanchet FG, Kindt R, et al. *Vegan: Community ecology package*, 2015.
- Lloret-Linares C, Faucher P, Coupaye M, et al. Comparison of body composition, basal metabolic rate and metabolic outcomes of adults with Prader-Willi syndrome or lesional hypothalamic disease, with primary obesity. *Int J Obes* 2013;37:1198–203.
- Theodoro MF, Talebizadeh Z, Butler MG. Body composition and fatness patterns in Prader-Willi syndrome: comparison with simple Obesity*. *Obesity* 2006;14:1685–90.
- Sode-Carlson R, Farholt S, Rabben KF, et al. Body composition, endocrine and metabolic profiles in adults with Prader-Willi syndrome. *Growth Hormone & IGF Research* 2010;20:179–84.
- Després J-P, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006;444:881–7.
- Zhang C, Yin A, Li H, et al. Dietary modulation of gut microbiota contributes to alleviation of both genetic and simple obesity in children. *EBioMedicine* 2015;2:968–84.
- Forslund K, Hildebrand F, Nielsen T, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* 2015;528:262–6.
- Cotillard A, Kennedy SP, Kong LC, et al. Dietary intervention impact on gut microbial gene richness. *Nature* 2013;500:585–8.
- Dao MC, Everard A, Aron-Wisniewsky J, et al. *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* 2016;65:426–36.
- Yassour M, Lim MY, Yun HS, et al. Sub-clinical detection of gut microbial biomarkers of obesity and type 2 diabetes. *Genome Med* 2016;8:17.
- Plovier H, Everard A, Druart C, et al. A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med* 2017;23:107–13.
- Everard A, Belzer C, Geurts L, et al. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A* 2013;110:9066–71.
- Goodrich JK, Waters JL, Poole AC, et al. Human genetics shape the gut microbiome. *Cell* 2014;159:789–99.
- Visconti A, Le Roy CI, Rosa F. *Interplay between the human gut microbiome and host metabolism*, 2019.
- Soret R, Chevalier J, De Coppet P, et al. Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in rats. *Gastroenterology* 2010;138:1772–82.
- Liu R, Hong J, Xu X, et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med* 2017;23:859–68.
- Ottosson F, Brunkwall L, Ericson U, et al. Connection between bmi-related plasma metabolite profile and gut microbiota. *J Clin Endocrinol Metab* 2018;103:1491–501.
- Tailford LE, Crost EH, Kavanaugh D, et al. Mucin glycan foraging in the human gut microbiome. *Front Genet* 2015;6:81.
- Belzer C, Chia LW, Aalvink S, et al. Microbial Metabolic Networks at the Mucus Layer Lead to Diet-Independent Butyrate and Vitamin B Production by Intestinal Symbionts. *MBio* 2017;8.
- Crost EH, Tailford LE, Monestier M, et al. The mucin-degradation strategy of *Ruminococcus gnavus*: The importance of intramolecular trans-sialidases. *Gut Microbes* 2016;7:302–12.
- Hall AB, Yassour M, Sauk J, et al. A novel ruminococcus gnavus clade enriched in inflammatory bowel disease patients. *Genome Med* 2017;9:103.
- Wexler HM. Bacteroides: the good, the bad, and the Nitty-Gritty. *Clin Microbiol Rev* 2007;20:593–621.
- Lagkouvardos I, Pukall R, Abt B, et al. The mouse intestinal bacterial collection (miBC) provides host-specific insight into cultured diversity and functional potential of the gut microbiota. *Nat Microbiol* 2016;1.
- Byndloss MX, Olsan EE, Rivera-Chávez F, et al. Microbiota-activated PPAR-γ signaling inhibits dysbiotic enterobacteriaceae expansion. *Science* 2017;357:570–5.
- Koortte RS, Levin E, Salojärvi J, et al. Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metab* 2017;26:611–9.