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Research article

Microbiome profiling reveals gut dysbiosis in a transgenic mouse model of Huntington's disease

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ABSTRACT

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a trinucleotide repeat expansion in the *huntingtin* (HTT) gene, which is expressed ubiquitously throughout the brain and peripheral tissues. Whilst the focus of much research has been on the cognitive, psychiatric and motor symptoms of HD, the extent of peripheral pathology and its potential impact on central symptoms has been less intensely explored. Disruption of the gastrointestinal microbiome (gut dysbiosis) has been recently reported in a number of neurological and psychiatric disorders, and therefore we hypothesized that it might also occur in HD. We have used 16S rRNA amplicon sequencing to characterize the gut microbiome in the R6/1 transgenic mouse model of HD, relative to littermate wild-type controls. We report that there is a significant difference in microbiota composition in HD mice at 12 weeks of age. Specifically, we observed an increase in Bacteriodetes and a proportional decrease in Firmicutes in the HD gut microbiome. In addition, we observed an increase in microbial diversity in male HD mice, compared to wild-type controls, but no differences in diversity were observed in female HD mice. The gut dysbiosis observed coincided with impairment in body weight gain despite higher food intake as well as motor deficits at 12 weeks of age. Gut dysbiosis was also associated with a change in the gut microenvironment, as we observed higher fecal water content in HD mice at 12 weeks of age. This study provides the first evidence of gut dysbiosis in HD.

1. Introduction

Huntington's disease (HD) is a progressive neurodegenerative disorder involving cognitive, psychiatric and motor symptoms. It is caused by the trinucleotide (CAG) repeat expansion in the *huntingtin* (HTT) gene, which is widely expressed in various cell types throughout the brain and peripheral tissues including the skeletal muscles, heart, and gut (Moffitt et al., 2009; Sathasivam et al., 1999; Sharp et al., 1995; The Huntington's Disease Collaborative Research Group, 1993). The expression of this mutant protein leads to a cascade of molecular dysregulation and cellular dysfunction. HD patients typically exhibit motor, cognitive and affective symptoms which are highly comorbid with skeletal muscle atrophy, progressive weight loss, altered metabolic homeostasis and gastrointestinal (GI) dysfunction (Andrich et al., 2009; van der Burg et al., 2011). Additionally, the presence of the mutant protein in the enteric nervous system, as well as intestinal epithelial cells along the GI tract, leads to the loss of neuropeptides in the gut, which are associated with abnormalities in gut function (van der Burg et al., 2011).

The mammalian GI tract hosts a complex ecosystem of microbes which work symbiotically with the host. It has been established that gut microbiota are key modulators for the bi-directional communication between the gut and the brain, also known as the gut-brain-axis. Intestinal microbiota have been shown to influence neurodevelopment, brain function and behaviour (Clarke et al., 2013; Hoban et al., 2017; Ogbonnaya et al., 2015; Stilling et al., 2015). The undesirable shift in microbiota composition, also known as gut dysbiosis, has been associated with the development of various gastrointestinal and metabolic diseases, including inflammatory bowel disease (IBD), obesity and diabetes (Greenblum et al., 2012; Qin et al., 2012; Turnbaugh et al.,

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2006). Moreover, gut dysbiosis has been reported in several neurological and psychiatric illnesses, including autism spectrum disorder, major depression, Parkinson's disease (PD) and Alzheimer's disease (AD) (Foster and Neufeld, 2013; Golubeva et al., 2017; Scheperjans et al., 2015; Vogt et al., 2017). Notably, numerous reports have shown that gut dysbiosis is not just a comorbidity but could also play a role in modulating the physiological, behavioural, and motor abnormalities in neurological diseases (Hsiao et al., 2013; Sampson et al., 2016; Zheng et al., 2016).

To date, there are no studies exploring the gut microbiota composition in HD. However, there are several lines of evidence pointing towards possible gut dysbiosis in HD. Notably, circulating gut microbiota-derived metabolites were altered in HD patients and transgenic animals, hinting that gut microbiota could be altered before the onset of the disease (Beal et al., 2006; Verwaest et al., 2011). Furthermore, weight loss, characteristic symptom in HD, could be affected by gastrointestinal dysfunction (van der Burg et al., 2011). In the present study, we aimed to characterize the gut microbiome of the R6/1 transgenic mouse model of HD. We hypothesized that the gut microbiome of HD mice would be different to WT mice with respect to bacterial diversity and taxonomic composition.

2. Materials and methods

2.1. Animal husbandry

Hemizygous R6/1 males were crossed with F1 (CBA x C57Bl/6) females to generate wild-type (WT) and R6/1 (hereafter referred to as HD) littermates. The animals were co-housed according to genotype, due to the coprophagic nature of mice, which could affect the gut microbiota. For male mice, there were a total of 10 WT mice, split between 3 cages, and 7 HD mice, split between 2 cages. For female mice, there were a total of 7 WT mice, split between 2 cages, and 11 HD mice, split between 3 cages. Animals had ad libitum access to water and chow in a temperature (22 °C) and humidity (45%) controlled room, with a 12 h light/dark cycle (lights on at 0700 h). Cage changes were conducted weekly and body weight was measured weekly. All experiments were approved by the Florey Institute of Neuroscience and Mental Health Animal Ethics Committee and performed in accordance with the animal research guidelines of the National Health and Medical Research Council.

2.2. Fecal sample collection for 16S rRNA sequencing

To collect fecal samples at 12 weeks of age, individual mice were placed in clean cages for 5–10 min. Fresh pellets were collected and immediately frozen with liquid nitrogen and stored at −80 °C until further processing. The fecal pellets were homogenised (FastPrep24) and genomic DNA was extracted (GenFind v2). The V4 hypervariable region (515–806) of 16S rRNA was amplified in quadruplicate, pooled and sequenced on the Illumina Mini-Seq platform (2 × 150 bp).

2.3. Food and water intake measurement

At 12 weeks of age, food and water intake was measured over the period of 72 h where the amount of consumed food and water was measured by weighing the portion in the beginning and at the end of the period.

2.4. Fecal output and fecal water content measurement

The fecal output and fecal water content were monitored once a week from 8 to 12 weeks of age. Briefly, animals were single-caged for 1 h and the number of fecal pellets expelled was counted. All the fecal pellets were collected and the total weight was measured before being dried at 95 °C for > 3 h. The percentage of difference between the initial total feces weight and the dry weight is taken as fecal water content.

2.5. 16S rRNA sequencing, bioinformatics and statistical analysis

Illumina Mini-Seq raw data were paired, demultiplexed and processed using Quantitative Insights into Microbial Ecology 2 (QIIME 2) pipeline (Caporaso et al., 2010). Briefly, 16S rRNA bacterial partial sequences were quality trimmed and reads were binned into operational taxonomic units (OTUs) using DADA2 default parameters in QIIME2 v2017.11 (Callahan et al., 2016). The representative sequence for each phylotype was aligned against Greengenes core set and a phylogenetic tree with midpoint root was constructed using FastTree (Desantis et al., 2006; Price et al., 2010). OTUs with < 10 frequencies in total were removed. Samples with < 4000 reads were also removed. The final number of samples for the subsequent analysis was 7–8 mice per group (7 mice in the male HD group and female WT group; 8 mice in both the male WT group and female HD group), with a total of 368 OTUs. Reads were rarefied to 10,000 reads to calculate several alpha-diversity metrics, including species richness (Observed), Shannon and Inverse Simpson metrics, using the 'Phyloseq' R package (Beal et al., 2006). Species richness (Observed) is the number of OTUs observed in a given sample. Shannon's and Inverse Simpson diversity index is a composite measure of richness (number of OTUs present) and evenness (relative abundance of OTUs). Kruskal Wallis test was used to compare the species richness and alpha-diversity measurements between the disease phenotypes.

The counts were normalized to their relative abundance by dividing raw counts from a particular sample by the total number of reads in each sample, before applying centered log-ratio (CLR) transformation for compositional data. To estimate beta-diversity, Bray-Curtis and unweighted UniFrac distances were calculated and used in principal coordinates analysis (PCoA). The unweighted UniFrac distance accounts for the phylogenetic relationship between the OTUs whereas the Bray-Curtis distance accounts for the abundance of the OTUs. To determine whether the visually observed differences were statistically significant, Adonis (Permutation multivariate ANOVA) from the 'vegan' R package was performed with 999 permutations (Anderson, 2001; Dixon, 2009). The R^2 value reported by Adonis indicates the amount of variance, on a scale of 0 to 1, in the data which can be explained by the factors tested.

Comparisons at the phyla level were tested using non-parametric *t*-tests with false discovery rate (FDR) correction. Sparse PLS discriminant analysis (sPLS-DA) from the 'mixOmics' package in R was used to identify a signature of discriminative OTUs associated with either different sexes or the disease phenotype (Lê Cao et al., 2016; Rohart et al., 2017). To assess the effects of cage within sex group, we used a linear model on the proportional counts transformed with CLR, with cage as random effect and genotype as fixed effect.

To gain insight into the possible functional pathways that differ between groups, we used Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) v1.1.3 (Langille et al., 2013). Briefly, the OTUs were picked against Greengenes v13.5 and, following the PICRUSt pipeline, the contributions of various OTUs to known biological pathways were calculated according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. To estimate the accuracy of PICRUSt's prediction, the weighted Nearest Sequenced Taxon Index (NSTI) scores, which represents the phylogenetic distance for each OTU to its nearest sequenced reference bacterial genome, were calculated for each sample. OTUs that have no close sequenced relatives will make a larger contribution to the NSTI score, indicating low accuracy of PICRUSt predictions. The output from the PICRUSt pipeline was analysed similarly, using sPLS-DA, to identify the pathway signatures which distinguish the different groups.

To calculate body weight gain, body weights were normalized to the weights at 5 weeks of age. In the case of assessing the effects of

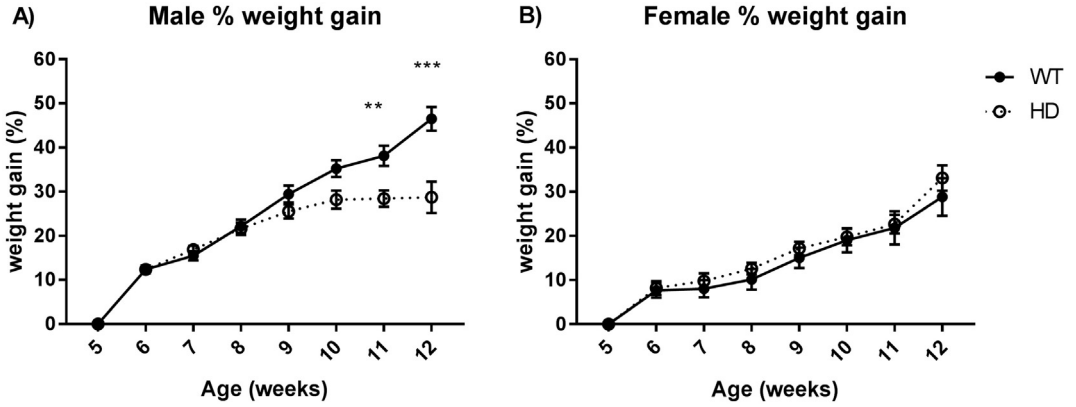

Fig. 1. Percentage of weight gain in R6/1 mice. (A) Male HD mice had decreased percentage of weight gain compared to WT, whereas there were no differences in weight gain in (B) female HD and WT. Data represents mean percentage of weight gain normalized to body weight at 5 weeks of age ± standard error mean (SEM) (**$p < 0.005$, ***$p < 0.0005$; $n = 7–11$).

genotype, age and their interactions on weight gain, fecal output as well as fecal water content, repeated measures two-way ANOVA tests were performed. To assess the effects of genotype on the food and water intake at 12 weeks of age, unpaired *t*-tests were performed. In all cases, significance level was set to 0.05.

3. Results

3.1. Reduction in weight gain despite higher food intake in male HD mice

Male HD mice had a reduction in weight gain compared to WT littermate controls beginning at 9 weeks of age and the reduction was significant at 11 and 12 weeks of age (Fig. 1A) ($p_{genotype} = 0.036$; $p_{age} < 0.0001$; $p_{interaction} < 0.0001$). There was no significant difference observed in weight gain for female HD mice compared to WT mice ($p_{genotype} = 0.529$; $p_{age} < 0.0001$; $p_{interaction} = 0.811$) (Fig. 1B). We then measured food and water intake at 12 weeks of age. There was a significant increase observed in food intake for male HD mice when compared to WT littermate controls, but no significant difference was observed in the female mice ($p_{male} = 0.019$, $p_{female} = 0.098$) (Fig. 2A). We also observed significantly higher water intake in both male and female HD mice when compared to WT littermates ($p_{male} = 0.002$, $p_{female} = 0.029$) (Fig. 2B).

3.2. Fecal output and fecal water content

We measured the fecal output of WT and HD mice (with the two genotypes housed in separate cages) from 8 to 12 weeks of age. A significant main effect of genotype ($p = 0.032$) was observed in the male mice but no significant effects of age or interaction were found on the fecal output per hour ($p_{age} = 0.438$; $p_{interaction} = 0.222$) (Fig. 3A). We found no significant effect of age, genotype or interaction between those two factors on fecal output per hour in female mice ($p_{age} = 0.036$; $p_{genotype} = 0.052$; $p_{interaction} = 0.462$) (Fig. 3B). In addition, we also measured the fecal water content of WT and HD mice from 8 to 12 weeks of age. In male mice, we found a significant age effect ($p = 0.024$), but no significant effect of genotype or interaction on the fecal water content ($p_{genotype} = 0.319$; $p_{interaction} = 0.907$) (Fig. 3C). For females, there were significant main effects of age and genotype ($p_{age} < 0.0001$; $p_{genotype} = 0.004$) but no significant effects of interaction were found on the fecal water content ($p_{interaction} = 0.201$) (Fig. 3D).

3.3. Gut dysbiosis in HD mice at 12 weeks of age

To characterize the microbiome, fecal samples were freshly collected by placing mice in clean cages for 5 min. The fecal samples were immediately flash frozen. The genomic DNA was extracted for 16S amplicon sequencing, followed by bioinformatics analyses.

When the entire population was examined together on a PCoA plot, using the Bray-Curtis distance, samples tended to cluster according to genotype as well as sex (Fig. 4). We firstly compared the effect of sex on each genotype separately. Significant differences were observed in the unweighted-unifrac distance (WT: $R^2 = 0.142$, $p = 0.019$; HD: $R^2 = 0.183$, $p = 0.004$) whereas no significant differences were observed in the Bray-Curtis distance between male and female mice (WT:

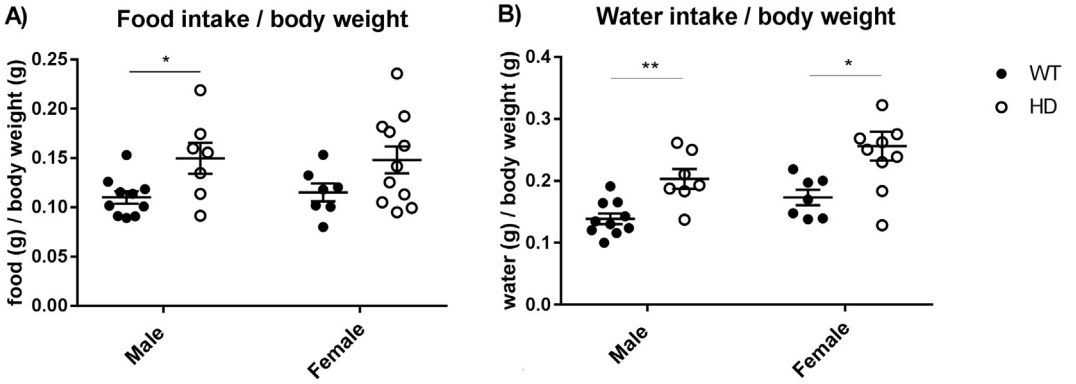

Fig. 2. (A) Food intake and (B) water intake of WT and HD mice at 12 weeks of age. Male HD mice had significantly higher food and water intake compared to WT mice at 12 weeks of age. Female HD were drinking more at 12 weeks of age compared to WT. Data represent average food and water intake per g of body weight ± SEM. (*$p < 0.05$, **$p < 0.01$, n = 7–11 mice).

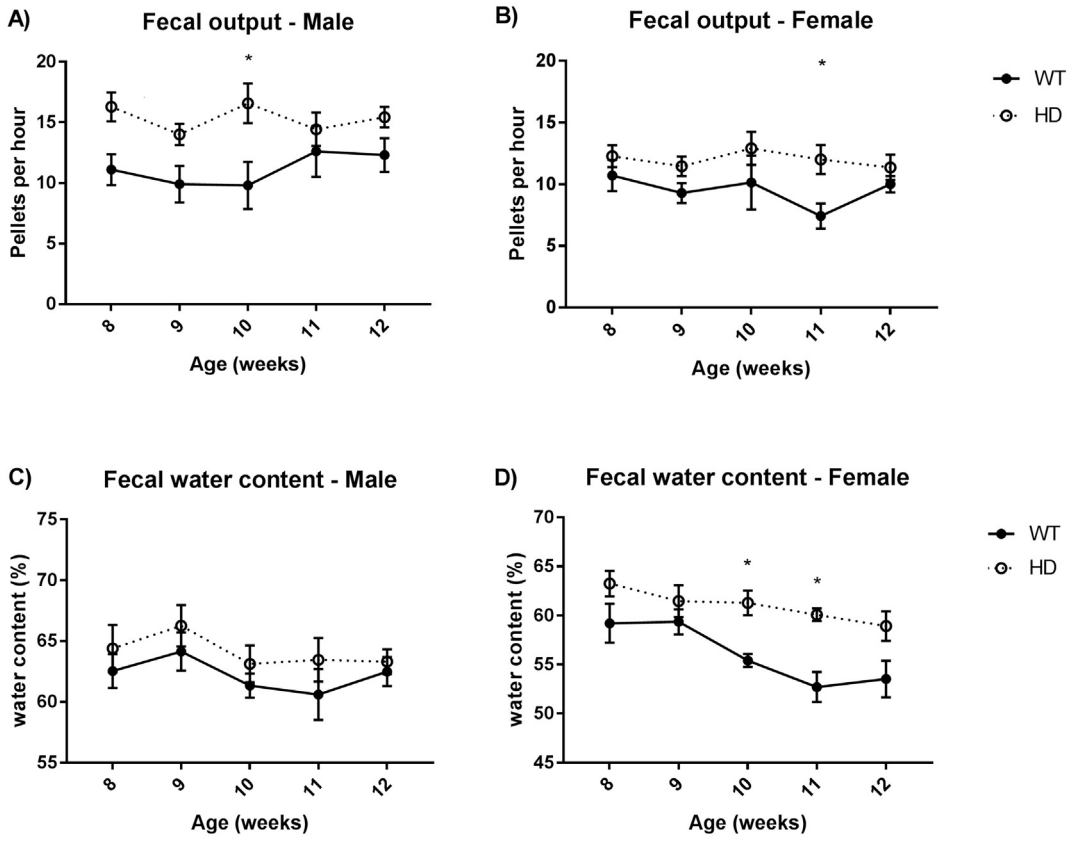

Fig. 3. (A,B) Fecal output and (C,D) fecal water content of WT and HD mice from 8 to 12 weeks of age. There was a significant main effect of genotype on the fecal output in males but no significant differences were found in the females. For fecal water content, there was a significant main effect of genotype observed in the females but not in the males. Values represent means ± SEM (*p < 0.05, n = 7–11 mice).

$R^2 = 0.1$, $p = 0.198$; HD: $R^2 = 0.118$, $p = 0.139$). We then tested whether microbiota composition differed between WT and HD. For both sexes, Bray-Curtis distance between HD and WT is significantly different (Male: $R^2 = 0.48$, $p = 0.001$; female: $R^2 = 0.39$, p = 0.001).

We then examined the bacterial composition and observed that, across all mice, the two dominant phyla are Bacteroidetes and Firmicutes, which together made up approximately 98% of total abundance. The remaining phyla were made up of Actinobacteria,

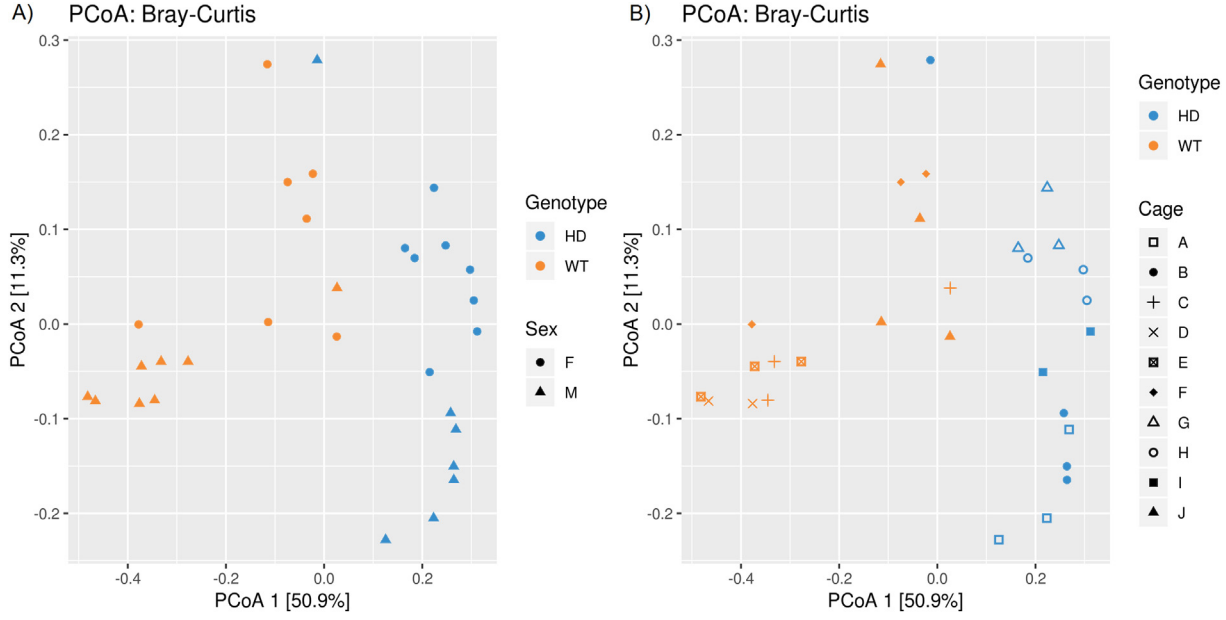

Fig. 4. Principal coordinate analysis of male and female data using Bray-Curtis distance with different combinations of metadata shown: A) genotype and sex, B) genotype and cage. Amount of explained variance per component is reported. The samples clustered according to sex as well as genotype, and to some extent, to cage. There were significant differences between the two genotypes for both sexes on the Bray-Curtis distance. No significant sex differences on Bray-Curtis distance but they were significant on the unweighted UniFrac distance (not shown here) (n = 7–8).

Proteobacteria, Cyanobacteria, Deferribacteres and Tenericutes. In particular, for the male WT mice, the most abundant phylum was Firmicutes (76.8%), followed by Bacteroidetes (22.3%), Proteobacteria (0.4%) and the remaining low-abundance phyla (Supplementary Tables). Conversely for male HD mice, Bacteroidetes (62.5%) is the most abundant phylum, followed by Firmicutes (36.1%), Actinobacteria (0.7%), Proteobacteria (0.6%) and the remaining phyla (Supplementary Tables). Similar to male WT mice, the most abundant phylum in female WT mice was Firmicutes (58.2%), followed by Bacteroidetes (39.8%), Actinobacteria (1.3%), Proteobacteria (0.7%) and other low-abundance phyla (Supplementary Tables). In the female HD mice, Bacteroidetes (61.6%) was the most abundant phylum, followed by Firmicutes (34.7%), Actinobacteria (2.9%), Proteobacteria (0.7%) and the remaining phyla (Supplementary Tables). The average relative abundance of each phylum per cage is shown in Supplementary Tables.

As there were sex differences indicated by the unweighted UniFrac distance, we separated the sexes for the subsequent analysis. We observed a significant increase in the Bacteroidetes phylum ($p_{male} < 0.001$, $p_{female} < 0.001$) and a significant proportional decrease in the Firmicutes phylum in the HD mice for both sexes ($p_{male} < 0.001$, $p_{female} < 0.001$) (Fig. 5A, C). In addition, we observed a significant decrease of Deferribacteres ($p = 0.044$) and significant increases in the proportion of Actinobacteria ($p = 0.023$) and Proteobacteria ($p = 0.044$) in male HD mice when compared to WT mice (Fig. 5B). However, there were no differences in the proportion of Actinobacteria, Proteobacteria or Deferribacteres between female HD and WT mice (Fig. 5D).

We found that the male HD group differed from the WT group in indices of α-diversity (Fig. 6). Particularly, Shannon and Inverse Simpson index were higher in HD mice compared to WT mice (Shannon, Kruskal Wallis, $p = 0.023$; Inverse Simpson, Kruskal Wallis, $p = 0.003$). However, there were no differences in the microbial richness (Observed) between male HD and WT mice (Kruskal Wallis test, $p > 0.05$). On the other hand, there were no differences in any of the three α-diversity indices measured between female HD and WT mice (Kruskal Wallis test, $p > 0.05$). We probed for differences in alpha-diversity indices in male and female WT mice, as well as male and female HD mice. There were no differences observed in the alpha-diversity indices measured.

sPLS-DA is a multivariate method based on the relative proportion of microbiome data used to identify microbial drivers discriminating particular phenotype groups. We identified a signature of bacteria discriminating male and female mice (Fig. 7). For WT, this signature consisted of Clostridiales, Bacteroidales, Deferribacterales, Erysipelotrichales, and Lactobacillales (Supplementary Tables). For HD, this signature consisted of bacteria from the order Clostridiales, Bacteroidales and Lactobacillales (Supplementary Tables). In addition, we also identified a signature of bacteria discriminating HD vs WT mice (Fig. 8). For males, this signature consisted of bacterial families from Clostridiales, Bacteroidales and Lactobacillales (Supplementary Tables). For females, this signature consisted of bacterial families from Coriobacteriales, Clostridiales, Erysipelotrichales, Bacteroidales, and Burkholderiales (Supplementary Tables). Long-term co-housing of mice may result in cage effects, which could potentially confound other factors of interest, for example, genotype effects in our study (Laukens

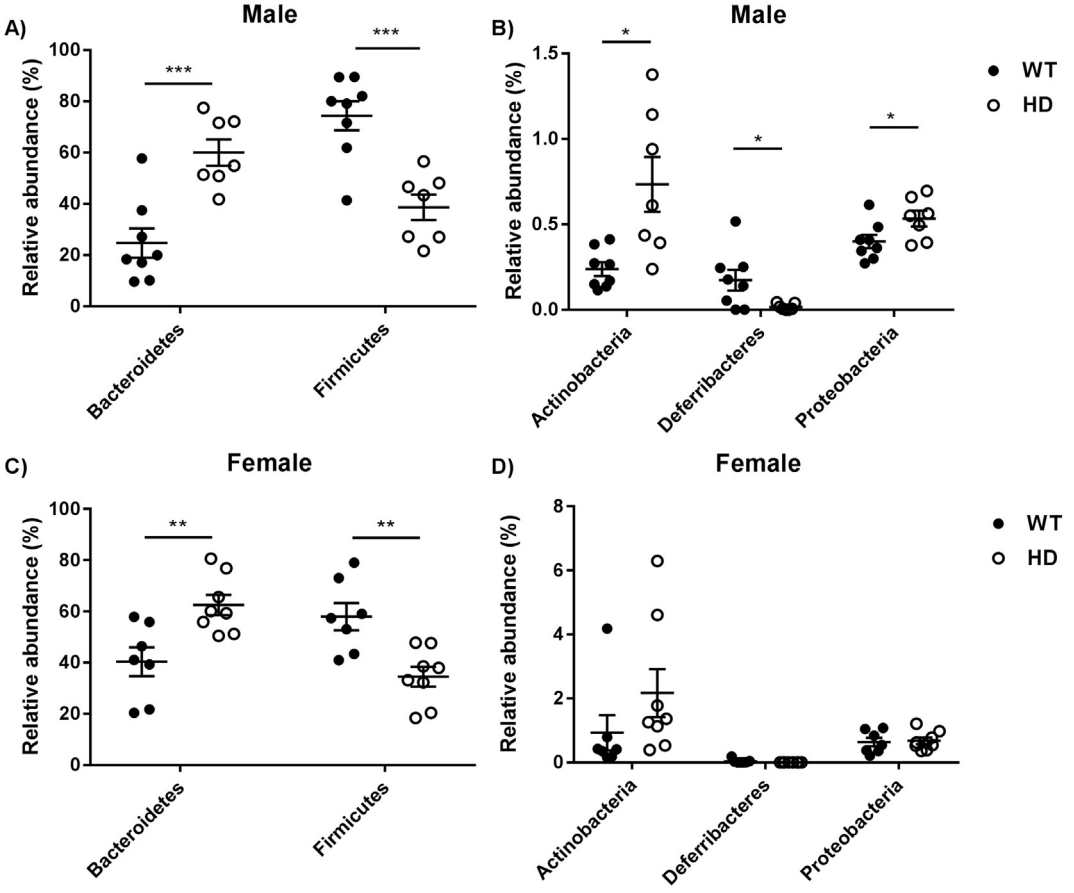

Fig. 5. The relative abundance of the major phyla, Bacteroidetes and Firmicutes, was examined for (A) male and (C) female mice. The relative abundance of minor phyla, Actinobacteria, Deferribacteres and Proteobacteria, was also examined for (B) male and (D) female mice. There was a significant increase in the Bacteroidetes phylum, with significant proportional decrease in the Firmicutes phylum, in HD mice for both sexes. There were also significant differences in the proportions of the minor phyla, but this was only observed in the males. The plots show mean ± SEM (n = 7–8).

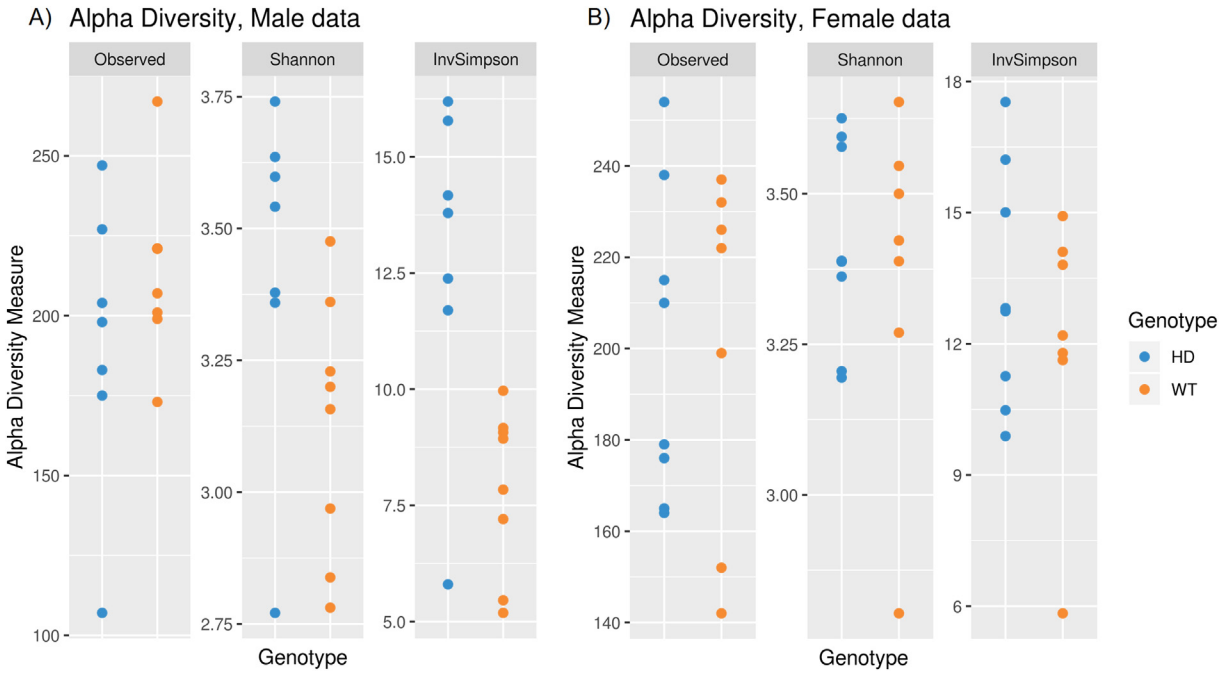

Fig. 6. (A) Alpha diversity metrics including Observed, Shannon and Inverse Simpson (InvSimpson) indices were probed for male and (B) female data. For male mice, there was no difference in Observed but the Shannon and Inverse Simpson indices were significantly higher in HD mice compared to WT. There were no differences in all three indices for female mice (n = 7–8).

et al., 2016). Therefore, we assessed the effect of cage within sex group on the proportional counts transformed with CLR. For males, 4 OTUs were identified as affected by cage, one (p_Firmicutes: g_Streptococcus) of which was selected by the sPLS-DA when identifying signatures that discriminate WT and HD. For females, 9 OTUs were identified as driving cage effect and one (p_Bacteroidetes: f_S24–7) was selected by sPLS-DA when identifying signatures that discriminate WT and HD.

We sought to predict the function of the microbiota structure using PICRUSt (Langille et al., 2013). The weighted NSTI scores were calculated for each sample to estimate the accuracy of PICRUSt's prediction. The mean NSTI score was 0.22 ± 0.04, indicating that the predictions of PICRUSt may be unreliable (Langille et al., 2013). PCA analysis of PICRUSt output revealed clustering according to genotype but not sex (Supplementary Results). Additional analysis on the predicted KEGG pathways using sPLS-DA revealed a signature of pathways that are affected including protein kinase, D-glutamine and D-glutamate metabolism, carbohydrate metabolism as well as transporters (Supplementary Results).

4. Discussion

In the present study, we provide the first evidence for gut dysbiosis in HD. There have been no previous reports characterising the gut microbiome in HD, either preclinically or clinically. In the present study, we performed bacterial 16S rRNA gene sequencing on DNA isolated from fecal samples in order to characterize the gut microbiome of HD mice.

We report that there is a significant difference in the gut microbiome composition of HD mice compared to WT littermate control mice at 12 weeks of age, which coincided with onset of a motor coordination deficit tested on the rotarod, but prior to onset of the hind-limb clasping phenotype (Supplementary Results). A significant change on the Bray-Curtis index, but not unweighted Unifrac index, suggest that the bacteria which distinguish between the WT and HD gut microbiomes are phylogenetically close to each other, but differ in abundance. We observed an increase in alpha-diversity in male HD when compared to WT, which is in contrast to studies reporting gut microbiome alterations in some other disorders, including AD and chronic fatigue syndrome (Giloteaux et al., 2016; Vogt et al., 2017). We also observed an increased abundance of Bacteroidetes, with a proportional decrease of Firmicutes in HD, similar to findings in AD, type 2 diabetes and chronic fatigue syndrome (Giloteaux et al., 2016; Vogt et al., 2017). The sPLS-DA analysis revealed that the gut microbiome signature of WT mice consists of mainly Clostridiales from Firmicutes phyla in WT mice, whereas the signature of HD mice consists of mainly Bacteroidales from the Bacteroidetes phyla. We tested for cage effects and that did not affect most of the signatures from sPLS-DA analysis. Drawing insights from obesity, Bacteroidetes is associated with weight loss and Firmicutes is associated with weight gain (Chakraborti, 2015; Ley et al., 2005). In addition, change in the B/F ratio is known to result in alterations of short-chain fatty acid (SCFA) levels, a by-product of the microbes from dietary fibre fermentation, which will have downstream effects on the host's metabolism (den Besten et al., 2013). Notably, the disturbance of the 'carbohydrate metabolism' pathway, as predicted by PICRUSt, may affect nutrient availability within the gut and may contribute to the observed weight loss. However, the PICRUSt pathway predictions for these samples are unreliable due to the relatively high NSTI scores, which also, indicated that the murine microbial community contained many poorly or un-sequenced genomes. As PICRUSt is based on a closed reference OTU picking approach, the function of novel OTUs could not be predicted in our analysis.

We also found sex differences in the gut microbiome in WT and HD mice. Significance on unweighted Unifrac index, but not Bray-Curtis, suggests that the bacteria which differ between the two groups are phylogenetically far apart but not different in abundance. Outside of HD, there are several reports of sex differences in the gut microbiome, which is likely mediated by sex hormones (Haro et al., 2016; Org et al., 2016). Sexual dimorphism is a common characteristic in a variety of diseases, including metabolic, psychiatric and other neurodegenerative diseases such as PD, AD and HD (Brooks et al., 2012; Farhadi et al., 2017; Jiao et al., 2016; Kessler et al., 1994; Li and Singh, 2014; Mo et al., 2014)

The gut dysbiosis observed in HD mice coincided with a deficit in the ability to gain weight despite higher food intake, especially in male

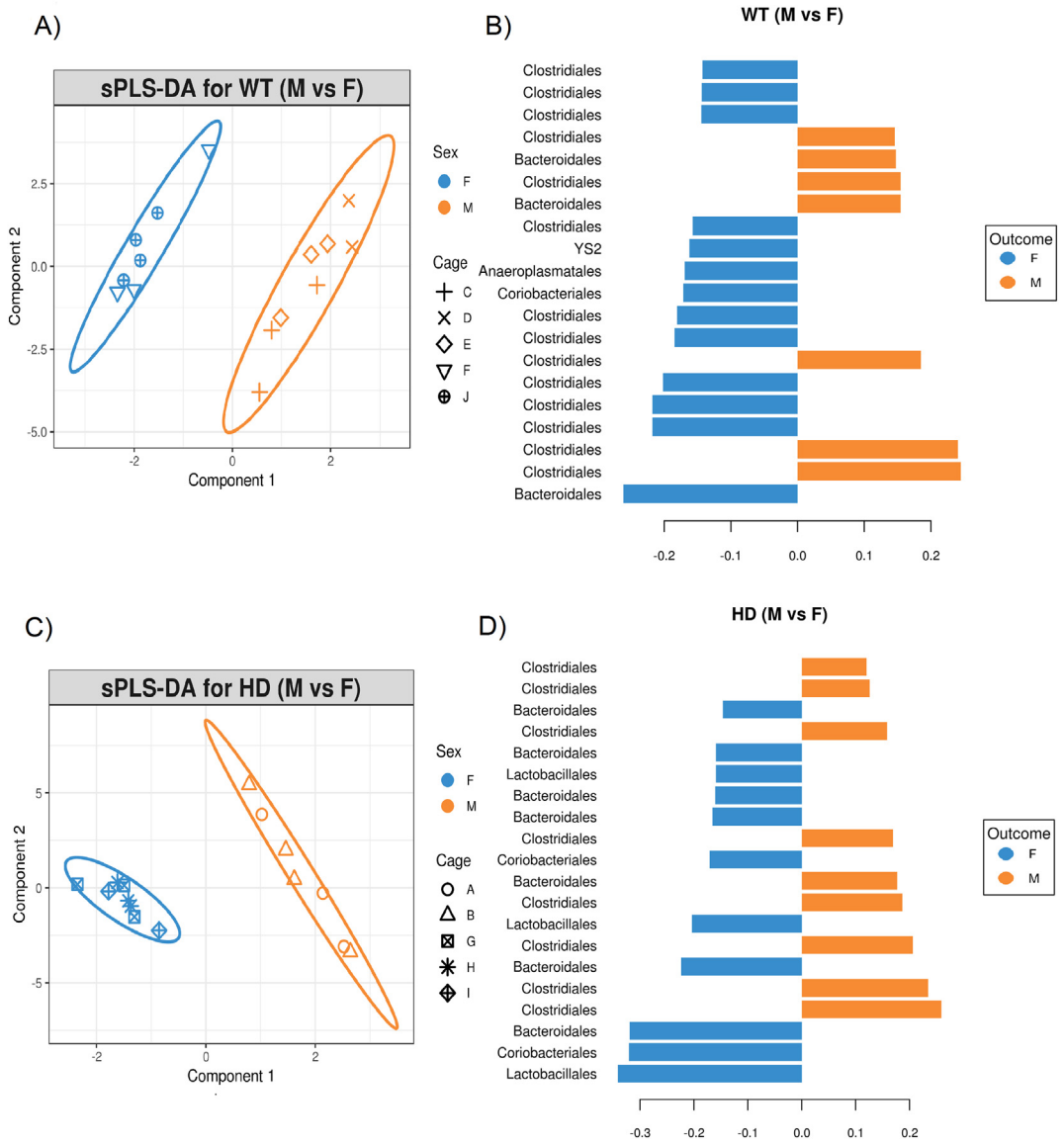

Fig. 7. Identification of a bacterial signature discriminating the two sexes with sPLS-DA. Sample plots with 0.95 confidence ellipse plots for (A) WT and (C) HD show a strong discrimination between HD and WT. The top 20 resulting bacterial signature contributing to component 1 is displayed in (B) for WT and in (D) for HD. The length of the bars indicates the importance of each OTU (at the Order taxonomic rank) in the signature (from bottom to top: decreasing importance) with color indicating the phenotype group with maximum median abundance. The OTUs shown are at the Order taxonomic level.

HD mice, which agreed with independent studies on HD patients and mouse models (Gaba et al., 2005; Kim et al., 2010; Koroshetz et al., 1997; Oliveira et al., 2007; Pratley et al., 2000). The gut microbiome is known to regulate the host metabolism and may contribute to this particular HD phenotype. In addition, we observed an increased fecal output per hour which may be caused by increased food intake. We also observed increased water intake in HD mice which is consistent with previous reports of xerostomia in HD patients and mice (Wood et al., 2008). The majority of excess water is excreted via urination in mammals whereas a fixed amount of water is excreted via defecation, so that stool water content is always regulated. As ingested water is rapidly absorbed by the body, mostly in the small intestine, the increase in fecal water content paired with increased water intake suggests the dysfunction of water absorption in the small intestine (Péronnet et al., 2012).

As the *HTT* gene is widely expressed throughout the periphery, including the gut, there are many ways in which the expression of the HD gene mutation may disrupt the gut microbiome in this mouse model. One of the factors affecting microbial diversity is colonic transit time, which can affect the bioavailability and absorption of various by-products and water by the microbes which could alter gut microbiota composition (Roager et al., 2016; Vandeputte et al., 2015). We observed higher fecal water content in HD mice, indicating that there is dysfunction in either gut transit time or colon water absorption, which could lead to a change in the gut environment, and in turn, cause the observed shift in microbiota composition.

In addition, a few studies have highlighted the fact that the intestinal epithelial cells (IEC) in the GI tract can release extracellular vesicles carrying miRNA from the host and could affect the gut microbiota gene expression (Liu et al., 2016; Van Niel et al., 2001). The cargo of extracellular vesicles is dependent on the cell of origin and it is well known that mutant HTT affects the cellular miRNA profile (Colombo et al., 2014; Hoss et al., 2015; Lee et al., 2011; Packer et al., 2008). It is possible that the IEC-derived vesicles contain altered miRNA cargo due to the presence of HTT in the parent cell, which in turn affects the expression of the gut microbiome.

Our results provide the first evidence of gut microbiome dysregulation in HD. The gut dysbiosis observed here may play a role as a

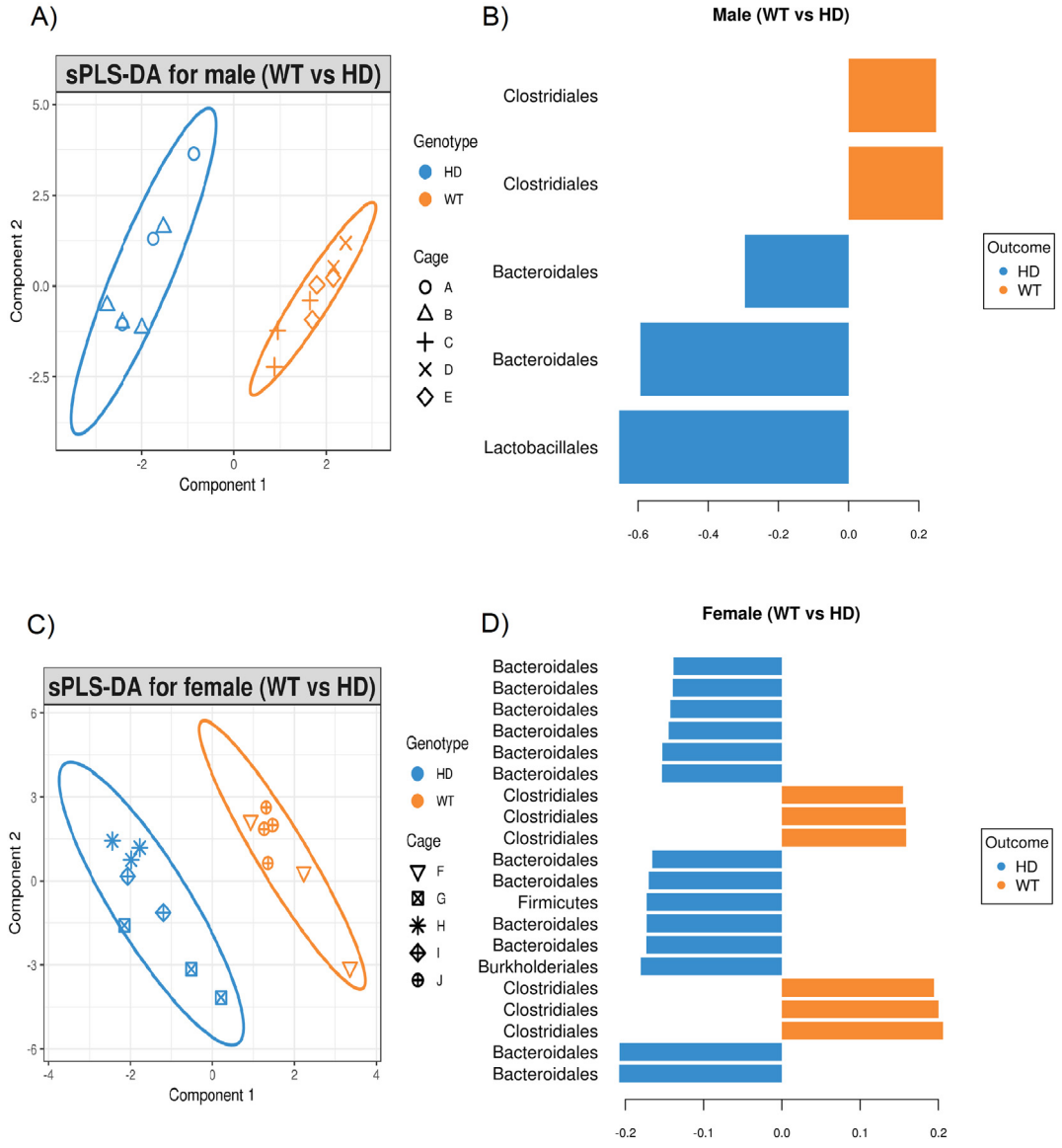

Fig. 8. Identification of a bacterial signature associated with HD vs WT with sPLS-DA. Sample plots with 0.95 confidence ellipse plots for (A) male and (C) female show a strong discrimination between HD and WT. The resulting bacterial signature contributing to component 1 is displayed in (B) for males and for females, the top 20 bacterial signature is displayed in (D). The length of the bars indicates the importance of each OTU in the signature (from bottom to top: decreasing importance) with color indicating the phenotype group with maximum median abundance. The OTUs shown are at the Order taxonomic level. In the case when the Order level is not assigned, the next highest level is shown.

modulator to the onset and progression of HD symptoms. Whilst these transgenic mice have strong construct validity as a model of HD, one urgent priority in following up the findings of the present study is to investigate the gut microbiome in clinical samples to validate gut dysbiosis in HD.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2018.09.001.

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