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Vasopressin deletion is associated with sex-specific shifts in the gut microbiome

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ABSTRACT

Brattleboro rats harbor a spontaneous deletion of the arginine-vasopressin (*Avp*) gene. In addition to *diabetes insipidus*, these rats exhibit low levels of anxiety and depressive behaviors. Recent work on the gut-brain axis has revealed that gut microbiota can influence anxiety behaviors. Therefore, we studied the effects of *Avp* gene deletion on gut microbiota. Since *Avp* gene expression is sexually different, we also examined how *Avp* deletion affects sex differences in gut microbiota. Males and females show modest but differentiated shifts in taxa abundance across 3 separate *Avp* deletion genotypes: wildtype (WT), heterozygous (Het) and AVP-deficient Brattleboro (KO) rats. For each sex, we found examples of taxa that have been shown to modulate anxiety behavior, in a manner that correlates with anxiety behavior observed in homozygous knockout Brattleboro rats. One prominent example is *Lactobacillus*, which has been reported to be anxiolytic: *Lactobacillus* was found to increase in abundance in inverse proportion to increasing gene dosage (most abundant in KO rats). This genotype effect of *Lactobacillus* abundance was not found when females were analyzed independently. Therefore, *Avp* deletion appears to affect microbiota composition in a sexually differentiated manner.

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16S rRNA; Brattleboro; gut microbiota; sex differences; rat

Introduction

The neuropeptide arginine-vasopressin (AVP) is released from hypothalamic neurons into the bloodstream of mammals, where it regulates water balance and other autonomic functions.[1] However, AVP is also released within the brain where it has been shown to influence social and anxiety-like behavior,[2] and modulate stress responses.[3] The Brattleboro rat, which contains a base-pair deletion in the *Avp* gene which prevents functional AVP expression, is a model for studying the effects of AVP on behavior.[4,5] Many of the behavioral abnormalities observed in Brattleboro rats, such as decreases in anxiety-like behavior[6] and abnormal social preference,[7] are assumed to result from a lack of direct activation of AVP-responsive behavioral circuits. However, systemic factors that may be influenced by AVP expression may also influence anxiety and social behaviors in this model. One such systemic factor may be the gut microbiome, which has recently been shown to influence both social and anxiety behaviors.[8]

Treatment of mice with antibiotics, which produces large-scale reconfiguration and depletion of gut microbiota, decreases hypothalamic AVP expression.[9] This suggests that microbiota may influence AVP expression. However, this axis may be bidirectional and there are multiple ways in which AVP expression could influence microbiota composition. For example, AVP expression influences stress responses, systemic inflammation, and behaviors that could subsequently influence microbiota composition. Furthermore, it is plausible that AVP expression and gut microbial compositional changes that are influenced by AVP expression could reinforce each other in a positive feedback loop.

This study seeks to establish whether there are compositional differences in gut microbiota between AVP knockout rats and wildtype rats. In addition, as AVP expression is sexually dimorphic, with male rodents expressing more than female rodents in centrally-releasing projections as well as in neurosecretory cells,[10,11] we sought to observe the effects of AVP deletion on sex differences in gut microbiota. Therefore,

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the objectives of this study were: i) to compare microbiota composition across AVP deletion genotypes (homozygous knockout, heterozygous, and wildtype Long Evans rats) and ii) to identify changes in sex differences of the microbiota upon haploid or diploid deletion of the AVP gene.

Results

Metadata. Long Evans rats with heterozygous expression of a functional and nonfunctional copy of the arginine-vasopressin gene (*Avp*) were bred to produce subjects expressing wildtype (WT), heterozygous (Het) and homozygous knockout (KO) variants of the *Avp* gene deletion. A total of 42 fecal samples (6 WT male, 8 WT female, 6 Het male, 7 Het female, 8 KO male, and 7 KO female) were collected with one sample per subject, from which DNA was amplified and sent for sequencing. After OTU picking and checking for chimeric transcripts, a total of 1,322,857 reads were assigned to 4,189 OTUs. Each sample had an average of 31,497 reads.

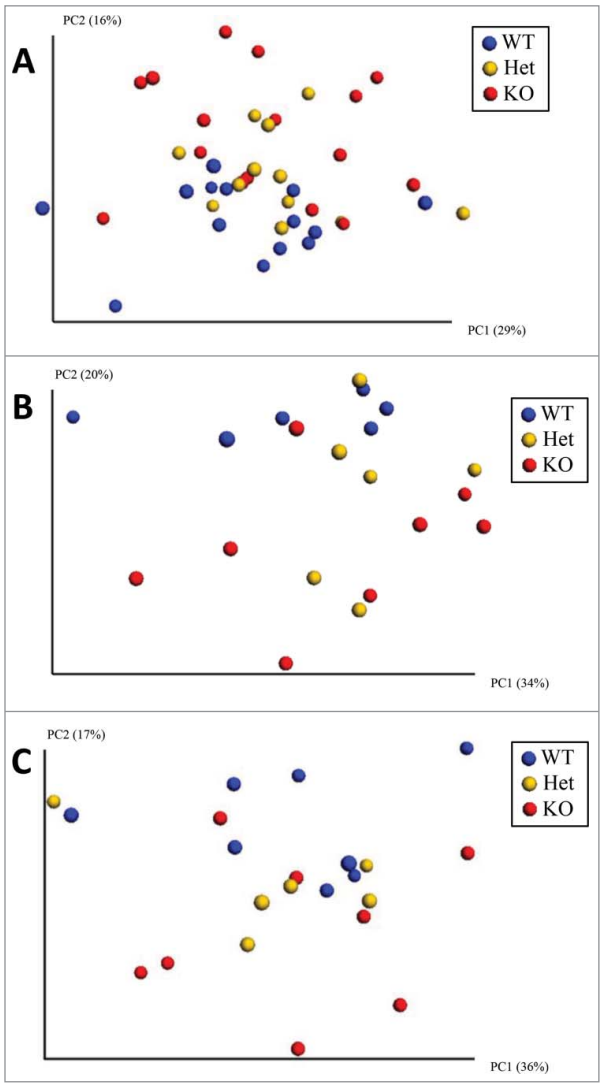

Figure 1. Covariation of community structure using weighted UniFrac distances demonstrates limited clustering of samples by genotype when (A) both sexes are analyzed together [KO are clustered in upper right, WT are clustered in lower left, while Het are found in the middle; PERMANOVA, $p <= 0.05$] and when (B) males [PERMANOVA, $p = 0.051$] and (C) females [PERMANOVA, $p = 0.071$] are analyzed separately.

Differences in bacterial communities between Avp deletion genotypes

Gut microbial richness was not statistically different across the 3 *Avp* deletion genotypes. Between genotypes, we found no difference in any of the 3 measures of α-diversity, which measures community richness (Shannon's diversity index, observed species and Chao1), when all data points were combined nor when genotypes were analyzed for each sex separately (data not shown).

The relationships between global microbiota compositions were examined using Principal Coordinate Analysis (PCoA) based on weighted UniFrac distances. With males and females combined, weighted UniFrac-based cluster analysis revealed modest but differentiated microbiota compositions for each genotype (Fig. 1A). The observed clustering of each group was confirmed by PERMANOVA ($p = 0.024$). Separation between WT and KO samples was particularly evident when Het samples were excluded (Supplementary Figure). When males and females were analyzed independently, clustering by genotype was observed (Fig. 1B and C), with trends in differentiation by genotype for both males and females ($p = 0.051$ and 0.071, respectively). These data suggest that the microbial community structures found in the guts of WT, Het, and KO Brattleboro rats are differentiated across a limited number of taxa.

We used LEfSe to identify specific bacterial taxa that are significantly differentiated between groups. All features identified by LEfSe exceed an LDA score of 2.0, which indicates significant differences between groups. Figure 2 shows bacterial taxa differentially represented between genotypes identified with the one-against-all algorithm, which identifies taxa that are only differentiated in one genotype relative to the other 2 genotypes. When both sexes were analyzed together, *Lactobacillus spp.* were most abundant in

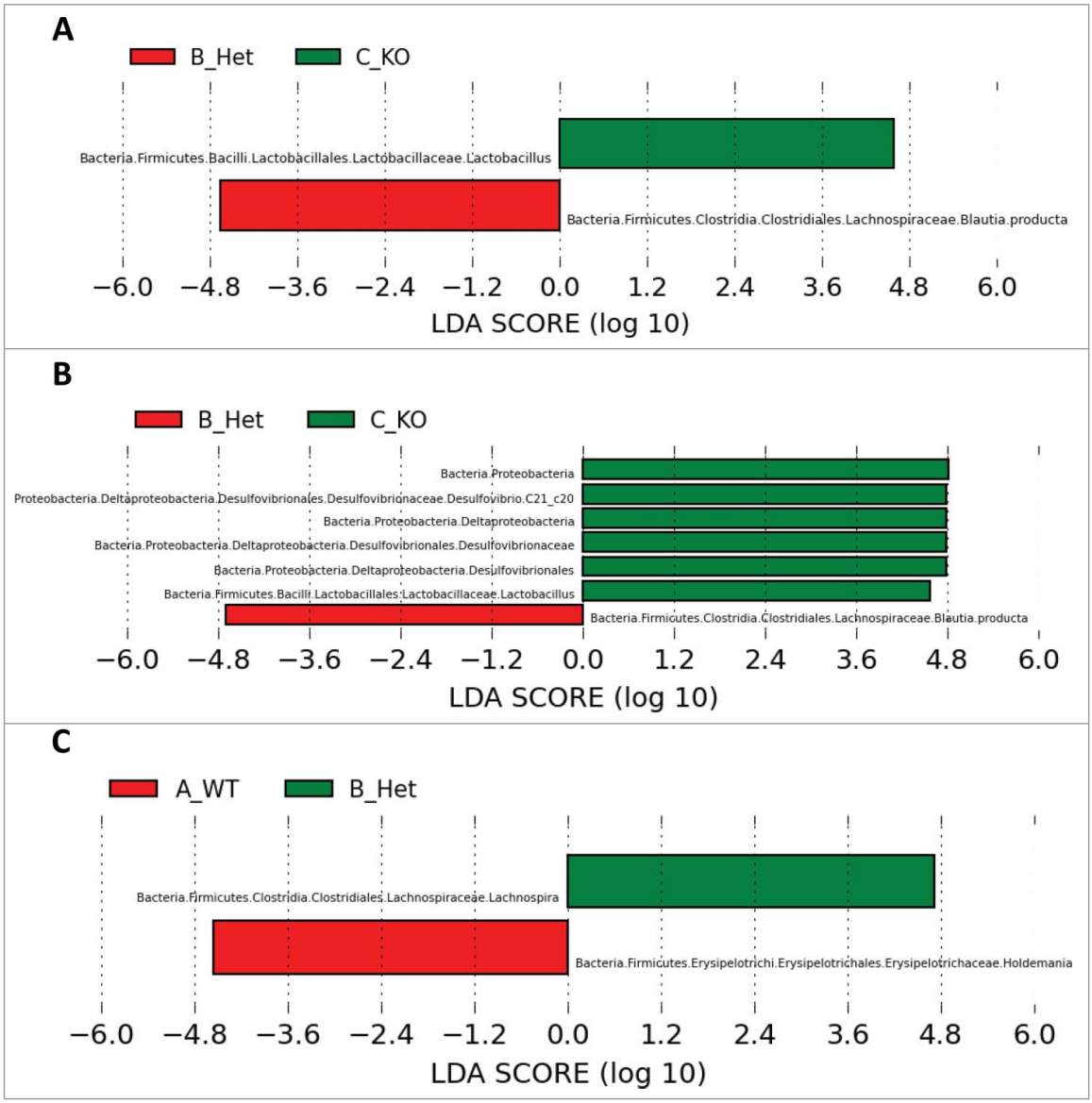

Figure 2. Bacterial taxa significantly differentiated between genotypes identified by Linear Discriminant Analysis (LDA) coupled with Effect Size (LEfSe). (A) shows differentiated taxa between WT, Het, and KO rats when males and females are combined. (B) and (C) show differences between genotypes for males and females, respectively. All LDA scores exceed 2.0, which is the threshold for significantly differentiated features.

KO rats and *Blautia producta* was most abundant in Het rats (Fig. 2A). When male samples were analyzed separately by genotype, the same taxa were differentiated, with the addition of *Desulfovibrio c21_c20* being more abundant in KO rats (Fig. 2B). When female samples were analyzed independently, *Lachnospira spp* were most abundant in Het rats while *Holdemania spp* were most abundant in WT rats (Fig. 2C). Using the all-against-all algorithm within LEfSe, which identifies features that are significantly differentiated among all pairwise comparisons, we found zero significantly differentiated taxa between genotypes when both sexes were combined. However, when the sexes were analyzed separately, significantly differentiated taxa between genotypes were identical to those identified with the one-against-all algorithm. For example, *Lactobacillus* is significantly differentiated between all 3 genotypes among male rats but is not significantly differentiated among female rats (Fig. 3). The all-against-all LEfSe algorithm indicates that the relative abundance of *Lactobacillus* is differentiated across all 3 genotypes for males (LDA score = 4.6), and the average abundance for each class increases with haploid and diploid deletion of

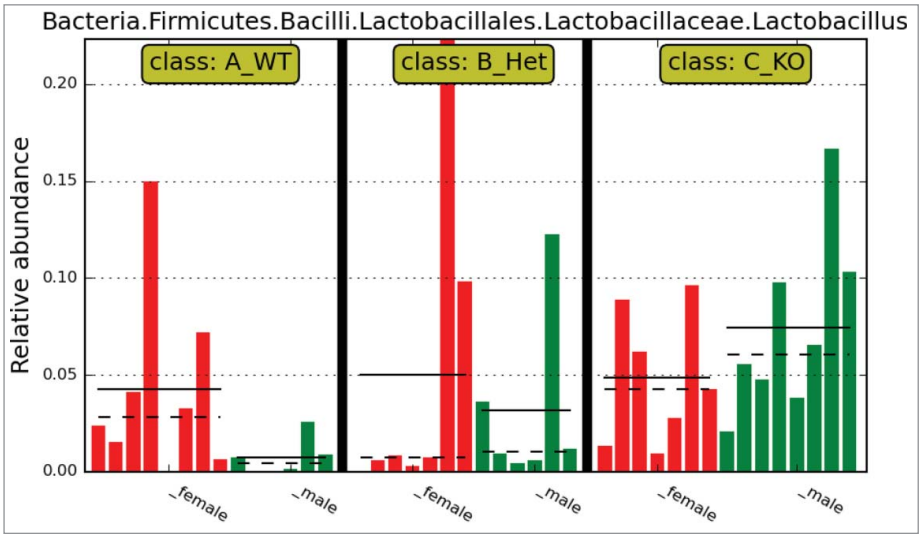

Figure 3. Relative abundance of *Lactobacillus* taxon between genotypes. All-against-all algorithm of Linear Discriminant Analysis (LDA) coupled with Effect Size (LEfSe) identifies this taxon as significantly differentiated between all genotypes [WT, Het, and KO] for male rats. (LDA score = 4.6, which exceeds the score threshold of 2.0, indicating statistical significance). Neither the all-against-all or one-against-all algorithms detect *Lactobacillus* as a significantly differentiated taxon between genotypes for female rats.

the *Avp* gene. In keeping with differentiated clustering of Het animals identified via PCoA plots, this LDA analysis suggests that Het rats harbor a microbiota that is differentiated from that found in WT and KO rats, particularly for these bacterial taxa.

Using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), we explored the predicted functional consequences of these compositionally differentiated microbiota for males and females separately. The OTU table was normalized by 16S rRNA copy number and gene pathways were predicted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. This generated the pathway abundance table that was analyzed by LEfSe. Males show more differentiation in pathways between genotypes. In males, 6 pathways were most abundant in KO rats (e.g. "Ribosome biogenesis in eukaryotes," aminoacyl tRNA biosynthesis," "Xylene degradation, " etc.), 9 pathways most abundant in Het rats (e.g., "Genetic information processing," "Translation," and "Ribosome," etc.) and 8 pathways most abundant in WT rats (e.g., "Other glycan degradation," "Sphingolipid metabolism," "Biosynthesis of other secondary metabolites," etc.) (Fig. 4A). Between female rats, "Glycolysis and gluconeogenesis" was most abundant in KO rats, "Amino acid metabolism," "Valine, leucine, and isoleucine biosynthesis," and "Pantothenate and CoA biosynthesis" were most abundant in Het rats, and "Phenylalanine, tyrosine, and tryptophan biosynthesis," "Arginine and proline metabolism," and "C5 branched dibasic acid metabolism" were most abundant in WT rats (Fig. 4B).

Differences in bacterial communities between sexes

When considering overall community composition via PCoA analysis, we observed no sex differences in gut microbiota. When we compared α-diversities between sexes with all of the genotypes combined, or between sexes for each separate genotype, no significant differences in species diversity were observed. No sex differences in overall community composition were identified via PCoA analysis of all of the samples combined, or for any of the 3 separate genotypes (data not shown).

At the level of individual taxa (as analyzed via LEfSe), we were able to identify sex differences across all 3 genotypes. Between WT males and females, *Dorea spp* and *Ruminococcus spp* were more abundant in female rats (Fig. 5A). This sex difference in community composition was altered in Het and KO rats. Among Het rats, *Odoribacter spp, Lactobacillaceae spp* and *Dehalobacterium spp* were more abundant in females, whereas *Granulicatella spp* and *Blautia producta* were more abundant in males (Fig. 5B). Among KO rats, *Lactobacillaceae spp,*

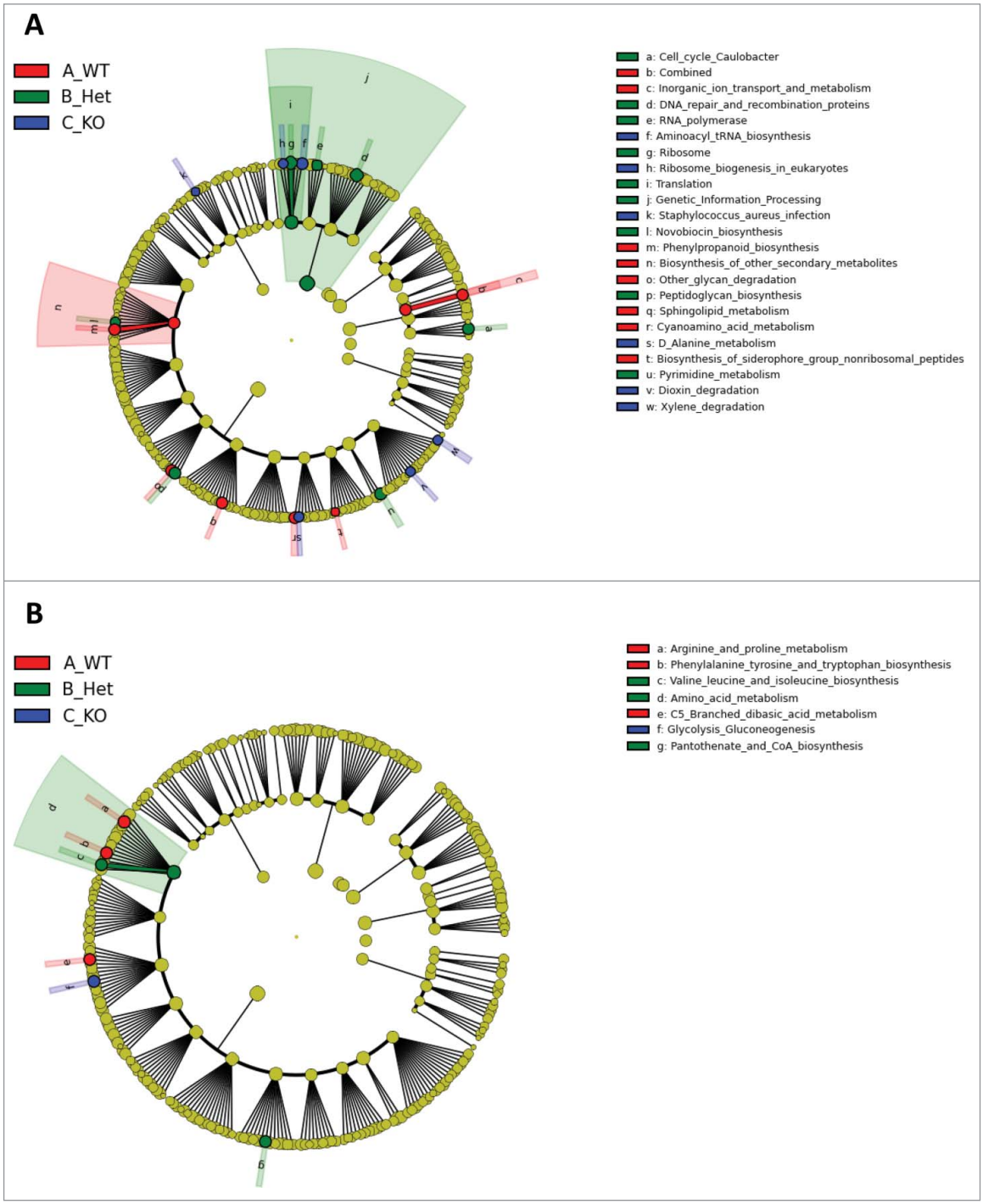

Figure 4. Cladogram of gene pathways significantly differentiated between genotypes identified by Linear Discriminant Analysis (LDA) coupled with Effect Size (LEfSe). The innermost ring represents KEGG Level 1 pathways, the middle ring represents KEGG Level 2 pathways, and the outermost ring represents KEGG Level 3 pathways. (A) and (B) show predicted functional differences between genotypes [WT, Het, and KO] for males and females, respectively. All highlighted pathways have LDA scores that exceed 2.0, which is the threshold for significantly differentiated features.

Dehalobacterium spp, and *Eubacterium dolichum* were more abundant in females (Fig. 5C).

The metabolic potentials between sexes for each genotype were explored using PICRUSt-generated BIOM tables analyzed via LEfSe. We were only able to identify one gene pathway category that was sexually differentiated across each of the 3 distinct genotypes. In WT rats, "Secretion Systems" predominated in females (Fig. 6A), whereas in Het rats, "RNA polymerase" pathways were more abundant in males (Fig. 6B).

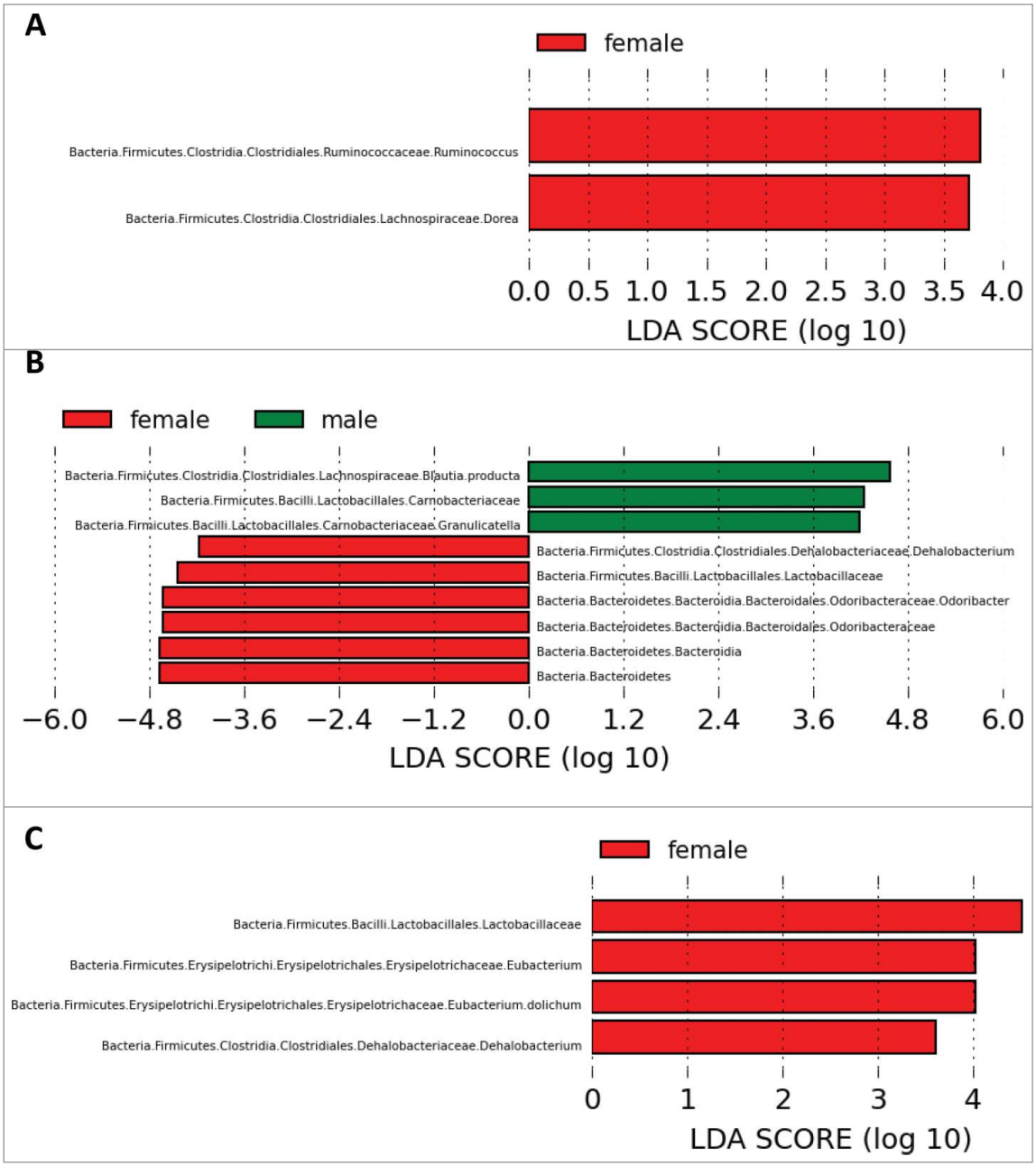

Figure 5. Bacterial taxa significantly differentiated between sexes identified by Linear Discriminant Analysis (LDA) coupled with Effect Size (LEfSe). Differentiated taxa between males and females of the (A) WT, (B) Het and (C) KO genotypes are shown. All LDA scores exceed 2.0, which is the threshold for significantly differentiated features.

These pathways were not sexually differentiated in KO rats, where an unclassified group of pathways was more abundant in females (Fig. 6C).

Discussion

This study is the first to identify differences in gut microbiota between arginine-vasopressin (AVP) deletion genotypes: namely homozygous (KO), heterozygous (Het) and wildtype (WT) Brattleboro rats. We found differences in microbiota across all 3 genotypes, suggesting that *Avp* is haploinsufficient to restore microbiota observed in WT rats. Interestingly, we also found that sex differences in gut microbiota were affected by *Avp* genotype.

Breeding genetic knockout and WT colonies in isolation may result in compositional differences in gut microbiota that are not truly reflective of genotype effects on microbiota composition.[12] We avoided this confounding effect by generating all genotypes used in

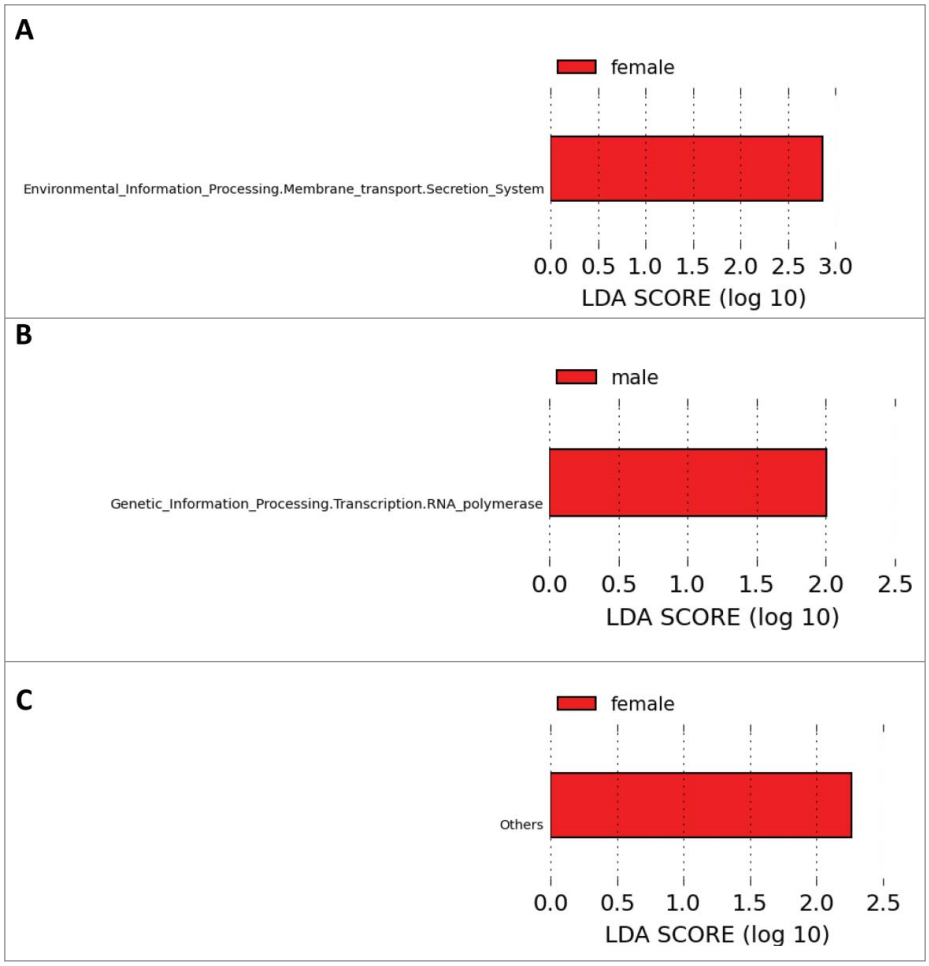

Figure 6. Gene pathways significantly differentiated between sexes identified by Linear Discriminant Analysis (LDA) coupled with Effect Size (LEfSe). Differentiated taxa between males and females of the (A) WT, (B) Het and (C) KO genotypes are shown. All highlighted pathways have LDA scores that exceed 2.0, which is the threshold for significantly differentiated features.

this study from heterozygous breeding pairs. To ensure that fecal samples were only collected from the subject animal and not from a cagemate, subject animals were single housed for 18–24 hours before sample collection. Single housing can affect stress reactivity,[13] and chronic stress exposure could potentially alter microbiota composition.[14] We reasoned that a 24-hour separation would not significantly alter microbiota composition, as large-scale differentiation of microbiota composition requires several days in other models[14–16] All animals were subjected to the same single housing protocol.

Our data suggest that haploid or diploid expression of the *Avp* gene differentially affects the abundance of specific bacterial taxa, and that it does so in a sex-specific manner. Microbial differences were detected with QIIME via PCoA analysis to determine whether large scale microbial population differences exist between groups[17] and with LEfSe, a very conservative biomarker discovery tool which detects the most robust taxa and pathway differences most likely to explain differences in host physiology and behavior between groups.[18,19] Both PCoA analysis and LEfSe indicate that each genotype is significantly differentiated from the others. Also, females exhibit a separate set of differentially abundant taxa between genotypes relative to those found in males. Unique findings of sex-specific compositional differences between genotypes are supported by analysis of microbiota composition by sex. The sexually differentiated taxa found in WT rats are not observed in Het and KO rats, and vice versa. PICRUSt analysis, which demonstrates differences in the functional capacity of gut microbiota, also suggests a differentiated microbiota for Het rats and highlights the effects of subject sex on genotype differences in microbiota composition. It is important to note that while PICRUSt has demonstrated a high level of predictive validity in mammalian microbial

samples, PICRUSt analyzes data from a "closed-reference" subset of the original community composition BIOM table, and the accuracy of PICRUSt predictions still lie between 60–90% for mammals.[20]

KO rats show less anxiety behavior than WT rats.[6] Our data suggest this may, in part, be driven by the higher abundance of *Lactobacillus spp* found in the gut microbiota of KO rats relative to WT rats. Oral administration of *Lactobacillus spp* decreases anxiety behavior in mice and rats.[21–27] Of note, male Het rats show levels of *Lactobacillus spp* intermediate to those found in male WT and male KO rats. As *Avp* has been shown to be haploinsufficient in restoring normal working memory[28] and in selective parameters of developmental behavior,[29] a thorough investigation of differences in other behaviors, such as anxiety behavior, between Het and WT rats is warranted. The anxiety modulating properties of *Desulfovibrio spp* and *Blautia producta*, most abundant in KO and Het rats respectively, have not been investigated in conventional WT rats. However, a gnotobiotic mouse model solely colonized with a *Blautia sp.* demonstrates decreases in marble burying behavior and moderate decreases in time spent in the periphery of the open field test relative to germ-free mice, suggesting decreases in repetitive and anxiety-like behaviors in these mice.[30] This is particularly notable, as germ-free mice show decreased anxiety-like behavior with respect to conventionally colonized mice.[31,32]

Some differentiated taxa that have been associated with weakened immune systems or with pro-inflammatory states are more abundant in KO or WT rats, respectively. AVP is important for shaping immune responses, and rats with a homozygous *Avp* deletion harbor a hyporesponsive immune system, showing deficits in macrophage activation, IgG antibody response, a smaller spleen and premature involution of the thymus.[33] *Desulfovibrio c21_c20*, found most abundantly in male KO rats relative to male WT rats, is a bacterial species of the Proteobacteria phylum, which has been found to be highly abundant in mice with a disruption in their innate immune system (namely, toll-like receptor 5 which recognizes flagellated bacteria).[34] Between female rats, *Lachnospira spp* are most abundant in Het rats and *Holdemania spp* are most abundant in WT rats. *Holdemania* is a genus of the Erysipelotrichales order; Erysipelotrichales bloom in response to a high-fat diet,[35] which has been shown to promote intestinal inflammation. Children with asthma have a lower abundance of *Lachnospira spp* in their gut microbiota, and germ-free mice colonized with a *Lachnospira* species show decreases in airway inflammation.[36] Given the 2-way relationship between microbiota and the immune system,[37,38] it is possible that *Lachnospira spp* suppress inflammation in a manner that promotes further replication of *Lachnospira spp* in female KO rats.

One mechanism by which *Avp* deletion may alter gut microbiota is via regulation of water consumption. Drinking water conditions, such as the pH of consumed water, can alter gut microbiota.[39,40] As AVP is important for water retention, Brattleboro rats display signs of *diabetes insipidus*, i.e. increased water intake and urine output. However, restoring systemic AVP levels via osmotic minipumps, which corrects water balance and diabetes symptoms, does not normalize anxiety and depressive behaviors in Brattleboro rats.[6] In addition, the heterozygous Brattleboro condition is sufficient to correct for outward signs of *diabetes insipidus*,[41,42] but the heterozygous condition is still unable to correct working memory deficits that are observed in homozygous knockout Brattleboro rats.[28] This suggests diabetes symptoms, such as water consumption, are not the sole driver of behavioral differences between Brattleboro and WT rats.

There are other potential mechanistic links between AVP expression and microbiota composition. It is possible that maternal behaviors such as pup licking/grooming affect microbiota composition. KO Brattleboro dams have been demonstrated to exhibit maternal neglect, spending less time licking/grooming their pups than Het dams.[43] However, all subjects in this study were raised by Het dams. Nevertheless, KO pups may elicit differing levels of maternal licking/grooming behavior than Het and WT rats. KO rats exhibit differing levels of ultrasonic calls relative to WT and Het rats[29] and pup ultrasonic calls may be associated with rates of licking/grooming,[44] which may potentially affect adult gut microbiota composition.

Moving from behavior to cellular biology, AVP may directly affect microbiota composition via receptors present on bacteria that may be structurally similar to host neurotransmitter/neuropeptide receptors.[45] Indeed, many neurotransmitters are suggested to derive from bacterial origins through lateral gene transfer into the metazoan lineage.[46] An *in vitro* study found that AVP was stable in a colonic environment

devoid of fecal microbiota.[47] Therefore, AVP may be metabolized by the microbiota in a manner that influences their growth, cell death, or functional output, and may subsequently affect the host.

AVP release, potentially both centrally and systemically, modulates the activity of immune cells,[48,49] and AVP-producing nuclei are responsive to inflammatory stimuli.[50] Many immune cells also express AVP receptors.[51] Similar to AVP, gut microbiota both regulate, and are shaped by, the immune system.[37,38] Therefore, there may be a bidirectional link between gut microbiota and AVP expression mediated by the immune system. Future studies could investigate differences in behavioral and cytokine profiles in germ-free rats administered microbiota from WT versus KO Brattleboro rats.

In summary, we characterized the gut microbiota of wildtype (WT) Long Evans rats and Long Evans rats carrying haploid (heterozygous, Het), or diploid (knockout, KO) deletions of the *Avp* gene (also known as Brattleboro Rats), and found a limited but potentially influential subset of significantly differentiated taxa that correspond with the immune status and anxiety behavior differences observed between WT and KO rats. Rats heterozygous for the *Avp* gene harbor a differentiated microbiota, which appears to be intermediate to that found in the guts of WT and KO rats. In addition, *Avp* gene deletion appears to affect the community composition of the gut microbiota of males and females in a sexually differentiated manner. Future studies should more fully explore the behavioral phenotype of Het rats relative to WT rats, and how sex differences in behavior are altered by *Avp* gene deletion.

Methods

Experimental design and fecal collection

Brattleboro rats carrying a homozygous (KO) or heterozygous (Het) deletion of the AVP gene against a Long-Evans background, along with wildtype (WT) Long-Evans rats, were bred from Het breeding pairs. Offspring from 11 litters resulting from 11 separate breeding pairs, all born within a 5-day span, were used in this study, yielding a total of 42 subjects. Upon weaning, all offspring were genotyped and pair-housed with the same genotype and sex. Prior to this study, at around 4 weeks of age, the rats were used in a play testing study.[29] These rats endured no further manipulations before the study. All of the animals were pair-housed with the same genotype and sex at the beginning of the study. We did not want to disturb this pairing to avoid the additional confound of introducing socially novel cage mates, which may independently affect microbial composition. The rats were housed in 2 separate subspaces of a housing room with generally regular exposure to the same set of researchers and environmental cues. The rats were housed in cages with ALPHA-Dri bedding (Shepherd Specialty Papers, Kalamazoo, MI), fed non-autoclaved rodent chow (5001 Diet, LabDiet, St. Louis, MO), and kept on a 12L:12D light cycle.

At 12 weeks of age, subjects from each cage were chosen at random and were single housed into clean cages for 16–24 h. Three to four fecal pellets per cage were then collected with ethanol-cleaned forceps and promptly stored at -80°C. From each litter, no more than one rat per experimental group was used, with 7 of the 11 litters producing animals from all 3 genotypes used in the study. With the exception of 4 animals, cage mates were not used (i.e., only one rat per pair housed cage was used in the study).

DNA extraction and 16s rRNA sequencing

Fecal microbial 16s rRNA was sequenced according to the protocol outlined in Chassaing et al. (2015).[16] Briefly, total bacterial DNA was isolated from feces using the QIAamp DNA Stool Mini Kit (Qiagen, Germantown, MD) according to manufacturer's instructions, and was stored at -80°C before further analysis. The 16S rRNA genes, region V4, were PCR amplified using the 515F/806R primer set (see Chassaing et al. [2015] for full sequence).[16] PCR reactions consisted of Hot Master PCR mix (Five Prime, San Francisco, CA), 0.2 μM of each primer, and 10–100 ng template. Reaction conditions were 3 minutes at 95°C, followed by 30 cycles of 45 seconds at 95°C, 60 seconds at 50°C and 90 seconds at 72°C on a Biorad thermocycler. PCR products were purified with Agencourt Ampure magnetic purification beads (Beckman Coulter, Indianapolis, IN). Sequencing was performed on an Illumina MiSeq sequencer (paired-end reads, 2 × 250 base pairs) at Cornell University, Ithaca.

Bioinformatics and statistical analysis

The sequences were demultiplexed, quality filtered using the Qualitative Insights Into Microbial Ecology (QIIME, version 1.8.0) software package, and

forward and reverse reads were joined using the fastq-join method (http://code.google.com/p/ea-utils).[52] Sequences were assigned to OTUs (Operational Taxonomic Units, a proxy for species classification, grouping closely related individuals) using the UCLUST algorithm with a 97% threshold of pairwise identity, and classified taxonomically using the Greengenes reference database (http://greengenes.lbl.gov) using uclust method with the suppression of new clusters (closed reference OTU picking strategy). FastTree was used to generate a phylogenetic tree and to compute unweighted UniFrac distances per sample (http://microbesonline.org/fasttree/). OTUs that were assigned to only one read for a sample were excluded from analysis. Principal coordinate analysis (PCoA) plots, constructed with weighted UniFrac distances, were used to assess the variation between experimental groups (β-diversity) and jackknifed β diversity was used to estimate the uncertainty in PCoA plots. Metagenomic data prediction of the functional profiles of fecal microbial composition was generated using PICRUSt.[20]

Measures of α diversity were compared across groups using the Mann-Whitney U test of significance. Significant tests of β diversity difference between sample groups were obtained using PERMANOVA in QIIME. The program Linear Discriminant Analysis (LDA) coupled with Effect Size (LEfSe) was used to identify significantly differentiated bacterial taxa.[18] LEfSe was also used to analyze differential abundance in gene pathways between microbial samples predicted by PICRUSt. Bootstrap Kruskal-Wallis-test was used to identify taxa or gene pathways with significantly differentiated abundance, with the LDA score computed with a bootstrapping algorithm repeated over 30 cycles, each sampling 2-thirds of the data with replacement. Unless otherwise stated, one-against-all multiclass analysis was used, and posthoc Wilcoxon pairwise comparisons among subclasses were only performed among identically named subclasses: in cross-genotype analyses, males were only compared with males and females only compared with females; in cross-sex analyses, subjects of the same genotype were compared with each other. The one-against-all algorithm detects whether at least one of the classes is significantly different from the other compared classes. However, the all-against-all algorithm detects whether all of the classes are significantly different from each other. The threshold on the logarithmic LDA (Linear Discriminant Analysis) score for discriminative features was set to 2.0 (indicating significant differential abundance between classes), and the α values for the factorial Kruskal-Wallis test among classes and the pairwise Wilcoxon test between subclasses were both set to 0.05.

Availability of data and material

The sequence data and mapping file for all the samples included in this study have been deposited in the European Nucleotide Archive and have been assigned accession number PRJEB19277.

Abbreviations

AVP	arginine-vasopressin
Het	heterozygous
KEGG	kyoto encyclopedia of genes and genomes
KO	knockout
LDA	linear discriminant analysis
LEfSe	linear discriminant analysis coupled with effect size
OTU	operational taxonomic unit
PICRUSt	phylogenetic investigation of communities by reconstruction of unobserved states
PCoA	principle coordinate analysis
QIIME	quantitative insights into microbial ecology
WT	wildtype

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Authors' contributions

CTF and BC performed the research and data analysis. CTF and MJP generated the colony. CTF wrote the manuscript. ATG and GJD funded the research and provided scientific guidance.

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