Cell

Human Genetics Shape the Gut Microbiome

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http://dx.doi.org/10.1016/j.cell.2014.09.053

SUMMARY

Host genetics and the gut microbiome can both influence metabolic phenotypes. However, whether host genetic variation shapes the gut microbiome and interacts with it to affect host phenotype is unclear. Here, we compared microbiotas across >1,000 fecal samples obtained from the TwinsUK population, including 416 twin pairs. We identified many microbial taxa whose abundances were influenced by host genetics. The most heritable taxon, the family Christensenellaceae, formed a co-occurrence network with other heritable Bacteria and with methanogenic Archaea. Furthermore, Christensenellaceae and its partners were enriched in individuals with low body mass index (BMI). An obese-associated microbiome was amended with *Christensenella minuta*, a cultured member of the Christensenellaceae, and transplanted to germ-free mice. *C. minuta* amendment reduced weight gain and altered the microbiome of recipient mice. Our findings indicate that host genetics influence the composition of the human gut microbiome and can do so in ways that impact host metabolism.

INTRODUCTION

The human gut microbiome has been linked to metabolic disease and obesity (Karlsson et al., 2013; Le Chatelier et al., 2013; Ley et al., 2005; Qin et al., 2012; Turnbaugh et al., 2009). Variation in host genetics can also underlie susceptibility to metabolic disease (Frayling et al., 2007; Frazer et al., 2009; Herbert et al., 2006; Yang et al., 2012). Despite these shared effects, the relationship between host genetic variation and the diversity of gut microbiomes is largely unknown.

The gut microbiome is environmentally acquired from birth (Costello et al., 2012; Walter and Ley, 2011), therefore it may function as an environmental factor that interacts with host genetics to shape phenotype, as well as a genetically determined attribute that is shaped by, and interacts with, the host (Bevins and Salzman, 2011; Spor et al., 2011; Tims et al., 2011). Because the microbiome can be modified for therapeutic applications (Borody and Khoruts, 2012; Hamilton et al., 2013; Khoruts et al., 2010; van Nood et al., 2013), it constitutes an attractive target for manipulation. Once the interactions between host genetics and the microbiome are understood, its manipulation could be optimized for a given host genome to reduce disease risk.

Although gut microbiomes can differ markedly in diversity across adults (Human Microbiome Project Consortium, 2012; Qin et al., 2010), family members are often observed to have more similar microbiotas than unrelated individuals (Lee et al., 2011; Tims et al., 2013; Turnbaugh et al., 2009; Yatsunenko et al., 2012). Familial similarities are usually attributed to shared environmental influences, such as dietary preference, a powerful shaper of microbiome composition (Cotillard et al., 2013; David et al., 2014; Wu et al., 2011). Yet related individuals share a larger degree of genetic identity, raising the possibility that shared genetic composition underlies familial microbiome similarities.

Support for a host genetic effect on the microbiome comes mostly from studies taking a targeted approach. For instance, the concordance rate for carriage of the methanogen *Methanobrevibacter smithii* is higher for monozygotic (MZ) than dizygotic (DZ) twin pairs (Hansen et al., 2011), and studies comparing microbiotas between human subjects differing at specific genetic loci have shown gene-microbiota interactions (Frank et al., 2011; Khachatryan et al., 2008; Rausch et al., 2011; Rehman et al., 2011; Wacklin et al., 2011). A more general approach to this question has linked genetic loci with abundances of gut bacteria in mice (Benson et al., 2010; McKnite et al., 2012), but in humans, a general approach (e.g., using twins) has failed to reveal significant genotype effects on microbiome diversity (Turnbaugh et al., 2009; Yatsunenko et al., 2012). Thus, heritable components of the human gut microbiome remain to be identified using an unbiased approach.

Here, we assessed the heritability of the gut microbiome with a well-powered twin study. Comparisons between MZ and DZ twin

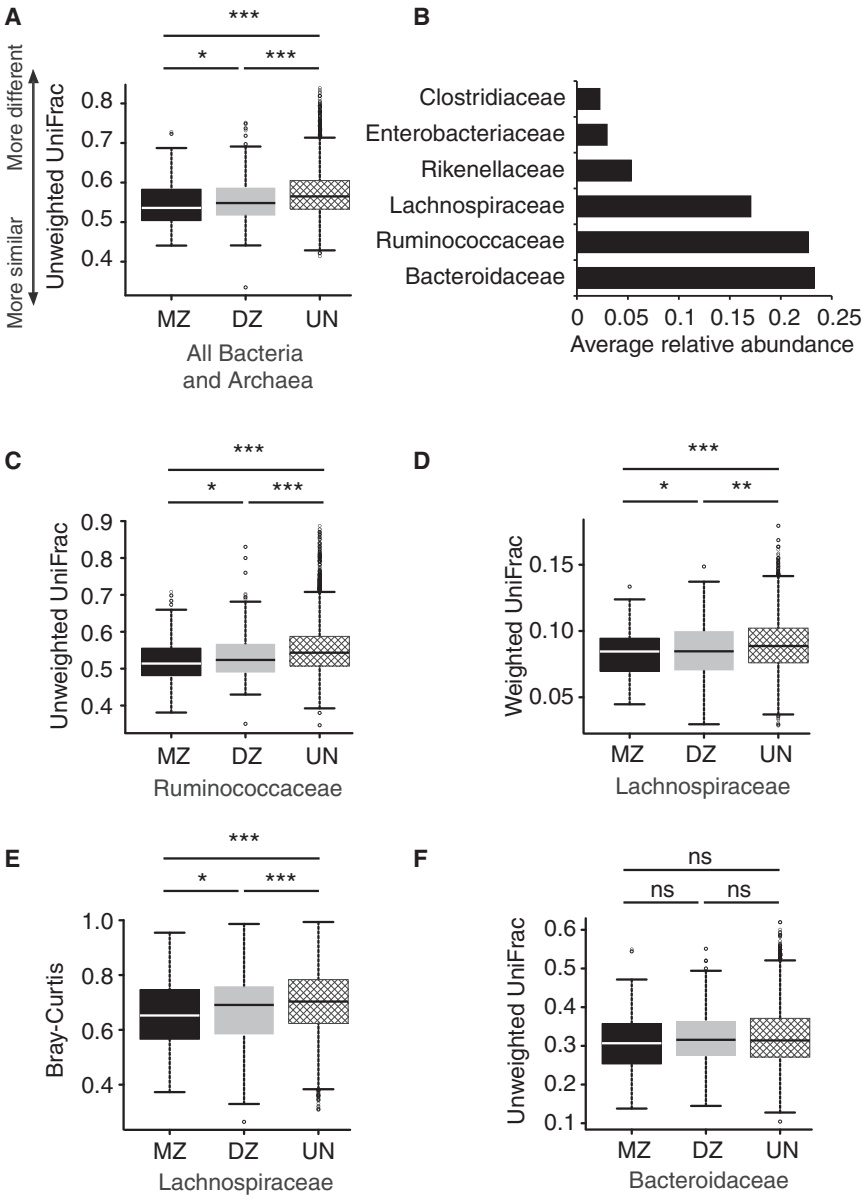

Figure 1. Microbiomes Are More Similar for Monozygotic Than Dizygotic Twins

(A and C–F) Boxplots of β diversity distances between microbial communities obtained when comparing individuals within twinships for monozygotic (MZ) twin pairs and dizygotic (DZ) twin pairs, and between unrelated individuals (UN). (A) The whole microbiome. (C) The bacterial family Ruminococcaceae. (D and E) The bacterial family Lachnospiraceae. (F) The family Bacteroidaceae. The specific distance metric used in each analysis is indicated on the axes. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for Student's t tests with 1,000 Monte Carlo simulations. (B) The average relative abundances in the whole data set for the top six most prevalent bacterial families (unrarefied data, see Experimental Procedures).

See also Figure S1 and Table S1.

pairs allowed us to assess the impact of genotype and early shared environment on their gut microbiota. Our study addressed the following questions: Which specific taxa within the gut microbiome are heritable, and to what extent? Which predicted metagenomic functions are heritable? How do heritable microbes relate to host BMI? Finally, we use fecal transplants into germ-free mice to assess the phenotype effects of the most heritable taxon.

RESULTS

Twin Data Set

We obtained 1,081 fecal samples from 977 individuals: 171 MZ and 245 DZ twin pairs, two from twin pairs with unknown zygosity, and 143 samples from just one twin within a twinship (i.e., unrelated). In addition, we collected longitudinal samples from 98 of these individuals (see Supplemental Information available online). Most subjects were female, ranging in age from 23 to 86 years (average age: 60.6 ± 0.3 years). The average BMI of the subjects was 26.25 (± 0.16) with the following distribution: 433 subjects had a low to normal BMI (<25), 322 had an overweight BMI (25-30), 183 were obese (>30), and 39 individuals in which the current BMI status was unknown. We generated 78,938,079 quality-filtered sequences that mapped to the Bacteria and Archaea in the Greengenes database (average sequences per sample: 73,023 ± 889).

Microbiome Composition and Richness

We sorted sequences into 9,646 operational taxonomic units (OTUs, ≥97% ID). Of these OTUs, 768 were present in at least 50% of the samples. Taxonomic classification revealed a fairly typical Western diversity profile: the dominant bacterial phyla were Firmicutes (53.9% of total sequences), Bacteroidetes (35.3%), Proteobacteria (4.5%), with Verrucomicrobia, Actinobacteria, and Tenericutes each comprising 2% of the sequences, and a tail of rare bacterial phyla that together accounted for the remaining 1% of the sequences.

The most widely shared methanogen was *M. smithii* (64% of people, using nonrarefied data), followed by vadinCA11, a member of the Thermoplasmata with no cultured representatives (~6%), *Methanosphaera stadtmanae* (~4%), and *Methanomassiliicoccus* (~4%, a member of the Thermoplasmata). Forty-six of the 61 samples in which we detected vadinCA11 also contained *M. smithii*, indicating that the two most dominant archaeal taxa are not mutually exclusive. Faith's PD was positively correlated with the relative abundance of the family Methanobacteriaceae (rho = 0.42 rarefied, 0.37 for transformed

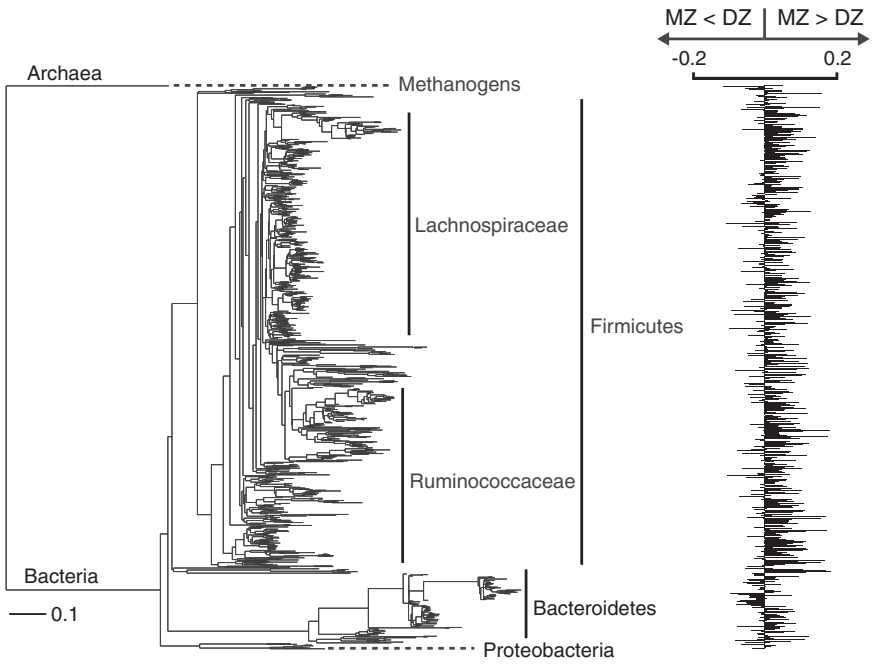

Figure 2. OTU Relative Abundances Are More Highly Correlated within MZ Than DZ Twin Pairs

Left: a phylogeny of taxa in the TwinsUK study (Greengenes tree pruned to include only OTUs shared by 50% of the TwinsUK participants).

Right: corresponding twin-pair intraclass correlation coefficients (ICCs). ICCs were calculated for each OTU and the difference in correlation coefficients for MZ twin pairs versus DZ twin pairs. Bars pointing to the right indicate that the difference is positive (i.e., MZ ICCs > DZ ICCs) and bars pointing to the left indicate negative differences (DZ ICCs > MZ ICCs). The scale bar associated with the phylogeny shows substitutions/site.

See also Figure S2.

counts, $p < 1 \times 10^{-11}$), which corroborates previous reports of higher richness associating with methanogens.

Broad Diversity Comparisons between MZ and DZ Twin Pairs

We observed that microbiotas were more similar overall within individuals (resampled) than between unrelated individuals ($p < 0.001$ for weighted and unweighted UniFrac and Bray-Curtis using a Student's t test with 1,000 Monte Carlo simulations) (Table S1A) and were also more similar within twin pairs compared to unrelated individuals ($p < 0.009$ for weighted and unweighted UniFrac and Bray-Curtis) (Figures 1 and S1; Table S1). MZ twin pairs had more similar microbiotas than DZ twins for the unweighted UniFrac metric ($p = 0.032$), but not the weighted UniFrac and Bray-Curtis metrics (Figures 1A and S1). As greater similarities for MZ versus DZ twin pairs are seen in unweighted UniFrac but not abundance-based metrics, MZ similarities are driven by shared community membership rather than structure.

We next constrained the distance metric analyses to the three most dominant bacterial families: the Lachnospiraceae and Ruminococcaceae (Firmicutes) and Bacteroidaceae (Figure 1B). We observed greater similarities for MZ compared to DZ twins using the unweighted UniFrac metric within the Ruminococcaceae family (Figure 1C). Within the Lachnospiraceae family, significantly greater similarity for MZ compared to DZ twins emerged using the weighted UniFrac and Bray-Curtis metrics (Figures 1D and 1E). In contrast, when restricted to the Bacteroidaceae family, we found that MZ and DZ twins had similar pairwise diversity using all three metrics (Figures 1F, S1B, and S1E).

MZ Twins Have More Highly Correlated Microbiotas

We next asked if the abundances of specific taxa were generally more highly correlated within MZ twin pairs compared to DZ twin pairs. For each twin pair we generated intraclass correlation coefficients (ICCs) for the relative abundances of OTUs. Mean ICCs were significantly greater for MZ compared to DZ twin pairs (Wilcoxon signed rank test on ICCs at the OTU level, $p = 6 \times 10^{-04}$; Figure 2). Because many OTUs are closely phylogenetically related, their abundances may not be independent, which may inflate levels of significance. To account for this effect, we maintained the structure of the phylogenetic tree but permuted the MZ and DZ labels in 10,000 tests to generate randomized ICCs. As an independent validation, we also applied these analyses to two previously published data sets generated originating in a population of twins from Missouri, USA: "Turnbaugh" (Turnbaugh et al., 2009), which described 54 twin pairs ranging from 21 to 32 years of age, and "Yatsunenko" (Yatsunenko et al., 2012), which included 63 twin pairs with an age range of 13–30 years of age. Mean ICCs of OTU abundances were significantly greater for MZ compared to DZ twin pairs in both of these data sets (significance by permutation: $p < 0.001$ and 0.047 respectively; Figure S2), corroborating our observations.

Heritability Estimates for OTUs and Predicted Functions

We estimated heritability using the twin-based ACE model, which partitions the total variance into three component sources: genetic effects (A), common environment (C), and unique environment (E) (Eaves et al., 1978). The largest proportion of variance in abundances of OTUs could be attributed to the twins' unique environments (i.e., E > A; Table S2). However, for the majority of OTUs (63%), the proportion of variance attributed to genetic effects was greater than the proportion of variance attributed to common environment (A > C; Table S2).

From the ACE model, we calculated 95% confidence intervals for the heritability estimates and determined the significance of the heritability values using a permutation method to generate nominal p values (Table S2). We found a high correlation between the tail probability for inclusion of zero in the confidence interval of heritability and the p values obtained from the permutation tests (rho = 0.872, $p < 10^{-15}$), indicating substantial consistency across these tests. Although heritability studies

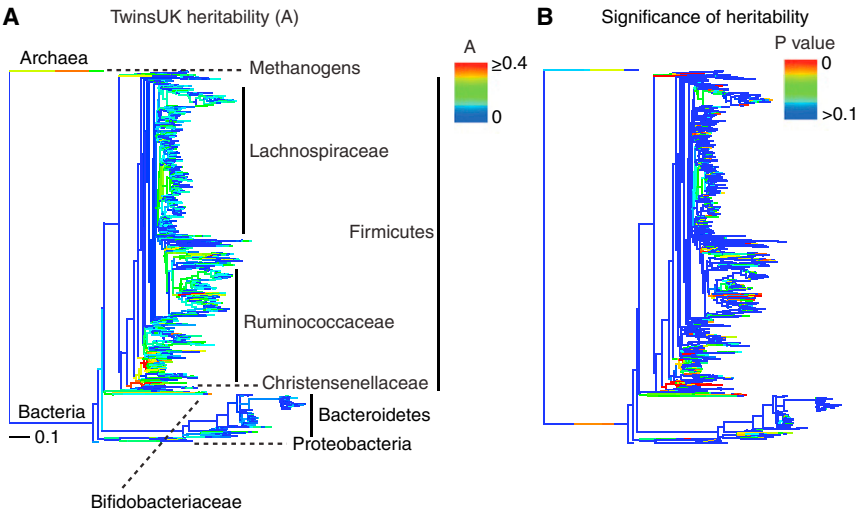

Figure 3. Heritability of Microbiota in the TwinsUK Data Set

(A) OTU Heritability (A from ACE model) estimates mapped onto a microbial phylogeny and displayed using a rainbow gradient from blue (A = 0) to red (A $\geq$ 0.4). This phylogenetic tree was obtained from the Greengenes database and pruned to include only nodes for which at least 50% of the TwinsUK participants were represented.

(B) The significance for the heritability values shown in (A) was determined using a permutation test (n = 1,000) and are shown on the same phylogeny as in (A). P values range from 0 (red) to >0.1 (blue).

See also Figure S3 and Table S2.

traditionally report confidence intervals and nominal p values only, we also generated FDR-corrected p values (Table S2).

We also applied the ACE model to the abundances of sequences mapping to each node in the phylogeny. Across the three studies, the nodes of the phylogeny with the strongest heritabilities lie within the Ruminococcaceae and Lachnospiraceae families, and the Bacteroidetes are mostly environmentally determined (Figures 3 and S3). Subsets of the Archaea are also heritable in the TwinsUK and the Yatsunenko studies (the Turnbaugh study did not include data for Archaea).

We characterized the longitudinal stability of each OTU by calculating the ICCs of the OTU abundance across repeat samples, which consisted of two samples collected from the same individual at different times. By permuting these repeat sample ICCs, we found that heritable OTUs (A > 0.2) were more stable (ICC > 0.6) than expected by chance (Figure S3E; $p < 0.001$, p value was determined as the fraction of permutations that had greater than or equal to the observed number of OTUs that are both heritable and stable).

We used PICRUSt (Langille et al., 2013) to produce predicted metagenomes from the 16S rRNA gene sequence data and applied the ACE model to estimate the heritability of predicted abundances of conserved orthologous groups (COGs). This analysis revealed six functions with heritabilities A > 0.2 and nominal p values < 0.05 (p values are generated by permutation testing; Extended Experimental Procedures; Table S2). Correcting for multiple comparisons, one category, "secondary metabolites biosynthesis, transport and catabolism" (Q), passed a stringent FDR (A = 0.32, 95% confidence interval [CI] = 0.16–0.44). We also tested α diversity for heritability and found that it was not heritable.

The Family Christensenellaceae Is the Most Highly Heritable Taxon

The most heritable taxon overall was the family Christensenellaceae (A = 0.39, 95% CI = 0.21–0.49, p = 0.001; Figure 4A; Table S2; this taxon passes a stringent FDR) of the order Clostridiales. Christensenellaceae was also highly heritable in the Yatsunenko data set (A = 0.62, 95% CI = 0.38–0.77; Figure 4B; Table S2). We repeated this analysis for the taxa abundances with the effect of BMI regressed out, and results were highly correlated (Pearson correlation = 0.95, $p < 1 \times 10^{-15}$).

Christensenellaceae Is the Hub in a Co-Occurrence Network with Other Heritable Taxa

We observe a module of co-occurring heritable families, and the hub (node connected to most other nodes) of this module is the family Christensenellaceae (Figures 5A and S4A). The heritable module includes the families Methanobacteriaceae (Archaea) and Dehalobacteriaceae (Firmicutes) and the orders SHA-98 (Firmicutes), RF39 (Tenericutes), and ML615J-28 (Tenericutes). The Christensenellaceae network is anticorrelated with the Bacteroidaceae and Bifidobacteriaceae families. We validated these results by applying this method to the family-level taxonomic abundances in the Yatsunenko data set (as this one is most technically similar to the TwinsUK data set), where we also found the same Christensenellaceae-centered module of heritable families anticorrelated to the Bacteroidaceae/Bifidobacteriaceae module (Figure S4B).

Christensenellaceae Associates with a Low BMI

The family Christensenellaceae was significantly enriched in subjects with a lean BMI (<25) compared to those with an obese BMI (>30; Benjamini-Hochberg corrected p value < 0.05 from t test on transformed counts; Table S2). Other members of the Christensenellaceae consortium were also enriched in lean-BMI subjects: the Dehalobacteriaceae, SHA-98, RF39, and the Methanobacteriaceae (Figure 5B). Overall, a majority (n = 35) of the OTUs with highest heritability scores (A > 0.2, nominal p < 0.05) were enriched in the lean subjects. A subset of OTUs classified as *Oscillospira* were enriched in lean subjects, and *M. smithii*, although not significantly heritable, was positively associated with a lean BMI.

Christensenellaceae Is Associated with Health in Published Data Sets

Because the names *Christensenella* and Christensenellaceae were only recently assigned to the bacterial phylogeny, we

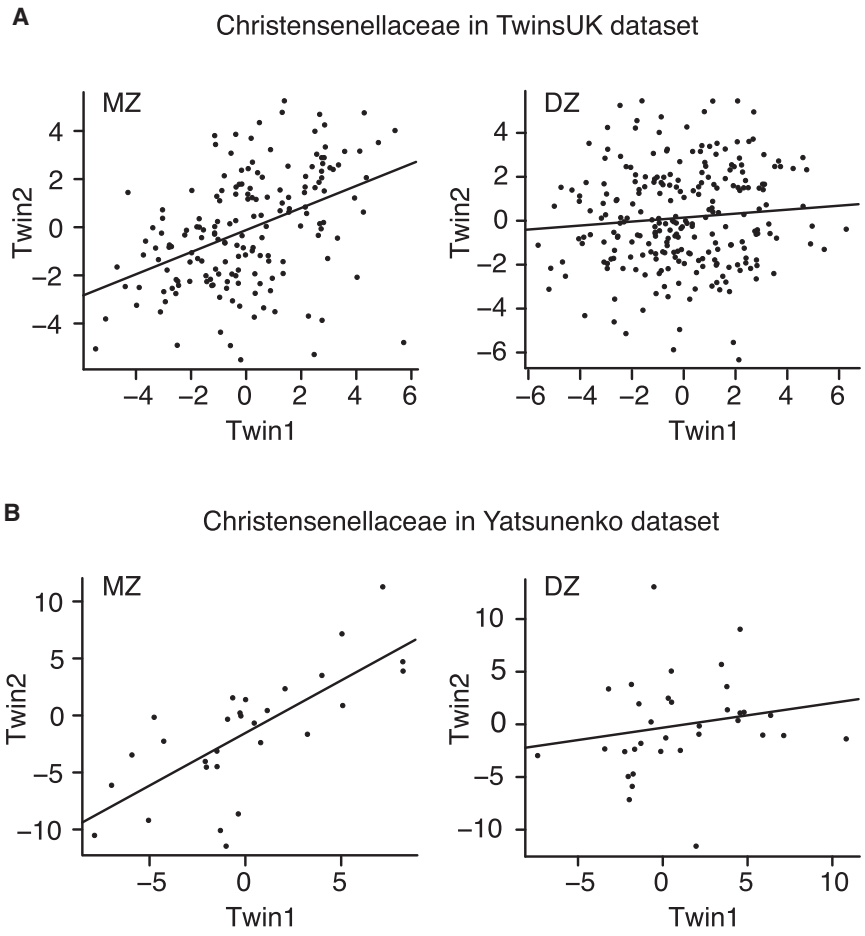

Figure 4. MZ Twin Pairs Have Higher Correlations of Christensenellaceae Than DZ Twin Pairs in TwinsUK and Yatsunenko Data Sets

Scatter plots comparing the abundances of Christensenellaceae in the gut microbiota of MZ and DZ co-twins. Christensenellaceae abundances were transformed and adjusted to control for technical and other covariates (Residuals are plotted, see Extended Experimental Procedures) and the data are separated by zygosity (MZ or DZ twins).

(A) TwinsUK data set.

(B) Yatsunenko data set.

assessed the abundances of sequences assigned to these taxa in previously published studies. This analysis revealed that members of the Christensenellaceae were enriched in fecal samples of healthy versus pediatric and young adult IBD patients ($p < 0.05$) (Papa et al., 2012). Christensenellaceae were at greater abundance in lean BMI compared to obese-BMI twins in the Turnbaugh data set, but the difference was not quite significant ("time-point 2" samples, $p = 0.07$). In a case study of the development of an infant's gut microbiome (Koenig et al., 2011), Christensenellaceae was present at 8.6% in the mother's stool at the time of birth and at 20% in the infant's meconium. We also noted that Christensenellaceae is enriched in omnivorous compared to herbivorous and carnivorous mammals (Muegge et al., 2011). However, we did not find a relationship between Christensenellaceae and diet information in human studies (Wu et al., 2011; Martínez et al., 2010; Koren et al., 2012).

Christensenellaceae Is Associated with Reduced Weight Gain in Germ-free Mice Inoculated with Lean and Obese Human Fecal Samples

Methanogens co-occurred with Christensenellaceae in this study and have been linked to low BMI in previous studies. Because of this previous association with a low-BMI, we wanted to ensure that methanogens were present in the Christensenellaceae consortium in an initial experiment exploring its effect on weight phenotypes. Therefore, we selected 21 donors for fecal transfer to germ-free mice based on BMI status (low or high) and presence or absence of the methanogen-Christensenellaceae consortium. Donors fell into one of four categories: lean with detectable methanogens (L+), lean without methanogens (L−), obese with methanogens (O+), or obese without methanogens (O−). The abundance of Christensenellaceae positively correlated with the abundance of methanogens in donor stool ($rho = 0.72$, $p = 0.0002$), indicating that methanogen abundance was a good proxy for the methanogen-Christensenellaceae consortium.

A 16S rRNA analysis of the fecal microbiomes before and after transfer to germ-free mice showed that although members of the Christensenellaceae were present throughout the experiment in recipient mice (Figure 6A), *M. smithii* was undetectable in the mouse fecal or cecal samples (the first sampling was at 20 hr postinoculation). At 20 hr postinoculation, the microbiota had shifted dramatically in diversity from the inoculation, but by day 5 had shifted back partially and remained fairly stable through day 21 (Figures 6B, 6C, S5A, and S5B). Abundances of *Christensenella* were correlated with PC3 (abundances rarefied at 55,000 sequences per sample versus unweighted UniFrac; Spearman $rho = 0.59$, $p < 2.2 \times 10^{-16}$), and PC3 captured the differences between the four donor groups (Figure 6D). We observed a trend for *Christensenella* abundances as highest in the L+ group and lowest in the O− group (Figure 6A), which mirrored the weight differences between those groups: the percent change in body weights of the recipient mice was significantly lower in the L+ group compared to the O− group (day 12, $p < 0.05$, t test; Figures 6E and 6F). Cecal levels of propionate and butyrate were significantly elevated in mice receiving methanogen-positive compared to methanogen-negative microbiomes controlling for the effect of donor BMI (two-way ANOVA, $p < 0.05$ for both SCFAs; Figures S5C–S5E). Stool energy content was significantly higher for the methanogen-positive microbiomes at day 12, when the percent changes in weight were greatest (two-way ANOVA, $p = 0.004$,

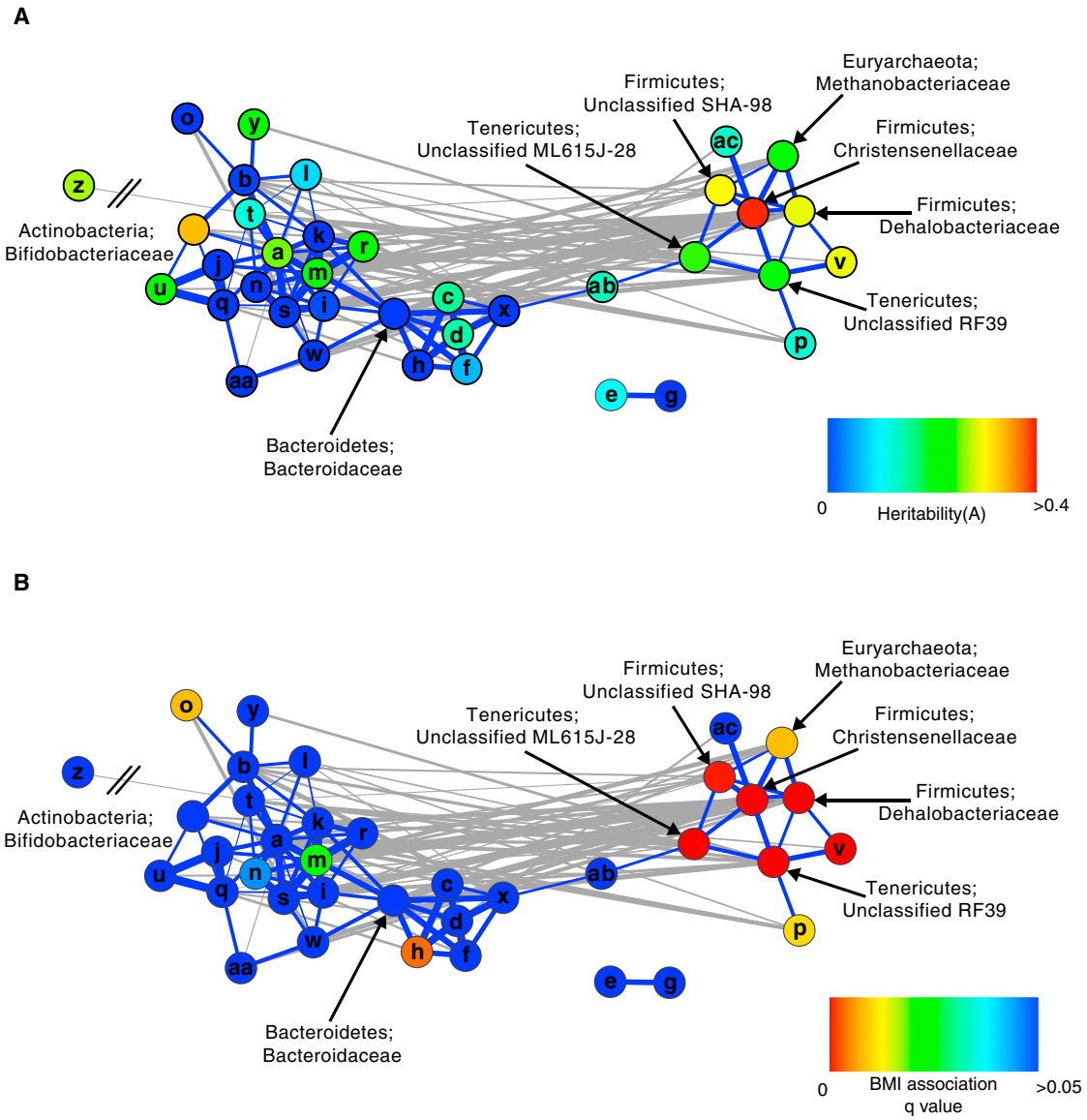

Figure 5. Christensenellaceae Is the Hub of a Consortium of Co-occurring Heritable Microbes that Are Associated with a Lean BMI

The same network built from SparCC correlation coefficients between sequence abundances collapsed at the family level. The nodes represent families and the edges represent the correlation coefficients between families. Edges are colored blue for a positive correlation and gray for a negative correlation, and the weight of the edge reflects the strength of the correlation. Nodes are positioned using an edge-weighted force directed layout.

(A) Nodes are colored by the heritability of the family.

(B) Nodes are colored by the significance of the association families and a normal versus obese BMI. Family names are either indicated on the panel, or nodes are given a letter code. Phylum Actinobacteria: (a) Actinomycetaceae, (b) Coriobacteriaceae; Phylum Bacteroidetes: (c) Barnesiellaceae, (d) Odoribacteraceae, (e) Paraprevotellaceae, (f) Porphyromonadaceae, (g) Prevotellaceae, (h) Rikenellaceae; Phylum Firmicutes: (i) Carnobacteriaceae, (j) Clostridiaceae, (k) Erysipelotrichaceae, (l) Eubacteriaceae, (m) Lachnospiraceae, (n) Lactobacillaceae, (o) Mogibacteriaceae, (p) Peptococcaceae, (q) Peptostreptococcaceae, (r) Ruminococcaceae, (s) Streptococcaceae, (t) Tissierellaceae, (u) Turicibacteraceae, (v) Unclassified Clostridiales, (w) Veillonellaceae; Phylum Proteobacteria: (x) Alcaligenaceae, (y) Enterobacteriaceae, (z) Oxalobacteraceae, (aa) Pasteurellaceae, (ab) Unclassified RF32; Phylum Verrucomicrobia: (ac) Verrucomicrobiaceae. See also Figure S4.

no effect of BMI or interaction; Figure S5F). In a replicated experiment, using 21 new donors, the same weight differences were observed (a significantly lower mean weight gain for the L+ compared to the O− mouse recipients at day 10 postinoculation; one-way t test, p = 0.047; Figure S5G).

Christensenella minuta Added to Donor Stool Reduces Adiposity Gains in Recipient Mice

Based on the observation that *Christensenella* levels in the previous experiment were similar to the weight gain patterns, we performed experiments in which a donor stool lacking

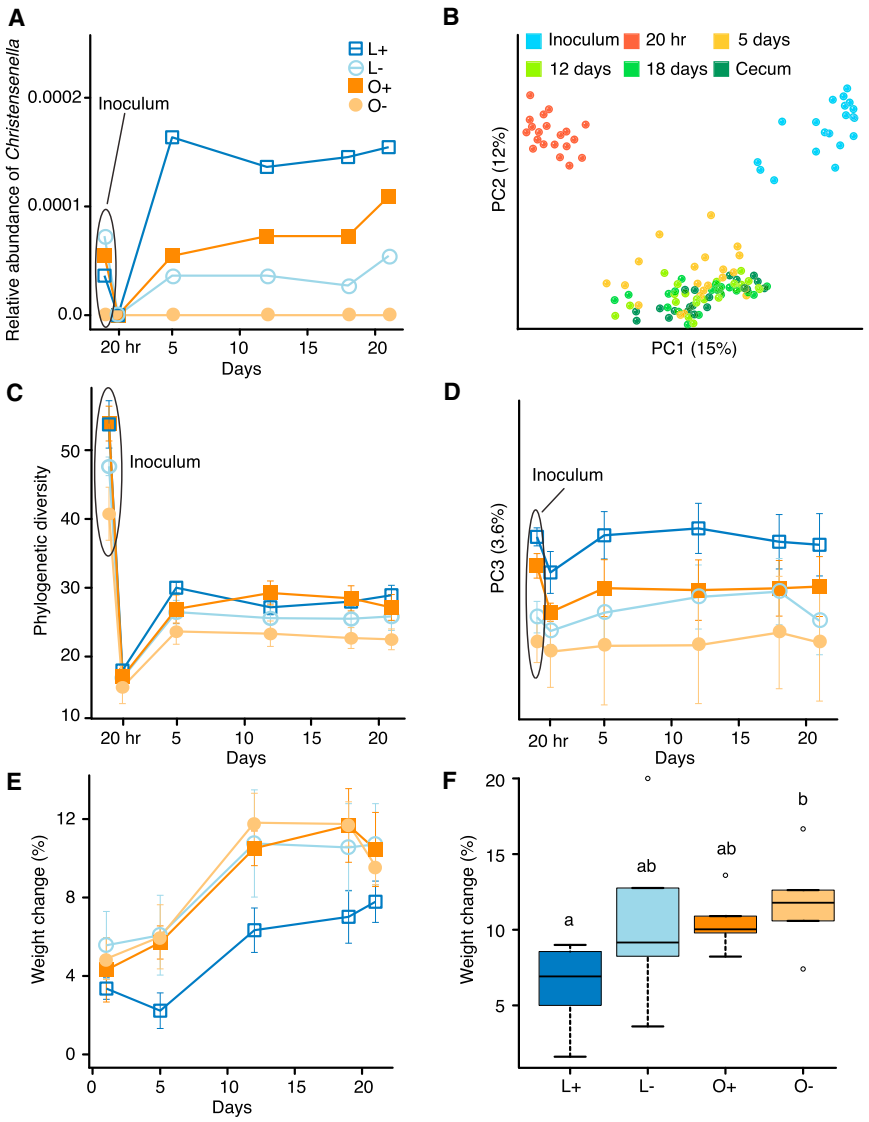

Figure 6. Fecal Transplants from Obese and Lean UK Twins to Germ-Free Mice Reveal Levels of Christensenellaceae Posttransfer Mirror Delayed Weight Gain

(A) Median relative abundances for OTUs classified as the genus *Christensenella* in the four donor treatment groups over time in the recipient mouse microbiotas.

(B) Principal coordinates analysis of unweighted UniFrac distances for (1) the inoculum prior to transplantation, (2) fecal samples at four time points, and (3) cecal samples at day 21 posttransplant; see panel legend for color key. The amount of variance described by the first two PCs is shown on the axes.

(C) Mean values ± SEM for richness (Faith's PD) for the microbiomes of the transplant mice plotted against time (days postinoculation, with day 0 = inoculation day).

(D) The mean values ± SEM for PC3 derived for the same analysis as shown in (B) are plotted against time (day 0 = inoculation day) for the four treatment groups. The amount of variance explained by PC3 is in parentheses.

(E) Percent weight change since inoculation for germ-free mouse recipients of 21 donor stools that were obtained from lean or obese donors with or without detectable *M. smithii*, which was used as a marker for the Christensenellaceae consortium. Means for each treatment group are plotted ± SEM.

(F) Boxplots for percent weight changes for the four groups at day 12 posttransplant, when maximal weight differences were observed. Letters next to boxes indicate significant differences if letters are different ($p < 0.05$). For all panels: dark blue, L+, lean donor with methanogens; light blue, L−, lean donor lacking methanogens; dark orange, O+, obese donor with methanogens; light orange, O−, obese donor without methanogens. We repeated this experiment with a set of 21 new mice and unique human donors and recovered the same effect.

See also Figure S5.

detectable *Christensenella* was amended with *C. minuta* and weight gain of recipient mice was monitored. One obese human donor was selected from the 21 donors from the first transplant experiment based on its lack of detectable OTUs assigned to the genus *Christensenella*. At day 21 postgavage, mice receiving the *C. minuta* treatment weighed significantly less than those that received unamended stool (nested ANOVA, $p < 0.05$; Figure 7A). Adiposity was significantly lower for mice receiving the *C. minuta* treatment (nested ANOVA, $p = 9.4 \times 10^{-5}$, Figure 7B). Energy content for stool collected at day 21 was not different between treatments (data not shown).

Analysis of the microbial community by 16S rRNA gene sequencing showed an impact on the overall community diversity that persisted over time (Figures 7C and 7D). After an initial acclimation (20 hr), the communities within recipient mice began to separate by treatment regardless of the effects of time and cocaging (Figures 7C, 7D, and S6). At 5 days postinoculation, the relative abundance of *C. minuta* was similar to that observed in the previous transplant experiment and persisted throughout the duration of the study. We identified two genera that discriminated the two treatments at day 21: *Oscillospira* and a genus within the Rikenellaceae were enriched in the *C. minuta* treatment (misclassification error rate of 0.06). *Oscillospira* abundances were significantly correlated with PC2 in the unweighted UniFrac analysis of the communities (rho = −0.71, $p = 0.0009$; Figure 7E), which is the PC that separates the *C. minuta*-amended and unamended microbiotas.

DISCUSSION

Our results represent strong evidence that the abundances of specific members of the gut microbiota are influenced in part by the genetic makeup of the host. Earlier studies using fingerprinting approaches also reported host genetic effects (Stewart

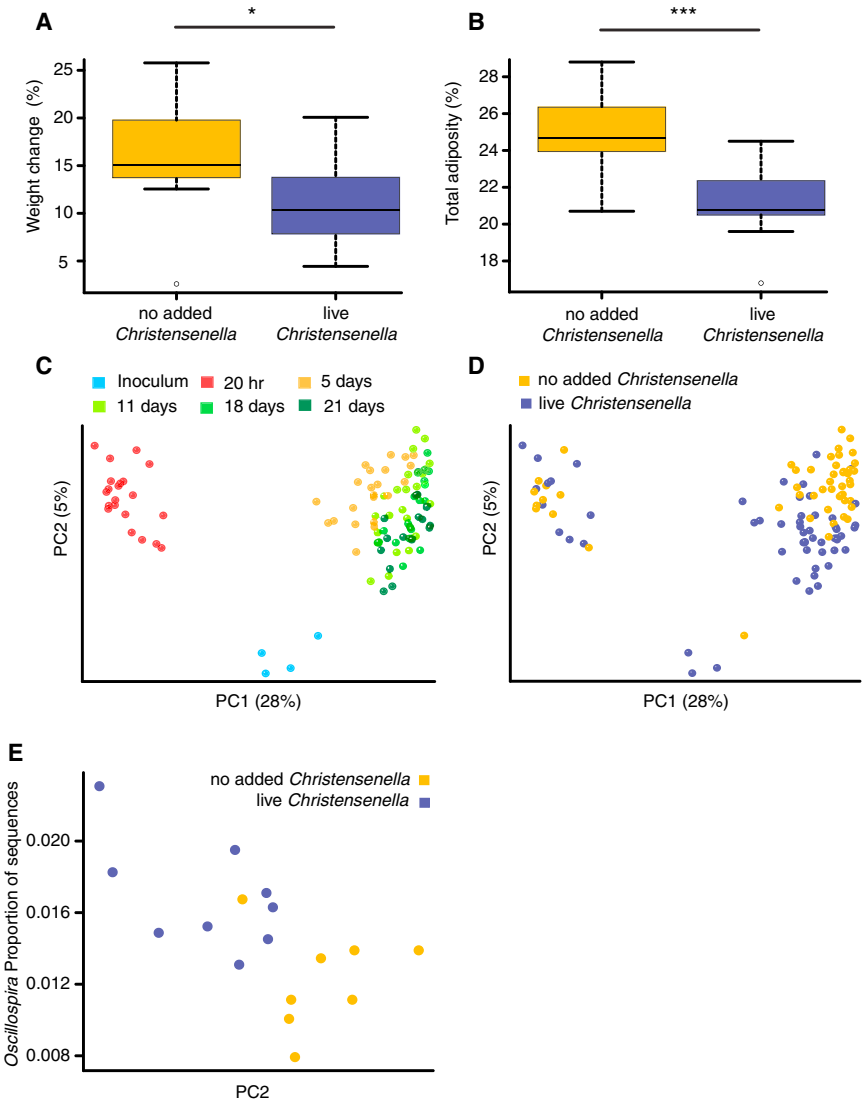

Figure 7. Addition of *Christensenella minuta* to Donor Stool Leads to Reduced Weight and Adiposity Gains in Recipient Mice

(A) Boxplot of percent weight change for germ-free mouse recipients of a single donor stool only (lacking detectable *Christensenella* in unrarefied 16S rRNA data) or the donor stool amended with live *C. minuta*.

(B) Boxplots showing percent body fat for mice in each group at day 21 (n = 12 mice per treatment).

(C and D) Principal coordinates analysis of unweighted UniFrac distances for (1) the inoculum prior to transplantation, (2) fecal samples at five time points posttransplant; see legend for color key. The amount of variance described by the first two PCs is shown on the axes. The same data projection is shown in (C) and (D); sample symbols are colored by time point (C) and by treatment (D).

(E) Relationship between PCs from the PCoA analysis and levels of *Oscillospira* at day 21 (rho = −0.71, p = p < 0.001). Symbols are colored by treatment.

See also Figure S6.

et al., 2005; Zoetendal et al., 2001), but without sequence data it is not possible to know if the taxa shown here to be heritable were also driving those patterns. The Turnbaugh et al. (2009) and Yatsunenko et al. (2012) studies, which are quite similar in experimental approach, reported a lack of host genetic effect on the gut microbiome, most likely because both studies were underpowered. Nevertheless, reanalysis of the data from both studies validated our observation that the abundances of taxa are more highly correlated within MZ than DZ twin pairs. Thus, host genetic interactions with specific taxa are likely widespread across human populations, with profound implications for human biology.

The most highly heritable taxon in our data set was the family Christensenellaceae, which was also the hub of a co-occurrence network that includes other taxa with high heritability. A notable component of this network was the archaeal family Methanobacteriaceae. Similarly, Hansen et al. (2011) had previously identified members of the Christensenellaceae (reported as relatives of *Catabacter*) as co-occurring with *M. smithii*. These co-occurrence patterns could derive from different scenarios: for instance, multiple taxa may be heritable and co-occur while each taxon is affected by host genetics independently, or alternatively one (or a few) taxa may be heritable and other taxa correlate with host genetics due to their co-occurrence with these key heritable taxa. Further experimental research will be required to elucidate if the co-occurring heritable taxa interact in syntrophic partnerships or simply respond similarly to host-influenced environmental cues in the gut.

Our results suggest that environmental factors mostly shape the Bacteroidetes community, because most were not heritable. These results are consistent with those of a recent study of Finnish MZ twins, in which levels of *Bacteroides* spp. were more similar between twins when their diets were similar (Simões et al., 2013). Members of the Bacteroidetes have been shown to respond to diet interventions (Wu et al., 2011; David et al., 2014)

Importantly, the family Christensenellaceae is heritable in the Yatsunenko data set and its network is also present. This validation did not involve a directed search using the taxa identified in this study but was made by applying the ACE model independently. In the TwinsUK as well as the Missouri twins data sets, the majority of OTUs with the highest heritability estimates fell within the Ruminococcaceae and Lachnospiraceae families. The Missouri and TwinsUK studies differed somewhat in the levels and structure of heritability, which may relate to study size (Kuczynski et al., 2010), participant age (Claesson et al., 2011), population (Yatsunenko et al., 2012), and/or diet (Wu et al., 2011), all of which have been shown to affect microbiome structure.

The high heritability of the Christensenellaceae raises questions about the nature of interactions between the host and members of this family, but to date there is little published work with which to infer their roles. *Christensenella minuta* is Gram-negative, nonspore forming, nonmotile, and produces SCFAs (Morotomi et al., 2012). A review of publicly available data suggests it is present from birth and associates with a healthy state but not with diet. Thus, although diet is a heritable trait in the same population (Menni et al., 2013; Teucher et al., 2007), it does not appear to be driving the heritability of the Christensenellaceae. Obesity is also strongly heritable in the TwinsUK population, raising the question of whether the heritabilities we saw for gut microbes were driven by BMI. To test this, we reran the heritability calculations using residuals after regressing out the effect of BMI and found that results of the two analyses were highly correlated. This suggests that the effect of host genetics on Christensenellaceae abundance is independent of an effect of BMI.

Our transplantation experiments showed a moderating effect of methanogen-presence in the human donor on weight gain of recipient mice, although strikingly, *M. smithii* did not persist in mice. In contrast, Christensenellaceae levels in mice mirrored their weight gain. Transfer to germ-free mice of microbiomes from obese and lean donors generally results in greater adiposity gains for obese compared to lean donors (Ridaura et al., 2013; Turnbaugh et al., 2008; Vijay-Kumar et al., 2010). These studies have not reported the methanogen or Christensenellaceae status of the donors, so whether these microbes affect the host phenotype is unknown. *M. smithii* has been associated with a lean phenotype in multiple studies (Million et al., 2012, 2013; Schwiertz et al., 2010; Armougom et al., 2009; Le Chatelier et al., 2013), raising the possibility that methanogens are key components of the consortium for regulating host phenotype. The results of our methanogen-Christensenellaceae transfer revealed that although methanogens may be a marker for a low BMI in humans, they are not required to promote a lean phenotype in the germ-free mouse experimental model. This result suggests that methanogens may be functionally replaced by another consortium member in the mouse, while the Christensenellaceae are not.

The results of the *C. minuta* spike-in experiments supported the hypothesis that members of the Christensenellaceae promote a lean host phenotype. Addition of *C. minuta* also remodeled the diversity of the community. Intriguingly, *Oscillospira*, which includes heritable OTUs in the TwinsUK data set and is associated with a lean BMI, was enriched in the *C. minuta*-amended microbiomes. How *C. minuta* reshapes the community remains to be explored. The relatively low levels of *C. minuta* and its profound effects on the community and the host may indicate that it is a keystone taxon. Together these findings indicate that the Christensenellaceae are highly heritable bacteria that can directly contribute to the host phenotype with which they associate.

Conclusions

Host genetic variation drives phenotype variation, and this study solidifies the notion that our microbial phenotype is also influenced by our genetic state. We have shown that the host genetic effect varies across taxa and includes members of different phyla. The host alleles underlying the heritability of gut microbes, once identified, should allow us to understand the nature of our association with these health-associated bacteria and eventually to exploit them to promote health.

EXPERIMENTAL PROCEDURES

Human Subjects and Sample Collection

Fecal samples were obtained from adult twin pair participants of the TwinsUK registry (Moayyeri et al., 2013). Most participants were women (only 20 men were included). Twins collected fecal samples at home, and the samples were refrigerated for up to 2 days prior to their annual clinical visit at King's College London, at which pointed they were stored at −80°C until processing.

Diversity and Phylogenetic Analyses

We amplified 16S rRNA genes (V4) from bulk DNA by PCR prior to sequencing on the Illumina MiSeq 2 × 250 bp platform at Cornell Biotechnology Resource Center Genomics Facility. We performed quality filtering and analysis of the 16S rRNA gene sequence data with QIIME 1.7.0 (Caporaso et al., 2010).

Predicted Metagenomes

PICRUSt v1.0.0 was used to predict abundances of COGs from the OTU abundances rarefied at 10,000 sequences per sample.

Heritability Estimations

Heritability estimates were calculated on the OTU abundances, taxon bins, nodes throughout the bacterial phylogenetic tree, α-diversity, and PICRUSt-predicted COGs using the structural equation modeling software OpenMx (Boker et al., 2011).

Microbiota Transfer Experiments

Stool samples from the Twins UK cohort were selected as described in the main text and inoculated into 6-week-old germ-free Swiss Webster mice via oral gavage, with one recipient mouse per fecal donor. Mice were single-housed. For the *Christensenella minuta* addition, three experiments were conducted: In the first experiment, one treatment group received only donor stool inoculum, whereas the other received donor stool amended with 1×10^8 *C. minuta* cells; for the second experiment, a heat-killed *C. minuta* control was added; in the third experiment, the heat-killed control was compared to live *C. minuta* only (no vehicle-only control). Mice were housed four per cage, with three cages per treatment. In all experiments, mice were provided with autoclaved food and water ad libitum and maintained on a 12 hr light/dark cycle. Body weight and chow consumption were monitored and fecal pellets were collected weekly. At sacrifice, adiposity was analyzed using DEXA. Body, mesenteric adipose tissue, and gonadal fat pad tissue weights were collected at this time. Gross energy content of mouse stool samples was measured by bomb calorimetry using an IKA C2000 calorimeter (Dairy One). Wet cecal contents were weighed and resuspended in 2% (v/v) formic acid by vortexing and measured using a gas chromatograph (HP series 6890) with flame ionization detection.

Statistical Analysis

Values are expressed as the mean ± SEM unless otherwise indicated. Full methods are described in the Supplemental Information.

ACCESSION NUMBERS

The European Bioinformatics Institute (EBI) accession numbers for the sequences reported in this paper are ERP006339 and ERP006342.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, six figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2014.09.053.

AUTHOR CONTRIBUTIONS

R.E.L. and A.G.C. supervised the study. J.T.B. and T.D.S. helped design study and provided comments and discussion. J.T.B. and T.D.S. oversaw collection of samples. J.K.G., R.E.L., O.K., J.L.S., A.C.P., and J.L.W. oversaw microbial data generation. J.K.G. performed the analysis with contributions from R.E.L., R.B., A.G.C., J.L.W., O.K., A.C.P., M.B., W.V.T., and R.K. J.K.G. and J.L.W. performed microbiota transfer experiments. J.K.G., J.L.W., and R.E.L. prepared the manuscript with comments from A.G.C., T.D.S., J.T.B., R.B., and R.K.

ACKNOWLEDGMENTS

We thank Wei Zhang, Sara Di Rienzi, Lauren Harroff, Largus Angenent, Hannah de Jong, Gabe Fox, Nick Scalfone, Aymé Spor, and Beth Bell for their help. We also thank three anonymous reviewers for their helpful comments and Mary-Claire King for her advice and encouragement. This work was funded by NIH RO1 DK093595, DP2 OD007444, The Cornell Center for Comparative Population Genomics, the Wellcome Trust, and the European Community's Seventh Framework Programme (FP7/2007-2013). The study also received support from the National Institute for Health Research (NIHR) BioResource Clinical Research Facility and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. R.E.L. is a Fellow of the David and Lucile Packard Foundation and of the Arnold and Mabel Beckman Foundation. J.K.G. is a National Academy of Sciences predoctoral Fellow. T.D.S. is holder of an ERC Advanced Researcher Award. R.K. is a Howard Hughes Medical Institute Early Career Scientist.

Received: April 3, 2014
Revised: July 10, 2014
Accepted: September 24, 2014
Published: November 6, 2014

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