Interactions between Gut Microbiota, Host Genetics and Diet Modulate the Predisposition to Obesity and Metabolic Syndrome

Graphical Abstract

- Host genetics determine changes in gut microbiota in response to high-fat diet
- Environmental history impacts on gut microbiota and response to dietary challenges
- Specific bacterial taxa correlate with metabolic phenotypes within and across strains
- Diet, host genetics, and gut microbiota interact in development of metabolic syndrome

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In Brief
In a longitudinal analysis of host genetics, diet, and gut microbiota interactions, Ussar et al. demonstrate how interactions between the gut microbiota, host genetics, and diet influence the development of metabolic syndrome. The authors find that specific bacterial taxa appear to be linked to specific phenotypes. Changing the environment in early life not only changes the microbiota but also changes development of metabolic syndrome.

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Interactions between Gut Microbiota, Host Genetics and Diet Modulate the Predisposition to Obesity and Metabolic Syndrome

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SUMMARY

Obesity, diabetes, and metabolic syndrome result from complex interactions between genetic and environmental factors, including the gut microbiota. To dissect these interactions, we utilized three commonly used inbred strains of mice—obesity/diabetes-prone C57Bl/6J mice, obesity/diabetes-resistant 129S1/SvImJ from Jackson Laboratory, and obesity-prone but diabetes-resistant 129S6/SvEvTac from Taconic—plus three derivative lines generated by breeding these strains in a new, common environment. Analysis of metabolic parameters and gut microbiota in all strains and their environment-normed derivatives revealed strong interactions between microbiota, diet, breeding site, and metabolic phenotype. Strain-dependent and strain-independent correlations were found between specific microbiota and phenotypes, some of which could be transferred to germ-free recipient animals by fecal transplantation. Environmental reprogramming of microbiota resulted in 129S6/SvEvTac becoming obesity resistant. Thus, development of obesity/metabolic syndrome is the result of interactions between gut microbiota, host genetics, and diet. In permissive genetic backgrounds, environmental reprogramming of microbiota can ameliorate development of metabolic syndrome.

INTRODUCTION

We are in the midst of worldwide epidemics of obesity, type 2 diabetes (T2D), and metabolic syndrome. These disorders present as a spectrum of overlapping phenotypes from metabolically healthy obese individuals to those with full-blown T2D and metabolic syndrome, arising from a complex set of interactions between genetic risk factors and environmental influences. Genome-wide association studies both in humans (Ng et al., 2014; Saxena et al., 2007; Speliotes et al., 2010; Xia and Grant, 2013) and rodents (Almind et al., 2003; Davis et al., 2012; Yazbek et al., 2011) have identified multiple loci that contribute to obesity and its associated metabolic abnormalities, each with a small effect. A number of environmental modifiers of disease expression have been defined, including caloric intake, dietary composition, levels of activity, and non-exercise-related energy expenditure (Franks et al., 2013; Kahn et al., 2006; Tuomi et al., 2014).

Recent studies in both rodents and humans indicate that the gut microbiota is also a contributor to metabolic disorders (reviewed in Khan et al., 2014). In general, obese humans and rodents have less diverse gut communities than their lean counterparts (Le Chatelier et al., 2013; Serino et al., 2012; Turnbaugh et al., 2009). Likewise, metagenomic studies have documented differences in the microbial gene repertoires represented in the gut communities of individuals with obesity (Greenblum et al., 2012), T2D (Karlsson et al., 2013; Larsen et al., 2010), or non-alcoholic fatty liver disease (Zhu et al., 2013). Evidence for a causal relationship between the gut microbiota and metabolic dysfunctions has come from studies showing that cohousing (Henao-Mejia et al., 2012; Upadhyay et al., 2012) or antibiotic treatment (Cox et al., 2014; Keeney et al., 2014) can modify obesity and metabolic phenotype in rodent models. Transplantation of fecal microbiota from obese versus lean mice, obese versus lean humans, and human twin pairs stably discordant for obesity into germ-free mouse recipients transmits adiposity and metabolic phenotypes (Le Roy et al., 2013; Million et al., 2013; Ridaura et al., 2013). Some of the effects of gastric bypass surgery on obesity and metabolic dysfunction have also been related to changes in gut microbiota (Liou et al., 2013). While cross-sectional studies and short-term experiments assessing effects of microbiota transfer have provided important insights into the role of gut microbiota in metabolic syndrome, additional approaches are needed to assess the long-term nature of normal environmental changes and the complex interaction between...
host genetics, diet, and the microbiota in the regulation of metabolism.

We and others have previously shown that C57BL/6J mice (B6J) gain more weight, have more insulin resistance, and show greater glucose intolerance than 129S1/SvImJ (129J) mice obtained from the same production facility. These phenotypic differences are further exacerbated by high-fat diet (HFD) (Almind and Kahn, 2004; Almind et al., 2007) or imposition of genetic defects in insulin signaling (Kulkarni et al., 2003), illustrating the strong impact of strain background on both genetic and environmentally induced insulin resistance. In the present study, we have characterized interactions between host genetics, diet, and the gut microbiota by conducting longitudinal analyses of the responses of three commonly used inbred strains of mice to long-term dietary challenges. Two of the strains were the B6J and 129J mice described above from the Jackson Laboratory (Jax). The third was the 129S6/SvEvTac strain from Taconic Farms (129T) (Almind et al., 2003; Mori et al., 2010), which is closely related to the 129J strain but is bred in a different facility and treated with a probiotic mixture, thereby creating a substantial difference in their microbiota (Ivanov et al., 2009). In addition, we have created three environmentally normalized groups by inbreeding each of the strains for three to six generations in our own animal facility under a common set of conditions.

We show that, in contrast to obesity- and diabetes-resistant 129J mice, genetically related 129T mice resemble B6J mice in their susceptibility to develop diet-induced obesity and enlarged livers on HFD, but retain normal insulin sensitivity and develop only mild glucose intolerance. Interestingly, these differences in weight gain and glucose tolerance between 129J and 129T mice were lost after rearing these mice in the same environment for more than three generations. Using bacterial 16S rRNA sequencing and fecal microbiota transplants to germ-free recipient mice, we demonstrate that both the original differences in phenotypes and their changes following environmental normalization and dietary challenge are closely related to the gut microbiota, and that the composition of microbiota is highly dependent on diet, environmental history, and host genetics. We also demonstrate strong mouse strain-dependent and strain-independent relationships between specific metabolic phenotypes and specific bacterial taxa or communities, indicating the strong, complex, and dynamic interactions between the microbiota, diet, environmental ancestry, and host genetics.

RESULTS

Susceptibility to Weight Gain and Insulin Resistance Is Influenced by Genetic Background and Environmental Origin

B6J and 129J mice exhibit major differences in their tendencies to gain weight and develop diabetes and metabolic syndrome with age or consumption of a HFD. As previously reported (Almind and Kahn, 2004; Almind et al., 2007), when 4-week-old, vendor-bred B6J male mice were placed on a NIH-31M chow diet (CD) and followed for 22 weeks, they gained significantly more weight than 129J mice, also from Jax Labs (Figure 1A, left panel). This difference in weight gain was further exaggerated when the mice were fed a HFD with 60% of calories from fat (Figure 1A, right panel). When these diets were given to 129T mice, the result was remarkably different with 129T mice being as prone to weight gain as the B6J animals (Figure 1A and see Figure S1A available online).

Among the three strains of mice, B6J animals had the highest fasting glucose levels and the greatest level of hyperglycemia following an intraperitoneal glucose tolerance test (GTT). This was true in CD-fed mice and exacerbated further by HFD (Figure 1B). 129J mice had a trend to lower fasting glucose levels and significantly lower excursions during GTT than B6J mice on both CD and HFD (Figure 1B). Interestingly, despite having similar body weights on CD, glucose tolerance was better in 129T than in 129J mice, whereas on HFD, both had similar impairments of glucose tolerance, although the 129T mice gained more weight than the 129J mice (Figures 1A, 1B, and S1A). Importantly, both 129 strains remained insulin sensitive compared to B6J mice with lower basal insulin levels and HOMA-IR levels, even after 22 weeks of HFD (Figures 1C, 1D, and S1B). On CD both 129 strains had higher serum levels of PYY and GIP compared to B6J mice, and 129T mice had higher levels of FGF21 (Figure S1C). When challenged with HFD, all three strains also showed 6- to 10-fold increases in hepatic triglyceride concentration (Figure 1E). However, there were major differences in degree of hepatomegaly among strains, with a 150% increase in liver weight in B6J mice, a 130% increase in 129T mice, and only a 30% in 129J mice (Figure 1F). Changes in tissue inflammatory markers in the three strains of mice, on the other hand, closely followed the differences in insulin resistance, with higher levels of TNF-α, IL-6, and F4/80 in B6J fat and liver compared to both 129 strains, especially on HFD (Figure 1G). Thus, B6J mice gain more weight and exhibit all of the features of metabolic syndrome when placed on HFD, whereas 129J are resistant to weight gain and protected from metabolic syndrome (Table S1). 129T mice, on the other hand, have a unique blend of features, being prone to obesity and hepatosteatosis on HFD but protected from insulin resistance, thus resembling the more metabolically healthy subgroup of obese humans (Samocha-Bonet et al., 2014).

“Environmental Normalization” Changes Susceptibility to Metabolic Syndrome

To determine to what extent genetic versus environmental differences at the commercial animal facilities contribute to these differences in susceptibility to metabolic syndrome, each of these vendor-bred mouse strains was imported into our animal facility, housed in separate cages in this common environment, and then inbred for three to six generations (Figure 2A). The resulting new lines were designated B6/Jos, 129/Jos, and 129T/Jos. The three Jos-born strains, as well as cohorts of mice imported directly from Jackson and Taconic, were fed either a CD or HFD for 22 weeks, starting at 4 weeks of age. Whereas B6/Jos and 129/Jos mice behaved similarly to the vendor-bred mice in terms of weight gain (i.e., B6/Jos mice gained more weight on both CD and HFD than 129/Jos mice), after three generations of inbreeding in a new environment, 129T/Jos mice gained significantly less weight, especially during the first 8 weeks of the study, on both CD and HFD than their vendor-bred counterparts (Figures 2B and 2C; Table S1).
Other components of metabolic syndrome were modified to different extents by environmental normalization. For example, the difference in glucose tolerance observed between 129J and 129T mice imported from the vendor disappeared completely after environmental normalization such that 129J/Jos and 129T/Jos mice had virtually identical glucose tolerance on CD and similar levels of glucose intolerance on HFD, both of which were significantly better than the B6J/Jos mice, which were quite glucose intolerant on both diets (Figure 2D). Likewise, although hepatic triglyceride content increased significantly in all three Joslin-bred strains when challenged with HFD, the differences in liver weight on HFD between the two 129J and 129T mice disappeared in the two environmentally normalized strains (Figures 2E, S2A, and S2B). By contrast, environmental normalization had little effect on inflammation. Both B6J and B6J/Jos mice fed a HFD showed marked increases in inflammatory markers in visceral adipose tissue and liver compared to all 129 strains, which showed little inflammation in these tissues (Figures 2E and S2C).

Both B6J and B6J/Jos mice had higher levels of insulin and HOMA-IR, especially on HFD, than did either strain of the vendor-bred or Joslin-bred 129 mice (Figures 2 G and 2H), indicating the higher level of insulin resistance in the B6J and B6J/Jos mice before and after environmental normalization. The differences in enteroendocrine hormones (GIP, PYY, and FGF21) also persisted in the Joslin-bred strains (compare Figure S1 C and Figure S2 D).

Thus, environmental normalization eliminated many of the differences observed in the two vendor-bred strains of 129 mice, such that both 129J/Jos and 129T/Jos mice showed similar
levels of glucose tolerance and were resistant to weight gain and hepatosteatosis on HFD (Table S1). By contrast, environmental normalization had no effect to ameliorate diet-induced obesity, insulin resistance, hepatosteatosis, or tissue inflammation in B6J animals, which remained prone to all aspects of the metabolic syndrome regardless of site of breeding.

Mechanisms Underlying Differences in Weight Gain and Inflammation

To better define the mechanisms underlying differences in weight gain, food intake, and energy expenditure, the strains were assessed in CLAMS metabolic cages 8 weeks into the study. Despite exhibiting less weight gain, both 129J and 129T mice tended to eat more on CD than B6J mice (Figure 3A). The differences in weight gain on HFD could not be explained by differences in caloric intake or by differences in spontaneous activity. B6J mice, which gained the most weight, were significantly more active than 129J, with 129T mice being intermediate with no difference between the original strains and their Joslin-bred counterparts (Figure 3B). Metabolic rates (i.e., oxygen consumption [VO₂] and carbon dioxide [VCO₂] production) were significantly higher in 129T than B6J mice especially on HFD, and intermediate in 129J mice (Figures 3C and 3D). The tendency to higher metabolic rate in 129J compared to B6J mice could contribute to their obesity resistance. Respiratory exchange ratios (RERs) were similar in all three original strains on CD, tended to be higher in 129J/Jos mice, and were significantly increased in 129T/Jos mice compared to the original strains. All mice showed similar reductions upon HFD feeding (Figure 3E). Thus, 129J mice tended to eat more, were less active than B6J mice, and were resistant to weight gain primarily due to a higher basal metabolic rate. 129T mice also had a higher metabolic rate than B6J mice, but were slightly less active and ate slightly more, allowing them to gain similar amounts of weight as B6J mice on HFD. Three generations of breeding in the new environment reversed the tendency to gain weight in 129T/Jos mice by inducing small decreases in food intake and small increases in metabolic rate.

Influence of Genetic Background, Birthplace, and Diet on Gut Microbiota

To characterize the fecal microbiota of mice with different genetic backgrounds, birthplaces, ancestral origins (i.e., descended from Tac mice or Jax mice), and diets, we sequenced...
variable region 4 (V4) of bacterial 16S rRNA genes present in the 287 fecal samples collected over the course of the experiments described in Figure 2A and analyzed as described in Supplemental Experimental Procedures (Table S2).

Principal coordinates analysis (PCoA) plot of unweighted UniFrac distances between all 287 samples showed that the first principal coordinate (PC1), which explains the greatest variance, segregated samples by diet, while the second coordinate (PC2) segregated them by “birthplace” (Jax, Tac, and Jos) and strain (B6, 129J, and 129T) (Figure 4A). These three major driving forces are even more apparent when samples are separated by experimental week (Figures 4B and S3A). At time 0, when all animals were 4 weeks of age and had been only on CD since weaning, the major separation was by site of breeding (vendor versus Joslin) with lesser but clear differences among the strains. Within 1 week of HFD feeding, both vendor- and Joslin-bred mice showed a major rightward shift along PC1, which persisted during the entire 22 week dietary challenge (Figure 4B). In addition, over time, there were shifts along PC2 such that the differences between vendor-bred and Joslin-bred mice of all strains tended to converge, although some differences remained (Figure 4B). Similar results were obtained when the PCoA analysis was performed for data collected each week: the plots separate Jax and Jos bred mice from the Tac bred 129T even more clearly, illustrating the unique phylogenetic composition of the microbiota in the Taconic vendor-derived animals (Figure S3A).

Permutational multivariate analysis of variance (PERMANOVA; see Supplemental Glossary and Supplemental Experimental Procedures) demonstrated that at week 0, when all three vendor-bred strains and the corresponding three Joslin-bred mice were consuming CD, birthplace accounted for 33.8% of the variance in microbial diversity (as measured by UniFrac distances), while “strain” (129 versus B6) and “ancestral origin” (Tac versus Jax) accounted for 10.2% and 4.3%, respectively. These influences were illustrated by the fact that Joslin-bred 129T/Jos and 129J/Jos mice harbored microbial communities that were more phylogenetically similar to each other than either was to B6J/Jos, whereas in vendor-bred animals, the B6J and 129J microbial communities were more similar to each other than either was to the 129T (Figure 4C). With increasing time at Joslin, CD-fed vendor-bred mice developed microbiota that manifested increasing phylogenetic similarity to their corresponding Joslin-bred strains (Figures 4B–4F, S3B, and S3C). This occurred despite the fact that none of the measured by UniFrac distances), while “strain” (129 versus B6) and “ancestral origin” (Tac versus Jax) accounted for 10.2% and 4.3%, respectively. These influences were illustrated by the fact that Joslin-bred 129T/Jos and 129J/Jos mice harbored microbial communities that were more phylogenetically similar to each other than either was to B6J/Jos, whereas in vendor-bred animals, the B6J and 129J microbial communities were more similar to each other than either was to the 129T (Figure 4C). With increasing time at Joslin, CD-fed vendor-bred mice developed microbiota that manifested increasing phylogenetic similarity to their corresponding Joslin-bred strains (Figures 4B–4F, S3B, and S3C). This occurred despite the fact that none of the
Figure 4. Phylogenetic Similarity and Phylogenetic Diversity of Fecal Microbiota

(A) PCoA of unweighted UniFrac distances for all 287 fecal samples, with fitted vectors indicating the effects of diet and birthplace calculated using weighted mean positions along principal coordinates 1 and 2 (PC1 and PC2).

(B) PCoA of unweighted UniFrac distances for all 287 fecal samples, separated by week.

(C) Percent phylogenetic similarity [calculated as 100 x (1 – unweighted UniFrac distance)] within each experimental group of mice and between the three vendor-bred strains and the three Joslin-bred strains at week 0 of the experiment. Comparisons of selected mean distances within and between experimental treatment groups were performed using permutation tests with 10,000 randomizations, followed by Holm’s corrections for multiple tests. Shown are means and bootstrapped 95% confidence intervals.

(D) Pie charts showing relative abundances of bacterial phyla in the fecal microbiota of mice at the indicated time points and diets.

(E) Phylogenetic diversity of the fecal microbiota of all strains at week 0 on CD and at week 22 on both CD and HFD. Differences between means were tested for significance using two ANOVAs, one for week 0 and one for week 22, followed by planned contrasts of means with p values adjusted using the single-step method.

(F) Relative abundances of Joslin-specific OTUs that colonize 129J, 129T, and B6J mice over time (± SEM). Differences between the mean relative abundances were tested within each mouse strain for CD-fed mice at weeks 0 and 22, and for CD-fed versus HFD-fed mice at week 22, using t tests and Holm’s corrections for p values. ***p < 0.001, **p < 0.01, *p < 0.05. See also Figure S3.
different groups of mice were ever cohoused. Despite this convergence of the microbial communities, birthplaces, ancestral origins, and strain/genetics still significantly contributed to the observed variance among fecal microbiota at week 22, but to a much lower extent (PERMANOVA, 9.5%, 3.4%, and 6.2%, respectively). As in the PCoA analysis of all samples, at week 22, diet (CD versus HFD) had the greatest influence on community structure, explaining 16.8% of the variance (Figures 4A, 4B, and S3A). Some of these patterns could also be seen in the relative abundances of bacterial phyla in the mice’s fecal microbiota (Figure 4D). For example, the fecal microbiota of CD-fed 129T mice at week 0 differed from all others in that the Firmicutes were more dominant, and the Bacteroidetes and Deferribacteres had roughly equal relative abundances, which were far below the Bacteroidetes representation in all other groups. During the 22 weeks on CD, most mouse strains showed only modest changes in microbiota structure at the phylum level, except for 129T mice, which demonstrated a significant increase in the relative abundance of Bacteroidetes (4.0% ± 1.1% to 19.6% ± 4.1%; p = 0.0007; Wilcoxon rank sum test) accompanied by decreases in Firmicutes (89.9% ± 2.0% to 77.5% ± 3.8%; p = 0.003; Wilcoxon rank sum test) and Deferribacteres (2.8% ± 1.3% to 0.2% ± 0.03%; p = 0.003; Wilcoxon rank sum test). On the other hand, all host strains showed major changes in microbiota configuration after 22 weeks on HFD, with an increase in Firmicutes (p = 6.6 × 10⁻¹¹, Kruskal-Wallis test) and a decrease in Bacteroidetes compared to CD (p = 1.0 × 10⁻¹², Kruskal-Wallis test; Figure 4D).

When sampled at week 0, phylogenetic diversity (PD) did not differ among the three vendor-bred strains or among the three Joslin-bred strains. However, the fecal microbiota of all vendor-bred mice had significantly lower PD than their Joslin-bred counterparts (Figure 4E; ANOVA, p < 10⁻⁶). After 22 weeks on CD at Joslin, the PD of the three vendor-bred strains increased, but remained lower than their Joslin-bred counterparts, especially in the B6J and 129J mice. HFD feeding resulted in significantly reduced PD in all mice. Interestingly, even after 22 weeks on HFD, the PD in the vendor-bred mice was significantly lower than their Joslin-bred counterparts (Figure 4E). The relative abundances of Joslin-specific OTUs that colonized 129J, 129T, and B6J mice increased over time in all three strains of mice; this colonization by Joslin-derived taxa was greatest in each mouse strain on CD (Figure 4F). Thus, early environmental exposures impacted the response to HFD; this diet prevented vendor-bred strains from developing fecal microbiota as phylogenetically diverse as their Joslin-bred counterparts.

**OTUs Associated with Birthplace, Ancestral Origin, and/or Strain of Mice**

To identify specific bacterial OTUs that differed in occurrence among B6J, 129J and 129T mice and their Joslin-bred counterparts, we performed multi-level pattern analysis (MPA) (De Caceres et al., 2010) (see Supplemental Experimental Procedures and Glossary). Of the 2,816 OTUs detected at week 0, 240 had significant associations with one or more of the groups (Figure 5A; Table S3 and Table S4). Hierarchical clustering of the mouse groups (strain × origin) based on the phi-coefficients of the most common, significantly associated OTUs showed vendor-born and Joslin-bred mice clustering apart from each other (Figure 5A). Interestingly, within vendor-born mice, B6J and 129J mice (both Jackson bred) clustered together, while after environmental normalization 129T/Jos and 129J/Jos mice clustered together. This suggests that host genotype may play a more important role in determining the occurrence of OTUs in mice bred at Joslin.

This analysis also demonstrated that the low phylogenetic diversity in the vendor-bred mice was due, in large part, to the fact that, in the vendor-bred strains, only six OTUs assigned to Bacteroidetes were present at a mean relative abundance >0.1%. Indeed, 129T mice had only one Bacteroidetes OTU (Parabacteroides goldsteinii) above this threshold level of abundance. By contrast, members of the phylum Bacteroidetes represented a substantial amount of the bacterial diversity in the Joslin-bred strains (Figure 5B; Table S4). Given the phenotypic differences between 129T and 129T/Jos mice, OTUs that differed between these two lines were of particular interest. MPA identified 21 OTUs strongly and uniquely associated with vendor-bred 129T mice at time 0. Interestingly, all of these were members of the Firmicutes, and included OTUs for Blautia producta and three Clostridial species. In addition, 53 OTUs were identified in the Joslin-bred 129T/Jos mice that were not present in the vendor-bred 129T animals (Figure 5A; Table S4).

Using nonparametric Kruskal-Wallis tests with p values adjusted for false discovery rate, under the Benjamini-Hochberg method (Benjamini-Hochberg-adjusted p values), we identified 226 OTUs that differed significantly in their relative abundances in the fecal microbiota of CD- versus HFD-fed mice in at least one strain after 22 weeks. One hundred forty-five of these OTUs, including 34 members of the Bacteroidetes, exhibited significantly higher relative abundances in mice fed HFD. By contrast, members of the phylum Bacteroidetes, exhibited significantly higher relative abundances in mice fed CD. This analysis also demonstrated that the low phylogenetic diversity in the vendor-bred mice was due, in large part, to the fact that, in the vendor-bred strains, only six OTUs assigned to Bacteroidetes were present at a mean relative abundance >0.1%. Indeed, 129T mice had only one Bacteroidetes OTU (Parabacteroides goldsteinii) above this threshold level of abundance. By contrast, members of the phylum Bacteroidetes represented a substantial amount of the bacterial diversity in the Joslin-bred strains (Figure 5B; Table S4). Given the phenotypic differences between 129T and 129T/Jos mice, OTUs that differed between these two lines were of particular interest. MPA identified 21 OTUs strongly and uniquely associated with vendor-bred 129T mice at time 0. Interestingly, all of these were members of the Firmicutes, and included OTUs for Blautia producta and three Clostridial species. In addition, 53 OTUs were identified in the Joslin-bred 129T/Jos mice that were not present in the vendor-bred 129T animals (Figure 5A; Table S4).

**Correlations between Bacterial OTU Abundances and Mouse Phenotypes**

Spearman’s rank correlations were used to identify bacterial OTUs whose relative abundances at week 22 were strongly correlated with one or more of the 36 phenotypes we quantified (see Experimental Procedures). Considering only the 383 OTUs with a relative correlation >0.1% in one of the 12 mouse-by-diet groups, 780 OTU-phenotype pairs, involving 135 OTUs, had Spearman’s correlations stronger than −0.5 or 0.5 (Figure 6A; Table S6). Of these, 771 involved phenotypes and OTUs that were significantly influenced by at least one shared variable such as HFD. Correlations with
Lactococcus lactis were also observed, but were not considered biologically significant, since the HFD, although twice irradiated, contained DNA of *L. lactis*, which is used in production of casein, a component of the diet. This *L. lactis* was nonviable by culture, as also previously described (Carmody et al., 2015), but was easily detected by 16S rRNA sequencing.

To identify OTU-phenotype correlations that persisted after controlling for the influences of mouse genetic background and diet, we calculated Spearman’s correlations using only 129 mice (129T, 129T/Jos, 129J, and 129J/Jos) on HFD. Only four correlations survived multiple test corrections (Benjamini-Hochberg-adjusted p value < 0.5), all in HFD-fed 129 mice (Figure 6C; Table S7). These correlations included a strong positive correlation between body weight and OTU 95638 (*Mucispirillum schaedleri*) and strong negative correlations between OTU 191491 (*Clostridium scindens*) and body weight and FGF21.

Figure 5. Representation of Bacterial Taxa in the Fecal Microbiota

(A) Heatmap showing the phi-coefficients of OTUs that have at least one significant phi-coefficient more extreme than −0.8 or 0.8 or mean relative abundance of at least 1% in at least one group of mice at week 0 of the experimental period.

(B) Abundance of OTUs assigned to the phylum Bacteroidetes in vendor-bred and Joslin-bred mouse strains. All Bacteroidetes OTUs with mean relative abundances of 0.1% or greater in at least one mouse group are included in the heatmap. Abundance values were log-transformed according to the formula log10(abundance/10,000 reads +1). Also see Figure S4.
Figure 6. Correlations between OTUs’ Relative Abundances and Phenotypes, Including All Mouse Groups

(A) A heatmap shows Spearman’s rank correlations between phenotypes and the relative abundances of selected OTUs. OTUs with at least four correlations more extreme than 0.5 or 0.5 or one correlation more extreme than 0.75 or 0.75 are shown. All phenotypes with at least one correlation more extreme than 0.5 or 0.5 are presented. Hierarchical clustering is presented for both OTUs and phenotypes, based on the Euclidean distances between their presented Spearman’s rank correlations.

(B) Linear correlations between the mean relative abundances (per 10,000 reads) of select OTUs and mouse phenotypes for each of experimental treatment groups.

(C) Heatmap of Spearman’s correlations between phenotypes and the relative abundances of OTUs using all HFD-fed 129 mice (129T, 129T/Jos, 129J, and 129J/Jos). p values were corrected for false detection rate using the Benjamini-Hochberg method and significant correlations after correction are highlighted with a yellow box. See also Figure S5.
and another strong negative correlation between OTU 166592 (Clostridiales) and free fatty acid.

We also calculated Spearman’s correlations focusing on the HFD-fed mice for each of the three genetic backgrounds separately, combining B6J and B6J/Jos, 129J and 129J/Jos, and 129T and 129T/Jos, thus allowing us to identify the specific associations for each genetic background. For 129T and 129T/Jos mice, 133 correlations stronger than −0.5 or 0.5 were identified (Figures S5A and SSB; Table S8). Seven OTUs were related to body weight (two positive, including OTU 95638 [Mucispirillum schaedleri] and OTU 470527 [Lactobacillus reuteri]), and five negatively, including four OTUs in the order Clostridiales and OTU 1698560 [Bacteroidales]). Four of the five OTUs that negatively correlated with body weight also negatively correlated with weight of the subcutaneous (inguinal) fat pad, as did OTU 293221, which was identified as the saccharolytic, butyrate-producing microbe Roseburia intestinalis. Five OTUs correlated with visceral (perigonadal) fat pad weight, but none of these were the same as those related to body or subcutaneous fat pad weight. On the other hand, insulin levels correlated with the abundance of six microbiota (five positively and one negatively), and of these, two also positively correlated with perigonadal fat pad weight, including OTU 266246 (Eubacterium plexicaudatum) and Clostridiales OTU 269418. Although 129T and 129T/Jos mice have much less adipose inflammation than B6J mice even at similar body weights, OTU 292302 (Lactobacillus murinus) and Lactobacillus OTU 422931 showed correlations to markers of inflammation (expression of IL-6 and MCP-1) in perigonadal fat (Figure S5A).

Despite the high level of genetic similarity between 129T and 129J mice and their respective Joslin counterparts, of the 182 OTUs that could be related to specific phenotypes in 129J and 129J/Jos mice, only two showed correlations in the same direction, including the Clostridiales OTU 321691, which negatively correlated with body weight and FGF21 levels in both strains (Figures S5A–S5D; Table S8). On the other hand, despite representing different genetic backgrounds and different vendors of origin, the two strain pairs that had some propensity to develop components of metabolic syndrome—the B6J and B6J/Jos pair and 129T and 129T/Jos pair—shared five correlations. Thus, in both groups, body weight was negatively correlated with the relative abundances of OTU 402711 (Clostridium lactatifermants) and Clostridium OTU 165762 (Figures S5A, SSB, SSE, and S5F). Likewise, in these pairs, FGF21 levels positively correlated with L. reuteri and negatively correlated with C. lactatifermants, while serum free fatty acid levels were negatively correlated with OTU 323257 (L. gasseri). Consistent with the group-wise analysis across all mouse strains on both CD and HFD (Figure 6B), L. gasseri was strongly positively associated with insulin levels in animals from both strain pairs originating from Jax (129J and 129J/Jos; and B6J and B6J/Jos), even when the analysis was restricted to mice on HFD only (Figure S5A; see Table S8 for a complete list of correlations).

In B6J mice, which is the most commonly used strain for the study of metabolic syndrome, inflammation in adipose tissue and liver has been linked to the pathogenesis of metabolic syndrome (Mori et al., 2010), and the gut microbiota has been linked to the inflammatory response (Chassaing et al., 2014). Importantly, in this strain pair, several OTUs showed robust correlations with multiple markers of inflammation, including strong positive correlations between levels of CD68, F4/80, CD11c, IL-6, and TNF-α in adipose tissue and the abundance of OTU 278234 (Akkermansia muciniphila), and weak correlations of these markers with OTU 302844 (Clostridium disporicum), OTU 258906 (Clostridium scindens), and another Clostridiales OTU 321691 (Figures S5E and S5F). Interestingly, none of these correlations were observed in the 129J-129J/Jos strain pair, which shows almost no inflammatory changes in adipose tissue when challenged with HFD (Figures S5C and S5D). In the 129T-129T/Jos pair, HFD induces a low level of inflammation in adipose tissue (Figure 2F), and in this pair, only two markers of inflammation in fat (IL-6 and MCP-1) showed any correlation with OTUs, (see Lactobacillus murinus OTU292302 and Lactobacillus OTU 422931 in Figures S5A and S5B).

Transplant Studies to Identify Microbiota-Transmissible HFD-Associated Phenotypes

To more directly investigate the causal relationships of these various microbiota in the development of metabolic syndrome, we colonized 6-week-old germ-free C57Bl/6 mice with the cecal microbiota from age-matched, vendor-born B6J, 129J, and 129T mice. Germ-free animals were started on HFD 1 week prior to colonization and maintained on this diet for an additional 8 weeks after receiving fecal transplants from the vendor-born donors (n = 9 recipients/donor microbiota). Recipients of all three microbiota gained significantly more weight throughout the study compared to germ-free controls that were not colonized. While weight gain did not differ between mice colonized with the different microbiota over the course of the 8-week experiment (Figure 7A), the mean value for nonfasting serum glucose 6 weeks after transplant was significantly greater for mice harboring a B6J microbiota than those colonized with a 129J microbiota, and marginally greater than those with a 129T microbiota (Figure 7B; ANOVA, p = 0.021). Differences in random-fed blood glucose did not differ at 8 weeks. However, assessment of glucose homeostasis at 8 weeks, assessed by oral glucose tolerance testing, revealed that animals colonized with a vendor-born 129T donor microbiota had significantly greater AUC values than recipients of a vendor-born 129J donor microbiota, with an intermediate phenotype exhibited by B6J recipients (Figure 7C; ANOVA, p = 0.001). Mice colonized with different microbiota also had marginally significant differences in their liver weights relative to their total body weights (ANOVA, p = 0.055), with mice harboring the transplanted B6J donor microbiota exhibiting marginally greater values compared to 129J-colonized mice (Figure 7D; Tukey’s honestly significant difference, p = 0.075).

16S rRNA sequencing of fecal samples obtained from gnotobiotic mice belonging to the different treatment groups revealed bacterial OTUs whose relative abundances correlated with the measured mouse phenotypes. Using indicator species values, MPA identified 119 97%ID OTUs that either differed significantly in their relative abundances or frequencies among the colonized gnotobiotic mice; 45 of these OTUs were previously identified as being associated with at least one of the conventionally raised mouse groups using phi-coefficients (Table S9). Of these, 31 (68.9%) were significantly associated with at least one of the conventionally raised strains and a corresponding gnotobiotic.
transferring the microbiota from 129T mice, which are prone to obesity but not metabolic syndrome, has less effect. In other words, reshaping the gut microbiota can prevent certain features of metabolic syndrome, and the resulting configuration of the microbiota determines which phenotypes are altered and to what extent.

**DISCUSSION**

Obesity, T2D, and metabolic syndrome are major health problems worldwide that pose financial and physical burdens to both affected individuals and society as a whole. Thus, understanding the causes of these disorders and developing new approaches to prevention and treatment is a high priority. This is made difficult, however, by the complex genetic and environmental drives of these diseases, including factors such as host genetics, diet, and the recently identified role of the gut microbiota. In the present study, we have explored the complex interaction between these factors and different components of metabolic syndrome using three inbred strains of mice from two different vendors and three environmentally normalized lines derived by inbreeding these strains in the same environment. Our results demonstrate the strong and complex interrelationships between host genetics, environment, gut microbiota, and diet. They also demonstrate how exposure to a common environment can remodel the gut microbiota and change the associated metabolic phenotypes in some strains of mice, while others maintain their phenotypes despite the changing gut microbiota, indicating a dominant genetic influence.

As previously observed (Almind and Kahn, 2004), C57Bl/6J (B6J) mice on HFD exhibit robust weight gain and characteristics of metabolic syndrome including insulin resistance, hyperglycemia, and fatty liver, whereas 129S1/SvImJ (129J) mice, also originating from the Jackson Laboratories, are resistant to HFD-induced weight gain and insulin resistance. On the other hand, a closely related 129 strain of mice from Taconic Farms, the 129S6/SvEvTac strain (129T), when challenged with HFD becomes as heavy as the B6J mice and develops hepatomegaly, but retains relatively good insulin sensitivity and glucose tolerance, resulting in a “healthier” obese phenotype compared to B6J mice.

To control for the differences in microbiota and other environmental factors that are present at the different commercial facilities (Harley et al., 2013), we inbred these three original strains in our mouse facility for at least three generations. Comparison of these environmentally normalized strains to their vendor-bred counterparts disclosed that regardless of site of breeding and housing, B6J mice were prone to obesity and metabolic syndrome, while 129J mice were resistant. 129T mice, on the other hand, were prone to obesity and hepatosteatosis when vendor-bred, but became resistant to these metabolic phenotypes after three generations of breeding in a new environment. Transfer of gut microbiota from the three vendor-born strains into germ-free C57Bl/6 mice revealed no significant differences in weight gain between the three recipient groups, but did demonstrate a significant microbiota-transmissible impact on glucose homeostasis, with 129J donor microbiota conferring the most glucose tolerant phenotype and the B6J donor microbiota producing the highest fasting glucose levels.

**Figure 7. Gnotobiotic Mouse Experiments Identify Microbiota-Transmissible Phenotypes in HFD-Fed Recipients**

(A) Weight gain of germ-free (GF) C57Bl/6 mice (n = 5) and GF mice colonized with fecal microbiota from B6J, 129J, and 129T (n = 9–10) mice fed a HFD (repeated-measures two-way ANOVA [time and donor strain], with Tukey’s post hoc test). (B) Random-fed blood glucose of mice from (A), after 6 weeks on HFD (one-way ANOVA with Tukey’s post hoc test). (C) AUC values calculated from data obtained during an oral glucose tolerance tests at 8 weeks postcolonization. One exceptionally high outlier value was removed from the mice colonized with the B6J microbiota prior to analysis. (D) Liver weights for mice described in (A) after 8 weeks of HFD feeding (one-way ANOVA with Tukey’s post hoc test). *P < 0.05, **P < 0.01, ***P < 0.001. All values presented as mean ± SEM.
16S rRNA-based analysis of the fecal microbiota of the vendor-bred strains revealed large differences between the three strains, with 129T mice being the most distinct. As expected, all gut bacterial communities became more similar after breeding in the common mouse facility; however, each line retained a unique pattern resulting in distinct microbiota-host phenotype correlations. The major differences in gut microbiota of mice obtained from different commercial vendors, between commercial and institutional animal facilities, and different time points after some environmental change, even when using the same diet, points out the need to control these variables as much as possible within any experimental study.

Some of the correlations between phenotypes and bacterial OTU representation that we identified in HFD-fed mice have analogs in human metabolism. For example, the relative abundance of \textit{Roseburia intestinalis}, a butyrate producer, was correlated with phenotypes in mice representing all three genetic backgrounds fed HFD, including negative correlations with subcutaneous adiposity, body weight, liver weight, serum insulin, FGF21 levels, and inflammation markers. In humans, members of the genus \textit{Roseburia} have been reported to be negatively correlated with plasma glucose (Larsen et al., 2010) and are less abundant in the microbiota of individuals with T2D (Qin et al., 2012). The abundance of \textit{Roseburia} has been shown to increase in the feces of individuals with metabolic syndrome who receive fecal transplants from lean metabolically healthy donors, and this has been associated with improved peripheral insulin sensitivity (Vrieze et al., 2012). Butyrate produced by \textit{Roseburia} can serve as an important carbon source for colonic epithelial cells and enhance mitochondrial function in peripheral tissues, but the exact mechanisms underlying the positive metabolic effects are not clear (Hartstra et al., 2015).

Other members of the Firmicutes also correlated with phenotypes. For example, in HFD-fed B6J and B6J/Jos mice, which develop the most severe manifestations of metabolic syndrome, \textit{L. reuteri} is strongly positively correlated with serum insulin levels, as well as FGF21 levels. Similar correlations are observed in the 129T-129T/Jos pair, which develops a mild form of metabolic syndrome on HFD, but not in 129J-129J/Jos pair, which are lean and insulin-sensitive on all diets. In humans, \textit{L. reuteri} has been observed to be more abundant in obese individuals than in lean individuals (Million et al., 2012). The strong positive correlation between the relative abundance of \textit{Lactobacillus gasseri} across all strains and diets is also of note, since \textit{Lactobacillus} species have also been shown to be correlated with glucose levels in humans (Larsen et al., 2010), and \textit{L. gasseri} was identified as the most discriminant taxon in the fecal microbiota of normal glucose tolerant compared to type 2 diabetic women (Karlsson et al., 2013). Moreover, \textit{Mucispirillum schaedleri}, a component of the Altered Schaedler’s flora, initially used to colonize animals stocking the Taconic barrier rooms, is significantly associated with 129T, but not 129T/Jos, mice and positively correlated with body weight gain in the 129T mice.

Although 129T and 129T/Jos mice have less adipose inflammation than B6J mice, even at similar body weights, in the 129T genetic background, markers of adipose inflammation also best correlate with two OTUs assigned to the genus \textit{Lactobacillus} (OTU 292302 [\textit{L. murinus}] and OTU 422931). In B6J mice, we find markers of adipose inflammation to correlate with levels of \textit{Akkermansia muciniphila}. However, this association of \textit{Akkermansia} with adipose tissue inflammation is restricted to B6J and is not observed in B6J/Jos mice (Figures SSE andSSF), indicating that other cosegregating microbes may be contributing to this correlation. This is further suggested by previous findings that administration of \textit{Akkermansia muciniphila} can have beneficial metabolic effects on body weight, adipose tissue inflammation, and glucose tolerance (Everard et al., 2013), with similar associations in humans (Tiig and Moschen, 2014). Therefore the correlation between this microbe and the immunological phenotypes may be part of changes in larger microbial communities that either directly or via effects on metabolism or the immune system modulate host physiology.

In line with previous studies (Harley et al., 2013), we could not detect any significant correlations between any of metabolic phenotypes and members of the segmented filamentous bacteria (SFB), which are known modulators of IL-17 secretion (Ivanov et al., 2009).

In summary, obesity, T2D, and metabolic syndrome are the results of complex interactions between genetic and environmental factors, including the gut microbial community. As a result, when genetically identical mice are raised in different environments and challenged with HFD, there may be major differences in the composition of the gut microbiota that can impact on the development of obesity, hepatosteatosis, insulin resistance, and other components of metabolic syndrome. Indeed, in some genetic backgrounds (such as 129S6/SvEvTac), environmental reshaping of the microbiota can significantly ameliorate development of features of the metabolic syndrome, whereas in other backgrounds (such as C57Bl/6J), strong genetic influences may be dominant. These interactions between diet, genetics, and the microbiota present a great challenge in any analysis of both preclinical models and human disease. Transplanting microbiota from donors with distinct phenotypes and microbial communities into mice representing different genetic backgrounds can be used to help establish a causal relationship between microbes and host biology, and also identify interactions between host genetic background and the microbiota. Developing strategies to manipulate the microbiota that drive specific components of the metabolic syndrome, especially those components that interact with a permissive host genetic background, may open the door to new strategies for effective and more durable ways of treating disease and ultimately preventing it.

**EXPERIMENTAL PROCEDURES**

Additional details of all methods are provided in Supplemental Experimental Procedures.

**Animals and Diets**

Animal care and study protocols were approved by the Animal Care Committees of the Joslin Diabetes Center and Washington University, and were in accordance with the National Institutes of Health guidelines. All mice were housed in the Joslin Diabetes Center mouse facility on a 12 hr light/dark cycle in a 22°C temperature-controlled room. C57BL/6J and 129S1/SvImJ animals were purchased from Jackson Laboratories (Bar Harbor, ME) at 3 weeks of age, and 129S6/SvEvTac mice were purchased from Taconic Farms (Germantown, NY), also at 3 weeks of age. Each of the three lines was inbred at the Joslin Diabetes Center for three to six generations. Animals were maintained on NIH-31 diet (Taconic, composed of 14% calories from fat, 24% from protein,
and 62% from carbohydrates) or a HFD (Research Diets D12492, composed of 60% calories from fat, 20% calories from protein, and 20% calories from carbohydrates).

Inbred colonies from 129.Jax and 129Tac mice were established in the Joslin animal facility for three consecutive generations. At the fourth generation, females were sampled from 3-week-old animals (B6 Jax/Jos, 129Tac/Jos, and 129 Jax/Jos) as well as from aged-matched vendor-born animals immediately upon delivery at the Joslin vivarium (B6Jax, 129Jax, and 129 Jos). Following initial sampling, mice from each colony were given either CD or HFD. Feces were collected for DNA extraction after 1, 8, and 22 weeks to assess short-term, medium-term, and long-term effects of diet and environmental exposure on microbiota composition. Great care was taken to perform physiological tests and collect samples at uniform time, since regulation of host metabolism and gut microbiota composition have been shown to have diurnal fluctuations (Thaisis et al., 2014; Zarrinpar et al., 2014).

Gnotobiotic Transfer Experiments

Germ-free (GF) male C57BL/6J mice were obtained from the Center for Metagenomics gnotobiotic core facility at Brigham and Women’s Hospital. Four groups of 5-week-old GF mice were fed HFD (60% calories from fat; Research Diet) for 1 week. Subsequently mice were divided into three groups and colonized with cecal microbiota collected from age-matched donors, or maintained as germ-free control. Donor microbiota were prepared from conventionally raised B6J, 129J, and 129T mice purchased from Jackson Laboratories or Taconic Farms at the date of transfer. Ceca from donor conventional mice were removed and transferred into a Coy anaerobic chamber, where they were placed in sterile prereduced PBS supplemented with 40 mM cysteine and manually homogenized with a sterile Dounce homogenizer to create a suspension. Materials were sealed in the hood and transferred to the gnotobiotic isolators to colonize gnotobiotic recipients by oral-gastric gavage with 200 µL of the materials. An additional 200 µL of material was spread over the fur and anus of mice. Mice were maintained in plastic flexible film gnotobiotic isolators (five mice per cage) on a strict 12 hr light cycle (lights on at 6 a.m.) and fed HFD for 8 weeks after colonization.

Microbiota Analysis

Multiplex sequencing of bacterial V4-16S rRNA amplicons was performed using an Illumina MiSeq instrument and DNA primer sets and protocols described elsewhere (Bokulich et al., 2013) with an average of 19,740 ± 254 reads (mean ± SEM) per sample (Table S1A). Reads were binned into operational taxonomic units sharing 97% nucleotide sequence identity (97%ID OTUs, or OTUs for short) and given a taxonomic assignment. The software package QIIME v.1.50-dev was used to pick 97% ID OTUs, filter, and rarefy the sequence data. A phylogenetic tree of the OTUs was constructed. Sequences were aligned with PyNast; sequences that could not be aligned were discarded (Caporaso et al., 2010). Taxonomy was assigned to each OTU using the RDP v. 2.4 classifier method (Wang et al., 2007).

To investigate the fecal microbiota of the colonized gnotobiotic recipient animals, we performed multiplex sequencing of bacterial V4-16S rRNA amplicons as described above, with an average of 71,030 ± 1,437 reads (mean ± SEM) per sample (Table S1B). The data were rarefied to 30,000 reads per sample prior to analysis. To identify OTUs that differed in their relative abundances or frequencies in the colonized gnotobiotic mice, we performed MPA as above, but used species indicator values instead of phi-coefficients. OTUs that occurred in at least 20% of the mice in a treatment group and had a mean relative abundance of at least 0.1% in a treatment group were included in the analysis. p values were FDR corrected. We also calculated Spearman’s rank correlations between the relative abundances of OTUs and the phenotypes measured in the gnotobiotic transplant recipients. These correlations were calculated across all treatment groups, and OTUs with a mean relative abundance of at least 0.01% across all samples were included in the analysis. p values were FDR corrected.

ACCESSION NUMBERS

16S rRNA data are accessible in the European Nucleotide Archive (ENA) database under accession ID PRJEB9702.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures, nine tables, Supplemental Experimental Procedures, and Glossary of Terms and can be found with this article at http://dx.doi.org/10.1016/j.cmet.2015.07.007.

AUTHOR CONTRIBUTIONS

S.U. designed the study, researched data, and wrote the manuscript. N.W.G. performed the microbiota analyses and wrote the manuscript. O.B. designed the study and researched data. S.F., S.V., and S.S researched data and helped in the analysis. L.D. and L.B. performed the gnotobiotic transfer experiments. J.I.G. helped design experiments and writing the paper. C.R.K. designed the study and wrote the manuscript.

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