A core gut microbiome in obese and lean twins

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The human distal gut harbours a vast ensemble of microbes (the microbiota) that provide important metabolic capabilities, including the ability to extract energy from otherwise indigestible dietary polysaccharides1–4. Studies of a few unrelated, healthy adults have revealed substantial diversity in their gut communities, as measured by sequencing 16S rRNA genes5–8, yet how this diversity relates to function and to the rest of the genes in the collective genomes of the microbiota (the gut microbiome) remains obscure. Studies of lean and obese mice suggest that the gut microbiota affects energy balance by influencing the efficiency of caloric harvest from the diet, and how this harvested energy is used and stored9–12. Here we characterize the faecal microbial communities of adult female monozygotic and dizygotic twin pairs concordant for leanness or obesity, and their mothers, to address how host genotype, environmental exposure and host adiposity influence the gut microbiome. Analysis of 154 individuals yielded 9,920 near full-length 16S rRNA sequences among faecal communities, plus 2.14 gigabases from their microbiomes. The results reveal that the human gut microbiome is shared among family members, but that each person’s gut microbial community varies in the specific bacterial lineages present, with a comparable degree of co-variation between adult monozygotic and dizygotic twin pairs. However, there was a wide array of shared microbial genes among sampled individuals, comprising an extensive, identifiable ‘core microbiome’ at the gene, rather than at the organismal lineage, level. Obesity is associated with phylum-level changes in the microbiota, reduced bacterial diversity and altered representation of bacterial genes and metabolic pathways. These results demonstrate that a diversity of organismal assemblages can nonetheless yield a core microbiome at a functional level, and that deviations from this core are associated with different physiological states (obese compared with lean).

We characterized gut microbial communities in 31 monozygotic twin pairs, 23 dizygotic twin pairs and, where available, their mothers (n = 46) (Supplementary Tables 1–5). Monozygotic and dizygotic co-twins and parent–offspring pairs provided an attractive model for assessing the impact of genotype and shared early environmental exposures on the gut microbiome. Moreover, genetically ‘identical’ monozygotic twin pairs gain weight in response to overfeeding in a more reproducible way than unrelated individuals10 and are more concordant for body mass index (BMI) than dizygotic twin pairs11. Twin pairs who had been enrolled in the Missouri Adolescent Female Twin Study (MOAFTS)12 were recruited for this study (mean period of enrolment in MOAFTS, 11.7 ± 1.2 years; range, 4.4–13.0 years). Twins were 21–32 years old, of European or African ancestry, and were generally concordant for obesity (BMI ≥ 30 kg m⁻²) or leanness (BMI = 18.5–24.9 kg m⁻²) (one twin pair was lean/overweight (overweight defined as BMI ≥ 25 and < 30) and six pairs were overweight/obese). Each participant completed a detailed medical, lifestyle and dietary questionnaire; study enrolees were broadly representative of the overall Missouri population for BMI, parity, education and marital status (see Supplementary Results). Although all were born in Missouri, they currently live throughout the USA: 29% live in the same house, but some live more than 800 km apart. Because faecal samples are readily attainable and representative of interpersonal differences in gut microbiota ecology, they were collected from each individual and frozen immediately. The collection procedure was repeated again with an average interval between sampling of 57 ± 4 days.

To characterize the bacterial lineages present in the faecal microbiota of these 154 individuals, we performed 16S rRNA sequencing, targeting the full-length gene with an ABI 3730xl capillary sequencer. Additionally, we performed multiplex pyrosequencing with a 454 FLX instrument to survey the gene’s V2 variable region13 and its V6 hypervariable region14 (Supplementary Tables 1–3).

Complementary phylogenetic and taxon-based methods were used to compare 16S rRNA sequences among faecal communities (see Methods). No matter which region of the gene was examined, individuals from the same family (a twin and her co-twin, or twins and their mother) had a more similar bacterial community structure than unrelated individuals (Fig. 1a and Supplementary Fig. 1a, b), and shared significantly more species-level phylotypes (16S rRNA sequences with ≥97% identity comprise each phylotype) (G = 55.2, P < 10⁻¹² (V2); G = 12.3, P < 0.001 (V6); G = 11.3, P < 0.001 (full-length)). No significant correlation was seen between the degree of physical separation of family members’ current homes and the degree of similarity between their microbial communities (defined by UniFrac15). The observed familial similarity was not due to an indirect effect of the physiological states of obesity versus leanness; similar results were observed after stratifying twin pairs and their mothers by BMI category (concordant lean or discordant obese individuals; Supplementary Fig. 2). Surprisingly, there was no significant difference in the degree of similarity in the gut microbiotas of adult monozygotic compared with dizygotic twin pairs (Fig. 1a). However, we could not assess whether monozygotic and dizygotic twin pairs had different degrees of similarities at earlier stages of their lives.

Multiplex pyrosequencing of V2 and V6 amplicons allowed higher levels of coverage compared with what was feasible using Sanger sequencing, reaching on average 3,984 ± 232 (V2) and 24,786 ± 1,403 (V6) sequences per sample. To control for differences
Analysis of 16S rRNA data sets produced by the three PCR-based methods, plus shotgun sequencing of community DNA (see below), revealed a lower proportion of Bacteroidetes and a higher proportion of Actinobacteria in obese compared with lean individuals of both ancestries (Supplementary Table 9). Combining the individual P values across these independent analyses using Fisher’s method disclosed significantly fewer Bacteroidetes (P = 0.003), more Actinobacteria (P = 0.002) but no significant difference in Firmicutes (P = 0.09). These findings agree with previous work showing comparable differences in both taxa in mice\(^2\) and a progressive increase in the representation of Bacteroidetes when 12 unrelated, obese humans lost weight after being placed on one of two reduced-calorie diets\(^3\).

Across all methods, obesity was associated with a significant decrease in the level of diversity (Fig. 1b and Supplementary Fig. 1c–f). This reduced diversity suggests an analogy: the obese gut microbiota is not like a rainforest or reef, which are adapted to high energy flux and are highly diverse; rather, it may be more like a fertilizer runoff where a reduced-diversity microbial community blooms with abnormal energy input\(^4\).

We subsequently characterized the microbial lineage and gene content of the faecal microorganisms of 18 individuals representing six of the families (three lean and three obese European ancestry monzygotic twin pairs and their mothers) through shotgun pyrosequencing (Supplementary Tables 4 and 5) and BLASTX comparisons against several databases (KEGG\(^17\) (version 44) and STRING\(^18\) plus a custom database of 44 reference human gut microbial genomes (Supplementary Figs 7–10 and Supplementary Results). Our analysis parameters were validated using control data sets comprising randomly fragmented microbial genes with annotations in the KEGG database\(^17\) (Supplementary Fig. 11 and Supplementary Methods).

We also tested how technical advances that produce longer reads might improve these assignments by sequencing faecal community samples from one twin pair using Titanium pyrosequencing methods (average read length of 341 ± 134 nucleotides (s.d.) versus 208 ± 68 nucleotides for the standard FLX method). Supplementary Fig. 12 shows that the frequency and quality of sequence assignments is improved as read length increases from 200 to 350 nucleotides.

The 18 microorganisms were searched to identify sequences matching domains from experimentally validated carbohydrate-active enzymes (CAZymes). Sequences matching 156 total CAZy families were found within at least one human gut microbiome, including 77 glycoside hydrolase, 21 carbohydrate-binding module, 35 glycosyltransferase, 12 polysaccharide lyase and 11 carbohydrate-esterase families (Supplementary Table 10). On average, 2.62 ± 0.13% of the sequences in the gut microbiome could be assigned to CAZymes (a total of 217,615 sequences), a percentage that is greater than the most abundant KEGG pathway (‘Transporters’; 0.13% of the sequences in the gut microbiome) and indicative of the abundant and diverse set of microbial genes directed towards accessing a wide range of polysaccharides.

Category-based clustering of the functions from each microbiome was performed using principal components analysis (PCA) and hierarchical clustering\(^19\). Two distinct clusters of gut microorganisms were identified based on metabolic profile, corresponding to samples with an increased abundance of Firmicutes and Actinobacteria, and samples with a high abundance of Bacteroidetes (Fig. 2a). A linear regression of the first principal component (PC1, explaining 20% of the functional variance) and the relative abundance of the Bacteroidetes showed a highly significant correlation (R\(^2\) = 0.96, P < 10\(^{-12}\); Fig. 2b). Functional profiles stabilized within each individual’s microbiome after 20,000 sequences had been accumulated (Supplementary Fig. 13). Family members had more similar profiles than unrelated individuals (Fig. 2c), suggesting that shared bacterial community structure (‘who’s there’ based on 16S rRNA analyses) also translates into shared community-wide relative abundance of metabolic pathways. Accordingly, a direct comparison of functional
of β-diversity with respect to the relative abundance of bacterial phyla (Fig. 3a), analysis of the relative abundance of broad functional categories of genes and metabolic pathways (KEGG) revealed a generally consistent pattern regardless of the sample surveyed (Fig. 3b and Supplementary Table 11); the pattern is also consistent with results we obtained from a meta-analysis of previously published gut microbiome data sets from nine adults (482; Supplementary Fig. 16). This consistency is not simply due to the broad level of these annotations, as a similar analysis of Bacteroidetes and Firmicutes reference genomes revealed substantial variation in the relative abundance of each category (see Supplementary Fig. 17). Furthermore, pairwise comparisons of metabolic profiles obtained from the 18 microbiomes in this study revealed an average value of $R^2$ of 0.97 ± 0.002 (Fig. 2a), indicating a high level of functional similarity.

Overall functional diversity was compared using the Shannon index (22), a measurement that combines diversity (the number of different metabolic pathways) and evenness (the relative abundance of each pathway). The human gut microbiomes surveyed had a stable and high Shannon index value (4.63 ± 0.01), close to the maximum possible level of functional diversity (5.54; see Supplementary Methods). Despite the presence of a small number of abundant metabolic pathways (listed in Supplementary Table 11), the overall functional profile of each gut microbiome is quite even (Shannon evenness of 0.84 ± 0.001 on a scale of 0–1), demonstrating that most metabolic pathways are found at a similar level of abundance. Interestingly, the level of functional diversity in each microbiome was significantly linked to the relative abundance of the Bacteroidetes ($R^2$ = 0.81, $P < 10^{-5}$); microbiomes enriched for Firmicutes/Actinobacteria had a lower level of functional diversity. This observation is consistent with an analysis of simulated metagenomic reads generated from each of 36 Bacteroidetes and Firmicutes genomes (Supplementary Fig. 18): on average, the Bacteroidetes genomes have a significantly higher level of both functional diversity and evenness (Mann–Whitney U-test, $P < 0.01$).

At a finer level, 26–53% of ‘enzyme’-level functional groups (KEGG/CAZY/STRING) were shared across all 18 microbiomes, whereas 8–22% of the groups were unique to a single microbiome (Supplementary Fig. 19a–c). The ‘core’ functional groups present in all microbiomes were also highly abundant, representing 93–98% of the total sequences. Given the higher relative abundance of these ‘core’ groups, more than 95% were found after 26.11 ± 2.02 megabases of sequence were collected from a given microbiome, whereas the ‘variable’ groups continued to increase substantially with each additional megabase of sequence. Of course, any estimate of the total size of the core microbiome will depend on sequencing effort, especially for

Figure 3 | Comparison of taxonomic and functional variations in the human gut microbiome. a, Relative abundance of major phyla across 18 faecal microbiomes from monozygotic twins and their mothers, based on BLASTX comparisons of microbiomes and the National Center for Biotechnology Information non-redundant database. b, Relative abundance of categories of genes across each sampled gut microbiome (letters correspond to categories in the COG database).
functional groups found at a low abundance. On average, our survey achieved more than 450,000 sequences per faecal sample, which, assuming an even distribution, would allow us to sample groups found at a relative abundance of $10^{-4}$. To estimate the total size of the core microbiome based on the 18 individuals, we randomly subsampled each microbiome in 1,000 sequence intervals (Supplementary Fig. 19d). Based on this analysis, the core microbiome is approaching a total of 2,142 total orthologous groups (one site binding (hyperbola) curve fit, $R^2 = 0.9966$), indicating that we identified 93% of functional groups (defined by STRING) found within the core microbiome of the 18 individuals surveyed. Of these core groups, 71% (CAZYs), 64% (KEGG) and 56% (STRING) were also found in the nine previously published, but much lower coverage, data sets generated by capillary sequencing of adult faecal DNA\textsuperscript{20,21} (average of 78,413 ± 2,044 bidirectional reads per sample; see Supplementary Methods).

Metabolic reconstructions of the ‘core’ microbiome revealed significant enrichment for several expected functional categories, including those involved in transcription and translation (Fig. 4). Metabolic profile-based clustering indicated that the representation of ‘core’ functional groups was highly consistent across samples (Supplementary Fig. 20), and included several pathways that are likely important for life in the gut, such as those for carbohydrate and amino-acid metabolism (for example, fructose/mannose metabolism, amino-sugar metabolism and N-glycan degradation). Variably represented pathways and categories include cell motility (only a subset of Firmicutes produce flagella), secretion systems and membrane transport (for example, phosphotransferase systems involved in the import of nutrients, including sugars; Fig. 4 and Supplementary Fig. 20).

The distribution of CAZY glycoside hydrolase and glycosyltransferase families was compared between each pair of microbiomes (see Supplementary Table 10 for CAZY families with a relative abundance greater than 1%). This analysis revealed that all individuals had a similar profile of glycosyltransferases ($R^2 = 0.96 ± 0.003$), whereas the profiles of glycoside hydrolases were significantly more variable, even between family members ($R^2 = 0.80 ± 0.01$; $P < 10^{-30}$, paired Student’s $t$-test). This suggests that the number and spectrum of glycoside hydrolases is affected by ‘external’ factors such as diet more than the glycosyltransferases.

To identify metabolic pathways associated with obesity, only non-core associated (variable) functional groups were included in a comparison of the gut microbiomes of lean versus obese twin pairs. A bootstrap analysis\textsuperscript{23} was used to identify metabolic pathways that were enriched or depleted in the variable obese gut microbiome. For example, similar to a mouse model of diet-induced obesity\textsuperscript{4}, the obese human gut microbiome was enriched for phosphotransferase systems involved in microbial processing of carbohydrates (Supplementary Table 12). All gut microbiome sequences were compared with the custom database of 44 human gut genomes: an odds ratio analysis revealed 383 genes that were significantly different between the obese and lean gut microbiome ($q < 0.05$; 273 enriched and 110 depleted in the obese microbiome; Supplementary Tables 13 and 14). By contrast, only 49 genes were consistently enriched or depleted between all twin pairs (see Supplementary Methods).

These obesity-associated genes were representative of the taxonomic differences described above: 75% of the obesity-enriched genes were from Actinobacteria (compared with 0% of lean-enriched genes; the other 25% are from Firmicutes) whereas 42% of the lean-enriched genes were from Bacteroidetes (compared with 0% of the obesity-enriched genes). Their functional annotation indicated that many are involved in carbohydrate, lipid and amino-acid metabolism (Supplementary Tables 13 and 14). Together, they comprise an initial set of microbial biomarkers of the obese gut microbiome.

Our finding that the gut microbial community structures of adult monozygotic twin pairs had a degree of similarity that was comparable to that of dizygotic twin pairs, and only slightly more similar than that of their mothers, is consistent with an earlier fingerprinting study of adult twins\textsuperscript{24}, and with a recent microarray-based analysis, which revealed that gut community assembly during the first year of life followed a more similar pattern in a pair of dizygotic twins than 12 unrelated infants\textsuperscript{25}. Intriguingly, another fingerprinting study of monozygotic and dizygotic twins in childhood showed a slightly reduced similarity profile in dizygotic twins\textsuperscript{26}. Thus, comprehensive time-course studies, comparing monozygotic and dizygotic twin pairs from birth through adulthood, as well as intergenerational analyses of their families’ microbiotas, will be key to determining the relative contributions of host genotype and environmental exposures to (gut) microbial ecology.

The hypothesis that there is a core human gut microbiome, definable by a set of abundant microbial organisinal lineages that we all share, may be incorrect: by adulthood, no single bacterial phylotype was detectable at an abundant frequency in the guts of all 154 sampled humans. Instead, it appears that a core gut microbiome exists at the level of shared genes, including an important component involved in various metabolic functions. This conservation suggests a high degree of redundancy in the gut microbiome and supports an ecological view of each individual as an ‘island’ inhabited by unique
collections of microbial phylyotypes: as in actual islands, different species assemblages converge on shared core functions provided by distinctive components. Our findings raise the question of how core functionality is assembled in this body habitat. Understanding the underlying principles should provide insights about microbial adaptation to, and mutualistic community assembly within, a wide range of environments.

METHODS SUMMARY

Faecal samples were collected from each individual. Community DNA was prepared and used for pyrosequencing (454 Life Sciences), as well as for PCR and sequencing of bacterial 16S rRNA genes. Shotgun reads were mapped to reference genomes using the National Center for Biotechnology Information ‘non-redundant’ database, KEGG\textsuperscript{17}, STRING\textsuperscript{18}, CAZY (http://www.cazy.org/) and a 44-member human-gut microbial genome database. Metabolic reconstructions were performed based on CAZY, KEGG and STRING annotations. The relative abundance of KEGG metabolic pathways is referred to as a ‘metabolic profile’.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Information This Whole Genome Shotgun project is deposited in DDBJ/EMBL/GenBank under accession number 32089. 454 pyrosequencing reads are deposited in the NCBI Short Read Archive. Nearly full-length 16S rRNA gene sequences are deposited in GenBank under accession numbers FJ362604–FJ372382. Annotated sequences are also available in MG-RAST (http://metagenomics.nmpdr.org/). 454-generated 16S rRNA sequences with sample identifiers are also available at http://gordonlab.wustl.edu/SuppData.html. Correspondence and requests for materials should be addressed to J.I.G. (jgordon@wustl.edu).

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METHODS

Community DNA preparation. Faecal samples were frozen immediately after they were produced. De-identified samples were stored at −80 °C before processing. Ten to twenty grams of each sample was pulverized in liquid nitrogen with a mortar and pestle. An aliquot (approximately 500 mg) of each sample was then suspended, while frozen, in a solution containing 500 μl of extraction buffer (200 mM Tris (pH 8.0), 200 mM NaCl, 20 mM EDTA), 210 μl of 20% SDS, 500 μl of a mixture of phenol/chloroform/isooamy alcohol (25:24:1, pH 7.9), and 500 μl of a slurry of 0.1-mm diameter zirconia/silica beads (BioSpec Products). Microbial cells were subsequently lysed by mechanical disruption with a bead beater (BioSpec Products) set on high for 2 min at room temperature, followed by extraction with phenol/chloroform/isooamy alcohol, and precipitation with isopropanol. DNA obtained from three separate aliquots of each faecal sample were pooled (≥200 μg DNA) and used for pyrosequencing (see below).

16S rRNA gene-sequence-based surveys. Complementary phylogenetic- and taxon-based methods were used to compare 16S RNA sequences among faecal communities. Phylogenetic clustering with UniFrac15 is based on the principle that communities can be compared in terms of their shared evolutionary history, as measured by the degree to which they share branch length on a phylogenetic tree. We complemented this approach with taxon-based methods27, which disregard some of the information contained in the phylogenetic tree of the taxa in question, but have the advantage that specific taxa are unique to, or shared among, groups of samples can be identified (for example, those from lean or obese individuals).

Before both types of analysis, we grouped 16S rRNA gene sequences into operational taxonomic units (OTUs/phylotypes) using both cd-hit26 and the furthest-neighbour-like algorithm, with a sequence identity threshold of 97%, which is commonly used to define 'species'-level phylotypes. Taxonomy was assigned using the best-BLAST-hit against Greengenes27 (E value cutoff of 10−10; minimum 88% coverage, 88% identity) and the Hugenholtz taxonomy (downloaded from http://greengenes.lbl.gov/Download/Sequence_Data/Greengenes_format/ on 12 May 2008, excluding sequences annotated as chamaerica).

Selection of operational taxonomic units. 16S RNA gene-derived pyrosequencing data were pre-processed to remove sequences with low-quality scores, sequences with ambiguous characters or sequences outside the length bounds (V6 < 50 nucleotides, V2 < 200 nucleotides), and binned according to sample-specific barcode (see, for example, ref. 13). Similar sequences were identified using Megablast26 and cd-hit, with the following parameters: E value 10−10 (Megablast only); minimum coverage 99%; minimum pairwise identity 97%.

Candidate OTUs were identified as sets of sequences connected to each other at this level using a maximum of 4,000 hits per sequence. Each candidate OTU was considered valid if the average density of connection was above threshold; otherwise, it was broken up into smaller connected components27.

Tree building and UniFrac clustering for PCA analysis. A relaxed neighbour-joining tree was built from one representative sequence per OTU using Clearcut28, employing the Kimura correction (the PH Lane mask was applied to V2 and full-length data), but otherwise with default comparisons. Unweighted UniFrac23 was run using the resulting tree. PCA was performed on the resulting matrix of distances between each pair of samples. To determine if the UniFrac distances were on average significantly different for pairs of samples (that is, between twin pairs, between twins and their mother, or between unrelated individuals), we performed a t-test on the UniFrac distance matrix, and generated a P value for the t-statistic by permutation of the rows and columns as in the Mantel test, regenerating the t-statistic for 1,000 random samples, and using the distribution to obtain an empirical P value.

Rarefaction and phylogenetic diversity measurements. To determine which individuals had the most diverse communities of gut bacteria, rarefaction plots and phylogenetic diversity measurements, as described by Faith31, were made for each sample. Phylogenetic diversity is the total amount of branch length in a phylogenetic tree constructed from the combined 16S RNA data sets, leading to the sequences in a given sample. To account for differences in sampling effort between individuals, and to estimate how far we were from sampling the diversity of each individual completely, we plotted the accumulation of phylogenetic diversity (branch length) with sampling effort, in a manner analogous to rarefaction curves. We generated the phylogenetic diversity rarefaction curve for each individual by applying custom python code (http://bmf2.colorado.edu/unifrac/about.py) to the Arb parsimony insertion tree32.

Pyrosequencing of total community DNA. Shotgun pyrosequencing runs were performed on the 454 FLX pyrosequencer from total faecal community DNA. Two samples were also analysed in a single run using Titanium extra-long-read pyrosequencing technology (see Supplementary Tables 4 and 5). Sequencing reads with degenerate bases (‘N’ s) were removed along with all duplicate sequences, as sequences of identical length and content are a common artefact of the pyrosequencing methodology. Human sequences were removed by identifying sequences homologous to the Homo sapiens reference genome (BLASTN E < 10−5, %identity > 75, score > 50).

CAZyme analysis. Metagenomic sequence reads were searched against a library of modules derived from all entries in the carbohydrate-active enzymes (CAZy) database (www.cazy.org using FASTY33, E < 10−6). This library consists of approximately 180,000 previously annotated modules (catalytic modules, carbohydrate-binding modules and other non-catalytic modules or domains of unknown function) derived from about 80,000 protein sequences. The number of sequencing reads matching each CAZy family was divided by the number of total sequences assigned to CAZy families and multiplied by 100 to calculate a relative abundance. An R2 value was calculated for each pair of CAZy profiles. We then compared the distribution of glycosyl hydrolase similarity scores with the distribution of glycosyl transferase similarity scores.

Statistical analyses. Xipe20 (version 2.4.2) was used for bootstrap analyses of pathway enrichment and depletion, using the parameters sample size = 10,000 and confidence level = 0.95. Linear regressions were performed in Excel (version 11.0, Microsoft). Mann–Whitney and Student’s t-tests were used to identify statistically significant differences between two groups (Prism version 4.0, GraphPad; Excel version 11.0, Microsoft). The Bonferroni correction was used to correct for multiple hypotheses. The Mantel test was used to compare distance matrices: the matrix of each pairwise comparison of the abundance of each reference genome, and the abundance of each metabolic pathway, were compared (Mantel program in Python using PyCogent34; 10,000 replicates). Data are represented as mean ± s.e.m. unless otherwise indicated.

Microbiome sequences were compared against the custom database of 44 gut genomes (BLASTX E < 10−5, bitscore > 50, and %identity > 50). A gene-by-sample matrix was then screened to identify genes ‘commonly-enriched’ in either the obese or lean gut microbiome (defined by an odds ratio greater than 2 or less than 0.5 when comparing the pooled obese twin microbiomes with the pooled lean twin microbiomes, and when comparing each individual obese twin microbiome with the aggregate lean twin microbiome, or vice versa). The statistical significance of enriched or depleted genes was then calculated using a modified t-test (q value < 0.05; calculated with code supplied by M. Pop and J.R. White, University of Maryland). We also searched for genes that were consistently enriched or depleted in all six monozygotic twin pairs. A gene-by-sample matrix was generated based on BLASTX comparisons of each microbiome with our custom 44-genome database, to calculate an odds ratio based on the frequency of each gene in each twin versus the respective co-twin. The analysis revealed only 49 genes (odds ratio > 2 or < 0.5); they represent a variety of taxonomic groups, including Firmicutes, Bacteroidetes and Actinobacteria, and did not show any clear functional trends.

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