Quantitatively different, yet qualitatively alike

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Quantitatively Different, yet Qualitatively Alike: A Meta-Analysis of the Mouse Core Gut Microbiome with a View towards the Human Gut Microbiome

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Abstract

Background: A number of human diseases such as obesity and diabetes are associated with changes or imbalances in the gut microbiota (GM). Laboratory mice are commonly used as experimental models for such disorders. The introduction and dynamic development of next generation sequencing techniques have enabled detailed mapping of the GM of both humans and animal models. Nevertheless there is still a significant knowledge gap regarding the human and mouse common GM core and thus the applicability of the latter as an animal model. The aim of the present study was to identify inter- and intra-individual differences and similarities between the GM composition of particular mouse strains and humans.

Methodology/Principal Findings: A total of 1509428 high quality tag-encoded partial 16S rRNA gene sequences determined using 454/FLX Titanium (Roche) pyro-sequencing reflecting the GM composition of 32 human samples from 16 individuals and 88 mouse samples from three laboratory mouse strains commonly used in diabetes research were analyzed using Principal Coordinate Analysis (PCoA), nonparametric multivariate analysis of similarity (ANOSIM) and alpha diversity measures. A reliable cutoff threshold for low abundant taxa estimated on the basis of the present study is recommended for similar trials.

Conclusions/Significance: Distinctive quantitative differences in the relative abundance of most taxonomic groups between the examined categories were found. All investigated mouse strains clustered separately, but with a range of shared features when compared to the human GM. However, both mouse fecal, caecal and human fecal samples shared to a large extent not only representatives of the same phyla, but also a substantial fraction of common genera, where the number of shared genera increased with sequencing depth. In conclusion, the GM of mice and humans is quantitatively different (in terms of abundance of specific phyla and species) but share a large qualitatively similar core.

Introduction

Shifts in the composition, known as dysbiosis, of the human GM have in several studies been associated with diseases such as allergies [1], asthma [2], inflammatory bowel disease [3], diabetes type 1 and 2 [4–6], and metabolic syndrome [7] - all indicating a causative role of the gut microbiota.

Rodents are the mammalian model most extensively used to investigate the relationship between GM and health and disease. The reason for the popularity of mouse models is their well explored genetic and relatively close physiological similarity with humans and the ability to control a wide range of environmental factors which reduces variation in the baseline gut microbiota between individual study objects [8]. However, despite the wide use of rodent models existing information about the human and laboratory mouse common GM core is still relatively scarce. One of the pioneering reports on this field, carried out before the age of next-generation sequencing, disclosed only 15% similarity between the human and mouse GM genera [9]. The majority of bacteria in the gut were shown to be members of the two phyla, Firmicutes and Bacteroidetes, and in both humans and mice, these two phyla together comprised more than 90% of the gut bacteria.

Many GM related disorders have been linked with bacterial dysbiosis on a higher taxonomic level proving the usefulness of sequencing the GM to e.g. phylum and family level. For example, Turnbaugh et al. showed that a switch from a low fat to a high fat, high sugar diet in mice, which was associated with obesity, lowered the Bacteroidetes/Firmicutes ratio in the gut within one day [10]. Also, alterations in the phylum Bacteroidetes and the Lachnospiraceae family have been suggested as possible biomarkers to help predict predispositions to inflammatory bowel disease [11]. In humans, patients suffering from T2D have been found to have significant reductions in the phylum Firmicutes and the class Clostridia compared to healthy controls in a 454 FLX based study [5]. In a later study comparing the gut metagenome of individuals...
suffering from T2D and healthy controls a group of butyrate producing bacteria and opportunistic pathogens that could serve as gut microbial markers for classifying type 2 diabetes were identified [12] underlining the possibility of identifying microbial markers at this taxonomic level associated with disease. Despite the advantages of deep metagenome sequencing it still remains a costly approach and a range of reports show that the relationship between many disorders and GM changes can be identified without the need for studying whole metagenomes [5,6,11,13,14]. However, the usefulness of mouse models for such studies would to a large extent also depend on similarities in their GM profiles at genus or species level with humans. The aim of the present study was therefore to demonstrate inter- and intra-individual differences and similarities between the GM composition of three laboratory mouse strains commonly used in research in chronic inflammatory diseases with those of humans based on more than 1.5 million high quality sequences of partial 16S rRNA gene verified with tag-encoded 454/FLX Titanium (Roche) pyro-sequencing.

**Materials and Methods**

**Dataset**

A total of 88 mice and 128 human (16 individuals with each individual sampled twice within 6 weeks and each sample sequenced 4 times) GM profiles determined using tag-encoded 16S rRNA gene 454/FLX Titanium (Roche) pyro-sequencing were included in the study (Table 1). All samples enrolled in the present meta-analysis have been treated according to the same protocols concerning DNA extraction, library preparation and sequencing [6,14]. Briefly, cellular DNA was extracted using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) basically following the manufacturer’s instructions, but with the addition of an initial bead beating step (FastPrep) for increasing cell lysis. Extracted DNA was stored at −80°C until analysis. Amplicons (466 bp) including the V3 and V4 regions of the 16S rRNA gene were amplified using the primers detailed in the electronic supplementary material (ESM) Table 1 [15] followed by a second round of PCR where primers with adapters and tags were used [16]. PCR amplification of the 16S rRNA gene plus purification and pyrosequencing of amplified PCR products were carried out as previously described [5]. The amplified fragments with adapters and tags were quantified using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and mixed in approximately equal concentrations to ensure most possible even representation of reads per sample. Two- region 454 sequencing runs were performed on a GS FLX Titanium Pico TiterPlates (70×75) using a GS FLX Titanium Sequencing Kit XLR70 according to the manufacturer’s instructions (Roche Diagnostics, Indianapolis, IN, USA).

All animal experiments were carried out in accordance with the Council of Europe Convention European Treaty Series (ETS) 123 on the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, and the Danish Animal Experimentation Act (LBK 1306 from 23/11/2007). The study was approved by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

Human specimens used in this meta-analysis come from the independent study that was approved by The Scientific Ethics Committee of Capital Region, Denmark (reference H-4-2010-137). Written informed consent was obtained from volunteers prior to recruitment.

**Data Treatment**

The dataset was analyzed using the Quantitative Insight Into Microbial Ecology (QIIME) open source software package [17]. All steps such as quality control, de-noising, chimera filtering and OTU picking were conducted as previously described [14]. High quality sequences purged from chimeric reads were further clustered at 97% relatedness using UCLAST (http://www.drive5.com/usearch/). The representative sequences from each cluster were aligned with pyNAST (http://qiime.org/pynast/) and subjected to the Ribosomal Database Project (RDP)-based 16S rRNA gene annotation. For intra-individual assessment all mouse and 16 human samples from the control group were subsampled to an equal number of reads per individual (4500 reads per sample which constitutes to 85% of the second most indigent sample in the dataset). For inter-group comparison the reads were merged according to host (mouse strain/human) and subsampled to an equal number of sequences per category, respectively 80000 reads for mice strains comparisons and 600000 reads for similarity assessment between mice and humans. Both numbers constitute approximately 85% of the least numerous category. Alpha diversity measures such as rarefaction curves based on the estimated species number (97% sequence identity threshold), Chao1 and Shannon indexes were calculated for OTU tables that were unified to 4000 (first most indigent sample) sequences per sample.

In order to investigate the influence of the sequencing method into variance between categories, which is caused mostly by the low abundant taxa, a set of 16 samples each sequenced in 4 independent runs were compared within their quadruplicates. A set of 1000 subsampled OTU-tables was generated for each sample (3000 reads per sample). Low abundant taxa were removed until taxa similarity of all replicates within a given sample crossed 99%. An average, minimum cutoff value was therefore calculated based on 16000 subsampled OTU tables using an in-house Matlab (Mathworks) script.

**Statistics**

Principal Coordinate Analysis (PCoA) plots were generated with the Jackknifed Beta Diversity workflow based on 10 distance metrics calculated using 10 subsampled OTU tables. The -e value (number of sequences taken for each jackknifed subset) was set to 85% of the sequence number within the most indigent sample. Analysis of similarities (ANOSIM) was used to evaluate group differences using weighted and unweighted uniFrac distance metrics that were generated based on rarefied (4500 reads per sample) OTU tables. The relative distribution of the GM genera registered in 88 mouse and 16 human samples was calculated for unified, summarized at the genus level OTU tables.

Differences in taxa abundances at phylum and genus level between categories were verified with Metastats (http://metastats. cbcb.umd.edu). From each group 14 samples (corresponding to the smallest category) were randomly chosen and combinations of group pairs were tested using 1000 permutations (p value threshold = 0.05; false discovery rate threshold = 0.5). The relationship between sequencing depth and shared GM, classified into phylum and genus level, between mice and humans was plotted based on multiple subsampled OTU tables composed of two categories collecting 794988 human and 714440 mouse GM 16S rRNA gene reads. Simulation of each sequencing depth was based on multiple subsampled OTU tables composed of two categories selecting 794988 human and 714440 mouse GM 16S rRNA gene reads. Simulation of each sequencing depth was repeated 100 times and an average proportion of shared taxonomic groups between the two categories were calculated (abundance threshold for unshared taxa = 0.19%).
Table 1. Data collection description.

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<tr>
<th>Total number of sequences</th>
<th>High quality reads</th>
<th>Sample type</th>
<th>NCBI accession number</th>
<th>Host’s age when sampled</th>
<th>Number of samples</th>
<th>Sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human (control)</strong></td>
<td></td>
<td>Feces</td>
<td>SRA058021</td>
<td>18–50 years</td>
<td>16</td>
<td>4</td>
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<td></td>
<td></td>
<td></td>
<td>469971</td>
<td>392726</td>
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<td></td>
<td>2010</td>
<td>446</td>
</tr>
<tr>
<td><strong>Human (placebo)</strong></td>
<td></td>
<td>Feces</td>
<td>SRA058021</td>
<td>18–50 years</td>
<td>16</td>
<td>4</td>
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<td></td>
<td>2010</td>
<td>446</td>
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<td><strong>NOD</strong></td>
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<td>Feces</td>
<td>SRA047328</td>
<td>14–30 weeks</td>
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<td>3590</td>
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<td><strong>GM</strong></td>
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<td>Feces</td>
<td>SRA057283</td>
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<td>157888</td>
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<td>1314</td>
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<tr>
<td><strong>GM</strong></td>
<td></td>
<td>Feces</td>
<td>SRA057283</td>
<td>16 weeks</td>
<td>19</td>
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<td></td>
<td></td>
<td>4159</td>
<td>1314</td>
</tr>
<tr>
<td><strong>BALB/c</strong></td>
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<td>Feces</td>
<td>SRA051317</td>
<td>13 weeks</td>
<td>21</td>
<td>232612</td>
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<td></td>
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<td></td>
<td></td>
<td>8818</td>
<td>450</td>
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</table>

Sequence collections representing five control groups from previously published studies used for this meta-analysis were stored in the Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/sra), National Center for Biotechnology Information (NCBI). The pool of human samples (SRA058021) composed of the control and the placebo group. Samples from these two categories were sequenced in four independent runs resulting in 16 human samples. The B6. V-<sup>Lep</sup> ob/J mouse groups could be classified as a clearly separated or partly separated. The B6. V-<sup>Lep</sup> ob/J mice became less distinct, and the frontier with the bacterial relative abundance (weighted UniFrac distance matrix) the differences become less distinct, and the frontier with the human GM distinctly separated from the mice, as also shown by ANOSIM analysis (Table 2). The two B6. V-<sup>Lep</sup> ob/J groups where age was the only varying factor were only partially separated (Fig. 2A and Table 2). When widening the information groups where age was the only varying factor were only partially separated (Table 2, p < 0.001**). As R values >0.75 generally are interpreted as clearly separated, R >0.5 as separated and R <0.25 as groups hardly separated [19] it was concluded that all categories with the exception of the two B6. V-<sup>Lep</sup> ob/J mouse groups could be classified as a clearly separated or separated.

Abundance Threshold

All samples in the human study SRA058021 have been sequenced four times in independent runs. However, when comparing the similarity within the same sample sequenced four times it was found that only an average of 77.1% of taxa was shared due to the nature of the sequencing method where low abundant taxa may or may be captured by pure chance [20]. Starting with the assumption that all four replicates representing a given sample should depict roughly the same relative distribution of bacteria this dataset was then used to calculate the most commensurate cut off value for low abundant taxa, that were not a true picture of low abundant microbial groups but which presence or absence was rather a result of a sequencing method/depth. Consequently, low abundant reads were removed until all 4 replicates of a single sample shared at least 99% of taxa. Sixty-four (16 × 4) samples from the above-mentioned group were included and 100 subsampled OTU tables were generated for each replicate (3000 reads per sample). The threshold for the low abundant taxa that needed to be removed in order to make all 4 replicates uniform was evaluated based on 16000 rarefied OTU tables and scored: 0.19%, which corresponded to approximately 10 reads per taxon. When a certain taxon was registered in all after filtering the low abundant OTUs (abundance threshold for unshared taxa = 0.19%) using the make_ouf_network.py script (QIIME). The visualization of the OTU-networks was performed with an open source platform –Cytoscape (version 2.8.3, http://www.cytoscape.org/).
replicates of a given sample and one or more were below the estimated threshold level, all values were kept.

Phyla Distribution and Abundance

All reads used in this study were classified into 9 phyla after applying the abundance threshold (0.19%), with one phylum noted as unclassified (Table 3). Generally Firmicutes and Bacteroidetes were the dominating phyla accounting for 89–97% of all reads with a clear preponderance of Firmicutes in all categories except the BALB/c mice (Table 3). Verrucomicrobia was the third most abundant phyla in NOD mice and the fourth most abundant in humans and B6.V-Lep/J (8 weeks of age). The three remaining mouse strains were either devoid of bacteria from this phylum, or the bacteria were below the detection limit.

Genera Distribution and Abundance

The relative abundance of genera depicted for all samples individually shows explicit alterations between studies with the human specimens being the most conspicuous category (Figure 3). In total 239 genera were registered for both groups but only 89 exceeded the threshold value (Table S1). The unclassified genus from the Lachnospiraceae family was the most dominant bacterial group in both mouse and human samples comprising on average 41% and 15% of the reads, respectively. The two consecutive most abundant genera in mouse samples were an unclassified genus from the Porphyromonadaceae (13%) and Alistipes (12%), while in human specimens – Roseburia (12%) and unclassified genus representing the Clostridiales order (10%) were the dominating genera.

Metastats analysis revealed widespread differences in the bacterial relative abundance at both phylum (Table S2) and genera (Table S3) level between all categories.

Gut Microbiota Qualitative Differences between Mice and Humans

Mice and humans shared 90% of bacterial phyla with Deferrribacteres and its only genus deputy - Mucispirillum causing the difference between mouse and human categories at the qualitative level (Figure 6A). Analysis on a deeper classification level showed that above the threshold value mouse and human samples shared 89% of bacterial genera (Figure 6B). The human GM cluster contained 9 unique genera compared to the mice, namely Faecalibacterium, Mitsuokella, Mogagobacter, Dialister, Asterolipid, Succinivibio, Sutterella, Paraprevotella and Phascolarctobacterium.

The collection of mice GM profiles presented one unique genus, Mucispirillum, while the remaining 80 genera despite differences in the relative abundance were common for both mice and humans (Figure S1).

The average similarity of the GM phyla and genera between mice strains verified using the raw dataset, without the abundance threshold, was respectively 20% and 56% lower than when using a cutoff value (Figures S2A and S2B). The similarity of the collective mouse and human microbiomes without the abundance threshold was correspondingly 40% and 57% lower at the phylum and the

Figure 1. Rarefaction curves based on the estimated number of OTUs. (97% sequence identity threshold), average Chao1 and Shannon indexes were calculated for 6 categories using rarefied OTU table (4000 reads per sample). Labels “BALB/c (f)” and “BALB/c (c)” stand for the GM microbiota profile of BALB/c mice determined using fecal and caecal samples respectively.

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In Figure 7 the function between sequencing depth and GM similarity at the phylum and genus level is illustrated. It was found that both phyla and - even more pronounced - genera resemblance between categories differs dramatically depending on number of reads used for analysis as increasing sequencing depth uncovers more genera that the two groups have in common (Figure 7).

### Discussion

Laboratory mice are commonly used as experimental models for diseases such as diabetes [6,21], inflammatory bowel disease [22,23] and allergies [24,25] where the GM composition and function has been found to be an important contributing factor [26]. However, at present a knowledge gap regarding the similarity of the human and mouse common GM core exists, especially on a deeper level of taxonomy, which might question the usefulness of these models. Therefore, in the present study, we

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**Table 2.** ANOSIM analysis between categories.

<table>
<thead>
<tr>
<th>Category</th>
<th>B6 (8 weeks)</th>
<th>B6 (16 weeks)</th>
<th>BALB/C (feces)</th>
<th>BALB/C (caecum)</th>
<th>NOD</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6. V-Lepob/J (8 weeks)</td>
<td>– – 0.644</td>
<td>&lt;0.001*** 1.000</td>
<td>&lt;0.001*** 0.999</td>
<td>&lt;0.001*** 1.000</td>
<td>&lt;0.001*** 1.000</td>
<td>&lt;0.001*** 1.000</td>
</tr>
<tr>
<td>B6. V-Lepob/J (16 weeks)</td>
<td>0.219</td>
<td>&lt;0.001*** – – 1.000</td>
<td>&lt;0.001*** 0.998</td>
<td>&lt;0.001*** 0.998</td>
<td>&lt;0.001*** 1.000</td>
<td>&lt;0.001*** 1.000</td>
</tr>
<tr>
<td>BALB/c (feces)</td>
<td>0.808</td>
<td>&lt;0.001*** 0.888</td>
<td>&lt;0.001*** – –</td>
<td>0.883</td>
<td>&lt;0.001*** 0.999</td>
<td>&lt;0.001*** 1.000</td>
</tr>
<tr>
<td>BALB/c (caecum)</td>
<td>0.663</td>
<td>&lt;0.001*** 0.876</td>
<td>&lt;0.001*** 0.850</td>
<td>&lt;0.001*** – –</td>
<td>0.994</td>
<td>&lt;0.001*** 1.000</td>
</tr>
<tr>
<td>NOD</td>
<td>0.531</td>
<td>&lt;0.001*** 0.564</td>
<td>&lt;0.001*** 0.901</td>
<td>&lt;0.001*** 0.475</td>
<td>&lt;0.001*** – –</td>
<td>1.000</td>
</tr>
<tr>
<td>Human</td>
<td>0.911</td>
<td>&lt;0.001*** 0.888</td>
<td>&lt;0.001*** 0.975</td>
<td>&lt;0.001*** 0.851</td>
<td>&lt;0.001*** 0.829</td>
<td>&lt;0.001*** – –</td>
</tr>
</tbody>
</table>

Analysis of similarity (testing whether two or more groups are significantly different) was calculated between all categories based on rarefied (4500 reads per sample) weighted (regular font) and unweighted (bold font) distance matrices. Each pairwise comparison of two groups was performed using 1000 permutations. R values >0.75 are generally interpreted as clearly separated, R >0.5 as separated and R <0.25 as groups hardly separated [17].
Table 3. The relative distribution of phyla among categories.

<table>
<thead>
<tr>
<th>Category</th>
<th>NOD BALB/c (cecum) B6 (16 weeks of age)</th>
<th>B6 (8 weeks of age)</th>
<th>BALB/c (feces)</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg. Max Min SD Avg. Max Min SD Avg. Max Min SD Avg. Max Min SD Avg. Max Min SD Avg. Max Min SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Actinobacteria</strong></td>
<td>0.11 1.20 0.00 0.32 0.03 0.12 0.00 0.03 0.05 0.30 0.02 0.07 0.03 0.06 0.00 0.02 0.07 0.16 0.00 0.04 1.15 5.18 0.02 1.55</td>
<td></td>
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</tr>
<tr>
<td><strong>Bacteroidetes</strong></td>
<td>22.47 45.40 7.36 12.09 22.07 34.40 11.40 7.55 32.85 54.50 20.72 9.14 35.54 58.54 19.24 12.14 51.37 71.18 20.54 11.89 16.73 26.08 2.94 7.16</td>
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<tr>
<td><strong>Deferribacteres</strong></td>
<td>66.43 90.82 47.92 15.90 69.99 78.48 56.82 6.84 64.38 77.66 41.56 9.63 58.93 78.04 35.20 14.06 43.90 75.26 27.08 12.33 74.92 95.34 44.54 12.41</td>
<td></td>
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<tr>
<td><strong>Firmicutes</strong></td>
<td>2.39 10.36 0.94 2.46 4.07 7.07 9.10 1.10 1.19 2.12 4.86 0.78 0.95 2.92 5.20 1.56 3.34 4.25 4.28 4.28 1.60 0.35 0.35 0.35 0.35 0.35 0.35</td>
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<tr>
<td><strong>Proteobacteria</strong></td>
<td>0.81 193.56 0.00 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06</td>
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<tr>
<td><strong>Tenericutes</strong></td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
<td></td>
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<tr>
<td><strong>Verrucomicrobia</strong></td>
<td>7.69 31.90 0.04 11.33 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
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**The relative distribution of the gut microbial phyla among single human (16 individuals) and five mouse groups (15, 14, 19, 19, 21 mice respectively). The abundance threshold within at least one of the category was set to 0.19%.

Relative value, this label was considered as shared. The calculated dissimilarities between the groups. Therefore a script was applied that whenever two categories shared a given taxon no matter its abundance. Consequently, despite ubiquitous species level differences, the bacterial relative distribution on higher taxonomic levels makes the GM of different mice strains more similar to each other compared to that of humans (Fig. 2 and 3).

Diet is an environmental factor known to strongly influence GM composition [32,33]. However all mice used in this study were fed with a similar chow diet (Altromin 1324); therefore diet was the least differing factor of microbial community between mouse strains but definitely a strong one when comparing with the human GM. Diet is therefore another force in addition to genetics pushing human GM cluster away from the mice.

Early priming of bacterial colonization during early life may also influence GM and immunity later in life [34–38], which might also be a driving force in the present study. Especially the environment of humans is undoubtedly the most dissimilar from that of laboratory mice, what again favors receding human bacterial profile away from the mice. Consequently, these differences are important to consider when performing mice-human translational studies.

Lastly, for adult humans and mice the influence of age on the GM profiles seems to be minor as neither human samples collected from patients at different age (18–50 years) nor NOD mice analyzed at different ages (14–30 weeks) clustered according to age. This supports the high level of GM composition stability in adult individuals which was previously documented among human samples included in the analysis that have been independently sequenced data sets, representing the same fecal sample reached an average qualitative similarity of only 77.1% was found. Therefore, in order to compare the categories in a more adequate manner, a commensurate abundance threshold was implemented. It was found that when applying a cut-off value of 0.19% at least 99% similarity was reached when comparing sequencing sets representing the same fecal sample. However, setting a threshold in a way that all numbers being below a given value would be excised (turned into zero) raised a problem of introducing false dissimilarities between the groups. Therefore a script was applied that whenever two categories shared a given taxon no matter its relative value, this label was considered as shared. The calculated

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[10.1371/journal.pone.0062578.t003]
threshold value was further implemented for all intra-groups comparisons.

In the present study the Firmicutes fraction was more abundant than the Bacterioidetes in most fecal and caecal samples, except for the BALB/c fecal samples, which showed an inverted proportion of these two phyla. Human fecal samples had relatively more Firmicutes and less Bacteriodes compared to most mouse fecal and caecal samples. This is in accordance with earlier studies reporting that the majority of bacteria in the gut are members of these two phyla and that in mice the Firmicutes fraction seems to be much larger than the Bacteriodes fraction [9,43–47].

Representatives of the TM7 phylum were clearly reduced in human samples (0.001%) compared to mouse fecal (0.1%) and caecal (0.8%) specimens. This is in correspondence with a study by Rawls et al. where human colonic samples were shown to be free of the TM7 phylum in comparison to the mouse cecum and zebrafish gut microbiota. However, a relatively low number of sequences was used (less than 3000) which would not be enough to detect representatives of this phylum at the similar abundance levels [48].

As seen from Fig. 6 increased sequencing depth disclose consecutive phylogroups (phyla and genus level) resulting in higher rates of similarity between the two categories, mice and humans with increased sequencing dept. Although the GM classified at the phylum level could be well explored with relatively few sequences [9], information at the genus level and its link with diseases in general require much deeper sequencing as also evident from Fig. 6.

For example, the Prevotella genus has been found to be inversely correlated with body weight gain, cholesterol accumulation, insulin resistance and diet-induced adiposity [49] and this genus was exclusive for the BALB/c fecal and caecal microbiota but not detected in any of the remaining mouse strains. In addition, the fecal microbiota of the NOD mice was the only one lacking representatives of the Odoribacter genus which relative abundance was recently shown to be increased in the caecum of mice exposed to grid floor induced stress [14]. Unclassified members from the Proteobacteria phylum were unique for the fecal and caecal samples of BALB/c mice and adult B6.V-Lepb/J group that on the other hand were lacking members of the Akkermansia genus. Akkermansia muciniphila has been suggested to possess anti-inflammatory properties as it was found to be present in lower levels in humans suffering from inflammatory bowel disease compared to the healthy control group [50] and greatly increased in vancomycin treated NOD whose cumulative diabetes incidence was significantly reduced [6]. A single unclassified genus was found to be the only qualitative difference between the fecal and caecal content of the two BALB/c groups with no differences at the phyla level and no major divergence in the species richness or diversity. It could be thus concluded that the main cause of differences between samples representing GM pictures of two parts of the BALB/c mice gastrointestinal track was the rearrangement in the bacterial relative abundance. It has previously been shown using the Denaturation Gradient Gel Electrophoresis (DGGE) that profiles of fecal and caecal microbiota do not cluster in the same way proving that the GM of an individual presents different proportions in species abundance along the GI track [51].

Pairwise comparison (mice vs. humans) using 0.6 million reads per category disclosed 89% similarity between mouse and human GM genera, with 9 genera being unique for human samples and not detected in any of the three mice strains (abundance threshold 0.19%). Among these 9 genera were Faecalibacterium from the Ruminococcaceae family and Asteroleplasma from phylum Tenericutes that both have been suggested as possible indicators of a healthy human GM since disturbances in the relative distribution of common species from these genera have been linked with the etiology of Crohn’s disease (CD) and ulcerative colitis (UC) [52–55]. The Megasphaera genus was also unique for humans not being detected in any of the three mouse strains. Megasphaera spp. have been imputed to support the growth of colonic mucosa [56,57].
The Mitsuokella genus has recently been identified as a GM member of lean as well as obese Indians [58]. Many bacterial communities from the Clostridia class including genera that were found unique for humans such as Faecalibacterium and Dialister but also Sutterella from the Proteobacteria phylum showed poor establishment after transplanting them from human into mice GI tracks [59]. It seems possible that mouse genetics disfavors their establishment.

**Figure 4. Number of GM phyla shared between given mouse strains after applying a cut off threshold (0.19%) for the low abundant taxa.** Whenever a given genus was present in both categories but the value for one or both was below the threshold level the label was kept and classified as shared. 80000 high quality 16s rRNA reads used to represent each mouse strain were annotated to the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu/) database. In three cases the single phylum Verrucomicrobia is reducing similarity to 90%. The only genus representing this subgroup is Akkermansia. Labels “BALB/c (f)”, “BALB/c (c)”, “B6.V-Lep^ob/J (16)” and “B6.V-Lep^ob/J (8)” stand for the gut GM of BALB/c mice determined using fecal and caecal samples and B6.V-Lep^ob/J mice using fecal specimens sampled in 16 and 8 weeks of age respectively.

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**Figure 5. Number of GM genera shared between given mouse strains after applying a cut off threshold (0.19%) for the low abundant taxa.** Whenever a given genus was present in both categories but the value for one or both was below the threshold level the label was kept and classified as shared. 80000 high quality 16s rRNA reads used to represent each mouse strain were annotated to the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu/) database. Genera differing between categories: a - Akkermansia, b – Prevotella, c- unclassified genus from Desulfovibrionales order, d – Odoribacter, e – unclassified genus from Proteobacteria phylum (source: Table S1).

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that despite immense differences in the bacterial relative abundance both mouse fecal, caecal and human fecal samples share to a large extent, not only representatives of the same phyla, but also a substantial fraction of common genera, which vindicates mice as a human experimental model.

Supporting Information

Figure S1 Shared and group-unique genera. The network presenting shared taxonomic GM groups between human (red node) and mouse (blue node) categories generated for normalized OTU tables (600000 reads per category) after filtering the low abundant OTUs (abundance threshold for unshared taxa = 0.19%) using the make_ou_network.py script (QIIME). The visualization of the OTU-networks was performed with an open source platform – Cytoscape (version 2.8.3, http://www.cytoscape.org/). (PDF)

Figure S2 Number of GM phyla and genera shared between given categories using raw data. Number of taxonomic labels shared between given mice strains using raw data where no abundance threshold was used (A) on the genus level and (B) on the phylum level. 60000 high quality 16s rRNA reads used to represent the GM of each mouse strain were annotated to the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu/) database. (C) Number of phyla and genera shared between collated categories of humans and mice using raw data (600000 reads per category). Labels “BALB/c g”, “BALB/c c”, “B6-V-Leb/WT/J” and “B6.V-Leb/F/J” stand for the gut GM of BALB/c mice determined using fecal and caecal samples and B6.V-Leb/F/J mice using fecal specimens sampled in 16 and 8 weeks of age respectively. (PDF)

Table S1 The relative distribution of bacterial genera among categories. The relative distribution of the gut microbial genera among single human (16 individuals) and five mouse groups (15, 14, 19, 19, 21 mice respectively). The abundance threshold with least one of the category was set to 0.19%. (PDF)

Table S2 Differences in the relative abundance of gut microbial phyla between categories. Differences in the gut microbial phyla relative distribution verified using Metastats (http://metastats.cbcb.umd.edu) for all combinations of categories. Each pairwise comparison was performed based on 1000 permutations (p value threshold = 0.05, q value threshold = 0.5). (PDF)

Table S3 Differences in the relative abundance of gut microbial genera between categories. Differences in the gut microbial genera relative distribution verified using Metastats (http://metastats.cbcb.umd.edu) for all combinations of categories. Each pairwise comparison was performed based on 1000 permutations (p value threshold = 0.05, q value threshold = 0.5). (PDF)

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Author Contributions

Conceived and designed the experiments: LK AKH DSN. Performed the experiments: LK FWJV. Analyzed the data: LK CHFH AKH DSN. Contributed reagents/materials/analysis tools: FWJV. Wrote the paper: LK CHFH DSN.
References


