**ABSTRACT:** Apolipoprotein E (APOE) genotype is the strongest prevalent genetic risk factor for Alzheimer’s disease (AD). Numerous studies have provided insights into the pathologic mechanisms. However, a comprehensive understanding of the impact of APOE genotype on microbiota speciation and metabolism is completely lacking. In this study, we investigated the association between APOE genotype and the gut microbiome composition in human and APOE–targeted replacement (TR) transgenic mice. Fecal microbiota amplicon sequencing from matched individuals with different APOE genotypes revealed no significant differences in overall microbiota diversity in group-aggregated human APOE genotypes. However, several bacterial taxa showed significantly different relative abundance between APOE genotypes. Notably, we detected an association of Prevotellaceae and Ruminococcaceae and several butyrate-producing genera abundances with APOE genotypes. These findings were confirmed by comparing the gut microbiota of APOE-TR mice. Furthermore, metabolomic analysis of murine fecal water detected significant differences in microbe-associated amino acids and short-chain fatty acids between APOE genotypes. Together, these findings indicate that APOE genotype is associated with specific gut microbiome profiles in both humans and APOE-TR mice. This suggests that the gut microbiome is worth further investigation as a potential target to mitigate the deleterious impact of the APOE4 allele on cognitive decline and the prevention of AD.—Tran, T. T. T., Corsini, S., Kellingray, L., Hegarty, C., Le Gall, G., Narbad, A., Müller, M., Tejera, N., O’Toole, P. W., Minihane, A.-M., Vauzour, D. APOE genotype influences the gut microbiome structure and function in humans and mice: relevance for Alzheimer’s disease pathophysiology. FASEB J. 33, 8221–8231 (2019). www.fasebj.org

**KEY WORDS:** apolipoprotein E • gut microbiota • metabolomics • butyrate • SCFAs

The gut microbiome is intimately involved in numerous aspects of human physiology. Emerging evidence links perturbations in the microbiome to neurodegeneration and Alzheimer’s disease (AD), with (neuro)inflammation proposed as an etiological link (1–3).

**ABBREVIATIONS:** AD, Alzheimer’s disease; APOE, apolipoprotein E; BMI, body mass index; CANN, Cognitive Ageing, Nutrition, and Neurogenesis; COB, chocolate, orange juice, and blackberry; COIA, coinertia analysis; LBP, LPS binding protein; OTU, operational taxonomic unit; PCoA, principal coordinates analysis; PERMANOVA, permutational multivariate ANOVA; QIIME, Quantitative Insights into Microbial Ecology; SCFA, short-chain fatty acid; TR, targeted replacement

1 Correspondence: Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom. E-mail: d.vauzour@uea.ac.uk

This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) (http://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

doi: 10.1096/fj.20190071R

This article includes supplemental data. Please visit http://www.fasebj.org to obtain this information.

The extent to which host genetic variation determines the microbiome composition is still currently debated. Indeed, although previous studies have reported that the microbiomes of humans and mice are associated with host genetic variation (4) and have identified several heritable bacterial taxa (5–7), other studies have reported a stronger environmental influence compared with host genetics in shaping human gut microbiota (8). Thus, the extent to which human genetics shape microbiome composition remains unclear.

Apolipoprotein E (APOE) genotype is the strongest prevalent risk factor for neuropathology and AD (9–11). ApoE was originally identified as a component of systemic circulating lipoproteins and a member of a family of apolipoprotein modulators of their metabolism. It has subsequently emerged as the almost exclusive lipid transporter in the CNS (12, 13). In humans, APOE exists in 3 different isoforms (apoE2, apoE3 and apoE4), arising from 3 different alleles (e2, e3, and e4). These alleles give rise to 3 homozygous (APOE2/E2, APOE3/E3 and APOE4/E4) and 3 heterozygous (APOE3/E2, APOE4/E3 and...
APOE/E2 genotypes in humans (14). Generally, 50–70% of populations present with the APOE3/E3 genotype, with the ε3 allele accounting for 70–80% of the gene pool, and the ε2 and ε4 alleles accounting for 5–10% and 10–15%, respectively (14). APOE4 carrier status is highly predictive of dementia and AD, with APOE3/E4 and APOE4/E4 being at 3–4- and 8–12-fold increased risk and a much earlier age of onset (9). Although the etiological basis of APOE4-neuropathological associations has been widely researched and reported, the main etiological mechanism has not been clearly defined. The ApoE protein is involved in multiple biologic processes, including lipoprotein metabolism (15), intracellular cholesterol utilization (16), cell growth (17), immunoregulation, (neuro)inflammation (18, 19), and neuroprotection (20). Although the role of ApoE in gut chylomicron metabolism and in inflammation has been described, and gut microbiota modulation improves the cardio-metabolic profile in ApoE-deficient mice (Apoë−/−) (21), the impact of APOE genotype on intestinal integrity and gut microbiome composition and metabolism is currently unknown (22).

In the present study, we explore the hypothesis that APOE variation influences the microbiome composition and its subsequent metabolism. Our experiments using human fecal samples and APOE–targeted replacement (TR) mice revealed significantly different relative abundance between bacterial taxa according to APOE genotypes. Furthermore, using a metabolomic approach, differences in microbe-associated amino acids and short-chain fatty acids (SCFAs) according to APOE genotypes were also observed. Taken together, our findings indicate that APOE genotype associates with specific gut microbiome profiles, which may affect the host metabolism and ultimately contribute to AD pathology.

**MATERIALS AND METHODS**

**Ethics approval and consent to participate**

The research involving human material has been performed in accordance with the Declaration of Helsinki. The study protocols were approved by the National Research Ethics Service Committee [13/EE/0066 for the chocolate, orange juice, and blackberry (COB) study, NCT01922869; 14/EE/0189 for the Cognitive Ageing, Nutrition, and Neurogenesis (CANN) study, NCT02525198], and all participants consented to provide stool samples and to the use of the stored samples for research purposes.

All experimental procedures and protocols involving animals were reviewed and approved by the Animal Welfare and Ethical Review Body and were conducted within the provisions of the Animals (Scientific Procedures) Act of 1986 (Reference 70/8710).

**Participants, sample collection, APOE genotyping, and biochemical analysis**

Fifty-six healthy participants, aged between 56 and 78 yr, were prospectively selected according to APOE genotype from the COB (NCT01922869) and the CANN (NCT02525198) studies for the analysis of their gut microbiota speciation. Participants were provided with fecal collection kits, which included a stool collection bag and an ice pack. They were asked to defecate directly into the bag, which was secured and placed with the ice pack into an insulated container and delivered to the study scientist. The samples were then homogenized by physical manipulation before aliquots were taken and stored at −80°C.

APOE genotyping was carried out as previously described (23). Briefly, DNA was isolated from the buffy coat layer of 8 ml of blood collected into sodium heparin mononuclear cell preparation tubes with the use of the Qiagen DNA blood mini kit (Qiagen, Germantown, MD, USA). Allelic discrimination of the APOE gene variants was conducted with TaqMan PCR technology (7500 Instrument; Thermo Fisher Scientific, Waltham, MA, USA) and Assay-on-Demand single nucleotide polymorphism genotyping assays (Thermo Fisher Scientific). The APOE haplotypes (E2/E3, E3/E3, E3/E4, and E4/E4) were determined from the alleles for the APOE single nucleotide polymorphisms rs7412 and rs929388. Twenty-four participants were selected as APOE4 carriers (APOE4/E4 and APOE4/E3; 12 men and 12 women), with 32 participants selected as APOE4 noncarriers (APOE2/3 and APOE3/3; 16 men and 16 women), with the selection process matching the genotype groups for age, body mass index (BMI), and gender.

Serum LPS binding protein (LBP) (ab213805; Abcam, Cambridge, MA, USA) and haptoglobin (ab108856; Abcam) plasmatic concentrations were detected by ELISA kits according to the manufacturer’s instructions. The assay range for the LBP and the haptoglobin ELISA kits was 1.56–100 and 0.078–20 μg/ml, respectively. Serum samples were diluted until the LBP or haptoglobin concentrations were in the range of these kits.

**Human fecal bacterial DNA extraction and 16S rRNA amplicon sequencing**

Total genomic DNA were isolated from human fecal samples using DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer’s instructions with some modifications following the repeated bead-beating method (24). The V3–V4 hypervariable region of the 16S rRNA gene was amplified to generate a fragment of 460 bp using the forward primer: 5′-TGCCTCAGCTACGAGATGTGATAAGAGACAGGACTACHVGGGTATCTAATCC-3′, and reverse primer: 5′-GITCCTGCGCTCCGGAGATGTGATAAGAGACAGGACTACHVGGGTATTCTAATCC-3′ (25). The Illumina overhang adapter sequences (Illumina, San Diego, CA, USA) were added to the 16S rDNA gene primer specific primer sequences. Each 30 μl PCR reaction contained 10 ng/μl microbial genomic DNA, 0.2 μM of each primer, 15 μl of 2× Phusion Taq High-Fidelity Mix, and 10.6 μl of nuclease-free water. The PCR conditions were initial denaturation 98°C for 30 s; 25 cycles of 10 s at 98°C, 15 s at 55°C, and 20 s at 72°C; and 72°C for 5 min for final elongation. The SpriSelect Reagent Kit (Beckman Coulter, Brea, CA, USA) was used to purify the amplicons. The Qubit double-stranded DNA High Sensitivity Assay Kit (Thermo Fisher Scientific) was followed for quantification and pooling. Library preparation was carried out by Teagasc (Oak Park, Ireland) on the Illumina MiSeq platform using paired-end Illumina sequencing run (2 × 250 bp).

**APOE-TR mice**

Twenty young (4 mo; n = 10/genotype) and 12 old (18 mo; n = 6 per genotype) male human APOE3 [B6.129P2-Apoë(APOE*3)Mae N8] and APOE4 [B6.129P2-Apoë(APOE*4)Mae N8] TR mice homozygous for the human APOE3 or APOE4 gene (Taconic Farms, Germantown, NY, USA) were used in these experiments (n = 10/genotype) (26). The model was created by Dr. Maeda (University of North Carolina, USA) by targeting the murine APOE gene for replacement with the human APOE3 and APOE4 allele in E14TG2a embryonic stem cells and injecting the targeted cells into blastocysts. Resultant chimeras
were backcrossed to C57BL/6 for 8 generations (N8). Mice were housed 2 per cage and were maintained in controlled environment (21°C; 12-h light/dark cycle; light from 7:00 AM) and fed a standard chow diet (RM3-P; Special Diets Services, Witham, United Kingdom) for the duration of the experiments.

**Mice genomic DNA extraction and 16S rRNA amplicon sequencing**

Bacterial genomic DNA was extracted from fecal samples using a FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) with 3 bead-beating periods of 1 min (27). Bacterial DNA concentration was normalized to 1 ng/μl by dilution with DNA elution solution (MP Biomedicals) to produce a final volume of 20 μl. Normalized DNA samples were sent to the Centre for Genomic Research (Liverpool, United Kingdom) for PCR amplification of the 16S rRNA gene and paired-end Illumina sequencing (2 × 250 bp) on the MiSeq platform. The V4 region of the 16S rRNA gene was amplified to generate a 254 bp insert product as previously described (28). The first round of PCR was performed using the forward primer: 5' - ACACCTTTCCTACAGGACGTGTTCCTCCAGATCTNNNNGTGCACAAGCMGGCGCGTTAA-3', and the reverse primer: 5' - GTGACTGGAGTTCAGACGT GTGCTCTTCCGATCTGGA-CTACHVGGGTWTCTAAT-3', which include recognition sequences that enable a second nested PCR, using the N501f and N701r primers, to incorporate Illumina adapter sequences and barcode sequences. The use of these primers enables efficient community clustering for the length of reads obtained through Illumina sequencing, and this method also allows for high-throughput sequencing. Sequencing data were supplied in fastq format with adaptors already trimmed.

**Metabolomic analyses**

Metabolites were analyzed and quantified by [1H] NMR analysis. The preparation method was similar to that previously described (29–32). Fecal contents were extracted from mice caeca and prepared for [1H] NMR using procedures validated and published in our laboratory for fecal metabolomics (33–35). Briefly, 20 mg of frozen fecal materials were thoroughly mixed on a vortex with 1 ml of saline phosphate buffer [1.9 mM Na2HPO4, 8.1 mM NaH2PO4, 150 mM NaCl (MilliporeSigma, Burlington, MA, USA)], and 1 mM trimethylsilylpropanoic acid [sodium 3-(trimethylsilyl)-propionate-d4] in deuterated water (Goss Scientifics, Crewe, United Kingdom), followed by centrifugation (18,000 g, 1 min). Supernatants were removed, filtered through 0.2 μm Fluoropore polytetrafluoroethylene membrane filters (MilliporeSigma), and stored at −20°C until required.

After mixing and centrifugation, 500 μl was transferred into a 5-mm NMR tube for spectral acquisition. High resolution [1H] NMR spectra were recorded on a 600-MHz Bruker Avance spectrometer fitted with a 5-mm TCI proton-optimized triple resonance NMR inverse cryoprobe and a 60-slot autosampler (Bruker, Billerica, MA, USA). Sample temperature was controlled at 300 K. Each spectrum consisted of 128 scans of 32,768 complex data points with a spectral width of 14 ppm (acquisition time 1.95 s). The noesyp1d presaturation sequence was used to suppress the residual water signal with low power selective irradiation at the water frequency during the recycle delay (D1 = 2 s) and mixing time (D2 = 0.15 s). A 90° pulse length of 8.8 μs was set for all samples. Spectra were transformed with a 0.3-Hz line broadening and zero filling, manually phased, baseline corrected, and referenced by setting the trimethylsilylpropanoic acid methyl signal to 0 ppm. Metabolites were identified using information found in the literature or on the Human Metabolome Database (http://www.hmdb.ca/), and by use of the 2-dimensional NMR methods, [1H]-[1H] correlation spectroscopy, [1H]-[13C] heteronuclear single quantum correlation, and [1H]-[13C] heteronuclear multiple bond correlation spectroscopy (36) and quantified using the software Chenomx NMR Suite 7.0 (Supplemental Fig. S1). Details on parameter settings for 2-dimensional NMR can be found in Le Gall (37).

**Analysis of 16S amplicon sequencing data from humans and mice**

Bioinformatics analysis of 16S amplicon sequencing data from humans and mice were performed using the Quantitative Insights into Microbial Ecology (QIIME) v.1.9.1 (38) and usearch v.8.1 (39) software and the following procedure. First, the paired-end reads were merged using Fast Length Adjustment of Short Reads (FLASH) v.1.2.8 (40); then, adapters were removed from reads using cutadapt v.1.8.3 (41). The sequences were demultiplexed and filtered using QIIME with the split_libraries_fastq.py script; all reads with a quality score below 19 were removed. Reverse primers were removed using QIIME with the truncate_reverse_primer.py script. An operational taxonomic unit (OTU) table was obtained using uSearch. Unique sequences were filtered (derep_fulllength) and sorted by length (sortbylength) with a length of 373–473 nt for the V3–V4 region and a length of 237–271 nt for the V4 region. After singleton removal (sortbysize), the remaining sequences were clustered into OTUs at a default 97% sequence identity (cluster_otus) and filtered for chimeras against the ChimeraSlayer reference database (uchime_ref) (42). All sequences were mapped against this database (usearch_global) to generate an OTU table. Classification of representative sequences for each OTU was carried out using mothur v.1.36.1 (43) against the 16S rRNA reference of Ribosomal Database Project database trainset 14 (44). To ensure an even sampling depth, we used QIIME to generate rarefied OTU tables with the single_rarefaction.py script and to compute α diversity metrics (chao1, phylogenetic diversity, Shannon’s diversity index, evenness) with the alpha_rarefaction.py script and β diversity metrics (weighted UniFrac, unweighted UniFrac, and Bray-Curtis distances) with the beta_diversity.py script.

**Statistical analysis**

Statistical analysis was carried out using R v.3.5.1 software packages (45). The significant differences in clinical measures, α diversity, and abundances of each taxonomic unit between ≥2 groups were detected using the Mann-Whitney U test or Kruskal-Wallis H test with Dunn’s multiple comparison test, respectively. The P values were corrected for multiple testing by Benjamini-Hochberg correction to control false discovery rate. Differences in β diversity were determined using permutational multivariate ANOVA (PERMANOVA) (v4.3.2 package; https://cran.r-project.org/package=vegan).

Multivariate statistical analysis (sparse partial least squares discriminant analysis and metabolite set enrichment analysis) of the [1H] NMR data was carried out using the MetaboAnalyst R package v.1.0.0 (https://www.metaboanalyst.ca/). Coinertia analysis (COIA) was used to investigate the relationships between the fecal metabolome and the composition of microbiota at OTU level using the coinertia function in the ade4 R package (46). Only OTUs present in at least 50% of the samples were used in COIA. Overall similarity in the structure between 2 data sets were measured by RV coefficient. The significance of the RV coefficient was tested using the Monte Carlo permutation test (47).
RESULTS

Descriptive statistics of human APOE-genotyped cohorts

A total of 56 fecal samples were analyzed from participants of the COB (NCT01922869) and the CANN studies (NCT02525198) (Norwich Clinical Centre, Norwich, United Kingdom) with the 4 APOE genotype groups selected to be matched for sex, age, and BMI (Table 1 and Supplemental Table S1).

Difference in human gut microbiota composition between APOE genotypes

The V3–V4 hypervariable region of the 16S rRNA gene was PCR amplified from fecal samples collected from participants to generate an amplicon of ~460 bp. Sequencing this amplicon allows determination of microbiota composition. The reads were clustered at a 97% similarity threshold into 3314 unique OTUs or sequence-based bacterial classification, approximating to species. The total OTUs were assigned to 15 phyla, 27 classes, 43 orders, 70 families, and 155 unique genera across the entire data set. The vast majority (99.5%) of all sequences were affiliated to 5 dominant phyla, mainly in the Firmicutes (82.2 ± 10.8%), with lower assignment to phyla Bacteroidetes (7.7 ± 6.3%), Actinobacteria (3.8 ± 4.5%), Proteobacteria (3.2 ± 9.5%), and Verrucomicrobia (2.6 ± 5.1%) (Supplemental Fig. S2A). After rarefaction with a depth of 8736 reads per sample, α diversity (net diversity within a single sample/subject) was measured by calculating 3 diversity indices, namely, cha01 (richness), phylogenetic diversity, and Shannon diversity index. None of these metrics was significantly different between APOE genotypes (Supplemental Fig. S3A). Similarly, there was no significant difference in any of the α diversity metrics between males and females (Supplemental Fig. S3B). However, the microbiota α diversity of obese subjects (n = 3) was significantly lower than that in normal weight and overweight subjects (P < 0.05, Supplemental Fig. S3C), in line with previous observations (48). β diversity analysis (which measures interindividual microbiota relatedness) was performed using principal coordinates analysis (PCoA) clustering based on unweighted and weighted UniFrac distances. A PERMANOVA test was employed for testing associations between clinical parameters and microbiota composition and results are given in Supplemental Table S2. There was no difference in β diversity of gut microbiota composition according to APOE genotype (Supplemental Fig. S4A). However, we observed a weak but significant association between microbiota composition and gender and BMI categories (Supplemental Fig. S4B, C).

Although α and β diversity analyses of the gut microbiota did not discriminate between APOE genotypes, these are global measures that detect relatively large differences in microbiota structure. We therefore questioned whether the relative abundance of any taxa might differ between these genotypes, using the Kruskal-Wallis H test to compare all taxa at various phylogenetic assignment levels across all genotypes. We observed that the relative abundance of the phylum Firmicutes and order Clostridiales was higher in subjects of the APOE2/E3 genotype than in APOE3/E3 or APOE4/E4 (P < 0.05; Fig. 1 and Supplemental Table S3). Furthermore, at the bacterial family level, the abundance of Ruminococcaceae (a family of fermentative anaerobes associated with fiber degradation and SCFA production) was higher in APOE2/E3 than in APOE3/E3 (P = 0.004), APOE3/E4 (P = 0.002), or APOE4/E4 (P = 0.072). On the other hand, the abundance of Prevotellaceae was lower in APOE2/E3 than the other 3 APOE genotypes (APOE3/E3, P = 0.008; APOE3/E4, P = 0.085; APOE4/E4, P = 0.015) and was slightly more abundant at close to significant levels (P = 0.088) in APOE3/E4 compared with APOE4/E4 with mean of relative abundance of 1.79 vs. 1.40% (Fig. 1C and Supplemental Fig. S2B and Supplemental Table S3). Within the Ruminococcaceae family, 3 genera, including Clostridium cluster IV, Clostridium cluster XIVa, and Gemmiger, were statistically significant and differentially abundant according to APOE genotypes. The abundance of Clostridium cluster IV was lower in APOE3/E3 than in APOE2/E3 (P = 0.027) and APOE4/E4 (P = 0.039), whereas the abundance of Clostridium cluster XIVa was higher in APOE4/E4 than in APOE2/E3 (P = 0.044) and APOE3/E4 (P = 0.078). Higher presence of Gemmiger was observed in fecal samples from APOE2/E3 compared with APOE3/E3 (P = 0.0499) and APOE4/E3 (P = 0.086). Moreover, we observed a higher abundance of Roseburia in fecal samples at close to significant levels (P < 0.1) in APOE3/E3 compared with 3 other APOE genotypes and in APOE3/E4 compared with APOE4/E4 (Fig. 1D and Supplemental Fig. S2C and Supplemental Table S3).

To determine possible associations between APOE genotypes and microbial translocation, we measured the plasma levels of 2 biomarkers of intestinal integrity, namely, haptoglobin and LBP. No significant differences were observed in the levels of haptoglobin and LBP (Supplemental Table S1) according to genotype. Furthermore, no significant correlation was observed between the

| Parameter                  | E2/E3 | E3/E3 | E3/E4 | E4/E4 |
|----------------------------|-------|-------|-------|-------|
| n                          | 14    | 18    | 18    | 6     |
| Age (yr)                   | 68.6 ± 4.6 | 68.5 ± 5.0 | 68.6 ± 3.0 | 67.7 ± 6.1 |
| Sex [male:female (n)]      | 7.7   | 9.9   | 3.3   | 3.3   |
| BMI (kg/m²)                | 25 ± 2.2 | 26.3 ± 2.6 | 26.1 ± 3.1 | 25 ± 1.9 |

Data presented as means ± sd. *Significance was calculated by the Kruskal-Wallis H test.
clinical parameters and both the weighted and unweighted UniFrac distances (Supplemental Table S2).

### Difference in murine gut microbiota composition between APOE genotypes

We next sought to investigate if APOE genotype–gut microbiota interactions in humans were evident in human transgenic homozygous APOE3- and APOE4-TR mice at 4 mo (young) and 18 mo (old) of age. Considering the average lifespan of laboratory mice, 18 mo of age would correspond to about 65 human years (49), which approximates our human participants’ age (68.5 ± 4.4 yr).

Their gut microbial communities were analyzed based on sequencing the V4 hypervariable region (~254 bp) of the 16S rRNA gene. There was no significant difference in a diversity between APOE3 and APOE4 genotypes. However, in line with our previous studies of microbiota in ageing humans (50) and rodents (51), both chao1 and phylogenetic diversity were much higher in young mice compared with old mice (P < 0.001 and Supplemental Fig. S5). Moreover, UniFrac distances (unweighted and weighted) PCoA showed that fecal microbial profiles of young mice separated significantly from those of old mice (PERMANOVA, P = 0.001; Fig. 2A). Within each age group, both UniFrac measures showed significant microbiota differences between APOE3 and APOE4 genotypes, with the P value from PERMANOVA analysis <0.005 (Fig. 2A). These differences could be explained by differences detected in the relative abundance of dominant taxa, of which the most dominant were Firmicutes (62.8 ± 14.4%) and Bacteroidetes (32.3 ± 14.8%), followed by Proteobacteria, Verrucomicrobia, and Deferribacteres accounting for <5% in total (Supplemental Fig. S6).

Analysis of differentially abundant taxa between APOE3-TR and APOE4-TR animals at the phylum level revealed that Deferribacteres in combined young and old mice were notably higher in the APOE4-TR mice compared with the APOE3-TR mice, whereas the opposite was true for Candidatus Saccharibacteria. In addition, lower relative abundance of Proteobacteria were seen in APOE4-TR young mice when compared with the APOE3-TR young mice (Supplemental Fig. S7 and Supplemental Table S4). Although no significant difference was found in aggregated Firmicute or Bacteroidetes phylum abundance between APOE genotypes, we observed an increase in Firmicutes:Bacteroidetes ratio in old mice when compared with young mice (P < 0.001; Supplemental Fig. S8), in agreement with a previous C57BL/6N mouse study (52). At the order level, Deferribacterales abundance in combined age groups was significantly higher in the APOE4-TR mice compared with the APOE3-TR mice. Additionally, Clostridiales, Erysipelotrichales, and Desulfovibrionales in young mice were significantly different in relative abundance between the 2 APOE genotypes. The increase of Lachnospiraceae and Deferribacteraceae abundance and decrease of Bacteroidaceae abundance at family
level in APOE4 transgenes compared with APOE3 was detected in combined age groups. Desulfovibrionaceae, Clostridiales Incertae Sedis XIII, Rikenellaceae, Prevotellaceae, and Erysipelotrichaceae were also found to be significantly different between APOE genotypes in young mice (Fig. 2B, Supplemental Fig. S7, and Supplemental Table S4). Those differentially abundant families by APOE genotype were reflected by Mucispirillum, Clostridium cluster XIVa, Butyrivibrio, Odoribacter, Enterorhabdus, and Bacteroides in combined age groups and by Mucispirillum, Desulfovibrio, Butyrivibrio, Bacteroides, Alistipes, and Johnsonella in young mice at the genus level (Fig. 2B, Supplemental Fig. S7, Supplemental Table S4).

**Fecal metabolite associations with APOE genotype and age**

In order to improve our understanding of the relationships between metabolite and microbiota composition in the gut, we performed metabolomic analyses of fecal water prepared from caecal contents. Sparse partial least squares discriminant analysis (sPLS-DA) showed a trend for separation according to age and APOE genotypes (Supplemental Fig. S9). Two-way ANOVA was therefore performed to investigate interactions between age and APOE genotype. Seven metabolites, AMP, α-ketoisovaleric acid, glucose, glycine, lactate, oxocaprate,
Figure 3. Fecal metabolome analysis of APOE3-TR and APOE4-TR mice. A) Heatmap and cluster analysis of 2-way ANOVA of significantly differentially abundant metabolites grouped by age and APOE genotype. Four clusters within significantly differentially abundant metabolites showed distinct APOE genotype and age correlations. Clustering was obtained following similarity analysis using the Ward hierarchical algorithm and Euclidean distance metrics. B) COIA of the association between metabolites and microbiota composition in the gut. The left panel shows the COIA of the microbiota principal component analysis (solid circle) at OTU level and the principal component analysis of metabolomics (empty circle); length of arrow indicates the divergence between 2 data sets. The right panel shows coinertia of metabolome and microbiota data, represented by (continued on next page)
and xanthine were present at significantly different levels in age-APOE genotype interaction, whereas 39 and 19 metabolites were significantly different in age groups and APOE genotype groups, respectively (Supplemental Fig. S10 and Supplemental Table S5). Four clusters of all significant metabolites had distinct correlations. Cluster A, comprising 5 metabolites (lactate, pyruvate, fumarate, hypoxanthine, and uracil), had inverse direct correlations with APOE4-TR old mice and had strong direct correlations with 3 other groups. However, cluster B and cluster C metabolites were associated with age. Ten metabolites in cluster B (methylamine, acetate, butyrate, propionate, arabinoose, xylose, succinate, glucose, AMP, GTP) were more abundant in young mice, especially in APOE3-TR young compared with old mice. Fourteen metabolites in cluster C (asparagine, alanine, tryptophan, threonine, tyrosine, lysine, phenylalanine, glutamate, histidine, leucine, glutamine, valine, isoleucine, methionine) showed an opposite trend. Cluster D metabolites were divided into 2 subclusters: cluster D1, comprising 4 metabolites (2-oxoisocaproate, α-ketoisovalerate, 3-methyl-2-oxovalerate, urocanate), had direct correlations with APOE4-TR young mice; cluster D2, including 14 metabolites (isobutyrate, 1,3-dihydroxyacetone, lactaldehyde, aspartate, ornithine, ribose, xanthine, choline, glycine, creatine, taurine, 2-methylbutyric acid, ethanol, and formate), had positive correlations with old mice (Fig. 3A). In addition, metabolite set enrichment analysis was used to identify significantly enriched pathways in metabolomics data associated with APOE genotype and age. Of the top 50 assigned pathways, the significant pathways in APOE genotype were ammonia recycling, urea cycle, and alanine metabolism (Supplemental Fig. S11 and Supplemental Table S6), whereas the significant pathways in age were ammonia recycling, urea cycle, glycine and serine metabolism, glutamate metabolism, and alanine metabolism (Supplemental Fig. S11B and Supplemental Table S7).

COIA was carried to explore the correlation between the composition of microbiota at OTU level and the fecal metabolome (Fig. 3B). The Monte Carlo permutation test revealed a high overall similarity in the structure between the 2 data sets, which was statistically significant (RV coefficient = 0.685; P = 0.01). The first 4 axes represented 72.5, 8.9, 7.0, and 2.6% of the explained variance, respectively, and so the analysis focused on the first axis. Each sample is represented by an arrow, where length of arrow indicates the divergence between 2 data sets. We observed that the aggregate arrow length was shorter in APOE4 mice compared with that in APOE3 mice, which indicated a higher consensus between microbiota composition and metabolites of APOE4 mice compared with APOE3 mice. The metabolites and OTUs that strongly correlated in the COIA axes were plotted on the first 2 COIA axes (Supplemental Fig. S12). Metabolites and bacterial OTUs were projected onto the same direction as samples, indicating that they were more abundant in those samples. There was an agreement between the metabolite abundance and the specific taxon abundance. Notably, SCFAs, including acetate, butyrate, and propionate, were located in the direction of butyrate-producing bacteria from Clostridium cluster IV genus and the families Ruminococcaceae and Lachnospiraceae.

DISCUSSION

Although several recent studies have implicated a link between the gut microbiome, SCFAs, and the development of AD (3, 53–57), there is no direct study that establishes a link between gut microbiota composition and the strongest genetic risk factor for AD, APOE genotype. The current study marks the first analysis that compares gut microbiota composition in humans and transgenic mice with different APOE genotypes. Analysis of 16S rRNA gene sequences and fecal metabolome showed that APOE genotype correlated with abundance differences of several gut bacterial taxa, which may drive the difference in amino acids and SCFAs levels.

Higher levels of Prevotellaceae were evident in APOE3/E3 carriers relative to other genotype subgroups, whereas higher levels of Ruminococcaceae were correlated with the APOE2/E3 genotype (Fig. 1 and Supplemental Table S3) relative to APOE4 carriers. Interestingly, loss of these bacteria has been reported to negatively correlate with neurodegenerative disorders and were noted as being less abundant in patients with Parkinson’s disease (58) and AD (3). A reduction of Prevotellaceae influenced mucin synthesis and increased mucosal permeability, allowing local and systemic exposure to bacterial endotoxin, which may lead to the accumulation of α-synuclein in the colon (59, 60). Aggregation-prone proteins such as β-amyloid and α-synuclein can propagate from the gut to the brain via the vagus nerve (61) and contribute to the pathogenesis of Parkinson’s disease, AD, and other neurodegenerative disorders (62–65). Ruminococcaceae are involved in the production of SCFAs, such that their depletion is causally linked to inflammation (66–68). These findings suggest that these bacteria might contribute to the protective effects of APOE2 and APOE3 alleles against AD relative to the APOE4 genotype (10, 69, 70).

The high abundance of Ruminococcaceae in subjects of the APOE2/E3 genotype was reflected by Gemmiger at the genus level. Gemmiger are strictly anaerobic bacteria that ferment a variety of carbohydrates to produce formic and N-butyrac acids, often with small amounts of acetic, lactic, succinic, malonic, and pyruvic acids (71). In
addition, we observed differences between APOE genotypes in *Clostridium* cluster IV, Roseburia, and *Clostridium* cluster XIVa, which are able to convert dietary fibers to SCFAs (72, 73). Although the butyrate-producing bacteria *Clostridium* cluster IV were modestly less abundant in human APOE3/E3 individuals, they could be substituted by Roseburia with an increased abundance of these bacteria in the APOE3/E3. A slight increase of *Clostridium* cluster XIVa from *Lachnospiraceae* was seen in the human APOE4/E4 genotype, which is consistent with the murine data. However, the increase of this genus in APOE4/E4 may not substitute for the reduction of other butyrate-producing bacteria. Additionally, several genera, which were not correlated with APOE genotype in human gut microbiomes, were significantly different between APOE3 and APOE4 genotypes in murine gut microbiomes. These taxa could not be detected in human data because of: 1) absence of some mouse gut microbiota in humans, such as *Murispirillum*, 2) differences in relative abundance of each individual taxon, and 3) complexity in interactions of human gut microbiota with genetics, diet, and other environmental factors.

Incorporating the gut microbiota data with the corresponding metabolites in fecal water resulted in fecal samples across the APOE genotype being discriminated based on their metabolomic profiles. The lower concentrations of several fatty acids, especially SCFAs and their precursors (lactate and succinate), that were detected in APOE4 old mice could be due to loss of butyrate-producing bacteria, similar to what we observed in humans with an APOE4/E4 genotype (Fig. 1 and Supplemental Table S3). Although lower levels of SCFAs were noted in old APOE3 mice, the lactate level was higher in this group, which is able to convert to butyrate by a subset of *Lachnospiraceae*, including *Eubacterium hallii* and *Anaerostipes caccae* (74). Similarly, *Bacteroides*, which were significantly more abundant in APOE3 mice, have been reported to have propionate-producing capacity through the succinate pathway (75). Several SCFAs have been shown to inhibit the formation of toxic soluble β-amylloid aggregates in vitro (76) and consequently decrease the risk of AD. Interestingly, cosegregation of the fecal metabolomic profiles and the gut microbiome profiles as revealed by COIA suggests that the differences in gut microbiota associated with APOE genotype and age in APOE-TR mice are reflected in the segregation of metabolites, which may be clinically relevant. Specifically, SCFA level and the relative abundance of certain species from *Clostridium* cluster IV, *Ruminococcaceae*, and *Lachnospiraceae* showed a positive correlation. Future studies should further investigate strain level differences between APOE genotypes, the metabolic capacity of the microbiome, and the metabolomic profiles of fecal water extracts.

The collective evidence here suggests a link between APOE genotypes and gut microbiome composition. Loss of butyrate-producing bacteria and SCFAs in APOE4 carriers might drive the impact of the APOE4 allele on neuropathology. Our findings suggest a possible role of gut microbiota butyrate-producing bacteria as an intervention point to mitigate the impact of APOE genotype in the development of AD.

**ACKNOWLEDGMENTS**

The authors thank the study participants for contributing samples and time to the studies used to generate the data. The authors also acknowledge the many CANN and COB trial colleagues who facilitated this study. This project was supported, in part by a Biotechnology and Biological Sciences Research Council (BBSRC) grant to the laboratory of A.-M.M. and D.V. (BB/M004449/1). Work in P.W.O.’s laboratory was supported, in part, by a Grant from the Science Foundation Ireland (APC/SFI/12/RC/2275) in the form of the Alimentary Pharmabiotic Centre (APC) Microbiome Ireland, the Food Institutional Research Measure (FIRM) Program, and the Immunomet Project of the Government of Ireland’s Department of Agriculture, Food, and the Marine. A.-M.M. and D.V. share senior authorship. The datasets used and/or analyzed during the current study are available at the NCBI Sequence Read Archive (SRA), under BioProject PRJNA533610. The authors declare no conflicts of interest.

**AUTHOR CONTRIBUTIONS**

P. W. O’Toole, A.-M. Minihane, and D. Vauzour conceptualized and designed the experiments and analytical approaches; S. Corsini and C. Hegarty carried out the experimental work; L. Kellingray and A. Narbad contributed to the sample preparation and microbial 16S rDNA sequencing; G. Le Gall performed the metabolomic analysis; N. Tejera conducted the clinical trial and helped with the sample preparation; T. T. Tran and D. Vauzour analyzed the sequencing and metabolomic data set and conducted the statistical analyses; T. T. Tran, P. W. O’Toole, A.-M. Minihane, and D. Vauzour wrote the paper with contributions from all authors; M. Muller critically revised the manuscript; and all authors read and approved the final manuscript.

**REFERENCES**

1. Tremlett, H., Bauer, K. C., Appel-Cresswell, S., Finlay, B. B., and Waubant, E. (2017) The gut microbiome in human neurological disease: a review. *Ann. Neurol.* 81, 369–382
2. Minter, M. R., Zhang, C., Leone, V., Ringus, D. L., Zhang, X., Oyler-Castrillo, P., Musch, M. W., Liao, F., Ward, J. F., Holtzman, D. M., Chang, E. B., Tanzi, R. E., and Sisodia, S. S. (2016) Antibiotic-induced perturbations in gut microbial diversity influences neuroinflammation and amyloidosis in a murine model of Alzheimer’s disease. *Sci. Rep.* 6, 30028
3. Vogt, N. M., Kerby, R. L., Dill-McFarland, K. A., Harding, S. J., Merluzi, A. P., Johnson, S. C., Carlson, C. M., Ashana, S., Zetterberg, H., Blennow, K., Bendlin, B. B., and Rey, F. E. (2017) Gut microbiome alterations in Alzheimer’s disease. *Sci. Rep.* 7, 13537
4. Spor, A., Koren, O., and Ley, R. (2011) Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat. Rev. Microbiol.* 9, 279–290
5. Turpin, W., Espin-Garcia, O., Xu, W., Silverberg, M. S., Kevans, D., Smith, M. I., Guttman, D. S., Griffiths, A., Panaccione, R., Otley, A., Xu, L., Shes topolof, K., Moreno-Hagelsieb, G., Paterson, A. D., and Croitoru, K.; GEM Project Research Consortium. (2016) Association of host genome with intestinal microbial composition in a large healthy cohort. *Nat. Genet.* 48, 1413–1417
6. Bonder, M. J., Kurishakov, A., Tigheelaar, E. F., Mujagic, Z., Imhann, F., Vila, A. V., Deelen, P., Vatanen, T., Schirmer, M., Smeekens, S. P.,
15. Raffai, R. L., Dong, L. M., Farese, R. V., Jr., and Weisgraber, K. H. (2019) The effect of host genetics on the gut microbiome. *Nat. Genet.* 48, 1407–1412

16. Beaumont, M., Goodrich, J. K., Jackson, M. A., Yet, I., Davenport, E. R., Vartiainen, E., Rieckmann, K., Kirjavainen, P., Pischon, T., Key, T. J., Kroke, A., Briel, M., Schmitz, N., and Weiland, S. H. (2008) High fat intake is associated with an altered gut microbiota composition among European adults. *Genome Biol.* 9, 210–215

17. Pontifex, M., Vauzour, D., and Minihane, A. M. (2018) The effect of APOE genotype on Alzheimer's disease risk is influenced by sex and docosahexaenoic acid status. *Neurobiol. Aging* 69, 209–220

18. Liu, C. C., Liu, C. C., Kanekiyo, T., Xu, H., and Bu, G. (2013) Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat. Rev. Neurol.* 9, 106–118; erratum: 184

19. Neufeld, E. P., Yuan, J., Jackson, M. A., Yet, I., Davenport, E. R., Vartiainen, E., Rieckmann, K., Kirjavainen, P., Pischon, T., Key, T. J., Kroke, A., Briel, M., Schmitz, N., and Weiland, S. H. (2008) Hepatic RXR activation in primary human hepatocytes is regulated by dietary fatty acids and vitamin D3. *FASEB J.* 22, 2551–2561

20. Jofre-Monseny, L., Minihane, A. M., and Rimbach, G. (2008) Impact of apoE genotype on oxidative stress, inflammation and disease risk. *Mol. Nutr. Food Res.* 52, 131–145

21. Rine, J., Rinaldi, S., Nielsen, D. S., Kanter, J. E., Bomfeldt, K. E., Lykkefeldt, J., Buschard, K., Kirk, R. K., Christoffersen, B., Fels, P. J., Joels, J., Hostetler, K., and Hansen, A. K. (2016) Modulating the Gut microbiota improves glucose tolerance, lipoprotein profile and atherosclerotic plaque development in ApoE-deficient mice. *PLoS One* 11, e0146394

22. Friedland, R. P. (2015) Mechanisms of molecular mimicry involving the microbiota in neurodegeneration. *J. Alzheimers Dis.* 45, 349–362

23. Calabuig-Navarro, M. V., Jackson, K. G., Walden, C. M., Minihane, A. M., and Lovegrove, J. A. (2014) Apolipoprotein E genotype has a modest impact on the postprandial plasma response to meals of varying fat composition in healthy men in a randomized controlled trial. *J. Nutr.* 144, 1775–1780

24. Yu, Z., and Morrison, M. (2004) Improved extraction of PCR-quality DNA from digesta and fecal samples. *Biotechniques* 36, 808–812

25. Klinworth, G. A., Pruesse, E., Schwier, T., Peplies, J., Quast, C., Horn, M., and Göcker, F. O. (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41, e1

26. Zhu, Y., Nwabuisi-Heath, E., Dumanis, S. B., Tai, L. M., Yu, C., Rebeck, G. W., and LaDu, M. J. (2012) APOE genotype alters glial activation and loss of synaptic markers in mice. *Glia* 59, 559–569

27. Maukonen, J., Määtä, J., Satokari, R., Söderlund, H., Mattila-Sandholm, T., and Saarela, M. (2006) PCR DGGE and RT-PCR DGGE show diversity and short-term temporal stability in the Clostridium cocoides-Eubacterium rectale group in the human intestinal microbiota. *FEMS Microbiol. Ecol.* 58, 517–528

28. Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., and Knight, R. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* 108 (Suppl 1), 4516–4522

29. Kosidis, S., Kokova, D., Dementeva, N., Salykova, I. V., Kim, H. K., Choi, Y. H., and Mayboroda, O. A. (2017) ^3^H-NMR analysis of feces: new possibilities in the helminthes infections research. *BMJ Infect. Dis.* 17, 275

30. Lamichhane, S., Yde, C. C., Schmedes, M. S., Jensen, H. M., Meier, S., and Bertram, H. C. (2015) Strategy for nuclear-magnetic-resonance-based metabolomics of human feces. *Anal. Chem.* 87, 5930–5937

31. Saric, J., Wang, Y., Li, J., Coen, M., Uzinger, J., Marchesi, J. R., Keiser, J., Veselkov, K., Lindon, J. C., Nicholson, J. K., and Holmes, E. (2008) Species variation in the fecal metabolome gives insight into differential gastrointestinal function. *J. Proteome Res.* 7, 352–360

32. Wu, J., An, Y., Yao, J., Wang, Y., and Tang, H. (2010) An optimised sample preparation method for NMR-based faecal metabolic analysis. *Analyst (Lond.)* 135, 1023–1030

33. Kellingray, L., Gall, G. L., Defernez, M., Beales, I. L. P., Fransen-Ehmuogo, N., and Narbad, A. (2018) Microbial taxonomic and metabolic alterations during faecal microbiota transplantation to treat Clostridium difficile infection. *J. Infect.* 77, 107–118

34. Le Gall, G., Cottutla, K., Kellingray, L., Tett, A. J., Ten Hoopen, R., Keiser, K. E., Savva, G. M., Ibrahim, A., and Narbad, A. (2018) Metabolite quantification of faecal extracts from colorectal cancer patients and healthy controls. *Onco Targets Ther.* 9, 33278–33289

35. Shi, Y., Kellingray, L., Zhai, Q., Gall, G. L., Narbad, A., Zhao, J., Zhang, H., and Chen, W. (2018) Structural and functional alterations in the microbial community and immunological consequences in a mouse model of antibiotic-induced dysbiosis. *Front. Microbiol.* 9, 1948

36. Le Gall, G., Noor, S. O., Fidgely, K., Swelll,L., Jamieson, C. G., Johnson, I. T., Colquhoun, I. J., Kershley, K. E., and Narbad, A. (2011) Metabolomics of fecal extracts detects altered metabolic activity of gut microbiota in ulcerative colitis and irritable bowel syndrome. *J. Proteome Res.* 10, 4208–4218

37. Le Gall, G. (2015) NMR spectroscopy of biofluids and extracts. *Methods Mol. Biol.* 1277, 29–36

38. Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 595–596

39. Edgar, R. C. (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2660–2661

40. Magoé, T., and Salzberg, S. L. (2011) FLASH: fast length adjustment of PCR amplicons. *Nat. Methods* 8, 275–278

41. Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* 17, 10–12

42. Haas, B. J., Gevers, D., Earl, A. M., Ffeldgarden, M., Ward, D. V., Giannoukos, G., Guilla, D., Tabbaa, D., Highlander, S. K., Sodergren, E., Methé, B., DeSantis, T. Z., Peterson, J. F., Knight, R., and Birren, B. W. Human Microbiome Consortium. (2011) Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Biol.* 21, 494–504

43. Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson,
C. J., Sahl, J. W., Stres, B., Thullinger, G. G., Van Horn, D. J., and Weber, C. F. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7357–7361

44. Camp, R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., Kulaan-Syed-Mohideen, A. S., McGarrell, D. M., Mars, T., Garrity, G. M., and Tiedje, J. M. (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 37, D141–D145

45. R Core Team. (2016) R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria

46. Dray, S., and Dufour, A. (2007) The ade4 package: implementing the duality diagram for ecologists. J. Stat. Softw. 22, 1–20

47. Moonseong, H., and Ruben Gabriel, K. (1998) A permutation test of variation across the lifespan of the healthy laboratory rat. Neurobiol. Aging 19, 341–346

48. Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Dore, J., Eckley, D. S., Hugon, J., and Nuttall, S. A. (2013) The impact of the environment on the human gut microbiome: an integrative analysis. Nature 500, 559–565

49. Dutta, S., and Sengupta, P. (2016) Men and mice: relating their ages. Life Sci. 152, 244–248

50. O’Toole, P. W., and Jeffery, I. B. (2015) Gut microbiota and aging. Science 350, 1214–1215

51. Finley, B., Gaci, N., Borrel, G., Sanderson, I. R., Chaudhary, P. P., Totey, W., O’Toole, P. W., and Grubesic, J. F. (2017) Fecal microbiota variation across the lifespan of the healthy laboratory rat. Gut Microbes 8, 495–499

52. Hoffmann, J., Parikh, I., Green, S. J., Chlipala, G., Mohney, R. P., Bhattacharjee, S., and Lukiw, W. J. (2013) Alzheimer disease brain: identification and topographic distribution—a pilot study. Acta Neuropathol. 120, 191–201; erratum: 588

53. Gewirtz, A. T., Xie, L., Jin, Y., and Relman, D. A. (2014) Metagenomic analysis of the human gastrointestinal tract. Cell 157, 1215–1226

54. Gollub, J., and Moore, W. C. (2007) Short chain fatty acids and gut microbiota differ between patients with Parkinson’s disease and age-matched controls. Parkinsonism Relat. Disord. 13, 60–67

55. Schepers, J., Olof, V., Pereira, P. A., Koskinen, K., Paulin, L., Pekkonen, E., Haapaniemi, E., Kaakkola, S., Eerola-Rautio, J., Pohja, M., Kunnunen, E., Murros, K., and Auvinen, P. (2015) Gut microbiota are related to Parkinson’s disease and clinical phenotype. Mov. Disord. 30, 350–358

56. Forsyth, C. B., Shannon, K. M., Kordower, J. H., Voigt, R. M., Shaikh, M., Jaglin, J. A., Estes, J. D., Dodyla, H. B., and Keshavarzian, A. (2011) Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson’s disease. PLoS One 6, e28092

57. Zhang, L., Wang, Y., Xiayu, X., Shi, C., Chen, W., Shi, C., Chen, W., Song, N., Fu, X., Zhou, R., Xu, Y. F., Huang, L., Zhu, H., Han, Y., and Qin, C. (2017) Short chain fatty acids and gut microbiota differ between patients with Parkinson’s disease and age-matched controls. Parkinsonism Relat. Disord. 32, 60–72

58. Gollub, J., and Moore, W. C. (2007) Short chain fatty acids and gut microbiota differ between patients with Parkinson’s disease and age-matched controls. Parkinsonism Relat. Disord. 13, 60–67

59. Schepers, J., Olof, V., Pereira, P. A., Koskinen, K., Paulin, L., Pekkonen, E., Haapaniemi, E., Kaakkola, S., Eerola-Rautio, J., Pohja, M., Kunnunen, E., Murros, K., and Auvinen, P. (2015) Gut microbiota are related to Parkinson’s disease and clinical phenotype. Mov. Disord. 30, 350–358

60. Forsyth, C. B., Shannon, K. M., Kordower, J. H., Voigt, R. M., Shaikh, M., Jaglin, J. A., Estes, J. D., Dodyla, H. B., and Keshavarzian, A. (2011) Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson’s disease. PLoS One 6, e28092

61. Holmqvist, S., Chutna, O., Bousset, L., Aldrin-Kirk, P., Li, W., Björklund, T., Wang, Z. Y., Roybon, L., Melki, R., and Li, J. Y. (2014) Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. Acta Neuropathol. 128, 805–820

62. Stefanis, L. (2012) α-Synuclein in Parkinson’s disease. Cold Spring Harb. Perspect. Med. 2, a009399

63. Jellinger, K. A. (2003) Alpha-synuclein pathology in Parkinson’s and Alzheimer’s disease brain: incidence and topographic distribution—a pilot study. Acta Neuropathol. 106, 191–201; erratum: 588

64. Crews, L., Tsigelny, I., Hashimoto, M., and Masliah, E. (2009) Role of synucleins in Alzheimer’s disease. Neurotox. Res. 16, 306–317

65. Bennett, M. C. (2005) The role of alpha-synuclein in neurodegenerative diseases. Pharmacol. Ther. 105, 511–531

66. Larsen, N., Vogensen, F. K., van den Berg, F. W., Nielsen, D. S., Andersen, A. S., Pedersen, B. K., Al-Soud, W. A., Sørensen, S. J., Hansen, L. H., and Jakobsen, M. (2010) Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One 5, e9085

67. Pryde, S. E., Duncan, S. H., Hold, G. L., Stewart, C. S., and Flint, H. J. (2002) The microbiology of butyrate formation in the human colon. FEMS Microbiol. Lett. 217, 135–139

68. Zhang, D., Fu, X., Dai, X., Chen, Y., and Dai, L. (2016) A new biological process for short-chain fatty acid generation from waste activated sludge improved by Clostridiales enhancement. Environ. Sci. Pollut. Res. Int. 23, 7297–7302

69. De-Almadra, B. V., de-Almeda, I. D., Camporez, D., de-Moraes, M. V., Morelato, R. L., Perrone, A. M., Belcavello, L., Louro, I. D., and de-Paula, F. (2012) Protective effect of the APOE-e5 allele in Alzheimer’s disease. Braz. J. Med. Biol. Res. 45, 8–12

70. Farver, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., Myers, R. H., Pericak-Vance, M. A., Risch, N., and van Duijn, C. M. APOE and Alzheimer Disease Meta Analysis Consortium, (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. JAMA 278, 1349–1356

71. Gossling, J., and Moore, W. C. (1975) Genniger formicilus, n. gen., n. sp., an anaerobic budding bacterium from intestines. Int. J. Syst. Bacteriol. 25, 202–207

72. Van den Abbeele, P., Belzer, C., Goossens, M., Keereezem, M., De Vos, W. M., Thas, O., De Weirdt, R., Kerckhoff, F. M., and Van de Wiele, T. (2013) Butyrate-producing Clostridium cluster XIVa species specifically colonize mucins in an in vitro gut model. ISME J. 7, 949–961

73. Duncan, S. H., Barcenilla, A., Stewart, C. S., Pryde, S. E., and Flint, H. J. (2002) Acetate utilization and butyryl coenzyme A (CoA)-acetate-CoAtransferase in butyrate-producing bacteria from the human large intestine. Appl. Environ. Microbiol. 68, 5186–5190

74. Louis, P., and Flint, H. J. (2009) Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol. Lett. 294, 1–8

75. Macy, J. M., Ljungdahl, L. G., and Gottschalk, G. (1978) Pathway of succinate and propionate formation in Bacteroides fragilis. J. Bacteriol. 134, 84–91

76. Ho, L., Ono, K., Tsuji, M., Mazzola, P., Singh, R., and Pasinetti, G. M. (2018) Protective role of intestinal microbiota derived short chain fatty acids in Alzheimer’s disease-type beta-amyloid neuropathological mechanisms. Expert Rev. Neurother. 18, 83–90

Received for publication January 10, 2019. Accepted for publication March 18, 2019.