Short Report

Synergistic effects of APOE and sex on the gut microbiome of young EFAD transgenic mice

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Abstract

Background: Alzheimer’s disease (AD) is a fatal neurodegenerative disease. APOE4 is the greatest genetic risk factor for AD, increasing risk up to 15-fold compared to the common APOE3. Importantly, female (♀) APOE4 carriers have a greater risk for developing AD and an increased rate of cognitive decline compared to male (♂) APOE4 carriers. While recent evidence demonstrates that AD, APOE genotype, and sex affect the gut microbiome (GM), how APOE genotype and sex interact to affect the GM in AD remains unknown.

Methods: This study analyzes the GM of 4-month (4 M) ♂ and ♀ E3FAD and E4FAD mice, transgenic mice that overproduce amyloid-β 42 (Aβ42) and express human APOE3+/+ or APOE4+/+. Fecal microbiotas were analyzed using high-throughput sequencing of 16S ribosomal RNA gene amplicons and clustered into operational taxonomic units (OTU). Microbial diversity of the EFAD GM was compared across APOE, sex and stratified by APOE + sex, resulting in 4-cohorts (♂E3FAD, ♀E3FAD, ♂E4FAD and ♀E4FAD). Permutational multivariate analysis of variance (PERMANOVA) evaluated differences in bacterial communities between cohorts and the effects of APOE + sex. Mann-Whitney tests and machine-learning algorithms identified differentially abundant taxa associated with APOE + sex.

Results: Significant differences in the EFAD GM were associated with APOE genotype and sex. Stratification by APOE + sex revealed that APOE-associated differences were exhibited in ♀E3FAD and ♂E3FAD mice, and sex-associated differences were exhibited in E3FAD and E4FAD mice. Specifically, the relative abundance of bacteria from the genera Prevotella and Ruminococcus was significantly higher in ♀E4FAD compared to ♀E3FAD, while the relative abundance of Sutterella was significantly higher in ♀E4FAD compared to ♂E3FAD. Based on 29 OTUs identified by the machine-learning algorithms, heatmap analysis revealed significant clustering of ♀E4FAD separate from other cohorts.

Conclusions: The results demonstrate that the 4 M EFAD GM is modulated by APOE + sex. Importantly, the effect of APOE4 on the EFAD GM is modulated by sex, a pattern similar to the greater AD pathology associated with ♀E4FAD. While this study demonstrates the importance of interactive effects of APOE + sex on the GM in young AD transgenic mice, changes associated with the development of pathology remain to be defined.

Keywords: Alzheimer’s disease, Gut microbiome, APOE genotype, Sex

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Background
The gut microbiome (GM), the collective genome of gastrointestinal bacteria, is an integral component of human physiology [1–5]. Recent studies link dysbiotic GM profiles with neurological disorders, with multiple sclerosis the first identified [6–12]. While subsequent studies have linked dysbiosis with Alzheimer’s disease (AD) pathology [13–22], the effects of AD risk factors, specifically APOE genotype, sex and their interaction, on the GM remain unclear.

The APOE4 genotype is the greatest genetic risk factor for AD, increasing risk up to 15-fold compared to the more common APOE3 genotype [23, 24]. Apolipoprotein E (apoE) is a member of the apolipoprotein family, the protein components of lipoproteins. Both humans and AD transgenic (−Tg) mice with APOE4 exhibit an increase in amyloid-β (Aβ) peptide accumulation, both as amyloid plaques, a hallmark of the disease, and small soluble aggregates. Thus, one explanation for the amyloid plaques, a hallmark of the disease, and small

Another risk factor for AD as females (♀) exhibit almost two-fold greater lifetime AD risk compared to males (♂) [38]. Additionally, sex plays an important role in the GM as the bacterial composition and metabolic function differ significantly between ♀ and ♂. [38–46]. Importantly, ♀APOE4 carriers have a greater lifetime risk for developing AD, an increased rate of cognitive decline and an accelerated accumulation of Aβ compared to ♂APOE4 carriers [47–61]. While the underlying mechanism is unclear, evidence suggests this interaction modulates the GM.

EFAD-Tg mice [62] overexpress Aβ42 via five familial AD (FAD) mutations [63] and express h-APOE3 or APOE4, allowing for the study of the interaction among AD risk factors [64–66]. EFAD mice expressing the APOE4/− genotype (E4FAD), compared to E3FAD mice, exhibit increased behavioral deficits, Aβ deposition and neuroinflammation. Importantly, these differences are reproduced in ♀ vs ♂EFAD mice, resulting in 4 pathologically-distinct cohorts when the EFAD mice are stratified by APOE + sex (♀E4FAD > ♂E4FAD = ♀ E3FAD > ♂E3FAD), a phenotype that develops with age [65, 66]. For this study, we focused on 4 M EFAD mice to evaluate the interactive effects of APOE + sex on the GM at an age prior to, or early in, the development of pathology. Microbial analysis of fecal samples demonstrated that APOE + sex have a significant effect on the GM at various taxonomic levels.

Methods
Mouse model
As previously described, the EFAD (5xFAD+/−/APOE3+/−) mice are homozygous for APOE2, APOE3, or APOE4 and heterozygous for the 5x familial AD (5xFAD) mutations [62, 63]. Although APOE2 is considered neuroprotective, 100% of APOE2+/+ mice have type III hyperlipoproteinemia, compared to only 15% of human ε2/2 carriers [67–69]; thus, E2FAD mice were excluded from the current study. At 4 M, fecal samples were obtained from the 4 cohorts (♀E3FAD, 8♀E4FAD, 19♀E3FAD, 12♀E4FAD) by individually placing mice in clean disposable Styrofoam cups. Feces were flash frozen and stored at −80°C until DNA isolation.

Bacteria identification
Fecal DNA was isolated using a PowerSoil DNA isolation kit (Mo Bio Laboratories) and DNA concentrations determined by UV absorbance (Nanodrop, Thermo-Fisher). The V4 variable region of 16S ribosomal RNA gene was PCR-amplified using target-specific primers containing bar codes and linker sequences [70]. PCR reaction conditions included an initial denaturation step of 30 s (s) at 98 °C, followed by 28 cycles of 10s at 98 °C, 15 s at 60 °C, 30s at 72 °C, and a final elongation step of 7 min at 72 °C. The PCR master mix (20 μl volume) contained 100 ng of DNA template, 0.5 μM forward and reverse primers, Phusion Hot Start DNA polymerase and high-fidelity buffer (New England Biolabs), dNTPs and sterile water. Results were checked by polyacrylamide gel electrophoresis and samples pooled in equimolar ratio. The samples were sequenced on an Illumina MiSeq sequencer at the University of Kentucky Advanced Genetic Technologies Center, with sequence merging, trimming, chimera removal, clustering and annotation performed using the software package QIIME [71]. The Greengenes database was implemented for Operational Taxonomic Unit (OTU) annotation at a threshold of 97% sequence similarity [72]. To avoid effects of uneven sequencing depth [73], datasets were rarified to 3000 sequences/sample prior to analysis. For statistical analyses, OTUs with a frequency below 0.1% across the dataset were removed [71].

Data analysis
The Shannon H α-diversity index was used to assess bacterial richness and evenness. The interaction between APOE + sex in α-diversity measures was evaluated using a mixed effects model, similar to a two-way analysis of variance (ANOVA), that analyzes repeated measures...
with missing values. This analysis was performed in the software package GraphPad Prism (version 8.2.0). For β-
diversity, permutational ANOVA (PERMANOVA) was used to compare microbial community structure within
and among the EFAD cohorts based on Bray-Curtis dis-
similarity [74, 75]. Pair-wise PERMANOVA was used to
assess the effect of the interaction among universal bio-
logical variables on the microbiome composition [76].
Principal coordinate analysis plots (PCoA; Bray-Curtis
distances) with 95% confidence ellipses were used to
visualize microbial communities [75, 77, 78]. The Mann-
Whitney U (MWU) test under the Monte Carlo simula-
tion, corrected with Benjamini-Hochberg False Discov-
ery Rate ($p < 0.05$), was used to identify differentially
abundant taxa associated with $APOE + sex$ at the taxo-
nomic level of genus. The Random Forest based Boruta
algorithm was used to determine OTUs significant in
distinguishing samples by $APOE + sex$ compared to ran-
domly generated probes or “shadow scores” [79]. Heat-
maps were generated using the R package, “pheatmap”,
calculating the Euclidean distance among cohorts.

Results and discussion

Mouse fecal microbial community structure was ana-
alyzed using high-throughput sequencing of 16S rRNA
gene amplicons, followed by sequence clustering (97% similarity) into a total of 2063 OTUs. No significant dif-
fERENCE in α-diversity (Shannon H index) was observed
between $E3FAD$ and $E4FAD$ mice ($p = 0.975$; Addi-
tional file 1: Figure S1A) or between $EFAD$ and $EFAD$
($p = 0.949$; Additional file 1: Figure S1B). In
comparing across cohorts stratified by $APOE + sex$,
Shannon H indices were significantly higher in $EFAD$
and $EFAD$, compared to $E3FAD$ and $E4FAD$ ($p < 0.05$; Additional file 1: Figure S1C). Additionally, the
interaction of $APOE + sex$ significantly modulated α-
diversity measures ($p < 0.05$; Additional file 1: Figure
S1C), suggesting that analyses by $APOE$ genotype or sex alone will mask effects on microbial community
structure.

Differences in microbial community structure between
EFAD cohorts (β-diversity) were examined with PER-
MANOVA (Additional file 3: Table S1) and visualized
with PCoA plots (Fig. 1). At the taxonomic level of
OTU, significant differences in microbial communities
were observed between $E3FAD$ and $E4FAD$ mice ($p < 0.05$; Fig. 1a) and between $EFAD$ and $EFAD$ mice ($p < 0.05$; Fig. 1b). Differences associated with $APOE$
genotype were also exhibited in the taxonomic levels of
Family and Genus (Additional file 3: Table S1A), sug-
gesting that $APOE$ genotype is an important modulator of
the GM, consistent with findings in $APOE-TR$ mice
[25]. Importantly, the interaction between $APOE + sex$
significantly modulated the GM across taxonomic levels
of Family, Genus and OTU ($p < 0.05$; Additional file 3:
Table S1A). Comparisons at the OTU level among sam-
ple stratified by $APOE + sex$ demonstrated significant
differences between $EFAD$ and $EFAD$ mice ($p < 0.05$; Fig. 1c), and between $EFAD$ and $EFAD$ mice
($p < 0.05$; Fig. 1c), indicating that the effect of $APOE$
genotype is consistent across sex. Furthermore, signifi-
cant differences associated with sex were observed be-
 tween $EFAD$ and $EFAD$ and between $EFAD$ and $EFAD$ ($p < 0.05$; Fig. 1c). These data demonstrate that
the $APOE$ genotype interacts with sex, leading to
sex differentiation in $E3FAD$ and $E4FAD$ mice. While a
recent paper by Dodiya and colleagues demonstrated no
sex effect on α- or β-diversity in FAD-Tg mice that ex-
press mouse $APOE$ [80], the current findings may sug-
gest that the sex effect is specific to carriers of human
$APOE$. This mirrors the synergistic effects of $sex$ and
$APOE4$ genotype on AD risk in humans, greatest in
$APOE4 > APOE4$ [47–50].

A taxon-by-taxon analysis at the genus level was per-
formed to identify microbial genera significantly different
between cohorts. The relative abundance of the genera
$Prevotella$, $Ruminococcus$ and $Sutterella$ were significantly higher in $E3FAD$ mice compared to $E4FAD$
mice, while the relative abundance of $Anaeroplasma$
was significantly lower (Fig. 2a). Interestingly, FAD-Tg mice
also exhibited significantly higher relative abundance of
$Anaeroplasma$ compared to wild-type mice [81, 82], sug-
gestting that $Anaeroplasma$ may have a role in AD path-
ology. Tran and colleagues demonstrated that $APOE4-
TR$ mice exhibit greater relative abundance of bacteria
from the genera $Murispirillum$, $Desulfovibrio$, $Butyri-
coccus$ and lower relative abundance of $Bacteroides$, $Alis-
tipes$, $Johnsonella$ compared to $APOE3-TR$ mice [25].
Thus, our results together suggest that the effects of
$APOE$ genotype on the GM is modulated by AD path-
ology. Additionally, Ong and colleagues determined that
$Allobaculum$, $Anaeroplasma$ and $Erwinia$ are the most
abundant genera in $♀$ mice relative to $♂$ mice [83]. Simi-
larly, $EFAD$ exhibited a significantly greater relative
abundance of $Allobaculum$ compared to $EFAD$
(Fig. 2b). Comparing the stratified cohorts, the fecal
microbiota of $EFAD$ mice had lower relative abundance
of $Sutterella$ and $Lactobacillus$ compared to $EFAD$.
$EFAD$ mice had lower relative abundance of
$Prevotella$ and $Ruminococcus$ compared to $EFAD$
(Fig. 2c). Similarly, these differences are significant at the
OTU level (Additional file 4: Table S2). Therefore, the
results suggest that the effect of $APOE$ genotype on dif-
ferentially abundant bacteria is modulated by sex, as spe-
cific genera and OTUs are significantly different in
males or females.

Compared to $E3FAD$ mice, $E4FAD$ mice exhibited a lower relative abundance of bacterial genera associated
with short chain fatty acid (SCFA) production, including *Prevotella* and *Ruminococcus* [84–89]. The GM is crucial for the production of SCFAs that, while the underlying mechanism is not completely understood, serve as energy sources for intestinal epithelial cells, regulators of plasma lipid levels, and modulators of immune cells. 

Fig. 1 Differences in microbial community between EFAD mice stratified by APOE, sex and APOE + sex. Analysis of β-diversity associated with (a) APOE, (b) sex and (c) APOE + sex in the GM of 4 M EFAD mice. PCoA plots with 95% confidence ellipses were generated based on the Bray-Curtis dissimilarity. Significant differences between cohorts were determined by PERMANOVA, with significance (bold) defined by *p* < 0.05. Additional file 1: Table S1 contains the complete PERMANOVA dataset.
Fig. 2 (See legend on next page.)
The current results suggest a metabolic dysfunction in the \( \varphi E4FAD \) GM. However, metabolomic and metagenomic analyses will be required to interpret accurately the interactive effects of \( APOE + \) sex on the metabolic function of the EFAD GM.

The Boruta algorithm identified 29 OTUs significant in distinguishing EFAD samples by \( APOE + \) sex (Additional file 2: Figure S2). These 29 bacterial OTUs were annotated at varying taxonomic levels, including the genera *Prevotella*, *Lactobacillus*, *Allobaculum*, *Anaeroplasmata*, and *Sutterella*, consistent with the results of differentially abundant bacteria (Fig. 2). Based on the abundance of these 29 OTUs, a hierarchical heatmap demonstrates that EFAD samples clustered by \( APOE + \) sex (Fig. 3). Clustering of \( \varphi E4FAD \) samples is further demonstration that the murine GM is affected by a specific interaction between \( APOE4 \) genotype and \( \varphi \) sex, consistent with human \( \varphi APOE4 \) carriers exhibiting greater AD risk compared to \( \varphi APOE4 \) carriers [47–50].

**Conclusions**

This short report demonstrates: 1) the EFAD GM is modulated by \( APOE + \) sex, 2) the synergistic effects of \( \varphi \) sex and \( APOE4 \) genotype yield a specific GM profile in \( \varphi E4FAD \) mice, and 3) clustering samples by only \( APOE \) genotype or sex masks the interactive effects of \( APOE + \) sex on the EFAD GM. Notably, these findings are consistent with AD readouts from EFAD mice varying in severity of pathology by \( APOE + \) sex, including behavioral deficits, \( \beta \) deposition and neuroinflammation greatest in \( \varphi E4FAD > \varphi E4FAD = \varphi E3FAD > \varphi E3FAD \) [65, 66]. Therefore, the GM would potentially serve as an AD...
readout, reflecting the interaction between APOE + sex. Although the use of 16S rRNA sequencing has more limited taxonomic resolution than shotgun metagenome sequencing [96], 16S rRNA sequencing is sufficiently robust to identify significant effects on the GM. This study demonstrates the importance of stratifying the EFAD population by APOE + sex to better understand the relationship between AD and the GM. Future studies will examine the composition and metabolic function of the GM throughout the development of EFAD pathology through the use of metagenomic and metabolomic analyses. In conclusion, the interactive effects of APOE + sex on AD play an important role in modulating the GM composition, and the current report is the first step in identifying and understanding these effects.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s13024-019-0352-2.

Additional file 1: Figure S1. Analysis of α-diversity of EFAD mice stratified by APOE, sex, APOE + sex. Based on bacterial evenness and richness, Shannon H index scores were generated and compared across EFAD mice stratified by (A) APOE, (B) sex and (C) APOE + sex with Mann-Whitney U test (*p < 0.05 vs sex; ♂p < 0.05 vs genotype). A mixed-model analysis was used to evaluate the interactive effects of APOE + sex on richness, evenness and α-diversity (***p < 0.05 vs APOE + sex).

Additional file 2: Figure S2. Boruta-identified bacterial OTUs from EFAD mice stratified by APOE + sex. Implementing the R package “randomForest”, Boruta is a feature-selection algorithm that determined the OTUs that were significant in distinguish samples by APOE + sex compared to randomly generated probes (“shadow scores” in blue). Significance is defined by a z-score > max shadow z-score (green; listed in the table). OTUs with a z-score that trends towards significance are labeled in yellow.

Additional file 3: Table S1. Permutational multivariate analysis of variance (PERMANOVA) of EFAD mice stratified by APOE, sex, APOE + sex. (A) PERMANOVA was used to assess the effect of the interaction between universal biological variables on the microbiome composition at various taxonomic levels. P-values were obtained using 9999 permutations under a reduced model. Pseudo-F ratio is defined by the difference between cohorts over the difference within each cohort and the degrees of freedom. Each term is contributing a fixed component to the overall model. Estimated sizes of components of variation are multivariate analogs to the classical ANOVA unbiased estimators. Significance (bold) is defined by a p < 0.05. (B) As the interaction between APOE + sex is significant, pair-wise PERMANOVAS at the OTU level evaluated the effects of APOE on β-diversity within 1EFAD and 2EFAD mice, and the effects of sex in 3EFAD and 4EFAD. Significance (bold) is defined by a p < 0.05.

Additional file 4: Table S2. Results of Mann-Whitney U tests at specific taxonomic levels in EFAD mice. Significantly different relative abundance of bacterial genera associated with APOE, sex, and APOE + sex, identified by Mann-Whitney U under the Monte Carlo Simulation corrected for false discovery rate (p < 0.05) at the levels of Phylum, Class, Order, Family, Genus and OTU.

Abbreviations
AD: Alzheimer’s disease; apoE: Apolipoprotein E; Aβ: Amyloid-β; FAD: Familial AD; GM: Gut microbiome; MWU: Mann-Whitney U; OTU: Operational taxonomic units; Perm: Permutation; PERMANOVA: Permutational multivariate analysis of variance; SCFA: Short chain fatty acid; Tg: Transgenic

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Authors’ contributions
ML and JMW analyzed the data and wrote the manuscript, IP and SE purified DNA and performed PCR, JMW and SG performed statistical analyses, JY bred the mice, SG and SE contributed to data analysis and manuscript editing. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Raw sequence data files were submitted in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI). The BioProject identifier of the samples is PRJNA556445.

Ethics approval
Animal studies were performed in compliance with IACUC (Institutional Animal Care and Use Committee) at University of Illinois-Chicago.

Consent for publication
All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Laukens D, Brinkman BM, Raes J, De Vos M, Vandebriel R. Heterogeneity of the gut microbiome in mice: guidelines for optimizing experimental design. FEMS Microbiol Rev. 2016;40(1):117–32.
2. Ursell LK, Mercafil JL, Parfrey LW, Knight R. Defining the human microbiome. Nutr Rev. 2012;70(Suppl 1):S8–44.
3. Collins SM, Surette M, Bercki P. The interplay between the intestinal microbiota and the brain. Nat Rev Microbiol. 2012;10(11):735–42.
4. Clarke G, Gilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: gut microbiota: the neglected endocrine organ. Mol Endocrinol. 2014;28(8):1221–38.
5. Lyte M, Cryan JF. Dealing with ability of the microbiota to influence the brain, and ultimately cognition and behavioral. Adv Exp Med Biol. 2014;817:ix-xi.
6. DuPont AW, DuPont HL. The intestinal microbiota and chronic disorders of the gut. Nat Rev Gastroenterol Hepatol. 2011;8(9):523–31.
7. Levy M, Kołodziejczyk AA, Thaisi CA, Elina V. Dysbiosis and the immune system. Nat Rev Immunol. 2017;17(4):219–32.
8. Chen J, Chia N, Kalari KR, Yao JZ, Novotna M, Paz Soldan MM, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. Sci Rep. 2016;6:28484.
9. Tremlett H, Fadroz DW, Faruqi AA, Zhu F, Hart J, Roalstad S, et al. Gut microbiota in early pediatric multiple sclerosis: a case-control study. Eur J Neurol. 2016;23(8):1308–21.
10. Bhargava P, Mowry EM. Gut microbiome and multiple sclerosis. Curr Neurol Neurosci Rep. 2014;14(10):492.
11. Jaggi S, Gandhi R, Cox LM, Li N, von Gehlen F, Yan R, et al. Alterations of the human gut microbiome in multiple sclerosis. Nat Commun. 2016;7:12015.
12. Ochoa-Reparaz J, Magori K, Kasper LH. The chicken or the egg dilemma: intestinal dysbiosis in multiple sclerosis. Ann Transl Med. 2017;5(6):145.
13. Vogt NW, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, et al. Gut microbiome alterations in Alzheimer's disease. Sci Rep. 2017;7(1):13537.
14. Cattaneo A, Cattane N, Galluzzi S, Provasi S, Lopizono N, Festari C, et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. Neurobiol Aging. 2017;69:60-8.
15. Mahmoudian-Dehkordi S, Arnold M, Nho K, Ahmad S, Jia W, Xie G, et al. Altered bile acid profile associates with cognitive impairment in Alzheimer's disease-an emerging role for gut microbiome. Alzheimers Dement. 2019; 15(1):76–92.
16. Fox M, Knor DA, Haptonstall KM. Alzheimer's disease and symbiotic microbiota: an evolutionary medicine perspective. Ann N Y Acad Sci. 2019;1448(1):22–34.
17. Jiang C, Li G, Huang P, Liu Z, Zhao B. The gut microbiota and Alzheimer's disease. J Alzheimers Dis. 2017;58(1):1–15.
18. Angellucci F, Cechova K, Amerlova J, Hort J. Antibiotics, gut microbiota, and Alzheimer's disease. J Neuroinflammation. 2019;16(1):108.
19. Garcez ML, Jacobs RR, Guillemin GJ. Microbiota alterations in Alzheimer's disease: involvement of the Kynurenine pathway and inflammation. Neurotox Res. 2019;36(2):194–204.
20. Zhuang ZQ, Sheng LL, Wu WW, Fu X, Zeng F, Gui L, et al. Gut microbiota is altered in patients with Alzheimer's disease. J Alzheimers Dis. 2018;63(4):1337–46.
21. Liu F, Wu L, Peng G, Han Y, Tang R, Ge J, et al. Altered microbiomes distinguish Alzheimer's disease from amnestic mild cognitive impairment and health in a Chinese cohort. Brain Behav Immun. 2019;80:633–43.
22. Li B, He Y, Ma J, Huang P, Du J, Cao L, et al. Mild cognitive impairment has similar alterations as Alzheimer's disease in gut microbiota. Alzheimers Dement. 2019;15(10):1357–66.
23. Reitz C, Mayeux R. Use of genetic variation as biomarkers for mild cognitive impairment and progression of mild cognitive impairment to dementia. J Alzheimers Dis. 2012;19(1):229–51.
24. Leon V. The effect of apolipoprotein E (ApoE) genotype on biomarkers of amyloidogenesis, tau pathology and neurodegeneration in Alzheimer's disease. Clin Chem Lab Med. 2011;49(3):357–83.
25. Tran TTT, Corsini S, Kellingray L, Hegarty C, Le Gall G, Narbad A, et al. APOE genotype influences the gut microbiome structure and function in humans and mice: relevance for Alzheimer's disease pathophysiology. FASEB J. 2019;33(7):20190071R.
26. Weisgraber KH. Apolipoprotein E: structure-function relationships. Adv Protein Chem. 1994;45:249–302.
27. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis. 1988;8(1):1–21.
28. de Knijff P, van den Maagdenberg AM, Frants RR, Havekes LM. Genetic modulation of plasma lipid and lipoprotein levels. Hum Mutat. 1994;4(3):178–94.
29. DeLongeille J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apolipoprotein E phenotype: a meta-analysis. J Lipid Res. 1992; 33(3):447–54.
30. Caparros-Martin JA, Lareu RR, Ramsay JP, Peplies J, Reen FJ, Headlam HA, et al. Statin therapy causes gut dysbiosis in mice through a PXR-dependent mechanism. Microbiome. 2017;5(1):95.
31. Weisgraber KH. Apolipoprotein E: structure-function relationships. Adv Protein Chem. 1994;45:249–302.
32. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis. 1988;8(1):1–21.
33. de Knijff P, van den Maagdenberg AM, Frants RR, Havekes LM. Genetic heterogeneity of apolipoprotein E and its influence on plasma lipid and lipoprotein levels. Hum Mutat. 1994;4(3):178–94.
34. Dallongeille J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apolipoprotein E phenotype: a meta-analysis. J Lipid Res. 1992; 33(3):447–54.
35. Caparros-Martin JA, Lareu RR, Ramsay JP, Peplies J, Reen FJ, Headlam HA, et al. Statin therapy causes gut dysbiosis in mice through a PXR-dependent mechanism. Microbiome. 2017;5(1):95.
36. Caparros-Martin JA, Lareu RR, Ramsay JP, Peplies J, Reen FJ, Headlam HA, et al. Statin therapy causes gut dysbiosis in mice through a PXR-dependent mechanism. Microbiome. 2017;5(1):95.
