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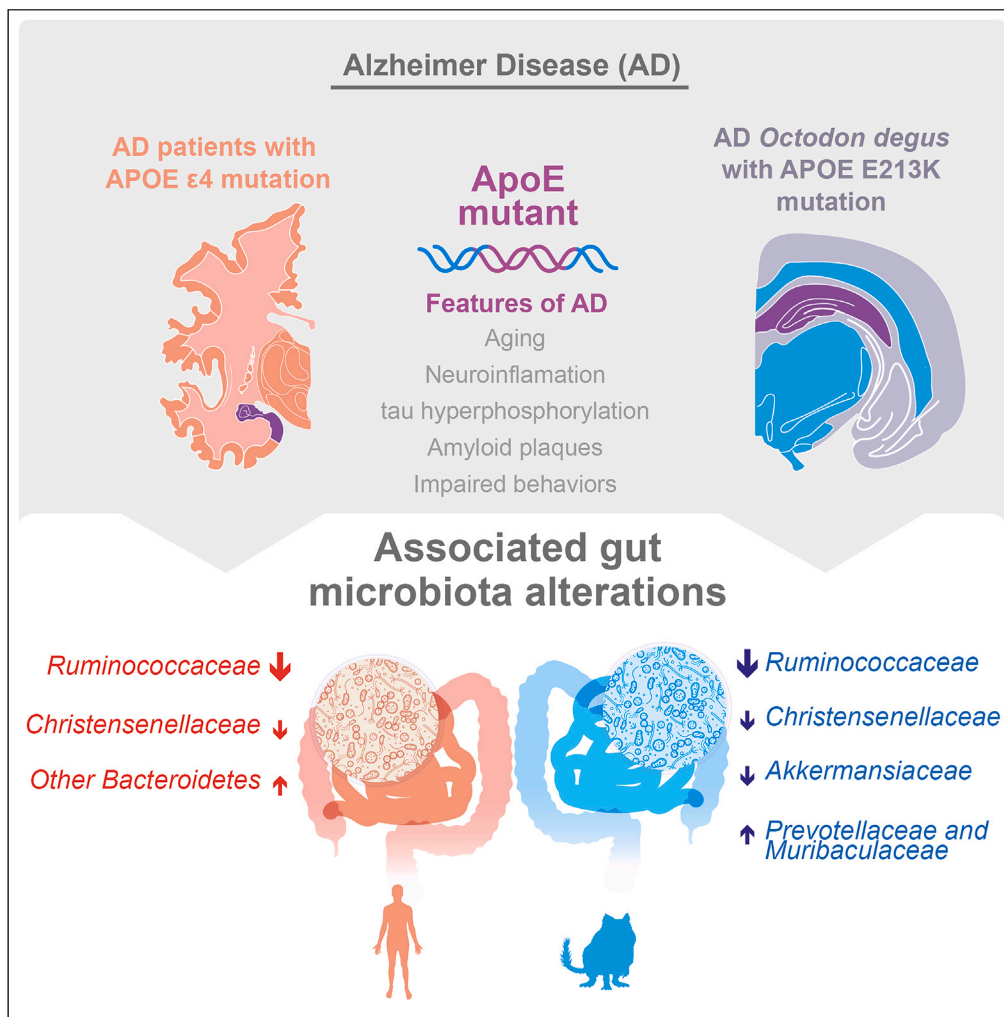

Guido Zampieri, Léa Cabrol, Claudio Urra, ..., Robert M.J. Deacon, Michael J. Hurley, Patricia Cogram

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Highlights

The APOE E213K mutation is linked to gut microbiome alterations in *Octodon degus*

These alterations mirror those observed in human patients with APOE ε4 mutation

Altered microbiome's metabolic potential may affect AD pathology in *O. degus*

The degu is an appealing unconventional animal model to study Alzheimer's disease

Zampieri et al., iScience *27*, 110348
August 16, 2024 © 2024 The Authors. Published by Elsevier Inc.
https://doi.org/10.1016/j.isci.2024.110348

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Microbiome alterations are associated with apolipoprotein E mutation in *Octodon degus* and humans with Alzheimer's disease

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SUMMARY

Gut microbiome dysbiosis is linked to many neurological disorders including Alzheimer's disease (AD). A major risk factor for AD is polymorphism in the apolipoprotein E (*APOE*) gene, which affects gut microbiome composition. To explore the gut-brain axis in AD, long-lived animal models of naturally developing AD-like pathologies are needed. *Octodon degus* (degu) exhibit spontaneous AD-like symptoms and *ApoE* mutations, making them suitable for studying the interplay between AD genetic determinants and gut microbiome. We analyzed the association between *APOE* genotype and gut microbiome in 50 humans and 32 degu using16S rRNA gene amplicon sequencing. Significant associations were found between the degu *ApoE* mutation and gut microbial changes in degu, notably a depletion of *Ruminococcaceae* and *Akkermansiaceae* and an enrichment of *Prevotellaceae*, mirroring patterns seen in people with AD. The altered taxa were previously suggested to be involved in AD, validating the degu as an unconventional model for studying the AD/microbiome crosstalk.

INTRODUCTION

The microbiota–gut–brain axis is now recognized as a significant modulator of behavior[1] and is increasingly implicated in various neurological disorders, including Alzheimer's disease (AD).[2–4] Gut dysbiosis may therefore contribute to the development and progression of AD.[5] A recent study revealed for the first time that AD symptoms can be transferred to healthy young rats via the gut microbiota, confirming a causal role of gut microbiota in AD.[6]

Polymorphism in the apolipoprotein E (APOE) gene is a major genetic risk determinant of AD.[7] APOE is a major cholesterol carrier that supports lipid transport and injury repair in the brain. Individuals with the *APOE4* allele are at an increased risk of developing AD compared to those with other *APOE* alleles.[8] Seo and colleagues (2020) recently showed *APOE* genotype and microbiome composition could influence tauopathy in a mouse model of AD.[9] Studies have shown that *APOE* genotype can influence the abundance of certain microbial species in the gut in humans and mice.[8] For instance, individuals with the *APOE4* allele tend to have a lower abundance of butyrate-producing bacteria, which are beneficial for gut health and may play a role in AD prevention.[8] Thus, the AD marker *APOE4* might play a crucial role in the complex interplay between the microbiome, the gut-brain axis, and memory decline in AD.[5]

Currently, there is a renewed emphasis on the use of appropriate animal models of AD. Thus far, most AD models use short-lived animals in which an AD-like phenotype is artificially driven via genetic manipulation, which can lead to intrinsic bias and a failure to recapitulate some aspects of the disease.[10] By contrast, long-lived animal models that naturally develop AD-like pathologies in an age-dependent manner offer many advantages in defining AD pathogenic mechanisms, especially for studying the role of the gut microbiome in AD.[11]

The *Octodon degus* (degu) is a long-lived rodent that lives for 9–10 years in captivity and, like humans, has a significant post-fertility lifespan. Age-dependent cognitive performance decline has been reported during natural aging in degus.[12] Some, but not all, degu spontaneously develop AD-like cognitive impairments and neuropathological features as they age, just like humans with AD, including amyloid-beta

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https://doi.org/10.1016/j.isci.2024.110348

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plaques, tau tangles in their brains, and neuroinflammation.[13–18] Cognitive deficits and memory impairment in affected degu are analogous to those in human patients with AD.[19–22] By the age of approximately 4 years, 30% of the degus captured from a natural population show impairments in episodic memory and hippocampal dependent activities of daily living such as burrowing performance, which was correlated with their expression profiles of AD markers.[18,22,23] The degu has thus been proposed as a non-conventional natural model to study AD. To date, the gut microbiome of the degu has not been investigated.

Recently we published the degu whole-genome and reported AD-related genetic variants that may have implications for AD risk and pathology in this model.[17,18] Among the 7 single nucleotide polymorphisms (SNP) detected in the degu *Apoe* gene, one (at position 213, denoted Mt4) is non-synonymous. At this site, there are three possible residues: glutamic acid (Glu: E) which corresponds to the wild type (without mutation); glutamine (Gln: Q), or lysine (Lys: K). Variants E213Q and E213K were associated with mild and severe AD-like phenotype, respectively.[17] Especially, the mutation E213K in the *Apoe* gene may be associated with an increased risk of developing AD-like neuropathology in the degu.[17,18]

The extent to which host genetic variation determines the composition of the gut microbiome remains unclear. Here we report for the first time the gut microbiome composition of 32 degu individuals. Our objective was to investigate the relationships between the degu gut microbiome, their AD-like phenotype, and their *Apoe* genotype, in comparison with the same relationships in human subjects. By revealing that human *APOE4* and degu *Apoe* E213K variants share specific microbiome dysbiosis traits, this work provides further evidence that the long-lived degu is a unique and valid non-conventional model for the study of age-related mechanisms of AD pathogenesis, including gut microbiome dysbiosis, and potentially useful for the further development of drugs to treat this disorder.

RESULTS

Characterization and comparison of degu and human gut microbiota

At the phylum level, the degu gut microbiota was characterized by a dominance of Bacteroidetes comprising on average 55 $\pm$ 6% of the whole bacterial community, followed by Firmicutes (33 $\pm$ 6%), Patescibacteria (5 $\pm$ 3%) and other less abundant phyla (Cyanobacteria, Proteobacteria, Spirochaetes, and Tenericutes) that comprised in variable amounts approximately 7% of the microbiota (Figure 1A). In our human dataset, Firmicutes represented 79 $\pm$ 10% of the community, Bacteroidetes represented 15 $\pm$ 9%, while Actinobacteria, Cyanobacteria, Proteobacteria, Tenericutes, and Verrucomicrobia were present in lower amounts (although Verrucomicrobia relative abundance spiked up to 25% in some samples). As a summary, the analyzed degu microbiota shared a prevalence of Firmicutes and Bacteroidetes with the human gut microbiota but -notably- in reverse proportions and presented higher heterogeneity including high-level taxonomic groups not identified in humans.

Overall, 66% of the identified genera in the degu microbiota were shared with the human microbiota, but only approximately 2% of the degu amplicon sequence variants (ASV) were shared with humans (Figure 1B). The percentage of classified ASV across lower taxonomical levels decayed faster in degu than in humans. Only 58% of the degu ASV have been classified at the genus level, in contrast to 74% of the human ASV, which can be interpreted as a less well characterized community in degu. At the ASV level, the degu microbiome was significantly more diverse than the human microbiome in terms of the Shannon diversity index (Figure 1C). The nonmetric multidimensional scaling (NMDS) analysis revealed strong differences in the gut microbiota structure between the two hosts, with two clearly separated clusters (PERMANOVA, $F = 170.68$, $p = 1.0 \cdot 10^{-4}$) (Figure 2A). The human gut microbiome presented stronger variability across individuals. The variation in the estimated metabolic potential of the degu and human microbiota reflects such compositional heterogeneity (Figure S1). Major specific dissimilarities in the predicted functional content of the microbiota between the two hosts are shown in Figure S2 and include biosynthetic and degradation pathways for nucleic acids, lipids, vitamins, and sugars.

Degu gut microbiota and Alzheimer's disease

Gut microbiome shifts associated with AD-like behavior in degu were compared with those found in human patients with AD. In the degu gut microbiota, significant differences in community structure were associated with AD-like behavior when considering Bray-Curtis distance ($F = 1.44$, $p = 0.043$), but not when bacterial phylogenetic relationships were taken into account based on weighted UniFrac similarity ($F = 1.65$, $p = 0.13$) (Figure 2A), suggesting that changes in community composition mostly involved low-abundance taxa and/or closely related taxa. In contrast, human gut microbiota samples exhibited marked differences between AD and healthy phenotypes in terms of both Bray-Curtis ($F = 2.70$, $p = 1.0 \cdot \times 10^{-4}$) and weighted UniFrac ($F = 4.78$, $p = 2.0 \cdot \times 10^{-4}$) distances. Overall, microbial differences associated with the AD phenotype in the host were smaller in degu than in humans. Gut bacterial diversity decreased in patients with AD compared to healthy controls, but this trend was not significant in AD-like degu (Figure 2B). Moreover, when aggregated at different taxonomic levels from genus to phylum, no significant global changes in taxon abundance could be detected in the degu microbiota in relation to the AD-like cognitive phenotype (data not shown).

The gut responses to AD were further examined at the ASV level. We identified 12 ASV from 6 different families that displayed significant abundance differences in AD-like degu (Figure 2C). The overlap between the shifts in the two host communities extended down to low taxonomic levels (Figure 2D). Remarkably, all the altered ASV belonged to taxa that have been previously linked to aging, age-related disorders, or brain functions in humans or mice (see discussion). The largest number of altered ASV belonged to the *Ruminococcaceae* family (Firmicutes phylum). Some common patterns of ASV alterations were observed between degu and human patients in response to AD, all in the Firmicutes phylum, such as a reduced abundance of *Ruminococcus* and *Ruminococcaceae* (e.g., *Ruminococcaceae*_UCG-014 group) (Figure 2C). Despite being the most abundant phylum, Bacteroidetes comprised a relatively limited number of significantly altered ASV in AD-like

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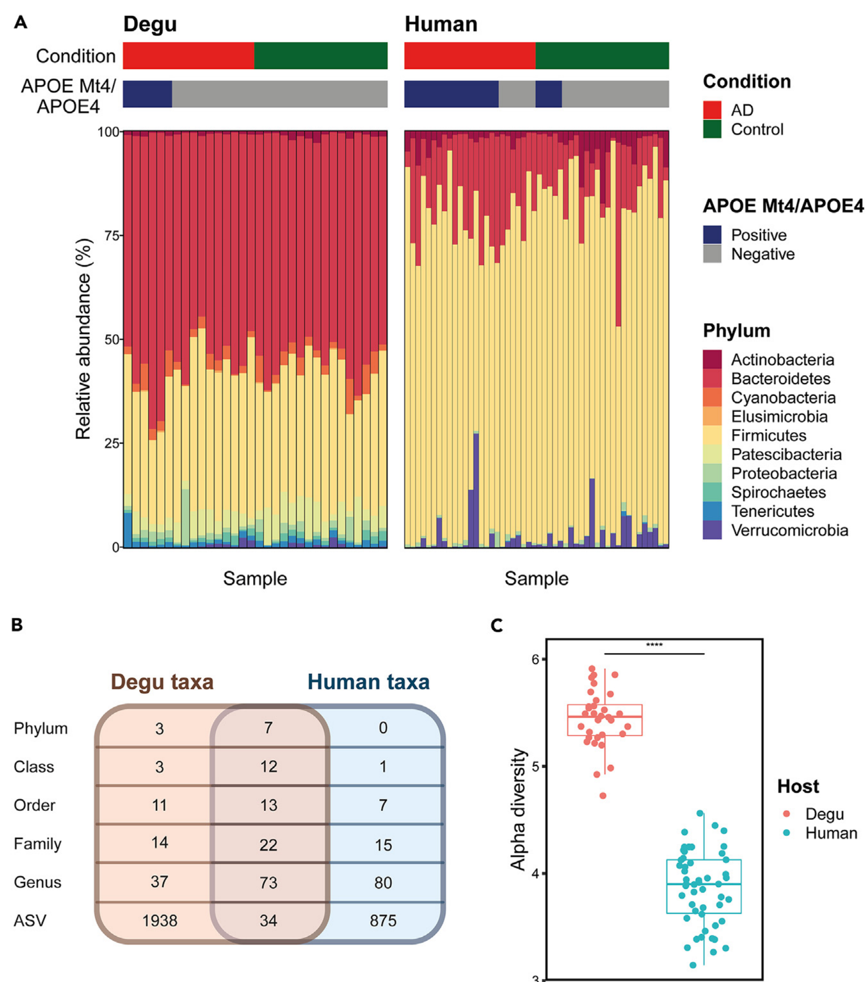

Figure 1. Comparison of degu and human gut microbiota

(A) Relative abundance of identified phyla across samples in degu and humans. The AD phenotype and *APOE* genotype of each sample are indicated as colored bars at the top.

(B) Microbial within-sample (alpha) diversity in degu and human gut bacterial communities calculated as the Shannon index. The significance was evaluated by a two-sided Wilcoxon rank-sum test ($p < 0.0001$).

(C) Number of unique and shared taxa between degu and humans among all taxonomical ranks. The last lane represents all the unique and shared ASV, independent of their taxonomic affiliation.

degu and human microbiota. Altered Bacteroidetes taxa were not the same in both hosts but they all exclusively increased in both hosts (Figure S3). Especially, *Muribaculaceae* and *Prevotellaceae* ASV were significantly over-abundant in AD-like degu microbiota.

In summary, the AD phenotype in the degu microbiota was associated with a global decrease in the abundance of several *Ruminococcaceae* ASV, probably leading to reduced short chain fatty acid (SCFA) production, and a global increase in Bacteroidetes ASV (*Muribaculaceae*, *Prevotellaceae*), which could indicate increased gut inflammation. Some of these patterns were also observed in the human AD patient gut microbiota and matched the findings from a range of previous studies, as further reviewed in the Discussion.

Degu apolipoprotein E E213K mutation is associated with an altered gut microbiota

The gut microbiota associated with the *Apoe* Mt4 E213K genotype (present in six of the 16 AD-like degu) were compared with those from degu without theE213K mutation. The observed microbiota shifts were further compared with the microbiota shifts in 18 human individuals carrying the *APOE4* genotype within the AD cohort.

Microbial differences associated with the *Apoe* E213K genotype were significant in the degu microbiome, considering both Bray-Curtis ($F = 1.94$, $p = 1.3 \cdot \times 10^{-3}$) and weighted UniFrac ($F = 2.69$, $p = 0.03$, Figure 3A) similarities, compared to degu without the E213K mutation. In the human microbiota, such differences were not statistically significant, which could be due to the fact that 20% of the control subjects had APOEε4 genotype yet.[2] A slight decrease in gut microbiota diversity was associated with *APOE4* and *Apoe* E213K mutations in human and degu hosts, respectively, but only significant in human (Figure 3B). Functional changes were predicted in association with the degu *Apoe*

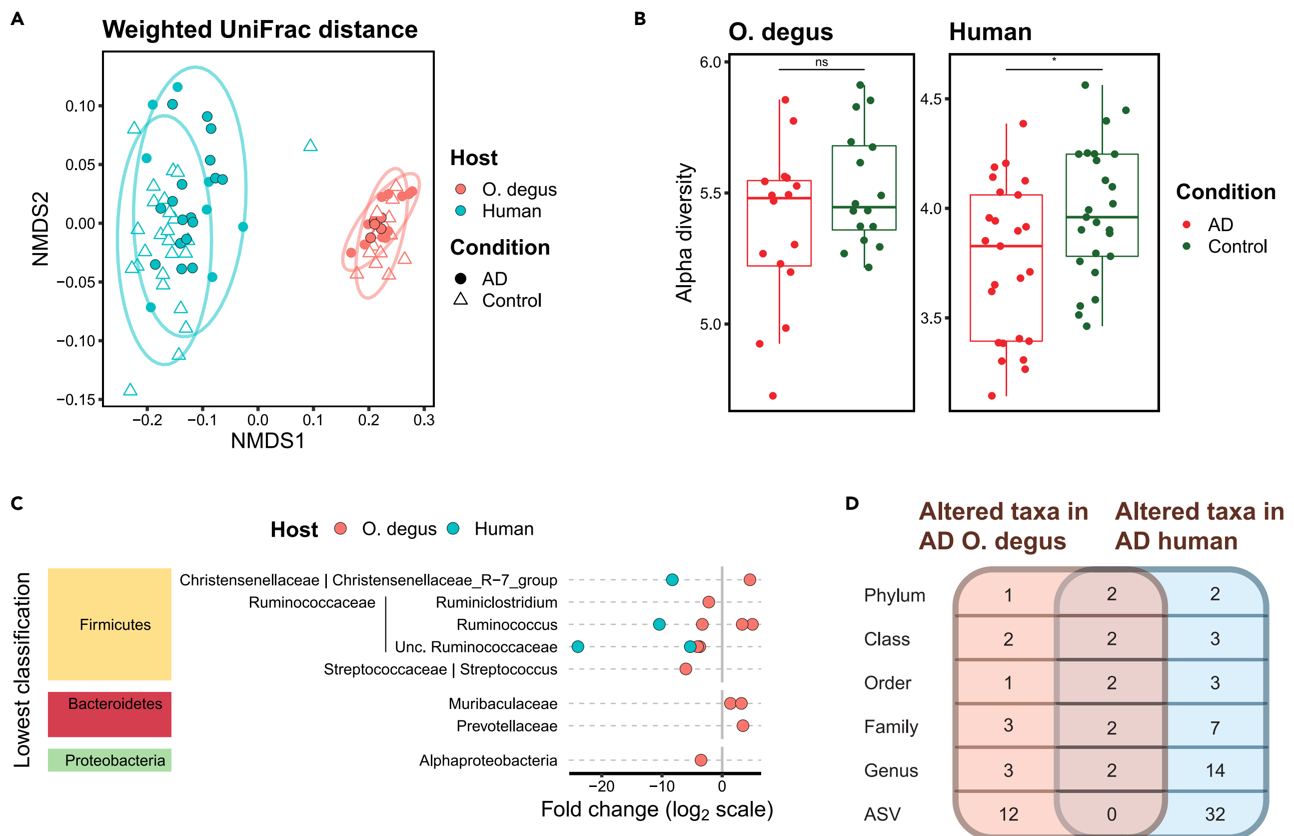

Figure 2. Gut microbiota alterations in AD-like degu involve bacterial groups also disturbed in humans with AD

(A) Nonmetric multidimensional scaling (NMDS) representation of degu (pink) and human (blue) gut bacterial communities based on weighted UniFrac distances (stress equal to 0.06). The shape of the symbols indicates the AD phenotype. Ellipses represent 95% confidence regions of each phenotype-host pair, assuming multivariate t-distributions.

(B) Gut microbiota alpha diversity of AD-like and control degu individuals and of patients with AD and control individuals, calculated as the Shannon index. The significance was obtained by a two-sided Wilcoxon rank-sum test ($p < 0.05$).

(C) Identification of ASV with significant differential abundances ($p < 0.05$) between AD-like and control degus (pink), and between patients with AD and control individuals (blue). The taxonomic affiliation of each ASV is indicated on the left at the lowest available level. For degu, all altered ASV are shown, while for humans only the altered ASV corresponding to the same genus or family as in degu are shown (the complete list of altered ASV in humans is shown in Figure S3).

(D) Number of shared and exclusive significant alterations associated with AD in the degu and human microbiota at each taxonomic level.

E213K genotype across several metabolic pathways (PERMANOVA, $F = 1.82$, $p = 0.046$, Figure S4). In particular, functional classes related to fermentation were strongly reduced in the microbiota of both degu and humans with the *Apoe* E213K/*APOE4* genotype, especially fermentation leading to SCFA production.

The analysis of bacterial differential abundance between *Apoe* mutant status groups in the degu microbiota (i.e., *Apoe* Mt4 E213K genotype versus other genotypes) identified 43 significantly altered ASV. Most of the altered ASV belonged to the *Ruminococcaceae* family and were under-abundant in the *Apoe* E213K degu (Figure 3C), as previously observed for the AD-contrasting analysis (Figure 2C). Most of these altered taxa were identified in other studies in association with AD or other neurodegenerative diseases or associated symptoms, suggesting that they might be involved in the brain-gut axis (see discussion). Specific taxa from Bacteroidetes (especially in the *Muribaculaceae* and *Prevotellaceae* families) and Proteobacteria were enriched in the degu microbiota (Figure 3C).

When ASV were aggregated at higher taxonomic levels, the *Apoe* E213K mutation was linked to a decrease in the abundance of the Verrucomicrobia phylum in the degu microbiota (adjusted $p = 0.048$, Figure 3D) compared to the no-E213K carriers. This observation was caused by a drastic decrease of *Akkermansia* abundance (BH-adjusted Mann-Whitney test, $p = 0.048$), which was the only member of the Verrucomicrobia phylum here. We also found a strong decrease in *Akkermansia* associated with human AD phenotype (Figure S3).

In human *APOE4* patients with AD, 36 significantly altered ASV were identified (Figure S5), 14 of them corresponding to taxa similar to the *Apoe* E213K degu and all belonging to the Firmicutes phylum (Figure 3C). Both hosts presented some common altered taxa in response to the *APOE* mutation genotype (Figure 3E), including a decreased abundance of members of the *Christensenellaceae*_R−7 group, *Ruminoclostridium*, *Ruminococcus,* and unclassified *Ruminococcaceae*, along with an increased abundance of members of unclassified *Lachnospiraceae*. Bacteroidetes ASV also increased in human patients with *APOE4* (but from different families than in *Apoe* E213K degu).

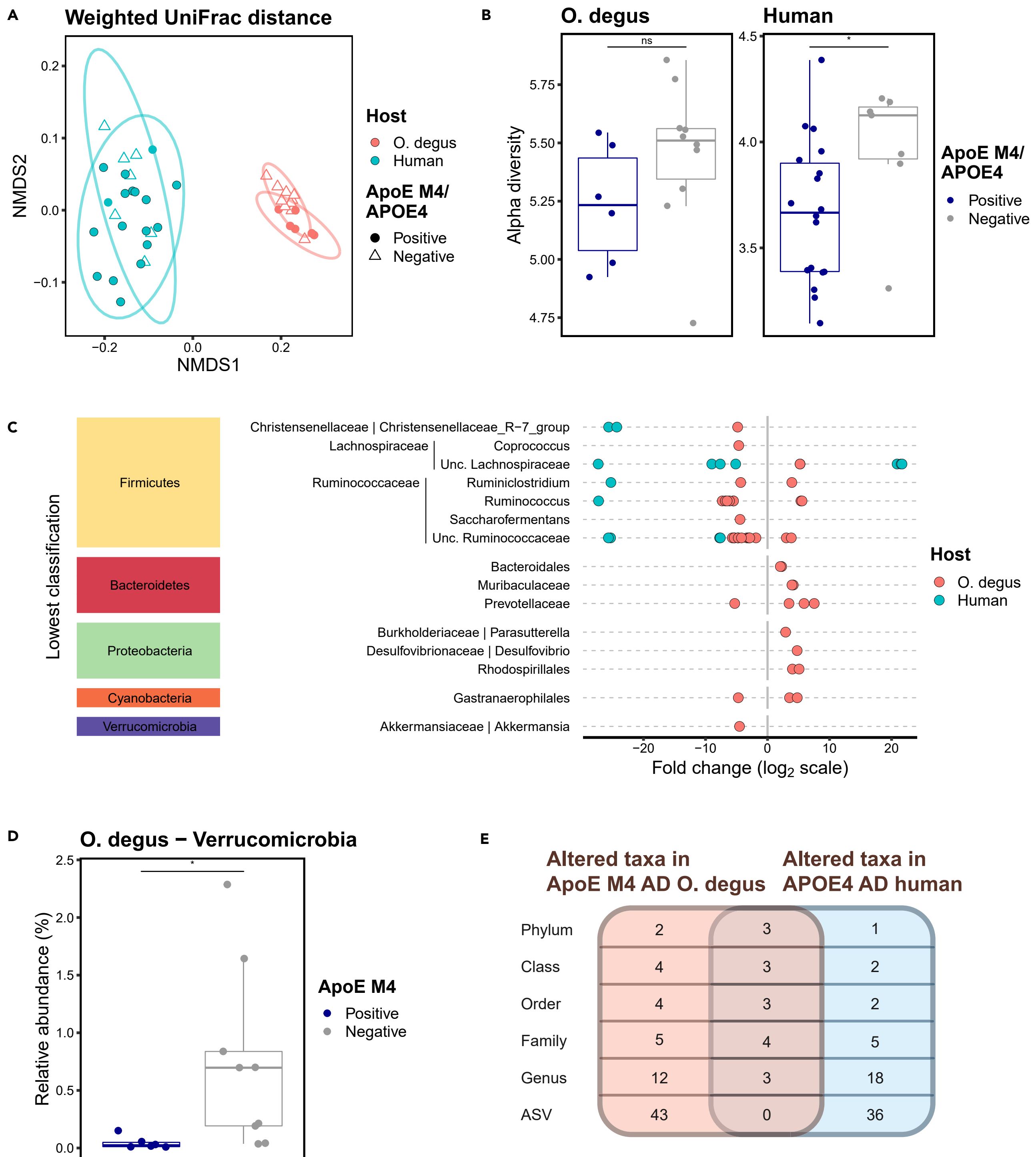

Figure 3. Gut microbiota alterations in *Apoe* E213K AD-like degu match key bacterial 0061lterations in *APOE4* AD humans

(A) Nonmetric multidimensional scaling representation of AD-like degu (pink) and AD human (blue) gut bacterial communities based on weighted UniFrac distance (stress equal to 0.05). The shape of the symbols indicates the *APOE* genotype: *Apoe* Mt4 E213K variant (circles) versus no-E213K carriers (triangles). Ellipses represent 95% confidence regions of each phenotype-host pair subpopulation, assuming multivariate t-distributions.

(B) Gut microbiota alpha diversity of AD-like degu with and without the *Apoe* E213K mutation and of patients with AD with and without the *APOE4* mutation, calculated as the Shannon index. The significance was evaluated by a two-sided Wilcoxon rank-sum test ($p < 0.05$).

Figure 3. *Continued*

(C) Identification of ASV with significant differential abundances ($p < 0.05$) between AD-like degu individuals with and without the *Apoe* E213K mutation (pink) and between patients with AD with and without the *APOE4* mutation that are in the same genera or families (blue). The taxonomic affiliation of each ASV is indicated on the left at the lowest available level. For degu, all altered ASV are shown, while for human only the altered ASV corresponding to the same genus or family as in degu are shown (the complete list of altered ASV in humans is shown in Figure S5).

(D) Comparison of the total abundance of Verrucomicrobia in the microbiota of AD-like degu individuals with and without *Apoe* E213K mutation. The significance was evaluated by a two-sided Wilcoxon rank-sum test ($p < 0.05$), FDR-corrected for multiple testing.

(E) Total number of significant abundance alterations associated with the *Apoe* E213K genotype in degu and with *APOE4* in human for each taxonomic level, distinguishing between alterations that are shared or exclusive for each host.

In summary, in degu, the *Apoe* E213K mutation was significantly correlated with changes in the gut microbiota structure compared to degu without this genotype (i.e., no-E213K carriers), triggering a shift toward the underrepresentation of the *Akkermansia* genus (Verrucomicrobia phylum) and of several Firmicutes members (especially from the *Christensellaceae* and *Ruminococcaceae* families), while specific taxa from Bacteroidetes (especially in the *Muribaculaceae* and *Prevotellaceae* families) were enriched. These trends were globally similar in the *APOE4*-human gut microbiota. Moreover, most of these microbial shifts were common between AD-like and *Apoe* E213K based contrasts in the degu microbiota and have been shown to be involved in gut/brain associations in previous studies (see discussion later in discussion).

DISCUSSION

This work represents the first description of the gut microbiome of the degu, a long-lived diurnal rodent in which AD-like pathology and cognitive decline naturally develop in some outbred individuals. Following early investigations illustrating the relationship of AD symptoms and/or host genetics with gut microbiota,[24,25] we hypothesized that gut microbial alterations should occur in the gut microbiome of AD-like and/or *Apoe* E213K-carrying degu.

The taxonomic composition of the degu fecal microbiota (Bacteroidetes representing more than half the community, and Firmicutes one-third) was globally comparable to the mouse fecal microbiota previously reported by our group[26] and others[27] thus confirming the relevance of this alternative natural model in terms of the comparability and generalization of results. It shows stronger dissimilarities from the mouse microbiota from other studies, composed mainly of Firmicutes (reaching more than 90%) with low Bacteroidetes representation.[28–30] In addition to host specific effects, the distinction between the degu and murine microbiome can also be due to diet and husbandry issues.[27] Fecal microbiota is partially normalized by extended co-housing.[27]

We compared the shifts of the degu gut microbiota associated with AD-like pathology with the human ones. First, as a way to confirm the robustness of our analysis, we found that the gut microbiota composition of our human dataset was globally similar to previous findings from 16S rRNA sequencing analysis of human fecal samples,[2,31,32] and relatively consistent with recent estimates from shotgun metagenomics sequencing,[33] although it is known that Bacteroidetes tend to be more abundant than Firmicutes in the elderly.[34] Second, despite many common genera between human and degu microbiota, there was a very low proportion of common ASV, consistent with the low overlap previously observed between human and other rodent (mouse) microbiota.[28] The differences between the degu and human gut microbiota can be explained by differences in diet, which are among the most important factors underlying gut microbiome structure and diversity in a range of animals.[35] The higher diversity in the degu gut microbiota was consistent with the strict herbivore lifestyle of this rodent and with the generally higher diversity previously observed across different herbivore mammals.[36] Moreover, such diversity is likely attenuated by captivity.[37] The higher homogeneity in the captive degu microbiota can be linked to the homogeneous feed provided during the experiment.

In our human dataset, the AD phenotype and the *APOE* mutation genotype were both associated with a reduced gut microbiota diversity, consistent with previous studies in different models.[38] This observation was not significant in degu. Nevertheless, our analyses revealed specific microbiota signatures in AD-like and *Apoe* E213K carrier degu, many of them being common to those observed in relation to the human AD phenotype and *APOE4* genotype and linked to various AD-associated patterns across bacterial phyla, as follows.

First, the main ASV alteration in AD-like and *Apoe* E213K degu gut microbiome was the under-abundance *Ruminococcaceae* (Firmicutes phylum), similar to what we observed in *APOE4* AD humans. Members of this family, such as *Ruminococcus*, *Ruminoclostridium*, or *Ruminococcaceae_UCG-014*, are known producers of gut-brain signaling molecules and their decline has been previously linked to aging[39] and AD in mice,[30,40] or to other AD co-morbidities, such as gut inflammation and obesity in mice[41,42] and humans.[8,43,44] *Ruminococcaceae* were previously reported as reduced in the gut microbiota of human subjects displaying the *APOE4* mutation[8] and have been linked to the infiltration of peripheral immune cells and neuroinflammation development during AD progression in mouse.[45]

Second, the observed over-abundance of Bacteroidetes in AD-like and *Apoe* E213K degu gut microbiome could mark an increased systemic inflammation, since Bacteroidetes promote the release of proinflammatory cytokines.[40] Among this phylum, the over-abundant *Muribaculaceae* and *Prevotellaceae* have been previously linked to longevity,[29,46] to AD in human patients[47] and to the *APOE4* genotype in humans.[8] In other cases, *Prevotellaceae* has been associated to *APOE3* genotype,[48] highlighting the inconsistency of some observations, which may reflect differences in models, age, husbandry method, lineage and/or diet.[9] *Prevotellaceae* might play a role in mucin synthesis and/or degradation and mucosal permeability, thus modulating the exposure of the host to bacterial toxins.[8]

Third, *Akkermansia* under-representation was a significant signature of the *Apoe* E213K degu gut microbiome in this study. *Akkermansia* is usually considered as a health-associated genus, positively correlating with age[39] and is believed to be protective against metabolic disorders through its excretion of endocannabinoids that control inflammation, gut barrier function, and gut peptide secretion.[49] The reduction in *Akkermansia* abundance has been previously linked to AD in transgenic AD mouse models[40] as well as to potential risk factors for AD, such as

atherosclerosis,[40] obesity, type 2 diabetes[50] and progeria[51] in mice, and to obesity in humans.[52] In contrast with our observations in *Apoe* E213K degu, a decrease in *Prevotella* abundance and an increase in *Akkermansia* abundance have been reported in mouse models with neurodevelopmental disorder linked with autism (fragile X syndrome) compared with wildtype,[26] suggesting some specificities linked to AD.

Finally, analysis of the metabolic potential of the degu *Apoe* E213K fecal samples showed significant differences in microbe-associated amino acids and SCFA compared to degu without the mutation. The reduced representation of SCFA-producing pathways in the *Apoe* E213K degu microbiota could be linked to the global under-abundance of *Ruminococcaceae* members, which are known producers of SCFA through the fermentation of complex plant polymers.[53] SCFA are known to directly or indirectly modulate gut–brain interactions via immune, endocrine, vagal or other pathways.[54–56] Microbiota-derived SCFA are important signaling mediators of gut-brain interactions and are involved in the occurrence and development of many neurodegenerative diseases, including AD[57] especially by promoting Aβ plaque deposition[58] but some of them can play either beneficial or harmful role.[59]

Altogether, our results further support the existence of common mechanisms of microbiota dysbiosis in AD-like degu and human AD subjects, making the degu an interesting model for exploring the gut-brain axis in the context of genetic risk factors for AD, and increasing the likelihood that findings from natural models can translate to human AD. By understanding the role of the microbiome in this natural AD model, we can gain valuable insights into the underlying mechanisms of AD pathology. Moreover, this could have implications in the design and testing of microbiota-targeted interventions or transplantations focusing on the role of SCFA as potential mediators with the brain affecting cognitive functions.[54]

Limitations of the study

The first limitation of this study refers to targeting the V4 region of the 16S rRNA gene for gut microbiome analysis, which might not capture the whole microbial diversity, especially the Archaea. Nevertheless, this possible bias is limited due to the high coverage of the selected primers. In addition, inferring potential metabolic functions from the taxonomic composition of a community is a controversial approach due to the lack of pure culture representatives for most community members. Another limitation is related to the captivity of the degu which might modify their behavior as well as their gut microbiome composition, in relation to their diet, compared to wild ones. In our approach, we analyzed fecal samples and considered them representative of the gut microbiome. The age difference between the degu and humans used in this study could be considered as a possible limitation; however, there is no direct equivalence between rodent and human age, due to differences in lifespan and developmental trajectories. Generally, rodents mature much more rapidly than humans, but various factors such as size, metabolism, and growth rates influence the aging process differently in rodents and humans. There are no published works about the physiological, cognitive, or biomarkers basis that would enable to correlate degu age with humans. Age and sex may play an important role in gut microbiota composition and AD development. The human data from Vogt's study (Vogt et al., 2017) involved 70% female participants. However, despite this unbalanced gender representation, the human controls were age- and sex-matched with the AD subjects, which is the important point to consider when comparing AD and non-AD subjects. The influence (or association) of sex, gender, or both on the results of this work is beyond the scope of our study. Finally, our results suggest that *APOE* genotype and microbiome composition can interact to ultimately influence AD pathology in the brain, perhaps through altered SCFA production leading to increased neuroinflammation through altered microglia function.[60] However, further studies should evaluate the SCFA concentration and pro-inflammatory cytokine levels in AD-like E213K degu to further elucidate the role of the microbiome in AD pathogenesis.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110348.

ACKNOWLEDGMENTS

PC was funded by ANID-FONDECYT project n°1200928. GZ and CA are grateful for support from Research England's THYME project. We sincerely thank all the Genetics Lab team.

AUTHOR CONTRIBUTIONS

PC designed and supervised the study and acquired fundings. GZ, CU, and RD performed experiments and collected the data. GZ performed the sequencing analysis and illustrations with the collaboration of LC's guidance. LC, MH, CA, ECN, GS, DC, and PC participated in the result interpretation. GZ and LC wrote the original draft. All authors contributed to review and edit the final article version and approved the submitted version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: April 10, 2024
Revised: May 22, 2024
Accepted: June 20, 2024
Published: June 22, 2024

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Octodon degus faeces	This study	N/A
Critical commercial assays		
Qiagen PowerSoil DNA Isolation Kit	Qiagen	Cat#47014
Qubit dsDNA BR Kit	ThermoFisher	Q32850
Deposited data		
Octodon degus gut microbiome data	This study	NCBI BioProject PRJNA643874
Octodon degus host genomic data	Hurley et al., 2022[17]	NCBI BioProject PRJNA623609 and NW_004524773.1
Human gut microbiome data	Vogt et al., 2017[2]	Have been kindly provided by Vogt et al., 2017, upon request
Experimental models: Organisms/strains		
Octodon degus specimen	This study	N/A
Oligonucleotides		
Primer 341f: CCTACGGGNBGCASCAG	Takahashi et al., 2014[61]	N/A
Primer 806bR: GGACTACNVGGGTWTCTAAT	Apprill et al., 2015[62]	N/A
Software and algorithms		
Original R code	This study	https://github.com/gzampieri/Degu_gut_microbiome
DADA2	Callahan et al., 2016[63]	https://github.com/benjjneb/dada2?tab=readme-ov-file
DECIPHER	Wright ES, 2016,[64]	https://bioconductor.org/packages/release/bioc/html/DECIPHER.html
Other		
Commercial rodent diet	Prolab®	5P76, IsoPro® RMH 3000

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources involved in this study should be directed to and will be fulfilled by the lead contact, Patricia Cogram (patricia.cogram@gmail.com).

Materials availability

This study did not generate new unique reagents and materials.

Data and code availability

- The raw 16S rRNA gene sequences of gut microbiome generated in this study have been deposited on NCBI BioProject PRJNA643874 and are publicly available as of the date of publication. Accession numbers are listed in the key resources table. Additionally, this paper analyses two sets of existing and publicly available data. First, the degu genomic information (raw sequencing data and ApoE sequences), previously published,[17] is available at BioProject PRJNA623609 and NW_004524773.1, respectively. Second, even if the present study did not directly work with human subjects, the human subject data were previously published,[2] where all participants provided written informed consent to be involved in the study. The human gut microbiome sequences have been complimentary provided by Vogt and collaborators upon our request.
- All original code has been deposited at github repository (https://github.com/gzampieri/Degu_gut_microbiome) and is publicly available as of the date of publication. URLs are listed in the key resources table.
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animals

The analysis was performed on 32 degu (16 normal, 16 AD-like) taken from our outbred degu colony that is derived from wild caught animals. Behavioral assessment of the degu was performed to distinguish AD-like from normal degu using the burrowing test as previously described.[22] Degu were housed in standard metal cages (50 × 40 × 35 cm) with a layer of wood shavings as bedding, containing a small metal nesting box (25 × 15 × 10 cm with a single entrance) under a controlled photoperiod (7 am -7 pm) and temperature (23°C). Water and a commercial rodent diet (Prolab RMH 3000, USA) were provided *ad libitum*. All animals used were males aged approximately 4.5 years. Degu with different *APOE* genotypes were kept in the same cages. By using a cohort of non-family-related and not-crossed individuals from different backgrounds, the study aims to capture a broader representation of genetic variability and environmental influences, mirroring the heterogeneity observed in humans. Ethical approval for this project was provided by the Institute of Ecology and Biodiversity Ethics Committee and the experiments were performed in accordance with the UK Scientific Procedures Act (1986) and the NIH Guide for the Care and Use of Laboratory Animals (1978). All animals were handled consistently in accordance with ARRIVE guidelines.

Degu genomic information

The *Apoe* sequences of the 32 degu individuals are available at NW_004524773.1.[17] The degu were heterozygous for the *Apoe* genotype. There are seven single-nucleotide polymorphisms (SNP) in the degu *Apoe* gene compared to the reference sequence. One non-synonymous missense SNP in exon 3 (E213K) denoted Mt4 was associated with late-onset AD-like phenotypes in this model.[17] Among the 16 degu with AD-like behaviour used in the present study, six had the *Apoe4* Mt4 E213K genotype. Raw sequencing data can be accessed at BioProject PRJNA623609.

METHOD DETAILS

Human and degu gut microbiome sequencing

DNA was extracted from 0.25 g of faeces per animal using Qiagen PowerSoil DNA Isolation Kit following the manufacturer's instructions. DNA was quantified with the Qubit dsDNA BR Kit. The hypervariable regions V3 and V4 of the 16S rRNA gene were amplified by PCR with universal primers 341f (5′-CCTACGGGNBGCASCAG-3′) and 806bR (5′-GGACTACNVGGGTWTCTAAT-3′). The amplicons were sequenced on an Illumina MiSeq platform with 2 x 300 nt paired-end sequencing with V3 chemistry. Raw sequences are available on NCBI BioProject PRJNA643874.

In parallel, a 16S rRNA sequence dataset from the faeces of 25 human AD patients and 25 healthy age- and sex-matched asymptomatic control individuals (without dietary differences) was obtained from a previous study.[2] The human subjects were categorized as non-carriers (zero ε4 alleles) or APOE ε4 carriers (one or two ε4 alleles). The data were generated by sequencing the V4 hypervariable region of the 16S rRNA gene using the Illumina MiSeq platform and were reanalysed in our study through an updated pipeline.

Bacterial community data processing

All the 16S sequencing data were processed and analysed in R v3.5. The rodent paired-end 16S reads were trimmed and filtered using the R package DADA2[63] based on Phred quality profiles, setting the maximum number of N bases to 0, the maximum number of expected errors to 2 and truncation quality threshold to 2. This was followed by de-replication and error correction through DADA2 using the default error estimation function. After merging paired reads, chimeric sequences were identified and removed with DADA2. This procedure yielded approximately 6,000 unique ASV for the 32 degu samples. In parallel, we used the same workflow to re-process human reads provided by Vogt and co-workers (2017),[2] obtaining approximately 4,000 ASV from their 50 samples. To merge and statistically compare the two sets of sequences, we trimmed degu ASV so that they extended over the V4 region only, cutting off the V3 region using DECIPHER[64] and thereby reducing the number of unique ASV to roughly 3,600. The subsequent merging of human and rodent sequences resulted in nearly 7,300 ASV, which were further filtered by using phyloseq[65] based on their taxonomical classification and on their abundance within and across samples. The taxonomical affiliation was obtained by alignment of the identified ASV against the SILVA version 132 16s rRNA gene reference database.[66,67] After collapsing the resulting taxonomy to the phylum level, ASV assigned to extremely rare phyla (defined as the phyla whose ASV were observed in fewer than 3 samples on average and whose absolute frequency sum over all ASV was lower than 30) were removed. Additionally, we eliminated potentially spurious taxa by selecting ASV present in at least 7 samples out of the total 82. Upon the whole taxa filtering process, we finally obtained a total of 2,847 ASV that were used in the subsequent statistical analysis.

Analysis of bacterial community composition

Standard metrics for richness and alpha diversity (ACE, Chao1, Shannon and inverse Simpson indices) were calculated with vegan.[68] Beta diversity between communities was quantified by different distance metrics, either Bray Curtis (computed in vegan) or unweighted and weighted UniFrac distance, which captures the phylogenetic relationship among ASV.[69] UniFrac distances were computed starting from a neighbour-joined phylogenetic tree estimated via RaxML v8.2.10 and its R interface and were weighted by the ASV abundance proportions.[70] Summarisation and visualisation of beta diversity were carried out via non-metric multi-dimensional scaling (NMDS) through the phyloseq package.[65]

Statistically significant pairwise differences in ASV abundance between conditions were identified using the DESeq2 package, which tests for the significance of log_2 fold changes between relative abundances in two conditions using a negative binomial generalised linear model.[71] This approach can effectively cope with library size heterogeneities and biological variability, leading to improved differential abundance detection compared to normalisation and rarefying.[72]

Estimation of the functional potential of bacterial communities

We conducted a metabolic potential analysis as implemented in PICRUSt2,[73] using taxonomic assignments and ASV from DADA2. Inferences were made from MetaCyc pathway databases under default parameters.[74,75] Our degu and human microbiota samples overall present a nearest sequenced taxon index (NSTI) of 0.31 $\pm$ 0.08, indicative of well-characterised communities[73] and supporting the soundness of estimated functional profiles.

The principal component analysis (PCA) of MetaCyc pathway relative abundances was carried out by the FactoMineR package for R.[76] Specific functional differences between sample groups were conducted through the linear discriminant analysis (LDA) effect size (LefSe) method[77] as available in the galaxy framework[78] at http://huttenhower.sph.harvard.edu/galaxy/. Normalised MetaCyc pathway abundances were aggregated based on the MetaCyc ontology, and subsequently analysed with LefSe to identify biological classes most likely to explain differences between sample groups (host, AD phenotype and APOE4 mutation status). The samples were sub-grouped by phenotype and *APOE* genotype in the contrasts between hosts and AD status, respectively.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical differences in richness and diversity distributions were estimated with two-tailed Mann-Whitney U tests through the *stat_compare_means* function in *ggplot2*.

Statistical differences between beta diversity estimates were obtained by permutational multivariate analysis of variance (PERMANOVA) while ensuring the absence of variance inhomogeneity by permutational analysis of multivariate homogeneity of variances (PERMDISP2) through the vegan package.[68]

The significance of differences in ASV abundance between conditions was established based on a 0.05 threshold on Benjamini-Hochberg adjusted *P*-values. To determine overall taxa differential relative abundance at higher taxonomic levels, we used Wilcoxon rank-sum tests with Benjamini-Hochberg correction for multiple hypotheses over each considered taxonomical level (phylum, class, order, family and genus).

For the analysis of the potential functions of the community, the *P*-value thresholds for the factorial Kruskal-Wallis test among classes and for the pairwise Wilcoxon test between subclasses were set to 0.05. To identify biological classes most likely to explain differences between sample groups (host, AD phenotype and *APOE4* mutation status), the threshold for the logarithmic LDA score was set to 1.0 (except for the comparison between hosts where it was set to 2.0).