Gut microbiota and aging

F. MANGIOLA, A. NICOLETTI, A. GASBARRINI, F.R. PONZIANI

Division of Internal Medicine, Gastroenterology and Hepatology, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy

Abstract. – The hypothesis of an important role of gut microbiota in maintaining physiological state into the gastrointestinal (GI) system is supported by qualitative and quantitative alteration of the intestinal flora in a number of physiological and pathological condition as shown in several studies. The evidence of the inflammatory state alteration, highlighted in neurodegenerative diseases such as Parkinson's and Alzheimer's strongly recalls the microbiota disturbance, highly suggesting a link between the gastrointestinal system and cognitive functions. Given this perspective, looking at the mutual influence between microbiota products, inflammation mediators and immune system, the modulation of gut microbiota may help to facilitate a physiological and non-pathological aging process and, perhaps, to contrast the progression of degenerating mechanisms. Some studies have already characterized gut microbiota in elderly, with promising results. Future studies should be designed to better understand the correlation between the gut microbiota, the ageing process and degenerative diseases typical of the elderly.

Key Words:
 Gut microbiota, Aging, Inflammaging, Neurodegenerative diseases.

Introduction

Human beings are superorganism, consisting in host cells and microbes. The intestine houses most of these microorganisms that are called "gut microbiota" and include more than 15000 kinds of bacteria, for a weight equal to 1 kg[1].

Two bacterial phyla, *Firmicutes* and *Bacteriodetes,* are the most represented microorganisms within the human gut microbiota[2], but it is also possible to recognize Archaea, Eukaryotes, Fungi, and many viruses and bacteriophages[3-6]. At present, our knowledge mainly concerns the sphere of bacteria, which will then be mostly considered in our discussion.

The microbiota is also composed by fungi and viruses. The composition of this microbial populations has not yet well characterized as the bacterial population, even if numerous studies have been conducted in *in vivo* and *in vitro* models. Furthermore, it has been demonstrated a predominance of the taxa of the genus *Candida* with about 160 species, with particular abundance of *C. albicans*, *C. glabrata* and *C. parapsilosis*[7-10].

The gut microbiota changes markedly from the duodenum to the ileum, and the microbial load increases further up to 10^{12} germs within the colon[11]. This is due to the increase in pH, gastrointestinal transit, availability of nutrients, mucin secretion, immune function, host's age and health[12-14].

The functions exerted by the gut microbiota are varied. It promotes the constitution of intestinal barrier and its maintenance through the production of short chain fatty acids (SCFAs); these molecules exert a trophic action on enterocytes and stimulate mucus production[15,16]. The gut microbiota is also involved in the immune system priming, as it triggers innate immune response in early life leading to maturation of gut-associated lymphoid tissue (GALT) and shaping adaptive local and systemic immunity[17].

In adult life, the immune system is continuously kept under stimulation by gut microbiota, leading to a state of "low-grade physiological inflammation"[18], which is a rapid and effective mechanism of defense against pathogens. Also, the gut flora exerts its protective role competitively metabolizing those nutrients necessary for survival of pathogens, and producing molecules that inhibit the growth of these microbes[19].

The confirmation of the key role of the gut microbiota in the maintenance of host health is supported by several studies that correlate its qualitative and quantitative alterations with various gastrointestinal and systemic disorders[16,20-27]. Nevertheless, it is not surprising that changes in the gut microbiota have been asso-

Corresponding Author: Francesca Mangiola, MD; fra.mangiola@gmail.com

ciated with physiological conditions, such as aging, characterized by a reduced performance of the human organism.

Aging as an Inflammatory Condition

Aging is defined as a genetically-determined and environmental-modulated process that leads to a generalized decline of physiological functions. The gastrointestinal tract is involved in this process, undergoing degeneration of the enteric nervous system (ENS), alteration of intestinal motility and of the mucosal barrier with relative reduction of the defense function, favoring the development of gastrointestinal pathologies[28,29]. The alteration of these functions is associated with the change of the gut microbiota with consequences on the gut-brain axis.

Immunosenescence, defined as a deterioration of the immune system, plays a key role in the aging process. Through this mechanism, the establishment of a state of basal inflammatory activity seems to be correlated with both morbidity and mortality in the elderly people, being a major risk factor for most of the age-associated

pathologies[30]. The term "inflammaging" has been first coined by Franceschi et al[31] to describe a low-grade pro-inflammatory state characteristic of the aging process. Macrophages, cellular stress and genetic factors are the mainly involved mediators (Figure 1).

This pro-inflammatory state is characterized by the increased expression of cytokines such as IL-6 and TNF-α, and the activation of transcription factors such as NF-kB. This condition has been hypothesized to predispose the organism to the development of various diseases related to age, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, atherosclerosis, heart disease, age-related macular degeneration, insulin resistance and type 2 diabetes, osteoporosis, cancer and other diseases[32-35]. Conversely, centenarians show high levels of both pro- and anti-inflammatory mediators that may delay disease onset[36].

This low-grade inflammation also induces an anti-inflammatory response sustained by cortisol, which elicits insulin resistance and
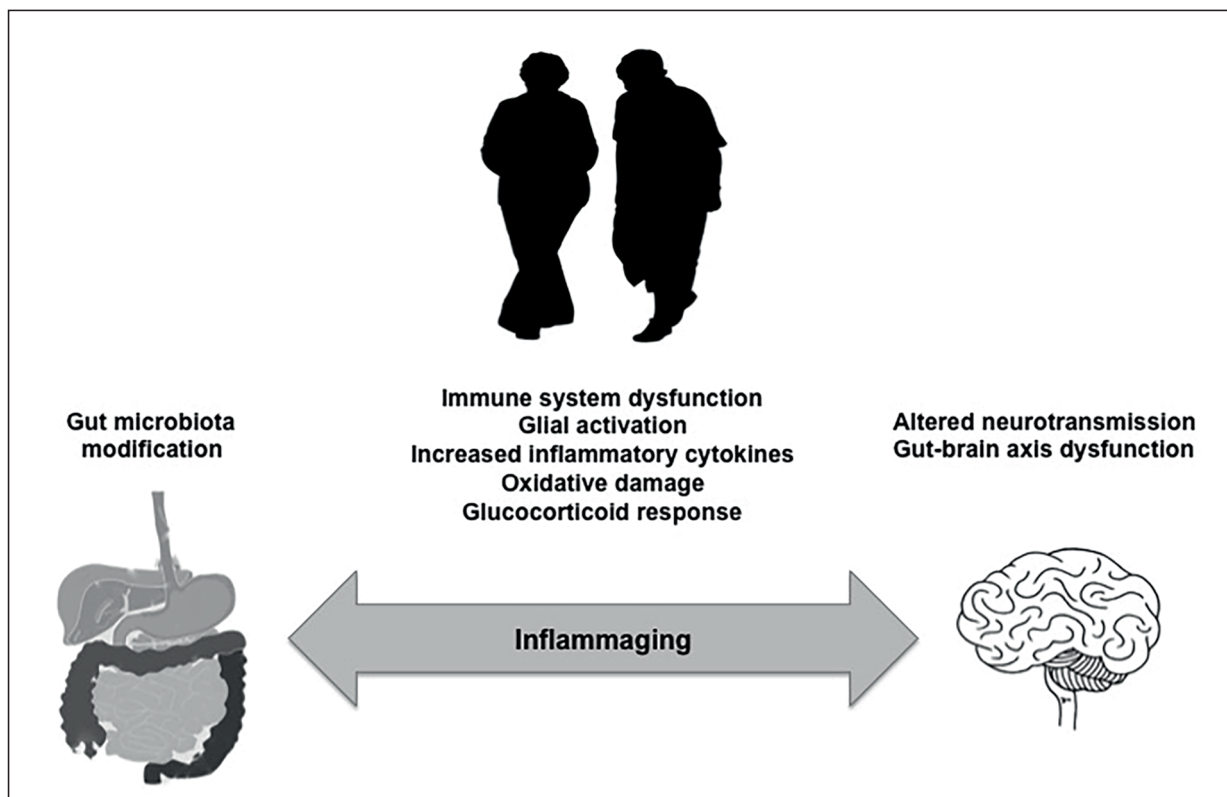

Figure 1. The inflammaging process in the elderly is associated with decline in physical and cognitive functions. The gut microbiota is involved in this process through the modulation of the immune system and brain function.

hepatic production of hormone-binding proteins, including cortisol binding globulin. This process results in cortisol inactivation and in the vanishing of the anti-inflammatory process due to the paradoxical coexistence of immunodeficiency and inflammation[34,35]. Over time, the anti-inflammatory response causes a marked reduction of immunological functions; this condition and the concomitant increase of circulating pro-inflammatory cytokines have a negative impact on metabolism, bone density, strength, exercise tolerance, vascular system, cognitive function, and mood[31], resulting in the inflammaging process.

Gut Microbiota and Aging

Microbial colonization of the human body begins before birth, and increases after delivery when the baby comes in contact with several microbial communities, including mother fecal, vaginal and skin microbiota[37]. Then, the composition of the gut microbiota is influenced by age, sex, development of the immune system and environmental factors, becoming more stable between 6 and 36 months of age[25,38,39]. Thereafter, it is possible to distinguish a constant endogenous flora, which is considered the "core microbiota", and bacteria that are still provisional, highly sensitive to external perturbations.

The age-related changes of the intestinal flora begin after a genetically and environmental determined age, depending on individual characteristics related to race and ethnicity, drugs, life style and diet habits[40]. Most of the members of the genus *Firmicutes* and *Bacteroidetes* remain dominant, although *Firmicutes* microorganisms are predominant in adults, and *Bacteroidetes* in the elderly. What mostly characterizes the gut microbiota composition in the elderly is a decreased diversity, a reduced abundance of species producing butyrate, and the presence of potential pathogens in the centenarians[13,41-49].

The production of SCFAs has been regarded as a central point in the relationship between the host and the gut microbiota, explaining the correlation between alteration of the flora and increase of frailty in the elderly.

Acetate, propionate and butyrate exert a modulating activity on the gut microbiota through the reduction of pH, the protective effect against the overgrowth of pathogens such as *Escherichia coli*, and the stimulation of the growth of beneficial bacteria belonging to the phylum *Firmicutes*[50-52].

Moreover, SCFAs have systemic actions, such as the modulation of intestinal transit time and insulin response, which is closely related to the metabolic syndrome[53].

Such characteristics of these compounds are permitted by the ability to influence cell proliferation and differentiation and hormone production[54,55]. This has been demonstrated also in colon cancer cell lines, where they have been shown to induce cell death by inhibiting histone deacetylase[56,57]. This feature of SCFA explains their role in modulating the immune response by inhibiting the production of inflammatory mediators such as TNF-α, IL-6, and NO or by promoting IL-10 production[58].

Biagi et al[59] reported the gut microbiota of the elderly has a peculiar composition. This study has been conducted in a restricted area of Italy, comparing four groups: 21 subjects aged 99 to 104 years, 22 aged 63 to 76, 20 aged 25 to 40 years and finally 21 offsprings of centenarians aged 59 to 78.

Results showed that bacterial diversity is low: in particular, *Bacteroidetes* and *Firmicutes* still dominate the gut microbiota of extremely old people, but the relative proportion of *Firmicutes* subgroups changes: *Clostridium cluster XIVa* is decreased, whereas an increase in *Bacilli*, and a rearrangement of the *Clostridium cluster IV* have been observed. Moreover, the gut microbiota of centenarians is enriched in *Proteobacteria*, a phylum including many pathobionts, or potentially pathogenic bacteria, while several butyrate producers can be found in lower amounts (*Ruminococcus obeum*, *Roseburia intestinalis*, *Eubacterium ventriosum*, *Eubacterium rectale*, *Eubacterium hallii*, all belonging *Clostridium cluster XIVa*, *Papillibacter cinnamovorans* and *Faecalibacterium prausnitzii* of the *Clostridium cluster IV*). Interestingly, *Faecalibacterium prausnitzii* is a species protective against gut inflammation. Conversely, the butyrate producers *Anaerotruncus colihominis* (*Clostridium cluster IV*), and *Eubacterium limosum* (*Clostridium cluster XV*) increase. This probably suggests that specific components of the gut microbiota are characteristic of the long life. A decrease in Bifidobacteria and increased levels of the mucin degrading *Akkermansia muciniphila* have also been detected in aged people compared to the young adults. As already discussed, the study also included a group of offsprings of the centenarian patients. Although the overall structure of the gut microbiota was similar to that of other subjects

of matching age (70 years-old in average), those offsprings who lived in the same house with their centenarian parent showed an increased occurrence of opportunistic or potentially pathogenic bacterial groups, with respect to those who did not share the living environment with their old parent. Therefore, co-housing can influence the composition of the gut microbiota in these subjects.

Factors Affecting the Gut Microbiota in the Elderly

Diet is one of the most well-known factors potentially able to change the composition of the intestinal microbiota and this has been confirmed in old subjects[29,60-67]. With aging, the diet undergoes drastic changes that can be attributed to the loss of taste and smell and difficulty in chewing. These conditions often lead to prefer foods rich in sugars and fats, reducing the intake of foods of plant origin[68]. Recently, the Healthy Food Diversity (HFD)[69], which values healthy foods within the diet, has been created. The ELDERMET consortium study confirmed the association between microbial components and a diet with low HFD score[70]. Claesson et al[70] observed an association between microbial diversity, the functional independence measure (FIM) and the performance in daily routine activities evaluated according to the Barthel index in elderly people in relation to their condition of stay in community, in day-hospital, in rehabilitation or in long-term residential care. In particular, changes in the dietary habits of the institutionalized elderly with respect to the community dwelling ones were found to affect the gut microbiota composition: a less diverse diet was linked to reduced gut microbiota diversity.

In another work[71], age-related differences in the gut microbiota composition between healthy subjects form different European Countries show a characteristic colonization pattern. This may be associated with country specific dietary habits and lifestyle, thus emphasizing the importance of not generalizing about the gut microbiota structure of subjects from different countries.

However, the diet is not the only factor involved in the variation in the composition of the microbiota in the elderly[72]. In the research by Claesson et al[70], the decrease in microbial diversity correlated not only with diet but also with increased frailty, inflammatory markers and impaired health parameters. Residence location and antibiotic treatment also had a role in the modulation of gut microbiota. Indeed, among patients on antibiotic treatment, the highest abundance of *Bifidobacteria* was found in the community-dwelling group and the lowest in those in long-term residential care. In the antibiotic-untreated group, *Lactobacilli* were higher in rehabilitation (hospital stay < 6 weeks) as compared to long-stay or community-dwellers. In a study conducted in Ireland on 161 healthy people aged 65 years or more[73], in most subjects the intestinal microbiota was distinct from that of the younger adults, with a composition that appeared dominated by the phylum *Bacteroidetes* followed by the *Firmicutes*, with inverse percentages compared to those found in younger adults. *Faecalibacterium* represented the most abundant genus with a prevalence of 6%, followed by *Ruminococcus, Roseburia and Bifidobacterium* (the latter around 0.4%).

These investigations have demonstrated that variability of microbial communities composition in elderly subjects may be due to several factors, such as the increased morbidity associated with age, the use of medications, and lifestyle changes (Figure 2).

Finally, it should be noted that some changes in the bacterial composition are less susceptible to external factors and may probably represent the core features of the gut microbial community in the elderly. Indeed, the decrease of *Ruminococcus* and *Blautia spp.* and of other butyrate-producing microorganisms (*Clostridium cluster XIVa* and *Clostridium cluster IV*) reported across studies in the elderly compared to young subjects seem to be less affected from diet[59,70,74]. Another frequent observation is the increasing prevalence of facultative anaerobes such as *Escherichia coli* with aging and inflammation[71]. Probably, modifications of the immune system function associated with immunosenescence overcome the variability related to dietary habits in determining these changes[30,75,76].

Gut Microbiota and Inflammaging

Recent evidence[77] suggests a role of the gut microbiota in the induction and maintenance of the inflammaging process (Figure 1).

Fransen et al[78] analyzed the effects of transferring the gut microbiota of elderly mice to young germ-free mice on the main lymphopoietic organs such as spleen, lymph nodes and small intestines. An important connection was found between the structure of the gut microbiota and inflammaging; in particular the lack of *Akkermansia* and the increased abundance of *TM7* bacteria and Proteobacteria was associated with the
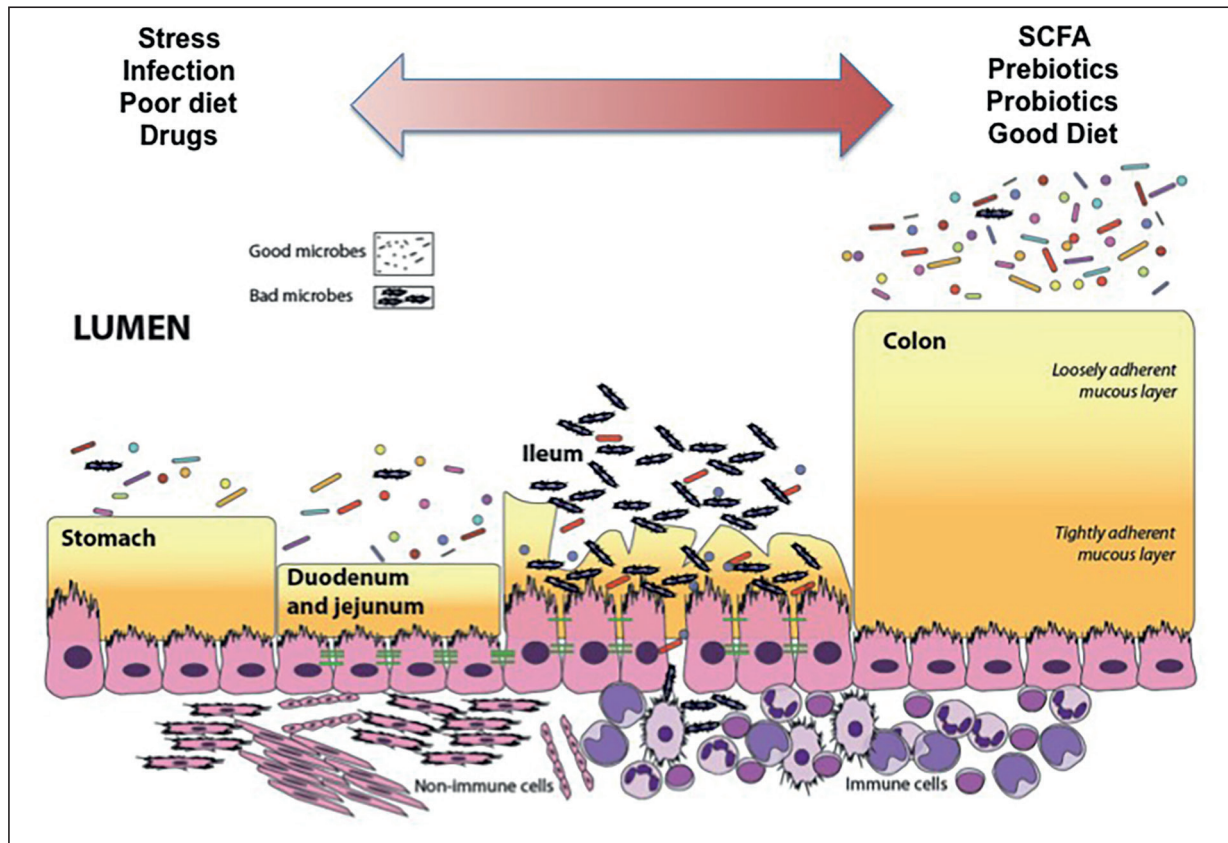
Figure 2. Gut microbiota modulation depends on several factors. Frailty, dietary habits, drugs (mainly antibiotics), and infections are associated with intestinal bacteria composition in the elderly. Conversely, a favorable microbial community may be selected through dietary implementation of fibers, or the use of pre- and pro-biotics.

activation of the local and systemic inflammatory response. This gut microbiota structure promoted small intestinal inflammation and the activation of T cells in the systemic compartment.

Inflammaging is correlated with the inflammatory response induced by lipopolysaccharide (LPS), a component of the Gram-negative bacteria wall.

In the colon of elderly mice, the expression of p16, a senescence marker, and of the sterile α-motif domain- and HD domain-containing protein 1 (SAMHD1), a regulator of cells cycle, was increased, as well as the *Firmicutes* to *Bacteroidetes* ratio and levels of circulating LPS; in addition, the nuclear factor kappa B (NF-kB) was activated. The lipopolysaccharide fraction of fecal lysates (LFL) was able to induce the expression of NF-kB and SAMHD1 in Toll-Like Receptor 4 (TLR-4) wild-type mice, but not in TLR-4 deficient mice. Similarly, p16 was induced more potently in TLR-4 wild-type mice than in knock-out mice[79].

The alteration of inflammatory cytokines associated with changes in the gut microbiota can also module the function of the central nervous system, in particular of microglia. Germ-free mice show altered maturation and global defect of the microglial system; nevertheless, temporary complete or partial gut decontamination can affect microglial function[80,81]. SCFAs are key regulators of the microglia integrity. This is extremely relevant in the elderly, and contributes to the cognitive decline[82,83].

Furthermore, there are some evidences that compounds derived from the gut microbiota, either constituents of bacterial structure or metabolism, entering the bloodstream can activate macrophages to a pro-inflammatory state responsible for atherosclerosis[84]. This process could cause the development of cardiovascular disease as well as vascular dementia.

Hence a modification of the gut microbiome in neurodegenerative disorders has been suggested[85]. Indeed, there is evidence that the modulation

of the gut microbiota has a strong impact on the inflammaging process. Indeed, oral administration of *Lactobacillus brevis OW38* to aged mice has been shown to strengthen intestinal barrier tight junctions, reduce the levels of circulating LPS and the expression of pro-inflammatory cytokines, inhibiting NF-κB activation. Furthermore, *Bacteroidetes* increased while senescence markers p16, p53, and SAMHD1 in the colon and the hippocampus were reduced. Consequently, hippocampal memory was restored as well as the expression of brain-derived neurotrophic factors[86].

Alzheimer's disease is characterized by the deposition of amyloid-β (Aβ) and tau in the brain, leading to degeneration of cognitive function. The genesis is not yet defined, but it seems that the intestinal microbiota can play a role in this process[87,88]. In particular, *in vivo* and *in vitro* studies have shown a correlation with the production of lipopolysaccharide (LPS), one of the most important products of the microbiota[89]. These proteins are considered strongly amyloidogenic[90,91].

Studies carried out on murine specimens have shown that LPS can promote the formation of amyloid bridles and their deposition[92,93].

Furthermore, brain tissue analysis of deceased patients with Alzheimer's disease has highlighted the presence of higher levels of LPS and *E. coli* fragments compared to controls within Aβ[94].

This hypothesis is supported by numerous studies that demonstrate how the production of peptides such as beta amyloid is generated by pro-inflammatory responses stimulated by the response to infectious agents via contact with LPS, also contributing to the neurotoxic capacity of this protein[95-98].

Scholars[96,98] have finally found how also viral infectious agents, such as HSV-1, can enhance the production of Ab42.

On the other hand, studies conducted on over-expressed mouse models – amyloid precursor protein and presenilin 1 (APPPS1), have shown that the alteration of the microbiota due to prolonged antibiotic therapy influences the formation and deposition of the amyloid protein[99], through alteration of the inflammatory cytokine-dependent pattern. These results were also confirmed by studies with antibiotic therapy performed at birth, asking the question of the effective timing of the influence of the microbiota on neurodegeneration[100].

Conclusions

Gut microbiota plays a key role in biological functions concurring in maintenance of host health. Age-related physiological and non-physiological changes, associated with dietary habits and lifestyle, affect microbial diversity and composition.

Chronic inflammation and changes in gut microbiota profile related to age follow a similar trajectory.

Many works have shown a correlation between the composition of the gut microbiota and cognitive performance, frailty and comorbidity of the elderly.

Given this perspective, the modulation of gut microbiota may help to facilitate a physiological and non-pathological aging process and, perhaps, to contrast the progression of degenerating mechanisms. Currently, available studies on the characterization and modulation of the gut microbiota in other diseases, such as IBS and diabetes, show promising results. It is possible to hypothesize that these interventions may be beneficial in the elderly (Figure 2). In particular, as direct and indirect effects on the immune response can be expected, this could modify the risk of infectious diseases that are frequently observed in the elderly, indirectly reducing hospitalization and long-term care.

However, whether a stable modification of the gut microbiota could be obtained and what its long-term effects on parameters such as frailty and cognitive tests could be still need to be clarified.

Considering the paramount interest of this topic, future researches should be designed to better understand the correlation between the gut microbiota, the ageing process and degenerative diseases typical of the elderly. This should be accompanied by a practical approach in the modulation of the gut microbiota easily applicable in the clinical context.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Clavijo V, Florez MJV. The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: a review. Poult Sci 2017; 97: 1006-1021.

2) SCHMIDT C, STALLMACH A. Etiology and pathogenesis of inflammatory bowel disease. Minerva Gastroenterol Dietol 2005; 51: 127-145.

3) ECKBURG PB, LEPP PW, RELMAN DA. Archaea and their potential role in human disease. Infect Immun 2003; 71: 591-596.

4) BREITBART M, HEWSON I, FELTS B, MAHAFFY JM, NULTON J, SALAMON P, ROHWER F. Metagenomic analyses of an uncultured viral community from human feces. J Bacteriol 2003; 185: 6220-6223.

5) BARKO PC, MCMICHAEL MA, SWANSON KS, WILLIAMS DA. The gastrointestinal microbiome: a review. J Vet Intern Med 2017; 32: 9-25.

6) NOVERR MC, NOGGLE RM, TOEWS GB, HUFFNAGLE GB. Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. Infect Immun 2004; 72: 4996-5003.

7) UNDERHILL DM, ILIEV ID. The mycobiota: interactions between commensal fungi and the host immune system. Nat Rev Immunol 2014; 14: 405-416.

8) SOLL DR, GALASK R, SCHMID J, HANNA C, MAC K, MORROW B. Genetic dissimilarity of commensal strains of Candida spp. carried in different anatomical locations of the same healthy women. J Clin Microbiol 1991; 29: 1702-1710.

9) HUFFNAGLE GB, NOVERR MC. The emerging world of the fungal microbiome. Trends Microbiol 2013; 21: 334-341.

10) SARTOR RB, WU GD. Roles for intestinal bacteria, viruses, and fungi in pathogenesis of inflammatory bowel diseases and therapeutic approaches. Gastroenterology 2017; 152: 327-339.e4.

11) SARTOR RB. Microbial influences in inflammatory bowel diseases. Gastroenterology 2008; 134: 577-594.

12) LIN CS, CHANG CJ, LU CC, MARTEL J, OJCIUS DM, KO YF, YOUNG JD, LAI HC. Impact of the gut microbiota, prebiotics, and probiotics on human health and disease. Biomed J 2014; 37: 259-268.

13) ECKBURG PB, BIK EM, BERNSTEIN CN, PURDOM E, DETHLEFSEN L, SARGENT M, GILL SR, NELSON KE, RELMAN DA. Diversity of the human intestinal microbial flora. Science 2005; 308: 1635-1638.

14) HUMAN MICROBIOME PROJECT C. Structure, function and diversity of the healthy human microbiome. Nature 2012; 486: 207-214.

15) BURGER-VAN PAASSEN N, VINCENT A, PUIMAN PJ, VAN DER SLUIS M, BOUMA J, BOEHM G, VAN GOUDOEVER JB, VAN SEUNINGEN I, RENES IB. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. Biochem J 2009; 420: 211-219.

16) JONES RM. The influence of the gut microbiota on host physiology: in pursuit of mechanisms. Yale J Biol Med 2016; 89: 285-297.

17) NELL S, SUERBAUM S, JOSENHANS C. The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. Nat Rev Microbiol 2010; 8: 564-577.

18) RAKOFF-NAHOUM S, PAGLINO J, ESLAMI-VARZANEH F, EDBERG S, MEDZHITOV R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 2004; 118: 229-241.

19) SEKIROV I, RUSSELL SL, ANTUNES LC, FINLAY BB. Gut microbiota in health and disease. Physiol Rev 2010; 90: 859-904.

20) NATIVIDAD JM, VERDU EF. Modulation of intestinal barrier by intestinal microbiota: pathological and therapeutic implications. Pharmacol Res 2013; 69: 42-51.

21) DE SILVA HJ, MILLARD PR, SOPER N, KETTLEWELL M, MORTENSEN N AND JEWELL DP. Effects of the faecal stream and stasis on the ileal pouch mucosa. Gut 1991; 32: 1166-1169.

22) ABREU MT, PEEK RM JR. Gastrointestinal malignancy and the microbiome. Gastroenterology 2014; 146: 1534-1546.e3.

23) MAYER EA, PADUA D, TILLISCH K. Altered brain-gut axis in autism: comorbidity or causative mechanisms? Bioessays 2014; 36: 933-939.

24) HSIAO EY, MCBRIDE SW, HSIEN S, SHARON G, HYDE ER, MCCUE T, CODELLI JA, CHOW J, REISMAN SE, PETROSINO JF, PATTERSON PH, MAZMANIAN SK. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 2013; 155: 1451-1463.

25) ROUND JL, O'CONNELL RM, MAZMANIAN SK. Coordination of tolerogenic immune responses by the commensal microbiota. J Autoimmun 2010; 34: J220-225.

26) MANGIOLA F, IANIRO G, FRANCESCHI F, FAGIUOLI S, GASBARRINI G, GASBARRINI A. Gut microbiota in autism and mood disorders. World J Gastroenterol 2016; 22: 361-368.

27) CARDING S, VERBEKE K, VIPOND DT, CORFE BM, OWEN LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis 2015; 26: 26191.

28) SOENEN S, RAYNER CK, JONES KL, HOROWITZ M. The ageing gastrointestinal tract. Curr Opin Clin Nutr Metab Care 2016; 19: 12-18.

29) CLAESSON MJ, CUSACK S, O'SULLIVAN O, GREENE-DINIZ R, DE WEERD H, FLANNERY E, MARCHESI JR, FALUSH D, DINAN T, FITZGERALD G, STANTON C, VAN SINDEREN D, O'CONNOR M, HARNEDY N, O'CONNOR K, HENRY C, O'MAHONY D, FITZGERALD AP, SHANAHAN F, TWOMEY C, HILL C, ROSS RP, O'TOOLE PW. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. Proc Natl Acad Sci U S A 2011; 108: 4586-4591.

30) OSTAN R, BUCCI L, CAPRI M, SALVIOLI S, SCURTI M, PINI E, MONTI D, FRANCESCHI C. Immunosenescence and immunogenetics of human longevity. Neuroimmunomodulation 2008; 15: 224-240.

31) FRANCESCHI C, CAMPISI J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 2014; 69: S4-9.

32) GIUNTA B, FERNANDEZ F, NIKOLIC WV, OBREGON D, RRAPO E, TOWN T, TAN J. Inflammaging as a pro-

drome to Alzheimer's disease. J Neuroinflammation 2008; 5: 51.

33) Boren E, Gershwin ME. Inflamm-aging: autoimmunity, and the immune-risk phenotype. Autoimmun Rev 2004; 3: 401-406.

34) Franceschi C, Valensin S, Lescai F, Olivieri F, Licastro F, Grimaldi LM, Monti D, De Benedictis G, Bonafè M. Neuroinflammation and the genetics of Alzheimer's disease: the search for a pro-inflammatory phenotype. Aging (Milano) 2001; 13: 163-170.

35) Lencel P, Magne D. Inflammaging: the driving force in osteoporosis? Med Hypotheses 2011; 76: 317-321.

36) Franceschi C. Inflammaging as a major characteristic of old people: can it be prevented or cured? Nutr Rev 2007; 65: S173-176.

37) Petursdottir DH, Nordlander S, Qazi KR, Carvalho-Queiroz C, Ahmed Osman O, Hell E, Björkander S, Haileselassie Y, Navis M, Kokkinou E, Lio IZL, Hennemann J, Brodin B, Huseby DL, Nilsson C, Hughes D, Udekwu KI, Sverremark-Ekström E. Early-life human microbiota associated with childhood allergy promotes the T helper 17 axis in mice. Front Immunol 2017; 8: 1699.

38) Scaldaferri F, Pizzoferrato M, Gerardi V, Lopetuso L, Gasbarrini A. The gut barrier: new acquisitions and therapeutic approaches. J Clin Gastroenterol 2012; 46 Suppl: S12-17.

39) Lee YY, Hassan SA, Ismail IH, Chong SY, Raja Ali RA, Amin Nordin S, Lee WS, Majid NA. Gut microbiota in early life and its influence on health and disease: a position paper by the Malaysian Working Group on Gastrointestinal Health. J Paediatr Child Health 2017; 53: 1152-1158.

40) Bibbò S, Ianiro G, Giorgio V, Scaldaferri F, Masucci L, Gasbarrini A, Cammarota G. The role of diet on gut microbiota composition. Eur Rev Med Pharmacol Sci 2016; 20: 4742-4749

41) O'Toole PW, Jeffery IB. Gut microbiota and aging. Science 2015; 350: 1214-1215.

42) Arboleya S, Watkins C, Stanton C, Ross RP. Gut bifidobacteria populations in human health and aging. Front Microbiol 2016; 7: 1204.

43) Saraswati S, Sitaraman R. Aging and the human gut microbiota-from correlation to causality. Front Microbiol 2014; 5: 764.

44) Kumar M, Babaei P, Ji B, Nielsen J. Human gut microbiota and healthy aging: recent developments and future prospective. Nutr Healthy Aging 2016; 4: 3-16.

45) Li M, Wang B, Zhang M, Rantalainen M, Wang S, Zhou H, Zhang Y, Shen J, Pang X, Zhang M, Wei H, Chen Y, Lu H, Zuo J, Su M, Qiu Y, Jia W, Xiao C, Smith LM, Yang S, Holmes E, Tang H, Zhao G, Nicholson JK, Li L, Zhao L. Symbiotic gut microbes modulate human metabolic phenotypes. Proc Natl Acad Sci U S A 2008; 105: 2117-2122.

46) Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ. Human colonic microbiota associated with diet, obesity and weight loss. Int J Obes (Lond) 2008; 32: 1720-1724.

47) Andersson AF, Lindberg M, Jakobsson H, Backhed F, Nyren P, Engstrand L. Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS One 2008; 3: e2836.

48) Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. Metagenomic analysis of the human distal gut microbiome. Science 2006; 312: 1355-1359.

49) Shoaie S, Ghaffari P, Kovatcheva-Datchary P, Mardinoglu A, Sen P, Pujos-Guillot E, de Wouters T, Juste C, Rizkalla S, Chilloux J, Hoyles L, Nicholson JK; MICRO-Obes Consortium, Dore J, Dumas ME, Clement K, Bäckhed F, Nielsen J. Quantifying diet-induced metabolic changes of the human gut microbiome. Cell Metab 2015; 22: 320-331.

50) Ghosh S, Dai C, Brown K, Rajendiran E, Makarenko S, Baker J, Ma C, Halder S, Montero M, Ionescu VA, Klegeris A, Vallance BA, Gibson DL. Colonic microbiota alters host susceptibility to infectious colitis by modulating inflammation, redox status, and ion transporter gene expression. Am J Physiol Gastrointest Liver Physiol 2011; 301: G39-49.

51) Zimmer J, Lange B, Frick JS, Sauer H, Zimmermann K, Schwiertz A, Rusch K, Klosterhalfen S, Enck P. A vegan or vegetarian diet substantially alters the human colonic faecal microbiota. Eur J Clin Nutr 2012; 66: 53-60.

52) Duncan SH, Louis P, Thomson JM, Flint HJ. The role of pH in determining the species composition of the human colonic microbiota. Environ Microbiol 2009; 11: 2112-2122.

53) Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes 2009; 58: 1509-1517.

54) Zaibi MS, Stocker CJ, O'Dowd J, Davies A, Bellahcene M, Cawthorne MA, Brown AJ, Smith DM, Arch JR. Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. FEBS Lett 2010; 584: 2381-2386.

55) Al-Lahham SH, Roelofsen H, Priebe M, Weening D, Dijkstra M, Hoek A, Hoek A, Rezaee F, Venema K, Vonk RJ. Regulation of adipokine production in human adipose tissue by propionic acid. Eur J Clin Invest 2010; 40: 401-407.

56) Deniz M, Bozkurt A, Kurtel H. Mediators of glucagon-like peptide 2-induced blood flow: responses in different vascular sites. Regul Pept 2007; 142: 7-15.

57) Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer 2001; 1: 194-202.

58) Vinolo MA, Rodrigues HG, Hatanaka E, Sato FT, Sampaio SC, Curi R. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. J Nutr Biochem 2011; 22: 849-855.

59) Biagi E, Nylund L, Candela M, Ostan R, Bucci L, Pini E, Nikkïla J, Monti D, Satokari R, Franceschi C,

BRIGIDI P, DE VOS W. Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. PLoS One 2010; 5: e10667.

60) CLEMENTE JC, URSELL LK, PARFREY LW, KNIGHT R. The impact of the gut microbiota on human health: an integrative view. Cell 2012;148: 1258-1270.

61) DE FILIPPO C, CAVALIERI D, DI PAOLA M, RAMAZZOTTI M, POULLET JB, MASSART S, COLLINI S, PIERACCINI G, LIONETTI P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 2010; 107: 14691-14696.

62) MINOT S, SINHA R, CHEN J, LI H, KEILBAUGH SA, WU GD, LEWIS JD, BUSHMAN FD. The human gut virome: inter-individual variation and dynamic response to diet. Genome Res 2011; 21: 1616-1625.

63) MORENO-INDIAS I, CARDONA F, TINAHONES FJ, QUEIPO-ORTUNO MI. Impact of the gut microbiota on the development of obesity and type 2 diabetes mellitus. Front Microbiol 2014; 5: 190.

64) RODRIGUEZ JM, MURPHY K, STANTON C, ROSS RP, KOBER OI, JUGE N, AVERSHINA E, RUDI K, NARBAD A, JENMALM MC, MARCHESI JR, COLLADO MC. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Health Dis 2015; 26: 26050.

65) WU GD, CHEN J, HOFFMANN C, BITTINGER K, CHEN YY, KEILBAUGH SA, BEWTRA M, KNIGHTS D, WALTERS WA, KNIGHT R, SINHA R, GILROY E, GUPTA K, BALDASSANO R, NESSEL L, LI H, BUSHMAN FD, LEWIS JD. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011; 334: 105-108.

66) FLINT HJ. The impact of nutrition on the human microbiome. Nutr Rev 2012; 70 Suppl 1: S10-13.

67) YATSUNENKO T, REY FE, MANARY MJ, TREHAN I, DOMINGUEZ-BELLO MG, CONTRERAS M, MAGRIS M, HIDALGO G, BALDASSANO RN, ANOKHIN AP, HEATH AC, WARNER B, REEDER J, KUCZYNSKI J, CAPORASO JG, LOZUPONE CA, LAUBER C, CLEMENTE JC, KNIGHTS D, KNIGHT R, GORDON JI. Human gut microbiome viewed across age and geography. Nature 2012; 486: 222-227.

68) JEFFERY IB, O'TOOLE PW. Diet-microbiota interactions and their implications for healthy living. Nutrients 2013; 5: 234-252.

69) DRESCHER LS, THIELE S, MENSINK GB. A new index to measure healthy food diversity better reflects a healthy diet than traditional measures. J Nutr 2007; 137: 647-651.

70) CLAESSON MJ, JEFFERY IB, CONDE S, POWER SE, O'CONNOR EM, CUSACK S, HARRIS HM, COAKLEY M, LAKSHMINARAYANAN B, O'SULLIVAN O, FITZGERALD GF, DEANE J, O'CONNOR M, HARNEDY N, O'CONNOR K, O'MAHONY D, VAN SINDEREN D, WALLACE M, BRENNAN L, STANTON C, MARCHESI JR, FITZGERALD AP, SHANAHAN F, HILL C, ROSS RP, O'TOOLE PW. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012; 488: 178-184.

71) MUELLER S, SAUNIER K, HANISCH C, NORIN E, ALM L, MIDTVEDT T, CRESCI A, SILVI S, ORPIANESI C, VERDENELLI MC, CLAVEL T, KOEBNICK C, ZUNFT HJ, DORÉ J, BLAUT M. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. Appl Environ Microbiol 2006; 72: 1027-1033.

72) MARIAT D, FIRMESSE O, LEVENEZ F, GUIMARAES V, SOKOL H, DORE J, CORTHIER G, FURET JP. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol 2009; 9: 123.

73) ZAPATA HJ, QUAGLIARELLO VJ. The microbiota and microbiome in aging: potential implications in health and age-related diseases. J Am Geriatr Soc 2015; 63: 776-781.

74) MAKIVUOKKO H, TIIHONEN K, TYNKKYNEN S, PAULIN L, RAUTONEN N. The effect of age and non-steroidal anti-inflammatory drugs on human intestinal microbiota composition. Br J Nutr 2010; 103: 227-234.

75) GUIGOZ Y, DORE J, SCHIFFRIN EJ. The inflammatory status of old age can be nurtured from the intestinal environment. Curr Opin Clin Nutr Metab Care 2008; 11: 13-20.

76) TIIHONEN K, OUWEHAND AC, RAUTONEN N. Human intestinal microbiota and healthy ageing. Ageing Res Rev 2010; 9: 107-116.

77) MAN AL, BERTELLI E, RENTINI S, REGOLI M, BRIARS G, MARINI M, WATSON AJ, NICOLETTI C. Age-associated modifications of intestinal permeability and innate immunity in human small intestine. Clin Sci (Lond) 2015; 129: 515-527.

78) FRANSEN F, VAN BEEK AA, BORGHUIS T, AIDY SE, HUGENHOLTZ F, VAN DER GAAST-DE JONGH C, SAVELKOUL HFJ, DE JONGE MI, BOEKSCHOTEN MV, SMIDT H, FAAS MM, DE VOS P. Aged gut microbiota contributes to systemical inflammaging after transfer to germ-free mice. Front Immunol 2017; 8: 1385.

79) KIM KA, JEONG JJ, YOO SY, KIM DH. Gut microbiota lipopolysaccharide accelerates inflamm-aging in mice. BMC Microbiol 2016; 16: 9.

80) MOSHER KI, WYSS-CORAY T. Go with your gut: microbiota meet microglia. Nat Neurosci 2015; 18: 930-931.

81) ERNY D, HRABE DE ANGELIS AL, JAITIN D, WIEGHOFER P, STASZEWSKI O, DAVID E, KEREN-SHAUL H, MAHLAKOIV T, JAKOBSHAGEN K, BUCH T, SCHWIERZECK V, UTERMÖHLEN O, CHUN E, GARRETT WS, MCCOY KD, DIEFENBACH A, STAEHELI P, STECHER B, AMIT I, PRINZ M. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci 2015; 18: 965-977.

82) HARRY GJ. Microglia during development and aging. Pharmacol Ther 2013; 139: 313-326.

83) IADECOLA C. Dangerous leaks: blood-brain barrier woes in the aging hippocampus. Neuron 2015; 85: 231-233.

84) CHEN X, ZHENG L, ZHENG YQ, YANG QG, LIN Y, NI FH, LI ZH. The cardiovascular macrophage: a missing link between gut microbiota and cardiovascular diseases? Eur Rev Med Pharmacol Sci 2018; 22: 1860-1872.

85) ROMANO L, GUALTIERI P, NICOLETTI F, MERRA G. Neurodegenerative disorders, gut human microbiome and diet: future research for prevention and supportive therapies. Eur Rev Med Pharm Sci 2018; 22: 5771-5772.

86) Jeong JJ, Kim KA, Hwang YJ, Han MJ, Kim DH. Anti-inflammaging effects of Lactobacillus brevis OW38 in aged mice. Benef Microbes 2016; 7: 707-718.

87) Masters CL, Bateman R, Blennow K, Rowe CC, Sperling RA, Cummings JL. Alzheimer's disease. Nat Rev Dis Primers 2015; 1: 15056.

88) Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, Carlsson CM, Asthana S, Zetterberg H, Blennow K, Bendlin BB, Rey FE. Gut microbiome alterations in Alzheimer's disease. Sci Rep 2017; 7: 13537.

89) Kell DB, Pretorius E. On the translocation of bacteria and their lipopolysaccharides between blood and peripheral locations in chronic, inflammatory diseases: the central roles of LPS and LPS-induced cell death. Integr Biol (Camb) 2015; 7: 1339-1377.

90) Hill JM, Dua P, Clement C, Lukiw WJ. An evaluation of progressive amyloidogenic and pro-inflammatory change in the primary visual cortex and retina in Alzheimer's disease (AD). Front Neurosci 2014; 8: 347.

91) Schwartz K, Sekedat MD, Syed AK, O'Hara B, Payne DE, Lamb A, Bole BR. The AgrD N-terminal leader peptide of Staphylococcus aureus has cytolytic and amyloidogenic properties. Infect Immun 2014; 82: 3837-3844.

92) Asti A, Gioglio L. Can a bacterial endotoxin be a key factor in the kinetics of amyloid fibril formation? J Alzheimers Dis 2014; 39: 169-179.

93) Kahn MS, Kranjac D, Alonzo CA, Haase JH, Cedillos RO, McLinden KA, Boehm GW, Chumley MJ. Prolonged elevation in hippocampal Abeta and cognitive deficits following repeated endotoxin exposure in the mouse. Behav Brain Res 2012; 229: 176-184.

94) Zhan X, Stamova B, Jin LW, DeCarli C, Phinney B, Sharp FR. Gram-negative bacterial molecules associate with Alzheimer disease pathology. Neurology 2016; 87: 2324-2332.

95) Calsolaro V, Edison P. Neuroinflammation in Alzheimer's disease: Current evidence and future directions. Alzheimers Dement 2016; 12: 719-732.

96) Lukiw WJ. The microbiome, microbial-generated proinflammatory neurotoxins, and Alzheimer's disease. J Sport Health Sci 2016; 5: 393-396.

97) Hill JM, Clement C, Pogue AI, Bhattacharjee S, Zhao Y, Lukiw WJ. Pathogenic microbes, the microbiome, and Alzheimer's disease (AD). Front Aging Neurosci 2014; 6: 127.

98) Zhao Y, Jaber V, Lukiw WJ. Secretory products of the human GI tract microbiome and their potential impact on Alzheimer's Disease (AD): detection of lipopolysaccharide (LPS) in AD hippocampus. Front Cell Infect Microbiol 2017; 7: 318.

99) Minter MR, Zhang C, Leone V, Ringus DL, Zhang X, Oyler-Castrillo P, Musch MW, Liao F, Ward JF, Holtzman DM, Chang EB, Tanzi RE, Sisodia SS. Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. Sci Rep 2016; 6: 30028.

100) Minter MR, Hinterleitner R, Meisel M, Zhang C, Leone V, Zhang X, Musch MW, Shen X, Jabri B, Chang EB, Tanzi RE, Sisodia SS. Antibiotic-induced perturbations in microbial diversity during post-natal development alters amyloid pathology in an aged APPSWE/PS1DeltaE9 murine model of Alzheimer's disease. Sci Rep 2017; 7: 10411.