Stroke: Vascular and Interventional Neurology

REVIEW

Understanding the Relationship Between Cerebrovascular Disease and the Gut Microbiome

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While traditional vascular risk factors (eg, hypertension, dyslipidemia, tobacco use) account for 20% of the explained variance in carotid atherosclerosis, they remain a prominent focus for primary and secondary ischemic stroke prevention strategies. Among other potential contributors to atheroma formation and cerebrovascular disease, the gut microbiome has become increasingly implicated as a mediator of vascular risk. The foods we eat, coupled with our physiology and exposures (eg, antibiotics, supplements), directly contribute to atherosclerotic disease in complex ways that are mediated by gastrointestinal flora and metabolic by-products. Proliferation of "pathogenic" gut microbes such as *Enterobacteriaceae* and *Streptococcus* spp, decrement of "commensal" species such as *Akkermansia* spp and the biodiversity of gut flora are directly related to an individual's dietary intake and exposure history. Each of these components of the gut microbiome correlate with the development or progression of many conditions including atherosclerosis. Moreover, the metabolism of certain substrates found in animal products (notably L-carnitine and choline) and of refined sugars by these microorganisms leads to buildup of circulating metabolites with known links to atherogenesis, platelet activation, atrial fibrillation, and other adverse vascular outcomes. Several of these toxic metabolites, including trimethylamine and trimethylamine N-oxide, have been extensively studied in cardiovascular and cerebrovascular disease. Trimethylamine and trimethylamine N-oxide represent not only biomarkers of gut dysbiosis and cardiovascular risk, but they are increasingly recognized as therapeutic targets for novel interventions in atherosclerotic vascular disease. The individualized targeting of one's microbiome, and perhaps more generalized targeting of toxic microbial metabolites, has the potential to revolutionize the treatment of vascular disease. In this review, we summarize the latest evidence illustrating the impact of the microbiome on cerebrovascular disease and highlight the potential applications of this information on individualized and global scales.

Key Words: atherosclerosis ■ carotid artery ■ innovation ■ microbiome ■ stroke

Mounting evidence implicates the microbiome as an independent, underappreciated contributor to ischemic stroke risk. According to ultrasound data from the Northern Manhattan Study, ≈20% of the variability in carotid atherosclerosis (CAS) can be associated with traditional vascular risk factors such as diabetes, dyslipidemia, and hypertension, with age being the most powerful contributor to plaque thickness.[1] Other nontraditional risk factors, including genetics, diet and lifestyle, and sociocultural factors (eg, race, ethnicity, access to care) likely comprise a majority of the variance in atherosclerotic and ischemic stroke risk (Figure 1).[2] Moreover, the impact of one's diet on stroke risk extends beyond the simple relationship between obesity and ischemic stroke, for which studies have shown an ≈5% increase in

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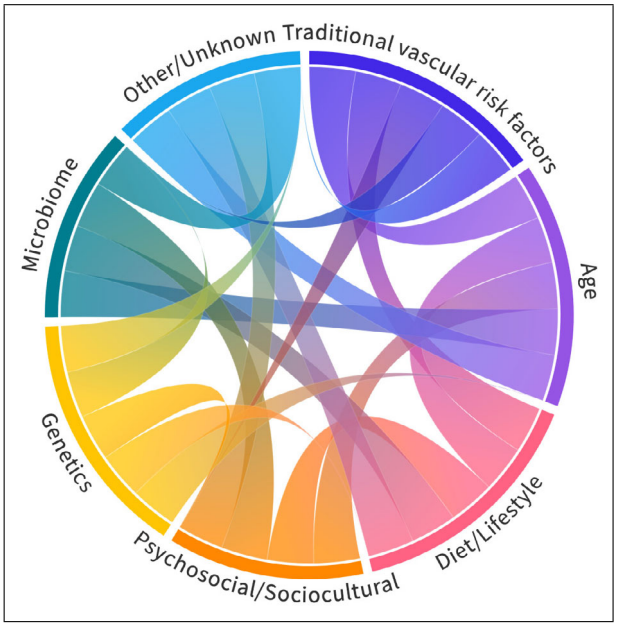

Figure 1. Chord diagram summarizing prevalence and interplay of major atherosclerotic risk factors. In this conceptual framework illustrated by a chord diagram, each color corresponds to a major atherosclerosis risk factor group, with all factors totaling 100% of the attributable risk of factors related to carotid plaque variability. For example, traditional risk factors (shown in dark blue) such as hypertension and diabetes may account for ≈20% of the variability in atherosclerotic plaque. However, the risk of developing several of these risk factors is impacted by age, diet/lifestyle, and psychosocial/sociocultural factors (eg, social determinants of health). It is unclear what proportion of atherosclerotic risk is mediated by the microbiome (dark green). Because of our incomplete understanding of stroke risk factors, estimates of risk factor prevalence and association between risk factors may not be accurate. Therefore, all elements are distributed proportionally. The visual should be thought of as a conceptual framework for risk factors that contribute to carotid atherosclerosis.

Nonstandard Abbreviations and Acronyms

CAS	carotid atherosclerosis
DIRECT-PLUS	Dietary Intervention Randomized Controlled Trial Polyphenols Unprocessed Study
FMT	fecal microbiota transplantation
KD	ketogenic diet
MEDIMACS	Impact of Mediterranean Diet, Inflammation, and Microbiome After an Acute Coronary Syndrome
PCSK9	proprotein convertase subtilisin/kexin 9
T2D	type 2 diabetes
PREDIMED	Prevención con Dieta Mediterránea
TMAO	trimethylamine N-oxide

CLINICAL PERSPECTIVE

What Is New?

- There is growing evidence that commensal and pathologic gut microbiota mediate a substantial effect of diet on atherosclerotic risk.

What Are the Clinical Implications?

- The gut microbiome represents an underappreciated target for nonpharmacologic and pharmacologic interventions aimed at reducing atherosclerotic vascular disease.

ischemic stroke for every greater unit of body mass index.[3] Heightened awareness of the impact of one's diet on atherogenesis may lead to novel pharmacologic and nonpharmacologic strategies aimed at reducing stroke risk. Further, the assessment of an individual's microbiome through the study of unique gastrointestinal floral species and circulating pathogenic metabolites in the serum may facilitate individualized therapeutic strategies aimed at promoting healthy, commensal gut flora and reducing inflammatory, atherogenic metabolic by-products. In this review, we summarize the latest evidence implicating the microbiome in atherosclerotic stroke risk, emphasizing the application of these findings on a global and individualized scale. A literature search of PubMed, Google Scholar, and Clinicaltrials.gov was performed by the 2 senior authors (W.R., J.E.S.) to identify relevant articles (and their references) until March 2024 using the key terms "gut microbiome," "atherosclerosis," "cerebrovascular disease," "metabolites," "stroke," "carotid atherosclerosis," "trimethylamine N-oxide (TMAO)," "inflammation," "Mediterranean diet," "ketogenic diet (KD)," "intermittent fasting," "probiotics," and "proprotein convertase subtilisin/kexin 9 (PCSK9)."

GUT MICROBIOME AND VASCULAR RISK FACTORS

While many modifiable vascular risk factors (eg, hypertension, diabetes) are a consequence of genetic predisposition, aging, lifestyle, and social determinants of health, these factors account for a minority (2%–30%) of the variance of vascular disease.[1,4] The interplay between each of these factors is more complicated than a linear sequence of events (Figure 2).

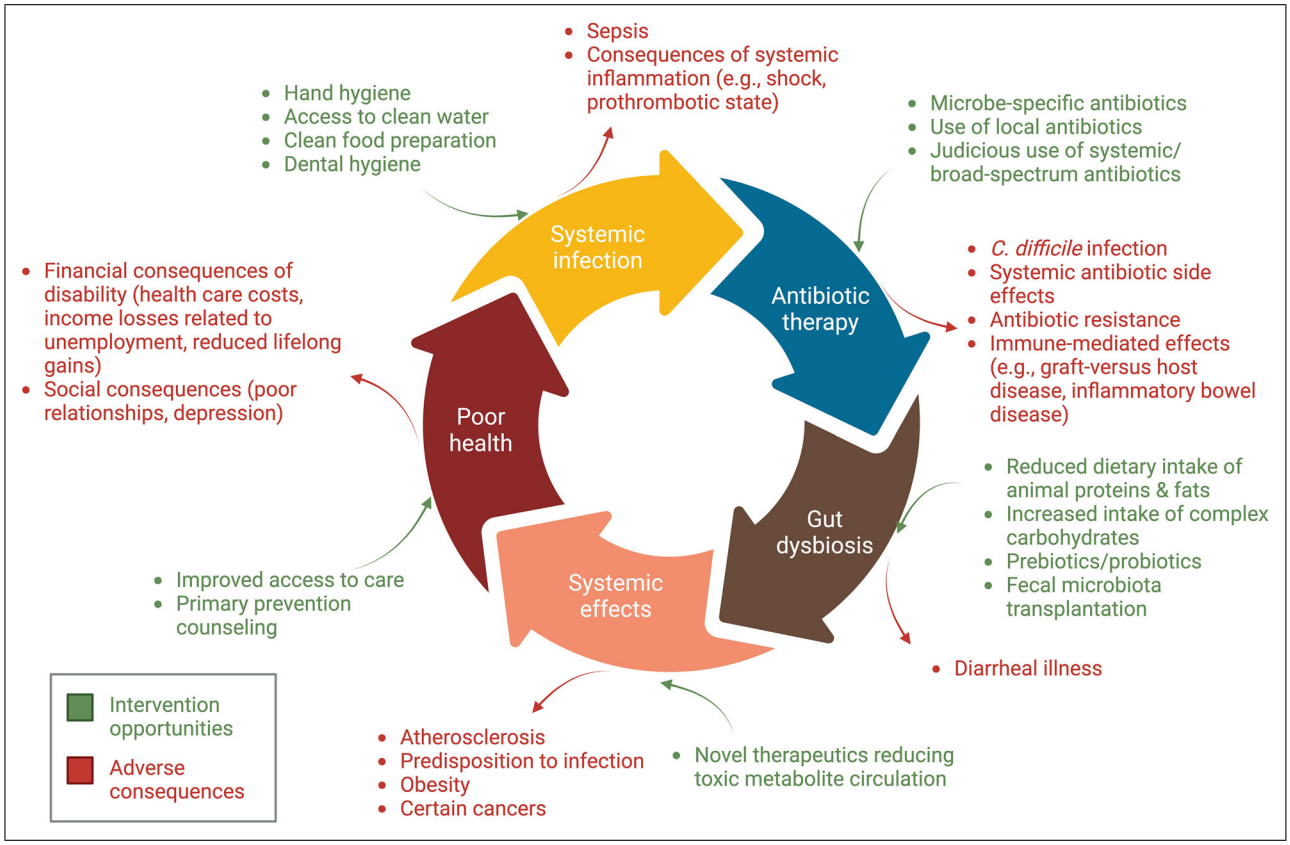

Figure 2. Feedback between the microbiome and systemic effects. Figure generated using biorender.com.

The gut microbiome represents a newly appreciated contributor to traditional risk factors for atherosclerosis, and itself is causally related to plaque formation. With a greater understanding of how the microbiome influences vascular risk factors, we may more effectively target the microbiome to treat systemic conditions using novel interventions.

Long considered an effect of genetics or high salt intake, there is increasing evidence that the microbiome contributes to meaningful changes in systemic vascular resistance. To date, the >900 unique genetic loci associated with hypertension account for <6% of the variance in systolic blood pressure.[5] Pathologic imbalance of gut microbial flora, or dysbiosis, is increasingly thought to contribute to hypertension independent of genetic predisposition and nutritional intake. An animal model study by Yang et al provided some of the earliest evidence that gut dysbiosis is associated with hypertension.[6] In their study, the investigators found that a reduction in the microbial richness and increase in prevalence of Firmicutes (with decrement in Bacteroidetes), now referred to as the Firmicutes/Bacteroidetes ratio, existed in rat models of hypertension. Additionally, a 2014 meta-analysis conducted by Khalesi and colleagues showed that consumption of probiotics could significantly reduce systolic and diastolic blood pressure.[7] While the effect was small (average reduction, 3.6 mm Hg), it was more pronounced with multiple species probiotics as opposed to single species. Lack of gut biodiversity has also been strongly tied to incident hypertension in young adults, according to 1 long-term prospective multicenter cohort study.[8] Moreover, interventions targeting nutritional content provide additional data supporting a causal relationship between gut diversity and hypertension.

Like hypertension, type 2 diabetes (T2D) has been historically attributed to dietary changes, obesity, and a genetic predisposition. The state of insulin resistance, however, may be mediated by other factors than these well-known contributors, which account for less than one third of the total variance of insulin resistance in population studies.[9] A reduction in gut microbial diversity has correlated with the later development of T2D,[10] while increase in the Firmicutes/Bacteroidetes ratio has been associated with obesity in animal studies.[11] One investigation of European women validated the inverse association between Bacteroidetes and T2D, and reported a high prevalence of certain pathologic Clostridiales species.[12] Fecal microbiota

transplantation (FMT) in human patients with T2D has also shown early success at improving the metabolic syndrome.[13]

As with other vascular risk factors, dyslipidemia may be driven by a variety of genetic and dietary factors in addition to gut dysbiosis. In 1 analysis of 893 subjects, Fu and colleagues reported 4% to 6% of the variance in serum lipid profiles and body mass index was attributable to the gut microbiome.[4] (Impressively, known genetic factors contributed to a smaller variance of lipid profiles, ≈2%–4%.) Some, if not most, of the influence of the microbiota on serum cholesterol levels appears related to the metabolism of lipids by commensal gut flora, which express unique genes responsible for converting ingested cholesterol to a poorly absorbed lipid (sterol coprostol) with resultant reductions in total host cholesterol absorption.[14]

Atrial fibrillation (AF) is a major risk factor for cardioembolic stroke. Alterations in the gut microbiome have been identified in patients with AF and have been proposed to play a role in its pathogenesis. One systematic review and meta-analysis on this topic identified 14 clinical studies, each with unique findings and outcomes.[15] Among them, gut dysbiosis was associated with AF recurrence following successful electrical ablation. Patients with AF often had higher TMAO production, inflammatory cytokine production, and reduced short-chain fatty acid (SCFA) production. These studies reveal similarities in gut microbiome composition and function between atherosclerosis and AF, a nonatherosclerotic stroke risk factor.

GUT MICROBIOME AND ATHEROSCLEROSIS

In addition to its influence on atherosclerosis risk factors, the gut microbiome also promotes atherogenesis *independently* (Table). Among the numerous metabolites from the gut microbial flora, trimethylamine and its hepatic metabolite TMAO are strongly implicated in atherogenesis. Multiple studies have linked serum levels of TMAO with increased cardiovascular risk and severity, correlating TMAO levels with atherosclerotic plaque size and cardiovascular events (Figure 3).[16,17] Among its other downstream effects, TMAO amplifies platelet hyperreactivity, foam cell formation within plaques, and induces inflammatory proteins like interleukin-6, cyclooxygenase-2, E-selectin, and intercellular adhesion molecule-1.[17,18,19] One case-control study involving 218 subjects with atherosclerotic cardiovascular disease (ASCVD) and 187 controls identified heightened levels of Enterobacteriaceae and *Streptococcus* in patients with ASCVD, accompanied by significant metabolic shifts, including increased bacterial genes encoding trimethylamine lytic enzymes and elevated TMAO production compared with healthy counterparts.[20]

CAS is 1 manifestation of atherosclerosis and comprises a major risk factor for ischemic stroke. Recent research has identified a proximate association between the gut microbiota and CAS.[21] Detection of genetic material from various bacteria originating from both the oral cavity and gut, along with the presence of live bacteria within the CAS atheroma, suggests a pathogenic microbial mechanism underlying local inflammation within these plaques.[22] One study involving 31 patients with CAS and 51 healthy controls revealed significantly elevated levels of pathogenic species *Bacteroides eggerthii*, *Escherichia coli*, and *Klebsiella pneumoniae* in the CAS group compared with controls. Control microbiota showed enrichment of Parabacteroides, *Prevotella copri*, *Bacteroides* sp 3_1_19, and *Haemophilus parainfluenzae*. [21] Additionally, associations between gut microbes and specific microbial metabolic pathways were identified, revealing the presence of a cyclic pathway involving TMAO concentrations in CAS patients compared with healthy controls.[23]

In 1 study of Chinese adults, increased consumption of fresh fruits and vegetables correlated with reduced CAS, as measured by intima media thickness, peak systolic velocity, and end-diastolic velocity.[24] In that study, the gut microbiome mediated 17% of the effect of lifestyle on CAS. Specifically, abundance of *Faecalicatena* was protective against CAS, while *Libanicoccus* spp was associated with increased carotid intima-media thickness. Symptomatic patients with atherosclerosis have an enriched gastrointestinal abundance of *Collinsella* compared with healthy controls.[25,26] The genus *Collinsella*, abundant in the microbiomes in individuals who consume low-fiber Western diets, are known to detrimentally affect host metabolism by decreasing glycogenesis in the liver, altering cholesterol absorption in the gut, and increasing triglyceride synthesis.[27] *Collinsella* also decreases the expression of tight junction proteins, leading to increased gut permeability and activating epithelial production of proinflammatory interleukins.[28] Emoto et al have also illustrated a significant rise in Firmicutes/Bacteroidetes ratio among patients diagnosed with coronary artery disease.[29] Circulating commensal bacteria, while uncommonly identified in the peripheral circulation, may also exacerbate atheroma formation. Early sequencing studies have even identified pathogenic bacterial DNA isolated from atheromatous plaques.[30]

Table. Gut Microorganisms Implicated in Atherogenesis

Pathogenic species	Notes	Commensal species	Notes
Enterobacteriaceae∗ spp (eg, *Escherichia coli*, *Klebsiella* spp, and *Enterobacter)*	Higher abundance of *Enterobacteriaceae* have been observed in patients with atherosclerotic disease compared with healthy controls[20]	*Prevotella copri*	The association between *P copri* and atherosclerosis is complex, with lower abundance of *P copri* having been observed in patients with atherosclerotic disease compared with healthy controls.[20] Further, carriers of *P copri* (often found in Western populations) may exhibit a limited response to the Mediterranean diet, whereas noncarriers of *P copri* may show a favorable cardiometabolic profile in response to a Mediterranean diet[118]
Streptococcus spp	Higher abundance of *Streptococcus* spp have been observed in patients with atherosclerotic disease compared with healthy controls[20]	Bacteroidetes spp	Lower abundance of Bacteroidetes have been observed in subjects with diabetes[11] and with atherosclerotic disease compared with healthy controls,[20] with higher levels being associated with lower insulin resistance.[119] Repletion of Bacteroidetes has been associated with a high-fiber diet[120]
Lactobacillus salivarius	Higher abundance of *L salivarius* have been observed in patients with atherosclerotic disease compared with healthy controls[20]	*Roseburia intestinalis*	*R intestinalis* is a butyrate-producing commensal microorganism found in less abundance in patients with atherosclerotic disease[20]
Solobacterium moorei	Higher abundance of *S moorei* have been observed in patients with atherosclerotic disease compared with healthy controls[20]	*Faecalibacterium* cf *prausnitzii*	*F* cf *prausnitzii* is another butryate producer found in lower abundance in patients with atherosclerotic disease[20]
Ruminococcus gnavus	Higher abundance of *R gnavus* have been observed in patients with atherosclerotic disease compared with healthy controls[20]	*Alistipes shahii*	Lower abundance of *A shahii* have been observed in patients with atherosclerotic disease compared with healthy controls[20]
Eggerthella lenta	Higher abundance of *E lenta* has been observed in patients with atherosclerotic disease compared with healthy controls[20]	*Akkermansia muciniphila*	While *Akkermansia* spp have been found in greater abundance in patients with diabetes in 1 study,[121] higher levels have been associated with a reduction in the risk of metabolic syndrome and inflammatory conditions[4,84,122]

Table is not comprehensive.

IMMUNE-MEDIATED ATHEROGENESIS AND THE GUT MICROBIOME

Inflammation is now well understood to drive atherosclerosis (Figure 4). In its immunomodulatory capacity, the gut microbiome can be both harmful and protective against atherosclerosis. Elements of the adaptive innate immune cascades, known to be modulated by gut microbiota and microbial metabolites, contribute to atheroma propagation and evolution. Gut microbial metabolites agonize toll-like receptors and are requisite for their expression in the small intestine.[31] Macrophages expressing toll-like receptors 2 and 4 are activated and promote myeloid differentiation primary response 88–dependent atherogenic inflammatory signaling. Several ligands produced by gut bacteria are known to activate toll-like receptor 2^+ and 4^+ macrophages isolated from carotid plaque.[32] Gut decontamination with antibiotics in 1 mouse model ameliorated the development of atherosclerosis in apolipoprotein E–deficient mice.[33] Additionally, colonization of germ-free mice with wild-type gut flora promotes plaque formation.[16,34]

The adaptive immune response may also exert influence on atherogenesis.[35] Certain adaptive immune cell subtypes, such as T helper 1 cells, are present in atheromatous plaque and exacerbate intraplaque inflammation and growth. Interferon-γ released from T helper 1 lymphocytes stimulate innate immune cells and foster atherogenesis. Regulatory T cells produce transforming growth factor-β and interleukin-10 and other T helper 2 cells that produce interleukin-10 suppress activation of nearby innate immune cells with subsequent suppression of atherogenesis.[36]

Other evidence linking the microbiome to immunomodulation and subsequent atheroma

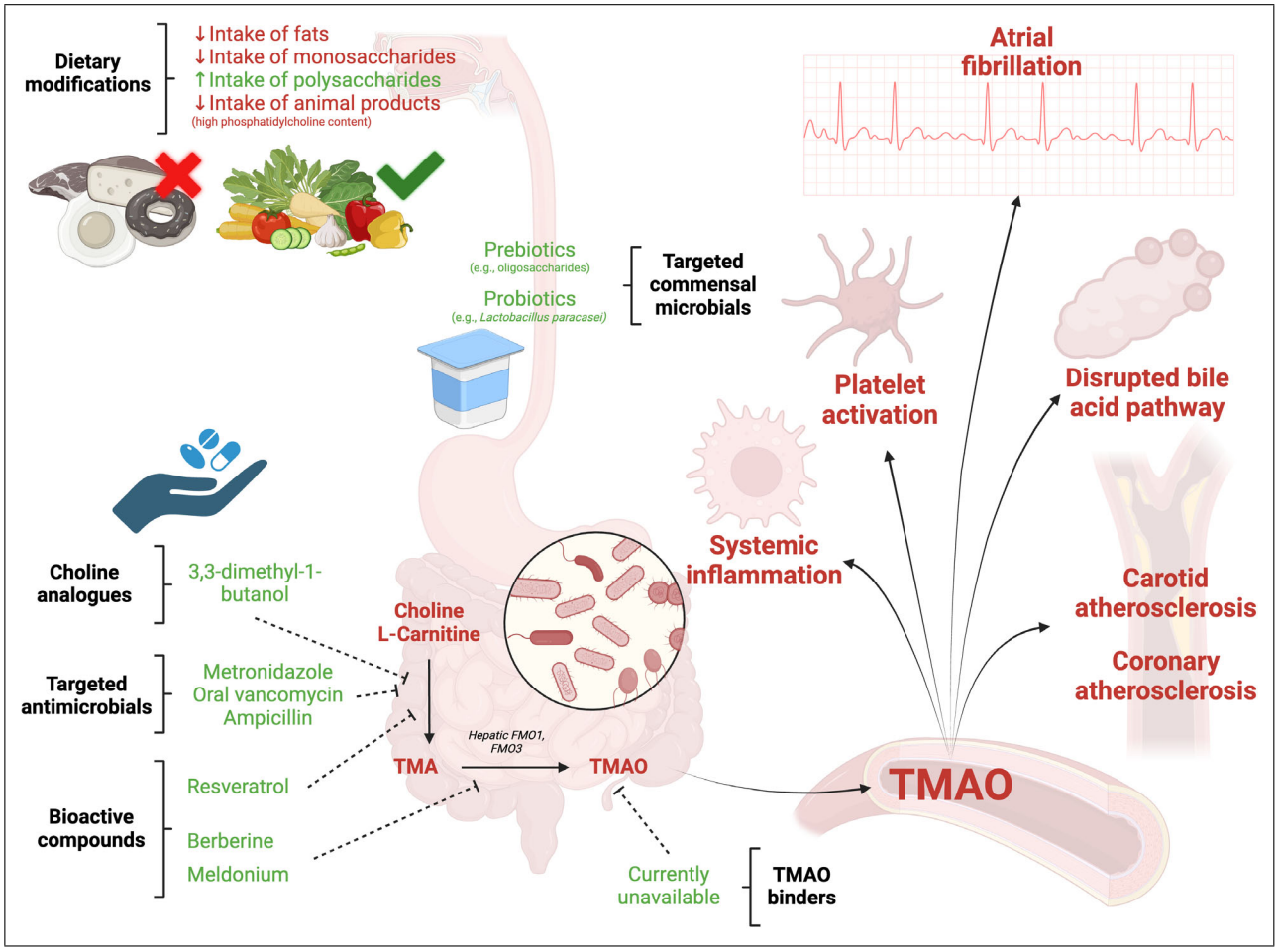

Figure 3. Potential therapeutic targets for stroke involving the microbiome. Text in green corresponds to potential therapeutic interventions to improve the microbiome whereas text in red corresponds to toxic exposures, bioactive compounds, or pathologic consequences. Figure generated using biorender.com. TMA indicates trimethylamine; and TMAO, trimethylamine N-oxide.

formation includes cellular and animal data reporting the role of short-chain fatty acids (including butyrate and propionate) in the induction of naïve T-cell differentiation toward the regulatory T-cell phenotype. Tryptophan is used and metabolized to several bioactive peptides that influence immune function. Bacteria harboring tryptophanase convert tryptophan to bioactive indole derivatives such as indole, indole-3-propionic acid, which can attenuate activation of macrophages. Other indoles exert immunosuppressive effects by activating aryl hydrocarbon receptors on innate immune cells and promote T-cell differentiation toward regulatory phenotypes.[37] Additionally, microbially modulated bile acids suppress inflammatory gene expression in multiple cell types through farsenoid X-receptor agonism and nucleotide binding and oligomerization domain-like receptor protein 3 suppression.[38] Further work is needed to fully establish the role of these immunomodulators in the pathogenesis of atherosclerosis.

DIET AND GUT MICROBIOME–ASSOCIATED RISK OF ATHEROSCLEROSIS

The nutritional components of one's diet fundamentally shapes the gut microbial community and its metabolic by-products. Diet provides nutrients that nourish the growth and metabolism of gut-resident organisms in addition to the human body. An analysis of twins found that heritability accounted for between 5% and 9% of microbiome taxa variability,[39] while another study reported that diet, environment, and anthropometric measurements accounted for 20% of microbiome variability between individuals (Figure 1).[40] It is unsurprising that diet and other exposures exert a greater influence on the microbiome than one's genetics, particularly given that the human body (including gastrointestinal tract) is sterile at birth.

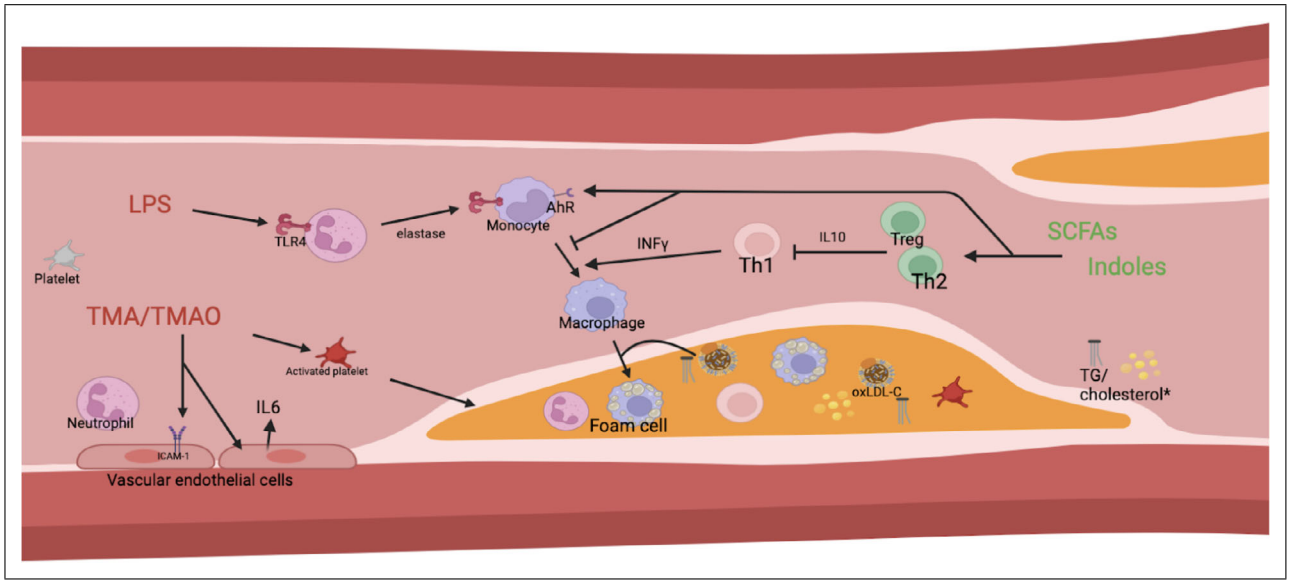

Figure 4. Diagram of gut microbial metabolites on immune–mediated atherogenesis. Influence of dysbiotic (red) and eubiotic (green) gut microbial effector metabolites on immune and molecular contributions to atherogenesis. ∗Circulating lipids and cholesterol bioavailability is influenced by luminal gut microbe consumption of these metabolites and by bile acids modulated by gut microbes (not depicted). Figure generated using biorender.com. AhR indicates aryl hydrocarbon receptor, ICAM-1, intercellular adhesion molecule-1; IL6, interleukin-6; IL10, interleukin-10; INFγ, interferon-gamma; LPS, lipopolysaccharide; oxLDL-C, oxidized low-density lipoprotein C; TG, triglyceride; Th2, type 2 helper T cell; TLR4, toll-like receptor 4; TMA, trimethylamine; TMAO, trimethylamine N-oxide; and Treg, regulatory T cell.

The Western diet (characterized by monosaccharide additives, ultraprocessed food, red meats, and products with high animal fat content) is highly associated with many atherosclerosis risk factors including hypertension, obesity, T2D, and dyslipidemia.[41] Migration from a non-Western country to the United States has been associated with loss of both α and β diversity in the gut microbiome. Across 3 human studies and 11 animal studies, high-fat diets have been associated with decreased SCFA production, increased bile acid production, and increased inflammatory markers.[42] A proposed mechanism for decreased SCFA production is through increased relative abundance of *Alistipes* and *Bacteroides*, concurrent with decreased *Faecalibacterium*, a fiber-degrading butyrate producer.[43] One systematic review of 6 interventional trials and 9 cross-sectional cohort studies reported that a diet rich in saturated or monounsaturated fat can decrease the total gut bacterial number and increase the relative amount of pathogenic species such as *Enterobacteriaceae*, *Turicibacter*, and *Prevotella*, which are associated with increased risk of cardiovascular disease.[44]

Although dietary recommendations are far from specific, they are frequently prescribed as interventions to reduce ASCVD risk. The most commonly recommended diets include plant-based and Mediterranean diets, with potentially intermittent fasting.[45,46] Vegetarian and vegan diets are associated with improved general health, and while there are microbiome differences between individuals adherent to a largely plant-based diet as compared with omnivores,[47,48] there is not a clear microbiome profile unique to each particular diet. The Mediterranean diet is looked to as a model of healthy eating and is recommended to people with high ASCVD risk following the seminal PREDIMED (Prevención con Dieta Mediterránea) study.[49] Hallmarks of the Mediterranean diet include high intake of vegetables, fruits, whole grains, legumes, nuts, olive oil, and moderate intake of fish and poultry. A randomized clinical trial testing the Mediterranean diet as a dietary intervention found that after 4 weeks individuals with strict adherence to the diet showed an increase in the gut microbial diversity, upregulation of microbial genes responsible for glucose metabolism, and increase in prevalence of *Faecalibacterium* species (a previously described commensal gut microbe with anti-inflammatory activity).[50] Without altering energy intake or physical activity, those who adhered to the Mediterranean diet also had reductions in plasma cholesterol and urine acylcarnitines, which have been associated with ASCVD.[50]

The KD is another well-described diet with pleiotropic effects, including anti-inflammatory mechanisms. This high-fat, low-carbohydrate diet has been used in the successful treatment of refractory epilepsy.[51] The neuroprotective nature of a KD is attributed to enhanced ketone production and modulation of γ-aminobutyric acid, though exact mechanisms are unclear. Animal studies have found that a KD drives increases in *Akkermansia muciniphila*, *Parabacteroides* spp, *Bacillota*, and decreases in

Bacteriodetes and α diversity, which have been associated with seizure protection.[52–54] β-Hydroxybutyrate, 1 of the major ketones produced through a KD, can suppress nucleotide binding and oligomerization domain-, and pyrin domain-containing protein 3, which may reduce systemic inflammation.[55] However, the KD is extremely restrictive to maintain the ketosis state. For a daily caloric intake of 2000 calories, carbohydrate intake cannot exceed 50 g. As a result, compliance with the KD is low. The recommendation to engage in a high-fat diet may also contribute to dyslipidemia, but the evidence supporting this claim is controversial.[56] There is also ample evidence of the adverse inflammatory effects of high-fat diets on the body, which may outweigh the protective effects of ketones.[52]

In addition to modulating fat intake, alteration of the type of carbohydrates consumed in one's diet may significantly influence the microbiome. In 1 randomized crossover trial, a diet rich in polysaccharides (eg, a whole-grain diet) as opposed to refined grains (eg, monosaccharide-heavy diet) was shown to significantly reduce body weight and biomarkers of systemic inflammation, without significantly altering the composition of fecal flora.[57] In a separate randomized trial, exposure to wheat bran extract, rich in arabinoxylan oligosaccharides, was associated with significant changes in gut flora, specifically an increase in the abundance of *Bifidobacterium* spp and reduction in *Bacteroides* spp, with an associated mild (nonsignificant) reduction in blood pressure among participants.[58]

The frequency of eating has also received considerable attention in primary ASCVD prevention. Intermittent fasting is used to describe fasting patterns that exceed typical overnight fasting. A literature review of 4 clinical and 18 preclinical studies concluded that intermittent fasting has been associated with increased α and β diversity.[59] A study of adults who fasted 17 hours for Ramadan found increased relative abundance in commensal gut microbial species *Akkermansia muciniphila* and *Bacteroides fragilis* when compared with baseline.[60] Both *Akkermansia* spp and *Bacteroides* spp are thought to be favorable bacterial species associated with reducing systemic inflammation and improving obesity, T2D, and hepatic steatosis in mice.[61,62]

The intricate relationship between the gut microbiome, ASCVD risk factors, and diet underscores the profound impact of nutritional choices on health and well-being. In addition to dietary patterns affecting microbiome-related risk factors for ASCVD, microbiome composition can also modulate anthropometric outcomes of dietary interventions. Over the course of a 24-week high-fiber dietary intervention with daily 500 kcal deficit, individuals with high *Prevotella* to *Bacteroides* ratio prior intervention lost, on average, 8.3 kg more body weight than individuals with low *Prevotella* to *Bacteroides* ratio.[63] This research can help provide insight into understanding the varying and seemingly inconsistent results of dietary interventions. The complex interplay between these factors emphasize the need for personalized dietary recommendations that consider individual microbiome profiles to optimize health outcomes and mitigate disease risks.

CURRENT AND FUTURE THERAPEUTIC TARGETS

Several methods to alter the gut microbiome have been tested in both preclinical and clinical settings including gut decontamination with antibiotics, prebiotics (metabolic substrates for eubionts), probiotics (live cultures of eubionts), synbiotics (mixtures of pre- and probiotics), and varieties of transplantation of fecal microbiota (FMT). To date, there have been no successful human trials of FMT for the treatment of ASCVD. However, the preclinical data are promising. Several murine models have shown safety and efficacy of FMT for the treatment of systemic atherosclerosis.[16,64] In humans, 1 double-blind randomized controlled pilot study of a single vegan donor FMT in 20 male patients with metabolic syndrome, investigators found no significant improvement in circulating TMAO levels, cytokine production, or change in uptake of ^{18}F-fluorodeoxyglucose in the aorta (which would correlate with morphologic plaque changes).[65] While there were significant changes in intestinal microbiota composition after 2 weeks of follow-up, this did not translate to meaningful improvements in biomarkers of systemic inflammation or atherogenesis. It is possible the lack of benefit with FMT in this trial was related to the inclusion of a highly specific demographic (western European White men), short duration of follow-up (2 weeks) or that the participants continued to engage in omnivorous diets (which may reconstitute trimethylamine-producing pathobionts).

Numerous preclinical trials of gut microbe-targeted therapeutic interventions in rodent models of atherosclerosis have been performed. In 1 systematic review and meta-analysis of these studies, 9 studies of probiotics in apolipoprotein E knockout mice showed, in toto, reduction in atherosclerotic plaque size, most significantly in those treated with *Pedicoccus acidilactici*, VSL#3, and male mice treated with *Lactobacillus mucosae*.[66] Many studies point to the prevalence of SCFA-producing bacteria as a key mechanism by which probiotic strains ameliorate atherosclerotic plaque. However, *Akkermansia muciniphila* (among the most extensively studied

probiotic strains) may also reduce circulating levels of TMAO in addition to its association with increased SCFA production. It is likely that probiotics may exert beneficial effects indirectly, through modulation of gut microbial network metabolism and fostering of beneficial consortia. More work is needed to validate these findings in patient cohorts and identify causal mechanisms from mechanism-oriented studies in preclinical models.

Other targets of the microbiome include agents that inhibit microbial production of trimethylamine such as resveratrol. In mice, administration of resveratrol has been shown to reduce trimethylamine and increase proliferation of commensal genera *Bifidobacterium* and *Lactobacillus*.[67] FMT from donor mice treated with resveratrol has also been shown to be effective at improving insulin resistance[68] and reducing blood pressure.[69] In human trials, oral resveratrol has been associated with improved insulin resistance among people with diabetes[70] and may have beneficial effects on endothelial function.[71] Many of these relationships have not been corroborated in other trials,[72] which may be related to nonstandardized doses of resveratrol or combination of resveratrol with other anti-inflammatory or bioactive compounds.[73] The effect of higher doses of resveratrol (eg, >150 mg/day) on insulin resistance is promising; however, the other cardiovascular effects are poorly substantiated at this time.

A related prebiotic compound, berberine, has received recent attention as a bioactive compound that can reduce plaque formation. Long used to treat bacterial causes of diarrhea in China,[74] with known efficacy in clinical trials,[75] berberine is a naturally occurring alkaloid found in several plant species (particularly *Coptis chinensis*) with a wide variety of pharmacologic properties, for which it has been used in traditional medicine for millennia. Its pleiotropic mechanisms as an anti-inflammatory agent, glucose-lowering therapy,[76] and antibiotic agent stem from its multiple interactions with various enzymes and macromolecules including telomerase, nuclear factor κ-light-chain-enhancer of activated B cells, and matrix metalloproteinases. It has also been shown in cellular studies to reduce the transcription of PCSK9 mRNA,[77] which can reduce atheroma formation. Furthermore, it has been shown to reduce the risk of AF in human trials,[78,79] which would expectedly lower cerebral embolic risk independently of its effect on the vascular endothelium. As it relates to the microbiome, berberine has been shown to significantly reduce gut microbial production of trimethylamine.[80] In animal studies, berberine attenuates trimethylamine production in the presence of a high-choline diet,[81] with multiple downstream effects including lowering body mass index, reducing P-selectin expression in the vascular endothelium (reducing platelet activation), and reducing thrombus formation. Moreover, it accomplishes this without showing evidence of major bleeding risk.[82] Berberine has also been shown in mice to reduce total cholesterol, low-density lipoprotein, and very-low-density lipoprotein levels, and to alter the composition of the gut microbial flora (increasing the amount of commensal microorganisms) in the setting of a high-fat diet.[83,84] Perhaps most impressively, in 1 human trial, berberine was shown to induce carotid plaque *regression* over time,[80] an effect that has not been seen with other diet/lifestyle modifications or high-intensity statin therapy. Other than its mild adverse effect of lowering serum glucose levels,[85] which could be beneficial in patients with diabetes, berberine has enormous potential to reduce the risk of vascular disease in patients with prior stroke (Figure 5).[86] Other methods of suppressing trimethylamine/TMAO production from choline have been proposed,[87] including CutC/D lyase inhibition and inhibition of flavin-containing monoxygenase-3, but have yet to be systematically studied in atherosclerosis models.

While "diet and lifestyle modifications" remain a mainstay of secondary prevention strategies in patients with cerebrovascular and cardiovascular disease, emphasis has been placed on the use of lipid-lowering pharmacologic agents in at-risk patients.[88] The mechanisms by which cholesterol-lowering pharmacologics improve vascular outcomes are thought to be related to inhibition of cholesterol synthesis, endothelial stabilization, and prevention of inflammation, however some of these agents may also mediate their effects by modulating the microbiome. For example, the variability in response to statin therapy (that is not fully accounted for by diet, lifestyle, or genetic predisposition[89]) may be explained in part by the microbiome.[90] In their analysis of >2000 patients, Wilmanski et al reported a higher prevalence of Bacteriodetes species and less biodiversity in the gut microbiome of patients with a greater statin response. How the diversity of the gut microbiome modulates the lipid-lowering effects of statins remains poorly elucidated but may be related to the way statins alter the composition of the gut flora and reduce dysbiosis.[91] The effects of the microbiome on responses to other lipid-lowering therapies (eg, ezetimibe, PCSK9 inhibitors) are less clear.[92,93] Caparros-Martin and colleagues recently characterized changes in the gut microbiota and metabolome following initiation of the PCSK9 inhibitor alirocumab and revealed a significant correlation between low-density lipoprotein cholesterol reduction and fecal secondary bile acid concentration and substantial elevation in circulating propionate.[93] Identification of the relevant changes in the microbiome that mediate the protective effects of statins (eg, NCT04215237) or other lipid-lowering therapies may improve our understanding of how nonpharmacologic interventions such as

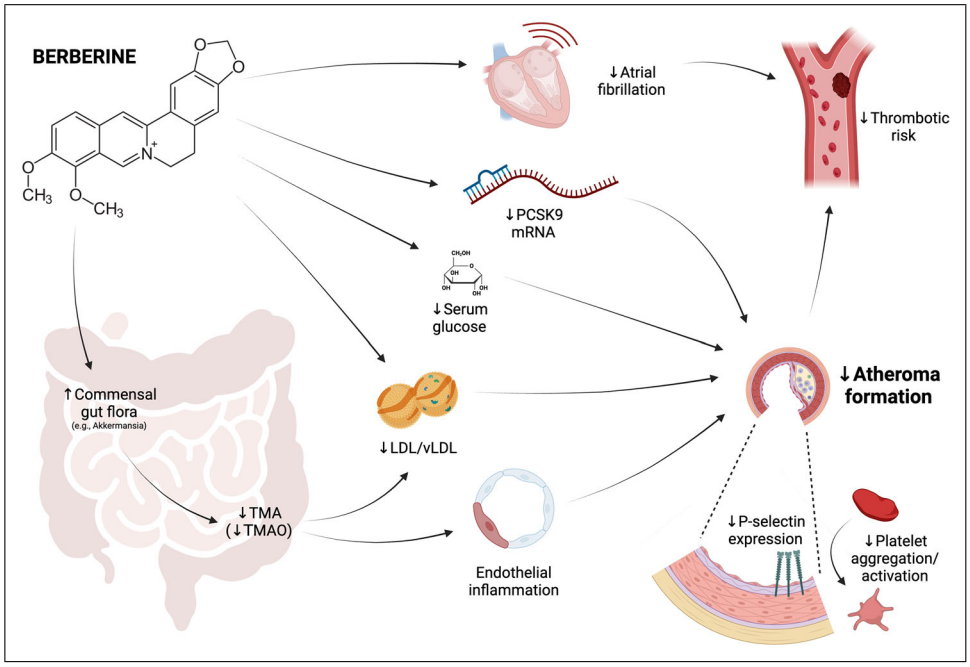

Figure 5. Berberine as a potential therapeutic agent in the treatment of vascular disease. Figure generated using biorender.com. LDL indicates low-density lipoprotein; mRNA, messenger ribonucleic acid; PCSK9, proprotein convertase subtilisin-kexin type 9; TMA, trimethylamine; and vLDL, very-low-density lipoprotein.

diet or probiotic treatments can produce similar health-enhancing effects.

In addition to the previously mentioned PREDIMED trial, other targeted interventions aimed at the microbiome have shown some success in the treatment of ASCVD. In one 3-armed trial evaluating a more plant-based Mediterranean diet versus other Mediterranean diet variations, the DIRECT-PLUS (Dietary Intervention Randomized Controlled Trial Polyphenols Unprocessed Study) investigators reported that restriction of meat intake coupled with daily green tea and a plant-based shake was associated with greater improvement in baseline lipid profiles, glycemic indices, blood pressure reduction, and greater reduction in waist circumference.[94] Further, the investigators observed proportional increases in commensal gut microorganisms such as *Prevotella* spp, and reductions in *Bifidobacterium*, which mediated the effect on improved patient cardiometabolic profiles.[95] The ongoing MEDIMACS (Impact of Mediterranean Diet, Inflammation, and Microbiome After an Acute Coronary Syndrome; NCT03842319) trial is currently randomizing patients with a recent acute coronary syndrome to a monitored, intensive Mediterranean diet versus standard of care.[96] These and other ongoing trials (NCT06220994, NCT04792320) will explore the impact of altering the microbiome in ASCVD.

Antibiotic therapy for the treatment of systemic infections is also known to alter the gut flora.[97] In addition to gut dysbiosis, there are other untoward effects of systemic antibiotic use including antimicrobial resistance, antimicrobial toxicity, and other emerging consequences such as inflammatory bowel disease and graft-versus-host disease (Figure 2).[98] With a growing awareness of commensal microbiota, there is an increasing emphasis on stewardship with targeted antibiotics to avoid detrimental effects related to losses of beneficial microorganisms.[99,100] Commensal gastrointestinal species not only aid in digestion, but they also impair the proliferation of pathologic species, a phenomenon known as "colonization resistance."[101] In supporting this complex and beneficial ecosystem, not only is it important to consider duration of antibiotic treatment but, when possible, selection of targeted antibiotics (eg, nitrofurantoin[102] or trimethoprim-sulfamethoxazole,[103] which have a limited influence on commensal gut anaerobes) ought to be considered.

APPLICATION AND SCALABILITY

As we continue to explore novel antithrombotic agents targeting known hemostatic pathways, we are finding marginal (if any) benefits with respect to secondary stroke prevention in atheroembolic stroke.[104,105] Moreover, the benefits of antithrombotic treatment appear time-dependent and decay rapidly after acute cerebral infarction.[106,107] More aggressive lipid-lowering therapeutics targeting PCSK9 have been associated with more significant, continuous relative risk reduction.[108]

Compared with these targeted strategies for reducing stroke risk, there may be greater untapped potential in leveraging the microbiome to modulate atherosclerosis, with other downstream advantages in promoting metabolic health, reducing systemic inflammation, and improving survival.[109]

Efforts to translate microbiome-based diagnostics into clinical practice have been challenging in part due to confounders of interpatient and interpopulation microbiome variability.[110] Commonly used 16S rDNA technology, which sequences a single bacterial gene and assigns taxonomy on the basis of nucleotide variants, captures only microbiome membership and abundance. Given the uniqueness of strains between individuals, 16S lacks the resolution to resolve many differences. While population variability remains high in terms of microbiota composition, function is conserved likely due to the universal processes each microbiome must provide (eg, digestion of complex carbohydrates and conversion of bile acids).[111] Genomic technologies that measure function include shotgun metagenomics and metatranscriptomics, wherein all bacterial genes or transcripts are sequenced. However, these technologies are time consuming to analyze, expensive, and, in the case of bacterial mRNA, highly labile. These limitations have prompted the microbiome field to increasingly adopt metabolomics to evaluate the status of the microbiome as it directly measures the functional output and thus the closest representation of phenotype.[112] Use of technologies such as 16S rRNA sequencing of fecal material and serum testing for known microbial metabolites are growing in the research setting. However, they have yet to significantly translate into clinical practice.

The application of these diagnostic tools and interventional strategies may be endless, particularly given the pleiotropic benefits of modulating the microbiome. There is also a growing precedent to personalizing health care at the individual level. Historically, we have tailored lipid-lowering therapies to individual low-density lipoprotein and triglyceride targets.[113] More recently, stroke physicians have tailored antithrombotic therapies on the basis of the metabolism/activation of P2Y12 inhibitors,[114] with evidence supporting personalized selection of ticagrelor over clopidogrel for certain individuals.[115] In fact, gut microbial sampling may help determine efficacy of antiplatelet regimens, as these have been shown to alter gut microbial composition in studies unrelated to atherosclerotic disease.[116] Outside of cerebrovascular disease, there is growing use of high-throughput genomic analyses for central nervous system infections,[117] leading to more accurate identification of pathogens and targeted antibiotic treatment. Such approaches could be easily applied to analyses of the intestinal microbiome. Only time will tell if and when our preclinical knowledge regarding the microbiome will translate into meaningful clinical applications for the next generation of health care.

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