NEURODEGENERATIVE DISEASES: LOOKING BEYOND THE BOUNDARIES OF THE BRAIN

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NEURODEGENERATIVE DISEASES: LOOKING BEYOND THE BOUNDARIES OF THE BRAIN

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EDITORIAL
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Editorial: Neurodegenerative Diseases: Looking Beyond the Boundaries of the Brain

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Editorial on the Research Topic

Neurodegenerative Diseases: Looking Beyond the Boundaries of the Brain

Neurobiological wisdom has long entertained the notion that the brain has functional primacy over the body. Under this presumption, the body serves as the "machine" through which the brain manifests its "sublime workings" in the form of overt behavior. The body, nonetheless, does much more than only being the brain's marionette. Indeed, it "dialogues, cares, nourishes and may even condition" the brain so that its functional morphology stays operative throughout life until, eventually, with increasing age, the body-brain whole irreversibly decays as death approaches.

As the saying outlines, "a healthy mind is a healthy body" and numerous instances support this simple yet profound notion. The occurrence of reciprocal altruistic/instructive trophic interrelationships between the body and the brain has existed for decades (Purves, 1992). Scientific awareness about the overpowering influence the body's inner sensing has on our conscious cognitive abilities and behavioral manifestations has also increased greatly. Indeed, embodied cognition and emotions are no longer denied facts (Seth and Friston, 2016; Holzer, 2017; Nummenmaa et al., 2018).

Despite this information, many scientists still believe that the origin of neurodegenerative processes rests within the brain itself. However, this neurocentric view is confronted by relatively new evidence suggesting that neuronal deterioration may be ignited, and its progression fostered by processes ongoing outside the boundaries of the brain. These include, but are not limited to, an impaired cardiovascular system, infection, systemic inflammation, cellular senescence, altered trophic interactions, endocrine disruption, and gut dysbiosis (Preciados et al., 2016; Castillo et al., 2019; Limphaibool et al., 2019; Winek et al., 2022). "*Neurodegenerative Diseases: Looking Beyond the Boundaries of the Brain*" is a volume that looks at brain diseases as consequences rather than as the result of primary neurological causes.

Gut microbiota, for instance, modulate the immune system in a variety of neurological diseases (Cryan et al., 2020; Willyard, 2021). The imbalance of its composition, distribution, and metabolic activity may lead, as commented by Maiuolo et al., to anxiety, depression, autism spectrum disorder, and multiple sclerosis. Studying the crosstalk between neurons, mucosal immunity, and gut microbiota may help us improve therapeutic measures aimed at lessening these disorders. Dumitrescu et al. evaluated the presence of biomarkers of intestinal inflammation and barrier permeability in the peripheral circulation and stool samples obtained from Parkinson's disease subjects. The correlation observed supported a causal link between dysbiosis, enhanced

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inflammatory response, and the progression of neurodegeneration. Rydbom et al. demonstrated that Tau is capable of disrupting gut motility, microbiome composition, and innate immune response in Drosophila. This opens the possibility that peripheral tauopathy may alter the availability of the antimicrobial peptides that oversee the elimination of pathogenic microorganisms that might promote neurodegeneration. Lastly, sirtuins, a family of histone deacetylases, modulate genome stability, stress cellular response, and nutrient and hormone sensing in response to various metabolites that signal aging, obesity, and diabetes. Chandramowlishwaran et al. revised the novel role of sirtuins on enteric neuronal growth and survival, and propose sirtuins as novel modulators of the gut-brain axis.

In addition to dysbiosis, viral infections are presumed to cause neurodegeneration (Limphaibool et al., 2019; Shinjyo and Kita, 2021). With the novel SARS-CoV-2 infection, growing evidence supports infections as an etiological path to neurodegeneration and cerebrovascular disease (Wenzel et al., 2021; Douaud et al., 2022). Römer reviews the epidemiological and experimental evidence that links viruses and endogenous retroviruses to neuro-immune degeneration.

Lifestyle and metabolic alterations during midlife are considered important risk factors for developing Alzheimer's Disease (AD) (Livingston et al., 2017). Memory dysfunction in AD patients results from the brain and peripheral glucose resistance (Arnold et al., 2018). Wei et al. revise the multiple pathogenic mechanisms induced by insulin resistance that are implicated in AD and discuss the use of antidiabetic and anti-inflammatory drugs to delay the onset of neurodegeneration. Sim et al. offer a comprehensive overview of the potential role of dipeptidyl peptidase-4 inhibitor and sodium-glucose cotransporter 2 inhibitors, both used in type-2 diabetes patients, as repurposing drugs against AD based on their antidiabetic effects.

As already mentioned, several lifestyle factors during midlife are important modulators of dementia onset later in life. In particular, unhealthy dietary habits influence neurodegenerative diseases through cellular inflammation and increased oxidation (Tan and Norhaizan, 2019; Winiarska-Mieczan et al., 2020). Nassir et al. provide a persuasive and plausible explanation that supports the role of cellular-derived circulating microparticles released after consuming unhealthy diets in promoting thrombotic events throughout the microcirculation. When thinking about the etiology of neurodegeneration, these microparticles must then be considered as risk factors and biomarkers of the brain-heart-gut axis.

The erythroid 2-related factor 2 (NRF2) forms part of the molecular machinery that supports the body's antioxidant defense system. Petrillo et al. performed a family study of Friedreich's ataxia, the most frequent autosomal recessive ataxia in Western societies. Although all family members were affected by frataxin depletion, those having activation of NRF2 were asymptomatic. The authors propose that the constitutive upregulation of NRF2 keeps the antioxidant defense above the threshold, a circumstance that protects against progressive oxidative damage.

The glymphatic circulation modulates neuroinflammatory responses in neurodegenerative diseases. Natale et al. argue that, besides waste elimination, the glymphatic system may also function to connect the brain with the periphery. It then facilitates communication between the immune system and the brain, thus modulating the brain's immune surveillance and neuroinflammation. The functional disruption of the glymphatic system must then be considered when unrevealing the mechanisms of neurodegeneration.

It becomes more and more clear that neurodegeneration progresses silently for many years or even decades before it becomes diagnosed. Therefore, the identification of early clinical biomarkers is urgently needed. Zhang et al. suggest that evaluating retinal degeneration may be a gateway to understanding, monitoring the progression, and diagnosing Parkinson's disease, as visual symptoms appear early in the disease.

Taken together, this Research Topic highlights the relevance of studying the etiology of neurodegeneration beyond the boundaries of the brain. Notably, this compilation of articles describes how peripheral alterations can impact brain function and health. Further research is needed to better understand these paths to develop more effective and early treatment options to stop the growing number of patients suffering from neurodegenerative diseases.

AUTHOR CONTRIBUTIONS

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REVIEW
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Role of Sirtuins in Modulating Neurodegeneration of the Enteric Nervous System and Central Nervous System

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Neurodegeneration of the central and enteric nervous systems is a common feature of aging and aging-related diseases, and is accelerated in individuals with metabolic dysfunction including obesity and diabetes. The molecular mechanisms of neurodegeneration in both the CNS and ENS are overlapping. Sirtuins are an important family of histone deacetylases that are important for genome stability, cellular response to stress, and nutrient and hormone sensing. They are activated by calorie restriction (CR) and by the coenzyme, nicotinamide adenine dinucleotide (NAD^+). Sirtuins, specifically the nuclear SIRT1 and mitochondrial SIRT3, have been shown to have predominantly neuroprotective roles in the CNS while the cytoplasmic sirtuin, SIRT2 is largely associated with neurodegeneration. A systematic study of sirtuins in the ENS and their effect on enteric neuronal growth and survival has not been conducted. Recent studies, however, also link sirtuins with important hormones such as leptin, ghrelin, melatonin, and serotonin which influence many important processes including satiety, mood, circadian rhythm, and gut homeostasis. In this review, we address emerging roles of sirtuins in modulating the metabolic challenges from aging, obesity, and diabetes that lead to neurodegeneration in the ENS and CNS. We also highlight a novel role for sirtuins along the microbiota-gut-brain axis in modulating neurodegeneration.

Keywords: central nervous system, enteric nervous system, gut microbiota, myenteric plexus, neuronal survival, neurodegeneration, sirtuin (SIRT)

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THE EFFECTS OF AGE, OBESITY, AND DIABETES ON NEURODEGENERATION IN THE CNS AND ENS

Neurodegeneration in the CNS is characterized by a progressive loss of distinct groups of neurons in specific regions of the brain, deposition of misfolded proteins in neurons, and alterations in astrocytes (Przedborski et al., 2003; Maragakis and Rothstein, 2006) which results in cognitive dysfunction, loss of synapses, impaired synaptic plasticity, disrupted neuronal signaling, and cell

death. The factors that contribute to neuronal stresses in the CNS are aging, neurodegenerative diseases (NDs), comorbidities such as obesity and diabetes, over nutrition via high calorie intake, a lack of physical activity, and genetic background (Popa-Wagner et al., 2020). These stresses and changing physiological demands from oxidative damage, protein aggregation, dietary changes, inflammation, high metabolic demands, are counteracted by cells to maintain cellular, protein, and metabolic homeostasis (Squier, 2001; Uttara et al., 2009). Similar to the CNS, the neurons of the enteric nervous system (ENS) or the "brain within the gut," are also prone to neurodegeneration. The ENS is a subdivision of the peripheral nervous system and functions independently of the central nervous system (Nezami and Srinivasan, 2010). The ENS is embedded within the walls of the intestine and directly controls gastrointestinal functions. The neurons and glia in the ENS are structurally organized into two interconnected layers, the myenteric and the submucosal plexi. The myenteric plexus, which is located between the circular and longitudinal muscle layers, regulates gastrointestinal motility whereas the submucosal plexus, which is located between the circular muscle and mucosa, regulates secretory activity. In these plexuses, the neuronal cells form groups of interconnected ganglions that are surrounded by glia. The individual ganglia are connected to each other and to the epithelium by neuronal projections (Nezami and Srinivasan, 2010). The ENS, intestinal epithelium, gut microbiota, and immune cells work in harmony together to ensure the proper functioning of the intestine (Walsh and Zemper, 2019). ENS-related neurodegeneration is particularly evident in individuals with aging and neurodegenerative disorders. Moreover, individuals with aging or NDs also experience symptoms related to gastrointestinal dysmotility especially chronic constipation due to loss of enteric neurons in the myenteric plexus leading to ENS dysfunction (Poirier et al., 2016; Rao and Gershon, 2016). Additional stresses from dietary changes and antibiotic treatments can alter the gut microbiota and also influence ENS function (Carabotti et al., 2015). Further understanding of the factors leading to neurodegeneration of the CNS and ENS is critical and can lead to new therapeutic targets.

Aging Associated Neurodegeneration

Aging is a multifactorial process accompanied by many changes at the cellular, tissue, and organismal level over time and has shown to be a major risk factor for neurodegeneration of the CNS and the ENS (Wade and Cowen, 2004; Hou et al., 2019). Cross-sectional, longitudinal, and quantitative magnetic resonance imaging (MRI) and voxel-based morphometry (VMI) analyses report reduced brain volume and brain atrophy especially in the hippocampus and the prefrontal cortex, in conjunction with a decline in cognitive functioning in older adults (Liu et al., 2003; Terribilli et al., 2011; Ramanoel et al., 2018). *In vitro* studies in rat primary cortical and hippocampal neurons, cultured long term, demonstrate irreparable DNA damage that underlies normal aging leading to proteostasis and cell senescence (Ishikawa and Ishikawa, 2020). This causes a loss of ability to repair tissues, chemokine and cytokine release, low grade inflammation, and results in age-related neurodegeneration. Population-based studies of cognitively unimpaired aged people reported an accumulation of abnormal protein deposits that positively correlated with age (Elobeid et al., 2016). While age-associated neurodegeneration is accompanied by a gradual loss of neurons, rapid progression in behavioral and cognitive changes have been attributed to chronic neurodegenerative diseases (ND) such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Wilson et al., 2010). Along with these functional declines, the cerebral levels of neurotransmitters such as dopamine, acetylcholine, serotonin, and norepinephrine, and neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are dramatically reduced in aging brains (Vecchio et al., 2018).

The impact of aging on the ENS is controversial. Enteric neurons from the colon of human tissues have been shown to decrease in the 4th year of age in both the plexuses with a further loss of 37% of total neuron population between the ages 20–65 (Gomes et al., 1997). Some studies have reported a loss of 50–60% of myenteric neurons has been reported in the aging guts and especially the colon of aging rats (Santer and Baker, 1988; Nezami and Srinivasan, 2010) while other studies contrasted that myenteric neuron numbers in the aging colon remain the same albeit with functional changes (Gamage et al., 2013). Neurotransmitters produced by myenteric neurons and neuronal reflexes mediate late neurogenesis and regulate intestinal motility (Cooke, 2000; Nezami and Srinivasan, 2010). Neurons that produce the choline acetyl transferase (ChAT) and neuronal NOS (nNOS) regulate intestinal motility by stimulating and inhibiting intestinal smooth muscles, respectively (Porter et al., 2002). An imbalance in the equilibrium between the nNOS- and ChAT-producing neurons can alter the ENS architecture and result in an altered bowel motility (Nezami and Srinivasan, 2010). These changes presumably alter the normally well-orchestrated crosstalk between the enteric neurons and glia. Some studies also report a loss of choline acetyltransferase (ChAT) and no changes in neuronal nitric oxide synthase (nNOS)- expressing myenteric neurons (Phillips et al., 2003; Nezami and Srinivasan, 2010) while other studies report the loss of nNOS and ChAT neurons in the myenteric plexus of aging mice (Becker et al., 2018; Sun et al., 2018). In rats, studies have reported a loss of submucosal neurons in the proximal and distal colon in 12 months old animals when compared to 3 month old animals with a greater loss occurring in the distal colon and maximum loss occurring at 24 months of age (Saffrey, 2013). In samples from human colon and ileum, the myenteric ganglia had a wider area overall, with a larger proportion of them with increased gaps within the ganglia and this correlated with increasing age, that might contribute to gut dysmotility seen in older individuals (Hanani et al., 2004). Factors and pathways cumulatively associated with neurodegeneration in the CNS and the ENS with aging (Wyss-Coray, 2016) are diagrammatically represented in **Figure 1**.

Abbreviations: Ab, Antibody; CR, Calorie restriction; CNS, Central nervous system; ENS, Enteric nervous system; GI, Gastrointestinal; HFD, High-fat diet; KO, Knock out; NDs, Neurodegenerative diseases; NF-κB, Nuclear factor kappa B; PGC-1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; ROS, Reactive oxygen species; Sirtuins, Silent information regulator genes: sirtuins; SCFA, Short chain fatty acid: SCFA; T2D, Type II diabetes; WT, Wild type.

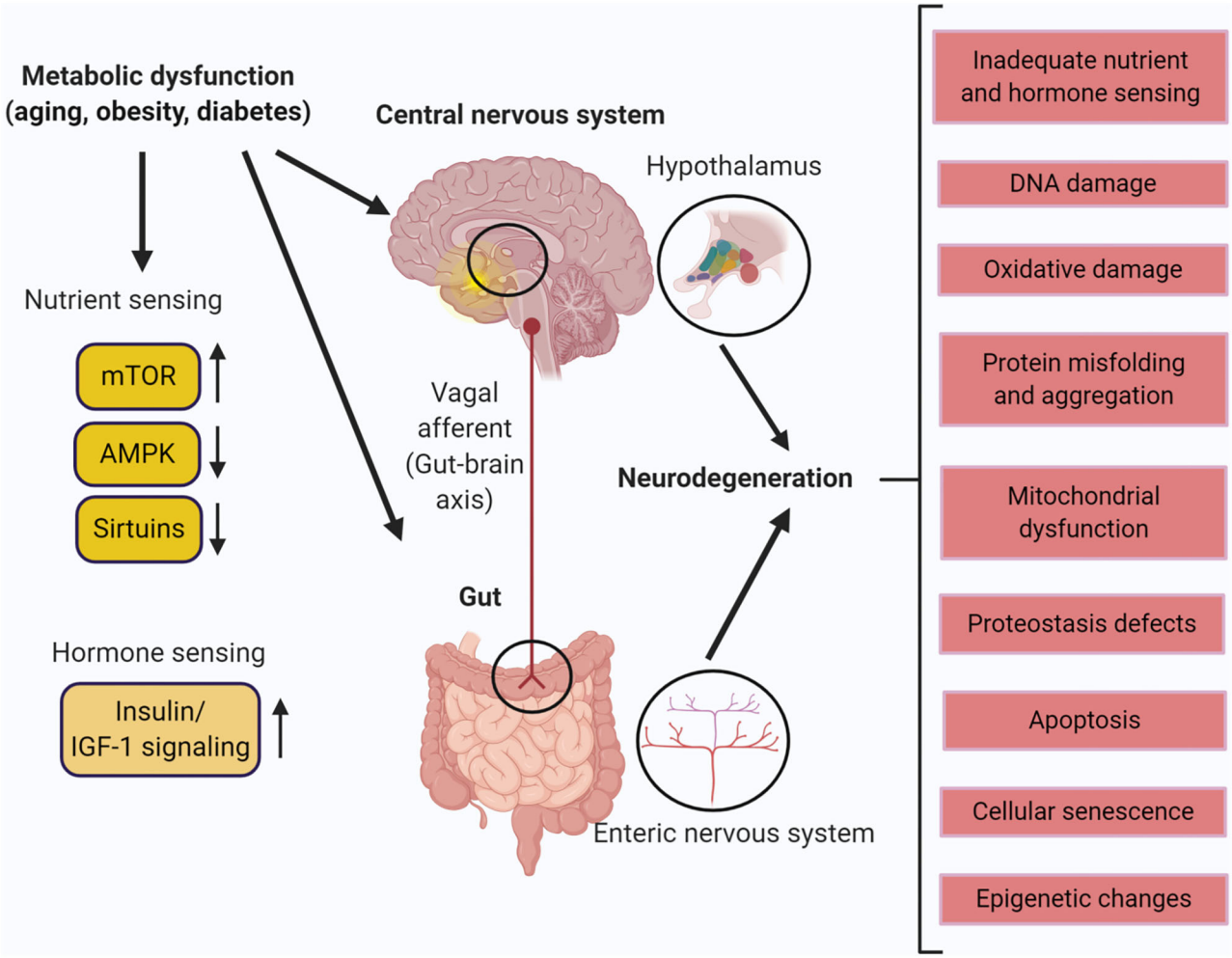

FIGURE 1 | Metabolic dysfunction in the central nervous system (CNS) and the enteric nervous system (ENS). Aging, obesity, and diabetes leads to different molecular and physiological changes that cause neurodegeneration in the hypothalamus of the CNS and the ENS of the gut. Created with BioRender.com.

Neurodegeneration Associated With Metabolic Disorders—Obesity and Diabetes

Obesity and type II diabetes (T2D) have been suggested to accelerate the physiological process of aging (Thorpe and Ferraro, 2004; Kalyani et al., 2017) and obesity is a known risk factor for T2D development (Al-Goblan et al., 2014). In age-dependent and age-independent studies, obesity has been shown to double the risk for mild cognitive impairment, dementia, and AD (Qiu et al., 2009; Hildreth et al., 2012). T2D causes brain atrophy, reduced cerebral glucose metabolism, and insulin resistance in the CNS, and this is also seen in AD (Arnold et al., 2018). Accumulation of misfolded phosphorylated tau and amyloid beta (Arnold et al., 2018) in the brain as well as the islet amyloid polypeptide (amylin) (Raimundo et al., 2020) co-secreted with insulin in the islet beta cells are major pathological features observed in T2D patients who develop AD. As seen in aging, computed tomography demonstrated structural changes in the obese brain such as atrophy in the hippocampus and decreased hippocampal volume (O'Brien et al., 2017). The prefrontal cortex and the hippocampus which are crucial for learning and memory are most vulnerable to obesity-related changes (Bischof and Park, 2015). The hypothalamus controls metabolic homeostasis by sensing nutrients and hormones via autonomic and neuroendocrine signaling to integrate the signals of satiety. Inflammation of the hypothalamus from high fat feeding induces Inhibitor Of Nuclear Factor Kappa-B (IKKβ)/NF-kB-dependent inflammation, changes satiety control, and increases the risk for developing obesity (Timper and Bruning, 2017). Magnetic resonance imaging (MRI) has shown an inverse relationship between Body Mass Index (BMI) as well as diabetes and brain volume, neuron viability, and gliosis in the hypothalamus (Thomas et al., 2019). Obese individuals with higher BMI with no cognitive defects also displayed decreased gray matter, brain atrophy in the frontal lobe, hippocampus and thalamus when compared to non-obese, thus demonstrating extensive neurodegeneration (Stillman et al., 2017). Vascular defects from obesity leading to cognitive decline include lipotoxicity, diabetes

impaired insulin metabolism and signaling pathway or defect in glucose transport mechanisms in the brain (Uranga and Keller, 2019). Adipose tissues including white adipose tissue (WAT) are important for metabolism and hormones derived from WAT such as leptin and adiponectin are involved in regulating obesity and diabetes (Stern et al., 2016). Leptin plays a major role in body weight regulation and reducing appetite (Ramos-Lobo and Donato, 2017). Leptin bound to its receptor Ob-Rb has been shown in the cortex and the hippocampus which are major sites of neurodegeneration in aging, AD and PD (McGregor and Harvey, 2018). Another adipokine, adiponectin has been shown to have an opposite effect to leptin in inflammation and insulin resistance, and the ratio of leptin to adiponectin is considered as a marker for developing T2D and obesity (Forny-Germano et al., 2018). High levels of circulating leptin caused by obesity has been shown to lead to leptin resistance in the hypothalamus and is linked to altered metabolism, inflammation, and neurodegeneration in the brain (Forny-Germano et al., 2018). Obesity and T2D also cause gastrointestinal dysmotility and lead to enteric neuronal degeneration (Yarandi and Srinivasan, 2014). Diabetic autonomic neuropathy has been shown to gastrointestinal disturbances including impaired esophageal transit, gastroparesis, and disorganized intestinal motility with constipation and diarrhea (Verrotti et al., 2014). Our studies in obese and overweight human subjects and in mice fed a high fat diet have demonstrated increased pyroptosis in nitrergic neurons, delayed colonic transit, and impaired electric field stimulation–induced colonic relaxation responses (Ye et al., 2020). In mouse Other studies in the ENS using mice models of high fat diet and obesity induced diabetic neuropathy reported a reduction in hormones such as ghrelin, cholecystokinin (CCK), and leptin levels; and inhibitory neurons expressing nitric oxide synthase (nNOS), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), and galanin as well as their expression levels (Chandrasekharan and Srinivasan, 2007; Li et al., 2011; Voukali et al., 2011; Stenkamp-Strahm et al., 2015). The neuronal changes characterized by the loss of important neurotransmitters and hormones resulted in altered gastric emptying, diarrhea and constipation that is characteristic of enteric neurodegeneration (Stenkamp-Strahm et al., 2015). Moreover, these alterations could be a compensatory mechanism to increase satiety and decrease food intake to balance weight gain in diet induced obese mice (Coll et al., 2007). A major orchestrator of pathways in response to stresses caused by age- and metabolism-associated neurodegeneration are the sirtuins (Duan, 2013). Factors and pathways cumulatively associated with neurodegeneration in the CNS and the ENS with metabolic dysfunction are summarized in **Figure 1**.

TABLE 1 | Overview of sirtuin localization, activity, substrates, functions, and activators.

Sirtuin	Specific examples relevant to metabolism
Localization	Sirtuins are present in all subcellular compartments and differ in their substrate specificities (Houtkooper et al., 2012). SIRT1, SIRT6, and SIRT7 are predominantly nuclear but also detected at lower levels in cytosol, membrane, and the cytoskeleton. SIRT2 resides in the cytoplasm (Houtkooper et al., 2012) though it is also found in the nucleus and the cell membrane (North and Verdin, 2007). SIRT3, SIRT4, and SIRT5 predominantly localize to the mitochondria although they are also found in the nucleus and the cytoplasm (Houtkooper et al., 2012).
Enzymatic activity	SIRT1, SIRT2, SIRT3, SIRT5, and SIRT7 predominantly deacetylate histone and non-histone proteins; SIRT4 and SIRT6 act as mono-ADP-ribosyl transferases (Canto et al., 2013).
Functions	SIRT1 and SIRT3—Neuronal protection and cell survival, DNA repair, chromatin remodeling, neuronal differentiation, apoptosis, energy and metabolic homeostasis, mitochondrial biogenesis, autophagy, glucose production and insulin secretion, lipid homeostasis, anti-inflammation (Yamamoto et al., 2007; Duan, 2013; Giblin et al., 2014). SIRT2—cell cycle regulation, modulation of microtubule deacetylation and myelination, tumorigenesis, neurodegeneration (Yamamoto et al., 2007; Gomes et al., 2015). SIRT4—insulin secretion, cell cycle regulation (Yamamoto et al., 2007). SIRT5—Mitochondrial metabolism, urea cycle (Yamamoto et al., 2007). SIRT6—glucose homeostasis, genome stability, DNA repair, anti-inflammation (Yamamoto et al., 2007; Zhong and Mostoslavsky, 2010). SIRT7—rDNA transcription (Wu et al., 2018).
Histone targets	H1, H3, H4 (H1K26, H1K9, H3K9, H3K56, H3K14, H4K16) by SIRT1; H4K16 by SIRT2; H3, H4 (H3K9, H4K16) by SIRT3; H2B, H3 (H2BK12, H3K9, H3K56) by SIRT6; and H2A, H2B, H3 (H3K18) by SIRT7 (Jing and Lin, 2015).
Non-histone targets	Transcriptional regulators (Martinez-Redondo and Vaquero, 2013; Jing and Lin, 2015) related to: • Stress - p53, Nuclear Factor kappa B (NF-κB), Forkhead Box (FoxO), Superoxide dismutase 2 (SOD2), Poly (ADP-ribose) polymerase (PARP), target of rapamycin (TOR) kinase (TORC2), bcl-2-like protein 4 (Bax), leucine zippers - c-Fos and c-Jun, Uncoupling Protein 2 (UCP2), Heat shock factor 1 (HSF1), b-catenin, E2F Transcription Factor 1 (E2F1), Period Circadian Regulator 2 (PER2), Circadian Locomotor Output Cycles Kaput (CLOCK) • Metabolism - Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), Liver X receptor (LXR), Farnesoid X receptor (FXR) • DNA repair - Ku70, Peroxisome proliferator-activated receptor gamma (PPARγ) • Structural protein - α-tubulin • Chromatin remodeling - p300, MOF
Agonists or stimulators	Resveratrol [activated SIRT1 and reduced signs of aging without changing the expression patterns of other sirtuins (Borra et al., 2005)], SRT1720 (Huynh et al., 2013) and oxazolo[4,5-b] pyridines (Bemis et al., 2009) [activated SIRT1 to treat diabetes and insulin resistance in mice (Bemis et al., 2009)], pyrrolo[3,2-b]quinoxalines [promoted SIRT1, SIRT2, and SIRT3-dependent anti-inflammatory properties *in vitro* (Villalba and Alcain, 2012)], and honokiol [activated SIRT3 and counteracted oxidative stress and mitochondrial damage in AD and diabetes studies (Ramesh et al., 2018; Zheng et al., 2018)].

FUNCTION AND DISTRIBUTION OF SIRTUINS

Localization, Activators, and Substrates of Sirtuins

Silent information regulator (Sirtuins) are a family of class III histone deacetylases with a conserved catalytic domain core of 275 amino acids (Houtkooper et al., 2012). The subcellular localization, enzymatic activities, transcriptional substrates, functions, and activators of sirtuins are briefly explained in **Table 1**. As cooperative sensors and regulators of nutrients and

energy metabolism in response to changes in diet and stress, they require NAD^+ for their enzymatic activity (Anderson et al., 2017). Energy deficits by calorie restriction or cellular stressors increase NAD^+ levels and activate sirtuins (Guarente, 2013).

Distribution of Sirtuins in the CNS and the ENS

All of the seven sirtuins are ubiquitously expressed in all human tissues (Yamamoto et al., 2007). Mass spectrometry and semi-quantitative studies have shown that all the sirtuins are expressed in the human and non-human brain and small intestine (Sidorova-Darmos et al., 2014; Jayasena et al., 2016). In the brain, SIRT1 (110 KDa) and SIRT2 (37 KDa) are the most abundant and widely expressed sirtuin subtypes. SIRT1 expression is highest in the neurons of the cerebellum, hippocampus, and the hypothalamus and lowest in the spinal cord. SIRT2 is highest in the spinal cord and brain stem and is also highly expressed in the cortex, frontal lobe, hippocampus, striatum, and cerebellum. The mitochondrial sirtuins, SIRT3, SIRT4, SIRT5, are also expressed in different regions of the brain, but at lower levels than SIRT1 and SIRT2. In the brain, SIRT6 and SIRT7 are expressed at the lowest levels compared to other sirtuins. All the sirtuins, except for SIRT7, are expressed at lower levels in the small intestine than is detected in the brain. SIRT7 is the most highly expressed sirtuin in the small intestine with 10-fold higher expression than in the brain. Considering that subcellular localization of sirtuins are cell type dependent, more sampling across different cell lines and tissue types can provide information about the anatomical contribution of the lesser abundant sirtuins. Recent studies have shown that both SIRT1 and SIRT3 are expressed by neurons of the ENS (Lakhan and Kirchgessner, 2011; Bubenheimer et al., 2016). However, the role of sirtuins in ENS neurodegeneration remains unknown.

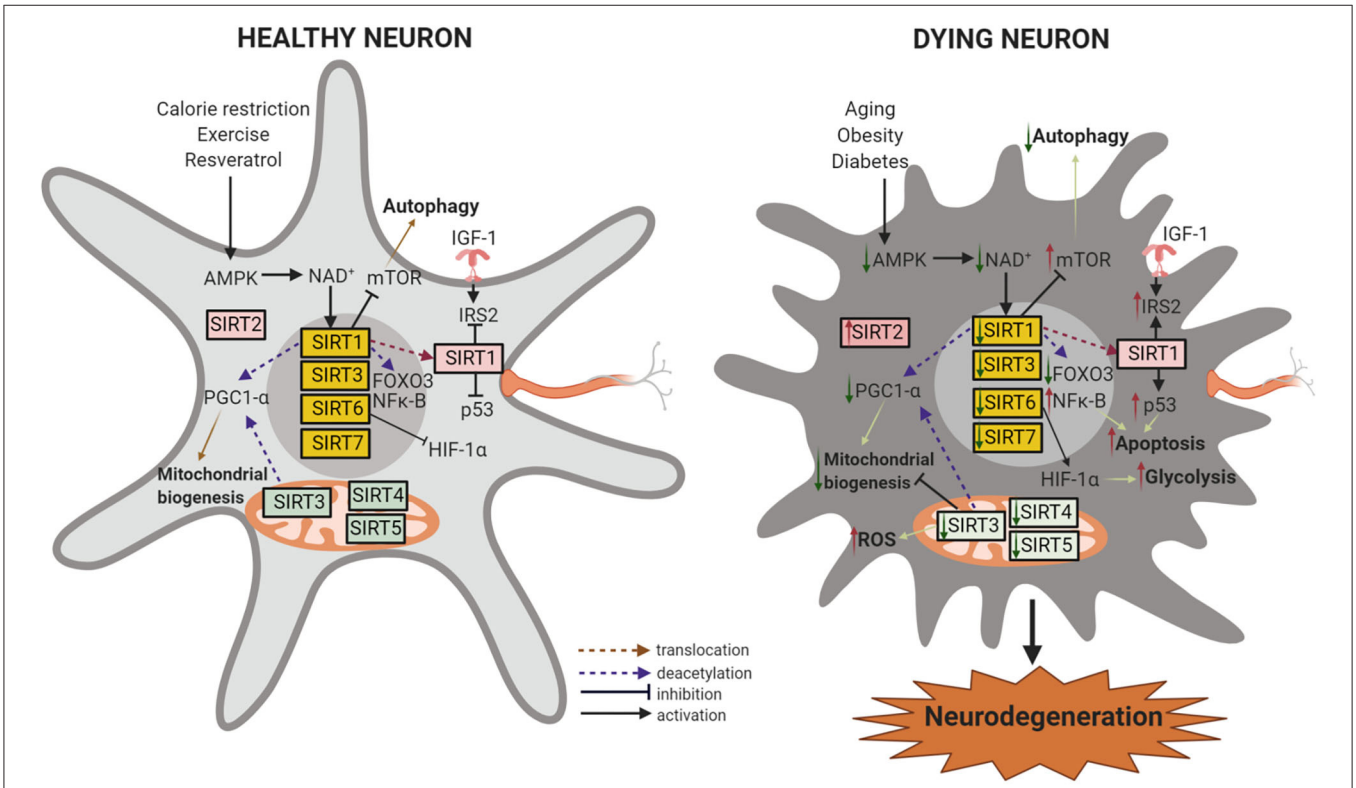

FIGURE 2 | Sirtuins in neuroprotection and neurodegeneration. In healthy neurons, downregulation of IGF-1 and activation of SIRT1 by the availability of NAD^+ induces the activation of FOXO transcription factors and the transcription of antioxidant genes in the nucleus. SIRT1 and SIRT3 activation by calorie restriction (CR) or by resveratrol also leads to PGC-1α modulation with improved mitochondrial function and decreased oxidative stress. PGC-1α and FOXOs can be directly activated through AMPK-dependent phosphorylation. SIRT1 or SIRT3 activation or SIRT2 inhibition can activate autophagy, leading to neuroprotection. SIRT4 and SIRT5 modulation of fatty acid oxidation and reducing oxidative stress contributes to mitochondrial homeostasis, and SIRT7 regulates nuclear encoded mitochondrial genes. SIRT6 represses the recruitment of HIF-1α to its target gene promoter and inhibits glycolysis and increases mitochondrial respiration. During aging, obesity, and diabetes, the reduced availability of NAD^+ causes decreased AMPK, SIRT1, and SIRT3 levels which in turn decreases the stimulatory effect of PGC-1α on mitochondrial biogenesis. Decreased SIRT1 reduces mTOR inhibition and reduces autophagy and decreases cell viability. SIRT1 can no longer suppress IGF-1, NF-κB, or p53, acetylates and stabilizes p53, and causes inflammation and apoptosis. Low levels of SIRT4 increases glutamine reflux, dysregulates insulin sensitivity, glucose metabolism, and fatty acid oxidation. SIRT5 and SIRT6 deficiency reduces ATP levels in the mitochondria. Moreover, low levels of SIRT6 leads to increased HIF-1α and results in increased glucose uptake and glycolysis. On the other hand, SIRT2 (or its isoforms) accumulate with age and promote cell death by deacetylating Foxo3a and upregulating a pro-apoptotic factor, Bim. These processes progressively lead to neuronal degeneration and cell death. Created with BioRender.com.

ROLE OF SIRTUINS IN MODULATING NEURODEGENERATION IN THE CNS

Role of Sirtuins in Modulating Neurodegeneration Associated With Aging and Neurodegenerative Diseases

Aging leads to damage of cellular organelles and accumulation of proteins that causes an imbalance in cellular homeostasis and accelerates neurodegeneration (Castelli et al., 2019). SIRT1 and SIRT6 levels increase and decrease respectively with age respectively despite similarities in cellular localization and their role in increasing lifespan (Lee et al., 2019). A reduction in SIRT1 activity was reported in post-mortem brain tissue of PD patients (Singh et al., 2017). SIRT1 has shown to be universally involved in multiple pathways associated with stress related to energy homeostasis and metabolism through epigenetic regulation and transcriptional modulation whereas SIRT6 is important for glucose metabolism and exerts neuroprotection from DNA damage (Ramadori et al., 2008; Zhong and Mostoslavsky, 2010). Overexpression of brain-specific SIRT1, ubiquitous overexpression of SIRT6, calorie restriction, or resveratrol, extended lifespan and prevented experimental AD amyloid neuropathology (Giblin et al., 2014). Resveratrol has been shown to inhibit the activity of a serine/threonine kinase called mammalian target of rapamycin (mTOR) which contrasts with nicotinamide, a SIRT1 antagonist enhanced mTOR activity and reduced age-induced autophagy (Ghosh et al., 2010). In neurons comprising of non-dividing cells, SIRT1 has been shown to foster DNA repair during double strand breaks and protect against genomic instability caused by aging (Oberdoerffer et al., 2008). Studies in rat brain, kidney, liver, and fat pad tissues showed that SIRT1 induced by calorie restriction maintained a DNA repair factor, Ku70 in a deacetylated state to sequester Bax from the mitochondria to attenuate apoptosis, thus shifting the balance from cell death toward cell survival (Amsel et al., 2008). SIRT1 and in some cases, SIRT2 and the signaling pathways of insulin and insulin-like growth factor-I (IGF-I), bidirectionally regulate each other (Sansone et al., 2013). IGF-1 is an important growth factor that has been shown to be important for neurogenesis and cell survival of neurons as well as inhibition of apoptosis during postnatal to adult stages (Nieto-Estevez et al., 2016). IGF-1 declines with age in the brains of humans and rodents, and treatment with IGF-1 agonists in preclinical models of AD and PD have shown to improve neuronal survival (Nieto-Estevez et al., 2016). Notably, SIRT1 deacetylates insulin receptor substrate 2 (IRS-2), a substrate protein for IGF-1 and activates Akt, an insulin receptor target of IGF (Sansone et al., 2013), highlighting SIRT1 importance in modulating IGF-1 signaling.

Overexpression of SIRT1 and the addition of resveratrol has shown to provide neuroprotective effects in various animal models of AD by reducing amyloid plaque formation and neurofibrillary tau pathology (Chen et al., 2005; Qin et al., 2006; Kim et al., 2007; Green et al., 2008; Karuppagounder et al., 2009; Min et al., 2010; Vingtdeux et al., 2010). SIRT1 was shown to target ADAM10, a retinoic acid receptor β target and induce Notch receptor cleavage to promote non-amyloidogenic processing of amyloid precursor protein (APP), thereby promoting neurogenesis (Donmez et al., 2010). Overexpression of SIRT1 protected SH-SY5Y neuroblastoma cells from toxin induced cell death by down-regulating NF-κB and cPARP-1 and reducing phospho-α-synuclein aggregates (Singh et al., 2017). Resveratrol acting via SIRT1/PGC-1α significantly protected dopaminergic neurons in the MPTP mouse model of PD (Mudo et al., 2012). Interestingly in other studies, SIRT1 failed to protect tyrosine hydroxylase (TH)-positive dopaminergic neuronal damage induced by MPTP (Kakefuda et al., 2009). SIRT2 inhibition was shown to reduce Aβ production and improved cognitive performance and microtubule assembly favoring cell survival (Biella et al., 2016; Silva et al., 2017). SIRT3 expression was decreased in AD patient's cerebral cortex and its dysfunction led to p53-mediated mitochondrial and neuronal damage in AD (Lee et al., 2018). Patients with AD showed a reduction in the expression of SIRT6. Increased signs of DNA damage, cell death, and hyperphosphorylated Tau, all features of neurodegenerative diseases, were observed in SIRT6-deficient mice brain (Kaluski et al., 2017) indicating the importance of SIRT6 regulation of DNA repair and maintenance of genomic stability to keep the brain healthy (Giblin et al., 2014; Kugel and Mostoslavsky, 2014). SIRT2 has largely been found as detrimental in several neurodegenerative disorders (Gomes et al., 2015). Polymorphisms in a SIRT2 intron increased susceptibility to AD and its knockout and inhibition studies improved outcomes in a PD model by reducing cytoskeletal pathology and increasing autophagy (Biella et al., 2016; Guan et al., 2016). SIRT2 inhibition may have beneficial effects for PD by rescuing α-synuclein mediated toxicity (Outeiro et al., 2007; de Oliveira et al., 2017). SIRT3 has been demonstrated to protect cortical neurons from various types of stress by increasing mitochondrial antioxidant capacity (Cheng et al., 2016). Mice with SIRT3 deletion was shown to have reduced neuron number, synaptic plasticity, and poor remote memory, thereby dramatically increasing neuronal vulnerability (Kim et al., 2011; Dai et al., 2014). SIRT3 and SIRT5 have the largest protective effects on neurons of the nigrostriatal pathway within the brain (Liu et al., 2015a,b). SIRT5 displays a protective role against MPTP-induced nigrostriatal dopaminergic degeneration by preserving mitochondrial antioxidant capacity (Liu et al., 2015b). Resveratrol and another polyphenol quercetin in mice models were shown to prevent motor neuron degeneration and polyglutamine-induced cell death in striatal neurons characteristic of motor neuron disorders such as amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and Huntington Disease (HD), respectively (Bhullar and Rupasinghe, 2013; Lazo-Gomez and Tapia, 2017). SIRT6 and SIRT7 were shown to deacetylate the nucleolar protein, nucleophosmin (NPM1) that is involved in DNA repair to regulate aging (Wu et al., 2018).

Role of Sirtuins in Modulating Neurodegeneration Associated With Obesity and Diabetes

Increased SIRT1 expression in dorsal root ganglion (DRG) neurons was shown to rescue mice from peripheral neuropathy induced by a high fat diet (HFD) (Chandrasekaran et al., 2019). SIRT1 is also regulated by the hypothalamus/pituitary axis that receives inputs related to nutrients and adiposity (Toorie and Nillni, 2014). SIRT1 inhibition in the hypothalamus, via the acetylation of FOXO1, increased neurons that express pro-opiomelanocortin and agouti-related peptide. These neurons produce satiety peptides to inhibit food intake after feeding and increase food intake in response to fasting and CR, respectively. This resulted in reduced feeding and body weight gain (Dietrich et al., 2010). This established the role of SIRT1 as an important regulator of nutrient sensing in the neural circuits that govern central and peripheral networks. SIRT3 deletion in the hippocampus of mice fed a high fat diet was shown to cause oxidative stress and impaired cognition (Tyagi et al., 2018). This was alleviated by SIRT3-mediated aerobic interval training that upregulated the antioxidant manganese superoxide dismutase (MnSOD) and inhibited neuronal apoptosis (Shi et al., 2018). SIRT6 and SIRT7 have been shown to be important for glucose production and metabolism. Studies in SIRT6 deleted mice have shown that SIRT6 deacetylates histone 3 lysine 9 (H3K9) to repress hypoxia-inducible factor, HIF-1α, in the promoter of Glucose transporter type1 (GLUT1) and Pyruvate Dehydrogenase Kinase, Isoenzyme 1 (PDK1) enzymes to facilitate glucose metabolism (Zhong et al., 2010). Knockout studies have shown that SIRT7 is an epigenetic modulator of glucose metabolism that regulates ribosomal biogenesis and promotes mitochondrial biogenesis via PRMT6 methylation and connects it to glucose availability in an AMPK dependent manner (Yan et al., 2018). These studies demonstrate the important functions carried out by SIRT6 and SIRT7 to regulate glucose homeostasis. The pathways relevant to neurodegeneration and modulated by sirtuins are summarized in **Figure 2**.

ROLE OF SIRTUINS IN REGULATION OF NEURODEGENERATION IN THE ENS

The ENS develops from enteric neural crest cells, a multipotent cell population that originates in the neural tube and migrates across the embryo to reach the developing intestine, where it proliferates and differentiates into enteric neurons and glia (Nagy and Goldstein, 2017). These neural progenitors eventually differentiate into several distinct neuronal subtypes that eventually comprise both the myenteric and submucosal plexi (Furness, 2012). Comparative studies between the intestines of young (3 months old) and old (>24 months) mice ($n = 6$) has shown that aging reduced the number of intestinal stem cells (ISCs) *in vivo* as well as the formation of intestinal organoids from the ISCs *ex vivo* that gives rise to differentiated cells of the gut (Igarashi et al., 2019). The plexuses are areas that are vulnerable to neurodegeneration from aging and high-fat or high sugar induced diets (Lakhan and Kirchgessner, 2011; Stenkamp-Strahm et al., 2015; McMenamin et al., 2018). Sirtuins are widely expressed in the gut (**Figure 3**, Zeisel et al., 2018) and neurons in the murine colon show immunoreactivity to SIRT1 where they localize to the nucleus, in the myenteric plexus (Lakhan and Kirchgessner, 2011). A knockout of SIRT1 in the gut of mice was reported to increase gastric emptying and intestinal contraction with suppressed villous apoptosis and increased crypt proliferation (Wang et al., 2012). This could indicate an altered cholinergic neuronal function. In the same study, the genes ghrelin and Period Circadian Clock 2 Gene, Per2, which regulate food intake and circadian rhythm respectively (Yannielli et al., 2007; Kim et al., 2018), were also found to be increased in the stomach and hypothalamus, implying a role for SIRT1 in regulating GI functions controlled by the circadian systems. Treatment of aged mice with SIRT1-dependent NAD^+ precursor, nicotinamide riboside restored ISC number and its functional defects in aged mice *in vivo* (Igarashi et al., 2019) but this was blocked by SIRT1 inhibitor EX527, suggesting a role for SIRT1 activators or precursors in maintaining the intestine during aging. Similar to the role of astrocytes in the CNS, enteric glia modulates the ENS by regulating motility and secretion by sensing neuronal reflexes by virtue of its plasticity (Gulbransen and Christofi, 2018). The glia is also important for epithelial health, and ablation of glia in transgenic mice has shown to cause alterations in motor and mucosal activity, resulting in intestinal inflammation, myenteric degeneration, hemorrhage, and necrosis (Aube et al., 2006). Obesity in the gut is characterized by persistent low-grade inflammation with alterations in gut motility (Hotamisligil, 2006). Experimental data show that gut inflammation, even if mild, could lead to persistent changes in GI nerve and smooth muscle function, resulting in dysmotility, hypersensitivity, and dysfunction (Mawe et al., 2009; Lakhan and Kirchgessner, 2010). Thus, the breakdown of mucosal barrier function as observed in obesity could cause alterations in the patterns of gut motility, abnormal secretion, and changes in visceral sensation that contributes to gastrointestinal symptoms. Whether the changes in GI motility observed in many obese patients are due to inflammation-related changes in the properties of enteric neurons is yet to be explored. Intestinal epithelium-specific knockout of SIRT1 in aged mice induced spontaneous inflammation and tissue damage in the colon and increased their susceptibility to colitis (Wellman et al., 2017). Increased proinflammatory cytokines and leukocyte infiltration, decreased colon lengths, elevated levels of LPS, and increased expression levels of anti-microbial proteins was observed in the SIRT1 KO mice compared to their age-matched controls. SIRT1 induced by resveratrol administration to rats has shown to be protective against acute intestinal inflammation from colitis by downregulating inflammation via NF-κB (Larrosa et al., 2009; Hofseth et al., 2010). In an experimental model of ileitis, oral administration of resveratrol increased the survival of resveratrol-treated mice after exposure to *T. gondii*, decreased mRNA expression of pro-inflammatory cytokine—IL-6, and increased the mRNA expression of anti-inflammatory cytokine—IL-10 in the ileum, compared to the control group (Bereswill et al., 2010). These studies highlight SIRT1 as a potential target in inflammatory diseases of the

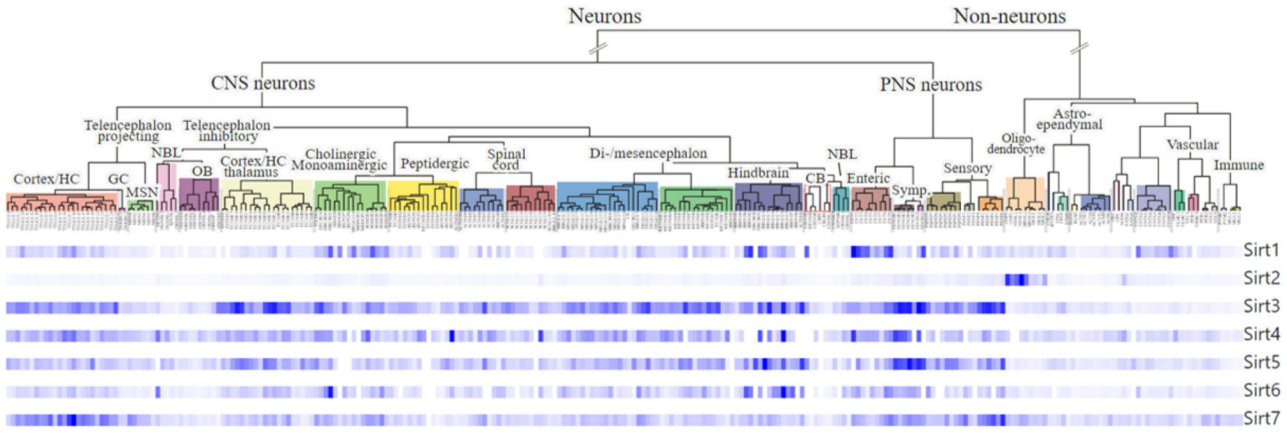

FIGURE 3 | Expression and cellular localization of sirtuins in the neurons of the mouse CNS and the ENS. Dendrogram showing gene expression of all the seven sirtuins in cell populations generated by single-cell RNA sequencing (scRNA-seq) in the central nervous system (CNS) neurons and the enteric neurons in the peripheral nervous system (PNS) using data from Zeisel et al. (2018). The plots were generated using the online database, Mouse Brain Atlas http://mousebrain.org/genesearch.html.

intestine. Studies in mice and cell cultures have shown that SIRT3 protects cortical and dopaminergic neurons from oxidative stress by regulating mitochondrial homeostasis (Kim et al., 2011; Dai et al., 2014; Shi et al., 2017). Unlike its protective role in the CNS, a SIRT3 knockout in mice exposed to dinitrobenzene sulfonic acid mode of colonic inflammation was shown to not have any effects in counteracting oxidative stress or the susceptibility of myenteric neurons to inflammation (Bubenheimer et al., 2016). Further research is required to explore the role of sirtuin proteins in enteric neurobiology during normal and inflamed states.

ROLE OF SIRTUINS AS MODULATORS OF GUT MICROBIOTA ALONG THE MICROBIOTA-GUT-BRAIN AXIS

The gut microbiota regulates many metabolic processes in addition to host energy homeostasis by taking part in the gut–brain crosstalk, a complex bidirectional communication system. This is mediated by gut microbiota produced signaling molecules like short-chain fatty acids (SCFAs: acetate, butyrate and propionate), lipopolysaccharide (LPS), 5-hydroxytryptamine (5-HT), biogenic amines (dopamine, norepinephrine), glutamate and γ-aminobutyric acid (GABA) (Nicholson et al., 2012; O'Mahony et al., 2015; Koh et al., 2016; Mazzoli and Pessione, 2016; Bhattarai et al., 2017; Sudo, 2019). The gut microbiota metabolites affect brain activity either through blood circulation or acting via vagus nerve afferent fibers, while vagal efferent fibers regulate gut permeability and inflammation influencing gut functions (Bonaz et al., 2018). Enteroendocrine cells (EECs) are in direct contact with the luminal contents and mediate the communication between gut microbiota and enteric innervations. They produce hormones and peptides including serotonin, ghrelin, cholecystokinin, glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and pancreatic polypeptide whose receptors are expressed in gut enteric neurons, vagal afferents, brain stem, and hypothalamus (De Silva and Bloom, 2012; Richards et al., 2014). EECs maintain gut homeostasis by regulating food intake and insulin secretion (Gribble and Reimann, 2016). SCFA stimulate the secretion of the leptin, GLP-1, and peptide YY(3–36), and lower body weight thereby contributing to gut-brain activation (Xiong et al., 2004; Tazoe et al., 2008; Tolhurst et al., 2012). The gut-brain bidirectional communication happens largely through the ENS which along with commensal microflora and immune cells, plays an important role in regulating intestinal epithelial barrier function (Snoek et al., 2010). Dysbiosis, an imbalance in the gut microbial community is linked to several metabolic diseases such as obesity, type-2 diabetes mellitus and inflammatory bowel diseases (Castaner et al., 2018; Zuo and Ng, 2018; Sharma and Tripathi, 2019). It is often associated with a reduction in the Bacteroidetes:Firmicutes ratio and increased gut permeability (Tremaroli and Backhed, 2012; Zuo and Ng, 2018; Sharma and Tripathi, 2019) with low-grade gut inflammation. Considerable shifts in human gut microbiota composition have been observed in several CNS disorders including neurodegeneration (Fung et al., 2017). Gut dysbiosis play an important role in modulating the gut–brain axis. An impaired gut barrier facilitate entry of bacterial endotoxins like LPS into the blood circulation, elicit inflammatory response, causing metabolic endotoxemia that eventually leads to insulin resistance and weight gain (van Olden et al., 2015). This impairment can also affect the blood brain barrier promoting neuro-inflammation and neurodegeneration including anxiety and depression (Liu, 2017).

Over recent years, accumulating evidence has suggested the role of sirtuins in obesity, diabetes, and various age-related neurodegenerative diseases by modulating gut microbiota at times, implicating the importance of gut-brain axis connections. Much of the studies involving sirtuins and gut microbiota have been done using resveratrol, which activates SIRT1. Resveratrol is thought to possess antibacterial activity against opportunistic pathogens of the digestive tract like *Escherichia coli*, *Salmonella*

enterica, and *Enterococcus faecalis* (Paulo et al., 2010), thereby contributing to maintenance of normal gut bacterial species. The epithelial barrier integrity and function is regulated by resveratrol by increasing the expression of intestinal tight junction proteins such as tight junction protein 2, occludin, and claudin 4 (Etxeberria et al., 2015; Ling et al., 2016; Wang et al., 2016). Resveratrol up-regulated SIRT1 display anti-inflammatory role in the gut by decreasing immune responses (Th1-type) and preventing bacterial translocation by maintaining gut barrier function (Bereswill et al., 2010), which is compromised in obesity (Cani and Delzenne, 2010). Resveratrol functions by modulating the composition of the gut microbiota (Chen et al., 2016; Komaroff, 2017). Mice studies have suggested that resveratrol can influence the relation between gut microbiota, diet, and obesity (Clarke et al., 2012; Komaroff, 2017) either by changing the expression of genes involved in central regulation of body weight homeostasis like fasting-induced adipose factor (Fiaf) or mTOR (Kim et al., 2010; Qiao et al., 2014; Jung et al., 2016), or by reversing the gut microbial dysbiosis caused by a high-fat diet by modifying the relative Bacteroidetes: Firmicutes ratio (Qiao et al., 2014; Sung et al., 2017). A recent study has shown that fecal microbiota transplantation from resveratrol treated mice to HFD mice reversed weight gain and improved gut microbiota composition and intestinal permeability (Wang et al., 2020). SIRT1 deficiency in the intestinal epithelium as studied with SIRT1 intestinal knock out mice, resulted in altered gut microbial composition, increased intestinal inflammation, and susceptibility to colitis implicating SIRT1 importance in maintaining intestinal tissue homeostasis through modulation of the gut microbiota (Wellman et al., 2017). In colonic biopsies from patients with inflammatory bowel disease (IBD), SIRT1 was downregulated by TNF-α and IL-21 in the mucosa (Caruso et al., 2014). Moreover, incubation with a SIRT1 agonist, Cay10591 reduced the acetylation of NF-κBp65 and suppressed the inflammatory cytokine production in the colon as seen in IBD. It also ameliorated experimental colitis induced in mice by reducing LPS-induced TNF-α production whereas a SIRT1 antagonist, Ex527 aggravated the same. On the contrary, another experimental study on mice and worms with an intestinal deletion of SIRT1 increased Paneth and goblet cell number and upregulated anti-bacterial peptides such as lysozyme and cryptidines resulting in a rearrangement of the gut microbiota, thus protecting them from colitis-induced colorectal cancer (Lo Sasso et al., 2014). The direct effects of resveratrol on SIRT1 or sirtuin activation in general is not completely conclusive as resveratrol as well as other sirtuin activators have many molecular targets that acts via diverse pathways on different sirtuin isoforms, depending on the substrate sequence and the type of acyl modification (Athar et al., 2009; Gertz et al., 2012; Britton et al., 2015; Gomes et al., 2019). With regards to SIRT1, resveratrol either directly binds and activates SIRT1 or increases the intracellular pool of NAD^+ via phosphorylation of AMPK by serine-threonine liver kinase B1 (LKB1) or calcium/calmodulin kinase kinase-β (CaMKKβ) kinases on its catalytic α-subunit, that can be utilized by SIRT1 (Lan et al., 2017).

The aberrant microbiota to CNS pathway is thought to result in the formation of insoluble protein aggregates within neurons in neurodegenerative disorders (Quigley, 2017). Toxic accumulation of misfolded and aggregated α-synuclein protein, Lewy bodies, is seen in both CNS and ENS of Parkinson's disease (PD) patients (Beach et al., 2010). Gram-negative bacteria in these patients are abundant producing LPS which contributes to α-synuclein aggregation leading to dopaminergic neuronal death, thereby causing motor impairments through inflammatory pathways (Sharma and Nehru, 2015). Using mice that overexpress αSyn, it is shown that gut microbiota promotes motor deficits and microglia activation. αSyn aggregation results in progression of the disease (Sampson et al., 2016). Studies show the correlation between increased gut permeability due to endotoxin exposure and alpha-synuclein staining in early Parkinson's disease (Forsyth et al., 2011). PD is frequently associated with impaired gastric motility (Fasano et al., 2015). Several studies have supported the hypothesis that PD may initiate in the gut since GI dysfunctions appear many years before motor impairments suggesting spread of α-syn pathology from the ENS to the CNS. Alzheimer's disease (AD) is characterized by an accumulation of protein aggregates composed of amyloid-β peptide (Aβ) and tau in CNS tissues impairing cognitive function and the pathogenesis is believed to be associated with increased permeability of the gut and blood-brain barrierinduced by microbiota dysbiosis (Jiang C. et al., 2017). Gut bacteria can secrete large amounts of amyloids and LPS which modulates the signaling pathways that lead to neurodegeneration and AD pathogenesis, as well as inflammatory response to Aβ accumulation in CNS (Pistollato et al., 2016). Amyloid precursor protein (APP) from which Aβ is derived, is expressed in the ENS suggesting its role in the pathogenesis of AD (Arai et al., 1991). Studies with transgenic mice overexpressing APP has shown progressive accumulation of Aβ within enteric neurons leading to a decreased number of enteric neurons, dysmotility and intestinal inflammation (Semar et al., 2013; Puig et al., 2015), implying that ENS dysfunction could occur in AD.

The concept of microbiota-gut-brain axis is extensively studied, with an emphasis being on the gut dysbiosis in the onset and/or progression of several metabolic diseases such as obesity, type-2 diabetes mellitus, and the most commonly studied forms of neurodegeneration, such as AD and PD. This review examines scientific literature addressing the possible role of sirtuins in regulating this axis thereby targeting themselves as molecules of importance therapeutically. Each sirtuin has different targets, located in different subcellular locations, and might function quite differently. Therefore, it is extremely important to develop selective activators or inhibitors that target a specific sirtuin. Sirtuins are known to modulate gut microbiota. It is critical for future studies to clarify using sirtuins as interventions to correct dysbiosis, which may provide safe and effective treatments to slow or halt the progression of clinical disorders.

FUTURE PERSPECTIVES

This review offers a consolidated overview of sirtuins and their important functions in modulating neurodegeneration in the CNS and the ENS. The precise functions of sirtuins

are still unclear, but they seem to be important players in age- and metabolism-associated neurodegeneration. Therefore, elucidating the molecular roles of sirtuins may enable the development of novel strategies for intervention in neurodegenerative diseases. Inhibition of SIRT2 while overexpressing SIRT1 is a potential strategy that could be used to treat certain age-related neurodegenerative diseases. The connection between sirtuins and dietary restriction also warrants further investigation on the precise role of sirtuins. However, the beneficial effect of dietary restriction on aging and various metabolic disorders is dependent on the activation of SIRT1 and can be mimicked by resveratrol. SIRT1 via resveratrol has shown neuroprotective effects against acute inflammation induced by colitis and are expressed by enteric neurons suggesting that it might help in gut motility and secretion. This could be a promising and previously unrecognized role of enteric sirtuins, especially SIRT1, in regulating energy homeostasis. Moreover, activation of enteric sirtuin pathways could offer a therapeutic approach to treating diabetes- and obesity-related gut dysfunction as well as age-induced neurodegeneration. Using genetically engineered reporter mice that illuminate the entire ENS (Jiang Y. et al., 2017), the effects of sirtuins on different neuronal subtypes can be better visualized and investigated when compared to traditional immunohistochemistry. Further research and identification of novel or repurposing of previously known small molecule activators and inhibitors of sirtuins could have a potential impact in the therapy of neurodegenerative disorders.

AUTHOR CONTRIBUTIONS

PC and SS designed the structure and contents of the review. PC, AV, and DA wrote and edited the manuscript. PC, AV, GL, SM, and SS provided critical revisions to the article. All authors contributed to the article and approved the submitted version.

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frontiers in Neuroscience

MINI REVIEW
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Glymphatic System as a Gateway to Connect Neurodegeneration From Periphery to CNS

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The classic concept of the absence of lymphatic vessels in the central nervous system (CNS), suggesting the immune privilege of the brain in spite of its high metabolic rate, was predominant until recent times. On the other hand, this idea left questioned how cerebral interstitial fluid is cleared of waste products. It was generally thought that clearance depends on cerebrospinal fluid (CSF). Not long ago, an anatomically and functionally discrete paravascular space was revised to provide a pathway for the clearance of molecules drained within the interstitial space. According to this model, CSF enters the brain parenchyma along arterial paravascular spaces. Once mixed with interstitial fluid and solutes in a process mediated by aquaporin-4, CSF exits through the extracellular space along venous paravascular spaces, thus being removed from the brain. This process includes the participation of perivascular glial cells due to a sieving effect of their end-feet. Such draining space resembles the peripheral lymphatic system, therefore, the term "glymphatic" (glial-lymphatic) pathway has been coined. Specific studies focused on the potential role of the glymphatic pathway in healthy and pathological conditions, including neurodegenerative diseases. This mainly concerns Alzheimer's disease (AD), as well as hemorrhagic and ischemic neurovascular disorders; other acute degenerative processes, such as normal pressure hydrocephalus or traumatic brain injury are involved as well. Novel morphological and functional investigations also suggested alternative models to drain molecules through perivascular pathways, which enriched our insight of homeostatic processes within neural microenvironment. Under the light of these considerations, the present article aims to discuss recent findings and concepts on nervous lymphatic drainage and blood–brain barrier (BBB) in an attempt to understand how peripheral pathological conditions may be detrimental to the CNS, paving the way to neurodegeneration.

Keywords: glymphatic system, lymphatic system, blood–brain barrier, neurovascular unit, neurodegenerative diseases

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INTRODUCTION: CLASSIC ANATOMICAL CONCEPTS

Apart from the general protection provided by the skull and dura mater, the brain environment is rigidly regulated by specialized structures, including leptomeninges, modified blood vessels and glial cells. In particular, selective capillaries, astrocyte end-feet, and pericytes represent the classic components of the blood–brain barrier (BBB). This barrier provides the brain with nutrients,

transports catabolites, and misfold-prone proteins out of the brain, maintains brain homeostasis, and regulates the immune function (Ballabh et al., 2004; Daneman and Prat, 2015; Hladky and Barrand, 2018).

This anatomical barrier, as postulated by Stern and Gautier (1921), regulates the molecular exchange between the blood flow and brain parenchyma, thereby controlling homeostasis within central nervous system (CNS). Apart from being a route of drainage for brain interstitial fluid (ISF) to lymph nodes, these structures provide the communication with the immune system modulating surveillance and immune-mediated responses to the brain. However, similar barriers also deputed to the regulation of molecular transport and immunologic protection are described outside the CNS. This is the case of the retina (part of the blood–ocular barrier), placenta, testis (seminiferous tubules), and thymus cortex. These barriers possess a well-defined anatomical substrate, since both endothelium and epithelial cells adjacent to capillaries exhibit special intercellular junctions (Fröhlich, 2002).

Morphological and functional findings allowed to look at the BBB from novel perspectives. For instance, the specialized metabolic interface of BBB can also act as a target for hormones and may secrete active compounds (Banks, 2019). The intimate relationship between CNS and blood vessels was deeply modified, when at the 2001 Stroke Progress Review Group Meeting of the National Institute of Neurological Disorders and Stroke the concept of the neurovascular unit (NVU) was formalized (Iadecola, 2017). Its cellular components include endothelial cells (ECs), basement membrane, (BM), perivascular astrocytes, neurons, pericytes, and microglia (**Figure 1**). As suggested by its name, this minimal functional unit emphasizes the relationship between CNS and blood vessels. In fact, a focally specific activity of a given NVU may alter locally the anatomy and physiology of the BBB, apart from controlling the amount of cerebral blood flow within the same specific region. ECs represent the major BBB component, being endowed with tight and adherent junctions between adjacent cells, which prevent paracellular diffusion of polar blood solutes while providing structural support (Daneman and Prat, 2015; Giorgi et al., 2020). Proteins such as occludins, claudins, and cadherins are expressed in these junctions. Endothelial transporters ensure mechanisms for both influx and efflux of either potentially beneficial or harmful substances. Surrounding the epithelium, the BM provides anchoring support to blood vessels and surrounding cells with its extracellular matrix rich in collagen and proteoglycan (Bell et al., 2020; Giorgi et al., 2020). Astrocytes lie between neurons and ECs, and with their end-feet surround blood vessels at precapillary and capillary level. Thus, astrocytes provide structural and functional connection between blood vessels and neurons. Neurons are particularly sensitive to changes of blood oxygen and nutrients, then acting as metabolic pacemakers. Apart from ion and neurotransmitter recycling, astrocytes are involved in BBB induction and maintenance through the release of several growth factors, regulation of dilation and constriction of blood vessels, as well as water balance within the interstitial space through the expression of aquaporin-4 (AQP4) at the level of their end-feet (Daneman and Prat, 2015; Giorgi et al., 2020). Pericytes also participate in BBB development, structural integrity, and function through the production and assembling of BM proteins, as well as regulation of tight junction expression, and EC proliferation (Armulik et al., 2010; Giorgi et al., 2020). Thanks to the presence of contractile proteins, pericytes have also been involved in blood flow regulation (Yamazaki and Kanekiyo, 2017; Bell et al., 2020; Giorgi et al., 2020). Finally, microglia and phagocytes in the extracellular matrix surrounding blood vessels play a waste-clearing and immunological role (Giorgi et al., 2020).

The belief of an absence of conventional lymphatic vessels in the CNS contributed to the concept that the brain, in spite of its high metabolic rate, represents an immune privileged region. This idea left questioned how cerebral interstitial fluid is cleared from waste products. It was generally thought that clearance depended on cerebrospinal fluid (CSF), acting as a pseudo-lymphatic system. CSF is generally formed by choroid plexuses, which are protrusions located in cerebral ventricles consisting of a single layer of secretory epithelial cells (modified ependymal cells) that surround a core of capillaries and connective tissue. While epithelial cells are provided with tight junctions, capillaries are fenestrated. Then, within the general concept of BBB, choroid plexuses are a highly vascularized tissue that represents a different functional interface between blood and ventricular as well as subarachnoid spaces, constituting the so-called blood-CSF (or liquor) barrier (Kratzer et al., 2020). Overcoming these classic anatomical concepts, outer brain barriers are indeed composed of at least 3 interfaces, blood-CSF barrier across arachnoid barrier cell layer, blood-CSF barrier across pial microvessels, and outer CSF-brain barrier comprising glial end-feet layer/pial surface layer (Brøchner et al., 2015). Again, both pioneering and recent evidence has been provided pointing at extra-choroidal CSF production as well as novel mechanisms for CSF clearance (Sato and Bering, 1967; Milhorat, 1969; Milhorat et al., 1971; Orešković et al., 2017; Fame and Lehtinen, 2020). The ongoing CSF production and solutes transport from the blood to the CSF in choroid plexectomized rhesus monkeys suggests that the choroid plexus is probably not the sole or even the major source of CSF within the primate ventricular system (Sato and Bering, 1967; Milhorat, 1969; Milhorat et al., 1971). Accordingly, CSF production and absorption are constant and present everywhere in the CSF system, and the CSF is mainly formed as a consequence of water filtration between the capillaries and interstitial fluid (Orešković et al., 2017).

Highly permeable capillaries are present in specific brain regions, where a typical BBB is lacking and molecules freely diffuse from the blood into the brain. Since these areas are mostly placed between neural tissue and ventricle lumen, they are known as "circumventricular organs." At this level, specialized ependymal cells, named tanycytes, equipped with a differential distribution of tight junction proteins, form a particular blood-CSF barrier. These circumventricular organs represent specialized neuro-epithelial regions, which include sensory (subfornical organ, area postrema, vascular organ of lamina terminalis) and secretory structures (median eminence, pituitary neural lobe, pineal gland). Then, these organs are important sites for communication with the CSF, as well as

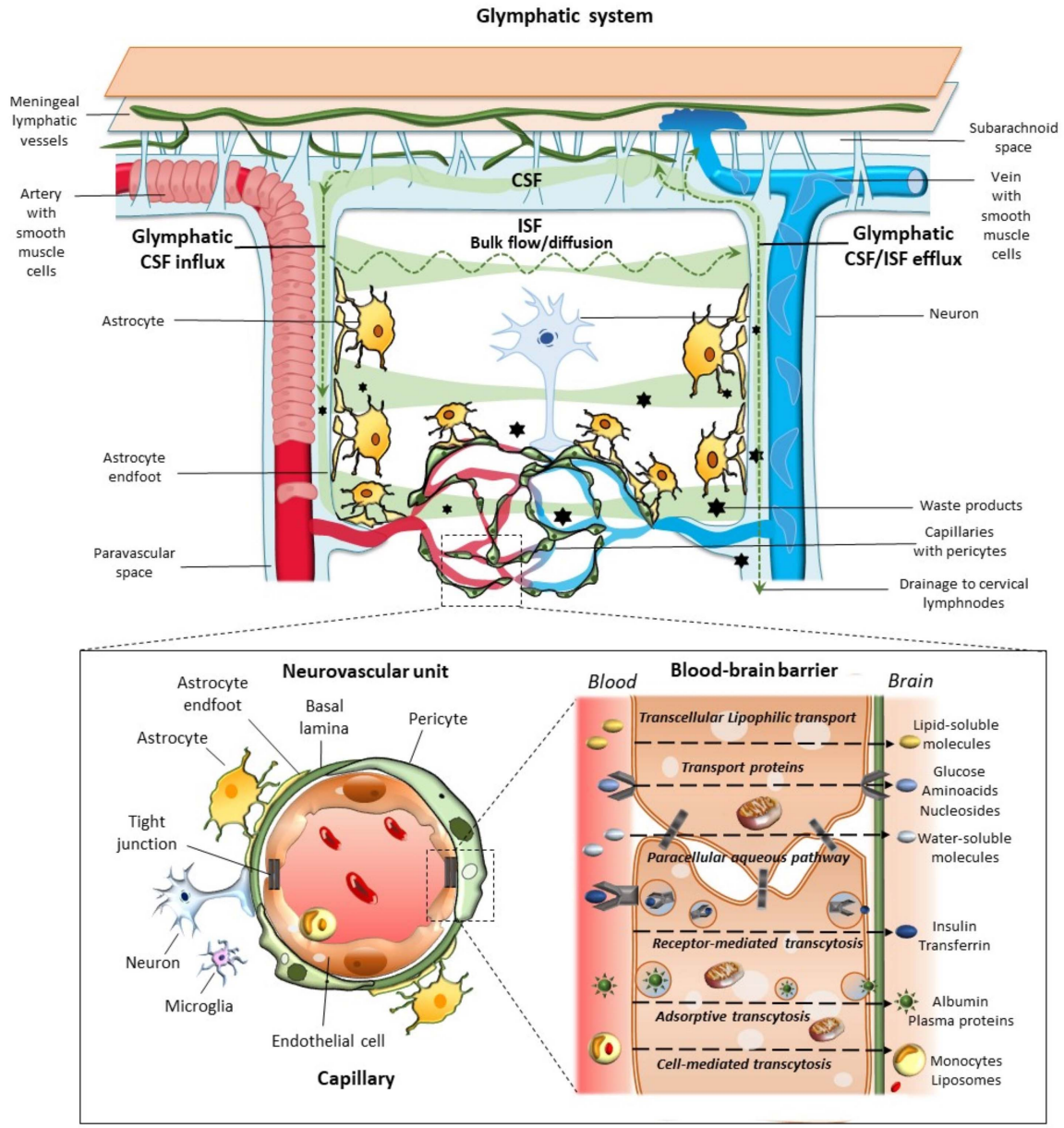

FIGURE 1 | Glymphatic system, neurovascular unit (NVU), and the blood–brain-barrier. The glymphatic system contributes to the transport of nutrients and signaling molecules into the brain parenchyma meanwhile promoting the clearance of proteins and interstitial waste solutes out of the brain. Subarachnoid CSF enters the brain parenchyma via para-arterial spaces and then mixes with the interstitial fluid (ISF) and waste solutes in the parenchyma. Whether this occurs through convective bulk flow or diffusion remains debated. The resulting CSF-ISF fluid exchange and the interstitial waste solutes enter the paravenous space through gaps between the astrocytic end-feet to be drained either back to the CSF-dural sinus-meningeal lymphatic vessels, or to the deep cervical lymph nodes. Green arrows and shades indicate the CSF and CSF-ISF fluid transport, while black stars indicate the interstitial waste solutes that exit the parenchyma via the paravenous efflux pathway. The insert depicts the main components of the NVU at the level of intraparenchymal capillaries, including perivascular astrocytes with their end-feet, neurons, microglia, pericytes, endothelial cells (ECs), and basement membrane (basal lamina). Capillary ECs are held together by tight junctions forming the blood–brain barrier (BBB), where the different transport routes are represented, including transcellular lipophilic transport, carrier protein-mediated transport, paracellular aqueous transport, receptor-mediated transcytosis, as well as adsorptive and cell-mediated transcytosis.

between brain and periphery by means of a rapid neuro-humoral exchange (Kaur and Ling, 2017).

The idea of a diffuse clearing process was replaced by the identification of anatomically and functionally discrete spaces surrounding the blood vessels of the brain (Bacyinski et al., 2017). These include the perivascular and paravascular spaces, where solute transport occurs in opposite directions. In detail, according to the perivascular model, ISF and solutes from the brain parenchyma enter the peri-arterial space in the BM of capillaries and within the tunica media of penetrating arteries

(Carare et al., 2008). From the peri-arterial space, solutes (soluble antigens but not cells) are cleared from the brain by dispersing in CSF or draining directly into cervical lymph nodes (Weller, 2005; Carare et al., 2008; Bacyinski et al., 2017). A failure of such a perivascular drainage is associated with β-amyloid accumulation (Carare et al., 2008; Bakker et al., 2016). Solute clearance from the brain parenchyma to the cervical lymphatic system through the perivascular pathway occurs in a direction which is opposite to that of both blood flow and paravascular pathway (Carare et al., 2008; Weller et al., 2009; Abbott, 2013; Bakker et al., 2016; Bacyinski et al., 2017).

The paravascular space (Virchow-Robin or Durant-Fardel space of the classic literature) was described in terms of a pathway for the clearance of interstitial molecules (Iliff et al., 2012). This was documented through *in vivo* two-photon microscopy and *ex vivo* confocal microscopy in mice, and consists of a three-step pathway: (1) CSF enters the brain parenchyma along with arterial paravascular (extramural) spaces; (2) CSF is mixed with ISF and solutes in a process mediated by AQP4; and (3) CSF exits through the extracellular space ("transparenchymal" convection) along venous paravascular spaces to be removed from the brain. Since this process includes the participation of perivascular glial cells with a sieving effect of their end-feet, and it resembles the classic peripheral lymphatic system, the group of Maiken Nedergaard coined the term of "glymphatic" (glial-lymphatic) pathway (Iliff et al., 2012). Thus, the glymphatic system consists of a unidirectional fluid current flowing from the paravascular space of penetrating arteries and arterioles to that of large caliber parenchymal draining veins (**Figure 1**).

As far as the classic lymphatic system is concerned, studies of the past decade provided the first morphological, phenotypical and functional characterization of lymphatic vessels in the cerebral dura mater draining to the cervical lymph nodes (Aspelund et al., 2015; Louveau et al., 2015; Da Mesquita et al., 2018). It is suggested that meningeal lymphatic vessels absorb CSF from the adjacent subarachnoid space and brain ISF via the glymphatic system, thus acting as a drainage route for CSF while contributing to immune surveillance of the CNS (Aspelund et al., 2015; Louveau et al., 2015; Raper et al., 2016; Da Mesquita et al., 2018; Tamura et al., 2020). These studies also allowed the work of Paolo Mascagni, who was the first to describe in the 1787 a potential lymphatic system in the dura of humans in his masterpiece "Vasorum lymphaticorum corporis humani historia et ichnographia," to be recognized and accepted by the scientific community (Natale et al., 2017; Irschick et al., 2019). This was reported by a number of additional works (Lukić et al., 2003; Bucchieri et al., 2015; Kumar et al., 2019; Sandrone et al., 2019; Mestre et al., 2020; Tamura et al., 2020). The presence of lymphatic vessels was demonstrated in the dura of both humans and non-human primates (Louveau et al., 2015; Absinta et al., 2017; Visanji et al., 2018). In humans, these vessels were detected at the level of the superior sagittal sinus and falx cerebri through immune-staining for podoplanin (a marker specific for lymphatic vessel ECs, Absinta et al., 2017; Visanji et al., 2018).

Thus, waste solutes may be ultimately cleared from the brain by draining into different compartments, including CSF-filled subarachnoid space and arachnoid villi, conduits along cranial and peripheral nerves, paravascular routes, as well as meningeal and cervical lymphatics (Iliff et al., 2012; Bedussi et al., 2015; Tarasoff-Conway et al., 2015; Raper et al., 2016; Benveniste et al., 2017; Ma et al., 2017). Similar to the perivascular clearance which requires cardiac output (Carare et al., 2008), cerebral arterial pulsation plays a pivotal role in driving glymphatic CSF influx into and through the brain parenchyma (Iliff et al., 2012). Thus, changes in arterial pulsatility may contribute to the accumulation of toxic solutes, including β-amyloid, in the aging brain (Iliff et al., 2013). Notwithstanding the importance of this discovery, characterization of such a highly organized CSF-ISF exchange pathway dates back to studies of the mid 80s by Patricia Grady's group (Rennels et al., 1985). In fact, early evidence for a paravascular fluid circulation in the mammalian CNS was provided by the rapid and widespread distribution of a CSF tracer (horseradish peroxidase protein) throughout the brain from the subarachnoid space.

Nowadays, the glymphatic model has been further confirmed and highly praised (Jessen et al., 2015; Nistal and Mocco, 2018; Plog and Nedergaard, 2018; Sun et al., 2018; Benveniste et al., 2019; Kumar et al., 2019; Thomas et al., 2019; Mestre et al., 2020), and it was recently described in humans (Ringstad et al., 2018). The glymphatic pathway is being widely investigated comparing healthy and pathological conditions, such as neurodegenerative disorders, including chronic Alzheimer's disease (AD), as well as hemorrhagic and ischemic stroke, hydrocephalus or traumatic brain injury. Descriptions in human disorders were backed up by experimental modeling of the system, which was developed to predict a potential site of therapeutic intervention (Rasmussen et al., 2018; Jiang, 2019; Ramos et al., 2019; Kaur et al., 2020; Reeves et al., 2020). Novel anatomical insights were provided indicating that the midbrain, due to the consistent thickness of its pial-glial BM, is better equipped for convective influx/glymphatic entry of the CSF compared with other brain regions. This may be a key for the intrathecal delivery of drugs into the brain (Dobson et al., 2017).

Nonetheless, the glymphatic model has been also revisited, and it represents a matter of debate. Many controversies and open issues exist concerning the perivascular and paravascular models, including the opposite directions of fluid flow, anatomical and functional differences, potential driving forces, and their role in health and disease (Bakker et al., 2016; Bacyinski et al., 2017). Some morphological and functional studies postulated an alternative hypothesis, which considers diffusion (not convective bulk flow) as the main mechanism regulating CSF-ISF exchange at the level of the NVU associated with brain capillaries, and throughout the interstitial space (Asgari et al., 2016; Jin et al., 2016; Smith et al., 2017; Abbott et al., 2018; Bakker et al., 2019; Kaur et al., 2020).

This is related to another major controversial aspect of the glymphatic hypothesis, which is centered on the role for AQP4 in CSF-ISF exchange under physiological conditions (Abbott et al., 2018). It has been argued that diffusion, rather than AQP4 expression, is an important regulator of paravascular flow, since CSF tracer uptake and interstitial flow rate are unaffected by ablation of the *Aqp4* gene (Smith et al., 2017). By using a cisternal infusion paradigm in mice similar to that

employed by Iliff et al. (2012) and Smith et al. (2017) argued that tracer movement in the brain parenchyma outside of the perivascular spaces was size dependent and consistent with diffusion as the main mechanism of transport. Both studies differ on the anesthetics used, to which a subsequent study accessed the correlations of different anesthetics, electroencephalogram (EEG) power, and CSF tracer influx (Hablitz et al., 2019). Again, arguing against a major contribution of the ISF bulk flow model, several studies showed that most of β-amyloid removal occurs via the BBB (Deane et al., 2004, 2008; Tarasoff-Conway et al., 2015; Hladky and Barrand, 2018). Nonetheless, several studies confirmed that AQP4 inhibitors or *Aqp4* gene deletion slows down or impairs both glymphatic CSF tracer influx and the clearance of several interstitial solutes, including β-amyloid, ApoE, tau, SOD1 oligomers, lactate, and viruses (Iliff et al., 2014; Achariyar et al., 2016; Murlidharan et al., 2016; Lundgaard et al., 2017; Mestre et al., 2018; Feng et al., 2020; Harrison et al., 2020; Hirose et al., 2020).

Mice expressing normal AQP4 levels but specifically lacking perivascular AQP4 localization also exhibit impaired CSF tracer influx (Mestre et al., 2018). In this frame, pericytes play a key role in regulating AQP4 polarization in astrocytes end-feet (Gundersen et al., 2014). As support to a key role of pericytes in glymphatic function, pericyte-deficient $Pdgfb^{ret/ret}$ mice feature both mispolarization of AQP4 from astrocyte end-feet, and defective glymphatic function (Armulik et al., 2010; Munk et al., 2019). In this same model, the development of the vasculature is more generally altered, including capillary dilation and impaired BBB function (Armulik et al., 2010). Several pieces of evidence now support a scenario in which pericytes influence the development of the glymphatic system through deposition of laminin 211 in the vascular BM, which via dystroglycan and dystrophin in astrocytes promotes polarization of AQP4 to its end-feet (Lendahl et al., 2019; Zheng et al., 2020).

Considering the vascular and metabolic importance of BBB and glymphatic system, alterations of these structures have been implicated in the pathogenesis of several neurological diseases. On the other hand, also in peripheral organs the human interstitial space has been revised and a novel concept of the space within and between cells has been proposed (Benias et al., 2018; Kumar et al., 2019). The present article aims to discuss recent findings in an attempt to envisage how perturbations of the glymphatic system can take a role in favoring or accelerating neurodegenerative processes within CNS. In particular, the involvement of peripheral alterations, central draining and clearing systems are considered in this intriguing relationship.

GLYMPHATIC SYSTEM AND CNS DISORDERS

The disruption of the brain (g)lymphatic system plays a crucial role in age-related changes of brain functions, as well as in the pathogenesis of neurovascular, neurodegenerative, neuro-inflammatory diseases, brain injury and tumors (Sun et al., 2018).

Several lines of evidence documented that β-amyloid and tau exit the brain via the glymphatic system, and glymphatic activity and CSF outflow decrease significantly in old mice (Iliff et al., 2014; Kress et al., 2014; Jessen et al., 2015; Ma et al., 2017). The glymphatic system removes potentially harmful metabolites from the CNS especially during sleep (Rasmussen et al., 2018; Hauglund et al., 2020). Accordingly, in animal models it was observed that during natural sleep or anaesthesia there is an enlargement of the interstitial space, which increases convective CSF exchange with ISF, and β-amyloid clearance rate (Xie et al., 2013). Again, obstructive sleep apnea increases cerebral β-amyloid aggregation and it is associated with increased prevalence of neurodegeneration, including AD (Ju et al., 2019). This is correlated with reduced slow wave activity (SWA). In fact, high SWA and certain types of anaesthesia support glymphatic activity, while norepinephrine signaling in the brain (and wakefulness, in general) has an attenuating effect (Xie et al., 2013; Hablitz et al., 2019; Hauglund et al., 2020). Disrupting the SWA is enough to abolish waste clearance (Ju et al., 2017), and sleep deprivation is correlated with increased levels of β-amyloid in the brain of both animals and humans (Kang et al., 2009; Shokri-Kojori et al., 2018). In line with this, the concentration of β-amyloid in CSF follows the sleep-wake cycle in AD human subjects, providing a correlation between bad sleep quality and β-amyloid deposition in the preclinical stage of AD (Ju et al., 2013).

In patients with AD, a decrease in both BBB and glymphatic function, accompanies a general dysfunction of NVU, including astrocytic end-feet atrophy, pericyte degeneration, alteration of endothelial tight junctions, and thickening of the basement membrane (Yamazaki and Kanekiyo, 2017). Consequently, CSF clearance of β-amyloid and tau tracers is reduced. This glymphatic dysfunction may be in part related to an altered AQP4 expression, as shown in different animal models of traumatic brain injury, AD, and stroke. In young APP/PS1 double transgenic mice, expressing chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant form of human presenilin-1 (PS1-dE9), it was shown a reduced glymphatic influx and clearance of β-amyloid, which worsens with aging. More in depth, glymphatic transport appeared suppressed in old APP/PS1mice, with β-amyloid deposits, and glymphatic clearance was reduced prior to the presence of β-amyloid deposits in younger APP/PS1 mice when compared to age-matched controls. As in a vicious circle, it was also shown that administration of wild-type mice with β-amyloid led to significant suppression of CSF tracer influx, suggesting that AD can cause a further reduction of glymphatic clearance (Peng et al., 2016). In fact, cerebral amyloid angiopathy consists of increased arterial stiffness, decreased arterial pulse, and reduction of perivascular spaces, due to extracellular β-amyloid accumulation (Peng et al., 2016; Plog and Nedergaard, 2018; Rasmussen et al., 2018; Reeves et al., 2020).

In the case of haemorrhagic stroke, the impairment of the glymphatic system is due to blood components, such as fibrin and fibrinogen deposits, which occlude perivascular spaces. In the ischaemic stroke there is an impaired CSF inflow and the release of several pro-inflammatory cytokines. Contrast-enhanced magnetic resonance imaging indicates that the glymphatic system is affected during stroke, although to a different

extent, depending on the specific disorder [subarachnoid or intracerebral hemorrhage, carotid ligature, and embolic ischemic stroke (Gaberel et al., 2014)]. Moreover, cerebral drainage appeared affected also during multiple microinfarction, with inhibition of AQP4 function, as demonstrated in a murine model (Wang et al., 2017). Clearance of solutes, including tau protein, from the interstitial space is reduced by ∼60% after traumatic brain injury in experimental animals, with this impairment persisting for at least 1 month (Iliff et al., 2014).

An altered glymphatic function has been advocated to account for AD, as well as for idiopathic normal pressure hydrocephalus. The latter condition affects up to 10% of patients affected by dementia, who concomitantly suffer from idiopathic normal pressure hydrocephalus, with progressive ventriculomegaly, and the clinical triad of gait ataxia, urinary incontinence, and dementia (Reeves et al., 2020). In this respect, intrathecal contrast-enhanced magnetic resonance imaging has been suggested to diagnose pre-clinical neurodegenerative disorders (Ringstad et al., 2018).

These pathological conditions are associated with a decrease in CSF influx to the glymphatic pathway or reduced clearance efficacy (traumatic brain injury, ischaemic stroke) or both (aging, AD, subarachnoid hemorrhage, idiopathic normal pressure hydrocephalus) (Plog and Nedergaard, 2018; Rasmussen et al., 2018). Nonetheless, it still remains difficult to establish to what extent a primary disruption of the glymphatic system is responsible for the onset of brain pathologies or rather it is a CNS disease that affects this delicate drainage pathway. Even, a mutual detrimental influence between noxious stimuli and interstitial fluid dynamics should be considered.

GLYMPHATIC SYSTEM AND PERIPHERY: IMPLICATIONS FOR CNS DISORDERS

Considering that a healthy human body depends on the correct communication among various integrated systems, it is essential to have a holistic view to better understand and interpret its dynamics under normal and pathological conditions. Then, the classic distinction between the CNS and the body periphery appears now inadequate. In this regard, another important issue to be discussed is the influence of peripheral pathologies on the integrity of CNS paravascular spaces, with possible negative consequences on neuronal activities. For instance, diabetes mellitus impairs glymphatic clearance of interstitial solutes within the hippocampus and hypothalamus of rats, which is correlated with cognitive decline (Jiang et al., 2017).

Again, accumulation of metabolic waste products and noxious substances in the brain ISF may result from liver disease, potentially contributing to neuronal dysfunction and cognitive impairment (Hadjihambi et al., 2019). This was confirmed in a rat model of chronic liver disease obtained through bile duct ligation, where altered glymphatic clearance and reduced AQP4 expression occurs in several brain regions, including the olfactory bulb, prefrontal cortex and hippocampus. These effects are aligned with cognitive/behavioral deficits (Hadjihambi et al., 2019). It has been speculated that, in the advanced phases of liver cirrhosis, glymphatic damage could be the end-stage phenomenon of a cascade of hydrodynamic events. These start from the onset of a vast number of artero-venous shunts in several organs and apparatuses and culminate into a reduction of jugular vein outflow (Gallina et al., 2019). This may in turn induce a reduction of cerebral-venous outflow and consequently, impairment of CSF circulation, derangement of AQP4-based clearance, accumulation of waste molecules and fluids, glymphatic congestion and inflammation.

A variety of general conditions can influence the efficiency of brain clearance from waste products. For instance, not only the level of consciousness, but also body posture (supine, prone, or lateral positions) contributes to glymphatic drainage. An experimental study indicates that the right lateral decubitus, which is natural in rodents at rest, is mostly efficient for glymphatic transport and elimination of waste products, including β-amyloid (Lee et al., 2015). One potential explanation for such an advantage is that the heart is positioned higher, which may favor pumping of blood and greater venous return to increase cardiac stroke volume; in turn, sympathetic tone is reduced, possibly improving glymphatic influx. However, more complex physiological adjustments to different head and body positions (including stretch on the nerves and vessels on the neck) are likely to be involved. Preliminary results were also obtained in patients, where postural changes seem to affect intracranial pressure (Andresen et al., 2016).

As previously mentioned, during sleep, the glymphatic system is highly active in removing waste products. Sleep disturbances are an early correlate of neurodegenerative diseases, including AD and Parkinson's disease (PD), where they often precede the onset of classic symptoms. In general sleep can be regarded as a neuroprotective factor acting through the glymphatic system (Sundaram et al., 2019). Sleep quality is controlled by circadian rhythms. Recent papers in rodents (Hablitz et al., 2020; Pulido et al., 2020) and Drosophila (Artiushin et al., 2018; Zhang et al., 2018) emphasized the circadian regulation of the glymphatic system, lymphatic drainage and BBB permeability. For instance, in mice, glymphatic CSF influx, and solute clearance from the brain, do vary according to circadian rhythms independent of arousal state (Hablitz et al., 2020). Glymphatic influx and clearance peak during the mid-rest phase of mice, while CSF drainage to the lymph nodes exhibits daily variation opposite to glymphatic influx. This is matched by the perivascular polarization of AQP4, which is highest during the rest phase. An intricate relationship has been documented between neuronal activity and the expression of circadian clock genes within brain ECs, which in turn, orchestrate the activity-dependent control of BBB efflux transport (Pulido et al., 2020).

Cervical Lymph Nodes and Brain Drainage

The glymphatic pathway is connected to a classic lymphatic network, associated with dural meninges covering the brain, as well as sheaths of cranial nerves and blood vessels, or drains via the olfactory route, then exiting through cranial foramina.

This network ultimately drains to deep and superficial cervical lymph nodes, then representing the next step in CNS drainage following the glymphatic system (Ma et al., 2017; Benveniste et al., 2019; Hershenhouse et al., 2019). In rats the uptake of Evans blue tracer from subarachnoid space (cistern magna) was shown to be drained into the meningeal lymphatic vessels and extracranial lymph nodes (Maloveska et al., 2018).

During aging, meningeal lymphatic vessels exhibit decreased vessel diameter and reduced drainage to cervical lymph nodes. Experimental studies in mice showed that ablated or ligated meningeal lymphatics led to an increase in β-amyloid deposition and macrophage recruitment to plaque sites, with a reduced extracellular clearance of altered proteins (Da Mesquita et al., 2018). Behavioral test, including spatial learning and fear memory, deteriorate along with impaired lymphatic function. These data suggest that an impaired efficiency of meningeal lymphatic vessels to drain toward peripheral lymph nodes play a significant role in the pathological accumulation of proteins implicated in neurodegeneration (Hershenhouse et al., 2019). Similar findings were reported for α-synuclein accumulation, a hallmark for a class of degenerative disorders (Zou et al., 2019). The presence in humans of meningeal lymphatic vessels connected with the glymphatic system arouse the hypothesis that clearance of macromolecules implicated in neurodegenerative proteinopathies, such as PD, might also occur through this efflux pathway. Then, an impairment of this drainage might result in α-synuclein accumulation, leading to neurodegeneration (Visanji et al., 2018).

The effects of an impaired drainage of cerebral lymphatic system in the pathogenesis of both ischemic and hemorrhagic stroke was examined. In a model of transient middle cerebral artery occlusion-induced stroke, the blockade of cervical lymphatics worsened cerebral edema and infarct size. Again, an obstruction of meningeal lymphatic vessels after a subarachnoid hemorrhage contributed to the exacerbation of the disease (Sun et al., 2018; Hershenhouse et al., 2019).

The bidirectional connection between the CNS and peripheral immune system through meningeal and cervical lymphatics is also relevant for autoimmunity. In fact, while assisting in the drainage of CSF components, meningeal lymphatics enable immune cells and self-antigen peptides to enter draining lymph nodes (Louveau et al., 2018). This may foster activation of T-cells in periphery while mounting CNS-directed adaptive immune responses (Limanaqi et al., 2019). In fact, peripherally activated T-cells can enter the brain parenchyma by crossing all CNS barriers including the blood-CSF, the blood-leptomeningeal, and the BBB (Shechter et al., 2013; Limanaqi et al., 2019). In line with this, resection of either meningeal lymphatics or deep cervical lymph nodes is beneficial in models of multiple sclerosis (MS), which is characterized by abundant inflammation and infiltration of brain-reactive immune cells throughout the CNS (Phillips et al., 1997; Furtado et al., 2008; van Zwam et al., 2009; Louveau et al., 2018; Hershenhouse et al., 2019).

It is intriguing that besides neuro-immune disorders such as MS, autoimmune mechanisms may also be implicated in classic neurodegenerative disorders such as PD. In fact, nigral dopamine (DA) neurons possess an enhanced sensitivity to the upregulation of major histocompatibility complex I (MHC-I) molecules (Cebrián et al., 2014). Thus, their susceptibility in PD may be related to cytotoxic, CD8 + T-cell-mediated death (Sulzer et al., 2017). This is bound to α-synuclein degradation and subsequent generation of self-antigen peptides for T-cell presentation through neuronal MHC molecules (Cebrián et al., 2014; Sulzer et al., 2017; Ugras et al., 2018). In fact, just like professional antigen presenting cells, DA neurons can internalize, process and load antigens onto MHC-I, especially during pro-inflammatory conditions (Cebrián et al., 2014; Limanaqi et al., 2019). This occurs following either administration of DA precursors, or microglial activation and subsequent cytokines release. In the presence of activated CD8 + T-cells, the cognate antigen/MHC-I complex exposed on the plasma membrane of DA neurons induces T-cells proliferation, and eventually, neuronal death via Fas/Fas ligand and perforin/granzyme pathways. Thus, an apparently paradoxical scenario configures, whereby drainage of α-synuclein to the peripheral lymph nodes may trigger autoimmune attack against brain DA neurons, which remains to be confirmed.

Glymphatic System and Brain-Gut Axis

Another interesting example of interaction between CNS and periphery is represented by the brain-gut axis. It is now well ascertained that there is a reciprocal communication between brain and gastrointestinal tract. At first, there is a direct transfer of peptides and regulatory proteins across the BBB. Furthermore, gastrointestinal hormones can alter the function of brain ECs, which compose the BBB. Finally, these hormones can affect the secretion from the BBB of substances involved in the regulation of feeding and appetite, such as nitric oxide and cytokines (Banks, 2008).

A large body of evidence shows how gastrointestinal pathologies can affect the CNS bypassing or altering BBB and related pathways, including the glymphatic system. In fact, according to Braak's hypothesis (Braak et al., 2003) neurodegenerative diseases, in particular PD, may have a peripheral origin. This may occur when putative pathogens enter the mucosa of the gastrointestinal tract, inducing misfolded/aggregated α-synuclein in specific neuron subtypes of the enteric nervous system. These α-synuclein aggregates may finally spread antidromically to the CNS via the vagal preganglionic fibers, up to the dorsal motor nucleus (Natale et al., 2008, 2010, 2011a). In other words, misfolded proteins can propagate via peripheral nervous system (Natale et al., 2011b, 2013; O'Carroll et al., 2020).

In spite of these findings, Braak's hypothesis is still debated. Liddle (2018) collected several data showing that PD may arise in the gut, whereas according to Lionnet et al. (2018) the human autopsy evidence does not seem to support this hypothesis. Finally, it is possible to agree on a compromise when a specific subset of patients affected by PD can be considered within the staging system of Braak (Rietdijk et al., 2017). In particular, two subtypes of PD patients can be recognized: a brain-first (top-down) type, where α-synuclein pathology initially arises in the brain with secondary spreading to the peripheral autonomic nervous system; and a body-first (bottom-up) type,

where the pathology originates in the enteric or peripheral autonomic nervous system and then spreads to the brain (Horsager et al., 2020) (**Figure 2**). Supporting this hypothesis, a novel experimental study showed that α-synuclein fibrils injected into the duodenal and pyloric muscularis layer can spread in the brain, first in the dorsal motor nucleus, and then in the

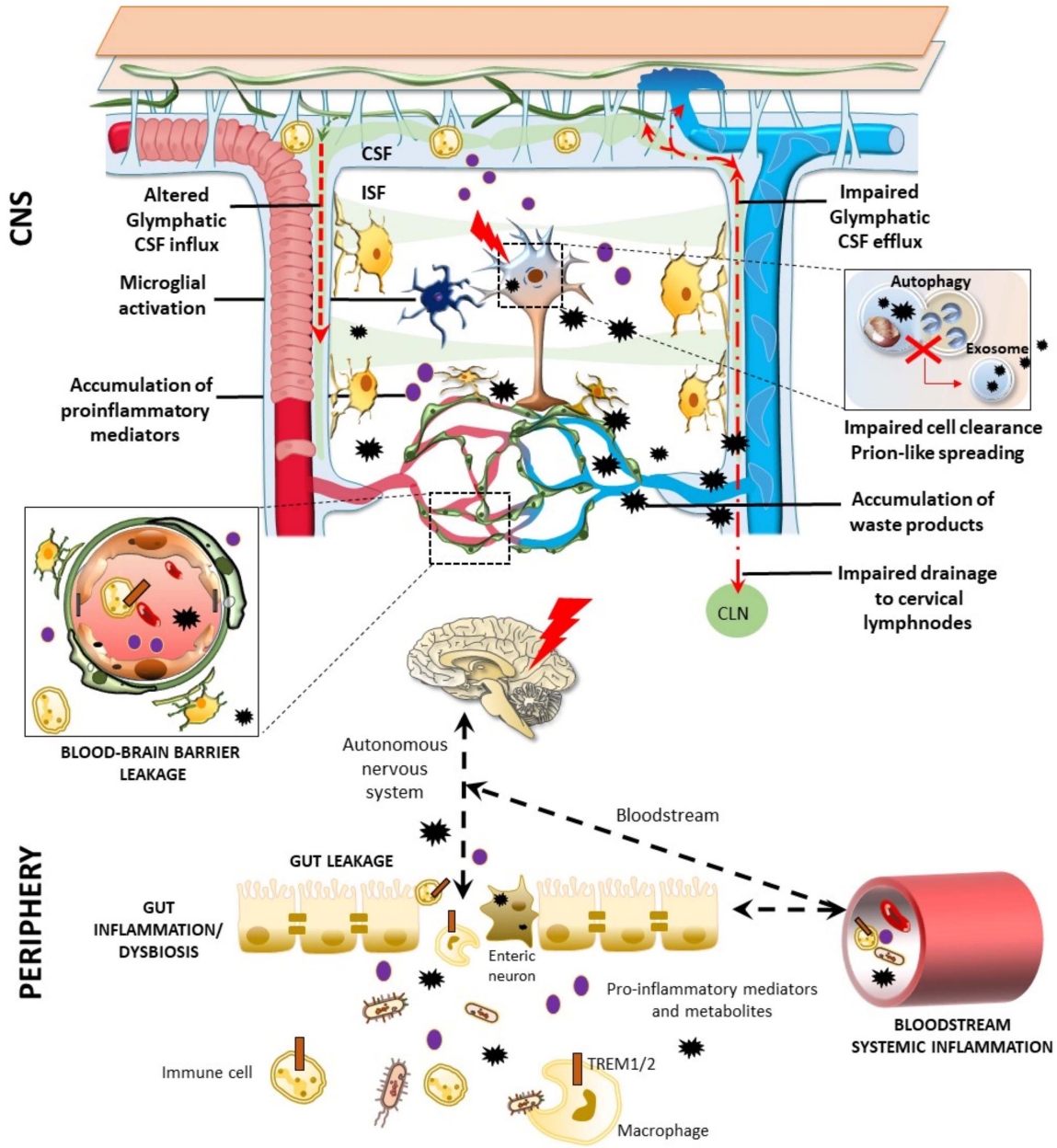

FIGURE 2 | Glymphatic pathway in pathological conditions: a role for the bidirectional gut-brain communication. Alterations of the glymphatic pathway may contribute to the extracellular accumulation of waste products, including altered protein in the brain (black stars). These include alterations in the morphology and drainage capacity of meningeal lymphatic vessels, impairment of CSF influx and efflux, along with the release of several pro-inflammatory cytokines and immune cells. Considering the reciprocal communication which occurs between the brain and the gastrointestinal tract, gut alterations can affect the CNS, and vice-versa. Potentially harmful solutes, including misfolded/aggregated proteins, may spread to the gut through the autonomic nervous system to induce inflammation [brain-first (top-down) type]. In turn, gut dysbiosis, inflammation, and leakage may promote the antidromic spread of potentially harmful molecules to the CNS via the vagal fibers or the bloodstream [body-first (bottom-up) type], bypassing and altering the glymphatic system and the BBB (left insert). These include misfolded/aggregated proteins such as α-synuclein, microorganisms, and also pro-inflammatory cytokines and activated immune cells, such as TREM TREM cells-positive activated macrophages. Extracellular accumulation of waste products related to an altered glymphatic drainage is exacerbated when intracellular clearing systems are impaired (right insert). This is the case of the autophagy pathway, which grants neuronal proteostasis and survival. When autophagy is impaired, extracellular release of undigested, potentially harmful substrates may occur via exosome release.

locus coeruleus, and later on, in basolateral amygdala, dorsal raphe nucleus, and substantia nigra pars compacta. Truncal vagotomy and α-synuclein deficiency prevent the gut-to-brain spread of synucleinopathy and associated neurodegeneration and behavioral deficits (Kim et al., 2019).

The role of the gastrointestinal microbiota and their metabolites in modulating brain functions and BBB integrity has rapidly increased over the past years (Cryan et al., 2020; Parker et al., 2020). Interestingly, in a recent work it has been observed that, following fecal microbiota transplant from aged into young mice, a down-regulation of proteins involved in glucose transport across the BBB, such as SlcA1 and A3, takes places, contributing to the dysfunctional bioenergetic system of the aging brain (D'Amato et al., 2020). Furthermore, in an up-to-date review, it was reported that, via the microbiota-gut-brain axis, Triggering Receptors Expressed on Myeloid cells (TREM)-positive activated macrophages along with inflammatory mediators may reach the brain through blood, glymphatic system, circumventricular organs, or the vagus nerve (**Figure 2**, Natale et al., 2019). This may foster pro-inflammatory reactions in the brain, bridging inflammatory bowel disease and neurological disorders. Similar hypotheses have also emerged on the correlation between gastrointestinal and neurological symptoms of SARS-CoV-2, which may apply indeed to a variety of microorganisms, and also "prionoid" proteins. Once the gastrointestinal tract is invaded, the virus may transit to the CNS through vascular and lymphatic systems, or through the vagus nerve (Bostancıklıoğlu, 2020a,b; Limanaqi et al., 2020). The virus can even infect leukocytes and migrate with them into the brain, or alternatively, viral particles can be directly transported across the BBB to the brain. Again, the virus can invade the peripheral lymphatic vessels which are connected with the glymphatic system, finding a route to the CNS. This suggests that lymph vessels around the gastrointestinal tract, the vascular system itself, or the gut-brain axis via the vagal nerve represent potential peripheral gateways for both pathogen neuroinvasion and prion-like spreading of potentially harmful catabolites to the CNS. If this is the case, accumulation of waste products in the brain would progressively foster pathology due to impairment of (g)lymphatic drainage activity or altered intracellular catabolite scavenge (for example, the autophagy pathway) (**Figure 2**).

At the same time, perineural spaces surrounding the cranial nerves, including the vagus, are known to provide some level of CSF drainage to peripheral lymphatics (Ma et al., 2017). When considering recent evidence that vagus nerve stimulation enhances CSF tracer influx (Cheng et al., 2020), the top-down hypothesis of neurodegeneration seems to take over. Although a correlation between glymphatic clearance of misfolded proteins and the vagus nerve remains to be investigated, some insights can be provided by the recently described ocular glymphatic system. Following up experimental data that documented retrograde CSF inflow to the paravascular spaces in the optic nerve, it was demonstrated that an eye-to-CSF pathway supports clearance of waste products from the retina and vitreous (Wang et al., 2020). This occurs in opposite direction as compared to CSF drainage, and neural activity seems to play a role on the rate of fluid fluxes, as light stimulation promotes fluid drainage and β-amyloid clearance. After traversing the lamina barrier through an ocular-cranial pressure difference mechanism, intra-axonal Aβ is cleared via the paravenous space and subsequently drained to lymphatic vessels. Apart from providing a potential link between neurodegenerative and ocular diseases, these findings open novel avenues for further experimental studies aimed at dissecting the role of the glymphatic system as a kernel connecting CNS and periphery.

AUTHOR CONTRIBUTIONS

GN drafted and wrote the manuscript. FL, CB, FM, FN, and SP-A contributed to the literature review, manuscript writing, and editing. FL made art-work. FF coordinator the manuscript, he critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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Nuclear Factor Erythroid 2-Related Factor 2 Activation Might Mitigate Clinical Symptoms in Friedreich's Ataxia: Clues of an "Out-Brain Origin" of the Disease From a Family Study

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Friedreich's ataxia (FRDA) is the most frequent autosomal recessive ataxia in western countries, with a mean age of onset at 10–15 years. Patients manifest progressive cerebellar and sensory ataxia, dysarthria, lower limb pyramidal weakness, and other systemic manifestations. Previously, we described a family displaying two expanded GAA alleles not only in the proband affected by late-onset FRDA but also in the two asymptomatic family members: the mother and the younger sister. Both of them showed a significant reduction of frataxin levels, without any disease manifestation. Here, we analyzed if a protective mechanism might contribute to modulate the phenotype in this family. We particularly focused on the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2), the first line of antioxidant defense in cells, and on the glutathione (GSH) system, an index of reactive oxygen species (ROS) detoxification ability. Our findings show a great reactivity of the GSH system to the frataxin deficiency, particularly in the asymptomatic mother, where the genes of GSH synthesis [glutamate–cysteine ligase (*GCL*)] and GSSG detoxification [GSH S-reductase (*GSR*)] were highly responsive. The *GSR* was activated even in the asymptomatic sister and in the proband, reflecting the need of buffering the GSSG increase. Furthermore, and contrasting the NRF2 expression documented in FRDA tissues, NRF2 was highly activated in the mother and in the younger sister, while it was constitutively low in the proband. This suggests that, also under frataxin depletion, the endogenous stimulation of NRF2 in asymptomatic FRDA subjects may contribute to protect against the progressive oxidative damage, helping to prevent the onset of neurological symptoms and highlighting an "out-brain origin" of the disease.

Keywords: Friedreich ataxia, oxidative stress, neurodegenerative disease, Nrf2, glutathione

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INTRODUCTION

Friedreich's ataxia (FRDA, OMIM #229300) is the most frequent autosomal recessive ataxia in western countries, with an estimated prevalence of 1:80,000 among Caucasian populations and a mean age of onset at 10–15 years (Cossée et al., 1999; Koeppen et al., 2009). Symptoms appear between 5 and 15 years of age in FRDA, and the brain atrophy begins early in the disease and plateaus in later stages, indicating that the neurodegenerative profile is an early-onset disease manifestation, with progressive mixed cerebellar and sensory ataxia, cerebellar dysarthria, and lower limb pyramidal weakness. However, other systemic manifestations, including hypertrophic cardiomyopathy, diabetes mellitus, kyphoscoliosis, pes cavus, optic atrophy, and sensory deafness can occur (Cossée et al., 1999; Koeppen et al., 2009; Pallardó et al., 2020). Late-onset (26–39 years) and very-late-onset (over 40 years) FRDA variants can also take place, usually presenting with a milder phenotype and lack of systemic manifestations (Koeppen et al., 2011). Frataxin (FXN) is a ubiquitously expressed protein, and its deficiency results in the decrease of mitochondrial copy number, iron accumulation, deficits of respiratory chain complex activities, and increased sensitivity to oxidative stress, thus affecting many different body districts (Vaubel and Isaya, 2013; Martelli and Puccio, 2014). The brain is the predominantly affected tissue in FRDA, but damage to cardiac myocytes and pancreatic beta-cells has also been evidenced (Delatycki and Corben, 2012; Loría and Díaz-Nido, 2015; Franco et al., 2017; Koeppen et al., 2017). Therefore, rather than a "brain disease," FRDA can be considered a "systemic disease," with implications that go beyond the brain itself.

This study moves from our previous report, where we described a family (**Figure 1A**) displaying two small expanded GAA alleles not only in the proband (II-1) affected by late-onset FRDA (LOFA) but also in the two asymptomatic family members: the mother (I-2) and the younger sister (II-2) (Santoro et al., 2020). Further studies revealed that both I-2 and II-2 were actually carriers of an expanded GAA allele and of an uncommon $(GAAGGA)_{66-67}$ repeat (Santoro et al., 2020), while the father (I-1) was a heterozygous carrier of an expanded allele of about 206 GAA repeats. Although expression studies showed that both the compound heterozygous carriers for the expanded GAA and the $(GAAGGA)_{66-67}$ repeat showed a significant reduction of *FXN* mRNA and protein levels in their leukocytes and fibroblasts (Santoro et al., 2020), none of them developed any disease manifestation, supporting that this array represents a benign variant as previously proposed by Ohshima et al. (1999).

To go deeper and understand if a protective mechanism might contribute to modulate the phenotype in this family, here, we report the results of the analysis of redox gene expression profiles in leukocytes and fibroblasts of all family members, particularly focusing on the nuclear factor erythroid 2-related factor 2 (NRF2) and on its glutathione (GSH)-related target genes.

Oxidative stress is a common condition in many neurodegenerative disorders (Barnham et al., 2004), and in FRDA, in particular, it represents one of the most peculiar, although not completely understood, aspects of the pathology (Lupoli et al., 2018). The GAA repeat-mediated FXN depletion leads to mitochondrial iron accumulation in the disease, causing reactive oxygen species (ROS) generation and lipid peroxidation (La Rosa et al., 2020c; Turchi et al., 2020a). As NRF2 regulates many genes directly involved in counteracting oxidative stress and NRF2 signaling axis is defective in FRDA (Paupe et al., 2009; Cuadrado et al., 2019; Petrillo et al., 2019), the evaluation of NRF2 expression in this family can help to open a window on new protective factors potentially buffering the FRDA symptomatology. NRF2 also modulates the cellular levels of GSH, which previously was found impaired in FRDA patients (Piemonte et al., 2001; Pastore et al., 2003) and whose equilibrated ratios between GSH and its oxidized form GSSG are crucial in maintaining the cellular redox balance (Schafer and Buettner, 2001). Thus, we further measured the GSH and GSSG content in family's members, to evaluate their ROS detoxification ability.

By this study, we ask if a differential expression of NRF2 or a dysregulated GSH homeostasis between symptomatic and asymptomatic family's members may represent a distinctive tract able to confer the clinical protection.

RESULTS

The Glutathione Homeostasis Is Dysregulated in the Family

The GSH content has been measured in blood (**Figure 1B**) and in fibroblasts (**Figure 1D**) of FRDA family's members (**Figure 1A**). As reported in **Figure 1B**, the GSH balance was dysregulated in blood, with the GSH levels surprisingly high in the affected proband II-1, approaching the controls' values (1,242 $\pm$ 23 vs. 1,302 $\pm$ 37 μM controls), whereas the asymptomatic mother I-2 (539 $\pm$ 53 μM) and sister II-2 (1,002 $\pm$ 8.2 μM) showed low GSH concentrations, as well as father I-1 (972 $\pm$ 0.6 μM). In parallel, the GSSG, which represents the oxidation product of GSH, was low in the proband II-1 (3.4 $\pm$ 0.08 μM), with respect to the consistently high GSSG levels found in the blood of the unaffected mother I-2 (13.4 $\pm$ 0.06 μM) and to the mild but significant rise in that of the younger sister II-2 (4.04 $\pm$ 0.09 μM, vs. 2.18 $\pm$ 0.10 controls, **Figure 1C**). The father (I-1) showed no significant differences with respect to the controls. This trend was confirmed in fibroblasts (**Figure 1D**), with high GSH levels in II-1 (50 $\pm$ 0.88 nmol/mg prot.) and low concentrations in I-2 (25 $\pm$ 0.33 nmol/mg prot.), II-2 (36 $\pm$ 0.37 nmol/mg prot.), and I-1 (27 $\pm$ 0.35 nmol/mg prot.).

The Glutathione-Related Genes Are Differently Expressed in the Family's Members

Given the different amounts of GSH and GSSG in affected and unaffected members of the family, we asked if the

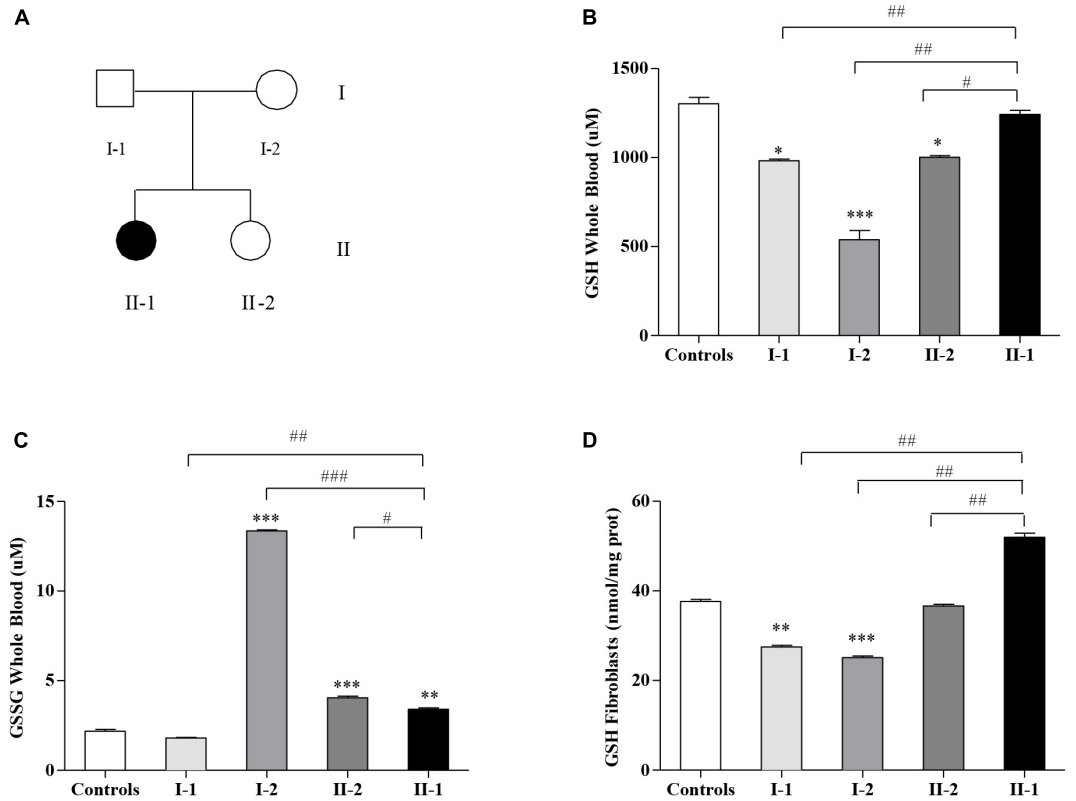

FIGURE 1 | Glutathione homeostasis in Friedreich's ataxia (FRDA) family's members. **(A)** Family tree: father (I-1), mother (I-2), younger sister (II-2), and affected proband (II-1) indicated by a black symbol. Reduced glutathione (GSH) **(B)** and oxidized GSSG **(C)** concentrations in the whole blood, and GSH content in fibroblasts **(D)** of I-1, I-2, II-2, and proband II-1 as measured by the enzymatic re-cycling assay. Values are expressed as median ± SEM. Statistical significance was defined as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ with respect to the controls; and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with proband II-1.

GSH-related genes, responsible for the GSH homeostasis in cells, could be dysregulated in the family. Thus, we analyzed the expression of glutamate–cysteine ligase (*GCL*), the gene coding for the step-limiting enzyme of the GSH synthesis, and the GSH S-reductase (*GSR*) gene, implicated in the re-cycling of the GSH from its oxidized form GSSG. As reported in **Figure 2**, while the GCL expression levels in I-1, II-2, and proband II-1 were comparable with those of the controls (**Figures 2A,B**), the asymptomatic mother (I-2) showed a significant upregulation of the GCL gene, either in leukocytes (**Figure 2A**) or in fibroblasts (**Figure 2B**), probably as a response to the low availability of GSH (**Figures 1B,D**). The expression of GSR, which reduces the GSSG re-establishing a correct GSH/GSSG ratio, was highly activated in I-2 and II-2, both in leukocytes (**Figure 2C**) and in fibroblasts (**Figure 2D**), thus reflecting the need to neutralize the GSSG overload (**Figure 1**). The GSR gene was activated even in the leukocytes (**Figure 2C**) and in fibroblasts of the proband II-1 (**Figure 2D**), who displayed mild but nevertheless significant increase in GSSG concentration (**Figure 1**). The I-1 showed no significant differences in GCL and GSR expression neither in leukocytes (**Figures 2A,C**) nor in fibroblasts (**Figures 2B,D**), with respect to the controls. Overall, these findings demonstrate a strong reactivity to the FXN deficiency of the GSH system, particularly in the I-2, where it was greatly responsive.

Nuclear Factor Erythroid 2-Related Factor 2 Is Activated in the Asymptomatic Members of the Family (I-2 and II-2)

Considering that the GSH-related genes are regulated by NRF2, whose expression is impaired in FRDA patients and in preclinical models of FXN deficiency (Paupe et al., 2009; D'Oria et al., 2013; Shan et al., 2013; La Rosa et al., 2019, 2020d; Petrillo et al., 2019; Turchi et al., 2020b), we evaluated if *NRF2* might be differently expressed in the family. Interestingly, as reported in **Figure 3**, *NRF2* was not induced in the fibroblasts of the proband II-1 (**Figure 3B**) but highly stimulated in leukocytes (**Figure 3A**). It is important to note that the symptomatic proband II-1 was under idebenone therapy at the time of blood collection, and idebenone is a well-known NRF2 inducer (Petrillo et al., 2019).

NRF2 was also significantly activated in leukocytes (**Figure 3A**) and in fibroblasts (**Figure 3B**) of I-2 and II-2, while its expression in the I-1 was comparable with that of the controls (**Figures 3A,B**).

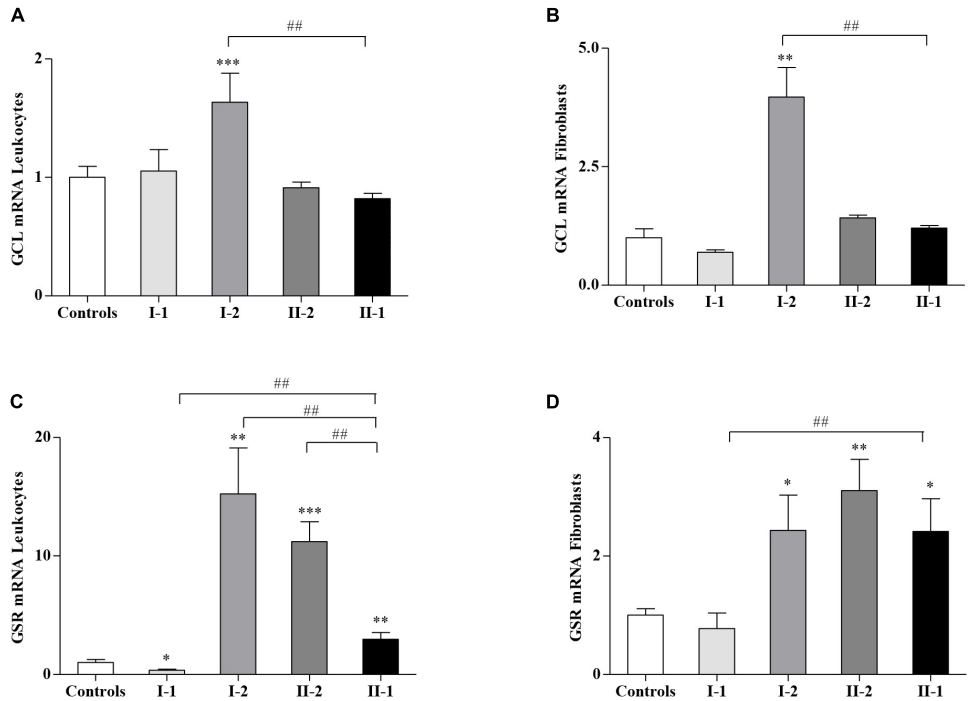

FIGURE 2 | Glutathione-related genes in Friedreich's ataxia (FRDA) family. The expression of glutamate–cysteine ligase (*GCL*) and glutathione S-reductase (*GSR*) was analyzed by quantitative real-time PCR (qRT-PCR), respectively, in leukocytes **(A,C)** and fibroblasts **(B,D)** of the I-1, I-2, II-2, and proband II-1. Values represent median ± SEM. Statistical significance was defined as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to the controls and $^{\#\#}p < 0.01$ compared with proband II-1.

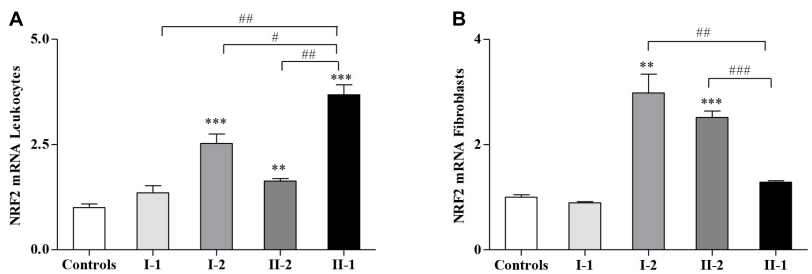

FIGURE 3 | Nuclear factor erythroid 2-related factor 2 (*NRF2*) gene expression in Friedreich's ataxia (FRDA) family. Quantitative real-time PCR (qRT-PCR) analysis of NRF2 transcripts in leukocytes **(A)** and in fibroblasts **(B)** of I-1, I-2, II-2, and proband II-1. Values represent median ± SEM. Statistical significance was defined as $^{**}p < 0.01$ and $^{***}p < 0.001$ with respect to the controls; and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with proband II-1.

MATERIALS AND METHODS

This study was conducted in agreement with the Declaration of Helsinki, and its design fulfilled the guidelines of all involved institutional ethical boards. RNA, and protein samples were extracted from peripheral blood leukocytes or cultured fibroblasts obtained from punch skin biopsies from all family members who gave a written informed consent authorizing storage and use of clinical data and biological samples for diagnostic and clinical research purposes.

Family Description

The proband (II-1) was a 43-year-old female whose symptoms started at the age of 35, with slowly progressive gait, balance, and mild speech impairment. Her family history was negative (**Figure 1A**). She first came to our attention at the age of 39 years, and neurological examination documented gaze evoked nystagmus, mild cerebellar dysarthria, gait ataxia, limb in coordination with positive Romberg sign, absent deep tendon reflexes, and bilateral Babinski sign; antibodies, serum alpha-fetoprotein, vitamins B12 and E, and lactic acid levels were all negative. Two pathological GAA expansions of approximately 206 (GAA1) and 473 (GAA2) repeats have been documented in the proband (Santoro et al., 2020).

The asymptomatic 36-year-old sister (II-2) displayed two expanded alleles apparently corresponding to 146 (GAA1) and 176 (GAA2) repeats (Santoro et al., 2020). During 3 years of

follow-up, symptoms slowly progressed in II-1, as expected; instead, II-2 did not develop any FRDA manifestation.

Finally, the 73-year-old mother (I-2) carried two GAA expansions of approximately 139 (GAA1) and 389 (GAA2) repeats, though detailed clinical neurological evaluation documented the absence of symptomatology (Santoro et al., 2020).

Blood Sample Collection

Blood samples from all family members were collected into 5% EDTA Vacutainer tubes (Becton Dickinson, Rutherford, NY) and fractionated as follows: 1 ml was stored at -80°C immediately after drawn for GSH determinations; 1 ml was destined to GSSG measurements and stored at -80°C, until analysis; and 5 ml of whole blood was used for isolation of leukocytes by 10% dextran.

After 45 min at room temperature, the upper phase containing leukocytes was centrifuged at 1,125 × g (5 min) and washed with 0.9% NaCl, until a clear pellet was obtained. Leukocytes have been stored at −20°C until the RNA extraction.

Cell Cultures

Skin biopsies were taken from all family members and three age-matched controls. Fibroblasts were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 50 units/ml of penicillin, 50 μg/ml of streptomycin, 0.4% (v/v) amphotericin B (250 μg/ml), and 1 mM of sodium pyruvate at 37°C in 5% CO_2, as reported in Pastore et al. (2003). Fibroblasts were grown to 70% confluence. The assays were performed in triplicates, and cells were used at similar passage numbers.

GSH and GSSG Determination

GSH and GSSG levels have been detected using the enzymatic recycling assay, as previously reported (Petrillo et al., 2019). Briefly, samples have been de-proteinized with 5% (w/v) sulfosalycilic acid (SSA; Sigma-Aldrich, St. Louis, MO, United States), and the GSH content was determined after dilution of the acid-soluble fraction in Na–phosphate buffer containing EDTA (pH 7.5). To prevent an overestimation of GSSG due to the oxidation of thiols during sample manipulation, blood samples have been collected in tubes prefilled with 30 mM of *N*-ethylmaleimide (NEM) (Giustarini et al., 2013). GSH and GSSG concentrations have been measured with the ThioStar® GSH detection reagent (Arbor Assays, Michigan, United States), using, respectively, GSH and GSSG as standards (Sigma Chemicals, St. Louis, MO, United States). The fluorescence has been measured using an EnSpire® Multimode Plate Reader (Perkin Elmer, Waltham, MA, United States). GSH levels in fibroblasts were expressed as nmol/mg proteins. Protein concentration was determined by the bicinchoninic acid assay (BCA) method (Thermo Fisher Scientific, United States).

Quantitative Real-Time PCR

Total RNA was extracted from leukocytes and fibroblasts using TRI Reagent (Sigma-Aldrich, St. Louis, MO, United States), according to manufacturer's protocol. One microgram of each RNA samples was reverse transcribed with the SuperScript™ First-Strand Synthesis system and random hexamers as primers (Life Technologies, Carlsbad, CA, United States). The mRNA of GCL, GSR, and NRF2 was measured by qRT-PCR in an ABI PRISM 7500 Sequence Detection System (Life Technologies, Carlsbad, CA, United States) using Power SYBR Green I dye chemistry. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method with TATA box binding protein (TBP) as a housekeeping gene and expressed as fold change relative to the controls. Primers used for qRT-PCR are reported in **Table 1**.

Statistical Analysis

Statistical analysis was performed using the GraphPad/Prism 5.0 Software (San Diego, CA, United States). Statistically significant differences between the controls and family's members were analyzed using Student's *t*-test for normally distributed variables. All data are presented as mean ± standard error. Statistical significance was defined as $^{*}p < 0.05$, $^{**}p < 0.001$, and $^{***}p < 0.001$ compared with the controls, and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with proband II-1.

DISCUSSION

This study moves from our previous paper focused on a peculiar family characterized by the presence in two first-degree relatives of the proband, affected by LOFA, of a compound heterozygosity for an expanded (GAA) repeat and a (GAAGGA) repeat at *FXN* locus; both compound heterozygotes are asymptomatic, supporting that the (GAAGGA) repeat would be indeed a benign variant. Yet FRDA studies (Santoro et al., 2020) showed that FXN mRNA and protein levels were markedly reduced not only in tissues of the proband but also in the two asymptomatic compound heterozygotes. This led us to hypothesize that some protective factors may mitigate detrimental effects of FXN deficiency in both subjects; thus, we decided to assess the status of the antioxidant response in that family.

A consequence of the FXN depletion in FRDA is the increase of oxidative stress, and the most credited pathogenic hypothesis is that the FXN-mediated impairment of the mitochondrial iron–sulfur cluster (ISC)-containing enzymes (respiratory chain complexes I–III and aconitase) contributes to the Fenton-mediated overproduction of ROS (Armstrong et al., 2010; Gomes and Santos, 2013; Vaubel and Isaya, 2013; Abeti et al., 2016; Lupoli et al., 2018).

TABLE 1 | Primers used for qRT-PCR.

Human genes	Sequence 5′>3′
NRF2	*Fw*-ACACGGTCCACAGCTCATC
	Rv-TGTCAATCAAATCCATGTCCTG
GCL	*Fw*-TTGCCTCCTGCTGTGTGATG
	Rv-ATCATTGTGAGTCAACAGCTGTATGTC
GSR	*Fw*-CACTTGCGTGAATGTTGGATG
	Rv-GATTTCTATATGGGACTTGGTG
TBP	Fw-CCGAAACGCCGAATATAATCC
	Rv-AAATCAGTGCCGTGGTTCGT

High susceptibility to oxidative stress has been demonstrated in FRDA patients' fibroblasts in early studies (Wong et al., 1999), and ROS overload was found in yeast (Bulteau et al., 2007; Irazusta et al., 2008), drosophila (Llorens et al., 2007; Anderson et al., 2008; Soriano et al., 2013), and mouse (Al-Mahdawi et al., 2006; Lupoli et al., 2018) disease models. In addition, elevated levels of oxidative stress markers have been found in the blood (Emond et al., 2000; Schulz et al., 2000; Bradley et al., 2004) and cells (Cotticelli et al., 2013; Abeti et al., 2015, 2016, 2018; Petrillo et al., 2019) of FRDA patients.

However, unlike the expected activation of the NRF2-mediated antioxidant defense, the NRF2 signaling pathway is defective in FRDA patients and in preclinical models of FXN deficiency (Paupe et al., 2009; Shan et al., 2013; La Rosa et al., 2020a,b), thus further exacerbating the susceptibility to oxidative stress and its induced defects in the disease (Abeti et al., 2018; La Rosa et al., 2020c,d).

In this family, we analyzed the antioxidant response in all members, particularly focusing on the GSH metabolism and *NRF2* expression, both pathways representing the first antioxidant defense lines in tissues. GSH is the main redox indicator in cells, and previous studies reported decreased levels of this molecule in the blood of FRDA patients.

NRF2 is the principal regulator of the GSH homeostasis by upstream modulating the GSH synthesis (*GCL* gene) and the GSH recycling from its oxidized form GSSG (*GSR* gene). All these actions may actively contribute to counteract the oxidative stress-mediated injury and, potentially, to slow down the onset of symptoms in FRDA.

Our findings demonstrate that the GSH homeostasis was dysregulated in the family (**Figure 1**), yet with unexpected significantly low GSH concentration in the asymptomatic compound heterozygous I-2 and high levels in the proband II-1. The amount of GSSG was also consistently high in I-2, and a moderate increase was even found in the other compound heterozygous II-2 and in the proband II-1, likely indicating a general activation of the GSH-mediated response. Also, the GSH-related genes were differently expressed in the family members (**Figure 2**), showing a great reactivity of the GSH system to the FXN deficiency, particularly in the I-2, where the genes of GSH synthesis (*GCL*) and of GSSG detoxification (*GSR*) were highly responsive. The *GSR* gene was activated even in the other compound heterozygous II-2, as well as in the proband II-1, reflecting the need of buffering the increase in GSSG.

However, as the imbalance of GSH levels did not allow explaining the lack of symptoms in the FXN-deficient compound heterozygous I-2 and II-2, we focused our attention on NRF2, the upstream regulator of GSH homeostasis, which is usually depleted under conditions of FXN deficiency.

Contrasting the reduced NRF2 expression documented in FRDA tissues, NRF2 was significantly activated in both leukocytes and fibroblasts of the two asymptomatic compound heterozygous I-2 and II-2 (**Figure 3**), suggesting that the occurrence of an endogenous stimulation of this transcription factor in these subjects might translate into protective and preventive effects on the symptomatology.

Instead, NRF2 was downregulated in the fibroblasts of the LOFA proband II-1, yet it was activated in her leukocytes (**Figure 3**), where it might be related to the effects of the idebenone treatment. Indeed, the proband was under idebenone therapy at the time of blood collection, and idebenone is known to activate *NRF2* expression in FRDA patients (Petrillo et al., 2019; La Rosa et al., 2020a).

Thus, in the LOFA proband, NRF2 is, as expected, constitutively low in fibroblasts, whereas it is exogenously activated in leukocytes by idebenone, but in both the asymptomatic compound heterozygous carriers, NRF2 is constitutively upregulated, although both of them would also show decreased FXN expression. So we hypothesize that the occurrence of a widespread upregulation of NRF2 expression in such individuals might contribute to protect the most susceptible tissues against the progressive oxidative damage and the onset of

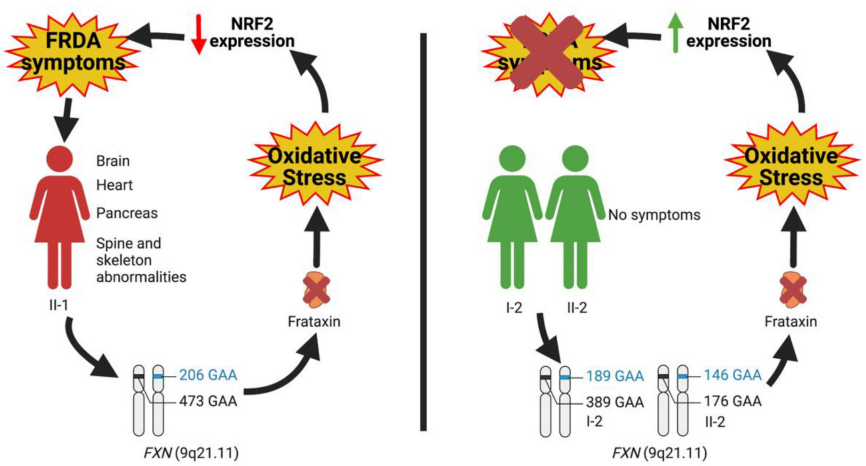

FIGURE 4 | Hypothesis on the role of nuclear factor erythroid 2-related factor 2 (NRF2) as a protective factor antagonizing the insurgence of Friedreich's ataxia (FRDA) symptomatology.

symptoms. Importantly, we suggest that the early administration of NRF2 inducers in patients, particularly in FRDA children, at the first onset of the disease could slow the progression of neurological damage, thus being of great therapeutic help.

Overall, by this study, we extend the spectrum of possible effectors responsible for the development of clinical symptoms, thus moving the origin of the disease outside the brain. In this regard, the family we analyzed is paradigmatic since, although all members displayed FXN deficiency, nonetheless some individuals appeared free of symptoms. Such as for Parkinson's (PD) and Alzheimer's diseases (AD), also for FRDA, alternative mechanisms, beyond the brain, can be hypothesized to contribute to the pathogenesis of the disease. In particular, our findings support the role of NRF2 as a protective factor whose constitutive upregulation can keep the antioxidant defense above a threshold, able to prevent the appearance of clinical manifestations (**Figure 4**). Future studies will be needed to expand the panel of NRF2 activities, in order to identify which pathways are more involved in clinical FRDA protection. It is important to note that NRF2 regulates the transcription of approximately 1% of the human genome (Cuadrado et al., 2019) and that beside maintaining the cellular redox homeostasis, multiple cellular processes, including regulation of inflammation, differentiation, proliferation, cell survival, protein homeostasis, and metabolism, are among the functions influenced by its activity (Corenblum et al., 2016; Robledinos-Antón et al., 2017; Cuadrado et al., 2019; Dodson et al., 2019; La Rosa et al., 2019; Turchi et al., 2020b). Two processes were recently shown to be deeply connected to FRDA pathogenesis: (i) ferroptosis, an iron-dependent cell death caused by impaired GSH metabolism, lipid peroxidation, and mitochondrial failure (Cotticelli et al., 2019; La Rosa et al., 2020d; Turchi et al., 2020b); and (ii) inflammation, a mechanism not yet fully understood in FRDA, but potentially involved, as demonstrated in fibroblasts of patients, where the anti-inflammatory heme-oxygenase 1 (HO-1) gene was found to be reduced (Petrillo et al., 2019) and in patients who showed beneficial effects upon treatment with an NF-kB suppressor (Lynch et al., 2019). Although it is undeniable that the NRF2 activation can ameliorate FRDA pathogenesis rescuing, at least in part, the detrimental effects generated by these processes, deeper and more complex regulations could be responsible for the NRF2-mediated protection observed in asymptomatic members of the family. Elucidating these defense mechanisms will be crucial not only in a mitochondrial and systemic disease such as FRDA but also in other oxidative stress-mediated disorders characterized by an out-brain origin (i.e., PD and AD).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Bambino Gesù Children's Hospital (code 1166/2016; date of approval 08/06/2016). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SP carried out the experiments and finalized the manuscript. MS, GS, and FP interpreted the data and wrote the manuscript. MG contributed in cell handling. PLR, AP, and EB performed the critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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REVIEW
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Diets and Cellular-Derived Microparticles: Weighing a Plausible Link With Cerebral Small Vessel Disease

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Cerebral small vessel disease (CSVD) represents a spectrum of pathological processes of various etiologies affecting the brain microcirculation that can trigger neuroinflammation and the subsequent neurodegenerative cascade. Prevalent with aging, CSVD is a recognized risk factor for stroke, vascular dementia, Alzheimer disease, and Parkinson disease. Despite being the most common neurodegenerative condition with cerebrocardiovascular axis, understanding about it remains poor. Interestingly, modifiable risk factors such as unhealthy diet including high intake of processed food, high-fat foods, and animal by-products are known to influence the non-neural peripheral events, such as in the gastrointestinal tract and cardiovascular stress through cellular inflammation and oxidation. One key outcome from such events, among others, includes the cellular activations that lead to elevated levels of endogenous cellular-derived circulating microparticles (MPs). MPs can be produced from various cellular origins including leukocytes, platelets, endothelial cells, microbiota, and microglia. MPs could act as microthrombogenic procoagulant that served as a plausible culprit for the vulnerable end-artery microcirculation in the brain as the end-organ leading to CSVD manifestations. However, little attention has been paid on the potential role of MPs in the onset and progression of CSVD spectrum. Corroboratively, the formation of MPs is known to be influenced by diet-induced cellular stress. Thus, this review aims to appraise the

body of evidence on the dietary-related impacts on circulating MPs from non-neural peripheral origins that could serve as a plausible microthrombosis in CSVD manifestation as a precursor of neurodegeneration. Here, we elaborate on the pathomechanical features of MPs in health and disease states; relevance of dietary patterns on MP release; preclinical studies pertaining to diet-based MPs contribution to disease; MP level as putative surrogates for early disease biomarkers; and lastly, the potential of MPs manipulation with diet-based approach as a novel preventive measure for CSVD in an aging society worldwide.

Keywords: cerebral small vessel disease, diet, microparticles, neurodegeneration, microthrombosis

INTRODUCTION

An acute cerebrovascular event due to an occlusion (or ischemia) of small blood vessels deep within the brain is a known manifestation of small vessel disease (SVD) involving the brain small end arteries, capillaries, venules, and arterioles (1–3). Of all ischemic stroke events, ∼30% are represented by cerebral SVD (CSVD) (1, 4). CSVD is a spectrum of complex and overlapping pathophysiological mechanism of various etiologies affecting the brain small vessel microcirculation that can trigger neuronal inflammation and the subsequent neurodegenerative cascade. However, it is generally viewed that CSVD represents pathological consequences of SVD on the brain parenchyma rather than the underlying diseases of the vessels (5). Prevalent with aging, CSVD is recognized as risk factor for stroke, vascular dementia, Alzheimer disease (AD), and Parkinson disease (PD) (6, 7). Despite being arguably the most common neurodegenerative disease (NDD) with predilection of the cardiocerebrovascular axis, there is only limited knowledge about CSVD underlying mechanisms.

Among the known modifiable risk factors for stroke, dietary patterns are recognized to modulate the non-neural peripheral events such as in the gastrointestinal tract (GIT) (i.e., GIT dysbiosis) and cardiovascular stress through cellular inflammations and oxidation. Moreover, diet plays a crucial role in maintaining the physiological systems responsible for homeostasis and hemostasis, whereby healthy dietary pattern has been classified as diet with lower concentration of plasma proinflammatory markers (8). Hence, certain dietary patterns could potentially lead to undesirable alterations in such systems as shown in the case of less or non-nutritious/unbalanced diets (9, 10). Moreover, unhealthy dietary habits have been reported to contribute to higher risk of developing metabolic disease, coronary heart disease, and stroke (11) and likely to modulate systemic peripheral events that can influence the development and progression of NDD such as CSVD. One key outcome from such events, among others, includes the cellular activations that lead to elevated levels of endogenous cellular-derived circulating microparticles (MPs). MPs can be produced from various cellular origins including leukocytes, platelets, endothelial cells (ECs), microbiota, and microglia. MPs could act as microthrombogenic procoagulant that could be detrimental to the vulnerable microcirculation, particularly the penetrating, poorly collateralized end-arteries in the brain parenchyma, leading to CSVD manifestations. However, little attention has been paid on the potential role of MPs in the onset and progression of CSVD spectrum. Corroboratively, the formation of MPs is known to be influenced by diet-induced cellular stress.

Thus, this review aims to appraise the body of evidence on the dietary-related impacts on circulating MPs from non-neural peripheral origins that could serve as a plausible microthrombogenic role in CSVD manifestation and hence a precursor of NDD. Here, we elaborate on the pathomechanical features of MPs in health and disease states; relevance of dietary patterns on MP release; preclinical studies pertaining to diet-based MPs contribution to disease; MP level as putative surrogates for early disease biomarkers; and lastly, the potential of MPs manipulation with diet-based approach as a novel preventive measure for CSVD.

MICROCIRCULATION NETWORK AND SMALL VESSEL DISEASE

The term *microcirculation* used to represent the terminal vascular branches or network of the systemic circulation that consist mainly of (small) microvessel (diameters of $<20\,\mu m$) (12). These microvessels comprised capillaries (including their subcellular components), arterioles, and postcapillary venules (13) (**Figure 1**). For example, in coronary blood supply (i.e., from right coronary artery, right coronary artery, and left main coronary artery), small muscular arteries are found throughout the myocardium that further branch into an extensive capillary bed (intramural arteries) that embraces the cardiac myocytes (14). In GIT, the small perforating arteries mainly originated from celiac trunk (arteries) that supply the foregut (i.e., esophagus, stomach, liver, gallbladder, superior pancreas, first and second part of duodenum), superior mesenteric artery supply the midgut (i.e., third part of duodenum, jejunum, appendix, cecum, ascending colon), and inferior mesenteric arteries that supply the hindgut (i.e., descending colon, rectum, upper part of anal canal) (15, 16). While renal microvasculature are smaller branches that form the afferent arterioles leading to the formation of glomerular capillaries, the distal glomerular capillaries form the efferent arterioles, followed by the peritubular capillaries that

supply the renal tubules (17). In the brain, ∼72% of cerebral blood flow (cBF) is contributed by anterior circulation that arises from the internal carotid artery (ICA) (18). cBF can be defined as the volume of blood that flows per unit mass per unit time in brain tissue [mL_{blood}/(100 g_{tissue} min)], or flow per unit volume of brain tissue [mL_{blood}/(100 mL_{tissue} min)] (19). From among the vast ICA branching network, the most significant pathophysiologically are the anterior cerebral arteries, middle cerebral arteries (MCAs), and anterior choroidal arteries. The branches of these arteries mainly supply the forebrain (i.e., frontal, temporal, and parietal lobes), as well as subcortical region of diencephalon and internal capsule. In addition, ∼30% of cBF is contributed by posterior cerebral circulation that is derived from tributaries of the vertebral and basilar arteries (13). These branches mainly supply posterior portion of brain, i.e., occipital lobes and posterior brainstems (see **Figure 1** for the illustration of blood supply to these major organs).

Pertaining the connection of vascular supply and drainage between these major organs, most of these organs receive their blood supplies locally or from the abdominal aorta (**Figure 1**). For example, some parts of the large intestine receive blood supply from the SMA (branching of the abdominal aorta) (16). The heart, on the other hand, consists of its own coronary vascular supply for oxygenated blood and coronary sinus for its venous drainage (14). All in all, most organs return deoxygenated blood either through superior or inferior vena cava for gaseous exchange through the pulmonary circulations. A direct connection between organs, for example, GIT–heart–brain axis, may be observed through the venous drainage but not through the arterial blood supply, whereby most of the GIT (visceral organs) circulation will return to inferior vena cava of the heart *via* the hepatic portal circulation (16). As for the cerebral circulation, the venous drainage will eventually reach the superior vena cava of the heart and, subsequently, the pulmonary circulation for gaseous exchange. For the brain, oxygen and nutrients from peripheral circulation are delivered through MCAs and their fenestrated capillaries that supply deep subcortical region (20). Hence, any initial peripheral event (from systemic and cellular insult or activation) may affect a specific organ through their local circulation or may even propagate *via* the abdominal aorta to other specific organ locations. Similarly, vascular drainage that eventually returns the deoxygenated blood from other organs of the body to the superior and inferior vena cava of the heart may also act as a "hitchhike" passageway for the systemic or cellular insults or activation by-products and lodged to other organs or blood vessels, including microcirculation network.

Consequently, microcirculation network is the most crucial compartment and terminal destination of the vascular systems, whereby it is the pinnacle site where the red blood cells (RBCs) in the capillaries directly transfer the oxygen to the surrounding parenchymal cells that require oxygen for energy metabolism (12). Apart from that, microcirculation helps to regulate intravascular-tissular space solute exchange, transporting all the nutrients and blood-borne hormones to the cells and tissues and moderating the functional activities of hemostasis and immune system (12). The vasculature of microcirculation consists primarily of lining of the ECs. The morphology and density of these endothelial structures varied between organs and vessels. However, endothelial lining generally consists of pores and fenestration that are held together by various adherent molecules such as cadherins and gap junctions (to carry current), hence allowing upstream electrical communication (12). Furthermore, ECs are symbiont with smooth muscle cells (SMCs) regulating the microvascular blood flow through the regulation of arteriolar vasotone with three different mechanisms, i.e., metabolic, myogenic, and neurohumoral control. The lumen of endothelium consists of gel-like structure (0.2–0.5 μm) synthesized by ECs, known as glycocalyx (e.g., proteoglycans, glycosaminoglycans, and plasma protein), which help in mediating endothelium functions, i.e., their microcirculatory functions (21, 22). Apart from glycocalyx, various subcellular substances are also present in the lumen of endothelium such as superoxide dismutase and antithrombin (23).

Therefore, the integrity of microvessel endothelium and its component is the main determinant for vascular barrier. Endothelial dysfunction is one of the ultimate cellular events that are responsible for hemodynamic changes seen in various pathological conditions (22). Microcirculation network is crucial for normal functioning of GIT, heart, and the nervous system, with the majority (up to 80%) of oxygen supplies to these organs is utilized for adenosine triphosphate production to aid sodium and potassium pumps maintaining the homeostasis. Thus, oxidative stress, hypoxia, nitro stress, and inflammatory mediators could potentiate the sequelae that lead to various SVD of these organs (24). Preclinical studies (including animal models) had shown that microcirculation and endothelial inflammation may serve as therapeutic targets to arrest microvascular-based organ or parenchymal injury (25, 26).

Small Vessel Disease—An Overview

SVD is a term used to represent the pathological process that damages the small end arteries, capillaries, venules, and arterioles (2). The condition may lead to alteration of microcirculation (i.e., blood flow or perfusion) of the affected organ. SVD is generally observed in major organs such as the brain, retinal, heart, and urinary system (i.e., kidney), due to fact that these organs primarily required a desirable amount of cardiac output for their functionality (27). However, the GIT arteries are rarely affected to vascular disease either SVD or large vessel disease (i.e., atherosclerosis) (28). In rare instances, especially following myocardial infarction or atrial fibrillation, thrombus may accumulate and cause occlusion in the artery resulted in ischemic colitis (with an acute onset of abdominal pain and blood in the stools) (28, 29). Moreover, the thrombus or arterial occlusions may cause the reduction of blood flow (chronically) in the colon that can trigger inflammation before turning gangrenous (tissue death due to lack of blood supply) (29).

The integrity of microvascular endothelium and its component plays a major role as a vascular barrier (i.e., between circulating blood and vessel wall). Therefore, SVD is frequently associated with the endothelium dysfunction that results in arteriolosclerosis and lipohyalinosis. In general, ECs

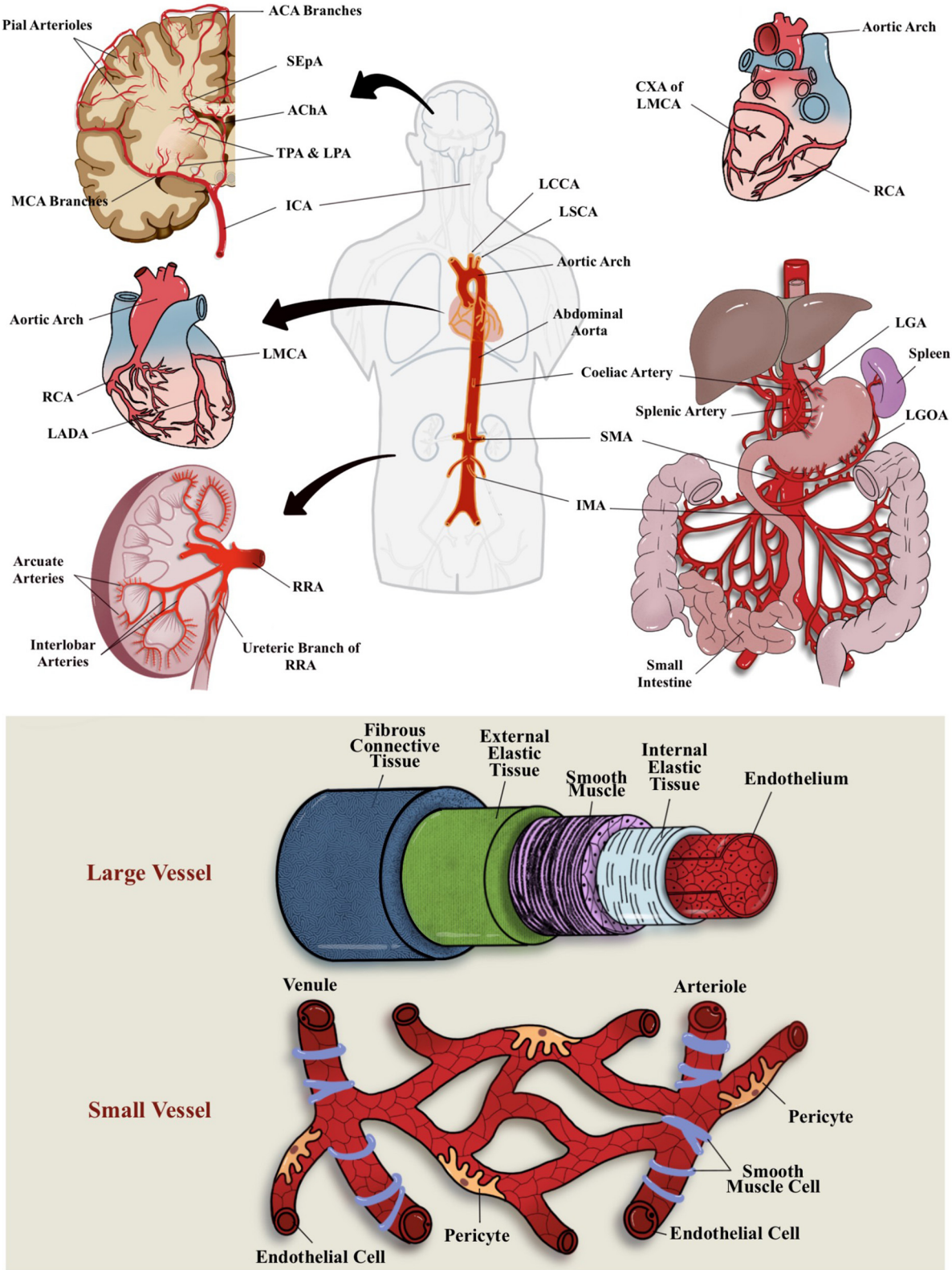

FIGURE 1 | Vascular blood (arterial) supplies to the brain, heart, GIT, and kidney and differential structure between large and small vessel. Most of these organs receives their blood supply locally or from the abdominal aorta. ACA, anterior cerebral arteries; AChA, anterior choroidal arteries; CXA, circumflex artery; ICA, internal carotid artery; IMA, inferior mesenteric arteries; LADA, left anterior descending artery; LCCA, left common carotid artery; LGA, left gastric artery; LGOA, left gastro-omental artery; LMCA, left main coronary artery; LPA; lenticulostriate perforating arteries; LSCA, left subclavian artery; MCA, middle cerebral artery; RCA, right coronary artery; RRA, right renal artery; SEpA, subependymal arteries; SMA, superior mesenteric artery; TPA, thalamic perforating arteries.

help maintain vascular barrier or health and blood flow (through capillaries and arterioles) in several ways including limiting the platelet or leukocyte aggregation, controlling the vascular permeability from plasma components, and regulating the vascular tone (30). Equally crucial for the ECs to function at their optimum is their interaction or crosstalk with the surrounding cells such as mural cells (i.e., pericytes and vascular SMCs), glial cells (i.e., astrocytes), and immune cells (31).

Risk Factors and Clinical Relevance of SVD

Previous report had confirmed that hypertension (i.e., systolic blood pressure ≥135 mm Hg), sex (i.e., male), type 2 diabetes mellitus (T2DM), smoking status, and aging (i.e., ≥70 years old) were the main risk factors that can lead to SVD (i.e., in the brain, retina, and heart) (32–34). Another contributing risk factor is the metabolic syndrome including obesity (as of dietary and lifestyle) due to accumulated fat in the abdominal location, hence abdominal obesity. The accumulated fat mediates the synthesis of inflammatory cytokines and causes further inflammation of GIT vasculature (35, 36). Moreover, microvascular complication such as increase of proinflammatory cytokines, vascular endothelial adhesion molecules (VCAMs), and intracellular cell adhesion molecules (ICAMs) has been associated with T2DM (37), hence increasing the risk toward multi-organ SVD.

Apart from that, endothelial dysfunction (in specific, related to cerebral microcirculation) has been associated with the impact of immune system related GIT microbiota, whereby the dietary pattern (i.e., high salt intake) potentially leads to neurovascular dysfunction through GIT initiated T helper cell 17—the cells responsible for tissue inflammation induction and destruction (38). Interestingly, recent evidence suggested that higher SVD incidence is associated with an increased systemic inflammation due to poor sleep quality (39), as well as societal-based depression and loneliness (40–42). Besides, individual(s) with SVD is suggested to suffer from "systemic" condition (27). This is so as SVD is commonly associated with nervous system disturbances such as stroke, cognitive decline, vascular dementia, and gait dysfunction (43–46). However, SVD possesses multiorgan and multidirectional predilection, whereby any organs with similar vascular risks may have the effects. For example, retinal SVD with neurodegeneration-related cognitive decline, retinal microvascular abnormalities associated renal failure, cardiac insufficiency, blindness, lungs, and GIT vascular-based disorders (47–54).

CEREBRAL SMALL VESSEL DISEASE

CSVD is a spectrum of complex and overlapping pathophysiological mechanism of various etiologies affecting the brain microcirculation that can trigger neuronal inflammation and the subsequent neurodegenerative cascade. However, it is generally viewed that CSVD represents pathological consequences of SVD on the brain parenchyma rather than the underlying diseases of the vessels (5). Therefore, the term *cerebral small vessel disease* is generally viewed as the state of brain parenchyma injury (often progressive) that is associated with distal leptomeningeal and intracerebral vessel pathology that resides in poorly collateralized subcortical gray and deep white matter. Moreover, it is mainly due to several focal or diffuse microvasculopathological processes that affect and cause occlusion to the small perforating cerebral capillaries (of sizes 50–400 mm), small arteries (mostly branches of MCAs), arterioles (diameter <0.1 mm), and venules that penetrate and supply the brain cortical and subcortical region (55, 56).

There are several etiopathogenic classifications of CSVD. However, the most well-recognized forms of CSVD are the amyloidal CSVD [e.g., sporadic and hereditary cerebral amyloid angiopathy (CAA)] and non-amyloidal CSVD including age-related and vascular risk-factor–related SVD (i.e., arteriolosclerosis and age) (56). Other less common forms of CSVD include inherited or genetic (monogenic) CSVD that is recognizably different from CAA [i.e., Fabry disease and cerebral autosomal dominant arteriopathy with subcortical ischemic strokes and leukoencephalopathy (CADASIL)], inflammatory and immunologically mediated CSVD, venous collagenosis, and other CSVD (i.e., non-amyloid microvessel degeneration in AD and postradiation angiopathy) (57). Clinical diagnosis of CSVD typically takes the form of acute lacunar infarct and, less commonly, as intraparenchymal hemorrhage, with neuroimaging findings such as white matter hyperintensities (WMHs) of presumed vascular origin, cerebral microbleeds (CMBs), cortical microinfarcts, lacunar infarcts, and recent subcortical brain infarcts (RSBIs) and enlarged perivascular spaces (PVS), or pathological phenomena with multifaceted etiologies (55, 58, 59). However, the lack of standardization and consistency in neuroimaging techniques leads to the development of STandards for Reporting Vascular changes on nEuroimaging (STRIVE), aiding in the imaging-based visual identification and classification of CSVD spectrum (60) (see **Figure 2** for neuroimaging correlates of different CSVD manifestation based on STRIVE method).

Risk Factors of CSVD Manifestation and Their Clinical Relevance

There are several and complex known risk factors toward development and progression of CSVD manifestation. For example, increased imaging loads of WMHs, lacunar infarcts, and RSBI were associated with lifetime exposure toward cardiocerebro(micro)vascular risks such as metabolic syndrome (i.e., hypertension, obesity, hyperlipidemia, dyslipidemia), lifestyle (i.e., smoking, alcohol abuse), and T2DM that posed a higher odd for acute ischemic (lacunar) strokes (62). However, age has served as one of the most significant determinants of the onset, proportion, and progression of all CSVD manifestations [for instance, being prevalent with healthy aging (∼6%) in the case of CMBs] (63). Higher risk of CMBs has been found in individuals with symptomatic cerebrovascular disease such as ischemic stroke and intraparenchymal hemorrhage (63). Meanwhile, genetic factors such as *NOTCH3* gene (chromosome 19) mutation as seen in CADASIL; mitochondria DNA mutation as seen in mitochondrial encephalomyopathy, lactic acidosis, and stroke-like syndrome (MELAS); Fabry disease; and familial CAA increase the burden and prevalence of CSVD (64, 65).

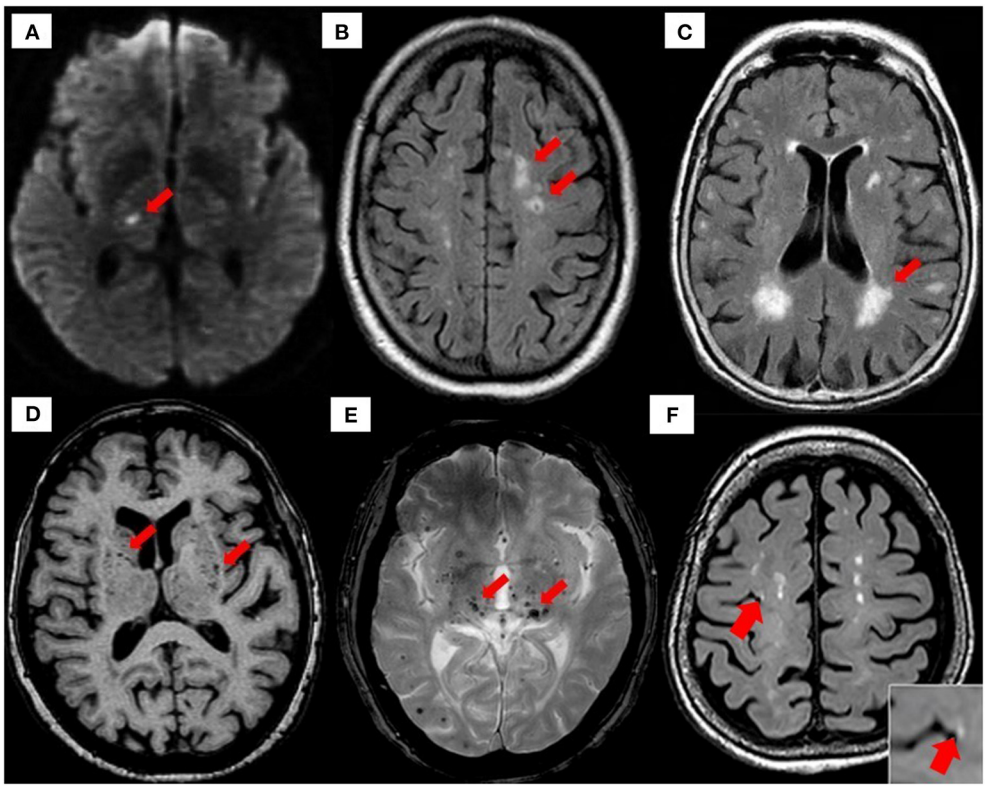

FIGURE 2 | Neuroimaging correlates of CSVD based on STRIVE method. **(A)** Recent small subcortical infarct (RSBI) on diffusion-weighted imaging (DWI) (red arrow). Usual diameter is around 3–15 mm, with hyperintense rim surrounding ovoid cavity. RSBI seen as increased T2-weighted, fluid-attenuated inverse recovery (FLAIR), and DWI signal intensities decreased T1-weighted signal and isointense in T2*-weighted gradient recoiled echo (GRE) signal and susceptibility-weighted imaging (SWI). RSBI is best identified through DWI with usual infarct diameter of ≤20 mm. **(B)** Lacunar infracts on FLAIR (red arrow). Lacunar infarcts appeared as increased hyperintensity in T2-weighted signal, decrease T1-weighted, and FLAIR signal and isointense in DWI. Usual diameter is around 3–15 mm, with hyperintense rim surrounding ovoid cavity. **(C)** White matter hyperintensities (WMHs) of presumed vascular origin on FLAIR (arrow). WMHS seen as increase intensity or hyperintensity on T2-weighted imaging, T2*-weighted GRE and FLAIR (best identified), isointense on DWI, and hypointense (decrease intensity) on T1-weighted imaging. **(D)** Enlarged perivascular spaces (PVS) on T1-weighted imaging (red arrow) with usual diameter of ≤2 mm. PVS is seen as decrease FLAIR and T1-weighted signal intensity, with increase T2-weighted signal. Meanwhile T2*-weighted GRE and DWI appeared isointense, and they also appeared in similar signal intensity with cerebrospinal fluid (CSF). **(E)** Cerebral microbleeds (CMBs) on T2*-GRE (red arrow). CMBs are small, rounded areas of signal void with blooming, whereby they were visualized as isointense T1- and T2-weighted signal, FLAIR, and DWI. They are best identified under T2*-weighted GRE or SWI as reduced signal intensities. Usual diameter is around ≤10 mm (mostly 2–5 mm). **(F)** 3-T MRI representation of cortical microinfarcts (red arrow) on T1-weighted (hypointense) [images A–E, reproduced with permission from Mustapha et al. (57), image F is adapted from Takasugi et al. (61)].

Hence, optimizing (micro)vascular risk factors for secondary stroke prevention is undoubtedly warranted.

In addition, most of CSVD manifestation has been demonstrated to increase the risk of vascular cognitive impairment and dementia. For example, previous report had shown that elderly person with hypertension who presented with confluent periventricular and hypoperfusion-based deep WMHs, respectively, had impaired executive function, short-term memory loss, and reduced processing speed, although other neurological and medical tests are normal (66). Moreover, elevated WMHs and CMBs were associated with gait disturbance, i.e., reduction in gait velocity, and stride strength (67, 68), higher urinary syndrome, or disturbance including urinary urgency, nocturia, and incontinence (67, 69). A significantly increased risk toward all subtypes of ischemic stroke (70) and neuropsychiatric syndromes (e.g., depression, anxiety, parkinsonism, mood disturbances, reduced processing speed, and sleep disturbance) also had been linked with the presence of WMHs, CMBs, and enlarged PVS (6, 66, 71, 72). Lacunar stroke had been reported as the outcome of small vessel occlusion-mediated lacunar infarcts (73). Moreover, many individuals with CSVD have been reported to have the occurrence of silent brain infarcts, a consequence of a lacunar stroke in a non-vulnerable brain region with unapparent clinical symptoms. Moreover, acute RSBI may cause secondary effects such as remote cortical thinning due to progressive degeneration of connecting white matter tracts (73). Alarmingly, CSVD manifestation can often be occult in nature and produce no clinical symptom (asymptomatic), hence referred to as "silent" brain infarcts.

Taken together, several cardiocerebrovascular risk factors such as T2DM, metabolic syndrome (i.e., hypertension, obesity), aging, and lifestyle (i.e., smoking and unhealthy diet) have

been correlated with and increased the risk toward onset and progression of CSVD. Hence, tackling these risk factors may be beneficial in the therapeutic and preventive measures to regulate the onset and progression of CSVD, ideally from early or young age.

CSVD as a Spectrum of Dynamic Microvascular Pathomechanism

Relatively small vessels/microvessels served an essential role as part of the neurovascular unit or the blood–brain barrier (BBB) in the central nervous system (CNS). To date, various and intensive investigations have been carried out to study the mechanism of interaction between cerebral parenchyma and its surrounding microvasculature (74). However, it is well-accepted that neurovascular unit or BBB owns the prior role in brain health and plasticity (capacity to recover) from insults that may initiate the pathologic cascade toward NDD. Two classical clinicopathologic representations of CSVD have been suggested: arteriolosclerosis or lipohyalinosis (thickening and/or damage the wall of arterioles), and occlusion of cerebral penetrating arteries (75). However, it is now recognized that most of the macrostructural manifestations in CSVD are reflections of the probable underlying of mesostructural responses such as cerebral microcirculation flow obstruction (intrinsic or extrinsic). For instance, the arteriolar occlusion or narrowing resulted in ischemia as seen in small lacunar infarct in the classical CSVD clinical spectrum.

Various physiopathologic changes (i.e., the mesostructural responses) of CSVD not only give rise to cerebral parenchyma damage (i.e., axonal injury, neuronal apoptosis, demyelination, and oligodendrocyte damage), but also to neurological symptoms, clinical signs, and multifaceted neuroimaging findings (76). Nonetheless, the underlying pathomechanism of CSVD remains contentious despite the growing insights from histopathological, epidemiological, physiological, and imaging studies. Insights on the current pathomechanism CSVD can be viewed from molecular and cellular consequences of several systemic dysregulations, which include coagulopathy, elevated microthrombosis, genetic mutation, increased cellular activation, inflammation, and oxidative stress, all of which contribute toward the corresponding cerebral microstructural changes such as endothelial dysfunction, altered cBF, and breakdown of BBB. **Figure 3** summarizes the current pathomechanism of CSVD through coagulation, cell activation, endothelial dysfunction, and inflammation. **Figure 3** also emphasizes on the proposed overlapping and multifaceted risk factors that may contribute to the detrimental macrostructural CSVD manifestations, with a specific highlight on the dietary patterns and MP formation as further elaborated in this review.

Coagulation and Microthrombogenesis

In general, the coagulation process or pathway serves to maintain hemostasis or to control bleeding, promote healing, and prevent spontaneous bleed (77). The coagulation pathway is controlled by certain naturally occurring inhibitory elements or anticoagulants such as protein S, protein C, antithrombin, and tissue factor pathway inhibitor (TFPI) that control and limit the formation of clot to prevent propagation of thrombus/microthrombus or further thrombosis/microthrombosis (77). Altered procoagulant properties of such coagulation factors would stir imbalance in the pathway, either with increased or decreased activities of a given factor (78). Generally, the thrombogenic elements of coagulation factors are produced from two sites: the vessel wall [i.e., tissue factor (TF), exposed endothelium, and collagen] and the circulating elements [i.e., platelets, platelet activating factor, prothrombin (factor II), fibrinogen (factor I), von Willebrand factor (vWF), and numerous clotting factors]. Certain events such as physiological disturbance, blood abnormalities, infection, elevated proinflammatory cytokines activities, and disturbance in the primary hemostasis (i.e., platelet plug formation at the insulted site of exposed ECs of the vessel wall) would result in the imbalance of the coagulation system, hence termed as coagulopathy (79, 80).

In microcirculation, whereby the arteriosclerosis and/or arteriolosclerosis is the major culprit in CSVD, the platelets may circulate in resting state. However, upon stimulation (i.e., by ruptured arteriosclerotic plaque or embolism from larger vessel) or activation (even at early stage of disease process), platelets can aggregate by intraplaque components such as TF, collagen, and vWF, or by soluble platelet agonists or vasoactive substances [i.e., thrombin, adenosine diphosphate (ADP), serotonin, or thromboxane A_2 or B_2] that promote microthrombogenesis (81). Moreover, platelet activation and aggregation lead to further release of thrombin, hence elevating the activation of coagulation cascade and subsequent synthesis of stable cross-linked fibrin clot or mesh. The formation of fibrin has been shown to increase the coagulation activity whereby the elevated level of alternative marker for thrombin generation such as fibrinopeptide-A has been associated with cerebral infarction (82). Systemic microcirculation coagulation cascade can be activated at early disease process, and platelet activation is the main player in microthrombi formation and its plausible effect on pathogenesis of CSVD.

Small transmembrane glycoprotein or TF facilitates the microthrombosis in microcirculation. In coagulation systems, the extrinsic pathway or the TF pathway is activated once ECs released the TF following damage to the vessel. The TF hence activates thrombogenic element factor VII into factor VIIa that will activate factor X into Xa, resulting in fibrin synthesis. TFPI can interfere and inhibit this pathway. Moreover, TFs are secluded in arteriosclerotic particulates, hence allowing the exposure of TF in microcirculation, leading to formation of microthrombus. Alongside TF, the exposed collagen also facilitates the microthrombosis through glycoprotein (GP-Ia/IIa)–mediated platelets–ECs adhesion, hence activating factor X into Xa leading to microthrombosis and fibrinogenesis (83). Thereby, the balance between prothrombotic factors and endogenous fibrinolysis determines whether the microthrombus progresses into larger thrombus, propagates, or dissolutes (84). Another important component that activates and enhances the contact and prothrombotic pathway, respectively, is the cell-free DNA and histone neutrophil extracellular traps with exposed TFs that present and propagate as part of the intravascular thrombi, hence triggering the generation of

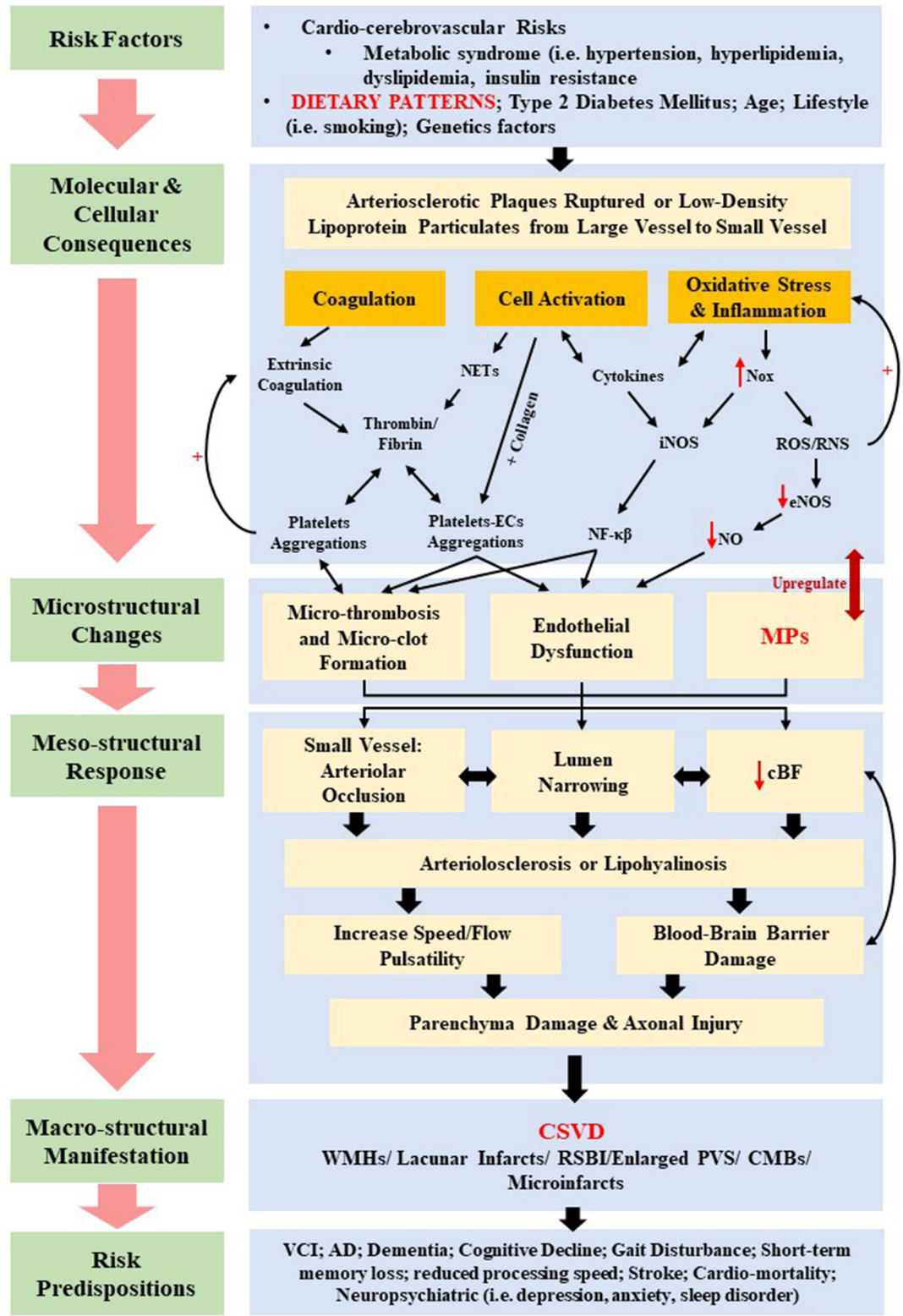

FIGURE 3 | Summary of the proposed overlapping pathomechanisms of cerebral small vessel disease (CSVD) through coagulation, cell activation, endothelial dysfunction, and inflammation. cBF, cerebral blood flow; CMBs, cerebral microbleeds; ECs, endothelial cells; eNOS, uncoupled endothelial nitric oxide; iNOS, inducible nitric oxide synthase; MPs, microparticles; NETs, neutrophil extracellular traps; NF-κβ, nuclear factor κβ; NO, nitric oxide; Nox, nitric oxide synthase oxidase; ROS, reactive oxygen species; RNS, reactive nitrogen species; RSBI, recent subcortical brain infarcts; PVS, periventricular spaces; WMHs, white matter hyperintensities.

thrombin (85, 86). Collectively, platelets and/or neutrophils activation and aggregation could give rise to generation of intra-arterial thrombus or microthrombus and form the basis for arteriomicrothrombotic disease such as CSVD.

In the case of CSVD, activated platelets and microthrombi formation initiate the narrowing of the arterial wall, as well as upregulating the proliferative arterial wall changes (87). Meanwhile platelet aggregation possibly releases the vasoactive substance, resulting in SMC constrictions, hence narrowing the arterial wall (88). Moreover, microthrombi consist of white thrombi of aggregated fibrin, and platelets have been observed to strengthen its association with intraparenchymal small vessel microclot or microthrombosis seen in cerebral ischemia or infarcts (89, 90). Microthrombosis-mediated cerebral microcirculatory dysfunction has been suggested as an outcome of intraparenchymal small vessel dilation to compensate the reduction in perfusion from peripheral pressure of larger arteries. This happened as small vessels trying to optimize the dilation process to maintain the cBF following the arterial lumen narrowing (82).

Moreover, increasing evidences have shown that reduced ability of small vessel to self-regulate cBF (due to aging and the presence of chronic hypertension) is subjected to various systemic blood pressure levels and increased arterial stiffness that would cause an increased speed and flow pulsatility in cerebral arteries and arterioles (91). In addition, the regulation of cBF is also mediated by nitric oxide (NO) signaling, whereby reduced NO is a marker for endothelial dysfunction and altered cBF (92). Thus, these hemodynamic changes may lead to microstructural and mesostructural changes and response, respectively, such as endothelial damage in the BBB and alter its permeability through an increase of the shear stress (93), which will be discussed in the foregoing section. Hence, the BBB breakdown is thought to be another pathogenesis feature of CSVD (93, 94), as hinted in **Figure 3**.

Circulating Cell Activation and Endothelial Dysfunction

As discussed, the cardiocerebrovascular and cardiometabolic risk factors such as T2DM and metabolic syndrome {i.e., dietary patterns, hypertension, abdominal obesity, dyslipidemia [elevated low-density lipoprotein (LDL) and triglycerides and reduced high-density lipoprotein]} had major global impact on development of arteriosclerosis and/or arteriolosclerosis disease, resulting in coronary heart disease and cerebral ischemia (95). Thereby, cellular activation and endothelial dysfunction have been described as the major implication of these risk factors.

It is known for larger vessel circulation that LDL can dissociate into smaller particulates or particles, hence embolizing to smaller vessel microcirculation, which is termed LDL modification (81). Therefore, the infiltration of these smaller particles causes the endothelial dysfunction in large or small vessel. This endothelial dysfunction is followed by EC activations that elevate the subsequent release of proinflammatory cytokines to potentiate host of leukocytes recruitment (i.e., monocyte, T lymphocytes, and macrophages) on the endothelium that further promotes the formation and stability of microthrombus (96). Moreover, monocyte can differentiate into macrophages, which aided in the mechanism of lipid uptake from the circulation. As the endothelial dysfunction ensued, the proinflammatory cytokines may further activate the ECs, hence increasing the expression of adhesion molecules such as VCAM-1, ICAM-1, and even EC-derived MPs (EDMPs) subpopulation such as cluster differentiation 62 (CD62E) or E-selectin. The adhesion process eventually acts on and weakens the ECs and its barriers that line the microvessels lumen. These activated cells distort the functions of EC barriers through the alteration of junctional protein of ECs cytoskeleton or along the width of intercellular junction (81).

Apart from leukocytes, platelet activation also largely contributes to the formation of microthrombus in arteriosclerosis and/or arteriolosclerosis. In response to inflammatory signal, damaged endothelium released the vWF, hence increasing the capacity of platelet activation and binding to vWF. Ensuing platelet activation is the releasing of platelet-derived MPs (PDMPs) CD40, and CD62P (or P-selectin) that bring surface adhesion molecules provoking the platelets and activated platelets by-product aggregation with leukocytes, hence adherence to endothelium promoting microthrombosis and arteriosclerosis (97). Moreover, activated platelets also elevate the synthesis of soluble vasospastic substance such as thromboxane A_2 or B_2 and ADP; the synthesis is possible after platelet binding with plasma fibrinogen. These substances elicit the platelets and platelets–monocytes aggregations from inside of arterioles vessel and have been used as markers for onset and progression of arteriosclerosis and/or arteriolosclerosis (82, 98). In addition, the ruptured arteriosclerotic plaques from larger vessel also may embolize and contribute to the instability of the aggregates and microthrombus and upregulate the small vessel systemic inflammation mediated by leukocytes and platelets (99). Aside from cellular activation, endothelial dysfunction can be initiated through the disturbance in the function of microvessel itself as a result of systemic or mechanical stress, leading to microthrombosis. For example, increase in P-selectin and NO in arteriolar endothelium has been associated with microthrombosis (100). Preclinical study had shown that the constriction of arteriolar lumen is due to microthrombosis whereby the intensity of the microthrombosis determined the level of constriction (100). Moreover, the damage in the function of arterioles can lead to local microthrombus formation.

Therefore, circulating cell activation and endothelial dysfunction have long been thought to be the main factors that contribute to the pathogenesis of CSVD. Several studies have shown elevated biomarkers of endothelial dysfunction related to CSVD such as reduced production of NO, resulting in arteriolar constriction (101, 102). Other known manifestations of endothelial dysfunction are hypoperfusion or reduced cBF (103) and increase BBB breakdown or permeability (104) (**Figure 3**).

Oxidative Stress and Inflammation

The risk factors and causes of oxidative stress and arteriosclerosis and/or arteriolosclerosis in the pathomechanism of CSVD are topics with active investigations. In addition, certain health conditions, diet, and lifestyles may contribute to the development and progression of arteriosclerotic and/or

arteriolosclerotic CSVD such as dyslipidemia, T2DM, aging, and unhealthy lifestyle (i.e., unhealthy diet, smoking, and sedentary living). Moreover, several studies had shown the association of detrimental effects of oxidative stress [i.e., through nicotinamide adenine dinucleotide phosphate (NADPH) on the endothelium-dependent NO signaling] toward pathogenesis of CSVD (105, 106).

As discussed, the inflammation and oxidative stress may result from increased inflammatory response from the endothelium (i.e., endothelial dysfunction) and cellular activation. Hence, oxidative stress has been associated with the pathogenesis of CSVD as in arteriosclerosis (107). Microthrombus and/or LDL particle aggregates on the small vessel endothelium are susceptible to oxidative and enzymatic modifications by reactive oxygen species (ROS) [i.e., superoxide ($O_2 \cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH)] and proinflammatory cells (95). ROS also induced the imbalance between antioxidants (i.e., EC-derived glutathione peroxidase, catalase, and superoxide dismutase) and pro-oxidants in age-related NDD, whereby the oxidative stress occurs due to NADPH oxidases (Nox)-mediated pro-oxidants overproduction and altered activity of antioxidants enzymes (108). Apart from ROS, the reactive nitrogen species (RNS) also contribute to cerebral vascular oxidative stress, as both ROS and RNS are mainly synthesized by mitochondria activity and certain pathways including NO synthase (NOS) and oxidase enzyme [i.e., NOS oxidase (Nox), uncoupled endothelial NOS (eNOS), cyclooxygenase (COX), lipoxygenase, xanthine oxidase, myeloperoxidase]. However, eNOS is essential in production of endothelium NO, hence also contributing to beneficial or protective role in the regulation of vascular tone, unlike eNOS dysfunction that results in the release of superoxide from ECs (107).

Furthermore, ROS elevate the inflammatory response that influences the progression of clots or thrombus, increase proinflammatory cytokines [i.e., interleukins (IL-6 and IL-8), tumor necrosis factor α (TNF-α), and monocyte chemoattractant protein 1 (MCP-1)] and endothelial function, and increase expression of vascular adhesion molecules (i.e., ICAM-1 and VCAM-1) (109). Subsequently, elevated level of RNS and ROS has been associated with oxidative stress–mediated cell migration and proliferation, DNA damage, necrosis and apoptosis, cellular autophagy, endothelial dysfunction, elevated level of oxidized LDL, and endoplasmic reticulum stress (110). Following overproduction of proinflammatory cytokines and inducible NOS (iNOS) is the activation of transcription factors [i.e., nuclear factor κβ (NF-κβ) and/or nuclear factor (erythroid-derived 2)-like 2 (Nrf2)] and signal transduction cascades (111) that further stimulate the release of cytokines and chemokines, hence increasing inflammation (112). However, NO is able to inhibit the expression of NF-κβ and adhesion molecules; hence, NO serves as crucial anti-inflammatory, antithrombotic, antihypertensive, and antiplatelet aggregation and important for vascular vasolidation (95). Apart from that, NO serves as a modulatory agent for the function of EC barriers whereby, NO modulates the activity of Rho-kinase in cerebral microvasculature and is associated with increase inhibition of NOS (113). Under pathological condition, reduced NO initiates the vicious cycle of reduced NOS to increase the Rho-kinase activation and *vice versa* (114). Hence, maintaining the adequate level of NO is crucial to reduce NO by eNOS to prevent endothelial dysfunction (i.e., elevate the EC monolayers permeability as a response following disruptions of adherent junction and stress fiber formation), whereas overproduction of NO by iNOS leads to an increased expression of proinflammatory factors (115).

Additionally, ROS may act on the ECs inducing the disruption of interendothelial junction, gap formation, actomyosin contraction, and altered phosphorylation or expression of junctional adhesion molecules (115, 116). Furthermore, released cytokines induce inflammation of ECs through extracellular matrix degradation followed by BBB breakdown (104). In addition to the endothelium, there exists cross-talk among cellular components of the BBB, such as pericytes, astrocytes, and oligodendrocyte precursor cells (OPCs) that are implicated in the microvascular damage as precursors for the onset and progression of CSVD (117, 118). In relation to this, reduced white matter integrity due to changes in oligodendrocytes has been shown in CSVD, whereby the ECs–OPCs signaling became compromised and altered the ECs' ability to secrete the releasing factor crucial for the growth and survival of OPCs that eventually caused oligodendrocytes prone to damage (119). An increased BBB damage and permeability further induced the degradation of basement membrane of ECs and accumulation of extracellular matrix components leading to stiffening of vessel wall (120). Moreover, BBB breakdown will intensify with the accompanying increased in the deposition of blood component such as platelets, MPs, and fibrin. Several studies showed that changes in walls of small vessels in the brain due to BBB breakdown lead to ischemic events, classified as WMH, lacunar infarcts, and CMB manifestation of CSVD (7, 93, 94) (**Figure 3**).

Therefore, the interactions of multiple BBB components are likely to play a crucial role in the discovery and development of new prevention steps and therapies for CSVD. Thus, endothelial dysfunction, BBB breakdown, altered cBF, and impaired cerebral autoregulation due to disturb coagulation system, cellular activation, oxidative stress, inflammation, and microthrombosis are thought to be the major players to the development and progression of CSVD, although another or other potential player(s) is still being sought. One such player is cellular-derived circulating MPs.

MICROPARTICLES—FROM PERIPHERAL TO CENTRAL

There has been growing recent interest in the identification and quantification of cellular debris such as MPs as biomarkers for their potential to inform the natural history of development and progression of several diseases including cardiocerebrovascular disease, GIT disease, cancer, metabolic disease, and sepsis. Flow cytometry (FC) is the most widely method to measure MPs and has major advantages over the other techniques in that each MP (and its subpopulations) is quantified individually based on

their antigen expressions (121). However, to date, there remains lack of consensus on such standardization between centers, in measuring MPs using FC due to complex and multifaceted nature of MPs. The development of standardized MPs technologies would permit a direct comparison of results between studies and would lead to a greater understanding of MPs in health and diseases.

Besides FC, other MPs assays include single-particle assays and bulk assays (**Table 1**). Single-particle assays include atomic force microscopy (122) and high sensitivity microscopy (123). These two procedures can be used for an accurate determination of MP size and shape but cannot be used for routine analysis of clinical samples as it can be rather costly to run and maintain (122). In contrast, bulk assays include immunoassays, functional assays, and hybrid assays that detect antigens expressed on MPs (124), PS/TF dependent procoagulant activity (125), and prothrombinase activity (126), respectively. However, bulk assays do not provide size information or single-particle counts (121). Other available MP analysis techniques, although much less popular, include dynamic light scattering (127), high-performance liquid chromatography (128), capillary electrophoresis (129), and mass spectrometry (130). Overall, FC has major advantages over the other techniques in that each MP is interrogated individually and allows for the identifications and quantification of MP subpopulation based on antigen expressions (as summarized in **Table 1**).

MPs—Definition, Formation, and Compositions

MPs represent one of the types and classifications of microvesicles—with an anucleated phospholipid bilayer. Apart from MPs, other classes of microvesicles include exosome and ectosome, which can be distinguished based on their size, composition, and origin. For instance, exosome is considered as the smallest microvesicles with the size ranging from 30 to 100 nm, whereas apoptotic bodies or large membrane blebs range from more than $\leq$5 μm in diameter (131, 132). However, this review focuses on MPs or ectosomes that are anucleate, small, and membrane-enclosed extracellular particles (133–136). Ranging from 0.1 to 1 μm in diameter, MPs are derived from direct deformation of cell plasma membrane and cell membrane phospholipid exocytic blebs that are released from the cell surface by proteolytic breakdown of the cytoskeleton, triggered by various mechanisms such as cellular activation, oxidative stress, inflammation, injury, or apoptosis. In this context, factors such as different agonists, thrombin, serine proteases, collagen, proinflammatory cytokines, and physiological shear stress, which are known to contribute to cellular activation, would further promote the secretion and aggregation of MPs (135, 137–139). In contrast, during apoptosis, the apoptosis-induced MP release is stimulated by the caspase-mediated Rho effector protein and the Rho-associated coiled-coil containing protein kinase 1 (ROCK 1), as well as by thrombin and TNF-α (140). **Figure 4** illustrates the general mechanism of MP formation and its mode of action, while it also introduces the proposed possible impacts of diets on MPs that could be linked with CSVD (as previously hinted in **Figure 3**). A converging proposed plausible link between diets, circulating MPs and CSVD manifestation is further delineated in *Diets and Circulating MPs—Proposing the Link With CSVD*.

MPs are heterogeneous and can be produced from multiple sources (or parental cells) within blood circulation, i.e., from platelets, erythrocytes (or RBCs), leukocytes (white blood cells), monocytes, ECs, and SMCs (141). Also, MPs can be present in various body fluids such as saliva, urine, bile, cerebrospinal fluid, and synovial fluid (142). MPs are identified by the presence of cell surface marker phosphatidylserine positive (PS^+), although PS negative (PS^-) is recently recognized (143). Moreover, in the blood circulation of healthy individuals, MPs are present in low level, with 70–90% of MPs represented by PDMPs (144). MPs are composed mainly of cytosol and enclosed by globose phospholipids bilayer, whereby their cytosol may include RNAs [i.e., non-coding small interfering ribonucleic acid, messenger RNA (mRNA), and micro-RNA (miRNAs)] (145, 146), enzymes, and cytoskeletal proteins of their parental cells, but are anucleate and lack synthetic capacity. However, to date, there is no evidence of DNA presence in MPs luminal space, although a trace of DNA had been found in exosomes and apoptotic bodies (147).

Given that MPs carry their own parental membrane proteins or markers, these are used to identify their cell of origin or subpopulations. For examples, cluster differentiation 41 (CD41) is to identify PDMPs, CD235/CD235a for RBCsderived MPs (RDMPs), CD31/CD146 for EDMPs, and CD45 for leukocyte-derived MPs (LDMPs) (148). Interestingly, PDMPs bring more than 40 membrane integral protein or glycoprotein characteristic of platelets, such as integrin β1 (CD29), αIIbβ3 (CD41), and P-selectin (CD62P). PDMPs and EDMPs also bring proinvasive or proinflammatory matrix metalloproteinase proteins (MMPs-2/9). Most of these proteins serve as adhesion molecules that stimulate the EVs internalization by these cells (144). Meanwhile, RDMPs are the smallest (∼0.15 μm) compared to other cell-derived MPs, whereby their surface consists of residual hemoglobin (20% from parent RBCs) (149, 150) (see **Table 2** for details).

In addition, previous studies reported that MPs consisted of identical lipid composition as plasma membrane. However, MPs may have augmented cholesterol or specific enrichment, sphingomyelin, or ceramide, which implies that MPs can be produced or shed from certain region of cellular plasma membrane, cell of origin, and/or pathophysiological properties (149). As aforementioned, majority (if not all) of MPs expose PS^+ at their outer membrane surface; hence, PS has been used as standard marker of MPs identification (149).

Notable Roles of MPs in Health

Recent evidence has shown that MPs extend some protective effects in health as part of maintaining the hemostasis. Hence, several subpopulations of MPs could also potentially play a role in mitigating the inflammatory effects. For example, EDMPs contain anticoagulant properties at their surface, which is important to bring balance in hemostasis by counterbalance the thrombosis driven by procoagulant MPs (151). Besides, an *in vitro* study has shown that EDMPs are crucial for maintaining

TABLE 1 | Profiles of multiple techniques for detection and characterization of MPs.

Technique	Quantification (bulk quantification)	Enumeration (single-particle counting)	Origin	Specificity	Sizing	Cost/complexity of instrumentation	Practicability
FC	++	+++	+++	++	+	−	++
Immunoassays	+++	−	+	+	−	+	+++
Functional assays	+++	−	+	+	−	+	+++
EM	−	+	+	+++	++	−	−
DLS	++	−	−	−	++	+	+
RICM	−	+	+	+	++	−	−
AFM	−	++	+	++	+++	−	−

Number of "+" represents the strength of the techniques, whereas "–" represents the weakness of the techniques. AFM, atomic force microscopy; DLS, dynamic light scattering; EM, electron microscopy; FC, flow cytometry; RICM, reflection interference contrast microscopy.

the integrity of vascular wall through the activation of vascular repair (134).

Moreover, in coagulation system (i.e., in common pathway), the activated protein C is able to induce the synthesis and release of EC–protein C receptor–derived MPs, whereby these MPs bring functional and actively bound protein C aiding in the inhibition of factor Va/VIIIa in the common pathway of coagulation cascade (152). Apart from that, certain subpopulations of MPs also possess anti-inflammatory properties; for example, monocyte-derived MPs (MDMPs) are known to influence the activity of macrophages and monocytes by enhancing the expression of peroxisome proliferator-activated receptor γ (PPAR-γ) protein (153). Furthermore, LDMPs also have been shown to possess an anti-inflammatory property, whereby they potentially aided in the downregulation of proinflammatory mechanism in coagulation cascade at an early stage of inflammation (154). Besides, LDMPs are also able to inhibit macrophages activation through the activation of anti-inflammatory macrophage response, i.e., the inhibition of cytokines (such as IL-8), inhibition of TNF-α, and releasing transforming growth factor β1 (154). Interestingly, low level of EDMPs was also found to correlate with thrombin and anticoagulant markers in healthy individuals, raising EDMPs' role in the inhibition of thrombosis (155).

MP Roles in Coagulation and Microthrombosis-Linking CSVD Correlates

Much of the MPs procoagulant and prothrombotic properties are due to their ability to bind to sub-endothelial matrix (and its components), adhesion with soluble and non-mobile fibrinogen, and coaggregation with platelet aided by a complex and dependent process involving GP-IIb/IIIa (156). As mentioned, PS presence on MPs surface acts as coagulation factors for assembly and binding agent or proteins in coagulation cascade that may lead to a prothrombotic state (137). PS binds to hematopoietic-derived clotting factors through electrostatic interactions between phosphate groups in phospholipids and Ca^{2+} in γ-carboxyglutamic (GLA) domain of clotting factors (157). Factors VII, IX, X, and prothrombin are the clotting factors that contain GLA domain. Therefore, the recruitment of PS bearing MPs and clotting factors aided the aggregation of platelet and synthesis of fibrin and hence for the formation of microthrombus (158). Furthermore, *in vitro* study had shown that combined PDMPs and EDMPs at low levels can also induce the generation of microthrombus (159). Of note, compared to activated platelets (parent cells), PDMP surfaces possessed up to 100 times higher procoagulant properties and higher affinity binding sites for activated coagulation cascade (160, 161). Hence, PDMPs would serve as a precursor for microthrombus formation by providing catalytic surface for the prothrombinase enzyme complex (i.e., involving factors IXa, Va, VIII, and Xa) (158).

Moreover, MPs also bring surface TF, where, for example, MDMPs have been reported to bring active TFs that potentially elevated the extrinsic pathway involving factors VII, VIIa, IX, and X in coagulation cascade (162, 163). In addition, LDMPs expressed P-selectin glycoprotein ligand 1 and platelet P-selectin on their surfaces that lead to the aggregation of TF-bearing leukocytes at the site of vascular or microvascular injury (164). In addition, the formation of EDMPs has also been associated with elevated level of endothelial dysfunction marker such as plasminogen activator inhibitor 1 (PAI-1) and elevated the procoagulant activity and prothrombotic state. This is so because EDMPs contain the expression of ULvWF multimer that enabled EDMPs to induce strong platelet aggregations (165). Therefore, it is plausible to deduce that TF-bearing MPs play an important part in macrothrombus and microthrombus formation. In fact, a study had shown that tumor cell–derived MPs bearing both PS^{+} and TF can be utilized as a biomarker for risk of venous thrombosis in cancer patients (139) (**Figure 4**).

Thus, in relation to CSVD clinical manifestations, numerous reports linking MP subpopulations as CSVD correlates may well reflect the fact that PS-bearing MPs and clotting factors aided the aggregation of platelet and synthesis of fibrin, which lead to the plausible microthrombus involvement in CSVD pathomechanism (see **Table 3** for MPs and CSVD correlates).

MPs and Inflammation

The release of MPs into the circulation that ensued tissue or cell inflammation can further aggravate the inflammatory activity (181). MPs can affect microcirculation by potentiating the production and expression of proinflammatory cytokines, chemokines, and ICAM-1 (182) (**Figure 4**). *In vitro* study had shown that ECs and monocytes' interaction with PDMPs able

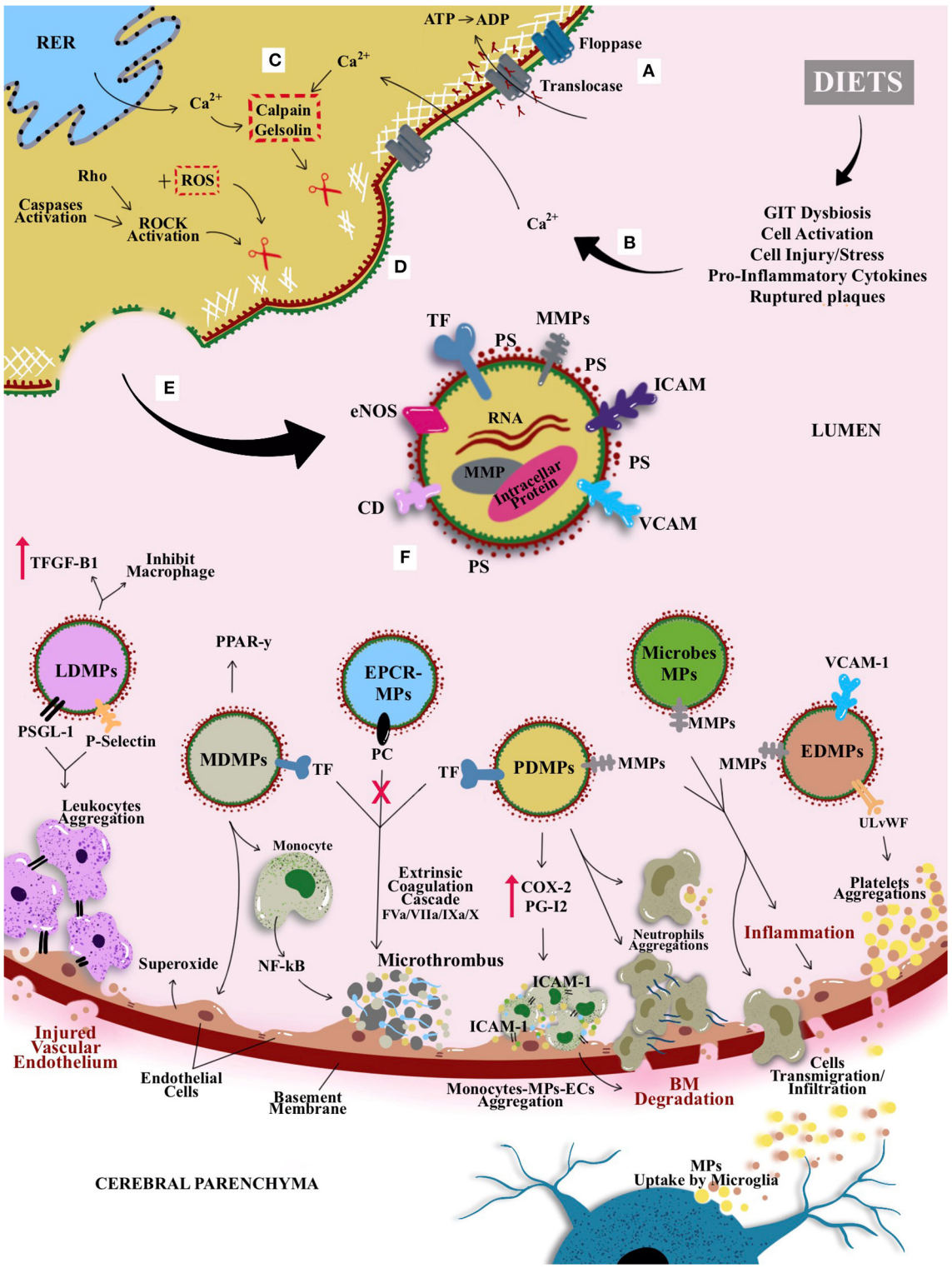

FIGURE 4 | The general mechanism of MP formation, its mode of action, and the proposed possible impacts of diets on MPs that could be linked with CSVD. **(A)** Active translocase transporting phosphatidylserine (PS) from outside to inside layer through adenosine triphosphate (ATP)–dependent manner. **(B)** Modifiable cardiocerebrovascular risk factors (with emphasis on dietary patterns in this review) are known to induce cellular activation or other cellular stressors (e.g., increased cytokines and from peripheral and GIT dysbiosis). **(C)** The activation causes an increase in intracellular cytosolic calcium release by stressed rough endoplasmic reticulum (RER) and acquired from extracellular space. Hence, activates enzymes calpain and gelsolin that cleave cell membrane cytoskeleton. **(D)** The cleaved cytoskeleton causes inactivation of translocase and hence induces phospholipid "flip-flopping." **(E)** Externalization of PS produces MPs that bring their parent surface

(Continued)

FIGURE 4 | molecules and protein antigens. **(F)** MP productions can trigger series of microthrombotic cascades that could be linked to the mechanism postulates on CVSD risk predisposition/prevention that could be modulated by dietary pattern. For example, leukocyte-derived MPs (LDMPs) expressed P-selectin glycoprotein ligand-1 (PSGL-1) and platelet P-selectin on their surfaces and hence aided the aggregation of TF-bearing leukocytes at the site of vascular or microvascular injury. Besides, LDMPs were also able to inhibit macrophages activation and releasing transforming growth factor β1 (TGF-β1). Monocyte-derived MPs (MDMPs) are known to influence the activity of macrophages and monocytes by enhancing the expression of peroxisome proliferator-activated receptor γ (PPAR-γ) protein. However, MDMPs also upregulated superoxide anion production on endothelial cells (ECs) and activation of nuclear factor κβ (NF-κβ) in monocytes that enhance microthrombosis. Most of the MPs, especially platelet-derived MPs (PDMPs) serve as precursor for microthrombus formation by providing catalytic surface for the prothrombinase enzyme complex (i.e., involving factors IXa/Va/VIII/Xa). PDMPs elicit the *de novo* expression and production of inflammatory molecule or agent such as cyclooxygenase (COX-2) and prostacyclin (PG12) that enable the monocytes-MPs-ECs aggregations through intracellular adhesion molecules (ICAM-1) to further elevate the basement membrane (BM) degradation and formation of microclot. Once PDMPs had a close contact with neutrophil, it can bind and increase neutrophil aggregations and elevate neutrophil phagocytic activity. This is followed by an activation of ECs or GIT dysbiosis, as they released endothelial-derived MPs (EDMPs) and bacterial or microbiota-derived MPs that express proteases proteins such as MMP-9 and MMP-2 to enable the invasion toward vasculature through disruption of BM. Disrupted BM enables cellular or molecules transmigration or infiltration; for example, MPs bridging through BBB, may undergo reuptake by microglia from cerebral parenchyma. Alongside proinvasive MMP-9, EDMPs bring ultralarge von Willebrand factor (ULvWF) monomers that upregulate the platelet aggregations to ECs and hence activate the ECs and endothelium dysfunction. Moreover, activated protein C induced the synthesis and release of ECs–protein C receptor (EPCR)–derived MPs that bring functional and actively bound protein C to aid the inhibition of factor Va and factor VIIIa in the common pathway of coagulation cascade leading to thrombogenesis.

TABLE 2 | Microparticles (MPs) subpopulation and their surface markers.

Parental cells	MPs	Surface markers/cluster differentiation (CD)
Platelets	Platelet-derived microparticles (PDMPs)	• CD62P or P-selectins (maker for platelet activation) • CD154 04 CD40L (maker for platelet activation) • CD42b (glycoprotein Ib) • CD42a (glycoprotein IX) • CD41/CD41a and CD63 • CD29 (integrin β1)
Endothelial cells (ECs)	Endothelial-derived microparticles (EDMPs)	• CD31/CD146/CD144/CD105 (maker for apoptotic-derived EDMPs) • CD54/CD106 (markers for EC activation) • CD62E or E-selectins and CD106 (marker for cellular inflammation) • EDMPs markers also expressed on other cell types, such as CD146 (expressed on pericytes and tumor cells), CD54 (expressed on leukocytes), CD105 (expressed on activated monocytes), and CD31 (expressed on activated platelets)
Leukocytes	Leukocytes-derived microparticles (LDMPs)	• CD45 (mostly all LDMPs) • CD14 (monocytes derived, MDMPs) • CD4 (lymphocytes) • CD15 (granulocytes)
Erythrocytes [red blood cell (RBC)]	Red blood cell–derived microparticles (RDMPs)	• CD47 • CD235/CD235a

CD, cluster differentiation; ECs, endothelial cells; MPs, microparticles.

to elicit the *de novo* expression and production of inflammatory molecule or agent such as COX-2 and prostacyclin (PG12), respectively (183). Another *in vitro* study had shown that EDMPs upregulated E-selectin, ICAM-1, and VCAM-1 and induced the expression and release of proinflammatory cytokines (i.e., IL-6 and IL-8) (184).

Furthermore, within the CNS, microglia are the innate immune cells with diverse roles and functions at their quiescent surveillance, as well as activated states (185–187). However, the traditional classification of M1-proinflammatory/M2–anti-inflammatory microglial phenotypes has been challenged with the emerging evidence, indicating a wide spectrum of microglial activation (188, 189). Microglial function and dysfunction have been indicated in aging and NDD such as AD (188, 190), PD (191), and stroke (192). Three types of microglia and CNS macrophages located around cerebral small vessels have been identified: (i) parenchymal microglia (distal to small vessels); (ii) vessel-associated microglia, which are parenchymal microglia proximal to cerebral vessels; and (iii) perivascular macrophages, which are located in perivascular spaces (193). Microglial activation was found to be associated with BBB leakages and cognitive impairment in angiotensin II–induced hypertensive mouse model (194), and subsequent study showed that inhibition of microglial activation reversed short-term memory impairment in mice (195). Distinct populations of extracellular vesicles have been identified in activated BV2 microglial cells in response to lipopolysaccharide challenge (196). Activated microglia release MPs carrying IL-1β, and these microglia-derived MPs enhanced inflammatory response by transferring inflammatory stimuli to other microglia (197–199). A study by Schindler et al. (200) using cultured human mononuclear phagocytes demonstrated that microglia-derived MPs induced

TABLE 3 | Microparticles (MPs) subpopulation, their surface markers and CSVD correlates.

Microparticles (MPs)	Changes in MP level	CSVD correlates
Platelet-derived microparticles (PDMPs)	• Increase $CD42^+$, $CD61^+$, $CD62P^+$, and CD42a	• 110 patients (mean age, 71.1 ± 7.9 years) with acute-phase cerebral infarction, 34 with small vessel occlusion (166)
	Increase $CD41^+$ and $CD41^+/A^+$	• Cerebral infarction attributable to vasospasm in 20 elderly subjects (mean age, 52.2 years), suggesting the consequences of microthrombosis (167) • 40 middle-age subjects (mean age, 44.4 ± 12.2 years) with metabolic syndromes (168)
	• Increase level of CD40L and soluble P-selectin	• Silent brain infarct in subcortical white matter in 15 male healthy obese subjects and 50 male obstructive sleep apnea subjects (more prevalent) (169)
	• Increase $CD41^+$	• Middle cerebral artery occlusion in a rat model with cerebral infarction (170)
	• Increase total PDMPs	• In individuals with micro-embolic cerebral ischemia and associated with recent cerebrovascular events as seen in DWI (171)
Leukocytes-derived microparticles (LDMPs)	• Increase CD14	• Related to higher WMHs and the progression of brain atrophy in individuals ($n = 534$, 4 years' follow-up) with vascular disease manifestation (172)
	• Increase $CD45^+$ and $CD45^+/A^+$	• An increased risk of arteriothrombotic stroke with individuals with obstructive sleep apnea (173, 174) • Cerebral infarction attributable to vasospasm in 20 elderly subjects (mean age, 52.2 years), suggesting the consequences of microthrombosis (167).
	• Increase $CD4^+/TF^+$	• In individuals with cardiometabolic risk factors such as T2DM and dyslipidemia (175)
	• Increase $CD45^+$, $CD14^+$, $CD4^+$ and $CD15^+$	• 76 elderly individuals with ischemic cerebrovascular diseases (176)
Endothelial-derived microparticles (EDMPs)	• Increase $CD105^+/PS^+$, $CD54^+$, and $CD144^+$	• 41 elderly individuals with mild, moderate to severe ischemic stroke (177)
	• Increase level of $CD144^+$, $CD31^+$ and CD62E	• 129 elderly individuals [68 with acute ischemic stroke (mean age, 63.59 ± 13.33)] (178)
	• Elevated $CD31^+/A^+$ and lower $CD62E^+$	• 101 middle-age individuals with metabolic syndrome (with and without chronic heart failure), suggesting the relevance to neurohumoral and inflammatory activation (133)
	• Increase EDMP bearing VCAM-1 and soluble P-selectin	• 18 individuals with subcortical and periventricular subcortical lesion (179)
Red Blood Cells-derived microparticles (RDMPs)	• Increase $CD235^+$ and $CD235^+/A^+$	• Cerebral infarction attributable to vasospasm in 20 elderly subjects (mean age, 52.2 years), suggesting the consequences of microthrombosis (167)
	• Increase CD47* * Least data on its association with CSVD, compared to other MP subpopulations above	• Induced cerebral neuronal cell death *in vitro* (180)

CD, cluster differentiation; CSVD, cerebral small vessel disease; DWI, diffusion-weighted imaging; ECs, endothelial cells; MPs, microparticles; T2DM, type 2 diabetes mellitus; WMHs, white matter hyperintensities.

NF-κB activation, leading to the release of proinflammatory cytokines (200). The role of microglia-derived MPs was further substantiated in a study investigating neuroinflammation following brain traumatic injury whereby the MPs (identified through $P2Y12/CD45^+$) derived from neuroinflammation that developed in the brain were released into the circulation and initiated neuroinflammation in naive control animals (201). Collectively, these findings highlighted the role of MPs and microglia-mediated neuroinflammation in the CNS.

MPs and Cell Signaling

Alongside with procoagulant and proinflammatory abilities of MPs, they can also serve as mediators for cell-to-cell interactions and signal delivery between cells. As MPs bring along specific parental membrane receptors, cytosolic proteins, and RNAs, they can stimulate certain target cells to transform and communicate with microcirculation in a way programmed by these contents of MPs (202). For example, PDMPs can stimulate B cells to synthesize specific antibodies such as immunoglobulin G (IgG) by delivering CD154 IgG (203). In addition, PDMPs assisted in monocytes to EC interaction through ICAM-1 that could elevate chemotaxis of monocytoid cells (204). Furthermore, a previous study showed that once PDMPs had a close contact with neutrophil, they can bind and increase neutrophil aggregations and promote neutrophil phagocytic activity (205). Likewise, MPs can be phagocytosed by certain cancer cells (i.e., in lung cancer), hence stimulating the cell to further proliferate, inducing the expression of mRNA for the proinvasive MMP-9, and upregulating the adhesion to ECs, which activated the EC and endothelium dysfunction (206). Following the activation of ECs, they released EDMPs that express proteases proteins such as MMP-9 and MMP-2, leading to vessel invasion through the disruption of basement membrane (207) (**Figure 4**).

The Roles of MPs in GIT–Brain Axis

As discussed, although most of the organs are anatomically distinct, they shared a common systemic circulation and blood

supply mainly from abdominal aorta, which relates to the brain–heart–GIT axis. This is particularly the case given the emerging debates on the contribution of MPs through GIT-microbiota–derived MPs for GIT immune system and the connection with the heart and the brain.

Certain insult in GIT microbiota (i.e., through substance abuse or infection) has been associated with disturbed immune response and eventually GIT dysbiosis that preceded with metabolic and inflammatory disease (208, 209). Several studies suggest the involvement of systemic GIT-microbiota–derived MPs for these changes. For instance, Shen and colleagues had shown the association of *Bacteroides fragilis*–derived MPs with GIT disease (210), whereas Kang and colleagues linked saccharibacteria or TM7 (i.e., *Akkermansia muciniphila*) bacteria-derived MPs with progression of colitis (211). Therefore, it is plausible to deduce that microbiota-derived MPs may serve as the link to connect between these major organs, i.e., the brain–heart–GIT axis. Similarly, it is plausible that MPs derived from peripheral circulation would assume similar systemic circulation route to reach microcirculation network and hence contribute to the pathogenesis of SVD and NDD including CSVD.

The association or crosstalk between the system in peripheral organ, i.e., GIT microbiota and the brain, is of active research interests (212). Several studies had also described that circulating cells and/or microbiota-derived MPs generated from the peripheral system that enter the systemic circulation and assisted in crosstalk between the cerebral BBB and inflammatory pathways as a trigger for CNS insults (201, 213–215). However, despite the recognized role of peripheral MPs in pathomechanism of CNS disease, the detailed mechanism of MPs breaching the BBB remains elusive, with some insights involving proinvasive or proinflammatory MMP release, reorganization of extracellular matrix, recruitment of inflammatory cells, and regulation of epithelial barrier (216).

In addition, the interaction between the brain and the periphery is a bidirectional communication. This is supported by the evidence from the detection and enumeration of brain-derived MPs in the blood that are likely to have reached cerebral microcirculation and breached into cerebral parenchyma following uptakes by microglial cells (217, 218). For example, GIT or microbiota-derived MPs may bring proinflammatory and degradative enzymes such as MMPs, whereby this molecule enables MPs to be transmigrated into epithelial layer, be circulated in systemic circulation, and reach multiple organs including the brain. Moreover, the disrupted BBB and GIT epithelial layer enhance the inflammatory cargo deposition and cell signaling by MPs (**Figure 4**). This evidence lends support on the role of MP-mediated transport or breach through BBB as a putative insight on MP-mediated GIT-directed NDD such as CVSD.

MPs and Related Clinical Syndrome

It is well-accepted that the elevated level of MPs in blood circulation is reflective of their multifaceted roles; for example, higher level of MPs was found in hypertensive patients (219), abdominal obesity (220), myocardial infarction (221), tumor progression and metastasis (222), atherosclerosis (223), and cardiopulmonary bypass patients (160). Previous *in vitro* study had shown that elevated T lymphocytes–derived MPs induced arterial endothelial dysfunction (i.e., reduce expression of NOS) in immunocompromised states (224, 225). Moreover, another studies had shown that MPs can contribute to acute lung injury (226) and inflammatory airway disease (227); in this case, elevated level of MDMPs was enumerated to associate with upregulated proinflammatory IL-8, ICAM-1, MCP-1, superoxide anion production, and activation of NF-κβ in monocytes (153, 227). Interestingly, elevated EDMPs also had been correlated with the severity of endothelial dysfunctions in heart disease, i.e., coronary artery disease and acute coronary syndromes with worst clinical outcomes (133, 228, 229).

In the case of brain disease, MPs have been shown to contribute to both proinflammatory and anti-inflammatory responses in inflammation-mediated NDD including PD, AD, amyotrophic lateral sclerosis, and dementia (230), whereby CNS-derived MPs have been shown to circulate in peripheral circulation and hence may play a role in cerebral immune status by transferring peripheral proinflammatory molecules to CNS (218, 231, 232). Recent evidence also suggested that MP-mediated release of proinflammatory cytokines, miRNAs, and microbial by-products is associated with the onset, progression, and resolution of inflammation-based cerebral injury and NDD (233–235). Therefore, these associations make circulating MPs as pertinent and potential biomarkers of numerous disease onset and/or progression with CNS diseases (228, 236), in particular with microcirculation involvement as observed in CSVD manifestations.

DIET AS RISK FACTORS FOR MICROTHROMBOSIS AND SVD

It is well-acknowledged that healthy diet is crucial, and for it to be appealing, such a diet must be nutritious, pleasing, and indulging. As all foods contain variable degree of nutrients or additives, these food elements may be beneficial or detrimental (i.e., increase risk toward chronic disease) to our health. For the past decades, research had focused on a single nutrient consumption by the individual, i.e., protein, fat, carbohydrates, fiber, and sugar. However, as humans, we do not consume a single nutrient as such, but take food as whole. Moreover, nutrients also are associated with one another; hence, focusing on the effect of a single nutrient in food is rather incomplete. Thus, to date, growing research is now focusing on multinutrient interplay in foods and their effects on health, termed as *dietary patterns*. Dietary pattern has been described as the overall diet, type/groups of food and the nutrients therein, the combination/variety, and the quantity/frequency with which the food are habitually consumed (237, 238).

In addition, diet plays an important role in maintaining the homeostasis and hemostasis systems, whereby healthy dietary pattern has been classified as diet with lower concentrations of plasma proinflammatory markers (8). Certain modifications in the dietary pattern could potentially lead to alterations in these systems, notably in individuals who consume less

or non-nutritious or unbalanced diets, often linked to the typical Western-type diet, i.e., meat-based with elevated level of proinflammatory markers (9, 10). Modern lifestyles (with physical inactivity and smoking) and unhealthy dietary patterns are recognized modifiable risk factors for metabolic disease, coronary heart disease, and stroke (11) and likely to trigger systemic peripheral events that can influence the development (from early age) to progression (in middle age and elderly) of NDD such as CSVD. A recent systematic review has also linked unhealthy diets with neuropsychiatric disorder such as mental illness (239).

In the current globalization era, metabolic syndrome (syndrome X) (i.e., abdominal obesity, hypertension, insulin resistance, dyslipidemia, and hyperlipidemia) has become a major global health burden as a new non-communicable disease and a risk factor for cardiocerebrovascular disease. This scenario continues to coexist with the social standard of living and influences dietary pattern as a consequence from this social pressure (240). Hence, the foregoing paragraphs will discuss on the range of dietary patterns to date, with their likely effects on the onset and progression of non-communicable diseases such as CSVD.

Western Pattern Diet

The Western pattern diet (WPD) or modern dietary pattern is classified as a high intake of processed food [i.e., processed meat, red meats, prepackaged foods, and sugary desserts (candy and sweets), refined grains or carbohydrates, fried foods], conventionally raised animal products, eggs, corn (i.e., high-fructose corn syrup), potatoes, high-fat dairy products, and high-sugar drinks. All in all, these consumptions are classified as high intake of saturated and omega-6 fatty acids (SFAs) (241). Moreover, WPD is accompanied by no or low intake of omega-3 FA such as vegetables, fruits, whole grains, nut, grass-fed animal products, fish, and seeds (242). Components in WPD diet tend to be proinflammatory in nature, causing GIT dysbiosis (i.e., alteration in the diversity of GIT microbiota and reduced total bacterial load) and disrupting epithelial barrier structure and function in the GIT system (243).

Additionally, WPD has been widely associated with metabolic syndrome, arteriosclerosis and/or arteriolosclerosis, and T2DM (81, 244). Gross and colleagues reported that refined carbohydrate (i.e., in corn syrup) is associated with T2DM (245). Recent meta-analysis also concluded that higher intakes of food with refined or high-glycemic carbohydrates (seen as high-glycemic index, GI) increased the harmful effects toward T2DM (246). The risk of myocardial infarction also increases with high GI and high SFA by 33% (247). Moreover, highly refined carbohydrate with reduced fiber content found in corn starch, white rice, and white wheat flour has been associated with 55% higher prevalence of T2DM in East Asian population (248, 249). A higher incidence of hypertension and metabolic syndrome has been reported among Asian Indians with higher intakes of refined grain and increased waist circumference (250).

Furthermore, a higher intake of SFA has been associated with an increased endogenous thrombin related to metabolic syndrome (251). Alongside thrombin is the increment of vitamin K–dependent factors (i.e., factors II, VII, IX, and X) and extrinsic TF pathway in coagulation cascade with reduced TFPI, which facilitated microthrombosis formation. Apart from that, high intakes of red meat that is rich with heme iron also increased oxidative stress, epithelial proliferation, and iron-induced hypoxia signaling. Heme iron is known to increase the formation of harmful endogenous *N*-nitroso compound and heterocyclic amine content in GIT (252). Therefore, high intake of processed or unprocessed red meat is associated with higher incidence of vascular microthromboembolism, hence a higher burden of T2DM, risk of metabolic syndrome, colorectal cancer, and stroke (with an increased risk of ischemic stroke by 24%) (253, 254).

High-Fat Diets/Low-Carbohydrate Diets

High-fat (HFD) or low-carbohydrate diet (LCD) or ketogenic diet is a diet that is rich in fat contents such as SFA (i.e., myristic and palmitic acids) found in animal or tropical oils. HFD also included the low polyunsaturated FA (PUFA) such as linoleic acids (LAs) and α-linoleic acids (ALAs) and monounsaturated FA (MUFA) such as oleic acids (255). Dietary ALA and LA synthesized arachidonic acids (AAs) and docosahexaenoic acids (DHA) in the liver and brain (<1%) (256). The association between the high SFA intake and development and progression of vascular disease is complex because of modulatory effects of fat in both prevention and progression of vascular disease (81). However, habitual HFD individuals had been found to have increased WMH load (i.e., CSVD manifestation) (257). Furthermore, SFA triggers microglial activation to release proinflammatory stimuli by interacting with toll-like receptor 4 (TLR-4) (258). Activated microglia release MPs (197–199), and these microglia-derived MPs have been implicated to exert negative impact in cognition and synaptic plasticity in HFD mice (259).

In contrast, multiple studies had shown the beneficial effects of diets enriched with PUFA and/or MUFA (260, 261). In unesterified forms, AA and DHA cross the BBB through passive transports, and upon entering the brain, they regulate the neuroreceptor-coupled signaling and transcription that serve in modulating the cerebral immunity as they are the mediators for bioactive lipid (262, 263). Sun and colleagues had reported that DHA is beneficial in stroke protection, therapy, and prevention (264). This is due to fact that DHA aided in reducing the neuronal and white matter loss, reducing proinflammatory cytokines, MMP expression, and BBB damage, and regulating the activation of microglial (264). Moreover, DHA reduced platelet aggregation and lag time in healthy individuals (265), hence reducing the risk of microthrombosis. High-MUFA (i.e., oleic acids) diets helped to reduce thrombogenic factor (i.e., factor VIIa and factor VIIc) (266), whereas increased HFD (i.e., higher SFA intake) has been associated with an elevated level of proteobacteria species such as *Bilophila wadsworthia* (GIT dysbiosis), unlike high MUFA that reduced total bacteria in fecal content (267, 268).

Therefore, the interactions between dietary lipid (fats) with microbiota are crucial in the regulation of metabolic changes and systemic and peripheral inflammation. Previous studies proved that the inflammatory pathway from GIT to the brain occurred

following the changes in the GIT microbiota (269). This is made possible because SFA (i.e., palmitic acids) can activate the inflammatory response after desensitization of the GIT vagus nerve as seen in microglia-activated TLR4 in hypothalamus (270). In addition, *in vivo* and *in vitro* studies have shown that elevated expression of apoptotic genes and proinflammatory markers (i.e., TNF-α and ILs) with a reduction in brain-derived neurotrophic factor are associated with HFD (i.e., high SFA) (271, 272). Furthermore, Takechi and colleagues reported that BBB damage following high-SFA diets is attributable to elevated neuroinflammation after cerebral microvasculature leakage of peripheral proteins (273).

As mentioned, HFD implies low carbohydrate intakes and that LCD with high protein diets in mice model decreased the amount and function of circulating endothelial progenitor cells (EPCs) (274). However, if LCD (i.e., high unsaturated FA, low in fiber, vitamins, minerals, and polyphenols) is implemented with high PUFA and MUFA, this combination may turn out beneficial and cardioprotective instead. A previous study reported the reduced level of EDMPs (E-selectin), thrombomodulin, C-reactive protein (CRP), and PDMP (P-selectin) in individuals who practiced LCD (275), i.e., likely to reduce the risk toward T2DM and metabolic syndrome, two major risk factors for CSVD.

Mediterranean Diet

Mediterranean diet (MeDiet) is the type of diet that is characterized by the intake of high portion of vegetables, fruits, nuts, legumes (i.e., peas, lentils, beans, chickpeas, peanuts, and soybeans), olive oils, whole grains, and aromatic spices and moderate to high intake of marine origins (i.e., fish) and low intake of meat and sweetened products (276). MeDiet has been suggested as one of the healthiest and closest model diets toward a healthy diet (11, 277). It is associated with better control of cardiocerebrovascular risk factors such as hypertension (improve blood pressure), glucose metabolism, arrhythmic risk, metabolic syndrome (i.e., dyslipidemia), and GIT microbiota (278). The protective effects of MeDiet are attributable to its high level of PUFAs (from marine origins and plants), MUFAs, minerals, polyphenols [a dietary antioxidants from plants origins and beverages (i.e., green and black tea, coffee, and red wine)], and fiber, while low in SFA and sugar. All these components in MeDiet are associated with anti-inflammatory effects and reduced prevalence of vascular diseases (279), with underlying effects on modulating proatherogenic or arteriogenic and proinflammatory gene expression such as COX-2, MCP-1, and LDL receptor-related protein (LRP1) (280); lowering plasma level of prothrombotic coagulation and inflammation molecules such as ILs (i.e., IL-10, IL13, IL-18) and MMPs (i.e., MMP-9); and decreasing the NF-κβ activation in leukocytes (281, 282).

Marine origins such as fish in MeDiet is the major source of protein, vitamins (D, B), and long-chain omega-3 FA DHA and eicosapentaenoic acid (EPA). Individuals who consumed fish regularly had reduced risk of ischemic heart disease by 13% (283). In animal models, mice administered with fish oil diet showed reduction in platelet aggregation (284), whereas laboratory porcine fed with fish oil with PUFA showed inhibition of the synthesis of platelets thromboxane B_2, aiding in the prevention of microthrombosis (285). Vitamins such as folic acid, B_{12} and B_6 had been associated with a reduced risk of cerebrovascular disease such cerebral ischemia (286), whereas lower vitamin B_{12} intake had been associated with increased proportion of periventricular WMHs (287). Fish long-chain omega-3 PUFA helped to protect against vascular risk factors such as inflammation, endothelial dysfunction (with reduced circulating markers such as VCAM-1, E-selectin, and ICAM-1), and vascular resistance (i.e., improve flow-mediated arterial dilation) (288). DHA and EPA consumption had been reported to elevate PAI-1 in healthy individuals (289) and reduced the risk of RSBI and WMHs in older adults (290), while long-chain omega-3 PUFA supplementation in arteriothrombotic patients reduced the activation of prothrombin and increased TFPI (291, 292). Moreover, EPA and polyphenols helped to reduce the endogenous thrombin alongside TFPI and vitamin K–dependent factors (i.e., factors II, VII, IX, and X) and platelet aggregation, hence reducing thrombogenesis (251, 265). In addition, polyphenols helped to reduce leukocyte activation molecules such as NF-κβ and inflammatory adhesion molecules (293), ADP or collagen-mediated platelet aggregation and platelets–monocytes aggregation; reduce expression of P-selectin on platelets; and increase the release of platelet-derived NO (294).

Moreover, nuts had been reported to protect against the risk of hypertension (236) and T2DM (295), lowering cardiovascular risk, but surprisingly not against stroke (236, 260, 296). Nuts elevated the expression of TFPI in monocytes (280) and reduced TF-bearing PDMPs (297). A recent animal study revealed that mice with HFD supplemented with nuts (with high PUFA) showed a reduced plasma prothrombin level and expression of CD36 on atherosclerotic plaques in aortic region (298). Furthermore, legume (highly soluble fiber) consumptions also reported to reduce the risk of developing vascular disease, i.e., improve cholesterol level, lower GI, blood pressure, CRP, E-selectin, IL-6, TNF-α, VCAM-1, ICAM-1, and waist circumference and prevented T2DM (299–301). Previous study had reported that legumes possessed anti-inflammatory bioactive components such as inulin and oligofructose and modulated metabolic endotoxemia (302), whereas *in vivo* study showed that their secondary metabolites interacted with GIT microbiota to aid in modulating platelets hyperreactivity and potential thrombosis through the synthesis of trymethilamine N-oxide (303). A recent PREDIMED study had strengthened the fact that MeDiet possessed anti-inflammatory effects with reduced expressions of leukocyte adhesion molecules, VCAM-1, ICAM-1, reduced plasma levels of P- and E-selectin, proinflammatory cytokines (i.e., IL-1, IL-6, IL-8, CRP, TNF-α), MMPs, and chemokines (i.e., MCP-1, MIP-1) (304).

Dietary Approaches to Stop Hypertension Diet

Dietary Approaches to Stop Hypertension (DASH) diet is a dietary pattern that encourages reduction of sodium intake (2,300 mg or 1 teaspoon per day), SFA, red and processed meat, and sweet beverages and hence characterized as diet with high

intake of vegetables, legumes, fruits whole grains, nuts, low-fat dairy, fish, lean meats, and poultry (305). An increased sodium (i.e., table salts, salt additives) intake beyond the physiological requirement (high sodium-to-potassium ratio) has been shown to elevate blood pressure (306), raising the risk toward vascular disease and mortality (307). Moreover, recent systematic review had reported that an increased intake of dietary salts may increase the risk toward WMHs and ischemic stroke, i.e., lacunar stroke, and CMBs (308, 309). Previous studies had shown that DASH diet lowered the risk of developing and progression of metabolic syndrome up to 81% (310, 311), coronary heart disease by 20%, stroke by 29% (312), and the overall mortality (313). Moreover, DASH diet has been associated with improved endothelial function (314), body weight (315), inflammation grade (305), and GIT microbiota (316).

Multiple studies had also reported that DASH diet has high anti-inflammatory properties. In cross-sectional study of elderly individuals (aged 50–69 years) by Phillips and colleagues, DASH diet improved the measurement of adiposity (i.e., reduced BMI and reduced waist circumference) and lipoprotein and reduced proinflammatory, prothrombotic, and proatherogenic markers (i.e., IL-6, CRP, TNF-α, PAI-1, and leukocytes) (317). Another study showed improvements of obesogenic inflammatory markers such as reduced CRP, IL-6, and soluble ICAM-1 following DASH diet (318). A recent review also supported DASH diet beneficial effects in reducing the risk toward cancer such as breast and colorectal cancer (319). Collectively, many of these beneficial effects of DASH diet are attributable to its high-vegetable and high-fruit content, with a desirable risk reduction toward systemic and cardiovascular disease, including CSVD.

Gluten-Free Diet

Gluten (or *glue* in Latin) refers to a group of proteins mainly found in grains such as barley, wheat, spelt, and rye. Gluten added the sticky textures and consistency to the flour once mixed with water. Glutenin and gliadin are the major examples of gluten protein reported to cause a series of ill-health effects especially in individuals with celiac disease (CD) and gluten allergy or intolerance (320, 321). The consumption of gluten-containing diet has been linked with GIT dysbiosis and leakage and gluten-induced inflammation that can lead to pathogenesis of neurodegeneration (230, 322). Moreover, high-gluten diet also elevated the proinflammatory markers in young healthy individuals (323), and there was an increased rate of superoxide and nitrotyrosine synthesis in aortic root lesion of mice model (324). A high-gluten diet also has been linked with reduced expressions of anti-inflammatory and antidysbiotic genes such as *PPAR-γ* (in intestine, peripheral inflammation, and neuroinflammation) especially in individuals with CD. This is supported by preclinical study using macaques that shown the downregulation of *PPAR-γ*–mediated inflammation in intestines, followed by GIT dysbiosis (325).

Thus, gluten-free diet (GFD) has been suggested to restore the expression *PPAR-γ* gene in CD individuals. Moreover, *in vitro* study has reported that GFD, i.e., the consumption of foods with phytocannabinoids (low dose and naturally available), such as delta-9-tetrahydrocannabinol, aided in direct activation of *PPAR-γ* gene expression, hence inhibiting intestinal inflammation in CD (326). A recent review reported that GFD is associated with a reduced risk of endothelial dysfunction and oxidative stress especially in CD individuals (327). Furthermore, an animal study also revealed that mice with GFD had reduced proinflammatory cytokines (Il-6 and TNF-α) (328). Hence, GFD is a promising approach to prevent GIT inflammation and dysbiosis and restores the integrity of epithelial barrier, thus indirectly influencing the prevention strategy in reducing risk toward other potential cardiocerebrovascular disease such as CSVD.

Vegetarian Diets

Vegetarian diet is generally based on vegetables and fruits, and it is classified into four different styles, such as lactovegetarian (vegetarians with intake of dairy products but no eggs), ovovegetarians (intake of eggs but no dairy), ovolactovegetarians (no meat and fish, but consume both eggs and diary), and, lastly, vegan diet (absolute absence of all kind of animal-based food including seafood). Overall, vegetarian diet has been reported to reduce the risk of coronary heart disease and stroke and modulate GIT microbiota (329, 330). Meta-analysis of previous studies had shown the reduced risk factors that are linked to stroke, T2DM, and cardiovascular mortality with vegetarian diets (331–333). Moreover, a recent EPIC-Oxford study shows that vegetarian diets reduced the risk of ischemic heart disease by 22% compared to meat eaters, but with an elevated risk of hemorrhagic and total stroke (283).

Among the different types of vegetarian's diet, vegan diet has been proven to be beneficial for cardiocerebrovascular health (i.e., lower LDL cholesterol, triglycerides, and E-selectin) as it is rich with vitamins (except B_{12}), polyphenols, MUFA, and fiber. However, a limited supply of vitamin B_{12} (followed by elevated level of plasma homocysteine) in vegan diets is associated with arterial endothelial dysfunction and elevated thickness of carotid intima media (334). Moreover, a higher level of polyphenol such as flavanols improved cardiovascular function (i.e., endothelial function) and endogenous repair mechanism (i.e., increase flow-mediated dilation, and reduced systolic blood pressure) (335), which helped to reduce proinflammatory, leukocyte adhesion molecules and NF-κβ, platelet aggregation, and an increase in the release of platelet-derived NO (293, 294, 336). The level of CRP also has been shown to decrease following vegetarian diets (i.e., unrefined plant foods) (337, 338) with an elevated circulating EPCs (339).

The consumption of onion and garlic in vegetarian diets has been reported to have antiplatelet, anticoagulant, and antithrombotic properties as they possess sulfur-rich element (especially in garlic) that is known to reduce platelet function and aggregation through inhibition of COX and lipo-oxygenase, followed by the suppression of thromboxane B_2 production (340). In addition, an animal study had shown that administration of sesame seed whole grains in mice lowered the arterial thrombosis (341). Moreover, *in vitro* studies also showed that green beans extract, tomatoes extract, strawberry extract (dose-dependent: 0.1–1 mg/mL), garlic bolt, raw spinach, and

blanched garlic inhibited the AA and ADP-mediated platelet aggregation; the synthesis of platelets thromboxane B_2 reduced P-selectin and IL-1β levels (342–344) and thereby prevented thrombogenesis. These effects are believed mainly due to the presence of phenolic compounds (i.e., chlorogenic acid, ferulic acid, caffeic acid, and P-coumaric acid) in such vegetables (345). Of note, Framingham Heart Study Offspring Study reported that nutrients such as choline (precursor for acetylcholine, PS, and sphingomyelin) found in fruits (i.e., orange) and vegetables (i.e., broccoli) were associated with a lower WMHs load in relation to CSVD manifestation (257, 346).

DIETS AND CIRCULATING MPS—PROPOSING THE LINK WITH CSVD

For the past decades, research interests had grown on the relationship between dietary patterns and potential vascular disease including SVD pathomechanism such as cell activation and prothrombotic molecules release. Hence, treatment and management of cerebrocardiovascular disease risks such as modulating lifestyle habits and dietary pattern have been suggested as an important primary measure. Despite the advancement of understanding on the effects of diets on the release of endogenous circulating MPs toward major cardiovascular disease (i.e., atherosclerosis, coronary heart disease, and stroke), their relationships with the vascular integrity of microcirculation network and, in specific, and its roles in the pathogenesis of SVD (i.e., CSVD) require further deliberation. To date, there is no/limited study that had reported the direct impacts of diet-induced MP formation on NDD including AD and PD. At best, majority of previous studies focused mainly on the role of PDMPs and EDMPs, with scarce data available on other MP subpopulation, as well as their involvement in diet-based MP release, which may influence the risk and manifestation of SVD in general.

Previous studies had evaluated the role of diet-based circulating cell activation–derived MPs in healthy and disease populations. For example, Zhang and colleagues found that individuals with T2DM (major risk factor for CSVD) had a higher level of PDMPs and MDMPs ($CD11b^+$) compared to healthy non-diabetic individuals who practice healthy chronic diet (i.e., oats rich in polyphenols and low GI) as reported with MeDiets, DASH, and vegetarian diets. Additionally, they found that P-selectin, TF, and fibrinogen-positive PDMPs are higher in T2DM individual without obesity (347) and likewise in individuals who practiced WPD with higher EDMPs and PDMPs. Of note, WPD has higher SFA, GI, and refined carbs with low to no omega-3 fatty acids (348). In contrast, HFD and LCD with higher SFA lead to an increase in MDMPs, PDMPs, and EDMPs (349). Hence, diet-based PDMP release, especially in T2DM individuals, may contribute to microthrombosis (through GP-Ib-IX-V receptor complex binding) and inflammation. In such instances, PDMPs with surface P-selectin, fibrinogen, and TF enable leukocytes–platelets adhesion, platelet aggregation, and coagulation, respectively, in small vessel and could be more vulnerable to an early development of arteriosclerosis and/or arteriolosclerosis and hence plausible link to CSVD manifestation. However, polyphenols (i.e., avenanthramide and phenolic alkaloid) found in oats (i.e., in DASH diet and MeDiet) possess antioxidant and anti-inflammatory properties (350), whereby avenanthramide is known to reduce the levels of PDMPs with specific surface markers through inhibition of platelet activation by scavenging the free radicals (from oxidative stress mediated activation), or as antagonist on activation receptors, hence mimicking antiplatelet agents. A recent study by Sinegre and colleagues indicated that epicatechin (a major subclass of flavanols found in cocoa and fruits) supplementation typically in vegetarian diets may reduce the production and release of PDMPs ($GP\text{-}Ib^+$) and thrombin, respectively, without any impact on TF positive MPs which signified the effects of polyphenols on MP release and procoagulant status (351), which could influence the onset, progression, and even prevention of CSVD.

Moreover, individuals with high-gluten diet have been associated with higher systemic GIT-microbiota–derived MPs (230). However, polyphenols found in gluten-free black sorghum extract (BSE) also had been shown to possess an antioxidant property, which helped to reduce endothelial dysfunction, platelet activation or aggregation, and PDMP release mediated by oxidative stress (352, 353). Nignpense and colleagues reported that the consumption of BSE (with concentration no <40 g/mL), such as in GFD, MeDiet, and vegetarian diets, could reduce platelet aggregation (by 19%) and PDMPs (i.e., $CD42b^+$) release (by 47%). The antioxidative properties found in BSE polyphenols enabled the inhibition of PDMPs through the process of hydrogen peroxide (H_2O_2) neutralization, free radical scavenging, and/or interruption with intracellular signaling responsible for PDMP release (353). It seems that BSE polyphenol is a potential candidate to attenuate the thrombogenic effect of PDMPs. Besides, polyphenols also reduced PDMP release through the inhibition of COX-1–mediated platelet activation (354), hence modulating microvascular environment to improve endothelium function. Also, as mentioned in the previous section, as MPs can be generated after a physiological shear stress, polyphenols (i.e., spironolactone) could modulate the blood flow (*via* NO release) and endothelium relaxation to enable the inhibition of shear stress–mediated MP release and reduced the blood pressure. In addition, grapeseed (i.e., proanthocyanins) extract administration (400 mg/kg) in mice was also shown to reduce the production of P-selectins bearing PDMPs, proinflammatory molecules (i.e., IL-6, IL-8, and TNF-α), and vWF and adhesion molecules, whereas it increased the expression of CD34 on ECs and vascular endothelial growth factor receptor 2, which resulted in the inhibition of thrombosis (355) and thus could be protective against the onset and/or progression of CSVD.

MeDiet has been widely studied and associated with the improvements of endothelium structure and function of different vasculature and vascular territories (i.e., peripheral, central, and small/micro vessel) (277). Marín and colleagues reported that MeDiet such as the consumption of extra virgin olive oil (EVOO) possessed the antioxidative properties that aided the reduction of free radical release and protected against oxidative stress, hence mitigating the production of circulating MPs (356). A

TABLE 4 | Summary of the role of dietary patterns, its molecular/cellular response, MP release, and risk predisposition toward CSVD.

Dietary patterns	Molecular and cellular response	Diet-based MP correlates	Risk predisposition toward CSVD
Western Pattern Diets			
• (+) SFA • (+) GI • (+) Refined carbs • (–) Omega-3 fatty acid	• (+) Proinflammatory response • (+) Oxidative stress • (+) Thrombin • (+) Vitamin K-dependent factors (i.e., factors II, VII, IX, X) and extrinsic TF pathway in coagulation cascade • (–) TFPI	• (+) PDMPs • (+) EDMPs	• (+) Risk T2DM • (+) Cardiometabolic syndrome • (+) Microthrombosis • (+) Iron-induced hypoxia • (+) Ischemic stroke
High fat/low carbohydrate diets			
• (+) SFA • (–) PUFA (LA and ALA) • (–) MUFA	• (+) FVIIa/VIIc and extrinsic TF pathway in coagulation cascade	• (+) MDMPs • (+) PDMPs • (+) EDMPs • (–) EDMPs (specifically $CD31^{+}/CD41^{-}$) in LCD	• (+) GIT Dysbiosis (i.e., inflammatory response) • (–) BDNF • (–) EPCs • (+) BBB damages • (+) WMHs
Mediterranean diets			
• (+) PUFA • (+) DHA/EPA • (+) MUFA • (+) Polyphenols • (–) GI • (+) Vitamins (folic acid, B_{12}, B_6)	• (+) Anti-inflammatory response • (+) Antioxidant (i.e., in EVOO) • (–) Prothrombotic coagulation • (–) ILs/NF-κβ/MMPs/VCAM/ICAM • (–) Vitamin K dependent factors (i.e., factors II, VII, IX and X) • (–) Platelet aggregation • (–) Platelets thromboxane B_2 • (–) Prothrombin • (+) TFPI/PAI-1 • (+) NO	• (–) PDMPs • (–) EDMPs • (–) LDMPs • (–) SMCs-MPs • (–) Prothrombotic MPs • (–) Lymphocytes MPs	• (–) Risk of metabolic syndrome • (–) Microthrombosis • (–) Endothelial dysfunction • (+) Flow-mediated arterial dilation • (–) Risk of cerebrovascular disease • (–) Risk of ischemic heart disease • (–) Risk of RSBI and WMHs
DASH diets			
• (–) Sodium • (–) SFA • (+) Vegetarian diets	• (–) Proinflammatory, prothrombotic, and proatherogenic markers	• (–) PDMPs • (–) LDMPs • No effects on RDMPs and EDMPs	• (–) Risks of WMHs/CMBs/lacunar stroke/ischemic stroke • (–) Metabolic syndrome • (–) BMI and waist circumference • (+) Endothelial function • (+) GIT microbiota
Vegetarian diets			
• (+) PUFA • (+) DHA/EPA • (+) MUFA • (+) Polyphenols • (–) GI • (+) Vitamins (folic acid, B_6) • (–) B_{12}	• (–) LDL, triglycerides, and E-selectin • (–) Proinflammatory cytokines • (–) Leukocyte adhesion molecules • (–) NF-κβ • (–) Platelet aggregation • (+) Platelet-derived NO • (–) COX and lipo-oxygenase • (+) VEGF	• (–) EDMPs • (–) SMCs-MPs	• (–) Risk of coronary heart disease, stroke, T2DM • (+) GIT microbiota • (–) Arterial thrombosis • (+) Flow-mediated dilation • (–) Systolic blood pressure • (+) EPCs • Lower vitamin B_{12} associated with arterial endothelial dysfunction • (+) Phosphorylation of eNOS by ECs
Gluten based			
• (+) Gluten (glutenin/gliadin) • GFD • (+) Phytocannabinoids	• (+) Gluten-induced inflammation • (–) Expression of anti-inflammatory and antidysbiotic gene (i.e., PPAR-γ) • (+) MMPs • (+) Expression of PPAR-γ gene • (–) Oxidative stress • (–) Proinflammatory cytokines	• (+) Systemic GIT-microbiota derived MPs • (–) Systemic GIT-microbiota derived MPs	• (+) Pathogenesis of NDD • (+) GIT dysbiosis and leak • (–) Risk of endothelial dysfunction • (–) GIT inflammation and dysbiosis

(+) represents increase/elevate/higher/modulate/activate; (–) represents decrease/lower/reduce/absence. ALA, α-linoleic acids; BBB, blood brain barrier; BDNF, brain-derived neurotrophic factor; BMI, body mass index; CD, cluster differentiation; CMBs, cerebral microbleeds; COX, cyclooxygenase; DHA, docosahexaenoic acids; ECs, endothelial cells, EDMPs, endothelial derived microparticles; eNOS, endothelial nitric oxide synthase; EPA, eicosapentaenoic acid; EPCs, endothelial progenitor cells; EVOO, extra virgin olive oil; GI, glycemic indexes; GIT, gastrointestinal tract; ICAM, intracellular adhesion molecules; ILs, interleukins; LA, linoleic acids; LCD, low carbohydrates diets; LDL, low density lipoprotein; LDMPs, leukocytes derived microparticles; MDMPs, monocytes derived microparticles; MMPs, matrix metalloproteinase proteins; MPs, microparticles; MUFA, monounsaturated fatty acids; NDD, neurodegenerative disease; NF-κβ, nuclear facto kappa β; NO, nitric oxide; PAI-1, plasminogen activator inhibitor 1; PDMPs, platelets derived microparticles; PPAR-γ, peroxisome proliferator-activated receptor γ; PUFA, polyunsaturated fatty acids; RDMPs, red blood cell derived microparticles; SFA, saturated fatty acids; SMCs, smooth muscle cells; T2DM, type 2 diabetes mellitus; TFPI, tissue factor pathway inhibitors; VCAM, vascular endothelial adhesion molecules; VEGF, vascular growth factors; WMHs, white matter hyperintensities.

high MUFA found in EVOO also reduced the levels of PDMPs ($CD31^+/CD42b^+$) and EDMPs ($CD31^+/CD42^-$) (349) that enabled the reduction of subendothelium microthrombogenicity (measured as percentage of microvascular endothelium covered by platelets and the modification or arterial wall, i.e., wall area reduction and foam cell count) in animal model (357). Hence, it is tempted to posit that, through MPs modulation, MeDiet could be protective against the onset and/or progression of CSVD.

In contrast, Weech and colleagues reported that high-SFA diets (i.e., WPD and HFD) elevated the levels of PDMPs ($CD31^+/CD42b^+$) and EDMPs ($CD31^+/AV^+$ and $CD144^+/CD6E^+/AV^+$) (349). A recent preclinical study also indicated that mice treated with HFD had a higher level of MDMPs (CD36) (358). Hence, a higher level of prothrombotic diet–based MPs could potentially trigger the onset and advancement of CSVD. Interestingly, Marin and colleagues showed that MUFA (as attainable with MeDiet, DASH, and vegetarian diets) reduced the total count of EDMPs ($CD31^+/AV^+$ and $CD144^+/CD6E^+/AV^+$) in healthy individuals (356), whereas Chiva-Blanch and colleagues reported EVOO consumption in MeDiet produced a reduced level of PDMPs (PAC-1^+), SMCs-MPs (SMA-α^+), and lymphocytes-derived MPs ($CD3^+/CD45^+$) released in individuals with a high risk of cardiovascular disease (359). Moreover, MeDiet (i.e., nuts consumption) from asymptomatic individual with cardiovascular risk (but no cardiovascular event) had also been shown to have lower levels of prothrombotic PDMPs (PAC-1^+, $CD61^+$, $CD142^+/CD61^+$ and $CD62P^+$), EDMPs ($CD146^+$) and other MP subpopulations such as LDMPs ($CD63^+$ and $CD11a^+$), suggesting that nuts consumption could modulate endothelial function *via* MP level regulation (297). Finally, MeDiet, i.e., the consumption of EVOO and nuts, facilitated the reduction of prothrombotic MPs ($CD142^+/AV^+$), procoagulant MPs (TF bearing) and cell activation MPs ($CD11a^+/AV^+$) (360, 361), that could be beneficial in the setting of CSVD prevention.

In relation to HFD, Heinrich and colleagues found that HFD elevated the production of PDMPs and EDMPs (348), whereas LCD ($\geq$40 g/d) lowered EDMP (specifically $CD31^+/CD41^-$) levels (275). In contrast, the supplementation or consumption of fish oil (rich in EPA and DHA) such as in MeDiet and vegetarian diets reduced the EDMPs ($CD31^+/CD42b^-$) level, but not PDMPs ($CD31^+/CD42b^+$) in low to moderate cardiovascular risk individuals (362) with no effects on PDMPs ($CD41^+$) level, especially in healthy individuals (363). These differences may be due to the fact that healthy individuals may have a lower degree of cellular activation in their systemic circulation. Besides, an intervention using low-calorie diet such as DASH diet in obese individuals has been reported to reduce the level of PDMPs (GP-Ib^+) and LDMPs ($CD11a^+$ and $CD4^+$), but not RDMPs or EDMPs (364) despite the fact that the obese and overweight individuals possessed a higher baseline level of EDMPs ($CD144^+/CD42a^-/CD45^-$) (365). Moreover, weight loss in non-diabetic individuals has been associated with reduced PDMPs ($CD41^+$), suggesting that weight reduction may be independently mediating the inhibition of cell activation–mediated MP shedding (220). Furthermore, the consumption of cocoa flavonols (from cocoa drinks or natural cocoa), especially with DASH, MeDiet, and vegetarian diets, has been shown to reduce EDMPs ($CD31b^+/CD41^-$ and $CD144b^+$) and EDMPs ($CD42a/CD45^-/CD144b^+$) in individuals with coronary artery disease and in young asymptomatic obese individuals, respectively (365, 366). Finally, HFD supplemented with cocoa polyphenols (400 mg/kg per day) fed to rats showed a reduction in platelet aggregation and an elevated release of NO and phosphorylation of eNOS by ECs (367).

Taken together, this evidence provides persuasive and plausible roles of MeDiet, DASH, GFD, and vegetarian diets in the regulation MP systemic release in guarding against microthrombi formation, whereas the formation of MPs with procoagulant TF and proinflammatory properties following WPD, HFD, and LCD is recognized to heighten the risk for microthrombosis and arteriosclerosis and/or arteriolosclerosis (368, 369) and hence risk for CSVD manifestations. **Table 4** summarizes the role of dietary patterns, its corresponding molecular and cellular responses, underlying MP release, and putative predisposition toward CSVD.

CONCLUSION AND FUTURE PERSPECTIVE

CSVD is a complex pathophysiologic condition that originates from small vessel (microcirculation) insults with brain parenchymal lesions that feature as both asymptomatic (silent) and symptomatic neurological manifestations as we grow older. One of the probable risk factors toward the onset and progression of CSVD is the imbalance and undesirable dietary patterns such as WPD and HFD. Although the impact of diets on cerebrocardiovascular disease in general has been widely studied, to date, studies on the effect on dietary pattern in CSVD remain largely unexplored. Scientific evidence provides crucial pertinent leads on diets such as vegetarians, GFD, and MeDiet that are rich in vegetables and fruits, with moderate intake of fish reducing the prevalence of major cerebrocardiovascular disease. This review presents the deliberations on the plausible roles of circulating MPs (produced by oxidative stress, inflammation, GIT microbiota dysbiosis, and cell activation) and suggests their role as one of the novel risk factor and cell-based biomarkers in diseases related to the brain–heart–GIT axis, with an emphasis on CSVD and subsequent related NDD. In particular, the understanding of the role of diet-based MPs and their communications with and/or *via* microcirculation in relation to CSVD manifestations would stir further interests in the current limited understanding on the natural history of CSVD, as well as an opportunity to devise novel approaches for its preventive and therapeutic strategies. Given that MPs can be produced and released from numerous microvascular beds of various organs (i.e., in CNS, heart, GIT, or kidney) and circulate through common systemic circulation to accumulate and exert their thrombogenic effects (i.e., prothrombotic, procoagulant, and proinflammatory) in the small end arteries especially in the cerebral microcirculation, this could contribute as a novel pathomechanism of CSVD, within the background of specific diet pattern as a modifiable precursor. A more concerted multidisciplinary and transdisciplinary research efforts to

integrate the various aspects to advance our understanding of CSVD shall prove beneficial for the progressively aging society.

AUTHOR CONTRIBUTIONS

CMNCMN conceived the original idea, designed the outlines of the review, gathered the literature and resources, drafted, prepared the figures, tables and revised the manuscript. MM designed the outlines of the review, drafted, reviewed, and revised the manuscript. MMG, SH, NSI, WJH, HHN, CHG, LSY, NJ, YN, LF, LKO, HAH, HNA provided the resources, critically reviewed, revised, and improved the manuscript. All authors have read and approved the final manuscript.

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REVIEW
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Viruses and Endogenous Retroviruses as Roots for Neuroinflammation and Neurodegenerative Diseases

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Many neurodegenerative diseases are associated with chronic inflammation in the brain and periphery giving rise to a continuous imbalance of immune processes. Next to inflammation markers, activation of transposable elements, including long intrespersed nuclear elements (LINE) elements and endogenous retroviruses (ERVs), has been identified during neurodegenerative disease progression and even correlated with the clinical severity of the disease. ERVs are remnants of viral infections in the human genome acquired during evolution. Upon activation, they produce transcripts and the phylogenetically youngest ones are still able to produce viral-like particles. In addition, ERVs can bind transcription factors and modulate immune response. Being between own and foreign, ERVs are reviewed in the context of viral infections of the central nervous system, in aging and neurodegenerative diseases. Moreover, this review tests the hypothesis that viral infection may be a trigger at the onset of neuroinflammation and that ERVs sustain the inflammatory imbalance by summarizing existing data of neurodegenerative diseases associated with viruses and/or ERVs.

Keywords: HERV, LINE, virus, neurodegeneration, neuroinflammation

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INTRODUCTION

Viruses have long been linked with diseases of the nervous system. Several viruses, including human α-herpesvirus types 1, 2, and 3 (HHV-1 and HHV-2, known as herpes simplex viruses, and HHV-3, known as varizella zoster virus), human cytomegalovirus (CMV), human immunodeficiency virus (HIV), Epstein–Barr virus (EBV), Ebola virus, and rabies virus are capable of reaching the central nervous system (CNS) (Dando et al., 2014). Often, particular viral nucleic acids or proteins are found in the brain, cerebrospinal fluid (CSF), or peripheral blood of patients with a certain neurological disease. For example, HHV-3 and HHV-6 are present in the CSF (Mancuso et al., 2007; Alvarez-Lafuente et al., 2008), coronaviruses in the CNS of multiple sclerosis (MS) patients (Burks et al., 1980), and HIV and human T-cell leukemia virus-1 (HTLV-1) in the brains of amyotrophic lateral sclerosis (ALS) patients (Verma and Berger, 2006). HHV-6A DNA and transcripts, in turn, are increased in the brains of Alzheimer's disease (AD) patients and closely correlate with neuronal loss (Readhead et al., 2018). Tracing neuropathologies to viral infections can, however, be challenging. This holds particularly true when the virus becomes "slower" or "latent" following acute infection (Sigurdsson, 1954; Murphy and Yunis, 1976; Steiner et al., 2007;

Kennedy and Cohrs, 2010; Shu et al., 2015; Rodriguez et al., 2020). The tremendous research from the beginning of the HIV pandemic has greatly enhanced evidence and understanding of this slow action of viruses in the CNS (Garcia et al., 1999). Important to consider also is the long-term risk from accumulated infections during a lifetime that might lead to a cumulative and individual risk of developing neuropathology, such as stroke and dementia (Almeida and Lautenschlager, 2005; Ruprecht et al., 2006; Tai et al., 2009; Sico et al., 2015).

More recent research has shown that viruses, such as HIV, EBV, CMV, influenza, herpesviruses, and HTLV-1 can activate viral sequences originating from retroviral infections in the distant past of human evolution that have been incorporated into the human genome (Nellaker et al., 2006; Toufaily et al., 2011; Young et al., 2012; Leboyer et al., 2013; Li et al., 2014; Kury et al., 2018). While their ability to express viral products is mostly lost, some of these endogenous retroviruses (ERVs) have evolved to play important roles in physiological processes, such as placentation, early human embryogenesis, neurodevelopment, and immune response regulation (Kammerer et al., 2011; Wang et al., 2014, 2020; Chuong et al., 2016; Romer et al., 2017; Xue et al., 2020). Activation of ERVs, such as by exogenous viruses or environmental factors, can contribute to a multitude of neurodevelopmental, neurodegenerative, and neuroinflammatory disorders (Balestrieri et al., 2019; Gruchot et al., 2019, 2020; Tam et al., 2019; Evans and Erwin, 2020; Groger et al., 2021), including HIV-associated neurodegenerative disorder (HAND), AD, MS, ALS, schizophrenia, stroke, and neuropathogenesis of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) as well as to accelerated neurological decline in aging.

This review highlights the interplay between endogenous viruses and retroelements, on the one hand, and exogenous viruses, on the other hand, and aims at revealing underlying mechanisms in aging, and neurodegenerative and neuroinflammatory diseases summarizing recent advances in this field.

VIRAL INFECTION OF THE CENTRAL NERVOUS SYSTEM

The central nervous system (CNS) is not a common target organ for viruses. It is neither easily accessible nor as advantageous in terms of contagiousness and successful viral transmission to new hosts as the respiratory or gastrointestinal tract. Shielded by the meninges, CSF, and blood brain barrier (BBB), the CNS is immunologically unique and privileged (Louveau et al., 2015). Although the CNS itself is armed with an array of immunological mechanisms, including support from the periphery, it may be considered as a sanctuary where viral replication occurs despite a complete viral suppression in the peripheral blood. This has been shown, for example, for the HIV (Walker et al., 2008). In addition to viruses with neurotropism, only minor mutations may be sufficient to create viruses that can access the CNS via various routes (Wiley, 2020). Permeability of the BBB may be increased by high viremia accompanied by elevated cytokine levels and also by direct interaction with tight junction proteins (Toborek et al., 2005; Chai et al., 2014). Viruses can infect endothelial cells of the BBB, allowing viral replicates to be released into the CNS (Verma et al., 2009; Fletcher et al., 2012), while infection of leukocytes or monocytes by, for example, HIV and SARS-CoV-2, that pass BBB physiologically, provides a "trojan-horse" mechanism to enter the CNS (Larochelle et al., 2011; Takeshita and Ransohoff, 2012; Bostanciklioglu, 2020). Attention is currently drawn to the CNS invasion through retrograde neuronal transport of infected peripheral nerve afferents, as SARS-CoV-2 and other coronaviruses are associated with CNS entry via the olfactory pathway, a mechanism that has been also described for other viral families such as influenza A virus, rabies virus, and herpesviruses (van Riel et al., 2015), and other peripheral nerves, for example, the sciatic nerve and vagus nerve (Ren and Racaniello, 1992; Ohka et al., 1998; Guadarrama-Ortiz et al., 2020; Liu et al., 2020). **Figure 1** depicts the mechanisms of viral entry into the CNS.

Once in the CNS, acute infections present with encephalitis, myelitis, or viral meningitis. Generally, virus-triggered immune reaction is limited in time and ends with the virus being combated; however, certain neurotropic viruses can continue to elicit progressive damage on brain structure, function, and cognition long after the clearance of virus from the peripheral blood. In addition to this type of chronic infections, viruses can enter a latent (dormant) phase, interrupted by occasional full awakening of the virus. Sometimes, the same virus can contribute to both. This is the case for the HIV (Rodriguez et al., 2020), measles morbillivirus (Murphy and Yunis, 1976), HHV-1 (Shu et al., 2015), and HHV-3, to name a few (Steiner et al., 2007; Kennedy and Cohrs, 2010). The high worldwide seroprevalence of some of these viruses, such as that of HHV-1 and HHV-2 being around 90% (Wald and Corey, 2007), indicates that facilitating factors must exist that ultimately decide upon disease development. In consideration are comorbidities such as traumas to latently infected neurons (Zhang et al., 2013), immune-depriving conditions such as AIDS (Rodriguez et al., 2020), leukemia (Koskenvuo et al., 2008; Lancman et al., 2020), or stroke-induced immunodepression (Deroux et al., 2012; Hetze et al., 2013; Romer et al., 2015; Bertrand et al., 2019), and cumulative infectious burden (Sico et al., 2015), and also environmental factors (Liu et al., 2013; Brutting et al., 2018; Mueller et al., 2018; Del Re and Giorgi, 2020). The role of aging as a facilitating factor and the interplay with ERVs are discussed in detail below.

ENDOGENOUS RETROVIRUSES AND RETROELEMENTS

Exogenous retroviruses from which ERVs originate, like other retroviruses, contain single-stranded (ss) anti-sense RNA and RNA reverse polymerase to generate double-stranded DNA (dsDNA). With the help of the retroviral integrase, this DNA copy may become endogenized into the host genome, essentially when infecting gametes (germ cells) with chromosomal insertion sites that will allow the birth of viable offspring over generations. Over the evolution, this type of infections and endogenizations

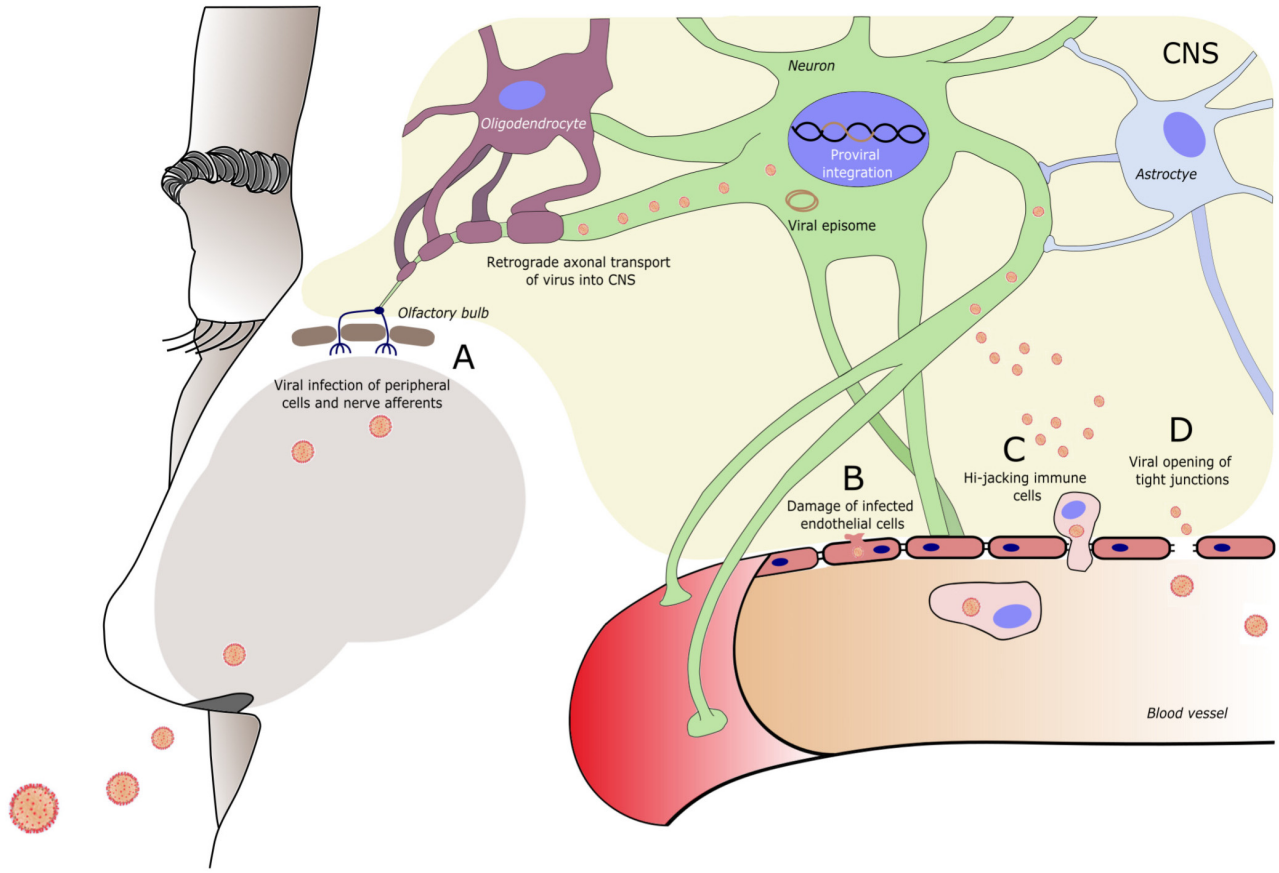

FIGURE 1 | Entry routes of exogenous viruses into the central nervous system (CNS). Virus can enter the CNS via **(A)** Peripheral nerve terminals (in this example of the olfactory nerve) and retrograde axonal transport. **(B)** Infection of and damage to endothelial cells of the blood brain barrier (BBB). **(C)** Infection of circulating immune cells that travel across the BBB. **(D)** Modulation of tight junction molecules of the BBB, increasing BBB permeability. A specific entry route can be typical for a specific virus, however, often more than one route is used. Entry route may vary during the course of infection (e.g., BBB damage in conditions of high viremia) and new CNS entry mechanisms can follow when the viral genome sustains mutational changes. A neurotrophic virus often infects specific cell types within the CNS. Reaching the CNS enables the virus to circumvent the peripheral immune response. To evade also the CNS immune response, viruses may enter a dormant state forming a viral episome or integrating into the host cell DNA.

of retroviruses have occurred multiple times (Kozak, 1985). Gradually, ERVs become non-infectious, lose the ability to exit the host cell, and adopt the nature of transposable elements. ERV sequences become transpositionally inactive, mutated, degraded, and epigenetically silenced as part of the host control in protection of genome stability. ERV sequences take up about 8% of the modern human genome (Gannet, 2019). ERV families that have been less prone to be degraded by the host, such as human ERV H (HERV-H) and HERV-K, have shaped the evolution and complexity of innate and adaptive immune pathways (Villarreal, 2011; Chuong et al., 2016, 2017). Regulation mechanisms to control the HERV activity, mainly via epigenetics (for example, cytosine methylation) form the basis for proper host–HERV interaction in controlling vital processes (Lavie et al., 2005; Turelli et al., 2014). The Krüppel-associated box domain (KRAB)-associated protein-1 (KAP1)-mediated silencing continues to be the key mechanism of ERV control in adult brain (Fasching et al., 2015). KAP1 deletion during brain development is lethal, and heterozygous deletion of KAP1 causes behavioral changes resembling those observed in human psychiatric conditions associated with HERV upregulation (Jakobsson et al., 2008).

Long interspersed nuclear elements (LINEs) are a group of non-LTR (long terminal repeat) retroelements that compose up to 21% of the human genome (Gannet, 2019). LINE-1 elements are a major source of structural polymorphisms in humans (Hancks and Kazazian, 2012). Higher LINE-1 activity is characteristic to brain areas of adult neurogenesis, in particular to the hippocampal dentate gyrus (Baillie et al., 2011; Kurnosov et al., 2015; Bachiller et al., 2017) and to human neural progenitor cells (Coufal et al., 2009). LINE-1 insertions often locate at neuronal genes, and LINE-1 activity can initiate neuronal differentiation (Muotri et al., 2005). Hippocampal LINE-1 activity and genomic mosaicism are involved in cognitive processes such as memory formation (Bachiller et al., 2017).

Alu family is the most common member of the short interspersed nuclear elements (SINEs) and accounts for about 13% of the human genome (Gannet, 2019). Alu elements are actively transposing. However, they do not encode a

functional reverse transcriptase protein and therefore rely on the machinery of other retroelements, especially LINEs (Wei et al., 2001; Dewannieux et al., 2003). Alu elements are involved in neurogenesis, brain connectome development, and in shaping cognition networks (Mehler and Mattick, 2007; Baillie et al., 2011; Bedrosian et al., 2016).

HERVs, LINEs, and Alus regulate gene expression networks at multiple levels, providing a rich pool for RNA diversification (Lev-Maor et al., 2003; Laperriere et al., 2007; Fujii, 2010; Gim et al., 2014; Goke and Ng, 2016), functioning as promoters and enhancers (Norris et al., 1995; Shen et al., 2011; Lee, 2012; Wu et al., 2013; Romer et al., 2017), and coordinating 3D genomic arrangements via topologically associated domains (Dixon et al., 2012; Zhang et al., 2019). These elements contribute significantly to defining neurobiological processes, including neuronal mosaicism and shaping brain development (Coufal et al., 2009; Baillie et al., 2011; Richardson et al., 2014; Bodea et al., 2018). Alteration of these networks are associated with neurodevelopmental, neurodegenerative, neuroinflammatory, and autoimmune diseases. HERVs, LINE, and Alu elements are subject to a multitude of environmental factors and xenobiotics, which can activate normally well-controlled HERV expression, such as hypoxia (Brutting et al., 2018), drugs (aspirin, caffeine, and valproic acid) (Diem et al., 2012; Liu et al., 2013), and hormones (Norris et al., 1995; Mueller et al., 2018). Also, LINE-1 retroelement activity is sensitive to a multitude of factors including social isolation stress, heavy metals, and anti-inflammatory and psychoactive drugs (Del Re and Giorgi, 2020). Together, (H)ERVs, LINE, and Alu elements regulate early human embryogenesis, neurodevelopment, neural diversity, and plasticity. All three are subject to a number of environmental factors, affecting a healthy brain.

INTERPLAY BETWEEN ENDOGENOUS AND EXOGENOUS VIRUSES

Inherent ERVs and exogenous viruses, being distant relatives, share common mechanisms but can also be opponents. When viruses first try to enter a cell, HERV, coming from inside the host genome, can provide protection by blocking the cellular receptors relevant for the exogenous retrovirus entry (Spencer et al., 2003). ERVs can protect against exogenous retroviral infections by receptor interference if both viruses share the specificity of the env glycoprotein (Weiss et al., 1985). Substantial similarity between HERV and exogenous retrovirus, such as that between HERV-K (HML-2) gag and HIV gag, could lead to fusion of viral proteins and production of defective viral particles (Monde et al., 2012). In addition, HERV antisense transcripts can interact with complementary exogenous retrovirus transcripts to block viral replication and generate dsRNA to be recognized as a pathogen-associated molecular pattern (PAMP) by the host immune system (Tang et al., 2012; Shekarian et al., 2017). Sensing PAMPs, such as viral proteins and nucleic acids, and danger-associated molecular patterns (DAMPs) derived from damaged cells, are part of the innate immune response. Cytoplasmic sensors for viral DNA include cyclic GMP-AMP synthase (cGAS), Z-DNA-binding protein 1 (ZBP1), and TLR9 (Rigby et al., 2014; Hayashi et al., 2015; Xia et al., 2016; Sandstrom et al., 2017; Jiao et al., 2020). Viral RNA are sensed by TLR8 (Heil et al., 2004), TLR3, melanoma-differentiated-associated gene 5 (MDA5), ZBP-1 (Gurtler and Bowie, 2013; Jiao et al., 2020), and retinoic acid inducible gene I (RIG-I) (Gurtler and Bowie, 2013). Innate immune response to viral infections leads to pro-inflammatory cytokine, chemokine, and type I interferon (IFN) release to stimulate adaptive immune response, the T lymphocyte-mediated cellular and B lymphocyte-mediated humoral immunity.

Activated innate and adaptive immune system cells both can stimulate ERV transcription (Bannert and Kurth, 2004). Generally, immune reactions are limited in time and cleared by the immune system. However, HERVs are continuously present and, under certain conditions, also continuously active. Aiming at clearing up the infection triggered by HERVs, TLR stimulation can, via IFN release, actually activate HERVs further (Bannert and Kurth, 2004). Dispersed at relevant immune genes, activated HERVs and in particular the polymorphic HERV-K (HML-2) loci, form another layer of immune response regulation (Nexo et al., 2011). Certain HERV insertions function as IFN-inducible enhancers, and type I IFN is one of the main innate immune response products to viral infection (Chuong et al., 2016). Neuroinflammation will awaken and activate HERVs in the human brain (Johnston et al., 2001; Manghera et al., 2015, 2016). In this feedback loop, HERV activity is upregulated by anti-viral immune response through inflammatory mediators and also by epigenetic dysregulation (Manghera and Douville, 2013; Hurst and Magiorkinis, 2015, 2017), leading to chronic stimulation of the immune system (Hurst and Magiorkinis, 2015, 2017; Grandi and Tramontano, 2017; Mameli et al., 2017; Ramasamy et al., 2017). Continuous ERV activation is associated with sustained neuroinflammation and predisposes to neurodegenerative and autoimmune diseases (Nexo et al., 2011).

Activation of ERV transcription can directly be achieved by several exogenous viruses, such as HIV, EBV, CMV, influenza, and herpesviruses, some of which can even induce a self-sustained HERV activation (Nellaker et al., 2006; Young et al., 2012; Leboyer et al., 2013; Li et al., 2014; Kury et al., 2018). Among exogenous retroviruses, HTLV-1 Tax can increase HERV-H, HERV-K, HERV-W, and HERV-E expression in T lymphocytes (Toufaily et al., 2011). HIV transactivator of transcription (Tat) protein can stimulate expression of HERV-K and HERV-W in astrocytes and peripheral blood cells and that of HERV-W also indirectly via TLR4 and proinflammatory cytokine (TNF-α, NF-κB) production (Uleri et al., 2014). Using mimicry, HIV rev, which mediates nuclear export of HIV messenger RNA (mRNA), also mediates the nuclear export of HERV-K mRNA, thereby promoting HERV-K translation (O'Carroll et al., 2020). Exogenous viruses can further facilitate expression of endogenous superantigens, linked in particular with the CNS-affecting autoimmune diseases (Acha-Orbea, 1992). This occurs, for example, between rabies virus and HERV-W (Lafon et al., 1992; Perron et al., 2001; Lafon et al., 2002) and between EBV and HERV-K18 (Sutkowski et al., 2001). In turn, ERVs may assist

their exogenous counterparts to escape immune surveillance, repair defects in exogenous retroviruses (Schwartzberg et al., 1985), and facilitate chronic viral replication (Rasmussen, 1997). Also, transcriptionally active ERVs provide a rich pool for recombinational events with exogenous retroviruses. When a host cell is infected by two different viruses, heterologous *trans*-activation can take place where transcription of one virus is initiated by factors produced by the other virus. When ERVs provide envelope glycoproteins to exogenous retroviruses, these could establish a new host cell repertoire and circumvent immune system response (Lusso et al., 1990). A certain degree of epitope similarity between ERV and exogenous retrovirus can lead to a weaker immune response against this virus (Miyazawa and Fujisawa, 1994).

Similar interplay between endogenous retroviruses and exogenous viruses exists in the periphery and may pave way to chronic inflammation. In fact, viruses and endogenous retroviruses have been linked with autoimmune disease pathology, such as systemic lupus erythematosus (Ogasawara et al., 2000; Moon et al., 2004), rheumatoid arthritis (Herve et al., 2002), and diabetes (Levet et al., 2017, 2019). Continued upregulation of HERV-H and HERV-K after the clearance of hepatitis C virus from the peripheral blood of chronic hepatitis C patients was recently associated with higher risks for cancer and autoimmunity in these patients (Tovo et al., 2020).

Peripheral inflammation can reach the brain via transversal of circumventricular organs, peripheral nerves, or through pro-inflammatory cytokine influx upon direct cytokine–endothelial interactions, resulting in reduced BBB integrity (Toborek et al., 2005; Chai et al., 2014). Moreover, peripheral inflammation processes can trigger major neurological events such as stroke via platelet aggregation, hypercoagulation, impaired endothelial function, and thrombosis (Elkind et al., 2020; Oxley et al., 2020).

Figure 2 draws common mechanisms in the interplay of exogenous and endogenous retroviruses leading to sustained neuroinflammation and subsequent CNS damage.

VIRAL AND ENDOGENOUS RETROVIRAL ASSOCIATED PATHOLOGIES

Table 1 summarizes exogenous viral and endogenous retroviral disorders discussed in the following sections.

HIV-Associated Neurodegenerative Disorder

HIV infection causes acquired immunodeficiency syndrome (AIDS) affecting multiple systems in the body. One of the complications of HIV infection is the HIV-associated neurodegenerative disorder (HAND) (Navia et al., 1986), which can develop into HIV-associated dementia (Nookala et al., 2017), the most common cause of dementia in young adults (Janssen et al., 1992) with higher prevalence among women (Duarte et al., 2020). HIV is transported to the brain with infected T-lymphocytes and monocytes (Wiley et al., 1986; Takahashi et al., 1996). These long-lived cells are referred to as sources of HIV chronic infection (Nookala et al., 2017). In the brain, HIV infects primarily the immunocompetent cells, perivascular macrophages, and microglia where it replicates (Watkins et al., 1990; Albright et al., 2000). HIV persists in the CNS, causing motor, cognitive, and behavioral deficits, which can be further aggravated by opportunistic infections by CMV, EBV, HHV-3, and HHV-6 (Almeida and Lautenschlager, 2005).

Neurodegeneration characteristic to HAND emanates from chronic inflammation, sustained by activated monocytes, macrophages and astrocytes, and neurotoxic HIV viral proteins (Ghafouri et al., 2006; Kraft-Terry et al., 2010). These include HIV Tat, HIV viral protein R (Vpr), and HIV env glycoprotein gp160 cleaved product gp120. HIV viral proteins induce neuropathology by aberrant calcium signaling, mitochondrial damage, oxidative stress, excitotoxicity, and inflammation (Nookala et al., 2017), collectively leading to neuronal death (Masliah et al., 1992).

Further augmentation of neurodegeneration and neuroinflammation in HAND comes from HIV and infection-induced cytokines' (IL-6, IL-1β, TNF-α, and IFN-γ) ability to dynamically activate HERVs, such as HERV-W (Uleri et al., 2014) and HERV-K (Bhardwaj et al., 2014; O'Carroll et al., 2020). In particular, HIV induces HERV-K transcription and can trigger adaptive immune response against the HERV-K capsid protein (de Mulder et al., 2017). A distinct temporal pattern between HIV and HERV-K activation has been observed in the brains of HIV-infected individuals, demonstrating increased HERV-K activation ahead of spikes in HIV replication in the peripheral blood (Contreras-Galindo et al., 2007) and ahead of clinical symptoms of neurocognitive impairment (Douville and Nath, 2017). HIV-associated motor neuron disease affecting upper and lower motor neurons is likewise escorted by increased HERV-K expression at the onset of neurological symptoms (Bowen et al., 2016). HIV can directly facilitate HERV-K expression, transcript transportation to cytoplasm, and viral particle production (O'Carroll et al., 2020) and regulate anti-viral gene expression through activating (H)ERV promoters (Srinivasachar Badarinarayan et al., 2020). Increased HERV-K env expression in cortical neurons of HIV-infected individuals has been linked with restricting HIV replication in these cells (Bhat et al., 2014). In the long term, however, neuronal HERV-K expression leads to neurite retraction and neuronal death (Dembny et al., 2020), in line with HAND. The antiretroviral therapy against HIV is effective also against HERV-K (Bowen et al., 2016). Overall, the neuropathology induced by HIV and HERV-K might have a certain overlap and is difficult to separate. It might be beneficial to monitor the level of HERV-K within the course of HAND.

Of note, incidence of HIV-associated dementia has reduced threefold after the combination antiretroviral therapy became available (Lawrence and Major, 2002). New medical concerns involve premature aging-related neurocognitive disorders (Robertson et al., 2007). HIV-associated dementia bares certain similarities with that of Alzheimer disease (AD) (Clifford et al., 2009). The neurons of HIV-associated dementia patients contain diffuse Aβ plaques, similar to the early stages of AD (Ortega and Ances, 2014), which could indicate a slower progression of HIV-associated dementia (Fulop et al., 2019).

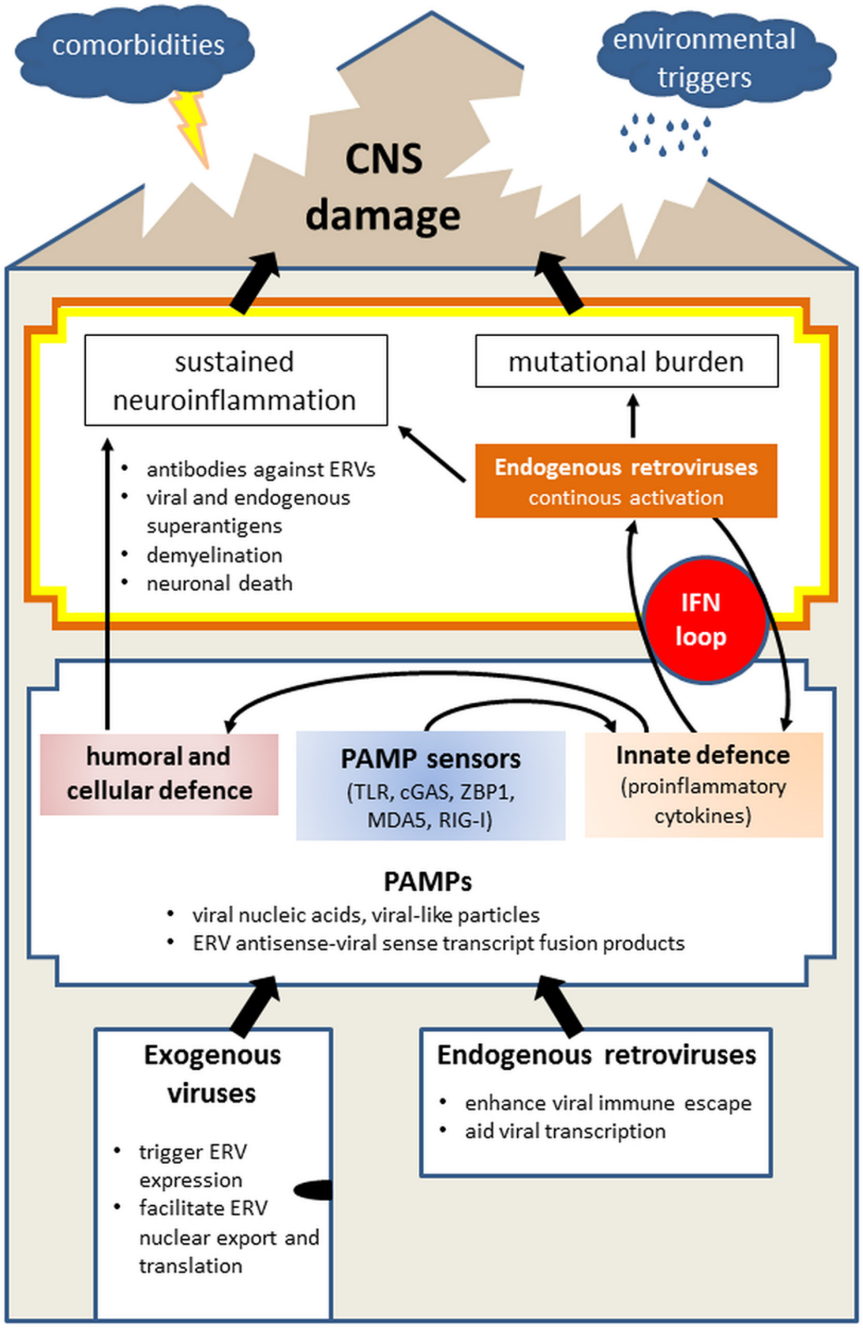

FIGURE 2 | Model of the role of exogenous virus and endogenous retrovirus (ERV) in initiating neuropathologies, illustrating the entry of viruses into the body ("house") and the presence of ERVs, the physiological immune response on the first level and continuous imbalance on the second, thus leading to damage of the CNS ("roof"). Infections with exogenous viruses often activate the transcription of endogenous retroviruses which are already present in the mammalian genome. This results from disruption of the host-control over ERVs, bordering between own and foreign. Separately and together, activated exogenous viruses and ERVs produce PAMPs, such as viral nucleic acids, viral-like particles, fused transcripts between exogenous virus, and ERV, which are sensed by PAMP sensors (TLR, cGAS, ZBP-1, MDA5, and RIG-I). Collectively, PAMP activation alarms the immune system by initiating innate immune response involving mainly IFN signaling which will trigger the adaptive (humoral and cellular) immunity. Viral infections are generally limited in time as they are combated by the immune system. However, the activation of ERVs and neurotrophic viruses which remain silent yet present within the body, can lead to sustained neuroinflammation via antibodies against ERVs, superantigen formation, demyelination, and neuronal death. In parallel, IFN-mediated innate immune response can further activate ERVs which contain IFN response elements (such as HERV-K), thereby creating an IFN loop. Next to the danger of chronic neuroinflammation, this additionally carries mutational burden for the host, collectively leading to the CNS damage. The probability and scope of the CNS damage is further determined by facilitating factors, including comorbidities and environmental triggers as well as age. Abbreviations: cGAS, cyclic GMP-AMP synthase; CNS, central nervous system; ERV, endogenous retrovirus; IFN, interferon; MDA5, melanoma differentiated associated gene 5; PAMP, pathogen-associated molecular pattern; RIG-I, retinoic acid inducible gene I; TLR, toll-like receptor; ZBP-1, Z-DNA binding protein 1.

TABLE 1 | Overview of disorders affecting the central nervous system (CNS) associated with an onset mediated by exogenous viruses and/or endogenous retroviruses.

Disorder	Clinical CNS symptoms	Associated peripheral pathology	CNS targets	Associated exogenous viruses	Associated endogenous retroviruses	Mechanisms
HIV-associated neurodegenerative disorder (HAND)	Cognitive, motor, and behavioral deficits similar to AD	AIDS	Microglia, astrocytes, and perivascular macrophages, neurons (motor and cortical)	HIV-1 (aggravation by CMV, EBV, HHV-3, and HHV-6)	HERV-K (HERV-E, HERV-T, two ERV9 subgroups)	• Neurotoxic viral products (Tat, Vpr, HIV-1 gp120) • Cytokine-induced HERV expression (IL-6, IL-1β, TNF-α, IFN-γ) • Viral induction of antibody against HERV-K capsid protein • Sustained inflammation • Increased pTau, neopterin, neurofilament light and diffuse Aβ plaques
Alzheimer's disease (AD)	Cognitive deficits (memory loss, learning difficulty, impaired logical thinking, confusion, speech problems, shortened attention span)	Systemic immune activation, and chronic peripheral inflammation	Microglia, hippocampal pyramidal neurons lymphocytes, neuronal, and endothelial cells parahippocampal, inferior frontal and superior temporal gyrus	HHV-1, HHV-6A, HHV-6B, HHV-7, EBV, and CMV	HERV-K, HERV-H, HERV-W, HERV-L, solitary long-terminal repeats (LTRs)	• Extension of inflammation to CNS (immune cells entry to brain via transversal of circumventricular organs, vagus nerve stimulation or pro-inflammatory cytokine influx) • Viral RNA sensor MAVS • Gliosis • Viral activation of HERVs • Higher rate of DNA damage and higher expression of pluripotency-related genes • HERV fusion products (ARC viral-like capsid protein overexpression) • HERV-induced TLR8 activation • Progressive neuronal death (PARP1-driven, caspase-independent apoptosis) • Dense Aβ plaques, Tau neurofibrillary tangles
Multiple sclerosis (MS)	Progressive physical and cognitive disabilities neurobehavioural deficits, such as weakness, gait unsteadiness, and altered executive functions	Hints for chronic inflammation, association with peripheral neuropathy	B cells, microglia, astrocytes, and macrophages that orchestrate damage to oligodendrocytes	HHV-1, HHV-2, HHV-3, HHV-6, EBV, CMV, JCV	HERV-W (HERV-K, HERV-H)	• Viral activation of HERV-W transcription • HERV-W env protein and syncytin expression • HERV-W env is a powerful superantigen • Syncytin induces neuroinflammation via oxidative stress • Stimulate anti-viral response associated with MS pathology by binding TLR4 and CD14 • Pro-inflammatory (anti-viral) response involving TLR4, CD14, IL-1beta • Increase in cellular protein oxidation, inhibition of oligodendrocyte maturation, myelin damage and antagonization of remyelination
Amyotrophic lateral sclerosis (ALS)	Fasciculation, cramps, muscle atrophy, and marked limb weakness	HERV-W env and gag are present in muscle biopsies from ALS patients, linked with macrophage activation and neurogenic atrophy of muscular tissue	HERV activity in prefrontal, sensory, motor, occipital cortex	Weak connection with HIV-1, and HTLV-1	HERV-K	• HERV-K transcription in ALS is stimulated by the TDP-43 • HERV-K transcription can be also initiated by HIV-Tat protein HERV-K env in cortical and spinal neurons • neuronal HERV-K activation is associated with the nuclear translocation of interferon regulatory factor 1 (IRF1) • Sustained neuroinflammation with progressive loss of cortical and spinal motor neurons
Schizophrenia spectrum disorders	Psychosis, hallucinations, delusions, apathy and disorganized thinking	Subclinical inflammation	Neurons of prefrontal cortex and (developing) hippocampus	HHV-2, perinatal influenza infection	HERV-W, LINE-1 (HERV-K, HERV-H)	• Impairment of synaptic genes • Upregulation of immune response genes • Lasting inflammatory dysregulation of the nervous system

(Continued)

TABLE 1 | Continued

Disorder	Clinical CNS symptoms	Associated peripheral pathology	CNS targets	Associated exogenous viruses	Associated endogenous retroviruses	Mechanisms
Neuropathogenesis of SARS-CoV-2	Dizziness, headache, encephalitis, seizures, intracerebral hemorrhage, and stroke, neuromuscular and autoimmune syndromes	Acute respiratory disease	Viral infection of neurons and glial cells	SARS-CoV-2	none	• Interacts with stress response, vesicle trafficking, lipid metabolism pathways, production of reactive oxygen species, RNA processing, RNA regulation, ubiquitin ligases and mitochondrial activity • Impaired lysosomal function combined with inhibition of ubiquitin-proteasome system • Protein misfolding and formation of protein aggregates • Expected neuroinflammation and neurodegeneration

Aβ, amyloid β; AD, Alzheimer's disease; AIDS, acquired immunodeficiency syndrome; ALS, amyotrophic lateral sclerosis; CD14, cluster of differentiation 14; CMV, cytomegalovirus; EBV, Epstein-Barr virus; gp120, glycoprotein 120; HAND, HIV-associated neurodegenerative disorder; HERV, human endogenous retrovirus; HHV-1, human alphaherpesvirus-1/herpes simplex virus-1; HHV-2, human alphaherpesvirus-2/herpes simplex virus-2; HHV-3, human alphaherpesvirus-3/varicella zoster virus; HHV-6, human alphaherpesvirus-6; HHV-7,human alphaherpesvirus-7; HIV-1, human immunodeficiency virus-1; HTLV-1, human T-cell leukemia virus-1; IFN-γ, interferon γ; IL, interleukin; IRF1, interferon regulatory factor 1; JCV, John Cunningham virus; LINE-1, long interspersed repetitive element 1; LTR, long-terminal repeat; MAVS, mitochondrial antiviral-signaling protein; MS, multiple sclerosis; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; Tat transactivator of transcription; TDP-43, trans-activation responsive TAR; DNA-binding protein 43; TLR, toll-like receptor; TNF-α, tumor necrosis factor α; Vpr, viral protein R.

Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by gradual cognitive decline. AD can start even decades before the appearance of clinical symptoms (Taylor et al., 2016; Fulop et al., 2018). AD is associated with systemic immune activation and chronic peripheral inflammation (Culibrk and Hahn, 2020). Neuropathologically, AD is characterized by presence of Aβ plaques, Tau neurofibrillary tangles, progressive neuronal death, neuroinflammation, and gliosis in the brain.

Growing evidence points to the role of pathogens, such as HHV-1, HHV-6A, HHV-7 (Lovheim et al., 2015; Readhead et al., 2018), EBV, and CMV (Carbone et al., 2014) in developing sporadic AD. As the worldwide seroprevalence of HHV-1 is around 90% (Wald and Corey, 2007), facilitating factors essentially contribute to the probability of HHV-1 triggering AD (Looker et al., 2015). HHV-1, HHV-6A, and HHV-6B viral glycoproteins can bind β-amyloid oligomers and accelerate Aβ plaque deposition (Eimer et al., 2018).

HERV-H, HERV-K, HERV-L, and HERV-W are transcriptionally active in the brains of AD patients (Johnston et al., 2001; Sun et al., 2018; Dembny et al., 2020). This activation could be directly mediated by HHV-1, HHV-3, and HHV-6 (Ruprecht et al., 2006; Brudek et al., 2007; Tai et al., 2009), or by heterochromatin relaxation and loss of epigenetic host control over HERVs (Sun et al., 2018), increasing DNA damage and expression of pluripotency-related genes (Frost et al., 2014). Upregulation of ERV-K family member in a streptozotocin murine model of sporadic AD was linked with upregulation of immune response genes and downregulation of genes involved in histone modifications and transmembrane transport and associated with cognitive impairments in contextual fear memory and spatial learning (Sankowski et al., 2019). HERV-K (HML-2) transcripts containing a motif 5′-GUUGUGU-3′ contribute to neuronal death and microglial accumulation associated with AD via TLR8 activation (Dembny et al., 2020). ERVs can be transmitted between neurons in the brains of AD patients, packed into an ARC viral-like capsid protein, which is overexpressed in AD patients and is associated with Aβ production (Wu et al., 2011).

Multiple Sclerosis

Multiple sclerosis (MS) is a neurodegenerative and neuroinflammatory CNS disease characterized by multifocal demyelinating lesions in the brain and spinal cord leading to progressive physical and cognitive disabilities. Development of MS has been associated with viral infections and activation of HERVs (Alvarez-Lafuente et al., 2008; Kriesel et al., 2017; Morandi et al., 2017; Gruchot et al., 2020).

Among viruses, higher transcription levels of HHV-3 and HHV-6 have been found in the CSF of individuals suffering from MS (Mancuso et al., 2007; Alvarez-Lafuente et al., 2008). Also, coronaviruses have been detected in the CNS of MS patients (Burks et al., 1980). EBV has been even suggested as a trigger for MS that activates HERV-W, which then sustains the disease (Mameli et al., 2012).

HERV-W is also the main HERV associated with MS pathology. Further, the expression level of HERV-W in the brain of MS patients correlates positively with the severity of disability and disease progression (Sotgiu et al., 2010). HERV-W and its env transcript and protein are upregulated in the brains (Perron et al., 1997; Antony et al., 2004) as well as in the peripheral blood and serum of MS patients (Garson et al., 1998; Perron et al., 2012a). HERV-W can be activated in MS by EBV (Mameli et al., 2012) and HHV-1 (Ruprecht et al., 2006; Marrodan et al., 2019). HHV-1-triggered HERV-W transcription occurs in immune cells central to the MS pathology, such as B cells, microglia, astrocytes, and macrophages (Ruprecht et al., 2006; Marrodan et al., 2019).

HERV-W env protein activates dendritic cells and boosts T helper lymphocyte type-1 (Th1) immune response, acting as a PAMP. It stimulates pro-inflammatory anti-viral response by binding TLR4 and CD14 (Rolland et al., 2006; Saresella et al., 2009). Upon binding the TLR4 on oligodendroglial precursor cells, HERV-W env stimulates release of pro-inflammatory cytokines, inducible nitric oxide synthase, and formation of nitrotyrosine groups leading to reduction of myelin expression in MS lesions (Kremer et al., 2013). In addition to the above, HERV-W env is a powerful superantigen linked with demyelination in MS (Perron et al., 2001; Rolland et al., 2005), perhaps associated with molecular mimicry with myelin oligodendrocyte glycoprotein (MOG) (do Olival et al., 2013; de Luca et al., 2019). Accordingly, treatment with HERV-W env antibody can effectively rescue myelin expression (Kremer et al., 2015).

Mechanistically, HERV-W env protein has been shown to induce microglial polarization and closely associate with myelinated axons in MS lesions ultimately leading to structural damage of these axons (Kremer et al., 2019). HERV-W env, encoded from a full-length provirus at locus 7q21.2, gives rise to a syncytin glycoprotein (Blond et al., 2000; Mi et al., 2000), the expression of which, similar to HERV-W env, is increased by threefold in the brain tissue of MS patients compared with the controls (Antony et al., 2004; van Horssen et al., 2016). HERV-W env and syncytin expression is confined to immunologically active cells, including cells resembling activated glia and phagocytic macrophages at acute and chronic MS demyelinating lesions (Antony et al., 2004; van Horssen et al., 2016). Syncytin activation leads to a myriad of MS-associated pathology, such as pro-inflammatory profile in astrocytes, interleukin-1β (IL-1β) production, cellular protein oxidation, inhibition of oligodendrocyte maturation, myelin damage, and antagonization of remyelination up to neurobehavioral deficits (Antony et al., 2004).

The central role of HERV-W env in MS neurodegeneration has led to the development of a specific monoclonal antibody, Temelimab (GNbAC1) (Curtin et al., 2012), an agent currently being tested in clinical phase II (ClinicalTrials.gov identifier: NCT02782858).

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, characterized by progressive loss of cortical and spinal motor neurons. While majority of ALS cases are sporadic, mutations in certain genes, such as trans-activation responsive (TAR) DNA-binding protein 43 (TDP-43), have been associated with ALS development (Yousefian-Jazi et al., 2020).

That ALS might be linked with viral infections, comes from finding HIV and HTLV-1 presence in the brains of ALS patients (Verma and Berger, 2006). Further, antiretroviral therapy of HIV-infected individuals with ALS-like syndrome reverses the symptoms related to ALS (Moulignier et al., 2001). The CSF of ALS patients negative of HIV, contains viral reverse transcriptase at levels seen in HIV-infected individuals (MacGowan et al., 2007; McCormick et al., 2008). This has led to investigation of HERVs and revealed the central role of HERV-K among the HERVs in ALS pathogenesis. HERV-K pol, gag, and env are all transcriptionally active in the prefrontal, sensory, motor, and occipital cortex of ALS patients (Douville et al., 2011; Li et al., 2015) and HERV-K env additionally in spinal neurons of sporadic ALS patients. Higher serum IgG and IgM reactivity toward HERV-K gag is also characteristic to ALS patients (Li et al., 2015). HERV-K in ALS can be activated by several mechanisms and occur from distinct cytogenic loci (at 7q36.1) (Douville et al., 2011). These mechanisms include neuronal injury and neuroinflammation through interferon-stimulated response elements in the viral promoter (Gonzalez-Hernandez et al., 2014; Manghera et al., 2016). Once activated, neuronal HERV-K upregulation contributes to sustained neuroinflammation through promoting nuclear translocation of IFN regulatory factor 1 (IRF1) and NF-κB isoforms p50 and p65 (Manghera et al., 2016). Also, TDP-43 activates HERV-K upon binding its DNA (Li et al., 2015). HERV-K and TDP-43 expression in ALS are strongly correlated (Douville et al., 2011). HERV-K env expression leads to neurite retraction, beading, and neurodegeneration (Chen et al., 2014; Li et al., 2015).

Schizophrenia Spectrum Disorders

Schizophrenia is a neuropsychiatric and neurodevelopmental disorder characterized by episodes of psychosis, hallucinations, and delusions. Disease typically starts in young adulthood and is strongly affected by genetic background and environmental factors (Owen et al., 2016). The neurobiology behind schizophrenia is poorly understood.

The likelihood of developing schizophrenia is increased by infections (most significantly evidenced with studies on HHV-2) (Arias et al., 2012), subclinical inflammation (Frydecka et al., 2018), and variation within brain-associated and immune genes (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Altered immune response and lasting inflammatory dysregulation of the nervous system are associated with chronic stress exposure (Pearce et al., 2019; Nettis et al., 2020). Upregulated immune response genes induce hyperactivation of LINE-1, which is common in schizophrenia patients (Bundo et al., 2014). Increased retrotransposition of LINE-1 is found in the neurons of prefrontal cortex, affecting intragenic regions and synaptic genes (Bundo et al., 2014).

In addition to LINE-1, expression of several HERV families, such as HERV-K, HERV-W, and HERV-H, has been shown to be dysregulated in the brains, cerebrospinal fluid, and blood of schizophrenia patients (Perron et al., 2012b; Li et al., 2019; Mak et al., 2019). This could involve activation of distinct HERV loci. For example, activated HERV-W env transcripts in schizophrenia have been shown to differ from these activated in bipolar disorder or MS. Combined with HERV-W copy number differences between schizophrenia patients and healthy controls, this might point to perinatal HERV-W activation (for instance by infections such as influenza), potentially leading to inflammation and subsequent neurotoxicity (Limosin et al., 2003; Perron et al., 2008, 2012b). HERV-W env protein expression in developing hippocampus was recently shown to alter the N-methyl-d-aspartate receptor (NMDAR)-mediated synaptic organization and plasticity. This was associated with defective glutamate synapse maturation, behavioral impairments, and psychosis

(Johansson et al., 2020). Apart from HERV-W, lower DNA methylation levels at HERV-K sequences in peripheral blood have been shown to be specific to early stages of schizophrenia (Mak et al., 2019).

Neuropathogenesis of SARS-CoV-2

Highly pathogenic severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a single-stranded RNA virus from the coronavirus's family (Su et al., 2016).

SARS-CoV-2 enters host via its immunogenic spike glycoprotein binding to ACE2 receptor on endothelial and smooth muscle cells (Lan et al., 2020; Kaneko et al., 2021). Resultant coronavirus disease 2019 (COVID-19) mainly presents as an acute respiratory disease; however, also neurological symptoms have been reported, including dizziness, headache, encephalitis, seizures, intracerebral hemorrhage, and stroke (Benger et al., 2020; Guadarrama-Ortiz et al., 2020; Huang et al., 2020; Lu et al., 2020; Moriguchi et al., 2020). In addition, neuromuscular and autoimmune complications are associated with COVID-19. These include most frequently Guillain Barré syndrome but also Miller Fisher syndrome, polyneuritis cranialis, acute myelitis, oculomotor paralysis, and Bell's palsy have been reported (Guadarrama-Ortiz et al., 2020; Katyal et al., 2020). SARS-CoV-2 uses the olfactory nerves and possibly the vagus nerve to access the brain (Guadarrama-Ortiz et al., 2020; Liu et al., 2020) where ACE2 receptor is expressed on neurons and glial cells (Zou et al., 2020). The neuromuscular invasion of SARS-CoV-2 likely involves retrograde axonal transfer of the virus in trans-synaptic pathway and cytokine storm (Katyal et al., 2020).

SARS-CoV-2 viral proteins interact with human proteins that regulate cellular longevity and aging, and are involved in stress response, vesicle trafficking, lipid metabolism, production of reactive oxygen species, RNA regulation, ubiquitin ligases, and mitochondrial activity (Gordon et al., 2020). Impaired lysosomal function combined with inhibition of ubiquitin–proteasome system can cause protein misfolding and protein aggregation in affected cells, including neurons, a common mechanism of many neurodegenerative diseases (Lippi et al., 2020).

Up to one third of COVID-19 patients develop neurological symptoms beyond the acute stage of the disease, mainly manifesting with chronic fatigue syndrome and myalgic encephalomyelitis (Nath and Smith, 2021). That several coronaviruses (CoV-OC-43, CoV-229E, and HCoV) are found in the brains of MS patients or have been associated with MS pathology (Burks et al., 1980; Murray et al., 1992) could indicate MS-like demyelinating neuropathology as a possible long-term complication of COVID-19. Further, it remains to be investigated how SARS-CoV-2 affects the expression of HERVs, LINE-1, and Alu elements and interacts with other viruses and environmental factors.

AGING

Aging is a progressive deterioration of physiological functions at the cellular, organ, and organism levels eventually leading to senescence. Aging disrupts the balance between the nervous and the immune system and increases risk for various neurodegenerative and neuroinflammatory diseases (Streit et al., 2004; Godbout et al., 2005; Valdes-Ferre et al., 2020).

Aging increases genomic instability (Lombard et al., 2005) by gradual loss of global DNA methylation and region-specific DNA hypermethylation (Jung and Pfeifer, 2015). Increased age-related activation of certain retrotransposon families is found in mice (ERVs) (Odaka, 1975) and Drosophila (LINE-like R2, LTR element gypsy transcripts, and env glycoprotein) (Li et al., 2013). In humans, aging causes profound de-repression of HERV-K, Alu, and LINE-1 elements (Bollati et al., 2009; Cardelli, 2018) with increasing chromatin openness at Alu, SVA, and LINE-1 elements in senescent cells (De Cecco et al., 2013). This affects most significantly evolutionarily younger elements (De Cecco et al., 2013). Transcription levels of HERV-H, HERV-K, and HERV-W change in distinct patterns during human life. HERV-H is highly transcribed in childhood, while HERV-K and also HERV-W transcription increases on reaching higher age (Balestrieri et al., 2015; Autio et al., 2020). ERV activation in aging Drosophila causes shorter lifespan, neurodegeneration, and memory deficits (Li et al., 2013). Similar effects of ERV activation on hippocampal memory and cognitive impairment are observed in mice (Sankowski et al., 2019). Particularly, in combination with chronic inflammation, the effect of HERV activation in aging brain can be detrimental and contribute to neuronal decline (Johnston et al., 2001; Sankowski et al., 2019). In addition to HERVs, LINE-1 hypomethylation has been described in the peripheral blood of elderly individuals (Mahmood et al., 2020). Some of the age-related epigenetic changes, such as those related to Alu methylation, seem to be regulated by longevity-associated genetic factors, including genes involved in nucleotide biosynthesis, metabolism, and signal transduction (Gentilini et al., 2013).

Aging can determine the outcome of interplay between endogenous and exogenous viruses. Interaction of the endogenous murine leukemia virus with the generally non-pathogenic murine togavirus lactate dehydrogenase-elevating virus leads to a fatal and progressive neurological disease in up to 100% of aged mice. This suggests convergence of age-related, genetic, immunological, and viral factors in the development of a neurological disease resembling ALS in humans (Contag and Plagemann, 1989).

CONCLUDING REMARKS

This review brings together studies that have described a role for exogenous viruses and (H)ERVs in CNS pathologies and thereby highlights the interplay between the inherent and the foreign. A contribution of exogenous and endogenous viruses, separately and together, is increasingly evident in common forms of dementia in young (HAND) and elderly population (AD), MS, ALS, and also schizophrenia. In other neurological complications of viral origin, such as SARS-CoV-2, it remains to be seen if and how HERV, LINE-1, and Alu expression may be involved. Viral CNS infections can be early triggers

of neuroinflammation; however, if viruses are successfully combated or entered in a latent state, a central role might be attributed to endogenous retroviruses. ERV activation during infections seems to be a common (physiological) mechanism that the host may not be able to control at some point. ERVs can become continuously activated and sustain the inflammatory imbalance. The crosstalk with IFN seems to play an important role here. Facilitating factors that are associated with continuous ERV activation such as aging, stress, and other comorbidities as well as re-awakening of a latent virus, cumulative or opportunistic infections, as seen in immune-deprived conditions, contribute to the progressive neurodegeneration or delayed CNS pathologies. We are only beginning to understand how exogenous viruses in connection with HERVs and other retroelements affect normal aging and development of neurodegenerative diseases and other neuropathologies. The central role of HERV-W in MS pathology has led to its targeting in clinical trials. It remains to be seen, whether other HERVs could provide key targets in other neurodegenerative diseases, such as HERV-K in ALS, to which there is currently no cure.

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REVIEW
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The Contribution of Gut Microbiota–Brain Axis in the Development of Brain Disorders

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Different bacterial families colonize most mucosal tissues in the human organism such as the skin, mouth, vagina, respiratory, and gastrointestinal districts. In particular, the mammalian intestine hosts a microbial community of between 1,000 and 1,500 bacterial species, collectively called "microbiota." Co-metabolism between the microbiota and the host system is generated and the symbiotic relationship is mutually beneficial. The balance that is achieved between the microbiota and the host organism is fundamental to the organization of the immune system. Scientific studies have highlighted a direct correlation between the intestinal microbiota and the brain, establishing the existence of the gut microbiota–brain axis. Based on this theory, the microbiota acts on the development, physiology, and cognitive functions of the brain, although the mechanisms involved have not yet been fully interpreted. Similarly, a close relationship between alteration of the intestinal microbiota and the onset of several neurological pathologies has been highlighted. This review aims to point out current knowledge as can be found in literature regarding the connection between intestinal dysbiosis and the onset of particular neurological pathologies such as anxiety and depression, autism spectrum disorder, and multiple sclerosis. These disorders have always been considered to be a consequence of neuronal alteration, but in this review, we hypothesize that these alterations may be non-neuronal in origin, and consider the idea that the composition of the microbiota could be directly involved. In this direction, the following two key points will be highlighted: (1) the direct cross-talk that comes about between neurons and gut microbiota, and (2) the degree of impact of the microbiota on the brain. Could we consider the microbiota a valuable target for reducing or modulating the incidence of certain neurological diseases?

Keywords: gut microbiota, neurological disorders, gut microbiota-brain axis, enteric nervous system, anxiety and depression, autistic spectrum disorders, multiple sclerosis

INTRODUCTION

The mammalian intestine hosts a microbial community of approximately 1,000–1,500 bacterial species called the "microbiota," destined to evolve over the course of the host's life and over the generations and subject to environmental changes. It has been amply demonstrated that the composition of the intestinal microbiota is also influenced by diet, age, lifestyle, and the presence of inflammatory processes (Na et al., 2017; Kim and Jazwinski, 2018), so it is accurate to say that the composition of the microbiota differs substantially from individual to individual. The microbial genome sequences contain approximately 3×10^6 genes, 150 times the length of the human genome. In addition, the commensal microorganisms that reside in the intestine exceed human somatic cells at a ratio of about 10:1 (Na et al., 2017). In healthy adults, the microbiota is primarily composed of five bacterial phyla: Firmicutes (79.4%), Bacteroidetes (16.9%), Actinobacteria (2.5%), Proteobacteria (1%), and Verrucomicrobia (0.1%) (Davenport et al., 2014). Normally, the gut microbiota consists of a high diversity and abundance of microbial populations, and this condition is known as "eubiosis." Over the span of a lifetime, a wide range of factors, including an incorrect diet, sleep disorders, obvious pathological conditions, drug abuse, pharmacological therapy, and many others, can alter diversity and abundance of the microbiota leading to a state of "dysbiosis" (Iebba et al., 2016). The symbiotic relationship between the gut microbiota and the host organism has been described as mutually beneficial: the host provides the nutrients and a suitable habitat for the microbiota, while the gut microbiota supports the host's intestinal development and maturation by providing nutrients. Therefore, a state of co-metabolism is generated between the microbiota and the host system (Obrenovich, 2018). Over the past 15 years, it has been highlighted how the microbiota is able to control and influence certain segments of the physiology of the host such as the immune system, the digestive system, and the brain (Morrison and Preston, 2016; Rooks and Garrett, 2016; Dinan and Cryan, 2017). For example, the microbiota plays a vital role in the formation of the host's immune system, and it can be said that there is real cross-talk between these two districts, which allows the development of the host's tolerance to the harmless antigens of the microbiota. Studies in germ-free animals (GF) have shown that the lack of the gut microbiota leads to significant deficiency in the functioning of the immune system (Belkaid and Harrison, 2017).

Until a few years ago, it was a common opinion that a fetus developed in a completely sterile uterine environment and that the first intestinal colonization occurred from birth onwards. However, recent studies have disproved this conception and have demonstrated the presence of microorganisms in the placenta, amniotic fluid, and umbilical cord (Aagard et al., 2014; Pelzer et al., 2017). It has been hypothesized that the fetus begins to colonize its own developing gastrointestinal tract by swallowing the amniotic fluid and the bacteria it contains in the uterus. In addition, fetal and newborn meconium contains microorganisms (Walker et al., 2017). It is only with childbirth, however, that infants are exposed to most of the microorganisms responsible for intestinal colonization and the development of the microbiota. Moreover, the type of delivery a newborn undergoes is very important since the initial intestinal microbiota of the baby could resemble, in terms of composition, the microorganisms with which it came into contact during delivery. For example, following a vaginal birth, the baby comes into contact with the vaginal microbiota, while following a caesarean section the child comes into contact with the epidermal microbiota (Shao et al., 2019). It has also been shown that babies born through natural childbirth could develop a more varied microbiota than babies born by cesarean section (Nagpal and Yamashiro, 2018; Jagodzinski et al., 2019). Despite microbial exposure *in utero*, most of the microorganisms that will colonize the infant's intestine are acquired after childbirth. The initial colonization pattern is believed to be chaotic, and numerous studies suggest that environmental factors and diet are responsible for major changes in composition (Savage et al., 2018). In a child, during the first phase of intestinal colonization, the microorganisms present are predominantly aerobic, such as Enterobacteria, *Staphylococci*, and *Streptococci*, many of which have a pathogenic potential. Subsequently, microorganisms become predominantly anaerobic. The composition of the intestinal community continues to change during the first year of life and thereafter in response to external factors such as diet and the use of antibiotics (Hill et al., 2017). It has been highlighted that a significant difference in the composition of the baby's intestinal microbiota occurs in relation to the type of milk he drinks, the type of weaning he undergoes, and the different types of foods he consumes (Brahm and Valdés, 2017). Breastfeeding (BF) is the nourishment conceived by nature for newborns and infants, although in the last decades, it is very frequently replaced with various milk formulations (formula-fed, FF). In general, it is possible to say that BF has proven to be a protective factor for many inflammatory bowel diseases as well as for neurodevelopment, while the use of the various types of milk formulated for children has been shown to increase the risk of intestinal diseases, following an incorrect formation of the intestinal microbiota (Le Doare et al., 2018). BF infants have a more uniform intestinal microbial population than FF infants. This aspect has very important implications for the future of the child: in fact, the study of the intestinal microbiota of a BF newborn could furnish fundamental information on the correct development of the immunitary system, the immune response and tolerance and for the tendency to develop fewer allergic, inflammatory, and autoimmune pathologies (Vieira Borba et al., 2018). The composition of breast milk includes proteins, fats and carbohydrates, as well as immunoglobulins, endocannabinoids, and indigestible polysaccharides. Some of these polysaccharides act as real prebiotics capable of selectively stimulating the growth of beneficial bacteria (Sayres and Visentin, 2018); most of these are Bifidobacteria, indispensable for strengthening the protection of the intestinal mucosa through their specific activity against pathogens and through the increase in immunoglobulin A, related to the modulation of the intestinal immune system. After weaning, the composition of the intestinal microbiota still varies in relation to the type of feeding, while after 3 years of life, in the absence of disturbances such as long-term dietary

changes or the repeated use of antibiotics and drugs, the bacterial composition of the intestinal microbiota remains approximately stable until old age. In general, over the course of life, the Bifidobacteria decrease while the Bacteroidetes and Firmicutes increase (Derrien et al., 2019).

Much scientific evidence has suggested that the intestinal microbiota maintains bidirectional interaction with critical parts of the central nervous system (CNS) as well as the immunitary system through both direct and indirect pathways (Petra et al., 2015; Fung et al., 2017). In addition, intestinal microbiota dysbiosis has been closely linked to various diseases, such as obesity, type 2 diabetes mellitus, hypertension, necrotizing enterocolitis, and many inflammatory bowel diseases (Weiss and Hennet, 2017; Allaire et al., 2018). Moreover, the existence of a close correlation between the intestinal microbiota and the brain has become increasingly evident even though the mechanisms involved are not completely clear: the existence of a gut–brain axis has become the main focus of neuroscience (Cryan et al., 2019). Evidence that dysfunction of the microbiota can play a key role in the development of certain neurological diseases is provided by the discovery that intervention that restores microbiota health and integrity may have a positive influence on the course, symptoms, and clinical conditions of said diseases (Julio-Pieper et al., 2014; Patel and DuPont, 2015; Fiorentino et al., 2016; Boyton and Altmann, 2019; Garg et al., 2019). This is the main reason why the intestine is called the "second brain" (Ali et al., 2019). In this direction, it would be interesting to consider neurological disorders and pathologies related to neurodegeneration, not as of being of "neural origin," but rather as being linked to other external factors, and the health of the intestinal microbiota could be one of these factors. In the light of what has been stated, it is clear how important the first phase of intestinal colonization is. A consecutive question is, "To what degree can proper intestinal colonization affect the possibility of developing neurological disorders?" Therefore, the main purpose of this review is to consider the alteration of the microbiota as a likely cause of numerous neurological and degenerative disorders.

In the following sections we will first deepen current knowledge on the dysfunction of the microbiota in several cerebral diseases, and their "non-neuronal origin"; later, we will compare these scientific data with the classical knowledge that identifies the brain as the primary cause of some specific disorders. Our attempt, as already mentioned, will be to shift the direction of the interpretation of these pathologies "from the microbiota to the brain" instead of "from the brain to the microbiota."

NEUROLOGICAL DISORDERS AND MICROBIOTA: FROM THE MICROBIOTA TO THE BRAIN

Neurological disorders are diseases of the central and peripheral nervous system that can impair the functioning of brain, spinal cord, cranial and peripheral nerves, autonomous nervous system, nerve roots, and neuromuscular plaque. The causes can be many: (a) diseases due to gene alteration; (b) degenerative diseases characterized by the progressive loss of populations of neurons that are selectively vulnerable; (c) diseases of blood vessels that may cause bleeding in the brain; (d) diseases due to problems in the development of the nervous system; (e) disorders due to injury to the spinal cord or brain; (f) convulsive disorders; (g) brain tumors; (h) more or less severe infections (Dugger and Dickson, 2017; Chi et al., 2018). Up to now, neurological disorders have always been considered to be a specific consequence of morphological and/or functional alterations of some neuronal segment. In this review, we will NOT consider them as such, but rather as the result of the alteration of the gut microbiota. Three neurological disorders will be explored below: anxiety–depression, autistic spectrum disorder (ASD), and multiple sclerosis. Despite the fact that these pathological conditions have completely different characteristics, they seem to have some points in common:

- involve the CNS;
- can present themselves in pathological form at a very early stage in life;
- are closely related to intestinal dysbiosis.

Can we identify intestinal dysbiosis as the actual cause of some of these neurological disorders? Could intestinal dysbiosis be considered to be the common denominator of the three neurological disorders that we are considering? We will try this approach with the help of current literary knowledge.

Anxiety and Depression

Anxiety and depression are psychiatric and neurological disorders that occur in 25% of the global population. In addition, these two pathological states seem to be closely related: in fact, 90% of patients with anxiety disorders also develop depression and 85% of patients with depression show significant anxiety (Bui and Fava, 2017). The phenomena of anxiety and depression can occur as early as childhood or adolescence, as well as at any other time in an individual's life. These two pathologies, both in early and late forms, considerably differ in terms of clinical symptoms (Groeneweg-Koolhoven et al., 2017). In recent decades, the increase in depressive symptoms has also led to an increase in the number of teenage deaths from suicide (Jorm et al., 2017; Matsumoto et al., 2017; Weinberger et al., 2018; Twenge et al., 2019). The states of anxiety and depression are constantly associated with changes in the composition and stability of the intestinal microbiota and this close correlation has been studied (Dinan and Cryan, 2013; Tognini, 2017; Zhao et al., 2018; Thomaz et al., 2019). In an important scientific study on animal models, it was found that the transfer of the microbiota from models with depression to that of other animals deprived of their microbiota also determined the transfer of the behavioral and physiological characteristics of the depression (Kelly et al., 2016). Since it is currently widely accepted that our intestinal microbiota is essential for brain processes (myelination, neurogenesis, microglia activation, and psychological processes such as mood and cognition) (Dinan and Cryan, 2016), the early formation of a well-balanced microbiota and its maintenance

throughout life seems to reduce the occurrence of a wide variety of diseases, including behavioral and neuropsychiatric disorders (Cenit et al., 2017). Childhood and adolescence are the most dynamic and vulnerable periods in the life of an individual for developing and achieving the composition of the microbiota and certain events and conditions may be important contributors including diet, drugs, stress, and infections (Borre et al., 2014; Erny et al., 2015). Still, although the composition of the gut microbiota may vary in adulthood as a result of the effects of harmful or negative factors, the symbiotic link between the host and microbiota are established early in life (Desbonnet et al., 2015). Stress-related disorders encourage the increase of some bacterial species (Kelly et al., 2015). A study conducted on a group of healthy students showed that, following an extremely stressful period, fewer species of *Lactobacillus* were present in the stool. Moreover, a condition of chronic stress induced in mice reduced *Lactobacillus, Eubacterium rectale*, *Lachnospira, Butyricicoccus, Sutterella*, and *Faecalibacteria* and increased the number of pathogenic species *Clostridium,* Proteobacteria, Actinobacteria, and *Enterobacteriaceae* (Tian et al., 2019). An important and well-organized experimental work revealed that an altered composition of the intestinal microbiota induced in mice through the use of massive doses of antibiotics in a period corresponding to the early adolescence of the animals, led to alterations in cognitive function and the appearance of symptoms related to anxiety and depression (Zeraati et al., 2019). It has been suggested that the type of diet can have positive or negative effects on depression: in particular, human studies have shown that an inversely proportional correlation exists between the consumption of omega-3 polyunsaturated fatty acids and anxiety–depression; that is, the more the assumption of omega-3, the less anxiety–depression (Grosso et al., 2014; Oppedisano et al., 2020). Finally, treatment with different probiotics has been particularly effective in reducing depressive behavior in animal models. For example, the administration of a probiotic cocktail, composed of *Lactobacillus rhamnosus* and *Lactobacillus helveticus,* reduced their symptoms of depression and anxiety.

Autism Spectrum Disorder

ASDs include a set of alterations in neurological development characterized by deficits in social interaction and communication, and repetitive and stereotyped behavior. ASD can vary in terms of symptomatic severity, varying from mild to very severe. The main symptoms of ASD appear as early as the first year of life and include delays in language development, repetitive movements, very few interests, limited or absent eye contact, limited sharing of feelings or interests, significant discomfort arising from a change in routine, failure to start and maintain conversations, strong attachments to specific objects, excessive reaction to sounds or visual cues, loss of interest in social relationships, and difficulty in engaging in imaginary play. To date, there is no specific treatment for these disorders and early medical behavioral therapy has been shown to improve but not resolve problems relating to mental capacity, language, and social ability (Howlin and Magiati, 2017; Lord et al., 2018). ASD is a pathology with an unclear and multifactorial etiology, yet several causes have been identified, which include genetic anomalies, dysregulation of the immune system, inflammatory processes, and interaction with environmental factors (Famitafreshi and Karimian, 2018).

ASD disorders are often associated with gastrointestinal co-morbidities and a large proportion of patients (23–70%) also suffer from constipation, diarrhea, abdominal pain, flatulence, and intestinal gas (Mulle et al., 2013). They are also associated with food restriction and eating problems (such as "selective and picky eaters" who show aversion to specific colors, textures, odors, or other food characteristics) (Cermak et al., 2010). The consequences of this are reduced dietary quality, nutritional deficiency, and altered composition of the intestinal microbiota (Berding and Donovan, 2016). Generally, the composition of the intestinal microbiota of autistic children shows substantial differences: the data in the scientific literature indicate overall a reduction of *Bacteroides* with a ratio (% ASD children/% control children) equal to 0.71; a reduction of *Bifidobacterium* with a ratio equal to 0.52; a reduction of *Escherichia coli* with a ratio of 0.3; an increase in *Faecalibacterium* with a ratio of 1.32; and an increase in *Lactobacillus* with a ratio of 2.17. The presence of *Clostridium* remains substantially unchanged (Tomova et al., 2015). Although it cannot be said that there are specific bacteria compatible and associated with the onset of ASD, it is clear that these neurological disorders are accompanied by lower levels of beneficial bacteria and higher levels of harmful bacteria (Iglesias-Vázquez et al., 2020). It has been hypothesized that the increase in *Faecalibacterium* in ASD children is responsible for the progression of inflammatory processes, with increased levels of type I interferon, and the alteration of the intestinal barrier. In addition, the reduction of *Bifidobacteria*, the main producers of lactic acid capable of suppressing the growth of pathogenic bacteria, leads to an alteration of the immunitary system (Hashemi et al., 2017). The reduction of *Bifidobacterium* also results in reduced levels of short-chain fatty acids (SCFAs), common in ASD children.

In ASD patients, an important correlation exists between the aforementioned active neuropeptides and disability. Their incorrect interaction involves a series of inflammatory disorders, autoimmune conditions, neurodegenerative and metabolic disorders, as well as problems regarding mood, behavior, cognitive function, autistic spectrum dysfunction, stress, and pain (Lerner et al., 2017). A likely mechanism could be that proteic transducers escape from the gastrointestinal tract and enter the bloodstream exerting a systemic effect (Mead and Ashwood, 2015).

The bacteria that make up the gut microbiota and their metabolites could play a critical role in the pathophysiology of ASD (Xu et al., 2019). In fact, some experiments have shown that patients who have had their intestinal microbiota remodeled through the administration of antibiotics or bacterial transfer therapy in the intestine, presented with attenuated symptoms of ASD (Kang et al., 2017). The administration of probiotics was sufficient to vary the composition of the microbiota and to guarantee greater control of the intestinal barrier (Doenyas, 2018). The mechanisms studied so far that correlate the intestinal microbiota with ASD disorders are manifold and concern the breakdown of the integrity of the intestinal barrier, the

production of toxins, and the formation of intestinal dysbiosis (Ding et al., 2017). Another extremely important aspect concerns the increase in neurotoxins produced by the intestinal system of ASD children that act distally on the brain (Yang and Chiu, 2017). It is also important to highlight the fact that the microbiota and the metabolites formed by it are indispensable for the maintenance of cerebral white matter and the integrity of the blood–brain barrier (Golubeva et al., 2017).

Autoimmunity and Multiple Sclerosis

Innate immunity is the host's first defensive line for eliminating invading and foreign pathogens. Through this type of immunity, in fact, critical mechanisms are activated for the rapid detection and elimination of pathogens. This type of immunity does not have immune memory and can only be based on specific receptors, which have been selected during the evolutionary process and which can only bind to the same unchanged antigens. Conversely, adaptive immunity has evolved with the aim of providing a vast repertoire of antigenic recognition of self- and non-self-molecules. Adaptive immunity uses the strictly regulated interaction between the cells presenting the antigen and the B and T lymphocytes. These cells consequently activate the immunological effector pathways in order to contrast the specific pathogen. In addition, adaptive immunity has an immunological memory capable of recognizing an antigen that has already been encountered and destroying it (Vatner and Janssen, 2019). Autoimmunity occurs when the immune system loses self-tolerance and begins to counteract its own molecules and cells. If this characteristic of immunological imbalance persists constantly in the body, more or less serious autoimmune diseases develop (Stetson, 2018). Multiple sclerosis (MS) is a demyelinating autoimmune disease of the nervous system and is characterized by chronic inflammation, breakdown of the BBB, and infiltration of immune cells into the CNS. The latter lead to the destruction of the myelin sheath with axonal loss and progressive disability (Matveeva et al., 2018; Maiuolo et al., 2019a,b). In general, it has been shown, in fact, that in MS, the inflammatory process involves T lymphocytes, CD4 and CD8, B lymphocytes, activated monocytes, and astrocytes. Oxidative stress was also a key factor in the pathogenesis of MS: in particular, macrophages and microglia produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) and secrete pro-inflammatory cytokines. These conditions develop neurodegeneration and excitotoxicity (Corasaniti et al., 2007; Oppedisano et al., 2020). It is clear, therefore, that MS is a multi-factorial pathology and genetic, environmental, and immunological factors are included in its etiology. Multiple sclerosis appears particularly in young women (female:male ratio = 3:1), especially in those women who have suffered intestinal disorders since birth (Jose et al., 2018; Sauma and Casaccia, 2020). It has been recently shown that an alteration of the intestinal microbiota leads to over-stimulation of immune cells with a higher incidence of the development of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and MS (Brown et al., 2019). In particular, there is increasing evidence (found in animal models) according to which there is a relationship between the type of intestinal microflora and the progression of MS. According to these scientific data, autoimmune reactions can be produced by molecular mimicry and by the excessive production of lymphocytes (Chu et al., 2018). Compared to healthy controls, patients with MS show a decrease in the proportion *of Faecalibacterium* and *Fusobacterium* and an increase in *Escherichia, Shigella, Clostridium, Eubacterium rectal, Corynebacterium,* and Firmicutes (Tremlett et al., 2016a,b,c; Tremlett and Waubant, 2017). Some metabolic by-products of the intestinal microflora activate the transcription of the gene *foxp3*, responsible for the codification of the FOXP3 protein, a transcriptional regulator also known as scurfin; FOXP3 binds to the promoters of the genes involved in the development and regulation of T-cell receptors, promoting the attenuation of the immune response (Zhang et al., 2019). These microbiota by-products include SCFAs, responsible for activating the FOXP3 pathway and modulating the immune response. When intestinal dysbiosis occurs, this whole regulatory process decays and pathways that lead to autoimmunity are triggered (Khan and Ghazanfar, 2018). In some scientific papers, it has been shown that Bacteroidetes, present in the intestinal microbiota, produce lipid 654, which behaves as a ligand for human and mouse Toll-like receptor 2 (TLR2), a toll-like receptor with a role in the immunitary system (Selmi, 2017). An important scientific study showed that lipid 654 was present in the serum of all the healthy subjects examined. Conversely, extremely low lipid levels were found in MS patients, indicating, for the first time, this lipid as a serum biomarker of MS (Farrokhi et al., 2013; Browne et al., 2019). There are some bacteria, such as *Clostridium perfringens*, which produce natural toxins that are involved in the early stages of MS (Wagley et al., 2019). These toxins are absorbed by the intestine, enter the bloodstream, and cause symptoms typical of those that occur in more or less the early stages of MS, such as blurred vision, lack of coordination, or spastic paralysis (Tsunoda, 2017). The suspicion that these toxins could be the potential cause of MS was already described in the 90s, since man is not a natural host of *C. perfringens*, but becomes so in the case of intestinal dysbiosis, which allows this bacterial family to gain the upper hand (Savva et al., 2019). The migration of these toxins to the CNS occurs precisely as a result of their binding to the receptors present in the vascular system and, in this way, they are conveyed to the myelinated and non-myelinated areas of the brain (Anwar et al., 2019). Experimental MS patients showed an exacerbation of their intestinal balance following the administration of first- and second-line drugs recommended for this pathology. This worsens the picture of the already-compromised microbiota. For example, the administration of the drug Glatiramer Acetate induces a reduction in the "good" *Bacteroidaceae, Faecalibacterium, Lactobacillaceae,* and *Clostridium* as compared to untreated patients (Abdurasulova et al., 2018). BF, as already mentioned, provides the child with a fundamental matrix of immune information regarding the formation of his microbiota. The data available in literature show a clear link between BF and the reduced development of some autoimmune diseases such as MS, diabetes, and celiac disease (Vieira Borba et al., 2018). However, further scientific studies are needed to understand

the mechanisms behind this link. To date, this correlation is also supported by the discovery that in patients with MS the administration of specific probiotics manages to increase several bacterial taxa of the intestinal microbiota that are normally reduced and/or absent. At the immune level, the administration of specific probiotics induces an anti-inflammatory response with the consequent reduction of inflammatory cells, such as monocytes and dendritic cells (Tankou et al., 2018).

CLASSICAL KNOWLEDGE OF ANXIETY–DEPRESSION, ASD, AND MS: BRAIN ORIGIN OF DISEASES

Neurodegenerative diseases are characterized by progressive dysfunction and loss of neurons, which leads to the distinct involvement of a particular functional system. In normal physiological conditions, the death of neuronal cells is mainly limited to the result of aging. In fact, mature neurons have the ability to manage and overcome different stressful conditions in order to maintain cellular homeostasis (Kole et al., 2013; Maiuolo et al., 2020). However, in diseases, the loss of specific neurons of the brain is a fundamental pathological characteristic (Kovacs, 2017) and cell death is the final destiny for a neuron that has accumulated more stressful events than it can recover from: this condition is commonly present in neurodegenerative diseases (Hollville et al., 2019).

For this reason, neurodegenerative diseases can be classified according to (1) the clinical characteristics they present; (2) the anatomical distribution of the neurodegeneration in act; and (3) the main molecular abnormalities encountered (Chi et al., 2018). A common element of many neurodegenerative diseases are aberrant protein aggregates, and their location and composition vary in different diseases (Dugger and Dickson, 2017). Loss of neurons can be appreciated in most neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, and amyotrophic lateral sclerosis (SLA). Until now, anxiety–depression, AD, and MS have all been considered to be disorders, the onset of which is mainly neurological in origin and have been addressed and treated as such. Below, we will describe our principal knowledge of these pathological disorders, the recommended therapies, and the scientific limitations known so far. Anxiety and depression are considered neuropsychiatric disorders and are found in normal cerebral conditions—that is, in the absence of morphological alterations—yet with reduced activity (Marwood et al., 2018). Although the causes of anxiety–depressive disorders are not yet known, it is believed that stress and genetic predisposition are essential factors. In general, stress activates the adrenal glands and leads to the overproduction of cortisol, which chronically stimulates certain brain structures such as the hippocampus and amygdala. This hyperstimulation reduces the volume and functionality of these districts by favoring the onset of the symptoms of anxiety and depression (Fiksdal et al., 2019). Some scientific studies have reported volumetric modifications of parts of the brain in patients suffering from anxiety–depressive symptoms: conducted measurements through nuclear magnetic resonance (NMR) and positron emission tomography (PET) showed a reduction of the amygdala–hippocampus complexes and prefrontal cortexes of these patients as compared to the control group (Gupta et al., 2019). Following pharmacological therapy, the morphological and functional recovery of the aforementioned anatomical structures was found, and in animal models, the antidepressant therapy determined the multiplication of stem cells in the hippocampus and in the amygdala (Ebrahimi-Ghiri et al., 2019). Nevertheless, it is important to emphasize that pharmacological therapy does not completely solve anxiety–depressive symptoms (Akil et al., 2018). The anxiety-depressive syndrome is considered prodromal for numerous neurological diseases of degenerative, inflammatory, or vascular nature: in fact, patients suffering from "neurological depression" may develop these diseases more frequently than the general population. Some epidemiological studies have shown the existence of a bi-directional relationship between neurological disorders and depressive disease: PD, AD, and epilepsy are often preceded by episodes of anxiety–depression (Jacobson and Newman, 2017; Pede et al., 2017; Steffens, 2017). Therefore anxiety–depression can be considered to be a risk factor for neurological disorders. To date, behavioral–cognitive therapy is the first-line treatment for depression and anxiety disorders, although it has been shown to be ineffective in 50% of patients (Cuijpers et al., 2014; Leuzinger-Bohleber et al., 2019), since few patients receive high-quality therapy. In fact, most affected people obtain non-optimal results in terms of inadequate dosages, the appearance of side effects, and interaction with drugs taken for other diseases. Many are not treated at all (David and Gourion, 2016). Psychological intervention through drug therapy is particularly recommended, and these associated therapies have shown benefits for the treatment of both depression and anxiety (Tolin, 2017; Marwood et al., 2018).

As has already been described, ASD is a pathology with an unclear and multifactorial etiology: in fact, among the causes considered to date, there are genetic abnormalities, dysregulation of the immune system, inflammatory processes, and interaction with environmental factors. Precisely for this reason, the diagnosis of this disorder is particularly problematic. Neural systems, involved in ASD, include the upper right temporal area, amygdala, prefrontal cortex, hippocampus, and Broca area, responsible for emotions, memory motor coordination, phonological processing, and executive functions. It follows that a certain vulnerability in the socio-behavioral system may be a risk factor for ASD (Ecker et al., 2015). To date, there is no specific treatment for these disorders and early medical behavioral therapy has been shown to improve, but not solve, deficits in mental capacity and linguistic and social abilities (Howlin and Magiati, 2017; Lord et al., 2018). In addition, support services are overburdened or insufficient (Shattuck et al., 2012; Hollocks et al., 2019). ASD not only involves affected patients and their families but also has an economic impact on overall spending: in fact, there are significant direct costs associated with ASD, which include expenses related to provision of special education, housing, and medical care programs, and indirect costs such as loss of productivity affecting individuals with ASD (Buescher et al., 2014). At present, the prevailing

practice follows a pattern of "wait and see" (whether delays worsen the situation or allow it to resolve itself) or "wait to fail" (identification occurs when ASD is established). It has been shown that over 50% of children with ASD do not receive a correct diagnosis before the sixth year of age and today scientific knowledge is aimed at determining a more precocious intervention (Baio et al., 2018). For most people, the obvious symptoms of ASD will not become apparent until childhood or later with the first problems being reported at around 32 months of life. Differences in social communicative characteristics will gradually emerge during the second year, so it would be difficult to act more quickly than the known practice (Estes et al., 2015; Sacrey et al., 2019). The North American Prodrome Longitudinal Study (NAPLS) has considered the possibility that young patients with ASD may develop a full-blown psychosis during their lifetime. The results obtained showed a correlation between ASD and psychosis in 18% of patients with ASD. However, due to the difficulty of the study, which tends to follow enrolled patients for a long time, there is a need to expand research (Foss-Feig, 2019).

Multiple sclerosis, as has already been said, is an autoimmune inflammatory disease that affects the CNS, brain, and spinal cord, characterized by demyelination and axonal loss. Although the etiology of MS remains unknown it is a common opinion that the disease is caused by an immune dysregulation triggered by genetic and environmental factors. The loss of function of the axons classifies MS as both a progressive and degenerative disease. Current pharmacological therapy includes the administration of anti-inflammatory corticosteroids, immunomodulators, or humanized monoclonal antibodies, all of which could help to alter the course of the disease. In summary, we can say that significant progress has been made in the area of MS therapy, but testing should continue in order to increase the arsenal of new therapeutic agents that can prevent or minimize the neuronal and/or axonal degeneration that occurs. Because of the innumerable, indefinite causes of this disease, there is a tendency to consider it neurological in origin, since the main symptoms lie in this seat. It would be well, however, to start to change the classic point of view.

MICROBIOTA–BRAIN COMMUNICATION

Recent scientific literature has highlighted the close correlation existing between the intestinal microbiota and brain development as well as a correspondence between alteration of the intestinal microbiota and the onset of some neurological pathologies such as anxiety and depression (Strandwitz, 2018), PD, AD (Sun and Shen, 2018), multiple sclerosis (Berer et al., 2017), cerebral ischemia (Nam, 2019), and ASD (Fattorusso et al., 2019). Based on these scientific findings, it is clear that any form of intestinal dysbiosis is able to favor the development of neurological diseases. For just this reason, it is fundamental to know and understand the instruments of dialogue that exist between the intestine and the brain. The intestine can interact with the brain through direct communication, which includes three main mechanisms:

- the enteric nervous system;
- the enteroendocrine cells (EECs) of the gut;
- neurotransmitters produced by the gut microbiota.

Enteric Nervous System

Functional aspects of the gastrointestinal tract such as peristaltic movements, the transport of substances, and the local flow of blood are all regulated by a network of neuronal ganglia known as the enteric nervous system (ENS) (Furness, 2000; Furness et al., 2004). It is known that the neurons of the ENS communicate with each other using the same "language" as in the CNS (Giuffrè et al., 2020). The ENS consists of two ganglion plexuses composed of neurons and glia that regulate a variety of gastrointestinal functions and are essential for life (Furness, 2006). These plexuses are located between the layers of the gastrointestinal tract and are characterized by about 20 subtypes of neurons that differ by the expression of several neuropeptides (Furness, 2000; Furness et al., 2004). The ENS shares many features with the brain, including the production of neurotransmitters that are used for synaptic transmission, the ultrastructural features present in neuron–glia interaction, and transcriptional programs (Rao and Gershon, 2016; De Vadder et al., 2018). The ENS is capable of operating independently of the brain and spinal cord, but, in healthy subjects, it works in collaboration with them together with input from the vagal, sympathetic, and parasympathetic systems. This is in order to regulate many gastrointestinal functions, such as motility. This direct cross-talk makes the ENS an important target for the pathogenesis of many neurological disorders (Liddle, 2018), and its dysfunction is related to gastrointestinal disorders including severe constipation, anorexia, and gastroparesis. It is also interesting to note that these symptoms are all common in patients with neurological conditions (Chalazonitis and Rao, 2018). The hypothalamic–pituitary–adrenal axis interacts with intestinal epithelium cells via the vagus nerve. Some preclinical studies have shown that the vagus nerve plays a central role in neural communication between the microbes of the intestine and centrally mediated behavioral effects. In particular, following a vagotomy performed early in childhood, these subjects had a lower risk of developing neurological disorders (Svensson et al., 2015). Vagus nerve stimulation is a medical treatment used to treat epilepsy and other neurological conditions and consists in the application of appropriate electrical impulses to the nerve. It is assumed that these electrical impulses exert antiepileptic (Fornai et al., 2011), antidepressant (Sackeim et al., 2007), and anti-inflammatory action by altering the nerve excitability in the cells involved (Breit et al., 2018). A close correlation between the ENS and the microbiota has been demonstrated by the reduced number of enteric neurons and intestinal motility observed in GF mice (McVey Neufeld et al., 2012). In addition important experiments have shown an intrinsically attenuated excitability in afferent primary neurons together with a defective intestinal mucosa in GF mice, despite the development and continuous influx of the ENS (Kabouridis et al., 2015). It is interesting to note that with the administration of the conventional microbiota, the recovery of GF mice saw the density and physiology of the ENS in the intestine normalized (Kashyap et al., 2013). Every microorganism can have a different effect on the ENS: some

commensal bacteria may have a local effect interacting with the ENS, while pathogenic bacteria benefit from the ENS by creating an environment more suited to their growth and advantageous for their effects (Giuffrè et al., 2020). The control exercised by the gut microbiota takes place through the vagus nerve and the ENS (Borre et al., 2014; Kaelberer et al., 2018): classic examples are provided by the bacteria *Lactobacillus rhamnosus,* which can modulate anxious behavior, and *Bifidobacterium longum* NCC3001, which exerts anxiolytic effects. It has been shown in mice that these effects are lost after vagotomy (Bercik et al., 2011; Bravo et al., 2011). The microbiota supports the ENS formed at birth and participates in its homeostasis throughout adult life. In fact, in GF mice, it has been shown that the ENS is highly compromised especially in those areas where bacteria are normally found. Increasing evidence shows that some neurodegenerative diseases such as PD can originate in the intestine and spread to the brain by means of the vagus nerve (Klingelhoefer and Reichmann, 2017). The possibility of a close correlation between the dysfunction of the ENS, the microbiota, and the diseases of the CNS has been considered, even if this hypothesis must be further analyzed and deepened.

Enteroendocrine Cells

EECs reside within the mucosa of the gastrointestinal tract and are electrically excitable. These cells produce more than 20 peptides/hormones in response to signals generated by nutrients, non-nutrient chemicals, food-born toxins, and microorganisms in the bowel lumen (Furness et al., 2013). They influence a variety of physiological functions including digestion and absorption of nutrients, defense responses against harmful/toxic substances, and food aversions (Latorre et al., 2016). These secreted products can act locally, through a paracrine mechanism that activates other EECs, they can be released into the bloodstream, reaching distant targets, or they can act directly on nerve endings near the release site. It is well known that EECs possess many characteristics similar to those of neurons: among these, it is appropriate to remember the receptors of neurotrophins, a family of proteins that induces the survival, development, and function of neurons, and pre- and post-synaptic proteins (Janssen and Depoortere, 2013). The expression of synaptic proteins increases the possibility that the EECs will come into contact with the nerves; there is also a neural circuit that connects the intestinal lumen with the nervous system (Kaelberer et al., 2018). Therefore, it can be said that the EECs represent the first level of integration from the intestinal lumen to the brain capable of generating appropriate functional responses. In particular, the vagal afferent pathways transmit stimuli generated by the EECs to the brain, representing an intermediate station in the bidirectional communication of the brain–intestine axis (Al Omran and Aziz, 2014). A detailed list of EECs, their location, and secreted hormones is shown in **Table 1**.

TABLE 1 | EEC subtypes, localization, and secreted hormones (taken and modified from Grochowska et al., 2019).

Major cell types	Localization	Major secretory hormones	Function
A (X-like)	Pancreas	Ghrelin	• Appetite control
G cell	Stomach, duodenum	Gastrin	• Gastrin secretion
D cell	Pancreas, stomach, intestine	Somatostatin	• Gastrointestinal hormone release; • Gastrointestinal motility; • Mucosal immunity
L cell	Ileum, colon, duodenum	Glucagon-like Peptide-1; Glucagon-like Peptide-2; Peptide YY (PYY);	• Appetite control; • Gastrointestinal motility; • Energy homeostasis
K cell	Stomach	Gastric inhibitory peptide (GIP)	• Insulin secretion
I cell	Duodenum	Cholecystokinin (CCK);	• Appetite control; • Gastrointestinal motility; • Bile acid and digestive enzyme release; • Mucosal immunity
Enterochromaffin cell	Small intestine, colon, appendix	Serotonin (5-HT)	• Appetite control; • Gastrointestinal motor and secretory function; • mucosal immunity
N cell	Small intestine	Neurotensin	• Gastrointestinal motility; • Mucosal immunity
M cell	Peyer's patches	Motilin	• Gastrointestinal motility
S cell	Small intestine, duodenum	Secretin	• Acidity; • Body fluid homeostasis
Enterochromaffin-like cell	Gastric glands	Histamine	• Acidity; • Mucosal immunity

Neurotransmitters Produced by the Gut Microbiota

The intestinal microbiota is also able to synthesize many neurotransmitters such as dopamine, serotonin, norepinephrine, and δ-amino butyric acids (GABA) that also exercise their own effects on the brain. For example, *Bifidobacterium infantis* has been shown to elevate tryptophan levels in blood plasma and thus influence central serotonin transmission; *Lactobacillus* and *Bifidobacterium* can produce GABA; *Escherichia*, *Bacillus*, and *Saccharomyces* spp. can produce noradrenaline; *Candida, Streptococcus, Escherichia*, and *Enterococcus* spp. can produce serotonin; *Bacillus* can produce dopamine; *Lactobacillus* can produce acetylcholine (Lyte, 2014). These neurotransmitters can go through the mucous layer of the intestine and enter the bloodstream, but they are not able to cross the blood–brain barrier. The impact on brain function, therefore, could be indirect by acting on the enteric nervous system (Dinan and Cryan, 2017). SCFAs, which include butyrate, propionate, and acetate, are essential metabolic products of gut microbial activity and may affect the brain, energy balance, and metabolism (Dinan et al., 2015). In addition, SCFAs have neuroactive properties. High doses of propionate, in young rats, induced a neuroinflammatory response and behavioral alterations while

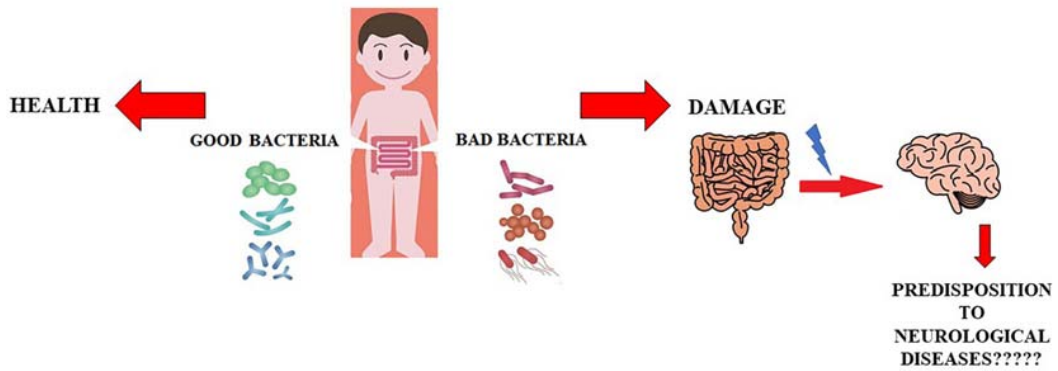

FIGURE 1 | Role of early colonization. In this cartoon the role of early colonization is shown. In particular, when the colonization is caratecrized by appropriate and beneficial bacteria the health status is mantained. On the contrary, whether bad bacteria early colonize the intestinal tract we can have damages in this organ and probably probably a predisposition to onset of some neurological diseases.

butyrate reduced the depressive behavior, exerting an effect on the CNS (Foley et al., 2014). To date, it is known that SCFAs act preferably as epigenetic modulators through histone deacetylases (Stilling et al., 2014). The gut–brain axis has another signaling pathway that involves immunity through cytokines. Cytokines, produced in the intestine, can flow into the bloodstream and, under altered conditions, can affect areas of the brain such as the hypothalamus (El Aidy et al., 2014).

DISCUSSION AND CONCLUSION

This review summarizes the knowledge, to date, on the importance of the intestinal microbiota and how the intestinal bacterial component manages to communicate with the brain (Quigley, 2017). In particular, the continuous cross-talk existing between the intestine and the brain and how the intestinal microbiota maintains constant and continuous interaction with the nervous system is highlighted. Intestinal dysbiosis, in fact, is directly involved in many brain disorders (Westfall et al., 2017; Russo et al., 2018). The causes of neurodegenerative diseases are still unknown, but it is certain that several factors including genetics, lifestyle, and aging play key roles. For example, healthy intestinal barrier function seems crucial for maintaining neurological health (Di Meo et al., 2018) and studies have been conducted to assess microbial composition in patients suffering from neurodegenerative diseases (Mohajeri et al., 2018). The microbiota is able to determine the severity of neurodegenerative diseases through two mechanisms:

- immuno-mediated neurodegeneration (Chen et al., 2016; Dombrowski et al., 2017);
- direct effect of metabolites (GABA, histamine, dopamine, norepinephrine and serotonin) on cells of the CNS (Corasaniti et al., 2007; Bano and Ankarcrona, 2018; Strandwitz, 2018).

In PD, it is interesting to observe that the compromised parts are the most caudal of both the CNS and the enteric nervous system (Clairembault et al., 2015). For this reason, the intestine and its effects on the CNS were investigated and many researchers are trying to evaluate whether PD begins in this organ; what is certainly undisputed is the role of the gut microbiota in the pathology of PD. In humans there are data showing that in the pathophysiology of PD, truncal vagotomy reduces the risk of PD (Perez-Pardo et al., 2017; Lionnet et al., 2018). Several alterations in the composition of the microbiota have been found in patients with PD which include a reduction in Firmicutes, *Clostridium saccharolyticum, Clostridium leptum*, and *Faecalibacterium*. In addition, a reduction of *Prevotella* occurs in the early stages of PD and could work as a biomarker for PD (Keshavarzian et al., 2015; Hill-Burns et al., 2017; Hopfner et al., 2017; Petrov et al., 2017). In the light of this scientific evidence, we can state that the bacterial composition of the colon may be predictive for PD (Li et al., 2017), although further assessments should be conducted.

Several studies in recent years have been carried out and have highlighted the involvement of the intestinal microbiota in the onset and pathophysiology of AD (Hu et al., 2016; Jiang et al., 2017). In particular, significantly decreased *Clostridium leptum* and *Clostridium saccharolyticum* were observed in AD as well as an increased Bacteroidetes phylum and *Alistipes* genus (Gerhardt and Mohajeri, 2018). A disbiotic intestinal microbiota produces and releases a mixture of metabolic products that increase the production of cytokines and inflammatory mediators. These compounds induce the amyloid aggregation present in AD by accumulating Aβ, hyperphosphorylating the Tau protein, and inducing chronic inflammation in the brain. In addition, during aging, regenerative capacities are reduced, leading to an increase in neurodegenerative processes and the clinical manifestations of dementia (Penke et al., 2017).

Among neurological disorders, three pathological conditions have been examined that occur very commonly in the population, which are closely related to the alteration of the microbiota and which can appear at early or very early times in life. These three pathologies are anxiety and depression, autism spectrum disorders, and multiple sclerosis, and despite having completely

different specific characteristics, they have the following points in common:

- are diseases related to the malfunction of the nervous system;
- are closely related to intestinal dysbiosis;
- occur in pathological form in a very early period of life: the phenomena of anxiety and depression can occur as early as childhood or adolescence, but also at any time in an individual's life. Autism spectrum disorders develop and appear during the first year of life. Multiple sclerosis appears particularly in young women (female:male ratio = 3:1), especially in those women who have had intestinal disorders since birth (Sauma and Casaccia, 2020).

This review does not intend to focus attention only on the close gut–brain communication, which is already well-known and studied in-depth, but intends to look at this problem from an identical but opposite perspective, and that is: "What if these pathologies actually had a non-neural onset?" and "Is it possible that these pathologies develop due to an altered microbial composition in the gut?"

If so, an evaluation of the intestinal microbial composition would be fundamental as an early preventive tool against brain diseases. The moment of intestinal colonization, during the very early stages of life, could be fundamental and determinative; in fact, if this process were to occur inadequately, an imbalance in the composition of the microbiota would be set off, which could persist throughout life. So, another vital question is: "How important is the breastfeeding process?" This type of feeding could provide the infant with an already mature and balanced "immune culture" capable of reacting promptly to a wide variety of external pitfalls.

In this direction, it could try to cure not the neurological cause, but directly correct the composition of the microbiota. So, whenever there are any dysbiosis conditions present, it would be desirable to:

- carry out prenatal and neonatal screening to find out the exact composition of the microbiota and in case of alterations correct it by using specific prebiotics and probiotics;
- repeat this screening periodically in order to identify the onset of intestinal dysbiosis;
- start to consider these pathologies as intestinal diseases rather than nervous diseases;
- consider the intake of substances of natural origin capable of establishing a correct oxidative status of the organism.

Further studies and insight into this topic are needed to change the point of view from which these issues are being observed and studied. A conclusive and definitive evaluation is indispensable before automatically assuming that anxiety and depression, ASD, and MS have a strictly neural origin; the hypothesis that intestinal dysbiosis could be the real culprit should be investigated thoroughly. This hypothesis is represented in **Figure 1**.

AUTHOR CONTRIBUTIONS

JM and VMo conceptualized and designed the manuscript. JM, MG, and VMo wrote the manuscript. RM, FO, SN, FB, VMu, CC, FS, MS, EP, SR, MZ, and CM revised the manuscript critically. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Serum and Fecal Markers of Intestinal Inflammation and Intestinal Barrier Permeability Are Elevated in Parkinson's Disease

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Parkinson's disease (PD) is characterized by alpha-synuclein misfolding with subsequent intraneuronal amyloid formation and accumulation, low grade neuroinflammatory changes, and selective neurodegeneration. Available evidence suggests that the pathology usually begins in the gut and olfactory mucosa, spreading to the brain via the vagus and olfactory nerves, by a prion-like mechanism. A causal relationship has not been established, but gut dysbiosis is prevalent in PD and may lead to intestinal inflammation and barrier dysfunction. Additionally, epidemiological data indicate a link between inflammatory bowel diseases and PD. Calprotectin and zonulin are markers of intestinal inflammation and barrier permeability, respectively. We evaluated their serum and fecal levels in 22 patients with sporadic PD and 16 unmatched healthy controls. Mean calprotectin was higher in PD, both in serum (14.26 mcg/ml ± 4.50 vs. 5.94 mcg/ml ± 3.80, $p = 0.0125$) and stool (164.54 mcg/g ± 54.19 vs. 56.19 mcg/g ± 35.88, $p = 0.0048$). Mean zonulin was also higher in PD serum (26.69 ng/ml ± 3.55 vs. 19.43 ng/ml ± 2.56, $p = 0.0046$) and stool (100.19 ng/ml ± 28.25 vs. 37.3 ng/ml ± 13.26, $p = 0.0012$). Calprotectin was above the upper reference limit in 19 PD serums and 6 controls (OR = 10.56, 95% CI = 2.17–51.42, $p = 0.0025$) and in 20 PD stool samples and 4 controls (OR = 30, 95% CI = 4.75–189.30, $p = 0.000045$). Increased zonulin was found only in the stool samples of 8 PD patients. Despite the small sample size, our findings are robust, complementing and supporting other recently published results. The relation between serum and fecal calprotectin and zonulin levels and sporadic PD warrants further investigation in larger cohorts.

Keywords: calprotectin, Parkinson's disease, intestinal inflammation, intestinal barrier permeability, zonulin

INTRODUCTION

Parkinson's disease (PD) is an incurable disorder affecting more than 6 million people worldwide. Its incidence and prevalence increase with age, culminating in the eighth decade (Collaborators, 2018). Disability is related both to motor (i.e., parkinsonism) and non-motor symptoms (including hyposmia and constipation), slowly progressing over the course of many years (Postuma et al., 2015; Balestrino and Schapira, 2020).

The neuropathology of PD is defined by intraneuronal accumulation of alpha-synuclein amyloids, namely Lewy bodies and Lewy neurites, low grade neuroinflammation and selective neuronal dysfunction with subsequent neurodegeneration, involving mainly the aminergic neurocircuits (Braak et al., 2003a; Balestrino and Schapira, 2020). The development of sporadic PD is linked to several environmental or acquired factors that are thought to initiate and promote the disease, especially in genetically susceptible individuals (Hawkes et al., 2007, 2009; Balestrino and Schapira, 2020). The upstream pathogenic events that trigger the initial amyloid transformation of alpha-synuclein (i.e., misfolding, self-aggregation and cross-seeding of the conformational changes) are largely unknown. Increasing evidence suggests that the pathology may begin in the gut and/or the olfactory mucosa, spreading to the cortex by a prion-like mechanism (Braak et al., 2003a,b, 2006; Halliday et al., 2006; Hawkes et al., 2007, 2009; Masuda-Suzukake et al., 2013; Hilton et al., 2014; Svensson et al., 2015; Rietdijk et al., 2017). Intestinal inflammation and barrier dysfunction, as well as products of the gut microbiota (that may cause "leaky gut" and/or local inflammation and may trigger or enhance amyloidogenesis), are thus considered potential key players in the etiopathogenesis of PD (Devos et al., 2013; Houser et al., 2018; Schwiertz et al., 2018; Becker et al., 2019; Mulak et al., 2019; Rolli-Derkinderen et al., 2020; Romano et al., 2021).

Gut dysbiosis and inflammatory bowel disease (IBD) are associated with increased risk of PD (Hill-Burns et al., 2017; Minato et al., 2017; Camacho-Soto et al., 2018b; Heinzel et al., 2019; Park et al., 2019; Pietrucci et al., 2019; Villumsen et al., 2019; Weimers et al., 2019; Zhu et al., 2019; Baldini et al., 2020; Nishiwaki et al., 2020; Nuzum et al., 2020; Lee et al., 2021). Recent studies found that fecal markers of intestinal inflammation, such as fecal calprotectin (routinely used for diagnosing and monitoring IBD) and possibly markers of intestinal barrier permeability, such as fecal zonulin, are elevated in people with PD (Schwiertz et al., 2018; Mulak et al., 2019). Inflammatory shifts of stool immune profiles comparable to those seen in IBD, were also described in PD (Houser et al., 2018). Here we present our preliminary results on the association between sporadic PD and serum and fecal levels of calprotectin and zonulin. To the best of our knowledge, this is the first study evaluating serum calprotectin and zonulin in PD, previous studies assessing only their fecal levels.

MATERIALS AND METHODS

We performed a case-control study investigating the serum and fecal levels of calprotectin and zonulin in adult people with and without PD. Participants were recruited at Colentina Clinical Hospital (Bucharest, Romania) starting from April 2019. Study enrollment was based on predefined inclusion and exclusion criteria—see below. The protocol of the study was approved by the local Ethics Committee (EMI-BPs, 3/16.04.2019). Written informed consent, compliant with the Declaration of Helsinki and the European General Data Protection Regulation 2016/679, was obtained from all participants prior to study enrollment.

To be included in the sporadic PD group, patients had to fulfill the Movement Disorder Society (MDS) Clinical Diagnostic Criteria for either clinically established or clinically probable PD (Postuma et al., 2015); additionally, the patients must have had the onset of their motor symptoms after the age of 50 years, no autosomal dominant or recessive family history of PD and no other elements indicative for monogenic PD. The following exclusion criteria were applied: recent (less than 6 months prior to study sample collection) or concurrent gastrointestinal or systemic conditions, including infections or surgical interventions, or severe disability that may interfere with the results of the tests or preclude the clinical evaluation; antibiotic treatment within the past 3 months; and use within the past month of other drugs or supplements that may interfere with the results of the testing, such as steroidal or non-steroidal anti-inflammatory drugs (NSAIDs), including daily aspirin above 100 mg, and daily use of proton pump inhibitors (PPIs). Further exclusion criteria for the control group were the presence of clinical motor or nonmotor markers for prodromal PD (Berg et al., 2015; Heinzel et al., 2019) or other chronic neurological diseases. The initial design included additional control groups and subgroups and aimed to evaluate several other potential markers, but because of the ongoing pandemic study enrollment was lower than planned; considering the minute sample sizes, these subsidiary data are not discussed.

The clinical evaluation was performed by a neurologist, within a few days from sample collection (typically the day before the blood sample). It included medical history, full neurological examination and assessment of parkinsonism using the modified Hoehn and Yahr scale (Goetz et al., 2004) and the Unified Parkinson's Disease Rating Scale (UPDRS) part III (Martinez-Martin et al., 1994). Ancillary data were obtained from medical records.

Whole blood samples were collected à jeun on vacutainer clot activator tubes and immediately stored at 4–8°C. Serum was separated and removed within 24 h (typically less than 6) and either fully processed or stored at −20°C. Participants collected their own stool samples (5–10 g) in a sterile plastic container, using the kits they were provided with. Stool samples were collected no later than 3 days after the blood sample (except for the cases of more severe constipation) and kept at room temperature for a maximum of 6 h, then preprocessed using commercially available preparation and extraction tubes (K 6998SAS, K 6999, Immunodiagnostik AG, Germany) and stored at −80°C before being fully processed (up to 8 weeks). The serum and fecal levels of calprotectin and zonulin were determined by enzyme-linked immunosorbent assay (ELISA). For this, we used the commercially available IDK® Calprotectin ELISA K 6927 (stool) and K 6935 (serum) kits and the IDK® Zonulin ELISA K 5600 (stool) and K 5601 (serum) kits (Immunodiagnostik

AG, Germany). All samples were processed according to the instruction leaflets that came with the kit.

The results of calprotectin and zonulin levels are expressed as means and standard deviations (SD). Statistical analysis included odds ratio (OR) with 95% confidence intervals (95% CI) and Spearman's rank correlations coefficient (R). Statistically significant differences were considered at p-values < 0.05. For correlations we used IBM® SPSS® Statistics (subscription).

RESULTS

We fully evaluated 22 patients with sporadic PD (15 males, 7 females) and 16 unmatched healthy controls (9 males, 7 females). Another 4 patients were partially evaluated but could not provide stool samples in due time; their data are not included in the present analysis (see above). The demographic and clinical characteristics of the study population are presented in **Table 1**. Out of the 22 patients with sporadic PD, 12 fulfilled the MDS diagnostic criteria for clinically established PD (Postuma et al., 2015); the other 10 had the motor onset of PD within the past 5 years and met criteria for clinically probable PD (Postuma et al., 2015); additionally, they fulfilled criteria for clinically established early PD (Berg et al., 2018). Modified Hoehn and Yahr stage ranged from 1 to 4, almost two thirds of the patients having bilateral symptoms and balance impairment [i.e., stage 2.5 or above—see (Goetz et al., 2004)]. Hyposmia (self-reported) was present in 10 PD patients (45.5%). Constipation (self-reported, defined as less than 3 bowel movements per week in the absence of symptomatic treatment) was present in 9 PD patients (41%). Other nonmotor symptoms and concomitant medication are detailed in **Supplementary Table 1**.

The mean serum calprotectin level was significantly higher in the PD group than in controls (14.26 mcg/ml ± 4.50, SD 10.78 vs. 5.94 mcg/ml ± 3.80, SD 7.75; $p = 0.0125$). Serum calprotectin levels above the upper reference limit were found in samples from 19 out of the 22 PD patients (86.4%) and in 6 (37.5%) out of the 16 controls (OR = 10.56, 95% CI = 2.17–51.42, $p = 0.0025$). The mean fecal calprotectin level was also significantly higher in patients with PD than in controls (164.54 mcg/g ± 54.19, SD 129.68 vs. 56.19 mcg/g ± 35.88, SD 73.22; $p = 0.0048$). Fecal calprotectin levels above the upper reference limit (50 mcg/g) were found in samples from 20 (90.9%) out of the 22 patients and in 4 (25%) out of the 16 controls (OR = 30, 95% CI = 4.75–189.30, $p = 0.000045$); levels above 100 mcg/g were found in samples from 12 (54.5%) PD patients, levels above 150 mcg/g in samples from 8 PD patients (36.4%), above 200 mcg/g in samples from 7 PD patients (31.8%), and above 250 mcg/g in samples from 4 PD patients (18.2%)—with the caveats that 3 out of the 4 patients with the highest levels were treated with levodopa/carbidopa intestinal gel (LGCI) and that all of the patients that were treated with LCGI ($n = 3$) had fecal calprotectin levels above 250 mcg/g; fecal calprotectin exceeded the above cut off values only in 1 (6.25%) of the control samples (exact value: 322.27 mcg/g). Differences between the PD groups and controls remained significant when considering 100 mcg/g as cut off for those age 60 and older and 50 mcg/g for those below 60 (OR = 5.2, 95% CI = 1.15–23.54, $p = 0.028$); this trend also maintained when using 51 mcg/g and 112 mcg/g as age-dependent cut offs (OR = 4.33, 95% CI = 0.96–19.58, $p = 0.049$), as previously done (Mulak et al., 2019). The mean serum zonulin level was higher in the PD group than in controls (26.69 ng/ml ± 3.55, SD 8.51 vs. 19.43 ng/ml ± 2.56, SD 5.22; $p = 0.0046$). No serum zonulin levels above the upper reference limit were found in the study population, but levels below the lower reference limit were identified in 4 (18.2%) out of the 22 PD samples and in 10 (62.5%) of the 16 controls (OR = 0.13, 95% CI = 0.03–0.59, $p = 0.0068$). The mean fecal zonulin level was higher in the PD patient group than in controls (100.19 ng/ml ± 28.25, SD 67.61 vs. 37.30 ng/ml ± 13.26, SD 27.07; $p = 0.0012$). Fecal zonulin levels above the upper reference range limit were found in 8 (36.4%) out of the 22 samples from PD patients and in none of the controls. Levels below the lower reference limit were found only in 2 (12.5%) of

TABLE 1 | Demographic and clinical characteristics of the study population.

	Sporadic PD cases ($n = 22$)	**Healthy controls** ($n = 16$)
Sex	15 (68.18%) males / 7 (31.82%) females	9 (56.25%) males / 7 (43.75%) females
Age ($p = 0.0001$)	Mean: 68.7 ± 3.51 years, SD 8.4	Mean: 50.5 ± 8.5 years, SD 17.4
BMI ($p = 0.446$)	Mean: 26.7 kg/m^2 ± 1.77, SD 4.23 BMI 25–29.9 kg/m^2 (overweight): 8 (36.4%) BMI ≥ 30 kg/m^2 (obese): 5 (22.7%)	Mean: 25.6 kg/m^2 ± 2.21, SD 4.51 BMI 25–29.9 kg/m^2 (overweight): 6 (37.5%) BMI ≥ 30 kg/m^2 (obese): 3 (18.75%)
Tobacco smoking	Never smokers: 19 (86.4%) Former smokers: 2 (9.1%) Current smokers: 1 (4.5%)	Never smokers: 13 (81.25%) Former smokers: 0 Current smokers: 3 (18.75%)
PD diagnostic (MDS criteria) and motor characteristics	Clinically established PD: 12 (55.5%) Clinically probable PD with criteria for Clinically established early PD: 10 (45.5%) Modified HY stage below 2.5: 8 (36.4%) Modified HY stage 2.5 and above: 14 (63.6%) Mean UPDRS part III: 21.5 ± 5.81, SD 13.9 No. with motor complications: 13 (59.1%)	NA (see exclusion criteria)

TABLE 2 | Calprotectin and zonulin levels.

	Sporadic PD cases (n = 22)	Healthy controls (n = 16)	Significance
Serum calprotectin (reference: <3 mcg/ml)	14.26 mcg/ml ± 4.50, SD 10.78	5.94 mcg/ml ± 3.80, SD 7.75	p = 0.0125
Serum calprotectin ≥3 mcg/ml	19 (86.4%)	6 (37.5%)	OR = 10.56, p = 0.0025, 95% CI = 2.17–51.42
Fecal calprotectin (reference: <50 mcg/g)	164.54 mcg/ml ± 54.19, SD 129.68	56.18 mcg/ml ± 35.88, SD 73.22	p = 0.0048
Fecal calprotectin ≥50 mcg/g	20 (90.9%)	4 (25%)	OR = 30, p = 0.000045, 95% CI = 4.75–189.30
Fecal calprotectin ≥100 mcg/g	12 (54.5%)	1 (6.25%)	OR = 18, p = 0.002, 95% CI = 2.01–161.05
Fecal calprotectin ≥200 mcg/g	8 (36.4%)	1 (6.25%)	OR = 8.57, p = 0.034, 95% CI = 0.95–77.57
Fecal calprotectin ≥250 mcg/g	4 (18.2%)	1 (6.25%)	OR = 3.33, p = 0.2856, 95% CI = 0.34–33.11
Serum zonulin (reference: 20–48 ng/ml)	26.69 ng/ml ± 3.55, SD 8.51	19.43 ng/ml ± 2.56, SD 5.22	p = 0.0046
Serum zonulin <20 ng/ml	4 (18.2%)	10 (62.5%)	OR = 0.13, p = 0.0068, 95% CI = 0.03–0.59
Fecal zonulin (reference: 15–107 ng/ml)	100.19 ng/ml ± 28.25, SD 67.61	37.30 ng/ml ± 13.26, SD 27.07	p = 0.0012
Fecal zonulin >107 ng/ml	8 (36.4%)	0	NA

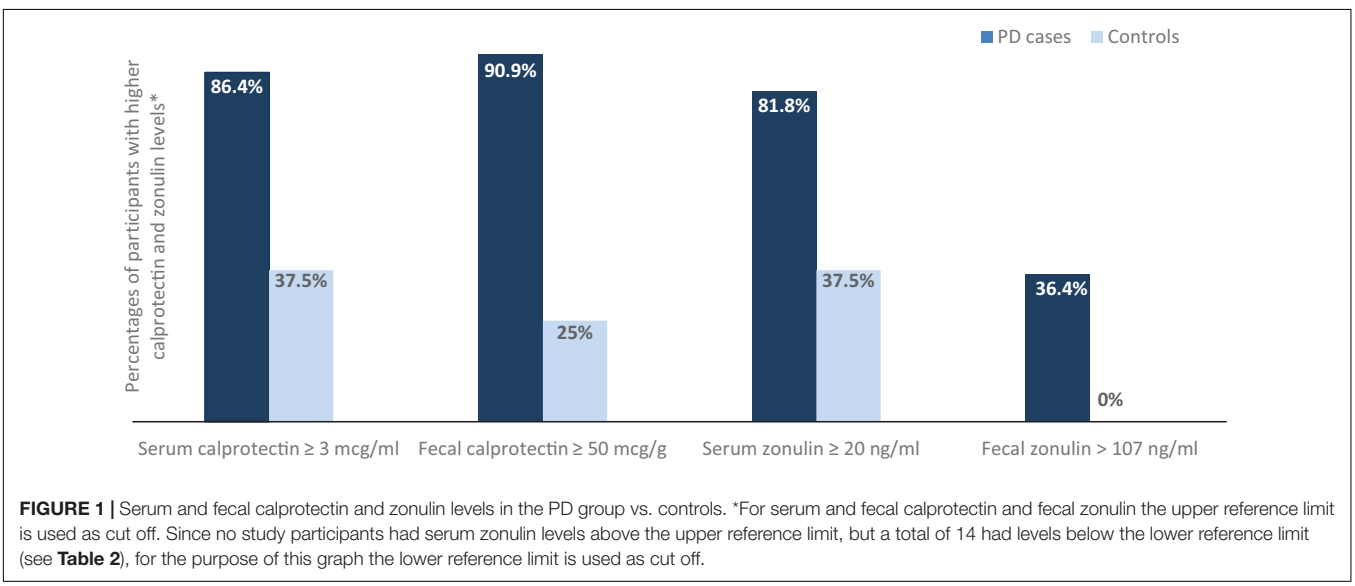

FIGURE 1 | Serum and fecal calprotectin and zonulin levels in the PD group vs. controls. *For serum and fecal calprotectin and fecal zonulin the upper reference limit is used as cut off. Since no study participants had serum zonulin levels above the upper reference limit, but a total of 14 had levels below the lower reference limit (see **Table 2**), for the purpose of this graph the lower reference limit is used as cut off.

the controls. For a summary of these results, see **Table 2** and **Figure 1**.

In the PD group, we found statistically significant and potentially relevant correlations between serum calprotectin and the modified Hoehn and Yahr stage (R = −0.528, p = 0.012). Disease duration correlated with parkinsonism severity, assessed with the modified Hoehn and Yahr stage (R = 0.775, p < 0.001) and the motor UPDRS score/UPDRS part III (R = 0.645, p = 0.001), as well as with the presence of constipation (R = 0.610, p = 0.003). Chronic constipation correlated with the motor UPDRS score (R = 0.423, p = 0.05) and with the daily levodopa equivalent dose (LED) (R = 0.43, p = 0.46). Motor UPDRS score correlated with female sex (R = 0.555, p = 0.007). Fecal calprotectin levels above 250 mcg/g correlated with female sex (R = 0.457, p = 0.042), LCIG treatment (R = 0.5, p = 0.018) and high serum C reactive protein (CRP) (R = 0.677, p = 0.011), while levels above 150 mcg/g inversely correlated with self-reported hyposmia (R = −0.567, p = 0.006); increased fecal calprotectin also correlated with body mass index (BMI) (R = 0.499, p = 0.018) and obesity (R = 0.490, p = 0.02). Serum calprotectin levels had negative correlation with the current smoker status (R = −0.463, p = 0.03). Except for the fecal calprotectin levels above 250 mcg/g, which correlated with female sex in the PD group (R = 0.457, p = 0.042), we found no significant correlations

between serum and fecal calprotectin and zonulin levels or between these and age or sex, neither in patients with PD, nor in controls.

DISCUSSION

We evaluated the serum and fecal levels of calprotectin and zonulin in people with sporadic PD vs. healthy controls. Despite the small sample size, we found that increased serum and fecal calprotectin levels (i.e., above the upper reference limit) are significantly associated with the risk of PD (OR 10.56 and 30, respectively, *p*-values below 0.005, null hypothesis outside the 95% CIs—see above). The serum zonulin levels were not increased above the upper reference limit in our study, but we found higher mean values in PD compared with controls ($p = 0.0046$); concurrently, levels below the reference range appear to be protective (OR = 0.13, 95% CI = 0.03–0.59, $p = 0.0068$). The mean value of fecal zonulin was higher in PD than in controls ($p = 0.0012$) and values above the upper reference limit were found only in PD ($n = 8$; 36.4%).

Calprotectin is a pleiotropic cytokine-like protein mainly involved in the recruitment of inflammatory cells; it also has bacteriostatic effects that are mediated by zinc-dependent enzymes (Kowalski and Mulak, 2019). It is released or secreted by activated neutrophils, monocytes and endothelial cells, its levels raising rapidly in the presence of bacteria (Diamanti et al., 2010; Jensen et al., 2011; Dhaliwal et al., 2015; Moein et al., 2017; Kowalski and Mulak, 2019). Interestingly, in some experimental settings calprotectin is protective against intestinal injury (Aranda et al., 2018), while in others it may promote amyloidogenesis (Kowalski and Mulak, 2019). Zonulin functions as part of the tight junction system of the mucosal intestinal barrier, modulating its permeability by promoting the disassembly of zona occludens (Ohlsson et al., 2017b). Its secretion or release is mainly triggered by bacteria, but also by some dietary components, such as gluten, higher levels resulting in increased intestinal barrier permeability, which is found along inflammatory changes in people with PD and other neurodegenerative conditions (Ohlsson et al., 2017b; Kowalski and Mulak, 2019).

Fecal calprotectin is a well-established biomarker of intestinal inflammation, routinely used in the diagnostic and monitoring of IBD. However, it is not specific for IBD. Other circumstances that may increase fecal calprotectin levels include the use of certain drugs, especially NSAIDs and possibly PPIs, and certain disorders, such as infectious enterocolitis, celiac disease and colorectal cancer (Khaki-Khatibi et al., 2020). In people with chronic diarrhea and other symptoms suggestive of IBD, fecal calprotectin below 50 mcg/g excludes clinically relevant intestinal inflammation, while levels above 250 mcg/g are highly specific (Diamanti et al., 2010; Jensen et al., 2011; Dhaliwal et al., 2015; Moein et al., 2017). Noteworthy, IBD is a risk factor for developing PD (Berg et al., 2015; Camacho-Soto et al., 2018a; Heinzel et al., 2019; Park et al., 2019; Villumsen et al., 2019; Weimers et al., 2019; Zhu et al., 2019; Lee et al., 2021) and a promising candidate risk marker for diagnosing prodromal PD (Heinzel et al., 2019). The role of serum or plasma calprotectin in the diagnostic and follow-up of chronic inflammation is not so well defined (Wang and Liang, 2019).

Interestingly, most people with sporadic PD develop gastrointestinal symptoms related to decreased transit time, constipation preceding the motor dysfunction with more than a decade in some cases (Postuma et al., 2015). Putatively, these symptoms are related to PD pathology in the enteric nervous system (Braak et al., 2006; Devos et al., 2013). Gut dysbiosis is common in PD and can result in local inflammatory changes and barrier dysfunction/disruption, which may increase alpha-synuclein expression and facilitate its exposure to amyloidogenic compounds found in the gut, thus possibly contributing to key pathogenic events in PD; prospective evidence is nevertheless scarce (Stolzenberg et al., 2017; Nuzum et al., 2020; Vascellari et al., 2021). The coexistence of an amyloidogenic gut microbiota with intestinal inflammation (leading to local overexpression of alpha-synuclein) and altered intestinal barrier permeability (exposing alpha-synuclein to the amyloidogenic xenobiotics) may play a key role in triggering the initial alpha-synuclein conformational changes in some people with sporadic PD (Sampson et al., 2020). Disturbances in the microbiota-gut-brain axis may also contribute to neurodegeneration, interfering with neuronal susceptibility to stressors and ultimately with neuronal survival (Devos et al., 2013; Stolzenberg et al., 2017; Nuzum et al., 2020; Vascellari et al., 2021).

In our study higher levels of calprotectin correlated with milder parkinsonism on the modified Hoehn and Yahr scale ($p = 0.012$), which would suggest that the intestinal inflammation is higher in the earlier stages of the disease. We also found that increased calprotectin levels (above 150 mcg/g) correlated negatively with self-reported hyposmia ($p = 0.006$); this cannot be interpreted solely based on our study, but fits with the hypothesis of a more heterogenous development of sporadic PD (Rietdijk et al., 2017), the initial pathogenic events being either intestinally-centered, or olfactory-centered, or both.

The results of our study are in line with those of two other previous studies that compared fecal calprotectin and zonulin levels in 35 PD patients and 20 controls, and in 34 PD patients and 28 age-matched controls, respectively (Schwiertz et al., 2018; Mulak et al., 2019). Both studies found significant associations between higher fecal calprotectin and PD, while only the latter found a significant association between higher fecal zonulin and PD. In addition to these studies, we also evaluated serum levels of calprotectin and zonulin and found significant associations between higher levels and PD. Although in people with IBD, serum calprotectin correlates with the fecal levels (Kalla et al., 2016), we found no such correlation. The present study poses a series of limitations that mandate caution when interpreting its results. Most important, the number of participants in each group was lower than planned and did not allow matching, therefore our results warrant confirmation on larger groups that are matched for age, sex and dietary habits (the latter being a possible confounding factor, especially in respect to zonulin levels). Except for the highest fecal calprotectin

levels being more common in females with PD, we found no significant correlations between serum and fecal calprotectin and zonulin levels and age or sex. Fecal calprotectin levels may increase with age, one study reporting median values of 18 mcg/g in healthy adults below 60 years old and 27 mcg/g in older individuals (Khaki-Khatibi et al., 2020). In our study, the association between increased fecal calprotectin and PD remained significant even when using age-dependent cut off values. As expected based on the available literature (Sapone et al., 2006; Khaki-Khatibi et al., 2020; Seethaler et al., 2021), we found no significant correlation between zonulin levels and age or sex. Dietary patterns were not assessed in our study, however, serum zonulin levels may correlate with macro- and micronutrient intake, including the total carbohydrate and vitamin D, respectively (Morkl et al., 2018). Increased BMI is another potential confounding factor, both for calprotectin and zonulin (Mortensen et al., 2009; Ohlsson et al., 2017a; Grand et al., 2020). In our study there were no significant differences in BMI between the PD group and controls (p = 0.446); in agreement with the available literature (Mortensen et al., 2009; Grand et al., 2020), increased calprotectin levels correlated with increased BMI and obesity in the PD group. All the PD patients included in our study had symptomatic treatments with dopamine agonists or levodopa-based drugs, which are possible confounding factors. We found no correlations between these drugs, LED, and calprotectin or zonulin levels, but this could be related to the small sample size; notably, very high fecal calprotectin levels correlated with LCIG (p = 0.018). Another limitation of our study is that serum and fecal calprotectin and zonulin levels were not confirmed on a different occasion or by further gastroenterological evaluation within the study, meaning that the results could reflect isolated circumstances (that were not accounted for by the exclusion criteria) rather than a chronic ongoing process related to PD.

Concluding, our findings suggest an association between sporadic PD and serum and fecal markers of intestinal inflammation and permeability. This is consistent with data from two other small studies and underlines the importance of further investigating the gastrointestinal tract to better understand the pathogenic events involved in the initiation and progression of sporadic PD. Provided future research confirms the relation between increased calprotectin levels and PD, serum and fecal calprotectin testing could help improve the accuracy of current clinical diagnostic criteria.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The protocol of the study was approved by the local Ethics Committee of Colentina Clinical Hospital (EMI-BPs, 3/16.04.2019). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LD drafted and revised the manuscript; she also designed and drafted the protocol of the study (as part of her Ph.D., under the supervision of BOP), critically revised the laboratory protocol used in the study, contributed to the ethics submission, to the recruitment and evaluation of some study participants and to data collection, and processing. DM performed the laboratory tests and revised the manuscript; she also drafted the laboratory protocol used in the study, critically revised the protocol of the study and contributed to the recruitment of study participants. AD critically revised the protocol of the study, the laboratory protocol and the ethics submission, contributed to the recruitment and evaluation of study participants and to data collection and processing, and revised the manuscript. AL critically revised the ethics submission, contributed to the recruitment and evaluation of study participants, data collection and processing, and revised the manuscript. DT and LC contributed to the recruitment and evaluation of study participants and revised the manuscript. EM, MG, and LC critically revised the laboratory protocol used in the study and the protocol of the study and revised the manuscript. BOP coordinated and critically revised all aspects related to the study and the manuscript, was responsible for PN 19.29.02.01, granting funding for this work, was the PI of the study, and the supervisor of LD's Ph.D. All authors approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

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MINI REVIEW
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Insulin Resistance Exacerbates Alzheimer Disease via Multiple Mechanisms

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Alzheimer disease (AD) is a chronic neurodegenerative disease that accounts for 60–70% of dementia and is the sixth leading cause of death in the United States. The pathogenesis of this debilitating disorder is still not completely understood. New insights into the pathogenesis of AD are needed in order to develop novel pharmacologic approaches. In recent years, numerous studies have shown that insulin resistance plays a significant role in the development of AD. Over 80% of patients with AD have type II diabetes (T2DM) or abnormal serum glucose, suggesting that the pathogenic mechanisms of insulin resistance and AD likely overlap. Insulin resistance increases neuroinflammation, which promotes both amyloid β-protein deposition and aberrant tau phosphorylation. By increasing production of reactive oxygen species, insulin resistance triggers amyloid β-protein accumulation. Oxidative stress associated with insulin resistance also dysregulates glycogen synthase kinase 3-β (GSK-3β), which leads to increased tau phosphorylation. Both insulin and amyloid β-protein are metabolized by insulin degrading enzyme (IDE). Defects in this enzyme are the basis for a strong association between T2DM and AD. This review highlights multiple pathogenic mechanisms induced by insulin resistance that are implicated in AD. Several pharmacologic approaches to AD associated with insulin resistance are presented.

Keywords: Alzheimer's disease, insulin resistance, amyloid beta, tau, drug

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INTRODUCTION

Alzheimer disease (AD) is a chronic degenerative brain disease characterized by memory loss, cognitive impairment, and loss of activities of daily living (Jha et al., 2019). It is the most common form of dementia and the sixth leading cause of death in the United States (Wilson et al., 2012; Heron, 2013). An estimated 5.8 million Americans suffered from AD in 2020 and this number will triple to nearly 14 million people by 2060 (Matthews et al., 2019). There are no treatments that effectively stop or reverse AD progression, although some medications temporarily improve symptoms (Hsu and Marshall, 2017). Notably, the United States Food and Drug Administration (FDA) approved Aducanumab on June 7th, 2021, the first antibody for the treatment of AD which reduces amyloid plaques. However, this drug had previously failed to gain FDA approval, because initial analysis of clinical trial data did not show a significant improvement in patients' mental abilities. Phase IV trials are still required to verify its clinical benefits.

There are two major forms of AD: the sporadic (late-onset) form, which accounts for most cases, and the familial (early-onset) form, which is generally associated with the inheritance of genetic mutations (Bekris et al., 2010). While the cause of most AD cases is poorly understood (Reitz and Mayeux, 2014), genes encoding amyloid precursor protein (APP), presenilin 1 and presenilin 2 account for the majority of early-onset familial AD cases (Cheignon et al., 2018), whereas apolipoprotein E (APOE) is the main genetic risk factor in sporadic AD, especially *APOE−ε4* (Morris et al., 2014; Clark and Vissel, 2018).

The pathogenesis of AD is multifactorial (Crous-Bou et al., 2017). Accumulating studies indicate a strong association between type II diabetes (T2DM) and AD (Kang et al., 2017). Neuronal insulin signaling pathways are disrupted in both T2DM and AD and over 80% of AD patients have T2DM or display abnormal blood glucose levels (Zhao and Townsend, 2009). Observational studies demonstrate that T2DM nearly doubles the risk of AD and increases the likelihood of dementia (Leibson et al., 1997; Luchsinger et al., 2001; Xu et al., 2009). In addition, APOE4 and insulin resistance were found to impair cognitive function in a study of human E4-targeted replacement mice (Johnson et al., 2017). Multiple studies have also established that insulin resistance leads to the progression of two main pathological hallmarks of AD—senile plaques from extracellular deposition of amyloid β-protein and tau-based neurofibrillary tangles (NFT) (Ardura-Fabregat et al., 2017). Consequently, AD may be considered a type of metabolic disease, and the development of AD therapeutics may benefit from an understanding of the relationship between AD and insulin resistance (Kang et al., 2017).

INSULIN RESISTANCE AND AD

Insulin is essential for metabolic homeostasis in the peripheral system (Tokarz et al., 2018), but has only been recognized for its role in regulating amyloid β-protein peptides and the generation of NFTs in the last few decades (Razay and Wilcock, 1994; Kroner, 2009). Under normal conditions, increased plasma glucose levels lead to stimulation of pancreatic β-cells to produce insulin, which decreases glucose levels. As blood glucose falls, counter-regulatory hormones including epinephrine, norepinephrine and cortisol from the adrenal glands arrest insulin-mediated glucose disposal. Insulin is then rapidly degraded in the liver, kidney and muscles by insulin degrading enzyme (IDE) (Watson and Craft, 2003). The pleiotropic biologic effects of insulin are mediated via binding and activating insulin receptors (IR) (Boucher et al., 2014), which are widely distributed in the periphery but selectively distributed in the central nervous system (CNS), including the cerebral cortex, hippocampus, hypothalamus and amygdala (Havrankova et al., 1978; Bosco et al., 2011; Soto et al., 2019). Insulin binding leads to a conformational change of the IR resulting in phosphorylation of intracellular IR substrate (IRS) proteins on tyrosine residues (Saini, 2010). Subsequently, IRS activates downstream pathways including mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K) (Gabbouj et al., 2019), which are important for mitogenic and metabolic functions (Plum et al., 2005).

However, in insulin resistance, cells fail to respond to insulin causing elevated blood glucose and effects on muscle, liver and brain (Kroner, 2009; Zhao and Townsend, 2009). Pancreatic β-cells produce more insulin in response to high blood glucose (hyperglycemia) resulting in hyperinsulinemia (high blood insulin), eventually leading to T2DM (Heydemann, 2016). Decreased levels of insulin and IR are found in the cerebrospinal fluid (CSF) of AD patients due to long-term peripheral hyperinsulinemia and decreased insulin transport across the blood-brain barrier (BBB) (Craft et al., 1998; Rivera et al., 2005; Steen et al., 2005; Gil-Bea et al., 2010; Stanley et al., 2016).

Accruing evidence shows that insulin facilitates memory and cognition under normal conditions (Watson et al., 2009; Tokarz et al., 2018) whereas chronic hyperinsulinemia impairs them (Lee et al., 2016). For instance, fructose-induced insulin-resistant rat models show impaired spatial learning in the water-maze test (Sachdeva et al., 2019). Moreover, intranasal insulin improves memory in humans (Benedict et al., 2008; Krug et al., 2010). Insulin resistance may accelerate the progression of senile plaques and NFTs via multiple mechanisms, resulting in cognitive decline, impaired long-term potentiation (LTP) and associated metabolic disease. A summary of the feed forward loop of insulin resistance and AD pathogenesis is provided in **Figure 1**.

Neuroinflammation Induced by Insulin Resistance in AD

The current consensus is that neuroinflammation plays a pivotal role in AD progression (Wang W. Y. et al., 2015), which is supported by results from APP transgenic mouse models in which injection of lipopolysaccharide (LPS, TLR4 activator) triggers neuroinflammation with two cellular hallmarks of AD in the brain, amyloid β-protein deposition (Lee et al., 2008; Go et al., 2016) and tau hyperphosphorylation (Kitazawa et al., 2005; Lee et al., 2010). Amyloid β-protein is the product of consecutive cleavage of APP by enzymes β-secretase (BACE) and γ-secretase. Processing of APP yields multiple forms of the protein; the 40 and 42 amino acid residue products are the most common forms (O'Brien and Wong, 2011). High levels of monomeric amyloid β-protein have a propensity to aggregate into fibrils and then plaques, resulting in neurodegeneration and induction of tau pathology (Mouchlis et al., 2020).

Inflammation is involved in activation of microglial cells, which are primarily responsible for amyloid β-protein phagocytosis. Microglia are brain-resident immune cells responsible for promoting phagocytotic clearance as well as providing trophic support to ensure tissue repair and cerebral homeostasis (Sarlus and Heneka, 2017). They also play a role in higher cognitive functions, such as learning and memory in the adult brain, and are involved in the pathogenesis of neurodegenerative diseases like AD. In the early stages of AD, activated microglia repair damaged tissue and decrease amyloid β-protein accumulation. However, chronic microglial activation induced by inflammation leads to release of inflammatory mediators and accumulation

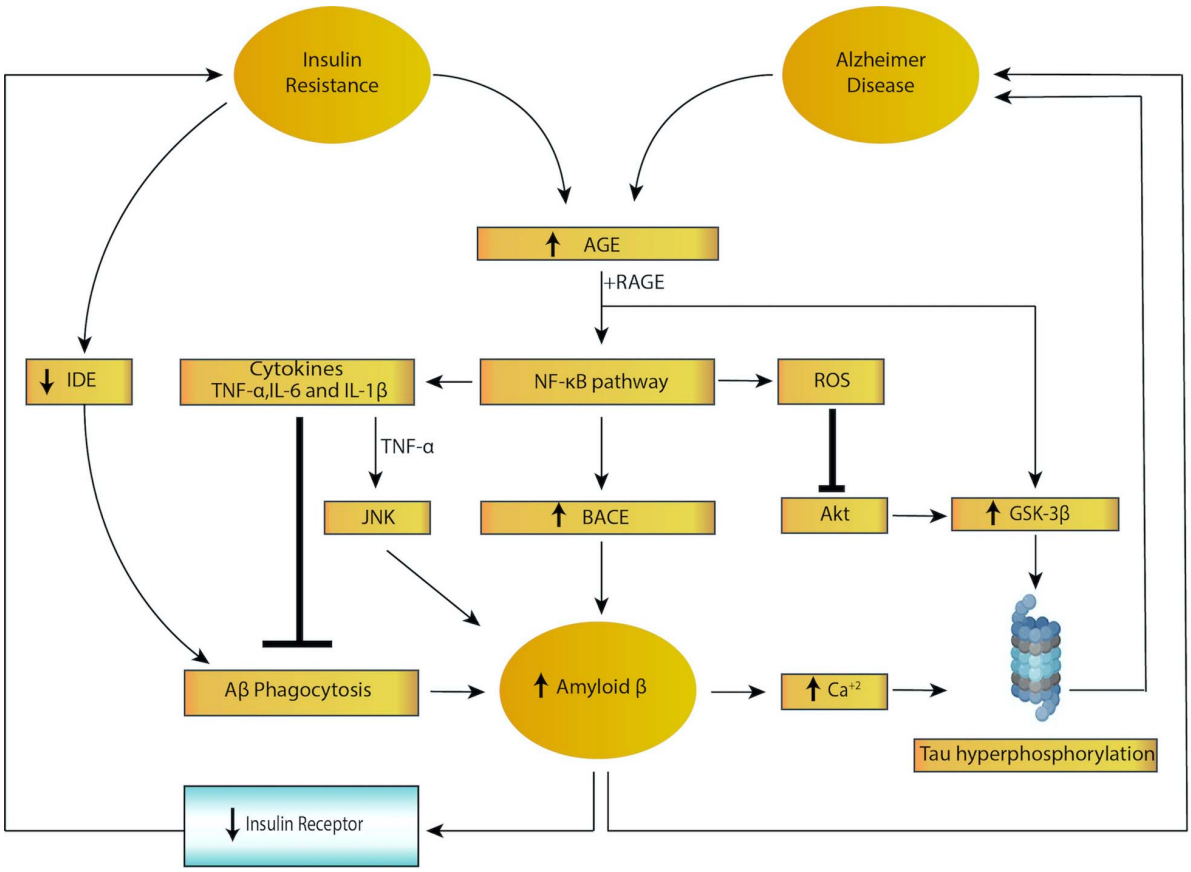

FIGURE 1 | Feed forward loop of insulin resistance and Alzheimer disease. Both insulin resistance and Alzheimer disease lead to activation of nuclear factor kappa B (NF-κB), increased cytokine secretion and increased reactive oxygen species (ROS) levels, triggering increased amyloid beta (amyloid β) and tau hyperphosphorylation. In addition, insulin resistance lowers levels of insulin degrading enzyme (IDE), resulting in impaired amyloid β phagocytosis. Higher levels of amyloid β, in turn, leads to decreased expression of the insulin receptor, which results in insulin resistance, creating a vicious cycle.

of danger-associated molecular patterns (DAMPs), which limits amyloid β-protein clearance, leading to more plaque accumulation, neuronal dysfunction and death (Clark and Vissel, 2015; Wang W. Y. et al., 2015; Brabazon et al., 2018). This hypothesis is supported by a longitudinal study showing increased levels of microglial activation in both mild cognitive impairment (MCI) and AD patients compared to controls, but a reduction in microglial activation following an initial peak in MCI patients (Fan et al., 2017). These data suggest that early microglial activation leads to a protective phenotype which can later turn into a pro-inflammatory picture due to failure of amyloid β-protein clearance and progressive neuronal damage.

Insulin resistance results in microglial activation and inflammation (McCaulley and Grush, 2017) by inducing the activation of resting (ramified) microglia and changes in cellular morphology, surface phenotype, secretary mediators and proliferative responses (Sarlus and Heneka, 2017). One common molecular pathology shared by insulin resistance and AD is increased levels of advanced glycation end products (AGEs) (Zhao and Townsend, 2009). Binding of AGEs to their cellular receptors (RAGE) not only upregulates glycogen synthase kinase 3β (GSK-3β), causing tau hyperphosphorylation (Peng et al., 2007; Li et al., 2012a,b), but also activates the NF-κB pathway, which produces reactive oxygen species (ROS) and pro-inflammatory cytokines [interleukin (IL)-6, IL-1β, TNF] (Kandimalla et al., 2017). These cytokines are observed to increase accumulation of amyloid β-protein in AD by two mechanisms: (1) increased levels of pro-inflammatory cytokines inhibit phagocytosis of amyloid β-protein in AD brains thereby hindering the removal of plaque by resident microglia; (2) TNF has been shown to upregulate the production of amyloid β-protein via activation of the c-Jun N-terminal kinase (JNK)-dependent MAPK pathway, which promotes phosphorylation and cleavage of APP (Liaoi et al., 2004; McAlpine and Tansey, 2008; Colombo et al., 2009; Montgomery et al., 2011; Cheng et al., 2014; Ahn et al., 2016; Decourt et al., 2017; Zhang et al., 2019). In addition, activation of the NF-κB pathway further increases BACE expression, resulting in increased production of amyloid β-protein (Guglielmotto et al., 2012; Cai et al., 2016). High levels of amyloid β-protein cause

IR downregulation via internalization, desensitization or direct substrate competition, which ultimately turn into insulin resistance (Xie et al., 2002; Mullins et al., 2017). Moreover, amyloid β-protein triggers Ca^{2+} influx, which not only causes hyperphosphorylation of tau protein (Bosco et al., 2011) via GSK-3β, but also inhibits IR tyrosine kinase signaling. The increased levels of Ca^{2+} stimulate Ca^{2+}-dependent serine/threonine protein kinases (PKC, Akt), which phosphorylate IRs and insulin resistance substrate (IRS) and thus negatively regulate IRs in the brain (Zhao and Townsend, 2009). Taken together, insulin resistance, neuroinflammation and exacerbation of amyloid β-protein and tau form a feed-forward loop in AD pathogenesis. Imbalance induced by any of these factors will facilitate AD progression, resulting in neurotoxicity, neurodegeneration and induction of a negative effect on IRs.

Oxidative Stress Induced by Insulin Resistance in AD

Growing evidence suggests that insulin/insulin-like growth factor (IGF) signaling is strongly associated with oxidative stress. Brain insulin/IGF resistance may contribute to impairments in glucose utilization and disruption of energy metabolism, resulting in production of ROS, DNA damage and mitochondrial dysfunction, eventually causing pro-apoptosis, pro-inflammation and amyloid β-protein cascades (de la Monte, 2014). Imbalance between the production of ROS and antioxidant defenses leads to oxidative stress which not only damages cells but also alters signaling pathways (Hurrle and Hsu, 2017). Oxidative stress has been implicated in AD and several studies have reported that it plays an important role in tau hyperphosphorylation and APP-amyloid β-protein accumulation (Huang et al., 2016).

Tau protein, a major microtubule-associated protein in the brain, functions mainly to maintain the stability of microtubules in neurons and other cells as well as facilitate cell differentiation and polarization (Mouchlis et al., 2020). According to the tau hypothesis, hyperphosphorylated tau pairs with other strands of tau protein and then forms NFT in neuronal cell bodies, which eventually induces microtubule dysregulation (Iqbal et al., 2005), causing impaired communication between neurons and even cell death (Bosco et al., 2011; Kametani and Hasegawa, 2018). As mentioned above, insulin resistance causes production of ROS via the activation of the AGE/RAGE pathway, inducing various stress sensitive signaling pathways, such as NF-κB, JNK/SAPK, p38 MAPK, and Akt pathway in particular (Rains and Jain, 2011). Increased oxidative stress inactivates the Akt pathway, concomitantly to downstream activation of GSK3 and subsequent hyperphosphorylation of tau protein (Bloch-Damti and Bashan, 2005; Hambright et al., 2015; Zhao et al., 2017; Ciotti et al., 2020).

Insulin resistance is also involved in APP-amyloid β-protein accumulation. APP-amyloid β-protein toxic fibrils, in turn, impair insulin signaling by downregulating IRs (Lee et al., 2013). Metal ions, such as zinc and copper bind to amyloid β-protein peptides and catalyze the production of ROS, which causes oxidative damage affecting both amyloid β-protein peptide and surrounding biomolecules, such as proteins and lipids (Cheignon et al., 2018). Both tau hyperphosphorylation and amyloid β-protein accumulation contribute to the positive feedback mechanism that exacerbates insulin/IGF resistance through increased oxidative stress, neurotoxicity and synaptic dysfunction (Lee et al., 2013).

Decreased Degradation of Amyloid β-Protein Induced by Insulin Resistance via IDE

Insulin is inactivated by IDE, also known as insulin protease (Manolopoulou et al., 2009; Song et al., 2018). IDE is widely distributed in many organs including liver, pancreas, brain and in diverse cellular compartments (Hulse et al., 2009). Accumulating studies have expanded the list of substrates and potential physiological roles of IDE, which includes degradation of multiple bioactive peptides, such as glucagon, IGF-2, and amyloid β-protein (Tang, 2016).

Amyloid β-protein forms various oligomers, leading to fibrils that then aggregate into plaques (Chen et al., 2017), which interrupt normal brain functions. Furthermore, soluble oligomeric forms of amyloid β-protein are the primary toxic species (Haass and Selkoe, 2007; Selkoe and Hardy, 2016) that have been shown to cause synaptic damage and neuronal cell death in both an APP knock-out mouse model and post-mortem human brains from patients with AD (Ding et al., 2019; Rolland et al., 2020). IDE is able to degrade both extracellular and intracellular amyloid β-protein, which protects against formation of these toxic oligomers. In addition, IDE functions as a "dead-end chaperone," preventing formation of toxic α-synuclein aggregates which can form a stable complex with amyloid β-protein (Sharma et al., 2015). α-synuclein is implicated in the pathophysiology of AD because high levels of α-synuclein are detected in the CSF of patients with MCI and AD (Twohig et al., 2018; Twohig and Nielsen, 2019).

Because insulin and amyloid β-protein are competing substrates for IDE, IDE defects are not only involved in the development of AD but also the basis for a strong association between T2DM and AD. Hyperinsulinemia may downregulate insulin uptake across the BBB and reduce levels of insulin in the brain because of saturation at supraphysiological levels (Reitz and Mayeux, 2014). This may result in decreased levels of IDE (Abdul-Hay et al., 2011; Protzek et al., 2016; Kang et al., 2017), causing decreased degradation of amyloid β-protein and increased deposits of amyloid β-protein (Li et al., 2018). In addition, increased levels of IDE are detected in post-mortem human brains from patients with moderate stage AD (Braak 3–4) whereas significantly reduced level of IDE are found in severe AD (Braak 5–6) (Delikkaya et al., 2019), suggesting that IDE is affected by insulin deficiency and insulin resistance in the early and moderate stages of AD. The development of IDE modulators may be a novel therapeutic approach to both T2DM and AD (Pivovarova et al., 2016).

TABLE 1 | Various potential treatments for Alzheimer's disease with insulin resistance.

Drug		Classification	Benefits
Anti-diabetic drugs	Metformin	Biguanide	First-line medication for T2DM; anti-inflammation; ↓ Aβ aggregation
	Liraglutide	GLP-1 agonist	↑ Insulin secretion; ↓ Aβ accumulation and ↓ tau hyperphosphorylation
	Intranasal insulin	–	Crosses BBB, improves cognitive functions and memory
Anti-inflammatory drugs	Tolfenamic Acid	Fenamate NSAIDs	Anti-inflammation via inhibition of NF-κB pathway; cognition enhancement via↓ Aβ and tau phosphorylation
	Mefenamic Acid	Fenamate NSAIDs	Anti-inflammation via inhibition of NLRP3 inflammasome; improve Aβ-induced learning and memory impairments
	Etanercept	TNF-α inhibitors	Anti-inflammation; ↓ Aβ to ↓ risk of AD
Antioxidant drugs	Vitamin C and E	Antioxidant	↓ Neuronal loss and Aβ; ↓ oxidative stress and tau-induced neurotoxicity
Thiazolidinediones (TZDs)	Rosiglitazone	–	↑ Insulin sensitivity; ↓ Aβ levels; improves cognitive functions
	Pioglitazone	–	↑ Insulin sensitivity; ↓ Aβ levels via downregulation of APP and BACE1

POTENTIAL TREATMENTS OF INSULIN RESISTANCE IN AD

Potential drug therapies for AD based on the association between insulin resistance and AD are listed in **Table 1**.

Anti-diabetic Drugs

Metformin, a biguanide antihyperglycemic agent which is the first-line medication for T2DM, attenuates inflammation, reduces risk of metabolic syndrome (Li et al., 2015) and may decrease risk of dementia and improve cognitive function. A meta-analysis showed that metformin was beneficial to diabetes patients with dementia or AD (Lin et al., 2018). Interestingly, T2DM patients with long-term use of metformin have been reported to slightly increase the risk of AD (Imfeld et al., 2012) due to metformin-induced vitamin B12 deficiency (Aroda et al., 2016; Campbell et al., 2018). Vitamin B12 deficiency has been reported to increase risk of AD, although the mechanism behind this association is uncertain (Abyad, 2002; Health Quality Ontario., 2013).

Liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist, is used to treat T2DM and obesity by increasing insulin release from the pancreas as well as decreasing excessive glucagon release (Femminella et al., 2019). Recent studies have indicated that liraglutide may attenuate cognitive impairment. *In vitro* investigation has shown that liraglutide regulates neuronal insulin signaling and BACE-1 activity to suppress accumulation of amyloid β-protein and hyperphosphorylation of tau protein (Jantrapirom et al., 2020). Also, it prevents loss of brain insulin receptors and synapses and reverses cognitive impairment induced by amyloid β-protein oligomers in mouse hippocampi (Batista et al., 2018).

Intranasal insulin provides a potential pharmacological strategy to treat AD. Although there are different routes of administration for insulin, such as subcutaneous, intramuscular, and oral (Henkin, 2010), intranasal insulin has the advantage of penetrating the BBB and accessing the CNS because of the direct neuroanatomical connections between the olfactory nerves and the brain (de la Monte, 2013) which are beneficial for treating neurodegenerative and psychiatric disease (Hanson and Frey, 2008). More and more clinical studies have shown that intranasal insulin effectively improves cognitive function and memory (Benedict et al., 2008; Hallschmid et al., 2008; Krug et al., 2010), although a newly released study contradicts this finding (Craft et al., 2020). Thus, more direct experimental and clinical evidence are needed to investigate the safety and efficacy of intranasal insulin.

Anti-inflammatory Drugs

In 2020, 18% of agents in Phase III trials and 15% of agents in Phase II trials targeted inflammation to treat AD (Cummings et al., 2020). This is because a number of epidemiologic studies have reported that anti-inflammatory medication lowers the risk of cognitive impairment and AD. Although the effect of non-steroidal anti-inflammatory drugs (NSAIDs) in AD is under debate (Wang J. et al., 2015; Zhang et al., 2018), fenamate NSAIDS have aroused people's attention. These compounds selectively inhibit the NLRP3 inflammasome, which is implicated in inflammatory diseases including AD and T2DM, via the inhibition of volume-regulated anion channels (VRACs). The anti-inflammatory effects of two drugs in this class, tolfenamic acid and mefenamic acid, showed benefits in a 3 × TgAD transgenic model of AD (Daniels et al., 2016).

TNF is a key pro-inflammatory cytokine involved in insulin resistance, systemic inflammation and upregulation of amyloid β-protein, which further affects tau hyperphosphorylation (Clark and Vissel, 2015, 2016). Considering the importance of TNF in T2DM and AD pathogenesis, are TNF inhibitors a promising approach to treat AD or AD with T2DM? Although insufficient data are available, TNF inhibitors have been shown to produce cognitive improvements and lower the risk of AD in clinical trials of infliximab and adalimumab (Shi et al., 2011; Zhou et al., 2020). Etanercept, a specific anti-TNF biological in wide clinical use (Clark and Vissel, 2021), has been reported to attenuate neuroinflammation and improve cognitive function in murine models of traumatic brain injury (Chio et al., 2010) and Japanese encephalitis virus (Ye et al., 2014) and in clinical studies (Chen et al., 2010). However, further investigations to evaluate the use and specificity of these agents for dementia needs to be conducted.

Antioxidant Drugs

Oxidative stress is involved in the pathogenesis of both AD and T2DM. Vitamins C and E, potent antioxidants, are believed to lower the risk of AD and dementia (Lam et al., 2016). This hypothesis is supported by a cohort study which showed a significant protective effect of combined vitamin C and E supplements on cognitive functions in elderly men (Masaki et al., 2000). Another study with 4,740 participants also showed that long-term use of vitamin C and E supplements in combination helped to reduce the incidence of AD (Zandi et al., 2004). In addition, lower plasma levels of vitamin C and E were detected in patients with MCI compared to controls (Rinaldi et al., 2003). However, other studies indicated that vitamins C and E did not reduce the risk of developing AD and vitamin E supplementation had no significant effect on the amyloidotic phenotype if the amyloid plaques were already deposited (Feng and Wang, 2012).

Thiazolidinediones (TZDs)

The peroxisome proliferator-activated receptor-γ (PPAR- γ), highly expressed in adipose tissue, has a pivotal role in regulating carbohydrate, protein, and lipid metabolism and inflammatory responses (de la Monte, 2017). Thiazolidinediones (TZDs) are synthetic PPAR- γ agonists and potent insulin sensitizers, approved to treat T2DM. TZDs are now considered an attractive treatment of AD because of their potential benefit in cognitive function and memory (Khan et al., 2019). Here, we discuss two prototype TZDs—rosiglitazone and pioglitazone.

Rosiglitazone not only increases insulin sensitivity but also regulates APP processing, leading to reduced plasma amyloid β-protein levels (Pardeshi et al., 2017). Rosiglitazone upregulates IDE levels and downregulates amyloid β-protein levels in a mixed transgenic APPSwe/PS1 mouse model exhibiting both AD and T2DM (Li et al., 2018). Patients with mild to moderate AD in clinical trials were found to significantly improve cognitive function when administrated rosiglitazone (Watson et al., 2005; Risner et al., 2006). However, a phase III trial of rosiglitazone showed no significant effect on cognition (Gold et al., 2010) and rosiglitazone had no effect on the risk of dementia in T2DM patients (Tseng, 2019).

Pioglitazone has been found to increase insulin sensitivity, downregulate levels of hippocampal amyloid β-protein oligomer and decrease pro-cognitive effects in insulin-resistant rats (Yin et al., 2013; Gad et al., 2015). Furthermore, pioglitazone improved cognitive performance in some patients with AD and T2DM (Hanyu et al., 2009; Sato et al., 2011). However, the adverse effects of TZDs, including edema and congestive heart failure, are major limitations for their use in the treatment of dementia and AD (Campbell et al., 2018).

DISCUSSION

AD is a well-known neurodegenerative disorder, which afflicts millions of people worldwide and places a huge financial burden on society (Jia et al., 2018). For decades, treatments targeting amyloid β-protein based on the amyloid-cascade hypothesis and oligomer-cascade hypothesis have failed (Morris et al., 2014, 2018; Panza et al., 2019). The FDA's approval of the amyloid β-antibody Aducanumab reflects a promising achievement in AD therapy despite uncertainty about this drug's clinical benefits and adverse reactions. Apart from amyloid targets, in 2020, according to the FDA registry, there were over 50 agents in clinical trials targeting tau protein, inflammation and metabolism (Cummings et al., 2020). Therefore, novel approaches based on recent insights into this disease are needed.

The role of insulin in AD pathogenesis has only recently gained attention. Insulin resistance may not be the primary cause of AD but it definitely exacerbates AD progression (Clark and Vissel, 2018). In this review, we summarize the mechanisms whereby insulin resistance worsens amyloid β-protein accumulation and tau hyperphosphorylation, including activation of neuroinflammation, activation of oxidative stress and downregulation of IDE. We highlight how insulin resistance and AD form a feed-forward loop in which insulin resistance increases the risk of AD and AD, in turn, exacerbates insulin resistance. Targeting insulin resistance may be a breakthrough strategy to treat AD and may avoid the pitfalls of past treatments targeting amyloid β-protein and tau protein. This review adds to the literature linking insulin resistance and AD by extending insights in this area to update the list of drug candidates that can be repurposed for AD. Further research into the mechanism of the metabolic drivers of AD is needed to identify novel therapeutic approaches for this devastating disease.

AUTHOR CONTRIBUTIONS

ZW and JK wrote the first draft of the manuscript. SR conceived the idea for the article and edited the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Role of DPP-4 and SGLT2 Inhibitors Connected to Alzheimer Disease in Type 2 Diabetes Mellitus

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Alzheimer's disease (AD) is characterized by memory loss and cognitive decline. Additionally, abnormal extracellular amyloid plaques accumulation and nerve damage caused by intracellular neurofibrillary tangles, and tau protein are characteristic of AD. Furthermore, AD is associated with oxidative stress, impaired mitochondrial structure and function, denormalization, and inflammatory responses. Recently, besides the amyloid β hypothesis, another hypothesis linking AD to systemic diseases has been put forth by multiple studies as a probable cause for AD. Particularly, type 2 diabetes mellitus (T2DM) and its features, including hyperinsulinemia, and chronic hyperglycemia with an inflammatory response, have been shown to be closely related to AD through insulin resistance. The brain cannot synthesize or store glucose, but it does require glucose, and the use of glucose in the brain is higher than that in any other organ in the mammalian body. One of the therapeutic drugs for T2DM, dipeptidyl peptidase-4 (DPP-4) inhibitor, suppresses the degradation of incretins, glucagon-like peptides and glucose-dependent insulinotropic peptide. Sodium-glucose cotransporter 2 (SGLT2) inhibitors, recently used in T2DM treatment, have a unique mechanism of action *via* inhibition of renal glucose reabsorption, and which is different from the mechanisms of previously used medications. This manuscript reviews the pathophysiological relationship between the two diseases, AD and T2DM, and the pharmacological effects of therapeutic T2DM drugs, especially DPP-4 inhibitors, and SGLT2 inhibitors.

Keywords: insulin signaling, insulin resistance, Alzheimer's disease, type 2 diabetes mellitus, DPP-4 inhibitor, SGLT2 inhibitor

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INTRODUCTION

Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) are two of the most common disorders affecting older adults. AD occurs in 60–80% of the elderly population as the most common neurocognitive disorder and type of dementia. Clinically, AD is characterized by progressive memory loss and decreased cognitive function, leading to premature death several years after diagnosis. The most common pathological features of AD are the abnormal accumulation of amyloid plaques due to the aggregation of amyloid β (Aβ) peptides and neurofibrillary tangles

(NFT) consisting of hyperphosphorylated tau protein. Recent studies have revealed that AD is associated with extracellular amyloid plaques, intracellular NFT, neuronal loss, and cellular damage. It is caused by oxidative stress, abnormal mitochondrial structure and function, inflammation, and aging (Kandimalla et al., 2017). This injury has also been associated with conditions related to insulin resistance, including hyperinsulinemia, chronic hyperglycemia, inflammation, and vascular changes. It was also confirmed that approximately 80% of AD patients are affected by insulin resistance or T2DM (de la Monte, 2014). This allows the mechanical relationship between T2DM and AD to be better understood. Research results have shown that AD can be regarded as a metabolic disorder with impaired brain glucose uptake and energy production. Therefore, studies on the causes of AD, based on the potential neuroprotective effects of anti-diabetic drugs and their direct and indirect mechanisms of action, are ongoing (Boccardi et al., 2019). In this review, we provide a summary of the mechanisms that link AD and T2DM; thereafter, we focus on the principal drugs of T2DM and explore their potential as suitable candidates for the treatment of AD.

INSULIN AS A MEDIATOR OF T2DM

The worldwide incidence of diabetes, which is a chronic metabolic disorder, is rapidly increasing. Diabetes can be classified as types 1 and 2. T2DM, which accounts for 95% of all cases of diabetes, is characterized by hyperinsulinemia and insulin resistance (Taylor, 2012). Another feature of T2DM is the formation of amyloid polypeptides, which induce pancreatic β cell dysfunction (Marzban et al., 2003). Insulin resistance and amyloid peptides reduce the absorption of blood glucose and ultimately induce chronic hyperglycemia, one of the pathological features of T2DM (Chatterjee and Mudher, 2018). Maintaining the insulin secretory function and ameliorating insulin resistance are important in the management of T2DM.

Insulin Signaling

Insulin is a hormone secreted by the β cells of the pancreas in response to high glucose levels. The binding of insulin to the insulin receptor (IR) initiates insulin signaling.

During insulin binding, IR auto-phosphorylates tyrosine residues in the intracellular portion of the receptor and then rapidly phosphorylates the tyrosine residues of C substrates 1 to 4 (IRS1-4; Lavan et al., 1997). IRS converts insulin *via* several pathways, the most well-known of which is the phosphoinositide 3-kinase (PIK3)/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) pathway of IRS1. When the serine residues of IRS1 and IRS2 are phosphorylated, these get separated from the IR, tyrosine phosphorylation of IRS is reduced, and the downward regulation of insulin signal is inhibited (Mothe and Van Obberghen, 1996).

In addition, insulin maintains glucose homeostasis by suppressing glycogen synthase kinase-3 β (GSK3-β), which regulates glucose production and glucose consumption by muscle and adipose tissue after passing through the glucose transporter type 4 (GLUT4; Guo, 2014; Roberts et al., 2014).

Insulin Resistance

Insulin resistance indicates a reduced function of insulin in target tissues, such as the liver, muscles, and adipose tissue. The ability of IRS to get activated and transmit downstream signals is diminished in insulin-responsive tissues, leading to impaired insulin secretion and insulin dysfunction, which are major causes of diabetes (Leng et al., 2004; Guo, 2014).

Insulin resistance in skeletal muscle reduces glucose intake, making it difficult to regulate muscle glycogen synthesis (Hunter and Garvey, 1998). This is considered to occur due to the suppression of *GLUT4* gene by excessive free fatty acids. High saturated fatty acid levels, which can suppress normal IRS1 tyrosine phosphorylation and induce insulin resistance in skeletal muscle, show a correlation with skeletal muscle insulin activity (Roden et al., 1996; Pan et al., 1997). Recent studies investigating the deformation of O-linked-β-N-acetylglucosamine (OGlcNAc) protein found that OGlcNAc transferase and β-anomalous variants of N-acetylglucosamine [mediated by O-GlcNAcase (OGA)] are IRS Ser/Thr residues. An important function of the liver is to produce and store glycogen in a glucose reservoir that is readily available to the body (Ma and Hart, 2013). Glucose production during normal postprandial state with glycogenolysis is sufficient to meet the energy needs of the brain and other body tissues. However, insulin resistance results in a systemic insulin resistance phenomenon (Beck-Nielsen et al., 2002; Bugianesi et al., 2005) which causes the body tissues to be deprived of glucose.

INSULIN IN THE BRAIN

Insulin receptors are expressed in all brain cells, but the differences in expression levels vary considerably from region to region and are most visible in the cerebral cortex, striatum, and cerebellum. This suggests that insulin signaling is important in the brain and plays various roles (Arnold et al., 2018). Insulin and insulin-like growth factor (IGF) signaling mechanisms in the brain are important for maintaining synaptic plasticity, and function (Boucher et al., 2014). When insulin binds to an IR, multiple tyrosine residues are auto-phosphorylated to activate IRS1 and IRS2, which mediate downstream signaling through PIK3. This PIK3 activates AKT and suppresses activity at serine 9 residues *via* GSK3-β phosphorylation, leading to glycogen synthesis (Avila et al., 2012). The PIK/AKT pathway stimulates excitatory and inhibitory cell membrane receptors to regulate synaptic plasticity, enhance N-Methyl-D-aspartic acid (NMDA) receptor-mediated long-term potentiation and neurotransmitter activity, and is important for learning and memory. Additionally, the PIK/AKT pathway increases cortical glucose metabolism (Farrar et al., 2005; Bradley et al., 2012). However, in an abnormal state, GSK3-β is overactivated and tau is phosphorylated; this hyperphosphorylated tau gets aggregated and entangled in nerve fibers (Avila et al., 2010). GSK3-β also acts as a mediator of cell death, increasing the production of Aβ (Qu et al., 2014; Meng et al., 2020).

Insulin activates the mitogen-activated protein kinase (MAPK) pathway, leading to Ras activation and activation of

rapidly accelerated fibrosarcoma (Raf), MAPK/ERK kinase (MEK), and extracellular signal-regulated kinase (ERK) in the protoplasmic membrane (Zhang et al., 2011). Although the direct role of the MAPK pathway components that mediate AD pathology has not yet been clarified, recent studies have reported that ERK plays an important role in synaptogenesis, learning, and memory function and has neuroprotective functions (Thiels and Klann, 2001).

Epidemiological studies and neuroimaging studies of the brain have indicated that insulin and IGF signaling pathways are important for the preservation and maintenance of learning and memory processes, and it can be confirmed that the function of learning and memory is improved in AD patients with intranasal insulin injection (Benedict et al., 2007).

AD CAUSED BY INSULIN RESISTANCE IN THE BRAIN

Alzheimer's disease can be classified into two clinical subtypes: familial AD (fAD) and sporadic AD (sAD). Although the two types of disease (fAD and sAD) exhibit similar pathological phenotypes such as presence of plaques, tangles, synaptic damage, and neuronal loss, the factors that induce the neurodegenerative process are completely different. Pathological accumulation in fAD occurs due to the presence of autosomal dominant mutations in one of the three genes: amyloid protein precursor (APP), presenilin-1, or presenilin-2 (Querfurth and LaFerla, 2010). However, the cause of sAD, which accounts for the majority of AD cases, is complex and multifactorial based on the combination of genetic factors, epigenetic factors, and lifestyle-related factors. Moreover, most sAD patients are elderly individuals with various comorbidities (e.g., stroke, stress, diabetes, seizures, osteoporosis, and kidney disease) that can significantly increase the complexity underlying the pathogenesis of sAD (Doraiswamy et al., 2002; Magaki et al., 2014; Aubert et al., 2015).

Many recent studies have confirmed that insulin signaling impaired due to insulin resistance also occurs in AD (Talbot et al., 2012). In fluorodeoxyglucose (FDG)–positron emission tomography (PET) studies of the brains of patients with "early-stage" AD, AD was referred to as "type 3 diabetes" because of reduced glucose intake (de la Monte and Wands, 2008).

The important role of insulin in the peripheral system is well known and has been widely studied, but studies on insulin function in the central nervous system are currently underway. Previously, it was believed that due to the size of insulin, insulin could not pass through the brain-blood barrier (BBB), and the brain was considered to be insulin-independent; however, some studies have shown that IRs are expressed in the brain, and there are several mechanisms to support the presence of insulin (Banks, 2004; Gray et al., 2014).

Recent studies have established that insulin is transported through the BBB *via* carrier-mediated, saturated, and temperature-sensitive active processes. All types of brain cells, including neurons, have insulin signaling pathways, and insulin regulates the concentration of neurotransmitters such as acetylcholine, recovery, differentiation, proliferation, regeneration, and neuronal cell death (de la Monte et al., 2003; Goberdhan and Wilson, 2003; Russo et al., 2005).

Studies have shown that ICR mice on a long-term high-fat diet (HFD) developed T2DM with insulin resistance in both the body and brain, along with Alzheimer's pathologies such as cognitive deficits, Aβ accumulation, and hyperphosphorylated tau. Aβ oligomers remove IRs in the protoplasmic membrane, and insulin also affects Aβ accumulation and systemic tau phosphorylation (Zhao et al., 2008).

The insulin-degrading enzyme (IDE) is also known to degrade other substrates such as Aβ (Farris et al., 2003). When insulin levels increase, IDE expression is activated, and it inhibits long-term insulin activity. However, in an insulin-resistant state, because IDE is used to remove insulin, senile plaques are formed in which IDE cannot lower Aβ (Shiiki et al., 2004).

GSK3-β, which is the most widely studied tau kinase, is also involved in Aβ production (Jeon et al., 2015). A study showed that GSK3-β, which is a multifunctional Ser/Thr kinase affected by tau phosphorylation and aggregation inhibition, improved learning and memory and reduced tau phosphorylation in an AD transgenic mouse model (Farr et al., 2016). In addition, GSK3-β expression was suppressed in an AD-pathology mouse model. In particular, intranasal insulin injection has been shown to help improve memory by maintaining serum insulin and glucose levels (Benedict et al., 2004), suggesting that insulin is a therapeutic target for AD.

In addition to PI3K/AKT and Ras/Raf/MAPK insulin signaling pathways, mTOR and its downstream targets that regulate neuronal survival and nutrient sensing play roles in AD pathogenesis; however, these roles are not well-defined. mTOR regulates protein synthesis by phosphorylating the key substrates of the translational machinery, namely, the eukaryotic initiation factor 4E-binding protein and p70S6 kinase. Rapamycin inhibits mTOR *in vivo* and halts cellular growth and proliferation (Showkat et al., 2014). Also, genetic inhibition of mTOR reduces the level of memory loss, improves cognitive function and reduces tan and Aβ deposits (Kaeberlein and Galvan, 2019). It is hypothesized that in an insulin-resistant state, these downstream signaling pathways are compromised, leading to increased levels of Aβ oligomers and hyperphosphorylated tau. These increased levels of Aβ oligomers and hyperphosphorylated tau occur not only due to a dysregulation of downstream kinases but also due to an impairment of autophagic clearance that arises as a result of an imbalance of the mTOR and autophagy pathways. Autophagic dysfunction which is recently gaining attention feature of AD causes the progressive accumulation of toxic proteins and eventually leads to neuronal death (Orr and Oddo, 2013).

LINKING AD AND T2DM

Alzheimer's disease, a degenerative brain disease, is the most common cause of dementia, with clinical features including gradual decline of cognitive function, amnesia, behavioral and personality changes, and pathological features including extracellular Aβ plaques and intracellular NFT intraneuronal

deposition, tau protein degeneration, and severe neuronal loss in the brain tissue (Saxena, 2010). Most AD treatments have focused on Aβ but have failed to look at AD from various perspectives, for example, relating AD to obesity and T2DM (Kang et al., 2017). Considering the relationship between AD and T2DM, it might be possible to treat AD with T2DM drugs. For instance, Thiazolidinedlones (TZDs), such as pioglitazone and rosiglitazone, are PPARγ agonists used as anti-diabetic drugs, that induce a decrease in plasma free fatty acid concentration and fasting hyperglycemia through an insulin-reducing effect. A recent pioglitazone-related study found that it may be of therapeutic benefit, showing a significant reduction in Aβ and tau pathology measured in cerebral blood flow from patients with early-stage and mild to moderate AD patients (Pérez and Quintanilla, 2015).

According to the Mayo Clinic Alzheimer Disease Patient Registry, 80% of patients with AD have impaired glucose tolerance or diabetes (Janson et al., 2004). Epidemiological studies have shown that T2DM induces cognitive impairment and that T2DM patients are 1.5−2 times more likely to be diagnosed with dementia than healthy individuals are (Biessels et al., 2014). There is also evidence of cellular insulin resistance or insulin deficiency in the brains of patients with AD, including non-diabetic patients (Vijan, 2015).

Type 2 diabetes mellitus is a chronic metabolic disorder that can damage blood vessels, nerves, eyes, and kidneys and causes serious complications. Typical symptoms of T2DM associated with insulin dysfunction, including hyperglycemia, insulin resistance, and relative insulin deficiency, also induce the accumulation of Aβ in the brain, contributing to AD pathogenesis (Ramos-Rodriguez et al., 2017).

Several pathogenic mechanisms overlap the two diseases, including dysregulation of glucose and insulin signals, increased inflammation, Aβ deposition, mitochondrial dysfunction, and oxidative stress (Liu et al., 2011). Insulin resistance and deficiency are increased by abnormally creating insulin signaling through PI3K/Akt/GSK3-β signals; GSK3-β activation is an important component of NFT and can lead to hyperphosphorylated tau (Zheng et al., 2015). In addition, IDE associated with insulin signaling plays an important role in insulin and Aβ clearance, so that impaired IDE function can cause AD and T2DM (Ramos-Rodriguez et al., 2017). IRS1 plays an important role in transferring insulin and IGF-1 receptor signals to signal adapter proteins and the intracellular pathway. PI3K/AKT kinase pathway and IRS1 dysfunction causes AD and T2DM (illustrated in **Figure 1**).

AD AND T2DM DRUGS

Expressing Organs and Function of Dipeptidyl Peptidase-4

When food is ingested, a series of hormones are secreted by epithelial cells of the small intestine to increase insulin secretion. When glucose is orally administered, insulin secretion from the pancreas increases, and this phenomenon is called the incretin effect. A typical incretin is an endogenous peptide that is mainly synthesized and secreted by enteroendocrine L cells into the gastrointestinal peptide hormone (GLP-1). Physiologically, it promotes β cell proliferation, improves β cell function, decreases β cell apoptosis, increases insulin secretion, and regulates glucose homeostasis (Graaf et al., 2016; Keshava et al., 2017). Glucagon-like peptide-1 receptor (GLP-1R) is widely expressed in the hippocampus, hypothalamus, cortex, nucleus basalis of the Meynert, choroidal plexus, and nucleus of the solitary tract (Erreger et al., 2012; Yildirim Simsir et al., 2018). This factor, which is overexpressed in the hippocampus of mice, also affects neurite growth, learning, and memory (McClean et al., 2011). In addition, GLP-1 analogs and GLP-1R agonists administered peripherally or centrally reduce Aβ deposition, prevent tau and NFT protein hyperphosphorylation, and have a neuroprotective effect against rodent AD-like neurodegeneration (Hölscher, 2018). There have also been reported to be effective for maintaining synaptic plasticity and learning and memory (McClean et al., 2011; Li et al., 2012; Xiong et al., 2013; McClean and Hölscher, 2014; Candeias et al., 2015; Wang et al., 2016).

However, GLP-1 has a short duration of reaction time as it is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) present in plasma and other body fluids, such as cerebrospinal fluid (Gong et al., 2014).

Dipeptidyl peptidase-4 is a type 2 transmembrane glycoprotein with various functions. Usually, the substrates of DPP-4 are peptides with a size of 80 amino acids or less, and there are more than 35 neuropeptides and chemokines, including GLP-1, GLP, neuropeptide Y, peptide YY, substance P, and stromal cell-derived factor 1, that serve as substrates for this peptide. It is found in epithelial cells, immune cells, including T lymphocytes, various cells, such as vascular endothelial cells, and almost all tissues, including kidney, liver, adrenal gland, skeletal muscle, pancreas, lung, small intestine, bone marrow, and spleen (Chen et al., 2019).

Expressing Organs and Function of Sodium-Glucose Cotransporter 2

Usually, 180 g of glucose per day is filtered by the kidneys and reabsorbed in the proximal tubules. Glucose reabsorption occurs *via* sodium-glucose cotransporter 2 (SGLT2) present mainly in the kidney, which comprises SGLT2 located in front of the proximal tubule and sodium-glucose cotransporter 1 (SGLT1) in the latter half. In a normal blood glucose level state, SGLT2 is responsible for approximately 97% of the reabsorption of filtered glucose, whereas and SGLT1 is responsible for approximately 3%.

Glucose reabsorption begins with active transfer of Na^+ the extracellular region by Na^+/K^+ ATPase in the proximal tubule. The electrochemical force generated while moving Na^+ extracellularly causes Na^+ and glucose to move intracellularly *via* SGLT. One Na^+ and glucose molecule move together through SGLT2, and two Na^+ and glucose molecules move together through SGLT1. When the glucose concentration increases by the glucose transferred into the cell, glucose is reabsorbed into the bloodstream *via* the glucose transporter based on the difference in glucose concentration between the cell and epilepsy.

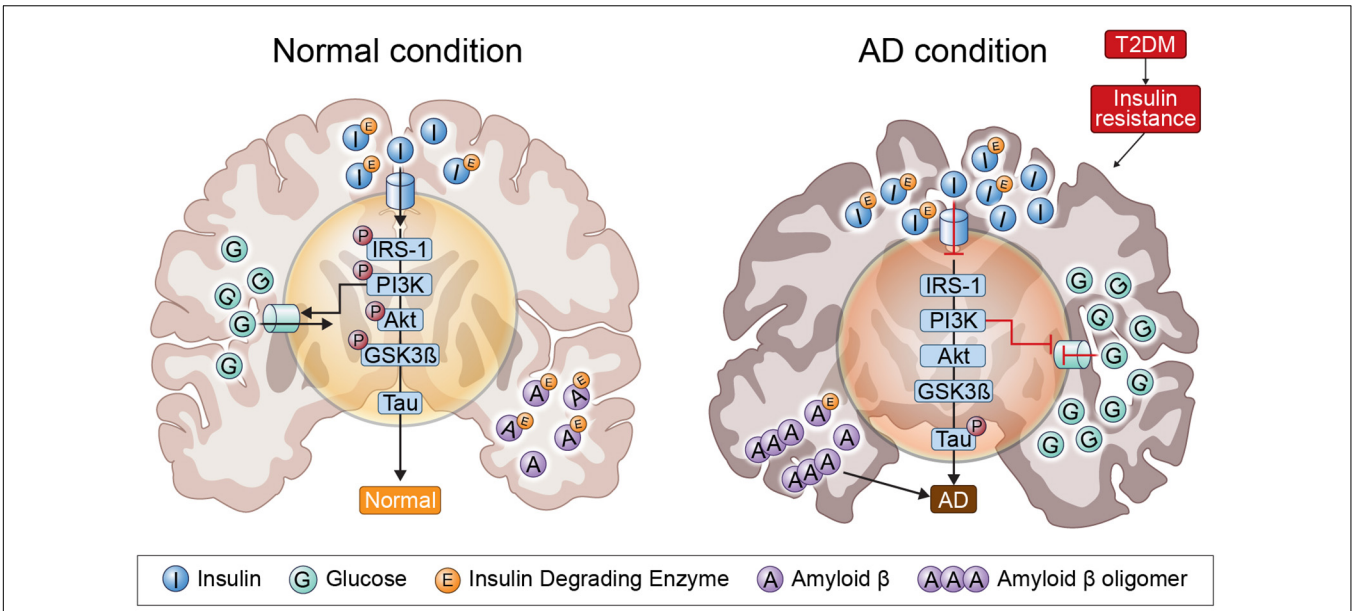

FIGURE 1 | Insulin signaling in the normal condition brain and AD brain. In insulin-resistant state induced by T2DM, as insulin signaling is impaired, insulin is unable to bind to an insulin receptor, and multiple tyrosine residues are not auto-phosphorylated to activate insulin receptor substrate 1, which mediates downstream signaling through phosphoinositide 3-kinase. Therefore, GSK3-β is overactivated and tau is phosphorylated.

Recently, there have been increasing reports of the presence of SGLTs in the mammalian central nervous system (Yu et al., 2010, 2013). The receptors for SGLT1 are expressed in CA1, CA3 (regions 1 and 3 of hippocampal cornu ammonis), and the dentate gyrus hippocampal subfields, and SGLT2 has been reported to be expressed in the hippocampus, cerebellum, and blood-hippocampal barrier endothelial cells (Poppe et al., 1997; Enerson and Drewes, 2006; Shah et al., 2012; Jurcovicova, 2014).

Pharmacological Role of DPP-4 and SGLT2 Inhibitors

Dipeptidyl peptidase-4 inhibitors (DPP-4i$_s$) include sitagliptin, vildagliptin, saxagliptin, linagliptin, alogliptin, and gemigliptin. As hormones that increase insulin secretion are degraded by DPP-4, DPP-4i$_s$ can be used to suppress this hormone degradation. In other words, the principle of DPP-4 is the increase in insulin secretion following food intake and the period of insulin secretion time can be improved, and blood glucose levels can be additionally improved by suppressing glucagon secretion without inducing hypoglycemia.

The SGLT2 inhibitors (SGLT2i$_s$) include dapagliflozin, canagliflozin, empagliflozin, ipragliflozin, tofogliflozin, luseogliflozin, and ertugliflozin. These inhibitors reduce glycated hemoglobin level by 0.3–0.9% and fasting blood glucose levels by 18–36 mg/dl, regardless of use of other drugs, and decrease body weight as well as blood pressures due to drug effects on glucosuria and natriuresis. In addition, since these have an insulin-independent hypoglycemic effect, SGLT2i$_s$ can reduce the blood glucose level even in an environment where the insulin secretory capacity is decreased. By increasing the excretion of glucose into the urine, insulin resistance can be improved, and by improving glucose toxicity, the function of pancreatic β cells can be maintained. These are diabetic treatment agents with a low risk of hypoglycemia because they facilitate the excretion of glucose in a hyperglycemic state without affecting insulin secretion.

Regulation of DPP-4i$_s$ in the AD Brain

Glucagon-like peptide-1 signaling in the brain regulates glucose metabolism. Inhibitors of DPP-4 improve neuronal insulin resistance by restoring insulin-induced phosphorylation of neuronal IR, IRS1 phosphorylation, and AKT/PKB-Ser phosphorylation, resulting in the brain mitochondrial dysfunction. Previous studies on diabetes-related AD rat models have demonstrated that GLP-1 positively affects learning and memory (Chen et al., 2012). In addition, a recent study has shown that DPP4i$_s$ can increase the levels of active GLP-1 in the brain and improve memory behaviors in AD mice models (D'Amico et al., 2010). These have also been shown to improve spatial learning and memory ability and protect synaptic proteins by increasing GLP-1 and GLP-1R expression levels in the hippocampus and cortex of AD mice (Pipatpiboon et al., 2013). Cognitive function was improved as a result of the administration of DPP-4i$_s$ and quercetin (3,3′,4′,5,7-pentahydroxyflavone) found in vegetables and fruits which is one of the major groups of polyphenols with effects on inflammation, diabetes and the nervous system in a study (Babaei et al., 2018; Li et al., 2019), and DPP-4i$_s$ was shown to ameliorate memory impairment, increase GLP-1 levels in the brain which acts as a neuroprotective agent (Holst et al., 2011) and could lead to improved brain and hippocampal mitochondrial function and reduced brain

TABLE 1 | Research of DPP-4 inhibitors in AD models.

Drug name	Drug type	Studies in AD models
Dipeptidyl peptidase-4 Inhibitors	Sitagliptin Vildagliptin Saxagliptin Linagliptin Alogliptin Gemigliptin	DPP-4 i improved spatial learning and memory ability and protected synaptic proteins by increasing GLP-1 and GLP-1R expression levels in the hippocampus and cortex of the brain in AD mice
		Cognitive function was improved as a result of administration of DPP-4 i and quercetin
		DPP-4 i ameliorated memory impairment, increased GLP-1 level in the brain, significantly reduced nitrosative stress, inflammation hallmarks, and Aβ deposits
		DPP-4 i significantly protected against Aβ-induced cytotoxicity, and inhibited the activation of GSK3-β and tau hyperphosphorylation by restoring insulin downstream signaling. DPP-4 i ameliorated Aβ-induced mitochondrial dysfunction and intracellular ROS generation, and upregulated *Sirt1* expression
		DPP-4 i showed a time-dependent improvement in memory retention and AD-associated proteins such as tau phosphorylation were decreased in the hippocampus with DPP-4 i administration
		The combination of DPP-4i and memantine could reduce the expression of APP, and phosphorylated tau protein
		DPP-4 i could alleviate cognitive deficits in 3xTG AD mice. It improved incretin levels in the brain and reduced Aβ, tau phosphorylation, and neuroinflammation

MDA (Pintana et al., 2013), and significantly reduce nitrosative stress, inflammation hallmarks, and Aβ deposits (D'Amico et al., 2010; Kosaraju et al., 2013a, 2017). These inhibitors showed a time-dependent improvement in memory retention and dose-dependent attenuation of Aβ, tau phosphorylation, and inflammatory markers, and AD-associated proteins were decreased in the hippocampus following DPP-4i$_s$ administration (Kosaraju et al., 2013b; Ma et al., 2018). The combination of DPP-4i and memantine could reduce the expression of APP and phosphorylated tau protein (Khalaf et al., 2019). Inhibitors of DPP-4 alleviated cognitive deficits in 3xTG AD mice. These improve incretin levels in the brain and reduce Aβ deposition, tau phosphorylation, and neuroinflammation (Thomas et al., 2008) and can significantly protect against Aβ-induced cytotoxicity, and inhibit the activation of GSK3-β and tau hyperphosphorylation by restoring downstream insulin signaling. Inhibitors of DPP-4 ameliorated Aβ-induced mitochondrial dysfunction and intracellular reactive oxygen species (ROS) generation and upregulated *Sirt1* expression (Kosaraju et al., 2017). HFD rats had brain mitochondrial dysfunction as shown by increased ROS production, mitochondrial depolarization, and mitochondrial swelling. In the mitochondria, it has been shown that increased levels of ROS could cause the opening of the inner membrane anion channel (IMAC), thus leading to mitochondrial membrane depolarization (Zorov et al., 2006). The depolarization of mitochondria could also lead to the dysfunction of mitochondria to produce ATP synthesis (Aon et al., 2006). Furthermore, increased ROS levels could play a role in the cognitive decline observed in HFD rats (**Table 1** for a summary, illustrated in **Figure 2**).

Regulation of SGLT2i$_s$ in the AD Brain

Inhibitors of SGLT2 not only improve peripheral insulin sensitivity and reduce body weight (Xu et al., 2017) but also improve brain mitochondrial function and insulin signaling, and reduce cell death. Furthermore, SGLT2i$_s$ prevent cognitive decline and protect synaptic plasticity in the hippocampus (Sa-Nguanmoo et al., 2017). Inhibitors of SGLT2 reduced the accumulation of Aβ in the cortical region of AD-T2DM mice (APP/PS1xdb/db mice) which is a genetically diabetic model of T2DM and showed the same effect on the amount of tau induced pathological cerebral atrophy (Wiciński et al., 2020). SGLT2i$_s$-mediated mTOR inhibition, through continuous loss of glucose in the urine, routinely restores a reliable, overnight catabolic-fasted state in older, inactive individuals and re-establishes the benefits associated with circadian catabolic/anabolic metabolism (e.g., reactivation of the endo-lysosomal pathway through inhibition of mTOR), removal and replacement of dysfunctional organelles/proteins, and lowering of blood pressure through mTOR-mediated modulation of sympathetic tone. Unrestrained chronic mTOR activation may be responsible for sustaining metabolic and mitochondrial dysfunction in AD, driving the breakdown of

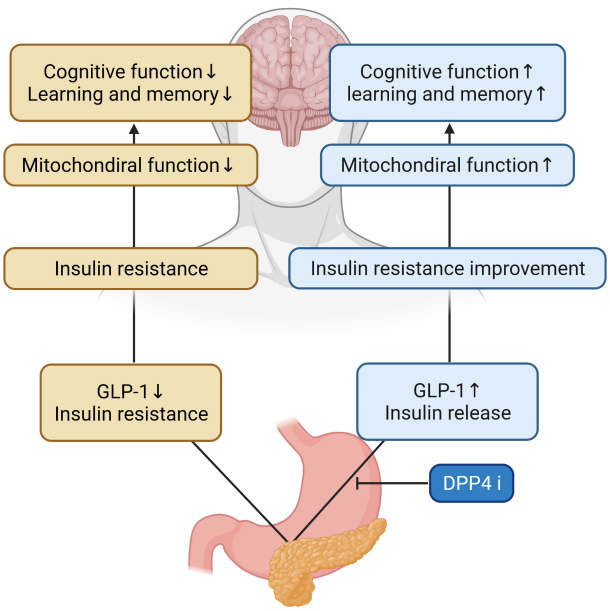

FIGURE 2 | Effects of DPP-4 inhibitor in the AD brain. During DPP-4 inhibitor treatment, GLP-1 increases and insulin is secreted, which improves insulin resistance and mitochondrial function in the brain. Therefore, cognitive function and learning and memory ability are improved.

TABLE 2 | Research of SGLT2 inhibitors in AD models.

Drug name	Drug type	Studies in AD models
Sodium-glucose cotransporter 2 inhibitors	Dapagliflozin Canagliflozin Empagliflozin Ipragliflozin Tofogliflozin Luseogliflozin Ertugliflozin	SGLT2 i not only improved peripheral insulin sensitivity and reduced increasing of body weight, but also improved brain mitochondrial function, insulin signaling, and reduction of cell death SGLT2 i prevented cognitive decline and protect synaptic plasticity in the hippocampus SGLT2 i reduced the accumulation of Aβ in the cortical region of Aβ precursor protein (APP)/PS1xdb/db mice and showed the same effect on the amount of tau pathological cerebral atrophy SGLT2 i restored mTOR signaling through mTOR inhibition and prevented the progression of the pathology of AD SGLT2 i physiologically elevates blood ketone bodies such as β-hydroxybutyrate, which can modulate NLRP3 inflammasome-IL-1β signaling, and a key pathologic pathway in AD SGLT2 i not only ameliorated albuminuria, and glomerular injury in db/db mice but also significantly prevented the impairment of cognitive function in db/db mice, which was associated with the attenuation of cerebral oxidative stress, and the increase in cerebral brain-derived neurotrophic factor SGLT2 i seems to be attributed to the attenuation of oxidative stress and since BDNF, the effect of SGLT2 i treatment promotes memory, and survival of neurons

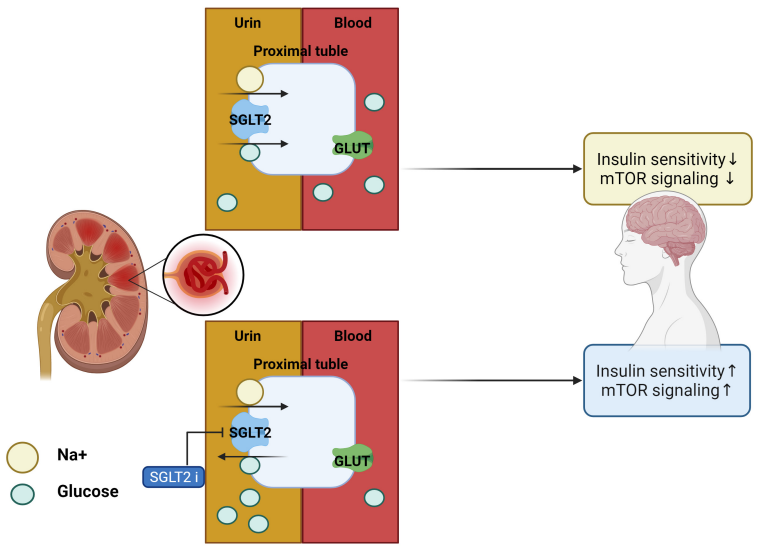

FIGURE 3 | Effects of SGLT2 inhibitor in the AD brain. With SGLT2 inhibitor, insulin sensitivity and mTOR signaling in the brain are improved, as excess glucose from insulin resistance is filtered out by the kidneys.

the BBB *via* endothelial cell dysfunction, as well as driving the hyperphosphorylation of tau, and formation of amyloid plaques in the brain (Mueed et al., 2018). These inhibitors can restore mTOR signaling through mTOR inhibition and prevent the progression of AD pathology (Esterline et al., 2020). In addition, SGLT2i$_s$ physiologically elevates blood ketone bodies such as β-hydroxybutyrate (Kim et al., 2019), which can modulate NLRP3 inflammasome-IL-1β signaling (Kim et al., 2020), a key pathologic pathway in AD (Heneka et al., 2013). Decreased blood glucose levels were seen in db/db mice after 10 weeks of treatment with SGLT2i$_s$ for T2DM. These inhibitors not only ameliorated albuminuria and glomerular injury in db/db mice but also significantly prevented the impairment of cognitive function, which was associated with the attenuation of cerebral oxidative stress and increase in cerebral brain-derived neurotrophic factor level (Lin et al., 2014). SGLT2i$_s$ treatment significantly attenuated cerebral oxidative stress and DNA oxidative damage in db/db mice, as shown by the reduction of cerebral superoxide and 8-OHdG, and this attenuation of cerebral oxidative stress was associated with the reduction of cerebral NADPH oxidase subunit. Therefore, the improvement of cognitive function by SGLT2i$_s$ seems to be attributed to the attenuation of oxidative stress. Moreover, the effect of SGLT2i$_s$ treatment on cerebral BDNF, since BDNF, a key protein promoting memory and survival of neurons, is significantly reduced in diabetic patients, and diabetic animals including db/db mice and the decrease in cerebral BDNF is shown to be associated with cognitive decline (Lin et al., 2014; **Table 2** for a summary, illustrated in **Figure 3**).

Clinical Evidence in Therapeutic Effects of DPP-4i and SGLT2i on Dementia

Majority of reports regarding the effects of anti-diabetic agents on dementia have been investigated from retrospective studies. In 240 elderly patients with T2DM affected by mild cognitive impairment (MCI), 2 years treatment group of DPP-4i

significantly improve cognitive functions measured by mini-mental state examination (MMSE), compared to the sulfonylurea which increases endogenous release of insulin from pancreatic β cells group (Rizzo et al., 2014). A prospective, non-randomized study showed that sitagliptin therapy prevented from the decline of MMSE during 6 months in old T2DM (Isik et al., 2017). Using a health insurance claim database in Korea, DPP-4i use demonstrated a significant 46% decrease in AD development among elderly T2DM (Kim et al., 2018). Although there are few data on SGLT2i, one randomized clinical trial reported no changes in MMSE after 12-month treatment of incretins vs. SGLT2i (Perna et al., 2018). Further larger and well-designed clinical studies are needed to evaluate the neuroprotective effects of DPP-4i and SGLT2i.

CONCLUSION

Recent studies have identified the key mechanisms by which the brain becomes resistant to insulin in AD and how impaired insulin signaling in AD is linked to memory impairment (Vieira et al., 2018). In this review, we describe the connections between AD and T2DM. Although the reason why many T2DM patients develop AD is not clear, the two diseases are associated with insulin resistance. Significant effort is required to identify the common pathological and molecular mechanisms between AD and T2DM, which will help better understand the onset and development of both diseases. Therefore, novel approaches to identify biomarkers for detecting early-stage of AD will likely increase the efficacy of anti-diabetic agents and allow treatment before severe neuronal dysfunction occurs in the AD brains.

AUTHOR CONTRIBUTIONS

AYS and SB wrote the manuscript. AYS, SB, JYK, Y-HL, and JEL participated in the discussion and revision. JEL designed and edited the final manuscript. All authors read and approved the final manuscript.

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BRIEF RESEARCH REPORT
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Altered Gut Microbial Load and Immune Activation in a *Drosophila* Model of Human Tauopathy

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Tau is a microtubule-associated protein that stabilizes the neuronal cytoskeleton. In the family of neurodegenerative diseases known as tauopathies, including Alzheimer's disease (AD), frontotemporal dementia (FTD), and chronic traumatic encephalopathy (CTE), abnormal tau aggregation destabilizes microtubule structure, contributing to a cascade of cellular processes leading to neuronal cell death. The gut microbiome has increasingly become a target of neurodegenerative disease research since gut microbiome imbalances have been linked to protein aggregation and inflammation through a bidirectional axis linking the gut and brain. Accordingly, the present study examined tau-mediated changes to gut microbiome composition and immune activation in a *Drosophila melanogaster* model of human mutant tauopathy. Fecal deposit quantification and gastric emptying time courses suggested an abnormal food distribution and reduced gut motility in tau transgenic flies compared to controls. Tau transgenic flies also showed an increase in gut bacteria colony forming units (CFUs) from diluted fly homogenate, indicating an increased bacterial load. Finally, we showed that tau transgenic flies have a trend towards elevated systemic levels of antimicrobial peptides targeting gram-negative bacteria using qPCR, suggesting an enhanced innate immune response to bacterial insult. These data demonstrate qualifiable and quantifiable gut microbial and innate immune responses to tauopathy. Furthermore, these results provide a framework for future studies targeting the gut microbiome as a modifier of neurodegenerative disease.

Keywords: tau, *Drosophila*, gut microbiome, motility, antimicrobial peptide (AMPs), innate immune activation, tauopathies

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INTRODUCTION

Accumulation of the microtubule-associated protein tau is the hallmark pathology of the family of neurodegenerative diseases known as tauopathies, which includes Alzheimer's disease (AD), frontotemporal dementia (FTD), and chronic traumatic encephalopathy (CTE). Upon hyperphosphorylation of tau, neurons undergo multiple changes leading to cell death, including alterations to cytoskeletal structure and mitochondrial function (Dias-Santagata et al., 2007;

Fulga et al., 2007; Steinhilb et al., 2007; Spillantini and Goedert, 2013; Arendt et al., 2016). Due to the incidence of tauopathies in the population, a greater understanding of the effects of neurotoxic tau protein could highlight critical therapeutic pathways. Recently, the gut microbiome has become of interest due to the connectedness of the brain and gut facilitated by the gut-brain axis (D'Argenio and Sarnataro, 2019).

The human gut microbiome, colonized at birth, encompasses the collective genome of approximately one-hundred trillion microorganisms residing in the gastrointestinal (GI) tract (Ghaisas et al., 2016). These gut microbes contribute to the preservation of human health through various mechanisms including the extraction and absorption of nutrients from food, protection against pathogen overgrowth, biosynthesis of vitamins, amino acids, and peptides, interactions with the intestinal epithelium, and modulation of the immune system (Hooper et al., 2012; Zhao et al., 2015). Additionally, the microbiome is susceptible to alteration due to factors ranging from antibiotic or probiotic exposure, diet changes, environmental factors, trauma, or disease (Hill et al., 2014; Pistollato et al., 2016). Consequently, the imbalance, or disruption, of the gut microbiome has been associated with numerous pathologies (Ghaisas et al., 2016; Ma et al., 2019). Of particular interest is the implication of microbiome dysbiosis on neurological disorders and neurodegenerative diseases, as mediated by the gut-brain axis.

Using the transgenic expression of mutant human FTDP-17–associated tau, tau^{R406W}, in *Drosophila*, we showed a reduced gut motility and subsequently increased gut bacterial load in aged tau transgenic flies compared to controls. We also showed an enhanced *Drosophila* innate immune response in tau transgenic flies using qPCR targeting specific antimicrobial peptide transcripts. Together, these data take advantage of the utility of transgenic *Drosophila* to show the widespread, systemic effects of tauopathy in an *in vivo* system. Furthermore, this work suggests that manipulation of the gut microbiome has potential to influence tau-mediated neurodegeneration, an important stepping stone to therapeutic approaches.

METHODS

Drosophila Crosses

Drosophila stocks were obtained from Bloomington Stock Center (pan-neuronal *elav-GAL4*) and Dr. Mel Feany at Harvard Medical School (*UAS-Tau*^{R406W}), respectively. Tau^{R406W} flies are referred to as "tau transgenic flies" for simplicity. All control (genotype: *elav-GAL4*/+) and tau transgenic flies (*elav-GAL4*/+;*UAS-Tau*^{R406W}/+) were the progeny of controlled genetic crosses using the GAL4/UAS bipartite expression system. Prior to testing, progeny were aged for 10 days to allow for the development of neurodegeneration. All flies were crossed and aged at 25°C in an incubator programmed on a 12 h light/dark schedule in cotton-plugged vials containing commercially available *Drosophila* food (Lab Express Fly Food M). Equal numbers of male and female flies were used for each experiment unless otherwise noted in the methods.

Fecal Deposits and Gastric Emptying Time Course

To examine gastrointestinal motility, fecal deposit counts were conducted. Vials were prepared by drawing a 2 cm × 2 cm box with a permanent marker on the outside of fly vials. The bottom of the box was drawn at the food line to reduce potentially confounding locomotor issues in tau transgenic flies. Each vial contained 7 mL of heated commercial fly food (Lab Express) mixed with 20 drops of blue food dye (Great Value brand). At 5 days post-eclosion, control and tau transgenic flies were placed into these dyed food vials and returned to the incubator for five more days of aging. At 10 days post-eclosion, flies were removed from the vials and fecal deposits within the marked boxes were quantified under a dissecting microscope. Counts were normalized to the number of flies within each vial.

To examine a time course of gastric emptying, 10-day-old control and tau transgenic flies were placed into separate blue food vials prepared as described above. Following a 24-h incubation period, flies were removed from the blue food and placed into individually labeled vials containing standard non-dyed *Drosophila* food. Flies were anesthetized at 4°C for 5 min prior to imaging. Based on previous work on anesthetization approaches, it is unlikely that the cold anesthesia approach would have preferential effects on the GI motility of control vs. tau transgenic flies (Badre et al., 2005; MacAlpine et al., 2011; Colinet and Renault, 2012; Bartholomew et al., 2015; MacMillan et al., 2016). Fly abdomens were imaged using a Leica Microsystems dissecting microscope with accompanying software and observed for the presence or absence of blue food in the abdomen at baseline and 2, 3, 4, 5, 6, and 7 h after the switch to standard non-dyed food. Flies were returned to the 25°C incubator between imaging sessions, and this process was repeated until the blue food was expelled from the abdomens of all control and tau transgenic flies. For this specific experiment, only female flies were used due to increased visibility of the abdomen.

Agar Plate Preparation

Acetobacter and *Lactobacillus* are the most frequently associated genera of the *Drosophila* microbiome (Broderick and Lemaitre, 2012). Thus, two mediums were used to assess bacterial growth: MRS agar, a medium selective for Lactobacilli, and nutrient agar, a general purpose medium that cultivates a wide-range of microbes, including *Acetobacter*. MRS agar plates and nutrient agar plates were prepared using sterile techniques and poured into separate 100 mm × 15 mm culture dishes.

16S rDNA Sequencing

Ten-day-old control and tau transgenic flies were placed into a −20°C freezer for rapid euthanasia for 10 min. Single flies were dipped in 70% ethanol three times to reduce contamination from cuticle and allowed to dry. Flies were then homogenized individually in a sterile microcentrifuge tube containing 100 μL of autoclaved water. The resulting homogenate solution was pipetted onto two separate mediums, MRS and nutrient agar plates, and incubated at 37°C for 48 h. All plating occurred using sterile tools and techniques.

Following incubation, unique cultured bacterial colonies on each plate were identified on the basis of colony morphology and color. Representative colonies were then inoculated into liquid broth and incubated at 37°C for 24–36 h until sufficient turbidity occurred. Microbial DNA was then isolated using the Qiagen DNeasy Microbial DNA Extraction Kit according to the manufacturer's instructions. Microbial 16S rDNA was then amplified through PCR reaction as described by the manufacturer (OneTaq, New England Biolabs) using the following cycling parameters: 5 min at 98°C, 32 1-min cycles at 94°C, 2 min at 55°C, 3 min at 72°C, 10 min at 72°C. Primer sequences were as follows: (Forward) 5′-GAGTTTGATYMTGGCTC-3′; (Reverse) 5′- GYTACCTTGTTACGACTT-3′.

PCR products were purified using the Qiagen PCR Purification Kit according to the manufacturer's instructions; purified products were run on a 1% agarose gel and visualized with ethidium bromide to confirm successful amplification prior to sequencing. Purified 16S rDNA samples were then sent to West Virginia University's Genomic Sequencing Core for sequencing, and the resulting sequences were identified using NCBI BLAST and Ribosome Database Project RDP[1] analysis.

Colony Forming Units Counts

Ten-day-old control and tau transgenic flies were euthanized by rapid cold exposure. Flies were then dipped three times in 70% ethanol solution and air dried. Individual flies were placed into sterile microcentrifuge tubes containing 200 μL sterile water and homogenized with disposable plastic pestles. Fly homogenates were briefly centrifuged to pellet the fly cuticle, which was discarded. Serial dilutions were then performed with the supernatant fly homogenate and sterilized broth, yielding 1:10, 1:100, and 1:1,000 diluted homogenates. Diluted (100 μL) and undiluted (50 μL) homogenates were spread onto MRS and nutrient agar plates. All plates were then incubated at 37°C for 48 h.

Following incubation, plates containing between 30 and 200 distinct bacterial colonies were used in the calculation of colony forming units (CFUs) per mL of plated homogenate solution, normalized to the dilution factor of the selected plate.

qPCR

qPCR was performed as previously described (Lohr et al., 2020). Briefly, pooled RNA samples were isolated using four 10-day-old flies (two per sex) homogenized in 500 μL Trizol/Qiazol (Qiagen Cat. 73906), followed by chloroform/isopropanol extraction and centrifugation. Pelleted RNA was then washed with 70% ethanol, resuspended in DEPC water, and quantified using a NanoDrop. DNase treatment was performed using DNase I (Ambion, Life Technologies Cat. 18068-015) according to the manufacturer's instructions. Reverse transcription was then conducted using an Applied Biosystems High Capacity cDNA RT kit (Fisher Cat. 4368814) as described by the manufacturer. The reverse transcription protocol was completed in a thermocycler for 10 min at 25°C, 2 h at 37°C, 5 min at 85°C, and held at 4°C. In a 96-well qPCR plate, 2 μL of diluted cDNA (1:2 in sterile water) was added to 14 μL of mastermix containing primer sets and 2X SYBR green mix (Fisher Cat. 4309155). The qPCR primer sets used in this study targeted the transcripts of the antimicrobial peptides attacin-A (Forward 5′-CACAACTGGCGGAACTTTGG-3′; Reverse 5′-AAACATCCTTCACTCCGGGC-3′), diptericin (Forward 5′-TACCCACTCAATCTTCAGGGAG-3′; Reverse 5′-TGGTCCACACCTTCTGGTGA-3′), and defensin (Forward 5′-AGTTCTTCGTTCTCGTGGCTA-3′; Reverse 5′-CCACATC GGAAACTGGCTGA-3′), the antifungal peptide drosomycin as a negative control (Forward 5′-CTGGGACAACGAGACCTGTC-3′; Reverse 5′-ATCCTTCGCACCAGCACTTC-3′), and the ribosomal housekeeping gene RpL32 (Forward 5′-GA CCATCCGCCCAGCATAC-3′; Reverse 5′-CGGCGA CGCACTCTGTT-3′). The plate was then run on an Applied Biosystems 7500DX qPCR machine and relative quantification (RQ) values were calculated for each transcript.

Statistics

With the exception of the gastric emptying survival curve, all statistical analyses were performed using two-tailed *t*-tests in GraphPad Prism 5.0 software, and reported as average ± SEM. The gastric emptying curve data were analyzed for statistical significance using the Log-rank (Mantel-cox) test in GraphPad Prism.

RESULTS

Following preliminary observations of distended abdomens and abnormal food distribution in tau transgenic flies compared to controls, a fecal deposit count was conducted to examine gastrointestinal function and motility. Tau transgenic flies showed significantly fewer fecal deposits per fly compared to controls (**Figure 1A**). To determine whether this reduction was due to decreased food intake or reduced gut motility secondary to neurodegeneration, a gastric emptying time course was conducted after feeding flies blue-dyed food. Images of fly abdomens (**Figure 1D**) showed a significantly increased time to gastric emptying for tau transgenic flies compared to controls (**Figure 1B**), as supported by the prolonged presence of blue food in the abdomens of tau transgenic flies (**Figure 1C**). These data suggest that tau transgenic flies show a reduced gut motility compared to controls. The reduced gut motility shown here is not due to neuronal protein overexpression alone as shown by a gastric emptying time course using elav-GAL4-driven eGFP transgenic flies (**Supplementary Figures 1A,B**). We showed no difference in gastric emptying between eGFP transgenic and control flies. Furthermore, delayed gastric emptying does not appear in all transgenic *Drosophila* neurodegeneration models. We showed no significant changes in motility in the SCA3 *Drosophila* model of spinocerebellar ataxia type 3 (SCA3; Machado-Joseph disease) (Warrick et al., 1998). However, significant reductions in gastric motility have been shown in alpha-synuclein transgenic models using both neuronal and glial drivers (Olsen and Feany, 2019), suggesting that these results may

[1]https://rdp.cme.msu.edu/classifier/classifier.jsp

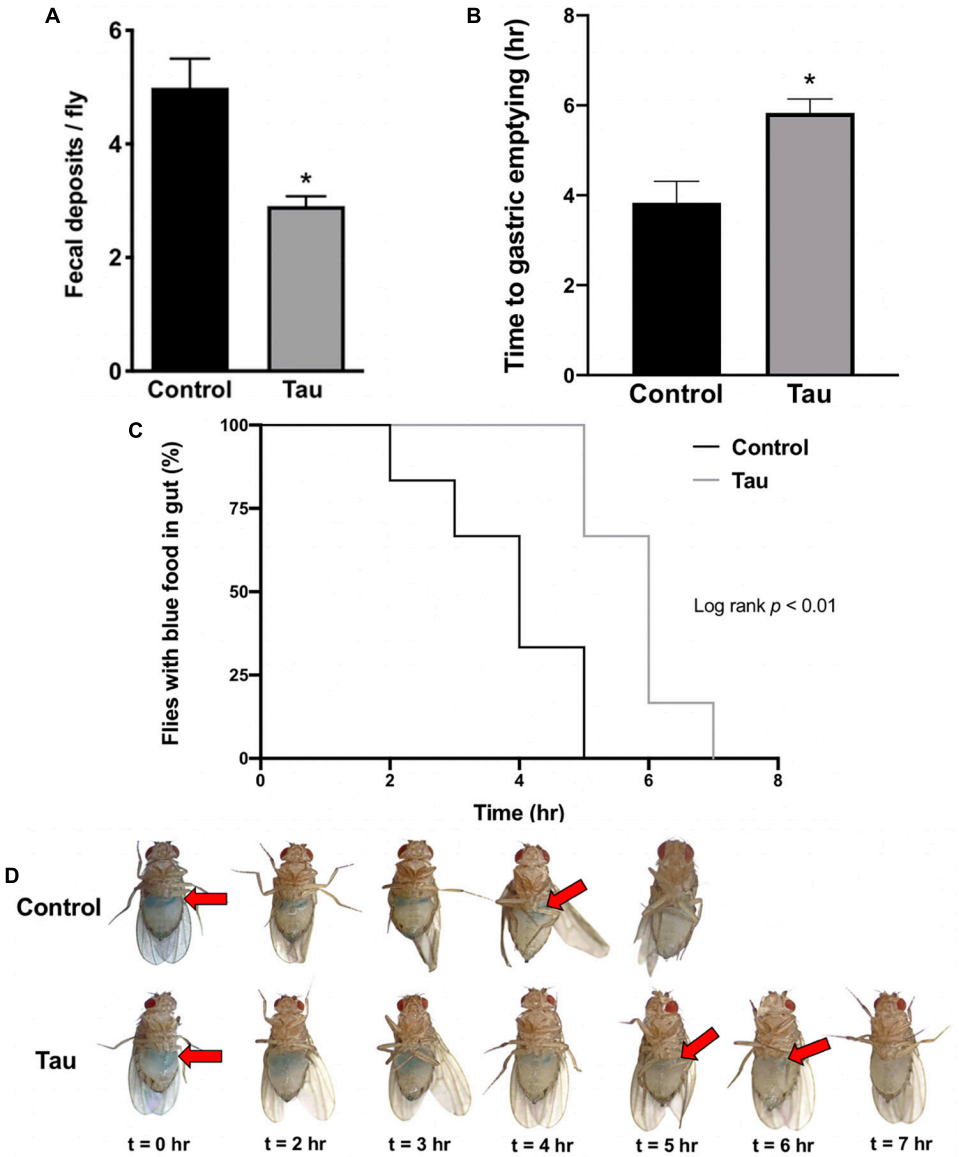

FIGURE 1 | Neuronal tau expression reduces *Drosophila* gut motility. **(A)** Tau transgenic flies show reduced fecal deposits per fly (n = 5–7, t-test $^*p < 0.05$). **(B–D)** Tau transgenic flies demonstrate an increased time to gastric emptying **(B)** (n = 6, t-test $^*p < 0.05$), supported by the prolonged presence of blue food in the abdomen in a gastric emptying time course **(C,D)**. Data are shown as average ± SEM.

be applicable to additional proteinopathies beyond tau-mediated neurodegeneration.

Attempting to address the effect of this reduced gastric motility in tau transgenic flies, we first analyzed the taxonomic classifications of gut bacteria in control and tau transgenic flies using 16S rDNA sequencing of morphologically distinct bacterial colonies from plated fly homogenate. Sequencing analysis revealed no difference in gut bacteria classification between tau transgenic and control flies, as both genotypes showed similar bacteria profiles containing the bacterial species *Lactobacillus brevis, Lactobacillus plantarum,* and *Acetobacter pasteurianus* (**Supplementary Table 1**).

With no observable difference in the types of bacteria within gut homogenate of tau transgenic and control flies, we next examined whether there was a quantitative difference in gut bacteria between the two genotypes. To examine gut bacteria quantity, CFU counts were performed on diluted fly homogenates from control and tau transgenic flies plated on both MRS and nutrient agar. Tau transgenic flies showed significantly higher CFU counts on both MRS (**Figure 2A**) and nutrient agar (**Figure 2B**) compared to control flies.

Due to this increased bacterial load in the guts of tau transgenic flies, we next examined whether tau transgenic flies also displayed an innate immune response. In response to

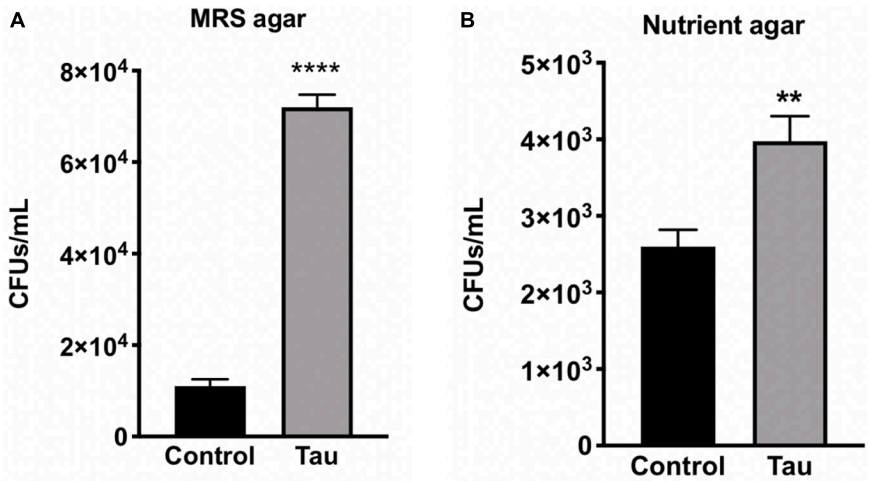

FIGURE 2 | Tau transgenic flies show an increased gut bacterial load compared to controls. CFU counts of diluted tau transgenic fly homogenates were increased on both **(A)** MRS agar ($n = 6$, t-test $^{****}p < 0.0001$) and **(B)** nutrient agar ($n = 5$, t-test $^{**}p < 0.01$). Data are shown as average ± SEM.

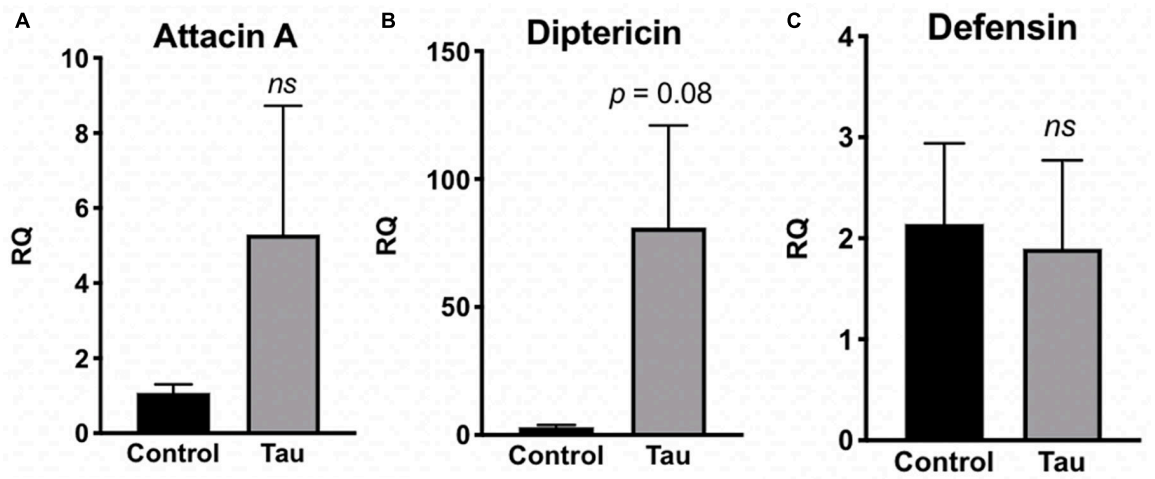

FIGURE 3 | Tau transgenic flies show trends of enhanced innate immune activation to gram-negative bacteria. qPCR targeting the antimicrobial peptide transcripts attacin-A **(A)**, diptericin **(B)**, and defensin **(C)**. RQ values suggest a non-significant elevation in innate immune expression of attacin-A and diptericin, but there is no trend observed in expression levels of the antimicrobial peptide transcript defensin. ($n = 6$, $p > 0.05$). Data are shown as average ± SEM.

bacterial insult, the Toll and Imd NF-kB signaling pathways of the *Drosophila* innate immune system are activated, regulating the production of antimicrobial peptides (AMPs) which target and degrade the cell walls of bacteria, facilitating microbial death (Hultmark, 2003; Hanson and Lemaitre, 2020). Accordingly, expression levels of AMP transcripts are often used to monitor innate immune activity. To measure innate immune activation for tau transgenic flies compared to controls, we performed qPCR on control and tau transgenic fly homogenate using specific primers for the AMP transcripts attacin-A, diptericin, and defensin, and the antifungal transcript drosomycin as a negative control. Tau transgenic flies showed a trend toward increased expression for the AMP transcripts attacin-A (**Figure 3A**) and diptericin (**Figure 3B**), both gram-negative response AMPs, compared to controls. No differences were seen in transcript levels of defensin, a gram-positive response AMP (**Figure 3C**) or the antifungal transcript drosomycin, as expected (**Figure 4**).

DISCUSSION

This study examined the effects of transgenic tau expression on gastrointestinal function and motility, the gut microbiome, and innate immune activation in *Drosophila melanogaster*. Tau transgenic flies showed significantly reduced gastric motility compared to controls. Widespread neurodegeneration in tau transgenic *Drosophila* is well characterized (Wittmann et al., 2001; Dias-Santagata et al., 2007; Fulga et al., 2007; Khurana et al., 2012; Lohr et al., 2020). Thus, it is not surprising that degeneration of neurons innervating the enteric nervous system

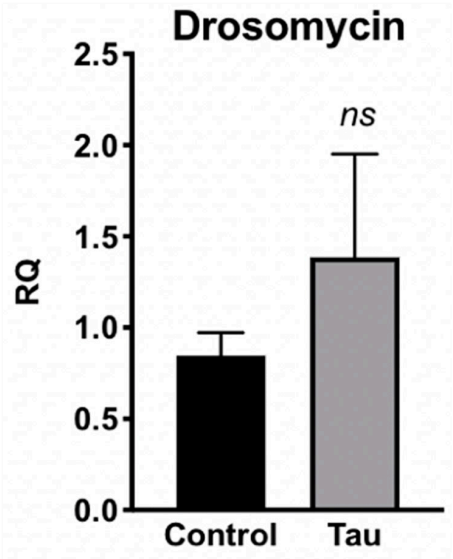

FIGURE 4 | Tau transgenic flies show no significant difference in antifungal AMP transcript levels. qPCR targeting the antifungal peptide transcript drosomycin showed no significant difference in expression levels between tau transgenic and control flies ($n = 6$, $p > 0.05$). Data are shown as average ± SEM.

of the gut would also occur in this aging fly model. This degeneration may contribute to the slowed gut motility and subsequent systemic changes shown in this study.

It has been suggested by multiple groups that *Drosophila* serve as an ideal model system for a microbial assessment due to the ease of environmental control and relative simplicity of the microbiota (Kenmoku et al., 2017; Trinder et al., 2017; Clark and Walker, 2018; Selkrig et al., 2018). While the *Drosophila* microbiota has 1–30 species dominated by *Lactobacillus* and *Acetobacter* (Blum et al., 2013; Erkosar et al., 2013; Chaston et al., 2014), it is estimated that the human GI microbiota is far more complex, with as many as 500 different bacterial species present (Quigley, 2013, 2017). Furthermore, both fly and human GI tracts also have similar structural anatomy, innervation, and function (Pitsouli et al., 2009; Apidianakis and Rahme, 2011).

Changes to gastrointestinal composition and function has become a point of interest in many types of neurodegeneration. Clinically, it is known that Parkinson's disease patients demonstrate delayed gastric emptying and reduced gut motility (Hardoff et al., 2001), and these gastrointestinal symptoms have been supported by deposits of alpha-synuclein within the enteric nervous system (Beach et al., 2010; Gelpi et al., 2014). α-Synuclein transgenic flies also show constipation as shown by a similar assay to the one used here (Olsen and Feany, 2019). Thus, the reduced GI motility and increased bacterial load shown in the present study may apply to other types of degeneration. Although digestive disorders, including irritable bowel syndrome, have also been associated with AD and related dementias (Liao et al., 2020), tau-mediated enteric nervous system changes remain poorly understood in tauopathies (Chalazonitis and Rao, 2018; Derkinderen et al., 2021). As such, the present study provides a potential role of tau in enteric nervous system degeneration, perhaps contributing to gastrointestinal symptoms, including reduced gut motility. Finally, there are additional variables to consider when interpreting the gut motility data from the current study. It should be noted that the retention of the blue-dyed food shown in the tau transgenic fly gut could be due to a leaky gut barrier and not just slowed motility of the tract. While it is also difficult to entirely eliminate potential differences in food consumption between control and tau transgenic flies, the gastric emptying time course suggests that tau transgenic flies eat similar amounts of food compared to controls as shown by similar starting levels of gastric filling.

Although 16S rDNA sequencing of gut homogenate showed no difference in gut bacteria classification between the genotypes, CFU counts revealed a significantly increased bacterial load in tau transgenic flies. It is possible that this increased bacterial quantity is due to the slowing of gastric emptying in the tau transgenic flies. Mammalian studies have shown that alterations to gastric motility can significantly alter proportions of bacterial types, contributing to gut dysbiosis (Sun et al., 2019). While the *Drosophila* gut has multiple differences in comparison to the mammalian GI tract, its structure and function are similar (Trinder et al., 2017). Thus, slowed gastric motility may be a contributing factor to the increased bacterial load seen in the tau transgenic flies.

Despite lacking the adaptive immunity characteristic of vertebrates, *Drosophila* has proven an important model in examining the interplay between gut microbiome homeostasis and innate immunity (Ryu et al., 2010). AMP expression increases in response to systemic bacterial infection as a way to destroy pathogens (Hanson and Lemaitre, 2020). In the current study, elevated AMP levels may be a systemic response to the enhanced bacterial load in tau transgenic flies (**Figure 2**). Furthermore, these AMPs may also be altering the relative levels of bacteria in the fly gut. Tau transgenic flies showed a trend toward elevated attacin-A and diptericin levels, two AMPs responsive to gram-negative bacteria such as *Acetobacter* (Imler and Bulet, 2005). This increased expression of gram-negative AMP responders may be contributing to the relatively low nutrient agar CFUs compared to that of MRS agar, which grows gram-positive *Lactobacillus* bacteria (**Figure 2**). This suggests that the elevated gram-negative responsive AMPs may be working to reduce the gram-negative bacterial load in tau transgenic flies, whereas less pathogenic types of gram-positive bacteria may be less regulated. As expected, expression levels of the antifungal peptide transcript drosomycin were unchanged.

These results are in line with the endotoxin hypothesis of neurodegeneration where endotoxin, a lipopolysaccharide (LPS) in the outer layer of gram-negative bacteria, contributes to neuronal dysfunction, particularly during infection and inflammation (Brown, 2019). Endotoxin treatment induces microglial activation, memory dysfunction, and neuronal changes in rodents and has been shown to promote formation of several neuropathologies, including aggregation of tau, amyloid β, and alpha-synuclein (Lee et al., 2008; Gardner et al., 2016;

Kim et al., 2016). Further adding to the gut-brain connection, some neurodegenerative diseases consistently present altered gut microbiomes compared to controls. For example, in Parkinson's disease, the gut microbiome is significantly altered and has been associated with gram-negative endotoxin-producing bacteria, including *H. pylori* (Scheperjans et al., 2015; Shen et al., 2017). These data suggest that gram-negative bacteria may contribute to neuronal dysfunction in some of these disease states.

The present study is significant in that it provides evidence for tau-mediated alterations to gut motility and microbiome composition. Furthermore, this work extends the links between AMP activity, innate immune mechanisms, and tau-mediated neurodegeneration. However, the precise manner through which AMPs may contribute to neurodegeneration remains poorly understood (Hanson and Lemaitre, 2020). As such, future studies should examine neurodegeneration and microbiome composition using germ-free *Drosophila*, reintroduction of specific gut bacterial species, antimicrobial conditions, or AMP transgenic flies. Further studies may also examine the gain or loss of innate immune signaling activity through an analysis of Toll and Imd receptor expression or analyze neuronal AMP expression through reporter assays. Such routes will undoubtedly strengthen our understanding of the interplay between AMP activity, innate immune signaling, and neurodegeneration.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

KL, JR, HK, and VH completed the experiments, analyzed the data, and edited the manuscript. JR and KL wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2021.731602/full#supplementary-material

Supplementary Figure 1 | Neuronal overexpression of eGFP and SCA3 shows no significant differences in gastric emptying at 10 day. eGFP and SCA3 transgenic flies show no difference in time to gastric emptying compared to controls **(A)** ($n = 6$, ANOVA, $p > 0.05$). These results are supported by similar retention of blue food in the abdomen in a gastric emptying time course **(B)**. Data are shown as average ± SEM.

Supplementary Table 1 | Bacteria identified by 16S rDNA sequencing in fly homogenate.

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REVIEW
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Retinal Degeneration: A Window to Understand the Origin and Progression of Parkinson's Disease?

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Parkinson's disease (PD), the second most prevalent neurodegenerative disorder, manifests with motor and non-motor symptoms associated with two main pathological hallmarks, including the deterioration of dopaminergic cells and aggregation of alpha-synuclein. Yet, PD is a neurodegenerative process whose origin is uncertain and progression difficult to monitor and predict. Currently, a possibility is that PD may be secondary to long lasting peripheral affectations. In this regard, it has been shown that retinal degeneration is present in PD patients. Although it is unknown if retinal degeneration precedes PD motor symptoms, the possibility exists since degeneration of peripheral organs (e.g., olfaction, gut) have already been proven to antedate PD motor symptoms. In this paper, we explore this possibility by introducing the anatomical and functional relationship of retina and brain and providing an overview of the physiopathological changes of retinal structure and visual function in PD. On the basis of the current status of visual deficits in individuals with PD, we discuss the modalities and pathological mechanism of visual function or morphological changes in the retina and focus on the correlation between visual impairment and some representative structural features with clinical significance. To consider retinal degeneration as a contributor to PD origin and progress is important because PD evolution may be monitored and predicted by retinal studies through state-of-the-art techniques of the retina. It is significant to integrally understand the role of retinal morphological and functional changes in the neurodegenerative process for the diagnosis and therapeutic strategies of PD.

Keywords: Parkinson's disease, visual deficits, morphological changes, neuropathology, retinal imaging technology

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INTRODUCTION

Parkinson's disease (PD) is a chronic and multisystemic neurodegenerative disease characterized by a series of motor symptoms (bradykinesia, resting tremor, rigidity, and postural instability) and non-motor neurologic phenomena (sleep disturbances, autonomic dysfunction, gastrointestinal, urogenital problems, cognitive decline, psychiatric symptoms, sleep abnormalities, as well as visual disturbances) (Santos Garcia et al., 2019; Xu et al., 2019). Age is a main factor of PD, and the global impact of the disease is emerging, with its prevalence at around >2% of all persons above 65 years of age, and >4% of all persons over the age of 80 years (Gbd 2015 Neurological Disorders Collaborator Group, 2017; Santos Garcia et al., 2019; Xu et al., 2019). Individuals suffering from PD

are increasing and are estimated to be 12 million patients by 2050 (Ggbd 2016 Parkinson's Disease Collaborators, 2018), and early diagnosis and intervention of PD pathology plays a very important role in medical health.

Elucidating the derivation of pathological changes is critical for the early diagnosis and intervention of PD. However, the pathological origin of PD is debated. Consistent with the dopamine depletion and pathologic a-Syn in the nigrostriatal pathway described in previous studies, the two pathologic hallmarks have observed in peripheral nervous system and various end-organs that lead to numerous non-motor manifestation of PD including autonomic impairment (Postuma et al., 2015), sleep dysregulation, mood disorder (Ortuno-Lizaran et al., 2018b) dementia, and visual alterations (Ortuno-Lizaran et al., 2018a). Increasing studies have now discussed a possibility of PD pathology initially arising outside of the central nervous system (CNS). Indeed, the spreading of a-Syn to the brain via peripheral inoculation (e.g., olfaction, gut) has been amply elucidated (Kim et al., 2019). Visual symptoms, including glaucoma (Pavlenko et al., 2018), dry eyes (Friedman, 2004; Tamer et al., 2005; Reddy et al., 2013), visual hallucinations (Onofrj et al., 2006; Williams et al., 2008), and deficits in color vision appears early in the disease in PD patients (Stenc Bradvica et al., 2015). As shown in **Table 1**, PD patients suffer from different eye disorders [Blinking (London et al., 2013; Seiple et al., 2016), eye movement dysfunctions (Anderson and MacAskill, 2013; Hanuska et al., 2015; MacAskill and Anderson, 2016), pupillary imbalance (Jain et al., 2011), nuclear cataract (Lai et al., 2015; Klettner et al., 2016)]. Ophthalmological examinations of subjects with PD also suggest a loss of color vision problems, visual acuity impairment as well as the deficiency of spatial contrast sensitivity (Uc et al., 2005; Bertrand et al., 2012; Weil et al., 2016; Guo et al., 2018). The cellular and molecular studies have demonstrated loss of dopaminergic amacrine cells and retinal ganglion cells are partially responsible for the reduced contrast sensitivity, impairment in visual acuity, or electroretinographic response in individuals with PD (Yenice et al., 2008; Esteve-Rudd et al., 2011; Koens et al., 2018; Ortuno-Lizaran et al., 2020). On post-mortem observation of PD patients, authors have found the loss of dopaminergic retinal cell and the aggregation of a-Syn (Diederich et al., 2014; Ortuno-Lizaran et al., 2018a). Interestingly, it has recently been proposed that animal models with retinal damage due to intravitreal injection of minimal doses of neurotoxins display symptoms of experimental PD (Willis et al., 2014). A further example is that the retinal exposure of welding flash is associated with increased incidence of PD (Willis, 2005). Conversely, an immune tolerance induced by eyes via administration of antigens into the anterior chamber could be used as a therapeutic approach to promote neuroprotection for neurodegenerative diseases (Farooq and Ashour, 2013; Toscano-Tejeida et al., 2016; Pineda-Rodriguez et al., 2017). Thus, it is reasonable to conclude that the retina may be intimately involved with the onset and progression of PD as a potential precipitating factor outside of the CNS.

The retina presents a unique opportunity to study the CNS. First, it shares a common origin, structure, and physiology with the brain in terms of nervous and microvascular systems (Cameron et al., 2017). Over the past decades, investigators have attempted to access the tools that leverage the accessibility of the retina to better understand and diagnose PD. The retinal degeneration, retinal ganglion cells (RGCs) loss, and retinal thinning as well as visual disorders were observed in PD and animal models. As the only portion of CNS, furthermore, the retina is capable of reliable and precise measures of high-resolution imaging, retinal neurons, and vascular morphology therefore begin to be analyzed by ocular measurements from large studies utilizing tools. For instance, using the optical coherence tomography (OCT) imaging, some authors have found retinal nerve fiber layer (RNFL) thinning (Garcia-Martin et al., 2014b; Mailankody et al., 2015; Satue et al., 2017), and lower capillary perfusion density (CPD) and capillary afflux index in the retinal vascular morphology revealed by OCT angiography (OCTA) and fundus imaging (Guan et al., 2013; Robbins et al., 2021).

In this review, we systematically assess evidence in the field of PD with a focus on the morphological changes and visual dysfunction in the retina. We first review the anatomical and functional relationship of the retina and the brain. Also, the introduction and development of new and highly sensitive ocular technology were described. Special care is taken to discuss up-to evidences on retinal morphological alterations and visual disorders in PD patients, with the highlight of the correlation between several representative structural features and visual impairment in PD patients. Finally, we emphasized the role of some retinal morphological changes in diagnostic and prognostic progression for visual neuropathology of the neurodegenerative disease.

STRUCTURE AND FUNCTION OF THE RETINA

In embryological origin, the retina is derived from the neuroectoderm. The retina shares a common origin and similar anatomy with brain tissue. Revisiting some of the basic anatomy of the retina is helpful for appreciating the impact of diseases on the retina.

Retina and optic nerve are essential parts of the neural conduction systems, which consist of different cell types and play a crucial role in visual imaging. As an innermost, light-sensitive layer of sensory tissue in most vertebrates and some mollusks, the retina possesses complex and multilayer structures and many cells with microcircuits features and different functions (**Figure 1**). Morphologically, five classes of neuronal cells play the important role in shaping the structure of the retina, encoding visual information and regulating vision function. These component cells include photoreceptors (rods and cones), horizontal cells (HCs), bipolar cells (BCs), amacrine cells (ACs), and RGCs. In the normal condition, the light energy is converted to membrane potential changes in rod (RC) and cone (CC) photoreceptors in the outer retina layer (ORL). Within the outer plexiform layer (OPL), the photoreceptors convey light information to BCs under the modulation of HCs. Then, as a sole output neurons of the retina, RGCs within the inner

TABLE 1 | Visual dysfunctions and manifestation in PD patients.

Organ	Mechanisms	Main manifestations	Morbidity	References
①Eyelid	Frontal DAN dysfunction	(1) Blinking: Bradykinesia of voluntary blinking, Abnormalities of reflex blinking, Reduced amplitude and blink rate		Postuma et al., 2015; Gbd 2015 Neurological Disorders Collaborator Group, 2017; Ggbd 2016 Parkinson's Disease Collaborators, 2018; Santos Garcia et al., 2019; Xu et al., 2019
		(2) Apraxia of eyelid opening		Ortuno-Lizaran et al., 2018b
		(3) Uncomfortable sensations, red eyes	53–60%	Ortuno-Lizaran et al., 2018a
		(4) Muscle disorder: eyelid retraction, eyelid ptosis, lepharospasm		Reddy et al., 2013; Kim et al., 2019
②Eyebulb	Extrapyramidal damage	(1) Eye movement dysfunctions: convergence insufficiency Abnormal saccades Smooth pursuit impairment		Friedman, 2004; Tamer et al., 2005; Onofrj et al., 2006
		(2) Diplopia	10–30%	Williams et al., 2008
③Pupil	Autonomic disorders	Pupillary imbalance: Reduced amplitude of contraction, Prolonged contraction time		Guo et al., 2018
③Lens	Mitochondrial dysfunction	Nuclear cataract	16–24%	Uc et al., 2005; Weil et al., 2016
④Retina	Retinopathy	(1) Visual acuity	70%	Bertrand et al., 2012; Ortuno-Lizaran et al., 2020
	DAN dysfunction	(2) Spatial contrast sensitivity		Esteve-Rudd et al., 2011
	a-Syn deposition	(3) Color vision		Esteve-Rudd et al., 2011; Willis et al., 2014
⑤Optic nerve	Macular thickness	Visual field defects	60–70%	Willis, 2005
⑥Visual cortex	Cortex impairment	(1) Visuospatial deficits	30–60%	Toscano-Tejeida et al., 2016; Pineda-Rodriguez et al., 2017
		(2) Visual hallucination		
		(3) Facial expression recognition		
⑦Other auxiliary apparatus	Retina DAN dysfunction	(1) Glaucoma	30–40%	Uc et al., 2005; Farooq and Ashour, 2013
		(2) Dry eyes	50%	Cameron et al., 2017; Satue et al., 2017; Ortuno-Lizaran et al., 2018b
		(3) Rapid eye movement sleep behavior		Garcia-Martin et al., 2014b

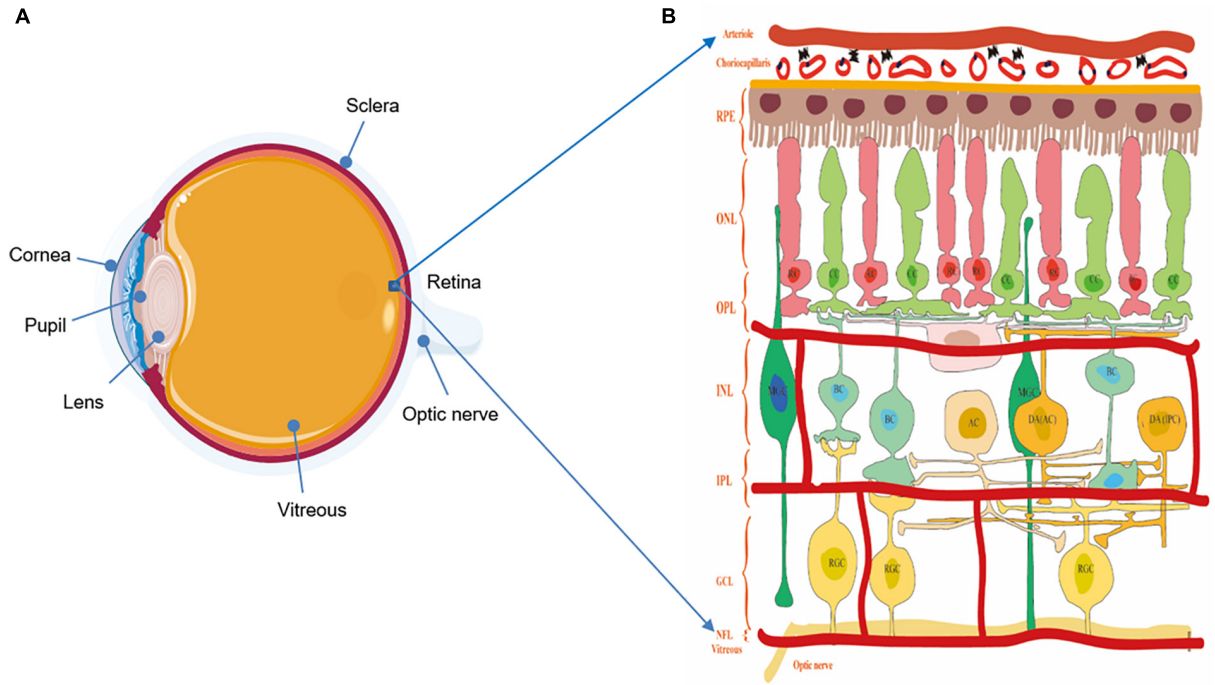

FIGURE 1 | (A) The structure of the eyeball. **(B)** The structure of the retina and diagram of the retinal neurons. Notably, understanding the physiologic structure and function is critical for better exploring the relationship of visual function and morphological changes. The retina possesses complex and multilayer structures and a large number of cells with microcircuits features and different functions. ILM, inner limiting membrane; RNFL, retina nerve fibers layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; ORL, outer retina layer; RPE, retinal pigmented epithelium; RC, rod cell; CC, cone cell; BC, bipolar cell; HC, horizontal cell; AC, amacrine cell; DA AC dopaminergic amacrine cell; DA IPC, dopaminergic interplexiform cell; RGC, retinal ganglion cell.

plexiform layer (IPL) contact BC and ACs at the inner nuclear layer (INL), projecting their axons to higher visual centers (Archibald et al., 2009; Schmidt et al., 2011). Aside from the above vertical and horizontal cell bodies, there are cells or related neurotransmitters mediating visual information, including the retinal pigment epithelium (RPE) with the capacity of visual pigment regeneration, and Müller glial cells (MGC) involved in neuronal metabolism, synaptic pruning, and neurotrophy (Vecino et al., 2016). These cells mediate retinal signaling in vertical and horizontal directions, and these are vital in shaping color vision, spatial resolution, and vision sensitivity (Weil et al., 2016).

Dopaminergic Neurons in the Retina

Malmfors (1963) first described the role of catecholamines in rat retinal function and regulation, involving in the light/dark adaptation and reshaping retinal circuitries. Subsequently, dopaminergic neurons have been identified in human retina, and overlap with neighboring DA cells as well as other retinal cells (the cone-rod, horizontal cells, and ganglion cells) (Frederick et al., 1982). In vertebrate retina, DA neurons contact two other types of amacrine cell (AII and A17) and interplexiform neurons (IPC) (**Figure 1**). The amacrine cells, via gap junctions, receive input from BCs and pass visual information to RGCs and the same cell types, modulating visual processing of the flow of photoreceptors-driven visual information. Compared with the AII amacrine cells, in scotopic conditions, the A17 amacrine cell receives GABAergic inputs instead of excitatory glutamatergic inputs from rod bipolar cells, and it plays a role in converging rod signals and amplifying the effects of low light stimulation (Hillman et al., 1995). DA cells are stimulated to release functional dopamine as an essential neuromodulator in photopic conditions, making synaptic contacts between DA cells and affecting on gap junction permeability both at the level of photoreceptors interactions with HCs and at the level of DA cells communication (Cameron et al., 2009; Zhang et al., 2014). In return, DA cells alter the action potential firing rate of their own cells and regulate DA release when receive excitatory or inhibitory feedback information.

In addition to this excitatory and inhibitory feedback system, the modulation of retinal DA cells conditions and dopamine concentration has diurnal variation, with low levels at night and higher levels during the day. From functional perspectives, some DA cells are light-evoked, involving in regulating the light/dark adaptation and electrophysiological communication between retinal cells in different layers depending on the circadian rhythm (Cohen et al., 1992). Also, some dopaminergic cells can activate spontaneously and alter DA level in the darkness. In turn, the DA effects on photoreceptor cells, BCs, ACs, and HCs (Popova,

2014), reshaping photomechanical movements and survival, to enhance flicker response of retinal rod pathway (Hampson et al., 1992), and regulate visual stimuli of cells communication as well as protects RNFL (Yavas et al., 2007). Knowledge of these anatomical connections and visual progressing demonstrated that DA is a chemical neurotransmitter in the retina, promoting synaptic effects and visual information to regulate electrical activity and retinomotor movements.

Ganglion Cells and Retinal Nerve Fiber Layer

In retinal physiology, RGCs, the output neurons that project visual information from the inner retina to the brain, extend to the lateral geniculate nucleus (LGN) via a nerve fiber tract complete with an oligodendrocytic myelin sheath (Henderson et al., 2008). There are numerous subtypes of retinal cells, such as photoreceptors, HCs, BCs, and ACs, making synaptic contact with RGCs in the inner plexiform layer through different communication systems, including acetylcholine, dopamine, glutamate, glycine, and gaba-aminobutyric acid. Influenced by light, RGCs receive photosensitive information through either direct or indirect circuitry, and act as the final common pathway in the flow of visual information to the optic nerve and brain cortex (**Figure 1**). Sparkly, the melanopsin-containing retinal ganglion cells (mRGCs), accounting for about 0.3–0.8% of the total ganglion cells within the retina, represent a specialized class of RGCs that respond to light without rod and cone information input (Hattar et al., 2002). Some authors therefore think that mRGCs constitute a third class of photoreceptors and are directly photosensitive. In addition, mRGCs are also responsible for the non-image forming pathways, mediating the circadian rhythm and pupil constriction that are involved in mood and sleep behaviors (Güler et al., 2008). Previous studies demonstrated that GCL thinning is relevant to lower visual acuity, contrast sensitivity loss, and color deficiencies.

The RNFL is the inner most layer of the retina and is composed largely of axons of RGCs. Many studies investigated the ganglion cells' death is inevitably reflected on thinning RNFL thickness, relatively presenting the number of RGCs axons loss (Henderson et al., 2008). Recently, the OCT and OCTA have been used in the investigation of structural changes and measures of the vertical retinal layers in the retina *in vivo*. RNFL thinning demonstrated the dopaminergic neuronal loss and decreased axons of RGCs, effecting retinal neuronal processing and electrophysiological function (Bodis-Wollner et al., 2014a).

Microvascular and Choroidal Structure in the Retina

In addition to retina nervous systems, evidence has implicated retinal small vessel plays an important role in structural and functional changes in the retina. Based on the potential risk the role of cerebral small vessel disease for the development of PD plays (Guan et al., 2013), some studies demonstrated retinal microvascular changes have been studied retinal capillary plexus vessel density (VD) and perfusion density (PFD) as well as structural changes in PD (London et al., 2013; Robbins et al., 2021). Thus, structural changes in retinal microvascular are seen as non-invasive biomarkers for the disease detection.

Considering the common embryologic and anatomic characteristics of retinal vascular with the cerebral circulation, microvascular changes in the retina may correlate with vascular changes in the CNS. The retinal vasculature is a window *in vivo* non-invasive assessment of microvasculature in the body (**Figure 1**). In embryology, the ophthalmic artery originates from the internal carotid artery gives off the central retinal artery, providing nutrients and oxygen to the inner layer of the retina. Metabolic waste and carbon dioxide from the retina are excreted into the sinus via the central retinal vein through the superior ocular vein. Central retinal arteries and veins form a terminal branch retinal circulation network on the surface of the retina. In addition, microvasculature of the retina shares similar neurobiology and electrophysiological function with those in CNS (Ge et al., 2021).

MORPHOLOGICAL AND FUNCTIONAL TECHNOLOGIES IN THE RETINA

As the eye is an extension of the brain, the retina displays similarities to the brain in anatomy, functionality, and pathological responses to environmental insult. So, to detect retinal morphological parameters of brain pathologies using imaging techniques seems reasonable.

Optical coherence tomography (OCT) is a non-invasive observational technique based on reflectance intensity of light, providing some real-time information of the retina on structure using infrared interferometric imaging (**Figure 2**). The OCT enables an optical biopsy of retina to provide two/three-dimensional cross-sectional images of the target tissue using the interference of infrared radiation (Langwinska-Wosko et al., 2016). In 1991, the first OCT image was described by David Huang in the anterior chamber of an *ex vivo* bovine eye (Huang et al., 1991). Subsequently, Fercher et al. (1993) and Swanson et al. (1993) showed the first *in vivo* measurements of human retinal structure using the non-contact and high resolution technique in 1993. Retinal OCT imaging detects and quantifies the structural correlates of these visual symptoms of patients, provides histologic level information about retinal nerve fiber layer, cells, and retinal blood vessels. With the growth of OCT scientifically and economically, OCT assesses the three-dimensional outer retina thickness in the higher image pixel density and quality of OCT (Fujimoto and Swanson, 2016). For instance, spectral domain OCT (SD-OCT) uses a wavelength of 820 nm, diminished the vitreous signal, and improved imaging of the macular choroid (Ha Usler and Lindner, 1998), reaching deeper structures of the retina. Swept-source OCT (SS-OCT) reaches deeper penetration using a wavelength of 1,020 nm. In addition, the special OCT, OCT angiography (OCTA) imaging, allows for blood flow visualization (Gulmez Sevim et al., 2019; Robbins et al., 2021). This is an emerging approach for imaging retinal vessels, can visualize microvasculature based on motion contrast from flowing blood to assess the blood pressure, intraocular pressure, vascular

density of the superficial capillary plexus, deep capillary plexus, and choriocapillaris (Zhang et al., 2018). Therefore, retinal OCT imaging not only costs lower, but provides insight into the underlying pathophysiology in the earlier disease process, compared to conventional neuroimaging methods, such as the fundus color photograph, fundus fluorescein angiography, and B-ultrasonography. These new, cost-effective, high-resolution imaging tools enabled increases in imaging speeds and quantity, further catering clinical need of diagnosis and therapeutics of diseases, and increasing clinical data demonstrated the important role of OCT in diagnostic and therapeutic applications of many diseases. The OCT has become a new and highly sensitive method for detecting and analyzing some classic ocular pathologies in diseases.

Aside from the over-mentioned OsCTs, some techniques are applied to evaluating the functional performances of retinopathy of PD, including electroretinogram (ERG) and visual evoked potential (VP). The ERG reflects retinal comprehensive potential caused by a brief light stimulation recorded from the cornea. There are flash ERG and graphic ERG based on different forms of light stimulation (Netser et al., 2021). The flash ERG consists of a negative A wave, a positive B wave, and the OPs waves superimposed on the B wave. Wave A mainly reflects the hyperpolarization activity of photoreceptors, while wave B is generated by the electrical activity of MCs and BCs in the retina (Takatsuna et al., 1992; Meng et al., 2012; Normando et al., 2016). The wave OPs on the B wave are related to the electrical activity of ACs. The VEP is generated by the electrical activity in occipital cortex after the visual stimulation. The structural and functional changes in the retina cause the change of waveform amplitude and/or latency in VEP.

These techniques detect retinal nerve fiber layer (RNFL) thickness (Inzelberg et al., 2004; Hajee et al., 2009; Kirbas et al., 2013; Jimenez et al., 2014), central macular volumes, morphology in foveal vision (Pilat et al., 2016; Nunes et al., 2019), inner and outer retinal layers (Pilat et al., 2016), and retinal pigment epithelium (Uchida et al., 2018), and also assess retinal blood flow and vascular alterations as well as other pathological features of retina in PD patients. The monitoring retinal morphology and function are used for exploring hallmark signs corresponding to pathological conditions in different degrees and stages of PD.

PATHOLOGICAL AND MORPHOLOGICAL CHANGES IN RETINA OF PARKINSON'S DISEASE

The retina is a simple model of the brain in the sense that some pathological impairment and morphological changes from the retina may be observed or applicable to the degenerative diseases as valuable models. In the retina of PD patients, there were dopaminergic deficiency (Schmidt et al., 2011; Vecino et al., 2016), misfolded a-synuclein (Weil et al., 2016), retinal ganglion cells loss (Malmfors, 1963), thinning of retinal nerve fiber layer (Frederick et al., 1982; Hillman et al., 1995; Cameron et al., 2009; Zhang et al., 2014), or neuroinflammatory (Cohen et al., 1992) at several levels of the visual pathway during pre-clinical stages. Moreover, studies on post-mortem of PD patients found the accumulation of misfolding α-synuclein, the main culprit of the disease, in the retinal layers, especially the OPN of patients with early PD (Hampson et al., 1992; Yavas et al., 2007; Popova, 2014). Furthermore, evidence has indicated microvasculature changes as some potential biomarkers of retinal pathological changes in subjects with PD. Compared to the control cases using immunohistochemical staining and image analysis, Guan et al. (2013) observed the decreased capillaries branching as well as shortening length and enlarging diameter in capillary network in the substantia nigra, middle frontal cortex, and other brain stem nuclei. As van der Holst et al. (2015) described, increased risk of Parkinsonism was observed in population with cerebral small vessel disease. So these structural changes of the retina of PD have been shown the association with the progression, severity, and duration of the disease (Ma et al., 2018; Hasanov et al., 2019).

A-Synuclein Deposits

A-synuclein (a-Syn) is a neuropathological landmark, and its abnormal accumulation can induce neuronal death, disturbance in the dopamine mechanism, and synaptic effects (Henchcliffe and Beal, 2008). In normal physiological state, a-Syn is encoded by the SNCA gene, and belongs to the synuclein family that is involved with the exocytosis and synaptic function. In retina, a-Syn exists at the OPL, mediating membrane fusion synaptic vesicle and neurotransmitter release, fatty acid binding, cell signaling, and cell growth (Iwai et al., 1995; Burre et al., 2010; Breydo et al., 2012). In contrast, because of inducing risk factors in PD, the a-Syn protein was transformed into truncation and multimerization from monomeric and tetrameric conformation, then converted to insoluble oligomers and amyloid fibrils, and eventually perturbed dynamic equilibrium of functional a-Syn (Kahle et al., 2001; Auluck et al., 2002; Liu et al., 2005). Moreover, aberrant aggregation of a-Synuclein has prion-like properties to trigger the intercellular transmission a-Syn fibrils (PFFs); it is time-dependent and changeable, implying that the propagation of a-Syn may be the key contributor to onset and progression of PD (Willis, 2008; Willis and Freelance, 2017; **Figure 3**).

Similarly, the conformational or metabolic changes of a-Syn polymer, such as phosphorylated a-Syn and abnormal accumulation into insoluble aggregates are cytotoxicity for cellular and molecular metabolism (Bodis-Wollner, 2009; Stenc Bradvica et al., 2015; MacAskill and Anderson, 2016) (**Table 2**). Some previous studies revealed the relation between retinal a-Syn aggregates and clinical and imaging manifestations of impaired vision in PD. Bodis-Wollner et al. (2014b) reported a-synuclein aggregations in the inner retina (GCL, IPL, and INL), and observed the loss of full retinal thickness in the retina of PD. These histopathological changes in retina provide a bridge between a-synuclein inclusions and RNFL thinning detected by OCT. In addition, based on the protein inclusions in GCL associated with the impaired GCs function, authors speculated a potential route of local transmission of the anormal protein between retina and central neurons systems. Similarly, Beach et al. (2014) has found that immunopositive phosphorylated a-Syn, a specific molecular marker of synucleinopathy, presence

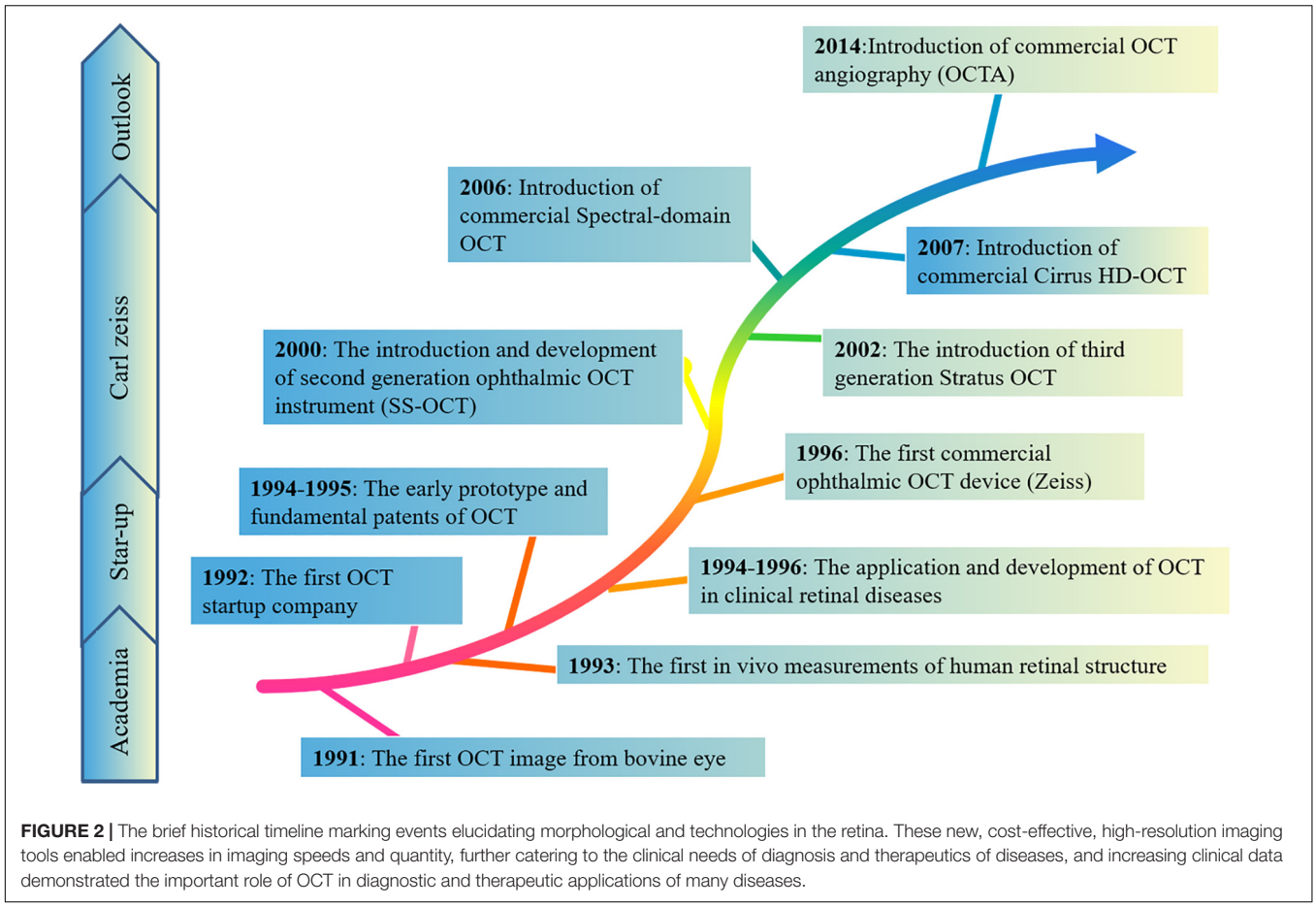

FIGURE 2 | The brief historical timeline marking events elucidating morphological and technologies in the retina. These new, cost-effective, high-resolution imaging tools enabled increases in imaging speeds and quantity, further catering to the clinical needs of diagnosis and therapeutics of diseases, and increasing clinical data demonstrated the important role of OCT in diagnostic and therapeutic applications of many diseases.

in the inner retinal surface of PD individuals paralleling to retinal thinning (GCL, IPL, and INL) has been reported with OCT (Altintas et al., 2008; Bodis-Wollner et al., 2014b). Further, Ortuno-Lizaran et al. (2018a) showed the p-α-syn deposits in retinal ganglion cells or intrinsically photosensitive ganglion cells in patients with PD. As a-Syn may spread from neuron to neuron, the a-synuclein may rely on the long axons of the GCs to spread from the retina and brain (Bodis-Wollner et al., 2014b). However, there is not enough evidence to answer some pivotal questions including whether multifocal initiation of a-Syn pathology exists and how some a-Syn species transmit through neural connections or non-neuronal cells.

Dopaminergic Deficiency

Considering the demonstrated implication of dopaminergic cells in retina functions like those previously mentioned, an impairment in the retinal dopaminergic system is linked to visual symptoms in patients with PD. In 1988, Nguyen-Legros (1988) described the loss of dopaminergic neurons in the retina, and changes in the ERG, VEPs, and contrast sensitivity were observed. Compared the retinal dopamine content in patients who received levodopa therapy(treatment with the DA precursor levodopa 2–15 h before death)and who had not, Harnois and Di Paolo (1990) showed decreased DA in the retinas of subjects with PD. Furthermore, Ortuno-Lizaran et al. (2020) reported the dopaminergic cell degeneration and the loss of synaptic contacts, revealing a failure in DA cells through gap junctions that are involved in visual function. Interestingly, it has recently been proposed that the retina plays an important role in the regulation of the circadian system and motor function. Some articles have reported the motor impairment and circadian disorders in PD animal models (Willis et al., 2014). In turn, using levodopa in the retina (Willis, 2008) and timed light therapy (Videnovic et al., 2017) [a dopamine release stimulation (Li and Tian, 2017)] showed an improvement of DA cells function, enhancing sleep, mood, and anxiety, and also improved motor function. Therefore, the retinal dopaminergic system is affected in PD and may explain the visual deficits, motor impairment, and circadian rhythm alterations described in patients.

With the development of ophthalmic techniques as mentioned above, updated retinal images in patients with PD exhibit reduced number of dopamine cells and dopamine concentration in the retina (Bodis-Wollner et al., 1987; Bodis-Wollner, 1990; Harnois and Di Paolo, 1990; Price et al., 1992). The measuring of the level of dopamine released by dopaminergic neurons is also beneficial for monitoring retinal morphological changes. Clinical data have demonstrated that dopamine and dopamine transporter (DAT) detected by single photon emission computerized tomography (SPECT) or positron emission tomography (PET) promise to be

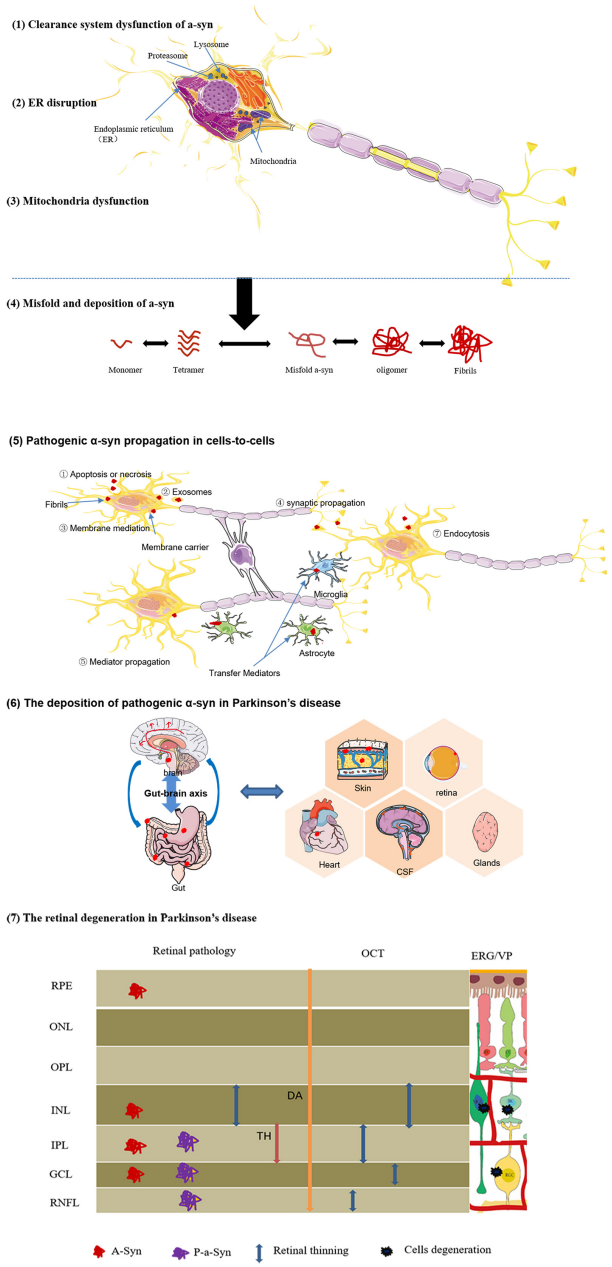

FIGURE 3 | Diagrammatic interpretation of the formation, propagation, and deposition of pathogenic a-synuclein. Certain risk factors effect on neurons, and initiate some pathological mechanisms associated with the etiology of PD, including (1) protein-clearance dysfunctions, (2) ER disruption, (3) mitochondria dysfunction. These pathological effects promote (4) abnormal a-Syn misfold and deposition. (5) Intercellular prion-like transmission of pathological a-Syn. Due to the propagative mechanisms of a-Syn similar to prions, abnormal proteins are released and transmitted via different mechanisms:Apoptosis or necrosis,exosomes,membrane mediation,synaptic propagation,mediator propagation, and so on. (6) The deposition of pathogenic α-syn in Parkinson's disease. For the definitive derivation of a-Syn, the brain-first hypothesis and gut-first hypothesis appear to be better received in current research. Especially, pathological a-Syn also was found in retina, skin tissue, heart, CSF, and glandular secretions. These pathological changes are critical for interpreting and understanding of clinical symptoms of patients with PD. (7) The retinal degeneration in Parkinson's disease.

objective and non-invasive markers to identify and determine retinopathy in PD patients (Nguyen-Legros, 1988; Wojtkowski et al., 2004; Biehlmaier et al., 2007). Also, abnormal transmitter production and atrophy in RGCs and RNFL caused lower dopaminergic cells to be identified using the OCT and be a promising marker to monitor the progression of the disease (Inzelberg et al., 2004). Thus, liked as pathological a-Syn, dopaminergic neurons loss may be the key factors triggering retinopathy in PD. Most experimental studies have shown that the formation and aggregation of a-Syn may induce a time dependent loss of DA neurons in the brain (Giordano et al., 2018). However, the specific pathophysiological mechanism of a-Syn in the retina and its relationship with the level of retinal dopamine remains to be further studied.

Retinal Ganglion Cells Loss and Retinal Nerve Fiber Layer Thinning

As previously mentioned, the ophthalmological examinations visualize on the surface of the retina, such as the RNFL and the retinal capillaries (attenuation, dilatation, aneurismal, and neovascular). Though it is not certain that all retinal thinning in PD is due to a-Syn aggregation or dopaminergic neuronal loss, the two main pathological hallmarks may damage retina structure (RGCs loss or RNFL thinning), interfere with signal transmission, and hence cause visual dysfunction.

Most non-invasive study of the retina in PD patients concentrated on the correlation of thinning RNFL detected by OCT. Inzelberg et al. (2004) first assessed with OCT and reported the RNFL thinning in patients with PD compared with controls. The results showed a decrease in the thickness of inferior quadrant RNFL near its entry to the optic nerve head (Inzelberg et al., 2004). Since then, it has been identified that the RNFL thickness was significantly thinner in four different quadrants, ranging superior, temporal (Altintas et al., 2008; Moschos et al., 2011), inferior (Inzelberg et al., 2004), and nasal (Shrier et al., 2012) in the retina of participants with PD. Likewise, the RNFL thickness in the macular region of PD was significantly lower than in the control groups (Moschos et al., 2011; La Morgia et al., 2013). Notably, the OCT quantification in macular seems to have a higher diagnostic yield than RNFL quadrants quantification (Polo et al., 2016). Sparkly, most studies revealed a significant thinning of the RNFL in the IRL (Hajee et al., 2009; Cubo et al., 2010; Albrecht et al., 2012) (constituting GCL, IPL, and INL) and in the central 5-mm quadrant of the macula (Albrecht et al., 2012; Garcia-Martin et al., 2014b), while no significant changes in the ORL of the retina (Hajee et al., 2009; Cubo et al., 2010; Adam et al., 2013).

The RNFL, as mentioned above, is formed from the axons of the ganglion cells and constitutes the output neurons of the retina. The thinness of the RNFL, specially GCL thinning, largely leads to decreased ERG responses (Cuenca et al., 2005). When comparing visual hallucination and OCT, Adam et al. (2013) reported that visual hallucinations positively correlate with retinal thinning in patients with PD. On the contrary, an inverse correlation of IPL thickness with central contrast

sensitivity was recently observed by Lee et al. (2014), and the correlation in PD is weaker that in control groups.

Moreover, there are studies in the literature reporting a correlation between the extent of the RNFL thinning and duration or severity of PD. For instance, Jiménez et al. (2014) demonstrated RNFL thickness correlation with disease severity, and reported a strong inverse correlation between RNFL thickness and the PD severity measured according to the Unified Parkinson's Disease Rating Scale (UPDRS) score. The result suggested the decreased RNFL thickness evaluated by OCT may be defined a simple biomarker for the clinical duration and average of PD. Similarly, Garcia-Martin et al. (2014a) demonstrated the more serious impairment of inner retinal layers in the patients with long disease duration, rather than healthy controls and PD with short disease duration.

Retinal Microvascular and Choroidal Structural Changes

Evidence has implicated the correlation of cerebral small vessel diseases with retinal microvascular changes detected by OCTA. In the Atherosclerosis Risk in Communities (ARIC) Study, Wong et al. (2001) detected that retinal microvascular abnormalities are associated with an increased incidence of stroke. Hughes et al. (2016) investigated associations between cerebral infarcts and white matter lesions and abnormalities of the retinal circulation, such as narrower arteriolar diameter, fewer arteriolar branching, and more tortuous venules. Further, clinical data demonstrated that these microvascular changes have been noted to have increased incidence of PD and retinal structural and functional alteration (Guan et al., 2013; van der Holst et al., 2015). Non-invasive tests of retinal vascular impairment are likely to serve as biomarkers for cerebral vascular changes in individuals with PD.

There are studies in the literature evaluating the retinal microvessel status in individuals with PD. In a prospective study (Kwapong et al., 2018), scholars evaluated macula microvasculature and intraretinal layer thickness using SD-OCT. The result demonstrated decreased microvascular density in retina and reported a strong correlation between RNFL thinning and the retinal microvascular abnormality. Also, Shi and collaborators (Shi et al., 2020) characterized lower retinal capillary density, decreased capillary perfusion density, and fractal dimension using OCTA, suggesting the role of retinal structural changes serving as a surrogate biomarker of cerebral changes in PD. On post-mortem analysis of brain tissue from patients with PD, Guan et al. (2013) also observed reduction in capillary branching, fragmentation of capillary, shortening vascular length, and larger diameter in the substantia nigra, middle frontal cortex, and brain stem nuclei. Recently, a cross-sectional study (Robbins et al., 2021) also compared relevant retinal parameters of individuals with PD and age- and sex-matched controls, found increased choroidal area, increased choroidal luminal area, and decreased capillary plexus vessel density and perfusion density in PD. Therefore, non-invasive retinal imaging, OCT, may detect structural changes in retinal microvascular as a novel technique for assessment and detection of PD.

However, the mechanism of retinal microvascular changes in PD is obscure. One may speculate that blood vessel regression effects retinal circulation network, disturbs energy metabolism and biochemistry functions. Retinal microvascular VD and PFD in individuals with PD, to some extent, may reflect the underlying blood vessel changes in neurodegenerative process of PD (Robbins et al., 2021). Further, comparing vascular regression and pathological features of PD in a-Syn overexpression mouse model, Elabi et al. (2021) observed dynamic changes in retinal microvascular morphology accompanied by a pathological accumulation of α-syn deposit. The result suggests the role of retinal microvascular pathology as an important pathophysiological marker in PD (Elabi et al., 2021). Moreover, early discoveries that the eye consists of unique surface molecules and cytokines, and presents some immune responses similar to those in CNS (Streilein, 2003), so retina may display similarities of microvascular changes to the brain.

VISUAL DYSFUNCTIONS ASSOCIATED WITH MORPHOLOGICAL CHANGES IN RETINA

The retina, as mentioned above, consists of different neurons, dendrites, and axons, and it is responsible for integrating response to the visual system to the cortex. Clinically, patients with PD often suffer from various functional disabilities in central, peripheral, or visuoperceptual vision. Although the visual system does not exist in isolation, we focus on the retina in this article and discuss the visual disorders associated with retinal dysfunction (**Table 3**).

Visual Acuity

Visual acuity (VA) is an ability to discriminate the details of a stimulus. It has been demonstrated that patients with PD present impaired VA in the prodromal phase of the disease (Jones et al., 1992). Compared with age- and sex-matched healthy people using the standard Snellen chart and computerized test, VA is impaired in individuals with PD. Similarly, Han et al. (2020) confirmed that VA in PD patients was worse than this in control groups. Especially, they also found that the worse VA groups have higher incidence of PD than individuals without visual disability, reflecting the visual disorder is one of the premotor symptoms for PD progression (Han et al., 2020).

Evidence has demonstrated a significant positive correlation between lower visual acuity and thinning RNFL thickness (Satue et al., 2017; Visser et al., 2020; Abd Hamid et al., 2021; **Table 3**). Sparkly, the thinness in the ganglion cell-inner plexiform layer in PD was strongly correlated with low contrast visual acuity via a comprehensive battery of visual function tests (Murueta-Goyena et al., 2019; Marrocco et al., 2020). Recently, Shi and his colleagues characterized retinal capillary complexity of retina in patients with PD, and found that lower retinal capillary and perfusion densities and capillary complexity was negatively correlated with VA (Shi et al., 2020).

According to the above, dopaminergic neuron cells in retina can release dopamine and contribute to functional VA.

TABLE 2 | Table outlining the features of native and phosphorylated a-synuclein in the retina.

	Subject	Retinal layers	Morphometric analysis	Aggregation propensity or toxicity	References
Native a-synuclein	Non-PD, PD patient	GCL, IPL, INL	Soluble a-synuclein, protein aggregates, Lewy body/neurite	±[a]	Guan et al., 2013; Mailankody et al., 2015; Robbins et al., 2021
Phospho-a-synuclein	PD patient	GCL, IPL, NFL	Protein aggregates, Lewy body/neurite	++[b]	Archibald et al., 2009; Schmidt et al., 2011

Non-PD, healthy control subject; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; NFL, nerve fiber layer.
[a] *±: none or low aggregation propensity and toxicity.*
[b] *++: increased aggregation propensity and toxicity.*

TABLE 3 | Retinal abnormalities in PD patients.

Visual abnormality	Morphological changes in Retina	Retinal mechanism defects	References
Visual acuity	RNFL thinning		Postuma et al., 2015; Gbd 2015 Neurological Disorders Collaborator Group, 2017; Ggbd 2016 Parkinson's Disease Collaborators, 2018; Santos Garcia et al., 2019; Xu et al., 2019
	Loss RGCs		Postuma et al., 2015; Gbd 2015 Neurological Disorders Collaborator Group, 2017; Ortuno-Lizaran et al., 2018b
	Decreased microvascular density		Ortuno-Lizaran et al., 2018a
Contrast sensitivity	RNFL thinning	①Retinal function in ERGs and VEPs; ② Retinal dopaminergic system impairment and dopamine reduction ③ Loss of synaptic contacts between retinal neurons	Ggbd 2016 Parkinson's Disease Collaborators, 2018
	RGCs loss		Ortuno-Lizaran et al., 2018b; Kim et al., 2019
	Thinning of foveal neural tissues		Friedman, 2004; Reddy et al., 2013
Visual hallucinations	RNFL thinning		Armstrong, 2017; Shi et al., 2020
Color vision	Loss RGCs		Ortuno-Lizaran et al., 2018b

Aggregates of misfolded a-synuclein and related retinal dopamine depletion lead to injury to light-adapted vision and VA. Nguyen-Legros noted that altered ERGs and VEPs are identical in PD patients and animal models with damaged dopaminergic retinal system (Wong et al., 1985; Olivier et al., 1986; Bodis-Wollner and Tzelepi, 1998; Afsari et al., 2014; Bonilha et al., 2015; Mammadova et al., 2019). In Archibald et al.'s (2011) research, 64 with PD, 26 with PD dementia (PDD), and 32 normal were evaluated using a series of diagnostic procedures about function on vision, cognition, and related pathology. The study reported the impairments in acuity in patients with PD or PDD, and poorer visual acuity in the last stage of untreated patients (Archibald et al., 2011). Likewise, Richard and his members beat out the correlation between poor VA and the lack of dopamine in the retina, but also the better acuity in PD patients receiving drugs (Jones et al., 1992).

Contrast Sensitivity

Contrast sensitivity is a special vision function involving in the regulation of visual resolution ratio and vision at a variety of spatial and light-black frequencies. Patients with diminished vision contrast sensitivity commonly are susceptible to falls, reading problems, and dark-adapted difficulty. In the 1980s, impaired contrast sensitivity was documented in PD patients in comparison with age-matched controls (Regan and Neima, 1984). Since then, an increasing number of studies have consistently offered proof for the impairment of contrast sensitivity in patients with PD (Langheinrich et al., 2000; Silva et al., 2005; Archibald et al., 2009; Armstrong, 2017). Current studies reported by Polo et al. (2016), showed that contrast sensitivity deficit is more common and severe than other visual disorders. Additionally, the impaired contrast sensitivity was associated with VH and cognitive impairment, as a useful biomarker in patients with PD (Diederich et al., 1998; Ridder et al., 2017).

Like VA, evidence revealed that the inner retinal thinning enhances the presence of contrast sensitivity (Pinkhardt et al., 2020), and the progressive changes in RNFL were associated with progression of abnormal visual function (Satue et al., 2017). Moreover, several studies demonstrated the correlation between impaired vision and remodeled foveal pit, showing the correlation of contrast sensitivity deficit with retinal parafoveal thickness (Miri et al., 2016; Pinkhardt et al., 2020).

In pathological mechanisms, it has been indicated that dopaminergic system impairment in the retina may explain and be partially responsible for the reduced contrast sensitivity in

patients with PD. Under physiological conditions, the contrast sensitivity and color vision are mainly modulated through D1 and D2 receptors differentially located in the retinal structure. When these receptors lack activation, there are the dispersion of visual signals and alterations in color vision and contrast sensitivity (Hajee et al., 2009). Also, dopamine reduction may result in loss of synaptic contacts with photoreceptor cells and mRGCs and to disturb contrast sensitivity (Hindle et al., 2013; Kaur et al., 2015; Ortuno-Lizaran et al., 2020). Bulens et al. (1987) reported, contrast sensitivity function of 10 patients with PD before and after levodopa treatment. The remission of contrast sensitivity deficit after the exogenous supplement of dopamine demonstrated the function of the retinal transmitter on the visual pathways. An updated study showed that dopaminergic system impairment and dopamine reduction may be responsible for the reduced contrast sensitivity in PD (Ortuño-Lizarán et al., 2020). In addition, these alterations are linked to loss of dopaminergic synaptic contacts or decline in mRGCs that contribute to circadian rhythm and sleep.

Visual Hallucinations

Visual hallucination (VH) is a specific feature of PD compared with other Parkinsonian disorders, accounting for 30–40% patients with the disease (Onofrj et al., 2007). Clinically, VH manifests various complex symptoms, including flashes of light, visual perception deficit, and color and motion perception impairment. Most studies in patients with VH demonstrated the disorder was associated with cortical visual discrimination involving in the changes at different visual pathways as well as other neural systems and motor function (Uc et al., 2005). It can explain why VH is also common in cognitive flaw PD patients, and the patients with dementia have the higher prevalence of VH than patients without dementia (Archibald et al., 2011). However, the underlying pathophysiological mechanisms are still unclear. Based on a hypothesis of VH titled the Charles Bonnet syndrome (Stebbins et al., 2004), we know that retinal damage is linked to poor signals in the brain regions, and lead to less visual cortical activation and play a crucial role in the neuropathophysiological function of VH in PD. Moreover, emerging studies stated that defective visual information processing involvement has been demonstrated in VH of PD patients, so retinal impairment appears to be one of mechanisms of the sign (Visser et al., 2020).

To date a limited number of studies reveals that VH appears to be associated with inner retinal thinning. Lee et al. (2014) identified RNFL thinning among the PD subgroups, and noted that RNFL thickness is thinnest in groups without dementia, suggesting RNFL thinning was associated with the occurrence of VH in PD. Similarly, a recent report confirmed the relationship between RNFL thinning and the presence of VH, revealing that individuals with VH had a thinner GCL-IPL than individuals without VH (Visser et al., 2020). It is believed that the old age, disease's duration, motor disorders, and other non-motor disturbances could worsen the VH as the risk factors in PD (Fénelon et al., 2000; Zhu et al., 2013).

Consistent with visual acuity and contrast sensitivity, retinal pathological changes contribute to the occurrence and development of VH. The dopaminergic system damages have been considered as the pathological basis of VH. Dopamine replacement therapy also supported the evidence for the effects of dopaminergic deficiency on the VH (Onofrj et al., 2002; O'Donnell et al., 2006). Like as the loss of DA, the LB pathology is also associated with the occurrence and progress of VH. Moreover, a number of studies have been proposed to explain VH in PD, noting that reduced levels of γ-aminobutyric acid (GABA) are associated with mechanisms of VH (Firbank et al., 2018).

Color Vision

As is known to all, color vision is the basic function mediated by the photoreceptor cones in the retina and related visual cortex via some specific visual pathways. In the early stages of PD, patients' color vision is impaired and deteriorates with disease duration (Price et al., 1992; Buttner et al., 1995). In the 1990s, scholars assessed the color vision in 35 patients with PD and 26 controls, and reported significant abnormality of color vision in PD compared to the healthy people (Price et al., 1992). Recent studies showed the color detection dysfunction affected movement. For instance, Penedo et al. (2018) reported the ability of obstacle avoidance was impaired due to the impairment of color. Likewise, the axial motor impairments were associated with the changes in color discrimination (Bohnen et al., 2017), suggesting shared pathophysiology between the alteration of color vision and motor or mobility dysfunctions in PD. Additionally, the disorders of visual pathways in PD patients might contribute to the occurrence of poor color vision, such as depressive symptoms (Li et al., 2018), idiopathic rapid eye movement sleep behavior disorder (Postuma et al., 2009), and other different retinal areas.

Likewise, RGCs loss and RNFL thinning may be also causes of impaired color vision in PD (Polo et al., 2016). In an observational cross-sectional study, Polo et al. (2016) evaluated visual dysfunction and its correlation with morphological changes in the retina in participants with PD, and found color vision was associated with most GCL thinning while not significantly correlated between RNFL thickness and other visual dysfunction.

As a pathological hallmark, dopaminergic deficiency in the retina showed an association with the impairment in color vision. In mammalian retinal layers, some dopaminergic receptors are in charge of color vision and contrast sensitivity, so alterations in these functions could be the result of lack of dopaminergic systems (Hajee et al., 2009). In patients with PD, deficiency in color vision influences retinal evoked potentials, reflecting abnormalities in dopaminergic synaptic activity in the retina (Bodis-Wollner et al., 1982). Silva et al. (2005) found color visual deficits within the parvo, Konio, and magnocellular pathways in the retina, especially the parvocellular pathway. Meanwhile, they also found the reduction of dopaminergic neurons around the fovea, suggesting that the dysfunctions of these pathways are possibly related to altered dopaminergic modulation.

TABLE 4 | Retinal abnormalities in PD animal models.

Animal	Model	Morphological changes	Retinal defects	References
Rat	Rotenone-induced	Decreased number of RGCs and DACs; INL and ONL thinning	Decreased scotopic and photopic a- and b-waves; Increased b-wave implicit time	Tamer et al., 2005; Onofrj et al., 2006
	6-OHDA-induced	Decreased DA levels	–	Onofrj et al., 2006
Mouse	MPTP-induced	Decreased number of DACs	Reduced oscillatory potentials, a- and b-waves;	Williams et al., 2008
	Retinal a-Syn overexpression	Decreased number of DACs	Decrease of light-adapted ERG responses and visual acuity	Guo et al., 2018
	Prnp- A53T- SNCA	Accumulation of α-synuclein, loss of photoreceptor cells		Weil et al., 2016
	TgM83 (A53T α-synuclein mutation)	Accumulation of a-Syn and phosphorylated tau, decreased number of photoreceptors	–	Weil et al., 2016
	DJ-1 knockout	RPE thinning, decreased number of dopamine	Increased amplitude of b-wave and ERG,	Uc et al., 2005
Rabbit	MPTP-induced	Decreased dopamine level	Decreased amplitude of b-waves and oscillatory potentials	Bertrand et al., 2012
	6-OHDA-inducd	Decreased dopamine level	Decreased amplitude of b-waves	Ortuno-Lizaran et al., 2020
Monkey	MPTP-induced	Decreased number of DACs, Deteriorated postsynaptic neurons	–	Esteve-Rudd et al., 2011
	MPTP-induced	Decreased number of DACs, RNFL thinning	Abnormal VEP and PERG responses	Willis et al., 2014
	6-OHDA-inducd	Decreased number of DACs	Abnormal PERG responses	
Drosophila	a-Syn over-expression	–	Decreased PERG responses	Willis, 2005
	LRRK2-G2019S	Loss of photoreceptor function	Decreased ERG response, loss of visual function	Toscano-Tejeida et al., 2016; Pineda-Rodriguez et al., 2017

FUTURE PERSPECTIVES

The increasing number of research explores morphological changes associated with retinal dysfunction in PD as summarized in **Table 4**. This evidence provides insight into the mechanism underlying visual dysfunctions and retinal changes in PD, mirroring PD brain pathology. Thus, morphological changes or dysfunction in the retina are regarded as a potential approach to diagnosis and monitor Parkinson's disease, and the successful use of the retinal technology in clinical trials is valid and reliable tools to explore neuropathies in the CNS. However, molecular changes and neuropathological mechanisms involved in retinal changes are obscure. More studies are needed to further validate the significance of retinal pathology and vision deficit to establish the causality of these relationships in PD.

AUTHOR CONTRIBUTIONS

TT and YY conceived the conception and design of the manuscript. YZ drafted the manuscript. TT was responsible for the revision of the article. YZ and XZ participated in the discussion about the article. All authors read and approved the final manuscript.

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