REVIEW ARTICLE

Emerging role of sulfur-containing amino acids in the etiology of neurological disorders

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Abstract

Neurological disorders are an important cause of disability and death worldwide. In the recent years hyperhomocysteinemia (hHcy) has gained a great deal of interest in the scientific society because of its high prevalence about 5% in the general population. Numerous evidences suggest relationship between high circulating homocysteine (Hcy) level and the development of brain disorders. Hcy may promote its detrimental effect by more than one mechanism. In this review we discuss the role of sulfur containing amino acids and especially hHcy as a risk factor, marker or both in the etiology and pathogenesis of Alzheimer´s disease (AD) and a stroke. We present the contribution of hHcy in AD generation in different animal studies, with emphasis on the main etiopathogenic AD hallmarks, such as amyloid-β and tau protein. We also focus on the hHcy involvement in the methylation hypothesis of AD pathogenesis. In addition, we describe detrimental effect of hHcy in ischemic animal models.

Abbreviations:
Hcy – homocysteine; hHcy – hyperhomocysteine; AD – Alzheimer´s disease; VaD – vascular dementia; Met – methionine; SAM – S-Adenosylmethionine; SAH – S-Adenosylhomocysteine; MS – methionine synthase; CBS – cystathionine β-synthase; CSE – cystathionine γ-lyase; Cys – cysteine; HTL – Hcy-thiolactone; MetRS – methionyl-tRNA syntethase; BHMT – betaine-homocysteine methyltransferase; BLMH – bleomycin hydrolase; BPHL – biphenol hydrolase-like enzyme; PON1 – paraoxonase 1; BBB – blood brain barrier; eNOS – endothelial nitric oxide synthase; NOX – NADPH oxidase; GSH – glutathion; SOD – superoxid dismutase; MMP – matrix metaloproteinase; TIMP – tissue inhibitor of metaloproteinase; PARP – poly-ADP-ribose polymerase; AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; NMDA – N-methyl-D-aspartate; APP – amyloid precursor protein; Aβ – amyloid-β; BACE – β-site APP-cleaving enzyme; PS – Cdk – cyclin-dependent kinase; CAA – cerebral amyloid angiopathy; PSEN-1 – gene PS1 promoter; NEP – neprilysin; 5LO – 5-lipoxygenase; PP2A – protein phosphatase 2A; PME-1 – protein phosphatase methylesterase 1; LCMT-1 – leucine carboxyl methyltransferase-1; IPC – ischemic preconditioning; CA1; Cornu Ammonis; ERK – extracellular-signal regulated kinase; MAPK – mitogen-activated protein kinase; MCAO – middle cerebral artery occlusion

Introduction

Neurological disorders are the leading cause of disability and second leading cause of death worldwide. As the human lifespan is prolonging and population is growing, a number of age-related neurological disorders such as Alzheimer´s disease (AD) and vascular dementia (VaD) or stroke is increasing (Group, 2017; Feigin *et al* 2014). Suffering people experience distinct neurological symptoms that affect their ability to perform everyday activities. The long duration of the patients´ disability and dependence on care has a great social and economic impact on public health. In 2015 there were 46.8 million people worldwide living with dementia and this number is estimated to increase to 131.5 million in 2050 (International 2016). High burden of patients, families and society attributed to AD highlights the importance of understanding the risk factors, especially modifiable risk factors in order to reduce their effect on these disorders (Hersi *et al* 2017). More than 90% of stroke burden is caused by modifiable risk factors, mostly behavioural (smoking, poor diet, low physical activity) and metabolic (high blood pressure, high body mass index, high fasting plasma glucose, high cholesterol). Air pollution is considered to be the significant contributor in global stroke burden especially in low income and middle-income countries (Feigin *et al* 2016).

One of the plausible modifiable risk factors is elevated level of plasma Hcy called hyperhomocysteinemia. In the recent years hHcy has gained a great deal of interest in the scientific society because of its high prevalence about 5% in the general population and close relationship with a number of disorders (Brustolin *et al* 2010; Kim *et al* 2018).

Association between moderate hHcy and AD, vascular dementia and stroke was communicated in McIlroy study (2002). Based on a search of epidemiological and experimental studies, a group of experts has marked hHcy as a modifiable risk factor for development of cognitive decline, dementia and AD in elderly people (Smith *et al* 2018).

Homocysteine metabolism

Homocysteine is a nonproteinogenic and nonessential amino acid naturally occuring in the body. The only source of Hcy is the dietary amino acid methionine (Met). Met is activated by ATP to produce S-Adenosylmethionine (SAM), a major methyl donor in the cells for methylation of DNA, RNA, proteins and phospholipids (Blom & De Vriese 2002). After demethylation by numerous methyltransferases, SAM is converted to S-Adenosylhomocysteine (SAH), a strong inhibitor of most transmethylation reactions (Blom & De Vriese 2002). SAH is eventually hydrolyzed by enzyme SAH hydrolase to Hcy and adenosine. Although this reaction is reversible and equilibrium dynamic prefers SAH synthesis to Hcy synthesis (Fuso & Scarpa 2011a), a permanent removal of Hcy via transsulfuration and remethylation pathway is essential and promotes Hcy synthesis.

Subsequent metabolism of Hcy follows three pathways: 1) remethylation back to Met catalyzed by vitamin B12-dependent methionine synthase (MS), occurs in every organ; 2) a two-step irreversible transsulfuration catalyzed by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (both vitamin B6-dependent) to cystathionine and cysteine (Cys) respectively, reaction is limited to the liver, kidneys and brain; 3) enzymatic conversion to Hcy-thiolactone (HTL) (generated in protein syntesis when Hcy is erroneously selected instead of Met by MetRS) catalyzed by methionyl-tRNA syntethase (MetRS) in every organ and requires ATP (Škovierová *et al* 2016; Jakubowski 2019). An alternative remethylation pathway is catalyzed by betaine-homocysteine methyltransferase (BHMT) that is mainly expressed in the mammalian liver and kidneys of primates and hogs (Blom & Smulders 2011; Finkelstein 2007).

HTL is metabolized by two pathways: 1) hydrolytic pathway yielding Hcy catalyzed by cytoplasmic bleomycin hydrolase (BLMH), in vitro by mitochondrial biphenol hydrolase-like (BPHL) enzyme or by serum paraoxonase 1 (PON1); 2) synthetic pathway yielding N-Hcy-protein by HTL reaction with protein lysine residue called N-homocysteinylation. HTL pathway is enhanced when remethylation or transsulfuration pathway are impaired due to genetic or metabolic impacts on enzymes. Also rich Met diet supports the flow through HTL pathway (Jakubowski 2019).

Maintaining low intracellular Hcy concentration is essential for cellular viability, instead, an accumulation of Hcy is toxic. Excess of Hcy and also HTL are exported out of the cells (James *et al* 2002; Jakubowski 2019).

In plasma, majority (98%) of Hcy is rapidly oxidized, bound by disulfide bonds to proteins and to small thiol molecules. Only 1–2% of Hcy remain in its reduced form. The term total plasma Hcy (tHcy) includes the sum of Hcy in its reduced form and Hcy obtained from the oxidized disulfide bound Hcy (Mudd *et al* 2000). Normal concentration of total Hcy in human plasma is in the range 5–15 $\mu mol.L^{-1}$, although level above 10.8 $\mu mol.L^{-1}$should be considered as a risk factor for human health (Sharma *et al* 2015). There are three categories of hHcy: moderate (16–30 $\mu mol.L^{-1}$), intermediate (31–100 $\mu mol.L^{-1}$) and severe (>100 $\mu mol.L^{-1}$). Severe hHcy reaching values up to 500 $\mu mol.L^{-1}$ is called homocystinuria (Medina *et al* 2001).

The main causes of hHcy are chronic deficiencies of vitamins B6, B9 and B12 and/or genetic polymorphisms in genes coding enzymes involved in Hcy metabolism. Excessive Met intake, chronic renal failure (Guldener 2006), autoimmune hypothyroidism (Cicone *et al* 2017), malignant tumors (Sun *et al* 2002) and side effects of

some drugs can be other causations of high circulating Hcy (Kim *et al* 2018). Unhealthy lifestyle may also play a role in modulating Hcy levels (Ansari *et al* 2014).

Hcy transport from blood into the cells is an essential step to exert its biological effects. Hcy can be transported into vascular endothelial cells and smooth muscle cells via at least three Na^+-dependent and Na^+-independent transport systems (X_{AG}, ASC and A) and taken up by different biological pathways. Hcy shares transport systems with cysteine and transport into endothelial cells is highly dependent on intact lysosomes (Jiang *et al* 2007). However, most of Hcy in plasma is disulfide-bound with proteins that cannot be transported via these cysteine transport systems (Budy *et al* 2006). In addition, Kamath *et al* (2006) have reported deleterious effect of elevated Hcy on brain microvessels and subsequent disruption of blood brain barrier (BBB) in mice. Endothelial dysfunction may compromise astrocytes and thereafter neurons. This detrimental effect of Hcy can imply the possible connection of atherosclerosis with neurodegenerative diseases (Kamath *et al* 2006).

HCY AND ITS TOXICITY

Induction of oxidative stress is one of the most detrimental toxic effects of elevated Hcy to the cell. Oxidative stress is defined as an imbalance between the production of reactive species and antioxidative defenses (Škovierová *et al* 2016). Oxidative stress involves direct Hcy autooxidation pathway (oxidation of the free thiol group of Hcy linked via a disulfide bound to plasma proteins or other low molecular thiols or to other Hcy) with generation of H_2O_2, or indirect pathway, indirect generation of O_2^- from uncoupled eNOS, activation of NADPH oxidases (NOXs) and xanthine oxidase. Excess Hcy leads to inhibition of the enzymatic activity of antioxidants in the cells (GSH, SOD, catalase, H_2S). Moreover, accumulation of Hcy decreases the binding of extracellular SOD to the vascular endothelial cell surface (Bhatia *et al* 2014; Lehotský *et al* 2016). Other reactive species are formed to further exaggerate toxicity, especially peroxynitrite. The increased production of reactive species induces subsequent oxidation of proteins, lipids and nucleic acids (Bhatia *et al* 2014; Petras *et al* 2014). Exposure of endothelial cells to Hcy leads further to endothelial dysfunction, inflammation, platelet activation and thrombus formation (Faraci & Lentz 2004; Petras *et al* 2014). In addition, Hcy induces endoplasmic reticulum (ER) stress and unfolding protein response (Perla-Kaján *et al* 2007).

Hcy also exerts its neurotoxicity by activation and proliferation of microglia (Cunha *et al* 2012) and by changes in morphology and activation of astrocytes with pro-inflamatory mediators release (Longoni *et al* 2018; Ostrakhovitch & Tabibzadeh 2019). Increased matrix metaloproteinases MMP2/MMP9 and decreased tissue inhibitors of metaloproteinases TIMP1/TIMP2 are caused by elevated Hcy, this dysbalance leads to the degradation of tight junction proteins resulting in BBB permeability (Kalani *et al* 2013). Hcy also modulates neuronal signaling via reducing Na^+K^+-ATPase activity and immunocontent on neuronal cells in cerebral cortex, hippocampus (Scherer *et al* 2013) and amygdala (Kolling *et al* 2016) in rats. Hcy was shown as an inductor of caspase-dependent neuronal apoptosis, by a mechanism involving DNA damage, poly-ADP-ribose polymerase (PARP) and mitochondrial dysfunction by caspase 3 activation (Lehotsky *et al* 2015).

In addition, Hcy can act as an agonist for both groups of glutamate receptors, metabotropic (groups I and III) and ionotropic (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)) and N-methyl-D-aspartate (NMDA) receptor. Overstimulation of these receptors leads to the enhanced intracellular Ca^{2+} level, higher production of free radicals and activation of caspases leading to apoptosis of neuronal cells (Škovierová *et al* 2016).

Harmful effect on proteins exerts the highly reactive Hcy thioester HTL. It incorporates via amide linkage into proteins in a reaction N-homocysteinylation (Perla-Kaján *et al* 2007). N-homocysteinylation results in inactivation of enzymes, aggregation of proteins and even protein precipitation (Sharma *et al* 2014).

ALZHEIMER´S DISEASE (AD)

AD is a progressive neurodegenerative disease characterized by loss of function and death of neurons in certain areas of brain leading to a decline in cognition and memory and ultimately leads to death (Amirrad *et al* 2017). It is the most common cause of dementia that accounts for 60–80% of dementia cases (Association 2018).

AD occurs in two forms. Early-onset, familial AD results from mutations in one of three genes encoding amyloid precursor protein (APP), presenilin 1 (PS1) or presenilin 2(PS2). It tends to develop symptoms before the age of 65 in less than 1% of cases. Late-onset, sporadic AD accounts for 90–95% of AD cases and occurs in people aged 65 or more (Ates *et al* 2016). According to Alzheimer´s association (2018) the prevalence of AD increases dramatically with age: 3 % of people age 65–74, 17 % of people age 75–84 and 32 % of people age 85 or older, making age the strongest risk factor for AD. Besides the age, other factors including genetic (carrying APOE-ε4 gene) and environmental (metabolic and lifestyle), are suggested to be implicated in a multifactorial pathogenesis of AD. (Lim *et al* 2016).

The neuropathological changes in AD brain are neuritic plaques originated from extracellular accumulation of amyloid-β peptides (Aβ), neurofibrillary tangles composed of intracellular hyperphosphorylated tau proteins, glial responses, neuronal and synaptic loss (Serrano-Pozo *et al* 2011; Association 2018).

In normal brain, Aβ peptides are present in a low concentration. Aβ peptides play divergent physiological roles, they could modulate a synaptic activity, mediate learning and memory formation, defense against oxidative stress, have a neurotrophic function (Garcia-Osta & Alberini 2009; Cárdenas-Aguayo *et al* 2014). Aβ peptides are continuously generated by neurons and non-neuronal cells from a larger precursor, a membrane glycoprotein, APP (Agnati *et al* 2007). In amyloidogenic pathway, APP is subsequently cleaved by β-secretase (also referred to as a β-site APP-cleaving enzyme, BACE) and γ-secretase to produce a 38 to 43 amino acid residue peptide, Aβ (Cárdenas-Aguayo *et al* 2014). In AD, Aβ peptides are the main components of senile plaques. Aβ monomers aggregate into different forms of soluble oligomers, which can spread throughout the brain and form larger and insoluble fibrils and then deposit into plaques (Chen *et al* 2017). Aβ42 and Aβ40 are considered to be the main toxic species (Zhang *et al* 2011). Accumulation of toxic extracellular and intracellular Aβ oligomers and their binding to various receptors have been suggested to be the most damaging for neuronal cells (Chen *et al* 2017).

In healthy neurons, tau, a soluble microtubule associated protein, is mainly distributed in axons (also in dendrites and nucleus of neurons and outside the neurons) and plays a major role in stabilization of microtubules by binding to their surface and promoting their self-assembly from tubulin (Mandelkow & Mandelkow 2012). It may undertake other functions in axonal transport, cell signaling, synaptic plasticity and regulation of genomic stability (Wang & Mandelkow 2016; Taleski & Sontag 2018; Guo *et al* 2017). Tau function is tightly regulated by the balance between phosphorylation and dephosphorylation forms (Obulesu *et al* 2011). In AD, increased tau phosphorylation, especially at selected sites, weakens tau affinity to microtubule and precedes its aggregation (Mandelkow & Mandelkow 2012; Taleski & Sontag 2018) into paired helical filaments and neurofibrillary tangles (Wang & Mandelkow 2016).

It is thought that multiple pathophysiological changes in AD may begin 15–20 years before clinical symptoms appear. Aβ plaques appear first in the neocortex and in later stages extend into other brain regions. Tangle formation starts in medial temporal lobe and gradually spreads to neocortex (Mufson *et al* 2016; Sperling *et al* 2013). Diverse symptoms advance over the years and vary among people. These symptoms reflect the degree of damage to neurons in different parts of the brain (Association 2018).

hHCY AND AD

Despite an enourmous effort an effective treatment of AD is still not available (Amirrad *et al* 2017). The attention of researches is focused on identifying modifiable risk factors and developing the means how to treat them in order to prevent or delay onset or to slower progression of AD (Beydoun *et al* 2014; Hersi *et al* 2017). Findings supporting the association between hHcy and AD have been accumulating over the past several years. Most studies suggested hHcy as a strong risk factor of AD (Seshadri *et al* 2002; Nazef *et al* 2014). But others did not find direct connection between hHcy and pathogenesis of AD (Luchsinger *et al* 2004; Nilsson *et al* 2012). Does hHcy play a causal or rather a marker role in AD pathogenesis? Several articles have summarized evidence of this relationship (Zhuo *et al* 2011; Cacciapuoti 2016). Based upon reviewing studies from the last years and according to Bradford Hill´s criteria of causation, a team of experts came to the conclusion that moderately raised tHcy above 11 $\mu mol.L^{-1}$ is a modifiable risk factor for development of cognitive decline, dementia and AD in elderly people. But elevated tHcy is just one component of the ´sufficient causes´ in development of multifactorial dementia. They suggested that hHcy could be both a direct cause of cognitive impairment and a marker of other causes and it might work in parallel with some of other risk factors. In return, hHcy might conversely affect these risk factors (Smith *et al* 2018).

Although an elevated tHcy is often considered as a biomarker of AD, underlying molecular mechanisms remain to be investigated. A large research in *in vitro* and *in vivo* studies has shown several potential mechanisms to explain manifold deleterious role of hHcy in the central nervous system.

The biological link between hHcy and amyloidogenesis has been investigated by different approaches. A positive relationship between elevated plasma Hcy and increased Aβ levels have been described in various transgenic AD-like or wild type mouse and rat models with gene, diet or injection induced hHcy (Pacheco-Quinto *et al* 2006; Zhang *et al* 2009; Zhuo *et al* 2010) but intrinsic mechanism is still unavailable.

Remarkably, the proper choice of an appropriate animal model is essential. Diet induced hHcy with either Met enriched diet or B-vitamin deficient diet results in moderate hHcy and enhanced Aβ level in AD-like animal models (Zhuo *et al* 2010; Fuso *et al* 2008). However, combination of these two diets in the same animal causes Hcy plasma levels reaching severe hHcy concentrations more than 150 μM without effect on Aβ level or deposition in the hippocampus or cortex. These findings suggest that different Hcy plasma levels could have a different biological mechanism. This combined diet inducing severe hHcy does not result in amyloidogenesis *in vivo* and may not be a suitable model for studying hHcy in association with AD amyloidogenesis (Zhuo & Praticó 2010b).

Growing evidence links hHcy to main pathological features of AD phenotype: accumulated Aβ peptides, hyperphosphorylated tau proteins and memory deficits. Increased Aβ levels and deposition, as well as behavioral impairment were detected in rat models with injection-induced hHcy (Zhang *et al* 2009) and in diet-induced hHcy transgenic, Tg 2576 and Tg CRND8

mouse models of AD (Zhuo & Praticó 2010a; Zhuo *et al* 2010; Fuso *et al* 2008). Association of hHcy with pathological alterations in tau protein was observed in rat models with vena caudalis injection of Hcy (Zhang *et al* 2008), in diet-induced hHcy in 3xTg mouse model of AD (Li *et al* 2014) and in P301L, Tg4510 mouse model of tauopathy (Shirafuji *et al* 2018).

A pleiotropic role of hHcy in AD pathogenesis has been revealed in triple transgenic (3xTg) mouse model with diet-induced hHcy. HHcy modulated all three key pathological features of AD phenotype. Increased Aβ levels and deposition caused by activation of γ-secretase pathway, raised insoluble fractions of tau and its phosphorylation at specific epitops resulted from activation of cyclin-dependent kinase (Cdk-5) and significant learning and memory deficits were observed in hHcy animals. In addition, it was shown that Hcy-induced tau phosphorylation might be Aβ-independent because suppression of Aβ formation did not influence tau phosphorylation (Li *et al* 2014).

Some studies detected direct connection of Hcy and its derivatives with Aβ. Direct binding of Hcy to Aβ 40 peptides was demonstrated in *in vivo* and *in vitro* experiments. After intrastriatal microinjection of Aβ in hHcy rats, Hcy bound to Aβ most likely due to noncovalent electrostatic interaction. This interaction promoted a β-sheet conformation of Aβ peptides and their aggregation (Agnati *et al* 2007). N-homocysteinylation of Aβ peptides by HTL led in the cell culture to conformational changes of Aβ resulting in decreased elongation of soluble Aβ oligomers and fibril formation and probably formation of new toxic protofibrils (Khodadadi *et al* 2012).

Recent clinical studies have revealed association of AD with cerebrovascular dysfunction (Iadecola 2003). Aβ can accumulate not only in brain parenchyma but also in cerebral blood vessel and contribute to the development of cerebral amyloid angiopathy (CAA). Elevated plasma Hcy/HTL can aggravate vascular impairment via enhanced interaction between Aβ and fibrinogen elucidated in *in vitro* and *in vivo* experiments (Chung *et al* 2016). Hcy/HTL can modify fibrinogen by homocysteinylation at fragment D region. Homocysteinylated fibrinogen more strongly interacts with Aβ42 that increases fibrinogen oligomerization. Hcy/HTL-modified fibrinogen forms enlarged fibrin clots with irregular clusters. Hcy/HTL-modified clots show delayed fibrinolysis *in vitro* (Chung *et al* 2016).

Agnati *et al* (2007) demonstrated in *in vivo* and *in vitro* experiments that Hcy can directly bind to Aβ 40 peptides presumably due to noncovalent electrostatic interactions between the Arg guanido-group of Aβ and the sulphur of Hcy. Aβ bound to Hcy can more easily create a β-sheet conformation and form fibrils. Intrastriatal microinjections of Aβ in hHcy rats confirmed this profibril action of Hcy.

In a search for a novel molecular mechanism connecting hHcy with AD pathogenesis, a methylation hypothesis has gained a great interest. Recent studies have provided a bulk of evidence about hHcy association with alteration of cellular methylation status. Hcy is a key intermediate in the methyl cycle and hHcy is connected with a global decrease in cellular methylation (Vafai & Stock 2002). HHcy increases cellular SAH levels which leads to inhibition of SAM-dependent methylation reactions that may regulate gene expression. Methylation impairment can include not only DNA methylation (Fuso *et al* 2008; Fuso *et al* 2011b; Fuso *et al* 2011c; Fuso *et al* 2012) but also protein methylation mechanism (Sontag *et al* 2007; Sontag *et al* 2008).

A site-specific demethylation of DNA could be one of possible underlying mechanisms of Aβ overproduction caused by hHcy. PS1 is one of four catalytic subunits of γ-secretase. PS1 expression is regulated by a methylation status of its gene (PSEN-1) promoter. Site-specific demethylation of PSEN-1 promoter leads to PSEN-1 overexpression (Fuso *et al* 2008; Fuso *et al* 2011c) and raised PS1 in mRNA and protein level. Stimulated PS1 upregulates γ-secretase and results in increased production of Aβ (Zhang *et al* 2009; Fuso *et al* 2011b; Li *et al* 2014). Fuso *et al* (2005, 2008) have also displayed association of altered Hcy/SAM cycle with DNA hypomethylation of BACE gene with subsequent BACE overexpression in neuroblastoma cells (Fuso *et al* 2005) and in a Tg mouse model of AD (Fuso *et al* 2008). Interestingly, Aβ itself can contribute to its own accumulation. In murine cerebral endothelial cells, Aβ may induce genome-wide hypomethylation and at the same time it can cause site-specific hypermethylation on gene promoter of neprilysin (NEP), an Aβ degrading enzyme, with subsequent suppression of NEP mRNA and protein expression. Lowered NEP expression may reduce Aβ clearance and increase Aβ accumulation (Chen *et al* 2009).

A novel biological link between hHcy and AD has been established involving the epigenetic modification of 5-lipoxygenase (5LO) (Li *et al* 2017b; Meco *et al* 2018a). 5LO, a monomeric enzyme, catalyzes the synthesis of leukotrienes (LTs) from arachidonic acid. LTs have strong pro-oxidant and pro-inflammatory activities. Besides cardiovascular system, 5LO is widely distributed in neuronal and glial cells of hippocampus and cortex. Its expression increases with age and although upregulation of 5LO might serve a physiological purpose, during the aging process it may also increase the vulnerability of the cardiovascular system and CNS to different insults/stressors. It is suggested that 5LO may be involved in age-related neurodegenerative diseases (Chu & Praticó 2009). A functional role of 5LO in the AD-like phenotype has been demonstrated in animal models of AD-like amyloidosis (Chu *et al* 2012b), 3xTg AD-like model (Chu *et al* 2012a) with viral-induced over-expression of 5LO and other 3xTg model studies (Giannopoulos *et al* 2014). 5LO promotes Aβ formation via activation of γ-secretase (Chu & Praticó 2011; Chu

& Praticó 2016) and tau phosphorylation via increased activity of Cdk-5 kinase (Chu *et al* 2012a).

5LO expression is regulated by DNA methylation and demethylation of its promoter (Uhl *et al* 2002). Several studies have revealed the association of increased Hcy and SAH levels with subsequent hypomethylation of DNA 5LO promoter and upregulation of brain 5LO enzymatic pathway. Li *et al* (2017) have established a crosstalk between Hcy and 5LO pathways and have demonstrated hHcy-dependent 5LO DNA hypomethylation, 5LO overexpression and Aβ formation in a diet-induced hHcy 3xTg AD-like mouse model, genetic-induced hHcy model and neuro 2A (N2A) cells (Li *et al* 2017b). In the next study in *in vivo* and *in vitro* models they have confirmed that the mechanism responsible for the Hcy-dependent worsening of AD-like phenotype is hypomethylation of 5LO gene (Li *et al* 2017a). A direct effect of hHcy via 5LO activation mechanism on tau phosphorylation and neuropathology has been presented in a human tau mouse model (Meco *et al* 2018b).

On the protein level, a posttranslational demethylation of protein phosphatase 2A (PP2A) might serve as a link between hHcy and AD pathogenesis. Reduced methylation of PP2A can enhance phosphorylation of tau protein and APP isoforms and accumulation of Aβ proteins in neuroblastoma cells and Tg AD-like mice (Sontag *et al* 2007). PP2A is a ubiquitous heterotrimeric (ABC) Ser/Thr phosphatase containing catalytic C, regulatory B and a scaffolding A subunit (Sontag & Sontag 2014). Methylation of C subunit in inactive dimers promotes binding with B subunit forming stable and active trimers (Sontag *et al* 2004), thus it is essential for assembly of PP2A. Bα is the major regulatory subunit in the brain. Trimeric PP2A composed of ABαC is the most efficient isoform that binds to and dephosphorylates tau protein at multiple serine and threonine sites (Martin *et al* 2013; Taleski & Sontag 2018; Sontag & Sontag 2014; Janssens & Goris 2001). PP2A activity is regulated by several mechanisms, including methylation and phosphorylation (Janssens & Goris 2001). Methylation of PP2A is controlled by leucine carboxyl methyltransferase-1 (LCMT-1) that methylates Leu^{309} residue on PP2A C subunit ($PP2A_c$) and conversely by the PP2A-specific methylesterase 1 (PME-1) that can remove methyl group from $PP2A_c$ and inactivate PP2A (Zhang *et al* 2008; Sontag & Sontag 2014). Low folate status and elevated Hcy can down-regulate LCMT-1 activity and that interrupts PP2A methylation (Sontag *et al* 2008; Sontag *et al* 2007). PP2A inhibition results in abnormal phosphorylation of tau protein and accumulation of phosphorylated tau species (Sontag & Sontag 2014). Increased level of PME-1 in Hcy-injected rats indicates that Hcy may activate PME-1 that could contribute to demethylation of $PP2A_c$ (Zhang *et al* 2009). Enhanced phosphorylation at Tyr^{307} site of $PP2A_c$ might also participate in inactivation of phosphatase. Simultaneously increased level of Tyr^{307} phosphorylated and Leu^{309} demethylated $PP2A_c$ led to inability of abnormally modified $PP2A_c$ in binding to B regulatory subunit (Zhang *et al* 2008).

Phospho-tau homeostasis is regulated by balance between phosphatase and tau kinases activities (Martin *et al* 2013). Hyperphosphorylation of tau can be achieved by either enhanced phosphorylation or reduced dephosphorylation. Cdk-5 and GSK-3 are the main kinases responsible for increased tau phosphorylation (Kimura *et al* 2014; Hernández *et al* 2010) and PP2A is considered to be the main phosphatase (Martin *et al* 2013). Abnormal tau phosphorylation with simultaneous activation of GSK3β and inhibition of PP2A, and tau cleavage and oligomerization via caspase-3 activation was detected in diet-induced hHcy P301L tau transgenic mice and human neuroblastoma M1C cells (Shirafuji *et al* 2018).

Stroke

Stroke is a leading cause of dementia and depression worldwide, the second leading cause of death and the third leading cause of disability. In the last four decades stroke incidence has more than doubled in low- and middle-income countries while in high-income countries it has declined by 42% (Johnson *et al* 2016).

Stroke is a medical condition when the blood supply to the part of the brain is interrupted or reduced and the brain cells without oxygen and nutrients start to die. There are two basic types of human stroke: hemorrhagic (an artery in the brain leaks blood or ruptures) and ischemic (an artery that supplies the blood to/in the brain becomes blocked). The majority of strokes (80%) are ischemic. The main etiologic subtypes of ischemic strokes are cardioembolic, atherosclerotic, lacunar, other specific strokes and strokes of undetermined etiology (Boehme *et al* 2017).

Hypertension, hyperlipidemia and atrial fibrillation belong to the traditionally recognized risk factors of the stroke. Other risk factors associated with stroke include non-modifiable (age, race) and modifiable (diet, comorbities) risk factors (Boehme *et al* 2017). HHcy appears to be a strong and independent risk factor in the development of stroke (Casas *et al* 2005). Number of clinical studies examining relationship and associating hHcy and increased risk, mortality and recurrence of stroke have been presented in the last years (Gajbhare & Juvale 2017; Zongte *et al* 2008; Kumawat *et al* 2018; Cui *et al* 2008; Kwon *et al* 2014; Kumral *et al* 2016; Wang *et al* 2017; Yao *et al* 2016) but the causal relationship has not been established yet. Connection of hHcy and stroke may be specific to certain pathophysiologic subtypes of stroke especially in stroke patients with large artery atherosclerotic subtype (Okubadejo *et al* 2008; Shi *et al* 2015; Ji *et al* 2015) and in much lesser extent with intracranial small artery disease (Eikelboom *et al* 2000).

In spite of an enormous effort of researchers, there are only a few effective treatments and a limited prog-

ress has been done in the search for novel approaches in the field of brain stroke. One of the ways to prevent stroke is preconditioning maneuver (Yang *et al* 2017). Preconditioning is a condition when subthreshold noxious stimulus triggers endogenous protection of tissue to subsequent injurious damage (Dirnagl *et al* 2009). There are numerous approaches of brain preconditioning. From these, ischemic preconditioning (IPC) has been extensively studied in experiments with animal models (Stetler *et al* 2014; Lehotský *et al* 2009; Lehotský *et al* 2016).

hHCY AND STROKE

Increased tHcy plasma levels associated with occurrence of human stroke support a hypothesis that hHcy may be causally associated with stroke and Hcy lowering therapy may reduce the stroke incidence. Numerous trials with B vitamin therapy (folate-B9, cyanocobalamin-B12 and pyridoxine-B6) have been run to lower elevated plasma Hcy. However, early studies did not show any benefit in reduction of stroke (Spence *et al* 2017). In 2011, Spence and Stampfer hypothesized that these findings might result from harmful effect of cyanocobalamin which overwhelmed benefit of B vitamins in participants with impaired renal function. Meta-analyses from two large clinical trials of B vitamin therapy (VISP and VITATOPS) performed by Spence *et al* in 2017 demonstrated that in stroke patients with good renal function and low doses or no cyanocobalamin, B vitamins reduced the risk of stroke. High doses of cyanocobalamin were considered harmful in participants with impaired renal function. Authors suggested the use of methylcobalamin or oxocobalamin instead of cyanocobalamin (Spence *et al* 2017; Spence 2018). In average, supplementation with B vitamins with minimal dosage of cyanocobalamin lowers blood tHcy by about 25% and reduces the risk of stroke by about 10%. In the countries without folic acid food fortification and with low folate consumption the risk of stroke lowers even to 25% (Hankey 2018). There is still a need for further studies to support the effect of vitamin therapy in stroke treatment (Kumral *et al* 2016).

Despite increasing evidence of the detrimental involvement of hHcy in several neurological disorders, only limited number of experimental studies have engaged to document the mutual influence of hHcy and brain damage in animal models of human ischemic stroke (Lehotský *et al* 2016).

Deleterious effect of subcutaneously induced hHcy in the brain has been documented in the study in rats with induced global cerebral ischemia (Kovalska *et al* 2015). Ischemic-reperfusion (IR) injury alone (15-minutes ischemia) caused selective cell death of vulnerable pyramidal neurons of the hippocampal CA1 (Cornu Ammonis) region and neurons of the cerebral cortex and striatum. It was demonstrated that ischemia with following 72-hour reperfusion could kill 75–80% of neurons in CA1 hippocampus with minimal changes in the cortex. Combination of induced hHcy with IR injury and 72-hour reperfusion was capable to increase the extend of neurodegeneration killing about 80–85% of neurons in CA1 hippocampal region. Interestingly, IPC with induced hHcy-IR-72h reperfusion significantly decreased the number of degenerated cells compared to hHcy-IR-72h reperfusion group. It was documented that IPC even if combined with hHcy markedly suppressed neurodegeneration of neuronal cells (Kovalska *et al* 2012; Kovalska *et al* 2015).

Hcy-induced neurotoxicity represents a unique signaling pathway that is different from glutamate-NMDAR mediated excitotoxic cell death (Poddar & Paul 2013). The extracellular-signal regulated (ERK) mitogen-activated protein kinases (MAPK) and p38 MAPK, serine/threonine kinases, are involved in Hcy-NMDAR induced neuronal cell death cascade. ERK MAPK plays a key role in cell survival and death depending on duration and/or magnitude of its activation. The duration of ERK MAPK activation depends on extracellular stimulus and may have different signaling pathways and cellular responses. While glutamate mediated NMDAR stimulation is higher and leads to activation of both NR2A- and NR2B-NMDARs with sequential activation (by NR2A-NMDAR) and inhibition (by NR2B-NMDAR) ERK MAPK pathway (transient pro-survival activation) and independent transient pro-apoptotic activation of stress activated p38 MAPK. Hcy activates only NR2A-NMDAR at lower level that leads to sustained two-tier (initial and delayed) activation of ERK MAPK pathway and downstream and on ERK MAPK dependent biphasic activation (the first phase – initial rapid and transient, the second phase – delayed and more prolonged) of p38 in neurons (Poddar & Paul 2009; Poddar & Paul 2013). Activation of p38 MAPK leads to activation of caspase 3 resulting in neuronal apoptosis (Poddar & Paul 2013). In the recent study the authors have highlighted the intermediary role of AMPAR in Hcy-NMDAR induced neurotoxicity. Active phosphorylated ERK MAPK decreases surface expression of Glu2A-APMAR subunit. Ca^{2+} influx through Glu2A-lacking, Ca^{2+}-permeable AMPAR leads to phosphorylation and activation of p38 MAPK and subsequent neuronal cell death. This study also demonstrates that Hcy-NMDA signaling is critical in the phenotypic switch of GluA2-containing to GluA2-lacking AMPARs in neurons. Whether this signaling pathway through GluA2-lacking AMPAR is unique to Hcy or could be triggered by other stimulating signals remains to be investigated (Poddar *et al* 2017).

In consistent with these results, combination of ischemia and IPC (Kovalska *et al* 2012; Kovalska *et al* 2015) mediated neuronal cell death triggering MAPK signaling pathways has been examined in rats with global cerebral ischemia. Changes in MAPKs protein levels were detected in M1 cortical region and CA1 of hippocampus. ERK MAPK was activated in the early stage of

IR reaching its peak 1 h after ischemia and then slowly declined in cortex while in hippocampus it decreased 15 min after stroke and then slowly increased. Protein level p38 showed similar prolonged activation with maximum 24 h after ischemia in both brain areas. Interestingly, after IPC maneuver the authors detected significantly different results in both brain regions compared to IR. After 24 h reperfusion ERK MAPK displayed its maximal level while p38 was detected in its lowest level (Kovalska *et al* 2012; Kovalska *et al* 2014). Hyperhomocysteinemic stimulus caused activation of both MAPKs in rats brains (Kovalska *et al* 2015). Joining of the two stressors, hHcy and ischemia significantly activated p38 MAPK with maximum at 24 h after reperfusion. IPC intervention in combination with hHcy and without hHcy from previous studies (Kovalska *et al* 2012; Kovalska *et al* 2014) showed to follow similar patterns 24 h after ischemia. The authors reported that IPC even in hHcy conditions still preserves the brain tissue (Kovalska *et al* 2015).

Autophagy is a regulated process that breaks down the organelles and macromolecules by the activation of lysosomal degradation (Zhao *et al* 2016). Activation of autophagy can contribute to cell survival in stress conditions or can be deleterious and promote cell death in other situations (Ginet *et al* 2014). Whether autophagy is a pro-survival or pro-death in neurons in cerebral ischemia has been a subject of long-term debate (Puyal *et al* 2013; Wang *et al* 2018). Further studies are required to reveal exact mechanism of Hcy involvement in autophagy process.

High level of Hcy could contribute to cortical neuronal cell death by autophagy overactivation after cerebral ischemia. Zhao *et al* (2016) in hHcy rats after middle cerebral artery occlusion (MCAO) reperfusion detected accumulation of autophagosomes and overexpression of autophagy related proteins, microtubule associated protein light chain 3 (LC3B) and Beclin-1 in neurons of brain cortex. They suggested that oxidative stress might be an underlying link between Hcy and autophagy. *In vitro* studies have demonstrated N-homocysteinylation of cytochrome-c (cyto-c), a mitochondrial peripheral membrane protein (Tyagi *et al* 2012). Tyagi *et al* (2012) determined that tetrahydrocurcumin treatment of genetic hHcy mice after MCAO suppressed homocysteinylation of cyto-c. The study showed potential of THC in cerebral ischemia therapy.

Conclusion

Nowadays, numerous studies relate hyperhomocysteinemia with chronic and acute neurodegenerative disorders including AD and stroke. HHcy is recognized to be an independent modifiable risk factor involved in these disorders. Concentration of plasma Hcy increases with age and together with accumulation of other noxious factors, metabolic changes and mutations over the lifetime it contributes to the development and progression of neurodegenerative disorders. Maintenance of low physiological plasma Hcy concentration might be beneficial in advancing age and in connection with CNS diseases. Homocysteine metabolism is largely dependent on B vitamins. Strategies with lowering of plasma Hcy through B vitamin supplementation have been performed in many trials but the beneficial effect has not been cleared yet.

Elucidation the link between hHcy and brain cell injury is vital for improving the prevention and treatment of Hcy-related CNS disorders. The precise role of hHcy in pathogenesis of neurological diseases still deserves further investigation.

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REFERENCES

1 Agnati LF, Genedani S, Leo G, Forni A, Woods AS, Filaferro M, *et al* (2007) Amyloid-beta peptides as one of the crucial volume transmission signals in the trophic units and their interactions with homocysteine. Physiological implications and relevance for Alzheimer´s disease. *J Neural Transm.* **114**: 21–31.

2 Amirrad F, Bousoik E, Shamloo K, Al-Shiyab H, Nguyen VHV, Aliabadi HM, *et al* (2017) Alzheimer´s disease: Dawn of a New Era? *J Pharm Pharm Sci.* **20**: 184–225.

3 Ansari R, Mahta A, Mallack E, Luo JJ (2014) Hyperhomocysteinemia and Neurologic Disorders: a Review. *J Clin Neurol.* **10**(4): 281–288.

4 Association, Alzheimer´s (2018) 2018 Alzheimer´s Disease Facts and Figures. *Alzheimers Dement 2018.* **14**(3): 367–429.

5 Beydoun MA, Beydoun HA, Gamaldo AA, Teel AT, Zonderman AB, Wang YW (2014) Epidemiologic studies of modifiable factors associated with cognition and dementia: systematic review and meta-analysis. *BMC Public Health.* **643**: 14.

6 Bhatia P, Gupta S, Sharma S (2014) Homocysteine Excess and Vascular Endothelium Dysfunction: Delineating the Pathobiological Mechanisms. *International Journal of Pharmacology.* **10**(4): 200–212.

7 Blom HJ and De Vriese S (2002) Why are homocysteine levels increased in kidney failure? A metabolic approach. *J Lab Clin Med.* **139**(5): 262–268.

8 Blom HJ and Smulders Y (2011) Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. *J Inherit Metab Dis.* **2011**(34): 75–81.

9 Boehme AK, Esenwa CE, Elkind MSV (2017) Stroke Risk Factors, Genetics, and Prevention. *Circ Res.* **120**(3): 472–495.

10 Brustolin S, Giugliani R, Félix TM (2010) Genetics of homocysteine metabolism and associated disorders. *Brazilian Journal of Medical and Biological Research.* **43**: 1–7.

11 Budy B, O´Neill R, DiBello PM, Sengupta S, Jacobsen DW (2006) Homocysteine transport by human aortic endothelial cells: Identification and properties of import systems. *Arch Biochem Biophys.* **446**(2): 119–130.

12 Cacciapuoti F (2016) Hyperhomocysteinemia for Alzheimer´s Disease: Risk Factor, Biomarker or both? *JSM Alzheimer´s Disease and Related Dementia.* 3(1): 1021.

13 Cárdenas-Aguayo M del C, Silva-Lucero M del C, Cortes-Ortiz M, Jiménez-Ramos B, Gómez-Virgilio L, Ramírez-Rodríguez G, *et al* (2014) Physiological Role of Amyloid beta in Neural Cells: The Cellular Trophic Activity. [book auth.] Thomas Heinbockel. s.l. : Neurochemistry, IntechOpen, 2014.

14 Casas JP, Bautista L, Smeeth L, Sharma P, Hingorani AD (2005) Homocysteine and stroke: evidence on causal link from mendelian randomisation. *Lancet.* **365**: 224–32.

15 Chen GF, Xu TH, Zhou YR, Jiang Y, Melcher K, *et al* (2017) Amyloid beta: structure, biology and structure-based therapeutic development. *Acta Pharmacologica Sinica.* **2017**: 1205–1235.

16 Chen KL, Wang SSS, Yang YY, Yuan RY, Chen RM, Hu CJ, *et al* (2009) The epigenetic effects of amyloid-beta 1-40 on global DNA and neprilysin genes in murine cerebral endothelial cells. *Biochemical and Biophysical Research Communications.* **378**: 57–61.

17 Chu J and Praticó D (2009) The 5-Lipoxygenase as a Common Pathway for Pathological Brain and Vascular Aging. *Cardiovascular Psychiatry and Neurology.* **2009**: 174657.

18 Chu J and Praticó D (2011) 5-Lipoxygenase as a endogenous modulator of amyloid beta formation in vivo. *Ann Neurol.* **69**(1): 34–46.

19 Chu J and Praticó D (2016) The 5-Lipooxygenase as Modulator of Alzheimer´s gama-secretase and therapeutic target. *Brain Res Bull.* **126**(Pt 2): 207–212.

20 Chu J, Giannopoulos PF, Ceballos-Diaz C, Golde TE, Praticó D (2012a) 5-lipoxygenase gene transfer worsens memory, amyloid and tau brain pathologies in a mouse model of AD. *Ann Neurol.* **72**(3): 442–454.

21 Chu J, Giannopoulos PF, Ceballos-Diaz C, Golde TE, Praticó D (2012b) Adeno-associated virus-mediated brain delivery of 5-lipoxygenase modulates the AD-like phenotype of APP mice. *Molecular Neurodegeneration.* **7**(1): 1.

22 Chung YC, Kruyer A, Yao Y, Feierman E, Richards A, Strickland S, *et al* (2016) Hyperhomocysteinemia exacerbates Alzheimer´s disease pathology by way of the amyloid beta-fibrinogen interaction. *J Thromb Haemost.* **14**(7): 1442–1452.

23 Cicone F, Santagulda MG, My G, Mancuso G, Papa A, Persechino R, *et al* (2017) Hyperhomocysteinemia in acute iatrogenic hypothyroidism: the relevance of thyroid. autoimmunity. *Journal of Endocrinological Investigation.* **41**: 831–837.

24 Cui R, Moriyama Y, Koike KA, Date C, Kukuchi C, Tamakoshi A, *et al* (2008) Serum total homocysteine concentrations and risk of mortality from stroke and coronary heart disease in Japanese: The JACC study. *Atherosclerosis.* **198**: 412–418.

25 da Cunha AA, Horn AP, Hoppe JB, Grudzinski PB, Loureiro SO, Ferreira AGK, *et al* (2012) Chronic Hyperhomocysteinemia increases Inflamatory Markers in Hippocampus and Serum of Rats. *Neurochem Res.* **37**: 1660–1669.

26 di Meco A, Li JG, Praticó D (2018a) Dissecting the Role of 5-Lipoxygenase in the Homocysteine-Induced Alzheimer´s Disease Pathology. *Journal of Alzheimer´s Disease.* **62**: 1337–1344.

27 di Meco A, Li JG, Barrero C, Merali S, Praticó D, *et al* (2018b) Elevated levels of brain homocysteine directly modulate the pathological phenotype of a mouse model of tauopathy. *Molecular Psychiatry.* 2018a. doi: 10.1038/s41380-018-0062-0. [Epub ahead of print].

28 Dirnagl U, Becker K, Meisel A (2009) Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. *Lancet Neurol.* **8**(4): 398–412.

29 Eikelboom JW, Hankey GJ, Anand SS, Lofthouse E, Staples N, Baker RI (2000) Association between high homocyst(e)ine and ischemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke. *Stroke.* **31**(5): 1069–75.

30 Faraci FM and Lentz SR (2004) Hyperhomocysteinemia, oxidative stress, and Cerebral Vascular Dysfunction. *Stroke.* **35**: 345–347.

31 Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, *et al* (2014) Global and regional burden of stroke during 1990–2010: findings from the Global Burden of Disease Study 2010. *Lancet.* **9913**(383): 245–254.

32 Feigin VL, Roth GA, Naghavi M, Parmar P, Krishnamurthi R, Chugh S, *et al* (2016) Global burden of stroke and risk factors in 188 countries during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet Neurol.* **15**: 913–924.

33 Finkelstein JD (2007) Metabolic regulatory properties of S-adenosylmethionine and S-adenosylhomocysteine. *Clin Chem Lab Med.* **45**(12): 1694–1699.

34 Fuso A, Seminara L, Cavallaro RA, D´Anselmi F, Scarpa S (2005) S-adenosylmethionine/homocysteine cycle alterations modify DNA methylation status with consequent deregulation of PS1 and BACE and beta-amyloid production. *Mol. Cell. Neurosci.* **28**: 195–204.

35 Fuso A, Nicolia V, Cavallaro RA, Ricceri L, D´Anselmi F, Coluccia P, *et al* (2008) B-vitamin deprivation induces hyperhomocysteinemia and S-adenosylhomocysteine, depletes brain S-adenosylmethionine, and enhances PS1 and BACE expression and amyloid-beta deposition in mice. *Mol. Cell. Neurosci.* **37**: 731–746.

36 Fuso A and Scarpa S (2011a) One-carbon metabolism and Alzheimer´s disease: is it all a methylation matter? *Neurobiology of Aging.* **32**: 1192–1195.

37 Fuso A, Nicolia V, Cavallaro RA, Scarpa S (2011b) DNA methylase and demethylase activities are modulated by one-carbon metabolism in Alzheimer´s disease models. *Journal of Nutritional Biochemistry.* **22**: 242–241.

38 Fuso A, Nicolia V, Pasqualato A, Fiorenza MT, Cavallaro RA, Scarpa S (2011c) Changes in Presenilin 1 gene methylation pattern in diet-induced B vitamin deficiency. *Neurobiology of Aging.* **32**: 187–199.

39 Fuso A, Nicolia V, Ricceri L, Cavallaro RA, Isopi E, Mangia F, *et al* (2012) S-adenosylmethionine reduces the progress of the Alzheimer-like features induced by B-vitamin deficiency in mice. *Neurobiology of Aging.* **33**: 1482e1–1482e16.

40 Gajbhare PT and Juvale NI (2017) The study of plasma homocysteine level as a risk factor for ischemic strokes in young patients. *International Journal of Advances in Medicine.* **4**(4): 1019–1025.

41 Garcia-Osta A and Alberini CM (2009) Amyloid beta mediates memory formation. *Learning & Memory.* **16**: 267–272.

42 Giannopoulos PF, Chu J, Joshi YB, Sperow M, Li JL, Kirby LG, *et al* (2014) Gene Knockout of 5-Lipoxygenase Rescues Synaptic Dysfunction and Improves Memory in the Triple-Trangenic Model of Alzheimer´s Disease. *Mol Psychiatry.* **19**(4): 511–518.

43 Ginet V, Spiehlmann A, Rummel C, Rudinskiy N, Grishchuk Y, Luthi-Carter R, *et al* (2014) Involvement of autophagy in hypoxic-excitotoxic neuronal death. *Autophagy.* **10**(5): 846–860.

44 Group, GBD (2015) Neurological Disorders Collaborator. 2017. Global, national and regional burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Neurol.* **2017**(16): 877–897.

45 Guo T, Noble W, Hanger DP (2017) Roles of tau protein in health and disease. *Acta Neuropathol.* **133**: 665–704.

46 Hankey GJ (2018) B vitamins for stroke prevention. *Stroke and Vascular Neurology.* **3**: e000156.

47 Hernández F, Gómez de Barreda E, Fuster-Matanzo A, Lucas JJ, Avila J (2010) GSK3: A possible link between beta amyloid peptide and tau protein. *Experimental Neurology.* **223**: 322–325.

48 Hersi M, Irvine B, Gupta P, Gomes J, Birett N, Krewski D (2017) Risk factors associated with the onset and progression of Alzheimer´s disease: A systematic review of the evidence. *NeuroToxicology.* **61**: 143–187.

49 International, Alzheimer´s Disease (2016) *World Alzheimer Report 2016. Improving healthcare for people living with dementia. Coverage, quality and costs now and in the future.* 2016.

50 Jakubowski H (2019) Homocysteine modification in protein structure/function and human disease. *Physiol Rev.* **99**: 555–604.

51 James SJ, Melnyk S, Pogribna M, Pogribny IP, Caudill MA (2002) Elevation in S-Adenosylhomocysteine and DNA Hypomethylation: Potential Epigenetic Mechanism for Homocysteine-Related Pathology. *J. Nutr.* **132**(8): 2361S–2366S.

52 Janssens V and Goris J (2001) Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem. J.* **353**: 417–439.

53 Ji Y, Song B, Xu Y, Fang H, Wu J, Sun S (2015) Prognostic Significance of Homocysteine Levels in Acute Ischemic Stroke: A Prospective Cohort Study. *Curr Neurovasc. Res.* **12**(4): 334–40.

54 Jiang X, Yang F, Brailoiu E, Jakubowski H, Dun NJ, Schafer AI, *et al* (2007) Differential Regulation of Homocysteine Transport in Vascular Endothelial and Smooth Muscle Cells. *Arterioscler Thromb Vasc Biol.* **27**(9): 1976–1983.

55 Johnson W, Onuma O, Owolabi M, Sachdev S (2016) Stroke: a global response is needed. *Bulletin of the WHO.* **94**: 634–634A.

56 Kalani A, Kamat PK, Givvimani S, Brown K, Metreveli N, Tyagi SC, *et al* (2013) Nutri-epigenetics Ameliorates Blood-Brain Barrier Damage and Neurodegeneration in Hyperhomocysteinemia: Role of Folic Acid. *J Mol Neurosci.* **52**(2): 202–15.

57 Kamath AF, Chauhan AK, Kisucka J, Dole VS, Loscalzo J, Handy DE, *et al* (2006) Elevated levels of homocysteine compromise blood-brain barrier integrity in mice. *Blood.* **107**(2): 591–593.

58 Khodadadi S, Riazi GH, Ahmadian S, Hoveizi E, Karima O, Aryapour H (2012) Effect of N-homocysteinylation on physicochemical and cytotoxic properties of amyloid beta-peptide. *FEBS Letters.* **586**: 127–131.

59 Kim J, Kim H, Roh H, Kwon Y (2018) Causes of hyperhomocysteinemia and its pathological significance. *Arch. Pharm. Res.* **41**: 372–383.

60 Kimura T, Ishiguro K and Hisanaga SI (2014) Physiological and pathological phosphorylation of tau by Cdk-5. *Frontiers in Molecular Neuroscience.* **7**: 65.

61 Kolling J, Scherer EBS, Siebert C, Longoni A, Loureiro S, Weis S, *et al* (2016) Severe Hyperhomocysteinemia Decreases Respiratory Enzyme and Na+,K+-ATPase Activities, and Leads to Mitochondrial Alterations in Rat Amygdala. *Neurotox Res.* **29**: 408–418.

62 Kovalska M, Kovalska L, Pavlikova M, Janickova M, Mikuskova K, Adamkov (2012) Intracellular signaling MAPK pathway after cerebral ischemia-reperfusion injury. *Neurochem Res.* **37**(7): 1568–77.

63 Kovalska M, Kovalska L, Mikuskova K, Adamkov M, Tatarkova Z, Lehotsky J (2014) p-ERK involvement in the neuroprotection exerted by ischemic preconditioning in rat hippocampus subjected to four vessel occlusion. *Journal of Physiology and Pharmacology.* **65**(6): 767–776.

64 Kovalska M, Kovalska L, Tothova B, Mahmood S, Adamkov M, Lehotsky J (2015) Combination of hyperhomocysteinemia and ischemic tolerance in experimental model of global ischemia in rats. *Journal of Physiology and Pharmacology.* **66**(6): 887–897.

65 Kumawat BL, Sharma CM, Shah MJ, Panchal MA (2018) Hyperhomocysteinemia and Vitamin B12 deficiency as a risk factor for ischemic stroke: A case-control study from Northwestern India. *Indian J Health Sci Biomed Res.* **11**: 228–33.

66 Kumral E, Saruhan G, Aktert D, Orman M (2016) Association of Hyperhomocysteinemia with Stroke Recurrence after Inicial Stroke. *Journal of Stroke and Cerebrovascular Diseases.* **25**(8): 2047–2054.

67 Kwon HM, Lee YS, Bae HJ, Kang DW (2014) Homocysteine as a Predictor of Early Neurological Deterioration in Acute Ischemic Stroke. *Stroke.* **45**: 871–873.

68 Lehotský J, Burda J, Danielisová V, Gottlieb M, Kaplán P, Saniová B (2009) Ischemic Tolerance: The Mechanisms of Neuroprotective Strategy. *The Anatomical Record.* **292**: 2002–2012.

69 Lehotsky J, Petras M, Kovalska M, Tothova B, Drgova A, Kaplan P (2015) Mechanisms Involved in the Ischemic Tolerance in Brain: Effect of the Homocysteine. *Cell Mol Neurobiol.* **35**: 7–15.

70 Lehotský J, Tóthová B, Kovalská M, Dobrota D, Beňová A, Kalenská D, *et al* (2016) Role of Homocysteine in Ischemic Stroke and Development of Ischemic Tolerance. *Frontiers in Neuroscience.* **10**: 538.

71 Li JG, Chu J, Barrero C, Merali S, Praticó D (2014) Homocysteine Exacerbates beta-Amyloid Pathology, Tau Pathology, and Cognitive Deficit in a Mouse Model of Alzheimer Disease with Plaques and Tangles. *Ann Neurol.* **75**(6): 851–863.

72 Li JG, Barrero C, Merali S, Praticó D (2017a) Five lipoxygenase hypomethylation mediates the homocysteine effect on Alzheimer´s phenotype. *Sci. Rep.* **7**: 46002.

73 Li JG, Barrero C, Gupta S, Kruger WD, Merali S, Praticó D (2017b) Homocysteine modulates 5-lipoxygenase expression level via DNA methylation. *Aging Cell.* **16**: 273–280.

74 Lim, *et al* (2016) The Eye as a Biomarker for Alzheimer´s Disease. *Frontiers in Neuroscience.* **10**: 536.

75 Longoni A, Bellaver B, Bobermin LD, Leite Santos C, Nonose Y, Kolling J, *et al* (2018) Homocysteine Induces Glial Reactivity in Adult Rat Astrocyte Cultures. *Mol Neurobiol.* **55**: 1966–1976.

76 Luchsinger JA, Tang MX, Shea S, Miller J, Green R, Mayeux R (2004) Plasma homocysteine levels and risk of Alzheimer disease. *Neurology.* **62**: 11.

77 Mandelkow EM and Mandelkow E (2012) Biochemistry and Cell Biology of Tau Protein in Neurofibrillary Neurodegeneration. *Cold Spring Harb Perspect Med.* **2**: a006247.

78 Martin L, Latypova X, Wilson CM, Magnaudeix A, Perrin ML, Terro F (2013) Tau protein phosphatases in Alzheimer´s disease: The leading role of PP2A. *Ageing Research Reviews.* **12**: 39–49.

79 McIlroy SP, Dynan KB, Lawson JT, Patterson CC, Passmore AP, *et al* (2002) Moderately elevated plasma homocysteine, methylentetrahydrofolate reductase genotype, and risk of stroke, vascular dementia, and Alzheimer disease in Northern Ireland. *Stroke.* **2002**(10): 2351–2356.

80 Medina MA, Urdiale JL, Amores-Sánchez MI (2001) Roles of homocysteine in cell metabolism. Old and new functions. *Eur. J. Biochem.* **268**: 3871–3882.

81 Mudd SH, Finkelstein JD, Refsum H, Ueland PM, Malinow MR, Lentz SR, *et al* (2000) Homocysteine and its Disulfide Derivatives: A Suggested Consensus Terminology. *Arterioscler Thromb Vasc Biol.* **20**: 1704–1706.

82 Mufson EJ, Ikonomovic MD, Counts SE, Perez SE, Malek-Ahmadi M, Scheff SW, *et al* (2016) Molecular and cellular pathophysiology of preclinical Alzheimer´s disease. *Behavioural Brain Research.* **311**: 54–69.

83 Nazef K, Khelil M, Chelouti H, Kacimi G, Bendini M, Tazir M, *et al* (2014) Hyperhomocysteinemia Is a Risk Factor for Alzheimer´s Disease in an Algerian Population. *Archives of Medical Research.* **45**: 247–250.

84 Nilsson K, Gustafson L, Hultberg B (2012) Elevated Plasma Homocysteine Level Is Not Primarily Related to Alzheimer´s Disease. *Dement Geriatr Cogn Disord.* **34**: 121–127.

85 Obulesu M, Venu R, Somashekhar R (2011) Tau Mediated Neurodegeneration: An Insight into Alzheimer´s Disease Pathology. *Neurochem Res.* **36**: 1329–1335.

86 Okubadejo NU, Oladipo OO, Adeyomoye AA, Awosanya GO, Danesi MA (2008) Exploratory study of plasma total homocysteine and its relationship to short-term outcome in acute ischaemic stroke in Nigerians. *BMC Neurology.* **8**: 26.

87 Ostrakhovitch EA and Tabibzadeh S (2019) Homocysteine and age-associated disorders. *Ageing Research Reviews.* **49**: 144–164.

88 Pacheco-Quinto J, Rodriguez de Turco EB, DeRosa S, Howard A, Cruz-Sanchez F, Sambamurti K, *et al* (2006) Hyperhomocysteimic Alzheimer´s mouse model of amyloidosis shows increased brain amyloid-beta peptide levels. *Neurobiology of Disease.* **22**: 651–656.

89 Panpalli Ates M, Karaman Y, Guntekin S, Ergun MA (2016) Analysis of genetics and risk factors of Alzheimer´s disease. *Neuroscience.* **325**: 124–131.

90 Perla-Kaján J, Twardowski T, Jakubowski H (2007) Mechanisms of homocysteine toxicity in humans. *Amino Acids.* **32**: 561–572.

91 Petras M, Tatarkova Z, Kovalska M, Mokra D, Dobrota D, Lehotsky J, *et al* (2014) Hyperhomocysteinemia as a risk factor for the neuronal system disorders. *Journal of Physkology and Pharmacology.* **65**(1): 15–23.

92 Poddar R and Paul S (2009) Homocysteine-NMDA receptor mediated activation of extracellular-signal regulated kinase leads to neuronal cell death. *J Neurochem.* **110**(3): 1095–1106.

93 Poddar R and Paul S (2013) Novel crosstalk between ERK MAPK and p38 MAPK leads to homocysteine-NMDA receptor mediated neuronal cell death. *J Neurochem.* **124**(4): 558–570.

94 Poddar R, Chen A, Winter L, Rajagopal S, Paul S (2017) Role of AMPA receptors in homocysteine-NMDA-receptor-induced crosstalk between ERK and p38 MAPK. *Journal of Neurochemistry.* **142**: 560–573.

95 Puyal J, Ginet V, Clarke PHG (2013) Multiple interacting cell death mechanisms in the mediation of excitotoxicity and ischemic brain damage: A challenge for neuroprotection. *Progress in Neurobiology.* **105**: 24–48.

96 Scherer EBS, Loureiro SO, Vuaden FC, Schmitz F, Kolling J, Siebert C, *et al* (2013) Mild hyperhomocysteinemia reduces the activity and immunocontent, but does not alter the gene expression, of catalytic alfa subunits of cerebral Na+,K+-ATPase. *Mol Cell Biochem.* **378**: 91–97.

97 Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological Alterations in Alzheimer Disease. *Cold Spring Harb Perspect Med.* **1**: a006189.

98 Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D´Agostino RB, *et al* (2002) Plasma homocysteine as a risk factor for dementia and Alzheimer´s disease. *N Engl J Med.* **346**(7): 476–483.

99 Sharma GS, Kumar T, Singh LR (2014) N-Homocysteinylation Induces Different Structural and Functional Consequences on Acidic and Basic Proteins. *PLoS ONE.* **9**: 12.

100 Sharma M, Tiwari M, Tiwari RK (2015) Hyperhomocysteinemia: Impact on Neurodegenerative Diseases. *Basic & Clinical Pharmacology & Toxicology.* **117**: 287–296.

101 Shi Z, Guan Y, Huo YR, Liu S, Zhang M, Lu H, *et al* (2015) Elevated Total Homocysteine Levels in Acute Ischemic Stroke Are Associated With Long-Term Mortality. *Stroke.* **46**: 2419–2425.

102 Shirafuji N, Hamano T, Yen SH, Kanaan NM, Yoshida H, Hayashi K, *et al* (2018) Homocysteine Increases Tau Phosphorylation, Truncation and Oligomerization. *Int J Mol Sci.* **19**: 891.

103 Škovierová H, Vidomanová E, Mahmood S, Sopková J, Drgová A, Červeňová T, *et al* (2016) The Molecular and Cellular Effect of Homocysteine Metabolism Imbalance on Human Health. *International Journal of Molecular Sciences.* **1733**(17): 3871–3882.

104 Smith AD, Refsum H, Bottiglieri T, Fenech M, Hooshmand B, McCaddon A, *et al* (2018) Homocysteine and dementia: An International Consensus Statement. *Journal of Alzheimer´s Disease.* **62**: 561–570.

105 Sontag E, Hladik C, Montgomery L, Luangpirom A, Mudrak I, Ogris E, *et al* (2004) Downregulation of Protein Phosphatase 2A Carboxyl Methylation and Methyltransferase May Contribute to Alzheimer Disease Pathogenesis. *Journal of Neuropathology and Experimental Neurology.* **63**(10): 1080–1091.

106 Sontag E, Nunbhakdi-Craig V, Sontag JM, Diaz-Arrastia R, Ogris E, Dayal S, *et al* (2007) Protein Phosphatase 2A Methyltransferase Links Homocysteine Metabolism with Tau and Amyloid Precursor Protein Regulation. *J. Neurosci.* **27**(11): 2751–2759.

107 Sontag JM, Nunbhakdi-Craig V, Montgomery L, Arning E, Bottiglieri T, Sontag E (2008) Folate Deficiency Induces In Vitro and Mouse Brain Region-Specific Downregulation of Leucine Carboxyl Methyltransferase-1 and Protein Phosphatase 2A B-alfa Subunit Expression that Correlate with Enhanced Tau Phosphorylation. *J Neurosci.* **28**(45): 11477–11487.

108 Sontag JM and Sontag E (2014) Protein phosphatase 2A dysfunction in Alzheimer´s disease. *Frontiers in Molecular Neuroscience.* **7**: 16.

109 Spence JD and Stampfer MJ (2011) Understanding the Complexity of Homocysteine Lowering With Vitamins. The Potential Role of Subgroup Analyses. *JAMA.* **306**(23): 2610–2611.

110 Spence JD, Yi Q, Hankey GJ (2017) B vitamins in stroke prevention: time to reconsider. *Lancet Neurol.* **16**: 750–60.

111 Spence JD (2018) Homocysteine Lowering with B Vitamins for Stroke Prevention – A History. *US Neurology.* **14**(1): 35–39.

112 Sperling RA, Karlawish J, Johnson KA (2013) Preclinical Alzheimer disease – the challenges ahead. *Nat Rev Neurol.* **9**(1): 54–58.

113 Stetler RA, Leak RK, Gan Y, Li P, Hu X, Jing Z, *et al* (2014) Preconditioning Provides Neuroprotection in Models of CNS Disease: Paradigms and Clinical Significance. *Prog Neurobiol.* **114**: 58-83.

114 Sun CF, Haven TR, Wu TL, Tsao KC, Wu JT, *et al* (2002) Serum total homocysteine increases with the rapid proliferation rate of tumor cells and decline upon cell death: a potential new tumor marker. *Clinica Chimica Acta.* **321**: 56–62.

115 Taleski G and Sontag E (2018) Protein phosphatase 2A and tau: an orchestrated ´Pas de deux´. *FEBS letters.* **592**: 1079–1095.

116 Tyagi N, Qipshidze N, Munjal C, Vacek JC, Metreveli N, Givvimani S, *et al* (2012) Tetrahydrocurcumin ameliorates homocysteinylated cytochrome-C mediated autophagy in hyperhomocysteinemia mice after cerebral ischemia. *J Mol Neurosci.* **47**(1): 128–138.

117 Uhl J, Klan N, Rose M, Entian KD, Werz O, Steinhilber D (2002) The 5-Lipoxygenase Promoter Is Regulated by DNA Methylation. *The Journal of Biological Chemistry.* **277**(6): 4374–4379.

118 Vafai SB and Stock JB (2002) Protein phospatase 2A methylation: a link between elevated plasma homocysteine and Alzheimer´s Disease. *FEBS Letters.* **518**: 1–4.

119 van Guldener C (2006) Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering? *Nephrol Dial Transplant.* **21**: 1161–1166.

120 Wang Y and Mandelkow E (2016) Tau in physiology and pathology. *Nature Reviews Neuroscience.* **17**(1): 5-21

121 Wang W, Gao C, Yu C, Liu S, Hou D, Wang Y, *et al* (2017) No Association between Elevated Total Homocysteine Levels and Functional Outcome in Elderly Patients with Acute Cerebral Infarction. *Frontiers in Aging Neuroscience.* **9**: 70.

122 Wang P, Shao BZ, Deng Z, Chen S, Yue Z, Miao CY (2018) Autophagy in ischemic stroke. *Progress in Neurobiology.* **163–164**: 98–117.

123 Yang T, Li Q, Zhang F (2017) Regulation of gene expression in ischemic preconditioning in the brain. *Cond Med.* 1(1): 47–56.

124 Yao ES, Tang Y, Xie MJ, Wang MH, Wang H, Luo X (2016) Elevated Homocysteine Level Related to Poor Outcome After Thrombolysis in Acute Ischemic Stroke. *Med Sci Monit.* **22**: 3268–3273.

125 Zhang CE, Tian Q, Wei W, Peng JH, Liu GP, Zhou XW, *et al* (2008) Homocysteine induces tau phosphorylation by inactivating protein phosphatase 2A in rat hippocampus. *Neurobiology of Aging.* **29**: 1654–1665.

126 Zhang CE, Wei W, Liu YH, Peng JH, Tian Q, Liu GP, *et al* (2009) Hyperhomocysteinemia Increases beta-Amyloid by Enhancing Expression of gama-Secretase and Phosphorylation of Amyloid Precursor Protein in Rat Brain. *AJP.* **174**: 4.

127 Zhang YW, Thompson R, Zhang H, Xu H (2011) APP processing in Alzheimer´s disease. *Molecular Brain.* **4**: 3.

128 Zhao Y, Huang G, Chen S, Gou Y, Dong Z, Zhang X (2016) Homocysteine Aggravates Cortical Neural Cell Injury through Neuronal Autophagy Overactivation following Rat Cerebral Ischemia-Reperfusion. *International Journal of Molecular Sciences.* **17**(8): E1196.

129 Zhuo JM and Praticó D (2010a) Acceleration of brain amyloidosis in an Alzheimer´s disease mouse model by a folate, vitamin B6 and vitamin B12-deficient diet. *Exp Gerontol.* **45**(3): 195–201.

130 Zhuo JM and Praticó D (2010b) Severe In Vivo Hyper-Homocysteinemia is not Associated with Elevation of Amyloid-beta Peptides in the Tg2576 Mice. *J Alzheimers Dis.* **21**(1): 133–40.

131 Zhuo JM, Portugal GS, Kruger WD, Wang H, Gould TJ, Praticó D (2010) Diet-Induced Hyperhomocysteinemia Increases Amyloid-beta Formation and Deposition in a Mouse Model of Alzheimer´s Disease. *Curr Alzheimer Res.* **7**(2): 140–149.

132 Zhuo JM, Wang H and Praticó D (2011) Is hyperhomocysteinemia an Alzheimer´s Disease (AD) risk factor, an AD marker or neither? *Trends in Pharmacological Sciences.* **32**(9): 562–71.

133 Zongte Z, Shaini L, Debbarma A, Singh TB, Devi SB, Singh WG, *et al* (2008) Serum homocysteine levels in cerebrovascular accidents. *Indian Journal of Clinical Biochemistry.* **23**(2): 154–157.