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Review

Degenerative protein modifications in the aging vasculature and central nervous system: A problem shared is not always halved

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ABSTRACT

Aging influences the pathogenesis and progression of several major diseases affecting both the cardiovascular system (CVS) and central nervous system (CNS). Defining the common molecular features that underpin these disorders in these crucial body systems will likely lead to increased quality of life and improved 'health-span' in the global aging population. Degenerative protein modifications (DPMs) have been strongly implicated in the molecular pathogenesis of several age-related diseases affecting the CVS and CNS, including atherosclerosis, heart disease, dementia syndromes, and stroke. However, these isolated findings have yet to be integrated into a wider framework, which considers the possibility that, despite their distinct features, CVS and CNS disorders may in fact be closely related phenomena. In this work, we review the current literature describing molecular roles of the major age-associated DPMs thought to significantly impact on human health, including carbamylation, citrullination and deamidation. In particular, we focus on data indicating that specific DPMs are shared between multiple age-related diseases in both CVS and CNS settings. By contextualizing these data, we aim to assist future studies in defining the universal mechanisms that underpin both vascular and neurological manifestations of age-related protein degeneration.

1. Introduction

Age-related decay of the circulatory system contributes to the pathogenesis of cardiovascular disease, diabetes, hypertension and stroke, diseases that together represent the global leading cause of death (Barquera et al., 2015). However, vascular pathology is not only a feature of circulatory disorders, since key disease elements also feature prominently in age-associated neurodegenerative disorders, including

Abbreviations: ACPAs, anti-citrullinated protein antibodies; AD, Alzheimer's disease; ALS, amyotrophic lateral ssclerosis; Cit, citrulline; cEpo, carbamylated erythropoietin; cHDL, carbamylated high-density lipoproteins; cLDL, carbamylated low-density lipoproteins; CNS, central nervous system; DPMs, degenerative protein modifications; HCit, homocitrulline; HDL, high-density lipoproteins; LDL, low-density lipoproteins; MD, mixed dementias; MPO, myeloperoxidase enzymes; MS, multiple sclerosis; MSpec, mass spectrometry; NOD, non-obese diabetic; PADs, peptidyl arginine deiminases; PPAD, *P. gingivalis*-derived peptidyl arginine deiminase; PD, Parkinson's disease; PiD, Pick's disease; PTMs, post-translational modifications; RA, rheumatoid arthritis; ROS, reactive oxygen species; TGs, transglutaminases; VaD, vascular dementia; VED, vascular endothelial dysfunction; CVS, cardiovascular System; VSMC, vascular smooth muscle cell

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Alzheimer's disease (AD), Mixed Dementias (MD) and Parkinsonism (Gallart-Palau, 2019; Iadecola, 2013; Rosso et al., 2018; Toledo et al., 2013). The molecular mediators of vascular disease have been extensively studied in individual disorders, but there remain fundamental gaps in knowledge about the shared mechanisms that lead to age-related damage in both the cardiovascular system (CVS) and central nervous system (CNS).

Degenerative Protein Modifications (DPMs) commonly refers to non-enzymatic post-translational polypeptide modifications (PTMs) (Gallart-Palau et al., 2015b), however, in this review article with the term DPMs we also refer to PTMs that specifically alter the proteins structure and function and tend to accumulate and exert cytotoxicity in age-related degenerative diseases. Although PTMs are naturally occurring biological regulators of protein-protein interactions and protein turnover (Snider and Omary, 2014), excess accumulation of DPMs in elderly individuals increase the risk for multiple major diseases (Gallart-Palau et al., 2015b; Santos and Lindner, 2017a). Thus, age-related proteome instability caused by decay, imbalance or impairment of cellular proteostasis systems (ubiquitin-proteasome and lysosome-autophagy, see (Clarke, 2003; Hoffman et al., 2017; Lopez-Otin et al., 2013; Vilchez et al., 2014) for extensive review) prominently determines the effects of the DPMs that will be discussed in this review. To date, approximately 300 PTMs have been identified (Liddy et al., 2013), but very few of these have hitherto been implicated in age-related pathologies (Santos and Lindner, 2017b). From these, those PTMs predicted to be most detrimental for protein function and structure include, although not exhaustively, oxidation, glycation, phosphorylation, nitration, citrullination, deamidation and carbamylation. PTMs strongly associated with aging proteome instability and diseases affecting the elderly (Adav and Sze, 2016). In particular, carbamylation, citrullination and deamidation form a core group of specific DPMs that appear to characterise age-associated pathologies in both the VS and CNS. However, before enter in detail on these three DPMs, we consider that phosphorylation requires a special mention here due to its vast implications in the regulation of aging and its diseases. Although phosphorylation in aging has been vastly reviewed previously (Lesnefsky and Hoppel, 2006; McCully, 2018), and it falls out of the scope of this review, this PTM is implicated in cell signalling, DNA transcription, cell cycle regulation, and metabolism, just to cite a very few (Ardito et al., 2017). Of note, recent findings indicate that altered phosphorylation dysregulates the insulin-signalling pathway with direct repercussions in mammals senescence, as well as, dysregulates energy metabolism, a central feature of unhealthy aging (see the volume Mattson, 2004) for extensive review on the implications of altered phosphorylation in aging health and disease).

Carbamylation is the irreversible, non-enzymatic addition of an isocyanic acid to the free amino groups of lysine (Lys), which in turn generates the non-coding amino acid homocitrulline (HCit) (Fig. 1A). Isocyanic acid is predominantly generated from urea dissociation or by myeloperoxidase catabolism of thiocyanate derived from the diet or smoking (Gorisse, Pietrement et al. 2016; Wang, Nicholls et al. 2007). Protein carbamylation affects intracellular proteins and appears to be involved in multiple processes associated with aging, atherogenesis and neurodegenerative diseases, with measurements of circulating HCit levels proving to be a useful predictor of cardiovascular risk and severity of coronary artery disease (Jaisson, Kerkeni et al. 2015; Verbrugge, Tang et al. 2015). Intriguingly, high levels of air pollution incorporating finite particulate matter that mimic effects of smoking can induce protein carbamylation and increase concentrations of neuroinflammatory markers in brain tissues of AD patients (Calderon-Garciduenas et al., 2004). While structurally similar to carbamylation, citrullination is instead generated by calcium-dependent catalysis of arginine (Arg) residues to form citrulline (Cit), in an irreversible process mediated by the peptidyl arginine deiminases (PADs) family of enzymes (Fig. 1**A**) (Fert-Bober and Sokolove, 2014). Dysregulation of PADs activity results in abnormal levels of Cit residues such as those commonly observed in the CVS of patients with type 1 diabetes, as well as in brain tissues affected by neurodegeneration (Ishigami et al., 2005; Jang et al., 2013; Rondas et al., 2015). Citrullination also affects intracellular proteins and shows specific hot-spots in the human proteome that depend on the amino acids flanking Arg residues (Gallart-Palau et al., 2016a, a). Accumulation of citrullinated proteins in the vasculature and generation of autoantibodies against these (anti-citrullinated protein antibodies; ACPAs), have been identified as potential biomarkers of cardiovascular risk in patients with rheumatoid arthritis (Fert-Bober et al., 2015). Similarly, circulating levels of citrullinated proteins may offer significant prognostic potential when assessing patient risk of neurodegeneration and CNS inflammation (Gallart-Palau et al., 2017a; Ishigami et al., 2005). Unfortunately, discriminating between Cit and HCit variants is not readily achieved using classical immunoassay approaches, since these residues only differ by a single side-chain carbon and the presence of a lone ureido group antigen (Fig. 1**A)** (Turunen et al., 2014). Consequently, mass spectrometry (MSpec) is the unique method currently able to successfully discriminate between HCit and Cit residues in critical tissue-derived proteins (Verheul et al., 2018), and it has therefore become the gold standard technology for characterizing DPMs in biological samples (Cox and Mann, 2011; Gallart-Palau et al., 2016a, b; Hao et al., 2017; Jin et al., 2013; MacCoss et al., 2002; Olsen and Mann, 2013; Serra et al., 2016a, b; Yates, 2017).

Deamidation DPMs are typically generated by spontaneous chemical reactions that occur in asparagine (Asn) and glutamine (Gln) residues via the respective formation of succinimide or glutarimide ring intermediates which subsequently undergo direct hydrolysis (Fig. 1**B-D**) (Capasso et al., 1991; Geiger and Clarke, 1987; Robinson and Robinson, 2001). However, Gln deamidation can also be mediated by transglutaminases (TGs), and NtQ-amidase, which promotes protein transamidation via ε-(γ-glutamyl)lysine cross-linking (Fig. 1**E**) (Kwon et al., 2000; Molberg et al., 1998; Serra et al., 2016b; Wang et al., 2009; Yao et al., 2012). Deamidation is known to play major roles in control of protein turnover (Gallart-Palau et al., 2016a; Zhang and Czupryn, 2003), cellular apoptosis (Zhao et al., 2004) and chronoregulation (Hao et al., 2017; Hasan et al., 2006; O'Connor et al., 2006; Robinson and Robinson, 2001; Stephenson and Clarke, 1989; Weintraub and Deverman, 2007b). Counterbalancing the deamidation of cellular Asn residues is the repair enzyme Protein L-isoaspartyl methyltransferase (PIMT), which catalyses the methylation of isoaspartyl residues to prevent accumulation of isoaspartic acid in host cells and tissues (Desrosiers and Fanelus, 2011). However, progressive aging can lead to decline in PIMT expression and function, as well as, is associated with deamidation of the enzyme itself, particularly in elderly brain tissues (Adav et al., 2016; Desrosiers and Fanelus, 2011). Deamidation also affects intracellular proteins and shows specific hot-spots in the brain proteome as we previously reported (Gallart-Palau et al., 2016a). In neurodegenerative diseases, deamidation of pro-aggregatory proteins can induce plaque formation (Schmid et al., 2009, 2011) and alterations in calcium-mediated binding, which together significantly contribute to neurotoxicity (Adav et al., 2016). Intriguingly, deamidation of extracellular matrix (ECM) proteins in the vascular bed can also confer paradoxical 'gain-of-function' changes by generating an isoDGR integrin binding sequence, which promotes leukocyte adhesion to atherosclerotic plaques (Dutta et al., 2017).

As aforementioned, a range of protein DPMs generated over the course of human aging are frequently detected in both VS- and CNS-linked pathologies. In this review, we focus on the roles of carbamylation, citrullination and deamidation, which represent core aging-associated DPMs that accumulate in both the vascular and neuronal compartments. In particular, we provide an update on the likely shared roles of these DPMs in the vast contexts of atherosclerotic cardiovascular disease and neurodegeneration.

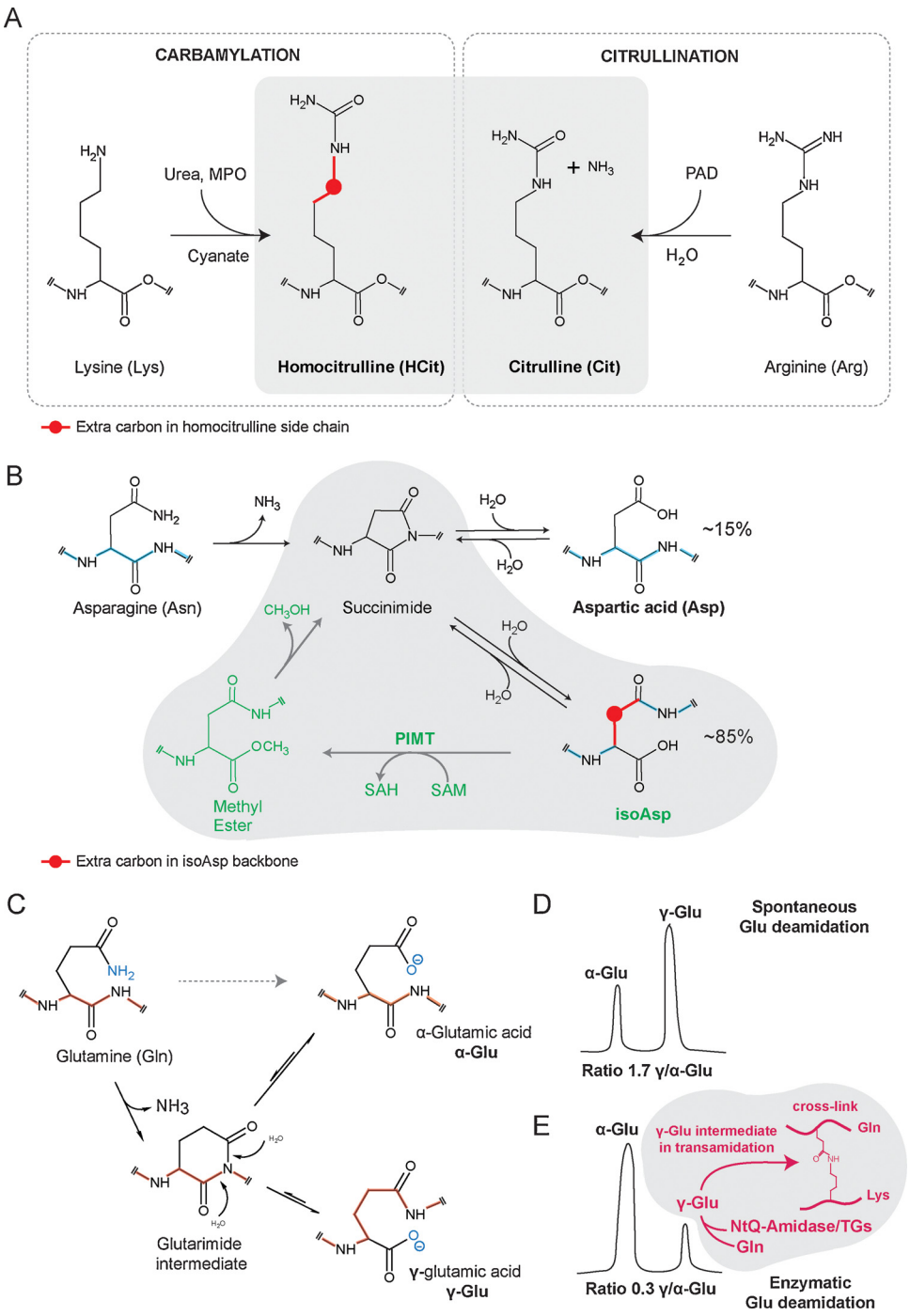

Fig. 1. Mechanisms of the DPM reactions carbamylation, citrullination and deamidation. **A.** Comparison of homocitrulline formation (by carbamylation of Lys residues) with citrullination of Arg residues. Homocitrulline and citrulline residues (shaded in grey) differ by only one carbon atom in their side chain (highlighted in red in the homocitrulline side chain). **B.** Reaction mechanism for Asn deamidation to generate the isomeric form isoAsp by hydrolysis (via formation of a succinimide intermediate). The amino acid backbones of Asp and isoAsp isomers are highlighted in blue. Shaded in grey is the reaction mechanism for enzymatic repair of isoAsp to succinimide mediated by PIMT enzyme (with S-adenosylmethionine [SAM] acting as methyl donor). In this reaction, isoAsp is methylated by PIMT and allows spontaneous formation of a methyl ester by hydrolysis, leading to reformation of the succinimide intermediate for hydrolysis into a mixture of Asp and isoAsp isomers. SAH indicates S-adenosylhomocysteine. **C.** Reaction mechanism for Gln deamidation, leading to the isomeric products α-Glu and γ-Glu via the formation of a glutarimide intermediate. The amino acid backbones are highlighted in orange. **D.** Representation of the γ/α-Glu ratio of 1.7 corresponding to a spontaneous deamidation process. **E.** Representation of altered γ/α-Glu ratio due to participation of γ-Glu in a transamidation reaction mediated by NtQ-amidase or transglutaminases (TGs). Cross-linkage resulting from transamidation an occur either intra- or inter-protein and involves both Gln and Lys side chains.

2. Carbamylation in the vascular system

2.1. Carbamylation of low-density lipoproteins

High circulating levels of low-density lipoproteins (LDL) are considered to be the primary cause of atherogenesis (Lichtenstein, 2003). Carbamylation of circulating apolipoprotein B is an irreversible process that generates carbamylated LDL (cLDL), which has been directly implicated in the formation of human atherosclerotic plaques (Apostolov et al., 2007). Specifically, cLDL enhances monocyte adhesion to endothelial cells via upregulation of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells (Apostolov et al., 2007; Delanghe et al., 2017; Sirpal, 2009). In addition, macrophage scavenger receptors including SR-A1 can mediate uptake of cLDL and promote cholesterol build-up in macrophages, leading to 'foam cell' formation and plaque development (Sirpal, 2009; Wang et al., 2007). Furthermore, cLDL can stimulate vascular smooth muscle cell (VSMC) proliferation, endothelial cell death, and progression of atherosclerosis/fatty atheroma (Asci et al., 2008; Delanghe et al., 2017; Sirpal, 2009; Verbrugge et al., 2015). Formation of cLDL has also been proposed to induce reactive oxygen species (ROS) generation and interact with the synthesis of nitric oxide in the vascular system (Speer et al., 2014; Verbrugge et al., 2015).

2.2. Carbamylation of high-density lipoproteins

High-density lipoproteins (HDL) possess anti-atherogenic effects that contribute to CVS homeostasis (Sirpal, 2009). Specifically, these

proteins are able to remove modified steroids from blood vessel walls and thereby inhibit foam cell formation (Fisher et al., 2012; Santana and Brown, 2018; Sirpal, 2009). HDL also displays anti-apoptotic properties that promote endothelial cell survival and impair the progression of atherosclerosis (Nofer et al., 2001). Accordingly, circulating HDL levels are negatively associated with cardiovascular risk in diverse human large (> 400 subjects) cohorts (Fisher et al., 2012; Khera et al., 2011). However, HDL can be modified via carbamylation of Lys residues to form cHDL, which can also be generated by reaction of apolipoprotein A with cyanate (derived either from urea breakdown or myeloperoxidase [MPO] activity) (Holzer et al., 2011). Similar to cLDL formation, carbamylation of HDL produces a dysfunctional molecule that lacks anti-apoptotic functions and allows endothelial cell death, thereby contributing to the pathology of atherosclerosis (Holzer et al., 2011; Sirpal, 2009; Verbrugge et al., 2015). Together, these data indicate that spontaneous protein carbamylation and MPO-mediated generation of cLDL and cHDL play critical roles in atherogenic initiation and progression in the CVS (Sirpal, 2009; Wang et al., 2007).

2.3. Carbamylation of collagens

Collagens are proteins with long lifespan that form the extracellular matrix of the CVS (Verbrugge et al., 2015), and comprise ˜60% of the total protein content in atherosclerotic plaques (Smith, 1965). Carbamylated collagen has been implicated in the formation of atherosclerotic plaques by enhancing monocyte adhesion and altering metabolic function in these cells (Garnotel et al., 2004). Enhanced monocyte adhesion to carbamylated collagen contributes to increased production and activation of the collagenase MMP-9 which confers increased ability to degrade local extracellular matrix constituents (Garnotel et al., 2004; Gillery and Jaisson, 2014). MMP-9 production and activation has also been implicated in cap rupture of atherosclerotic plaques, which in turn enhances risk of thromboembolism (Garnotel et al., 2004).

2.4. Homocitrulline (HCit) residues as biomarkers of cardiovascular risk

Patients with chronic and acute renal failure are prone to accumulate urea in the circulation and to exhibit increased levels of carbamylated haemoglobin in blood serum (Wynckel et al., 2000), which resembles the accumulation of carbamylated LDL in individuals with elevated risk of atherosclerosis (Apostolov et al., 2005). Protein carbamylation in serum may therefore represent an independent predictor of several age-associated diseases, particularly CVS disorders such as myocardial infarction and stroke (Jaisson et al., 2011). Several studies have reported that levels of HCit residues in blood serum positively correlate with severity of coronary artery disease in humans (Jaisson et al., 2015; Verbrugge et al., 2015; Wang et al., 2007). Thus, future high-accuracy detection of carbamylated serum proteins may uncover useful prognostic applications for measurement of HCit residues in a range of different vascular pathologies.

3. carbamylation in the central nervous system

3.1. Protein carbamylation in age-associated neurodegeneration

Proteinopathy is a characteristic feature of multiple age-associated neurodegenerative diseases including AD, Parkinson's disease (PD), Amyotrophic Lateral Sclerosis (ALS) (Gallart-Palau et al., 2016b) and Pick's disease (PiD). AD and PiD share the common feature of helical filament disruption in the microtubule-associated proteinTau (MAPT), resulting in aberrant structure and impaired ability to stimulate tubulin assembly into microtubules (Farías et al., 1997). Data on the role of carbamylation in neurodegeneration are currently scarce, although selective carbamylation of tau residues has been observed in protein aggregates in brain tissues (Farías et al., 1997). Similarly, we recently observed an accumulation of HCit residues in particulate brain fractions from post-mortem subjects with mixed dementia (AD combined with cerebrovascular disease) (Gallart-Palau et al., 2017a). These findings indicated that in dementia, particulate brain fraction might accumulate HCit-modified proteins capable of promoting neuroinflammation (Gallart-Palau et al., 2017a), consistent with the suggested pro-inflammatory role for protein sequestration in intraneuronal inclusions (Currais et al., 2017). Needless to say further validation of these findings will be required before the therapeutic potential of targeting this process in patients with mixed dementias can be established.

3.2. Carbamylation in neuroprotection

Potential neuroprotective effects of the hormone erythropoietin were first uncovered over a decade ago (Jelkmann, 2007), but therapeutic administration of this cytokine has since proved to be linked with various side effects ranging from hypertension and thrombosis to polycythemia and cancer (King et al., 2007; Thomas et al., 2013). The side effects of erythropoietin treatment are primarily induced via activation of the cognate receptor, which can be abolished by carbamylation of the protein to form cEpo, which retains the neuroprotective effects of the native molecule (Thomas et al., 2013). Consequently, cEpo has been proposed as a potential therapeutic tool for mitigating neurodegenerative disease progression (Lapchak, 2008; King et al., 2007). Although the molecular effects of cEpo on the cognate receptor were fully pointed (King et al., 2007), the mechanism of action of this molecule still remains poorly defined (Chen et al., 2015), thus the effectiveness of cEpo as a neuroprotective compound, despite promising, is still debatable and controversial. Most recent findings indicate that cEpo is not able to induce neurorestorative effects in postnatal proliferative subventrivcular zones of the brain (Osato et al., 2018). Thus, the therapeutic potential of cEpo in several pathological neurological conditions is still uncertain and remains under scientific scrutiny.

4. Common age-associated effects of carbamylation in the vascular and central nervous systems

Common age-associated effects of carbamylation in CVS and CNS are represented in Fig. 2 and summarized in Table 1. While the effects of carbamylation have not been yet thoroughly studied in the CNS, there is evidence to suggest that hyper-carbamylation of specific proteins may be associated with several age-associated pathologies. Jaisson and colleagues found that levels of protein carbamylation are increased in skin cells as they acquire other molecular features of aging (Jaisson et al., 2011). Additionally, Gorisse and colleagues observed that levels of protein carbamylation in elderly humans are negatively correlated with life expectancy (Gorisse et al., 2016). Other authors have also linked increased incidence of protein carbamylation with PD, AD, cerebrovascular diseases, and coronary heart disease (Stern et al., 2003; Hung et al., 2010). These data suggest that protein carbamylation may be an archetypal feature of age-related vascular pathology in a range of different tissues and disease settings. For example, the systemic plasma protein albumin possesses a potential carbamylation site at Lys 549 that becomes hyper-carbamylated under pro-atherogenic conditions (Berg et al., 2013; Verbrugge et al., 2015), and we have also identified hyper-carbamylation in human brain tissues from patients with dementia-associated aneurodegeneration (Gallart-Palau et al., 2017a). Similarly, elevated HCit concentrations have previously been linked with CVS inflammation and appear to precede atherogenesis and uremia (Delporte et al., 2018; Wang et al., 2007), but have also been detected in brain particulate fractions affected by dementia (Gallart-Palau et al., 2017a). Intriguingly, the affected proteins were primarily those already strongly implicated by neuropathology, suggesting a likely role in neuroinflammatory processes that is not yet fully understood. However, it has yet to be determined whether organ-specific or multi-tissue changes in DPM profile are routinely reflected in circulating plasma

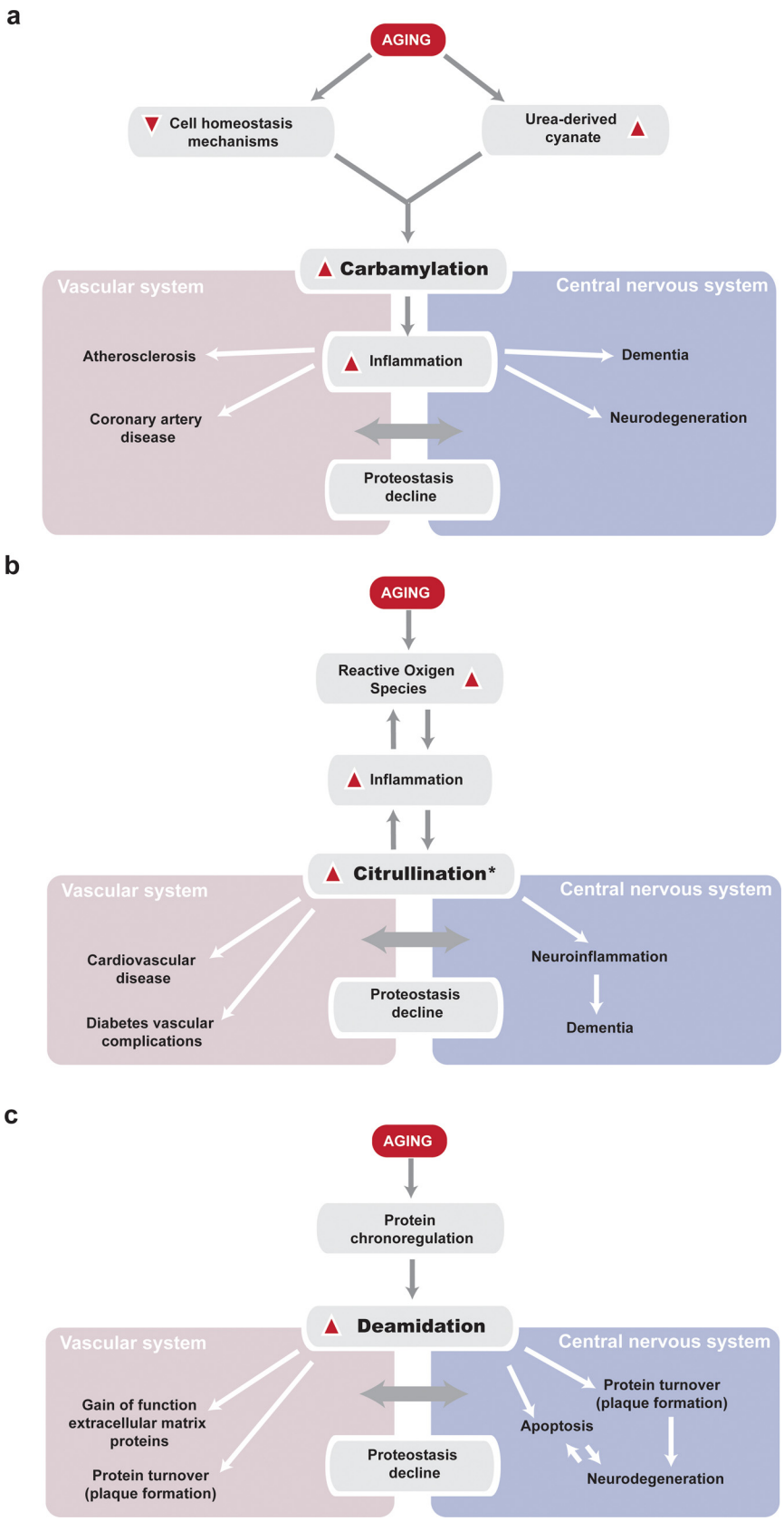

Fig. 2. Diagrams showing the relationship between aging and the DPMs analysed. **A.** Aging and carbamylation, as well as, the effects that carbamylation causes in the vascular and central nervous systems. **B.** Aging and citrullination, as well as, the effects that citrullination causes in the vascular and central nervous systems. **C.** Aging and deamidation, as well as, the effects that deamidation causes in the vascular and central nervous systems. * Indicates that citrullination and carbamylation are confounding factors at the time to be detected by traditional antibody-based biochemical methods.

Table 1
Common features of protein carbamylation in cardiovascular and neurodegenerative diseases.

Commonality	Reference
Causes structural and functional alterations of long-lived proteins in:	
Age-related diseases:	(Hung et al., 2010)
Parkinson's disease	
Alzheimer's disease	
Cerebrovascular disease	
Coronary Heart disease	(Stern et al., 2003)
Serves as biomarker in:	
Diabetes mellitus	(Berg et al., 2013; Jaisson et al., 2011)
Chronic renal failure	
Atherosclerosis	(Berg et al., 2013)
Coronary artery disease	(Jaisson et al., 2015; Verbrugge et al., 2015; Wang et al., 2007)
Cardiovascular risk in the aging	(Verbrugge et al., 2015)
Dementia	(Gallart-Palau et al., 2017a)
Promotes inflammation in:	
Atherosclerotic plaque	(Wang et al., 2007)
Alzheimer's disease	(Zhang and Jiang, 2015)
Alzheimer's disease with cerebrovascular disease	(Gallart-Palau et al., 2017a)

proteins.

5. Citrullination in the vascular system

5.1. Citrullination in cardiovascular diseases

Multiple citrullinated proteins are commonly found in atherosclerotic plaques, including enolase, vimentin, filaggrin and fibrinogen (Sokolove et al., 2013; Tilvawala et al., 2019), which have been reported to stimulate local inflammatory responses (Fert-Bober and Sokolove, 2014). In rheumatoid arthritis (RA) patients, increased levels of ACPAs in atherosclerotic plaques, was associated with higher cardiovascular risk (Cambridge et al., 2013; Fert-Bober and Sokolove, 2014). In particular, antibodies against citrullinated fibrinogen and citrullinated vimentin (but not cyclic citrullinated peptide 2), are associated with increased aortic plaque burden in RA (Sokolove et al., 2013).

Immunochemical analyses performed in RA patients have revealed colocalization of peptidyl arginine deiminase 4 (PAD4) and citrullinated proteins within coronary artery plaques (Sokolove et al., 2013). Although RA has long been regarded as an independent risk factor for coronary heart disease (Fert-Bober et al., 2017), there is now evidence that ACPA may also be associated with coronary heart disease in non-RA patients (Cambridge et al., 2013).Therapeutic intervention in this process may prove possible by targeting the PAD enzymes that catalyse citrullination, perhaps resulting in effective treatments for vascular damage and atherosclerosis, as already suggested by observations in murine models (Knight et al., 2014).

5.2. Citrullination in heart failure

Sarcomeric proteins including actin, heavy meromyosin (HMM), tropomyosin and troponin are involved in control of cardiac functions (Solaro et al., 2010). Generally, citrullination of sarcomeric proteins catalysed by PAD2 reduces calcium sensitivity in cardiomyocytes, leading to a loss-of-function phenotype and decreased contractile ability in response to intracellular calcium ions (Fert-Bober et al., 2015). Indeed, defective calcium homeostasis appears to be a key feature of heart failure hence several alterations in the release and uptake of $Ca2+$ ions have been already identified (see Yano et al., 2005; Luo and Anderson, 2013; Meyer et al., 1995 for extensive review). These findings, as previously suggested by Fert-Bober and colleagues, indicate that protein citrullination may directly contribute to cardiac dysfunction in heart failure patients (Fert-Bober et al., 2015), an hypothesis worthy of further exploration.

5.3. Citrullination in diabetic-vascular complications

Cytokine induced dysfunction of pancreatic islet cells in diabetes mellitus (DM) was associated with presence of citrullination in the proteome of these cells (D'Hertog et al., 2007). Patients affected by DM show exacerbated autoimmunity signals against citrullinated and transglutaminated peptides (McGinty et al., 2014). Furthermore, these authors found that T cell sub-populations were very specific at the time to respond against modified peptides by citrullination whereas these same T cells sub-populations were unresponsive against unmodified counterparts (McGinty et al., 2014). Similarly, PAD2 expression and activation is increased in non-obese diabetic (NOD) mice, in which pro-inflammatory cytokine production induces citrullination of glucose-regulated protein 78 (GRP 78) in beta cells and generates an autoantigen that can subsequently be targeted by antibodies and T lymphocyte responses (Rondas et al., 2015). These data illustrate a pivotal role for inflammatory stress in beta cell destruction, and suggest that the 'pre-diabetic' state may represent a critical window for citrullinated protein detection and potential use as a biomarker and innovative therapeutic approaches.

6. Citrullination in the central nervous system

6.1. Citrullination in aging-associated neurodegenerative diseases

Citrullination requires the calcium-dependent enzyme PAD to catalyse the irreversible removal of animino moiety, thereby converting positively charged arginine into neutral citrulline (Fert-Bober and Sokolove, 2014). Increased protein citrullination in AD and CVD appears to be promoted by alanine, proline and histidine residues immediately flanking the target arginine (Gallart-Palau et al., 2017a). In humans, there are five isoenzymes comprising the PAD family (PAD 1–4 and 6) (Bicker and Thompson, 2013; Ishigami et al., 2005). As the most common isoform in brain tissues, PAD2 is now recognised as contributing to the pathogenesis of many neurodegenerative diseases (Ishigami et al., 2005; Musse et al., 2008), perhaps due to dysregulated enzyme activity allowing increased citrullination in the aging CNS (Ishigami and Maruyama, 2010; Ishigami et al., 2005; Masutomi et al., 2017). Abnormal activation of PAD2 has previously been reported in prion-challenged mice as well as in the hippocampus of AD patients (Ishigami et al., 2005; Jang et al., 2013), where other colleagues have also detected abnormal build-up of citrullinated vimentin and glial fibrillary acidic proteins (GFAP) (Ishigami et al., 2005). Since GFAP is one of the most common substrates of PAD2, it is perhaps not surprising that citrullinated GFAP is abundant in brain tissues from patients with AD, PD, multiple sclerosis, and prion diseases (Ishigami et al., 2005; Nicholas, 2011).

Multiple sclerosis (MS) is a neurodegenerative disease caused by immune responses against myelin sheath that provides important clues as to the role of citrullination in neurodegeneration, inflammation, and autoimmunity (Jang et al., 2013; Yang et al., 2016; Gallart-Palau et al., 2016a). Myelin basic protein (MBP) interacts with negatively charged ligands such as calmodulin, Fyn-SH3, actin and tubulin, contributing to inter-myelin-axonal communication (Hu et al., 2004; Yang et al., 2016). However, citrullination of arginine residues in MBP results in loss of positive charge and a consequent reduction in affinity for negatively charged ligands, thus leading to significant disruption of crucial protein-protein interactions (Yang et al., 2016). Citrullinated MBP also lacks the ability to compact lipid bilayers, resulting in loss of myelin integrity and further structural damage (Yang et al., 2016). In particular, citrullinated MBP tends to unfold and become susceptible to proteolytic degradation by the metalloprotease cathepsin D, which

cleaves the protein at phenylalanine-phenylalanine linkages (Pritzker et al., 2000; Yang et al., 2016). In MS, these processes release immunostimulatory peptides that can activate T lymphocytes to mount autoimmune responses against the CNS (Pritzker et al., 2000; Yang et al., 2016). Intriguingly, epidemiological studies have indicated that women are more susceptible to MS than men (Compston and Coles, 2002), and post-mortem brain tissue from Vascular Dementia (VaD) patients exhibits higher levels of MBP citrullination in females than in males (Gallart-Palau et al., 2016a). These data suggest that brain protein PTMs could be partially responsible for the increased risk of neurological disorders observed in women, although other biological factors and environmental stimuli are also likely to be involved. Indeed, chronic periodontitis caused by bacteria such as *Porphyromonas gingivalis* has been linked with cognitive decline in the elderly and implicated in AD neuropathology (Miklossy, 2011a, b). Despite originating in the oral cavity, *P.gingivalis*is are able to enter the CNS of infected hosts via nerve fibers / trigeminal ganglia and can secrete a PAD enzyme that induces host protein citrullination (Miklossy, 2011a). Consequently, CNS infection by *P. gingivalis* has recently been proposed as a potential causative agent of hypercitrullination in the brains of subjects with dementia, a highly provocative concept that requires further evaluation (Olsen et al., 2018).

7. Common age-associated effects of citrullination in the vascular and central nervous systems

Common effects of citrullination in the CVS and CNS are represented in Figure 3 and summarized in Table 2. Dysregulation of PAD activity appears to be a shared feature of both CVS- and CNS-linked pathologies including type 1 diabetes (Rondas et al., 2015), atherosclerosis (Andrew et al., 2008), multiple sclerosis (Mastronardi et al., 2007; Moscarello et al., 2007; Musse et al., 2008), PD and AD (Ishigami et al., 2005; Jang et al., 2013; Nicholas, 2011).Whether *P. gingivalis*-derived PAD enzyme is a common mediator of Arg citrullination and inflammatory trigger in the CVS as well as the CNS is currently unclear (Olsen et al., 2018). However, future studies of hypercitrullination in the CVS will likely help to identify critical inducers of disease and potential prognostic markers of cardiovascular risk (especially in RA patients via detection of ACPAs) (Fert-Bober et al., 2015). Similarly, since abnormal activation of PAD enzyme and accumulation of citrullinated proteins are features of both prion-inoculated mice and hippocampal tissues from AD patients, citrullination may also prove to be a useful biomarker of multiple neurodegenerative diseases (Ishigami et al., 2005; Jang et al., 2013). Nicholas, in a study with excellent spatial resolution, found hypercitrullination in the vasculature and degenerative plaque proteomes of the frontal cortex in AD patients (Nicholas, 2013), which also suggests a link between the common presence of HCit in the VS and CNS in dementia. In order to determine these possibilities, future studies will need to employ systems biology approaches and state-of-the-art MSpec methodologies to discriminate between the specific proteins and amino acid combinations that possess prognostic abilities in VS/CNS pathology (Adav et al., 2016; Cox and Mann, 2011; Gallart-Palau et al., 2015b, 2016b, Gallart-Palau et al., 2019, 2017b; Olsen and Mann, 2013b; Serra et al., 2016b; Yates, 2017). Detection of patient autoantibodies recognising specific citrulline residues may also be suitable for prognostic applications in diseases of both systems (Acharya et al., 2012; Cambridge et al., 2013; Fert-Bober and Sokolove, 2014; Sokolove et al., 2013).

Table 2
Common features of protein citrullination in cardiovascular and neurodegenerative diseases.

Commonality	Reference
Dysregulated PAD activity:	
Circulatory system:	
Type 1 diabetes	(Rondas et al., 2015)
Atherosclerosis	(Andrew et al., 2008)
Neurodegenerative disease:	
Multiple sclerosis	(Mastronardi et al., 2007; Moscarello et al., 2007; Musse et al., 2008)
Parkinson's disease	(Nicholas, 2011)
Prion diseases	(Jang et al., 2013)
Alzheimer's disease	(Ishigami et al., 2005)
Promotes inflammation (mediated by PPAD enzyme):	
Central nervous system	(Olsen et al., 2018)
Vascular system	
Serves as biomarker and/or antibody target in:	
Circulatory system (Cardiovascular risk):	
Rheumatoid arthritis (assess aortic plaque burden)	(Fert-Bober et al., 2015)
Neurodegenerative disease:	
Prion-infected mice	(Jang et al., 2013)
Alzheimer's disease	(Ishigami et al., 2005)

8. Deamidation in the cardiovascular system

8.1. Deamidation in atherosclerotic plaques

Deamidation substantially alters the structure and function of affected proteins, leading to accumulation of these in the vascular wall and likely is conferring key roles to these proteins in the formation of atherosclerotic plaques (Weintraub and Deverman, 2007; Dutta et al., 2017). In particular, plaque formation is enhanced via deamidation of Asn-Gly-Arg (NGR) motifs in vascular bed extracellular matrix (ECM) proteins including Fibronectin and Tenascin C (Dutta et al., 2017). Spontaneous asparagine deamidation of NGR generates isoDGR structures (isoAsp-Gly-Arg) that mimic the cognate integrin-binding sequence Arg-Gly-Asp (RGD). This 'gain-of-function' modification is thought to enhance leukocyte recruitment to the developing plaque and may represent a key mechanism driving age-linked atherosclerosis (Corti et al., 2008; Dutta et al., 2017). Crucially, this aberrant pattern of integrin binding and atheroma progression can be reversed by treatment with anti-αvβ3 blocking antibodies or using the αv-specific peptide inhibitor cilengitide (Dutta et al., 2017). These data indicate that ECM proteins containing isoDGR motifs could represent viable drug targets for the treatment of human atherosclerosis, as well as other cardiovascular disorders in which essential CVS structural proteins are subject to age-linked modification and loss of structural integrity.

8.2. Deamidation effects on endothelial integrity

Endothelial cells cover the luminal portion of the vasculature and exert a wide range of functions that extend from regulation of vascular tone to control of capillary leakage (Galley and Webster, 2004). Impairment of these functions (collectively termed vascular endothelial dysfunction [VED]), confers both pro-thrombotic and pro-inflammatory properties to the endothelium, and is thought to be a critical step in various disorders including atherosclerotic plaque formation (Rajendran et al., 2013; Varadharaj et al., 2017). Deamidation has already been directly implicated in the molecular pathogenesis of VED via modification of the adipokine lipocalin-2, which subsequently accumulates in the artery wall and promotes hypertension, particularly in obese individuals (Song et al., 2014). Studying the effects of deamidation on vascular bed biology *in vivo* has only recently become possible via the development of the systems biology method termed 'DISDIVO', which has also been optimised for the study of DPMs in aging-associated VED in live animal models (Serra et al., 2018).

8.3. PIMT enzyme activity in heart disease

The repair enzyme PIMT catalyses the transfer of a methyl group from S-adenosyl-L-methionine to the free carboxyl group of isoaspartyl

residues (Desrosiers and Fanelus, 2011). Although little is known about the involvement of this enzyme in CVS health, it was recently demonstrated that ablation of PIMT in heart tissue causes lethal dilated cardiomyopathy (Jia et al., 2018). This finding suggests that PIMT expression and optimal function are essential factors in heart health.

9. deamidation in the central nervous system

9.1. Deamidation and proteinopathy

Histopathological analysis of CNS tissues from dementia patients reveals marked proteinopathy in the form of amyloid plaques and neurofibrillary tangles (Brion, 1998; Gallart-Palau et al., 2015b; Watanabe et al., 1999). In particular, senile plaques and insoluble portions of brain parenchyma from affected patients contain multiple deamidated proteins such as ferritin, collagen, S100 calcium-binding protein A9 (S100A9), creatine kinase and multiple haemoglobin subunits (Adav et al., 2016). Deamidation of S100A9 introduces a negative charge and alters the calcium-binding capacity of the protein, leading to increased propensity to form amyloid plaques in AD patients (Adav et al., 2016; Adav and Sze, 2016; Dunkelberger et al., 2012). Similarly, deamidation of α-synuclein promotes aggregation of the modified protein in cerebrospinal fluid of PD patients (Barbariga et al., 2015; Dunkelberger et al., 2012). Deamidated tau protein is also be involved in the formation of paired helices which are a hallmark feature of AD neuropathology (Watanabe et al., 1999).

Deamidation of key synaptic proteins including tubulin and synapsin-1 is associated with synaptic failure in vascular dementia, and may therefore play a major role in the cognitive decay characteristic of dementia syndromes (Gallart-Palau et al., 2015a; Koffie et al., 2011; Selkoe, 2002; Selnes et al., 2017). Indeed, loss-of-function changes induced by protein deamidation can also inhibit normal function of the channel pump Na + -K + ATPase, leading to dysregulated membrane excitability, defective signalling processes, and impaired neuronal function in vascular dementia (Adav et al., 2014). Similarly, abnormal levels of hydrogen peroxide in the cerebrospinal fluid of PD patients can induce oxidation and deamidation of ceruloplasmin, leading to conformational change and loss of ferroxidase activity, which in turn leads to abnormal iron metabolism in the brain, a characteristic trait of neurodegeneration hypothesized to be implicated in the neuropathogenesis of aging-associated dementias (Barbariga et al., 2015, 2014; Wang and Wang, 2018).

9.2. PIMT in neurodegenerative diseases

During natural cellular aging in the brain, PIMT expression levels decline and permit parenchymal build-up of abnormal L-isoaspartyl residues, eventually leading to neuronal metabolic dysfunctions (Desrosiers and Fanelus, 2011; Gallart-Palau et al., 2015a; Qin et al., 2015). Remarkably, deamidation of PIMT enzyme itself has been proposed to confer reduced capacity to process abnormal isoaspartyl residues in the proteome of dementia patients (Adav et al., 2014). Previous research efforts have largely focused on identifying endogenous substrates of PIMT in the brain (Zhu et al., 2006) and revealed that α-synucleins can undergo structural repair by this enzyme (Morrison et al., 2012). Further gender-specific findings have come from reports that PIMT activity in the mammalian brain differs between male and female individuals (Qin et al., 2013), consistent with the sex-specific differences observed in deamidation profile of the white matter proteome of vascular dementia patients (Gallart-Palau et al., 2016a).

10. Common age-associated effects of deamidation in the cardiovascular and central nervous systems

Common effects of deamidation in the CVS and CNS are represented in Figure 4 and summarized in Table 3. Since deamidation serves as

Table 3
Common features of protein deamidation in cardiovascular and neurodegenerative diseases.

Commonality	Reference
Cell aging and lifespan:	
Circulatory system:	
Atherosclerosis	(Dutta et al., 2017)
Heart disease	(Jia et al., 2018)
Neurodegenerative disease:	
Alzheimer's disease	(Desrosiers and Fanelus, 2011; Gallart-Palau et al., 2015a)
Vascular dementia	
Parkinson's disease	
Effects on plaque proteins:	
Atherosclerosis*	(Dutta et al., 2017)
Neurodegenerative diseases	(Adav et al., 2016)

* Requires further research.

'internal molecular clock' of natural cellular aging, it is perhaps not surprising that this process appears closely linked with organisms' lifespan (Hao et al., 2017; Liddy et al., 2013). With increasing age, PIMT expression levels gradually decrease, allowing damaged proteins to accumulate across multiple tissues and ultimately leading to neurodegenerative disorders and/or heart disease depending on the organs targeted (Desrosiers and Fanelus, 2011; Gallart-Palau et al., 2015a). While these mechanisms clearly have major roles to play in the long-term maintenance of human health, the impact of deamidation on tissue homeostasis – particularly in the heart – will require extensive investigation before effective therapies can be developed to target this axis.

11. Conclusions

Aging is characterised by structural and functional alterations in proteomes of multiple vital organs and tissue systems. Mechanisms of how DPMs influence age-associated diseases are now under intensive investigation, but the extent to which mechanistic roles in one organ system also apply to remote tissues and disease settings remains poorly understood. In this review article, we have attempted to contextualize the degenerative protein modifications that affect the two essential systems most commonly subject to pathological decay during aging, namely the circulatory and central nervous systems. We propose that key age-associated DPMs such as carbamylation represent pathological features shared by many human diseases affecting the elderly, including atherosclerosis, coronary artery disease, and neurodegeneration. While carbamylation is already a well-established biomarker of coronary artery disease, the data discussed herein suggest that this DPM may also prove clinically useful in the context of dementia syndromes. This may in part be due to the influence that carbamylation exerts on the common inflammatory responses that affect both the CVS and CNS. Similarly, citrullination has been implicated in both neuroinflammation and CVS inflammation in multiple age-related diseases, thus emphasizing the need to move beyond traditional biochemical methods to better distinguish how the effects of this DPM are distinct from carbamylation. Finally, based on our experience analysing DPMs, we found that protein deamidation is a highly frequent DPM in the human proteome and since it can promote plaque formation in the vasculature by inducing gain-of-function structural changes, it is important to consider the fact that these DPMs not only show ability to impair but can also confer novel biological roles on critical proteins that we have yet to fully understand.

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