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Alzheimer's Disease and NQO1: Is there a Link?

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease characterised by a progressive decline in cognitive function and represents a major healthcare challenge worldwide. Increasing evidence indicates that mitochondrial dysfunction-mediated oxidative stress plays a significant role in the pathophysiological process of AD. Therefore, the physiological activation of antioxidant enzymes that respond to increased oxidative stress is thought to prevent neuropathology. One of those endogenous defences is NADPH quinone oxidoreductase 1 (NQO1). NQO1 is a cytosolic homodimeric flavoprotein that catalyses the two electron reduction of quinones and related molecules aimed at increasing their solubility and excretion. In line with its role as a phase II stress response protein, altered NQO1 expression is associated with several pathological conditions and disorders including AD. This review summarizes the association between NQO1 and AD pathology. Understanding this association will provide further insight into the pathogenesis of the disease. More importantly, recent interest in drugs that affect NQO1 expression or its activity provide hope that this approach could lead to novel therapeutic options for the treatment of AD.

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterised by a progressive decline in cognitive function and represents a major challenge to healthcare worldwide [1]. The pathological hallmarks of AD include deposition of extracellular amyloid-β peptide (Aβ) plaques and intracellular neurofibrillary tangles (NFT) in the brain of AD patients. AD may be sporadic with typically late onset (mostly seen in patients ≥ 65 years of age) or may be familial with early onset (mostly seen in patients before 55 years of age). While more than 90-95 % of AD cases are sporadic, less than 10 % of the cases are associated with genetic mutations in genes such as amyloid precursor protein (AβPP) and presenilin (PS1, PS2) that are involved in the production of Aβ peptide [2]. Sporadic AD cases are found to be associated with a number of genetic risk factors. The best studied one is the ε4 allele of apoliprotein E (ApoE) [2] Prevailing evidence suggest that the ApoE ε4 allele may increase the risk of AD by a reduced Aβ metabolism that causes Aβ accumulation [3]. In this context, it has been reported that the ApoE ε4 allele is strongly associated with an increased risk of Aβ deposits in the brain of sporadic AD patients [4], while a lack of the APOE ε4 allele has been shown to decrease Aβ deposition in mice [5]. Overall, defective Aβ processing has been identified as the major factor responsible for the pathology of AD [1]. According to amyloidogenic pathway, neurotoxic Aβ (1-42) peptides are generated by proteolytic cleavage of the AβPP located in plasma membranes via proteases called secretases (β and γ) [6]. In particular, the 42 amino-acid form of Aβ is cytotoxic as it easily aggregates to form insoluble or fibrillary Aβ oligomers contributing to the loss of neuronal, synaptic function, and cognitive impairment [7, 8]. Recently, this amyloidogenic pathway has been referred as the Aβ cascade hypothesis. According to this, AD is linked to an increased production and decreased clearance of Aβ, which in turn leads to an accumulation of this peptide and triggers the subsequent pathogenesis of AD [9]. There is also some evidence that the formation of oligomeric Aβ is enhanced by oxidative stress [8].

2. OXIDATIVE STRESS AND AD

There is good evidence that Aβ aggregation and NFT formation in AD is mediated by reactive oxygen species (ROS) [10-15]. ROS are known to have beneficial as well as detrimental effects on cellular functions depending on their concentration [16]. At low concentration, ROS can regulate cellular functions through redox dependent signalling and redox dependent transcription factors [16, 17]. However, at high concentration ROS impair vital cellular processes as a consequence of their damaging effects on cellular macromolecules such as protein, lipids and DNA. Therefore, a balance between ROS production and removal is essential for normal cellular functions. Any imbalance in the homeostasis of ROS levels can result in oxidative stress and subsequent pathological conditions. In fact, aging populations are more vulnerable to oxidative stress due to a decreased performance of their endogenous antioxidant system [18]. Neurons in the brain are particularly susceptible to this event for several reasons and thus explain at least in part the high prevalence of neurological disorder like AD in the elderly [19]. First and foremost, post-mitotic cells by definition are unable to regenerate [20]. In addition, their high metabolic rate and the presence of large amounts of polyunsaturated fatty acids make them prone to ROS-induced self-propagating chain reactions of lipid peroxidation [20]. Finally, neurons harbour limited antioxidant defence systems compared to other organs [20]. Therefore not surprisingly, oxidative stress is thought to be

central to the pathogenesis of AD [8, 20]. In human neuroblastoma SH-SY5Y cells, hydrogen peroxide at 100-250 μM-induced oxidative stress caused a significant increase in intracellular Aβ levels [11]. In addition to Aβ deposition, oxidative stress also promotes NFT accumulation [21]. NFT are composed of bundles of paired helical filaments (PHF), with the microtubule associated protein tau as a major component [21]. Tau phosphorylation occurs as a result of oxidative stress and appears to be critical for abnormal aggregation and disrupted function of this protein in affected neurons in AD [21]. In an *in-vitro* model of chronic mild oxidative stress, ROS increased levels of tau phosphorylation at the PHF-1 epitope (serine 399/404) in a time-dependent manner [21]. Furthermore, animal models of AD repeatedly demonstrated that elevated levels of oxidative stress precede Aβ deposition, tau hyperphosphorylation and impairment of cognitive function [8, 12, 22-24]. Consistently, increased levels of oxidative damage biomarkers have been shown in brains and tissues of AD patients and elderly individuals [25-27]. These biomarkers indicate the presence of lipid peroxidation (TBARS: thiobarbituric acid reactive substance, malondialdehyde), protein oxidation (protein carbonyl and nitration), DNA oxidation (8HdG: 8-hydroxy-2-deoxyguanosine), defects in the GSH system, and impaired superoxide dismutase activity [25]. Intriguingly, these biomarkers of oxidative stress correspond to the brain areas affected by AD and the amount of these biomarkers correlate with the severity of the disease [28, 29]. It is believed that in AD, ROS-induced macromolecular damage leads to protein misfolding and the subsequent aggregation of misfolded proteins including Aβ and tau [29].

ROS as an integral part of AD pathology is supported by the presence of oxidative stress biomarkers in plasma, urine and brain tissue of transgenic AD animal models [24, 30]. Additional impairments to the antioxidant defences in these AD mouse models not only elevated oxidative stress levels but also enhanced Aβ deposition compared to normal AD mice [31]. In contrast, overexpression of endogenous antioxidant systems (Mn-SOD/Cu-Zn SOD) in transgenic AD animals decreased oxidative stress, reduce brain plaque burden and restored memory deficits [22, 32]. In addition to the elevated levels of oxidative markers in AD brains, significantly decreased levels of non-enzymatic antioxidants such as Vit E, C, A and reduced activities of anti-oxidative enzymes such as SOD, glutathione peroxidase and reductase have been reported [33]. In line with this connection, deficient levels of these antioxidants cause neurological symptoms, cognitive impairment and an increased risk of developing AD. Similarly another antioxidant CoQ10 (ubiquinone) has also shown to reduce oxidative stress and amyloid plaque burden and improve cognitive behaviour in transgenic AD mice [34-36]. However the applicability of these findings to the human situation remains unclear as the main physiological quinone in rodents is not CoQ10 but rather CoQ9. In fact, inherent limitations of CoQ10 absorption, uncertainty around blood-brain barrier transport and therefore access to its target site as well as absence of properly controlled clinical trials make this approach questionable at present [37, 38]. Another quinone with structural similarities to CoQ10 is idebenone, which possesses higher water solubility and better oral bioavailability compared to CoQ10 [39]. This drug was tested in multiple clinical trials in AD patients. Out of six clinical trials, five trials showed beneficial effect of idebenone in AD while one study failed to show a protective effect in AD patients. Despite the successful trials, the regulatory body deemed the data as insufficient to support the therapeutic use of idebenone in AD [40-44]. It has to be noted that there is still some controversy in the literature whether idebenone is merely an antioxidant like CoQ10 or a pharmaceutical drug with entirely different functions [39].

It is important to note the mitochondrial electron transport chain (ETC) generates the majority of cellular ROS (80-90 %). At the same time, the function of these organelles is highly susceptible to the effect of oxidative stress [45]. There is good evidence that mitochondrial function declines with aging [46]. Therefore not surprisingly, mitochondrial dysfunction has been implicated in many age-related neurodegenerative diseases including AD [8, 47]. ~~Defects in mitochondrial function~~Altered cerebral glucose metabolism ~~-~~has been detected in hippocampal and cortical neurons of AD patients [48-50]. Two aspects of altered cerebral glucose metabolism have been proposed that relate to abnormal glucose transport as a result of impaired brain insulin responsiveness and altered intracellular glucose metabolism as a result of mitochondrial dysfunction [49, 50]. Consistent with this idea, patients with type 2 diabetes show an increased risk of developing AD compared to healthy individuals [51]. In fact, insulin signalling is increasingly recognised to affect various brain functions including cognition and memory; functions that are also impaired in AD [51]. Mitochondrial dysfunction in AD is evident by reduced activity of key enzymes of mitochondrial oxidative phosphorylation (OXPHOS) such as cytochrome C oxidase ~~was reported to be reduced~~ in the brain of AD patients [52]. Furthermore, impaired Kreb's cycle enzymes including pyruvate dehydrogenase, isocitrate dehydrogenase and alpha-ketoglutarate dehydrogenase have been reported in AD brain tissue [53]. Similarly, in transgenic AD mice, excessive ROS production, reduced mitochondrial antioxidant activities, decreased mitochondrial membrane potential and apoptosis have been reported and are believed to contribute to neurodegeneration [54, 55]~~Furthermore, in transgenic AD mice reduced activity of Mn-SOD was described, which is responsible for mitochondrial dysfunction, oxidative stress, loss of mitochondrial membrane potential and finally apoptosis~~ [54]. In addition, the most direct evidence for mitochondrial dysfunction in AD is the presence of mitochondrial DNA (mtDNA) mutations in AD brains [33]. Brain tissue from AD patients in comparison to brain tissue from healthy individuals exhibit a striking increase in the rate of mutations within the control region (CR) of the mtDNA [56]. 65 % of the AD brains were positive for the CR mutation T414G whereas this mutation was absent in brains of healthy individuals. In fact several CR mutations are specific to AD including T414C, T4774 and other CR mutations have been reported in AD including T477C, T146C and T195C [56]. These mutations are located in the L-strand transcription (ND6) and/or H-strand replication region of mtDNA. Therefore, they are directly linked to reduced mtDNA copy numbers [56]. However there is some controversy whether some of these heteroplasmic mutations observed in AD patients are selective to AD pathology or also occur in the general population in a homoplasmic state [57]. Other mtDNA mutations include $tRNA^{Gln}$ gene mutation at nucleotide 4336 which is found in 5.2 % of AD patients and the ND1 gene mutation at nucleotide 3397 in 2 % of late onset AD patients [58, 59]. Quantification of the common 4977 mtDNA deletion showed that the levels of this mutation in the cortex of AD patients were on average 15-fold higher compared to control brains [58]. Intriguingly, Aβ itself has been demonstrated to alter mitochondrial function [20]. Aβ was detected in brain mitochondria of AD patients, transgenic AD mice, and neuroblastoma cells expressing mutant human AβPP [60, 61]. This mitochondrial Aβ was associated with impaired mitochondrial metabolism, diminished enzymatic activity of the mitochondrial respiratory chain, abnormal mitochondrial dynamics and as well as the presence of mtDNA mutations [61-63]. This direct effect is illustrated by the observation that the levels of Aβ directly correlated to the levels of hydrogen peroxide in a transgenic mouse model of AD [60]. Conversely, mitochondrial-mediated ROS may also increase Aβ production in *in-vitro* and *in-vivo* [64]. While mitochondrial

complex I and III inhibitors induce mitochondrial dysfunction and ROS production, they also enhance Aβ production. At the same time antioxidants can ameliorate mitochondrial dysfunction, ROS production and can reduce Aβ production [64]. Additionally, mitochondrial complex I defective mice or AD mice treated with a complex I inhibitor showed increased Aβ production, which supports the view that mitochondrially-derived ROS triggers Aβ production and can trigger the subsequent pathogenesis of AD [64]. Consistent with this sequence of events, mitochondrial respiratory impairment together with increased oxidative damage were reported to occur prior to the appearance of Aβ plaques, which strongly suggests that mitochondrial impairment occurs early in the disease progression and is likely a key pathological factor in AD [48, 60].

As a consequence of mitochondrial dysfunction and defective insulin signalling, it is believed that inflammatory and oxidative stress responses including the release of cytokines, activation of microglia and astrocytes, increased vasoconstriction, generation of Aβ and disturbances in the blood brain barrier are triggered. These events then decrease cerebral blood flow, synaptic function, neuronal integrity in both grey and white matter and subsequently lead to cognitive dysfunction and AD [65, 66].

Taken together, these findings suggest that mitochondrial-dysfunction-generated oxidative stress is a major risk factor for AD. Therefore the physiological or pharmacological activation of antioxidant enzymes that counteract increased oxidative stress are essential for the prevention of neuropathology. One of the acute physiological antioxidative responses is the upregulation of NADPH quinone oxidoreductase 1 (NQO1).

3. NQO1

NQO1 is a cytoplasmic FAD-dependent flavoprotein that catalyses the two electron reduction of quinones, quinoneimines, nitroaromatic, glutathionyl-substituted napthoquinones, dicholorophenolindophenol (DCPIP) and azo dyes by using NAD(P)H as electron donor. This two electron reduction prevents for example the formation of reactive semiquinones, which would otherwise result in oxidative stress [67]. In addition to this catalytic role, NQO1 has been reported to directly scavenge superoxide radicals in tissues with relatively low levels of SOD such as the heart muscle [68]. NQO1 is also essential to stabilize the tumour suppressor protein p53, by preventing its 20S proteasomal degradation, a mechanism that is essential to protect against carcinogenesis [68].

NQO1 is highly expressed in heart, liver, lung, breast, colon, vascular endothelia, adipose, corneal, lens epithelium, retinal pigment epithelium (RPE), optic nerve and nerve fibre tissue [69]. Despite elevated basal levels in some tissues, NOQ1 protein levels are generally highly inducible and NQO1 expression is regulated by a protein complex consisting of the nuclear factor erythroid 2-related factor 2 (Nrf2) and Kelch- like ECH-associated protein 1 (Keap1) **(Figure 1)** [67]. Under normal physiological conditions cytoplasmic Nrf2 binds to the ubiquitin ligase systems (Cullin 3-base-E3 ligase) via Keap1, which promotes continuous ubiquitination and proteasomal degradation of Nrf2 [67]. Under conditions of oxidative stress some of the highly reactive cysteine residues at positions 257, 273, 288 and 297 in Keap1 are easily attacked by radicals, which destabilizes the complex with Nrf2 [68, 70, 71]. As a result Nrf2 is not ubiquitinated but rather accumulates in nucleus, where it

binds to the antioxidant-response elements (ARE) in the promoter regions of its target genes. This binding promotes increased transcription of a broad range of cytoprotective genes such as hemeoxygenase (HO-1), γ-glutamyl-cysteine-ligase (GCL), glutathione S-transferase (GST), glutathione peroxidase (GPX) as well as NAD(P)H:quinone oxidoreductase 1 (NQO1) [70, 72]. A significant part of this cellular response to pro-oxidative conditions can be attributed to NQO1, since induction or knockdown of NQO1 is directly associated with decreased or increased levels of oxidative stress [68]. In line with its role as a stress response protein, NQO1 expression has been associated with several pathological conditions and disorders including Alzheimer's disease. Some studies have also reported neuroprotective effects of HO-1, which is typically co-expressed with NQO1. Although, HO-1 upregulation has also been reported in AD (reviewed by [73, 74]), others have reported a detrimental effect of HO-1 upregulation on glial cells due to increased iron-mediated oxidative stress [75-78]. Consistent with this detrimental role, inhibition of glial HO-1 hyperactivity attenuated AD-associated behavioural deficits and neuropathological changes in both *in-vitro* and *in-vivo* models of AD [79, 80]. Furthermore, unlike NQO1, the few reported association studies in human AD patients do not link HO-1 polymorphisms with increased AD risk [81, 82]. Therefore, this review focuses on the association between NQO1 and AD.

4. NQO1 AND AD

Numerous studies have investigated a possible role of NQO1 on the pathology of AD by examining levels and sites of NQO1 expression in AD. Overall, increased levels of NQO1 expression have been reported for the brains of AD patients [83-85]. This NQO1 up-regulation is generally regarded as a neuroprotective response to the oxidative stress associated with AD. When brains of AD patients were compared with age-matched and young controls, elevated NQO1 expression was mainly observed in areas affected by AD pathology such as in the cytoplasm of hippocampal neurons (47). At the same time, in healthy age-matched and young individuals, NQO1 expression was significantly lower in the same neuronal populations [83]. Similarly, histochemical staining of hippocampal sections from AD patients showed elevated levels of both NQO1 protein levels and enzyme activity in pyramidal neuron populations that exhibited tau immunostaining [84]. Further evidence that NQO1 enzyme activity co-localizes closely with areas of AD pathology comes from a study that measured NQO1 enzyme activity and immunohistochemical staining in the areas commonly affected by AD such as frontal cortex and compared them with the regions unaffected in AD such as occipital cortex, cerebellar and substantia nigra [85]. This study revealed that the ratio of frontal to cerebellar enzymatic activity of NQO1 was significantly increased in AD patients compared to controls. Moreover, NQO1 staining, which was present in the frontal cortex of AD patients, was absent in control individuals [85]. In fact, elevated NQO1 expression in 3xTg-AD mice preceded any intraneuronal Aβ immunoreactivity or increased expression of other antioxidant enzymes. This suggests that up-regulation of NQO1 is one of the first indicators of disease pathology, at least in 3xTg-AD mice.

In contrast to increased NQO1 levels in AD, some studies also reported reduced expression of Nrf2 and Nrf2 target genes such as NQO1 in AD patients [86, 87]. Analysis of Nrf2 expression demonstrated mainly nuclear

and some cytoplasmic localization in hippocampal neurons of control subjects. In contrast, only cytoplasmic localization of Nrf2 was observed in AD patients [88]. The activation of Nrf2-targeted genes is differentially regulated in AD patients depending on the stage of disease [29, 88]. During the initial stages, Nrf2-dependent gene expression is increased as a result of the initial defensive cellular mechanism against ROS. However as the disease progresses, oxidative stress continue to increase, while Nrf2-dependent gene expression is either reduced or remains stagnant [89]. In 3xTg-AD mice a significant increase in NQO1 protein in the hippocampus and cortex during the initial stages of the disease was observed at 2 months [86]. However, by 6 months of age, hippocampal NQO1 levels were significantly reduced [86]. This NQO1 expression pattern was supported by a significant increase in nuclear Nrf2 levels at 3 months and decreased levels in the cortex of the 3xTg-AD mouse model at 15 months [87]. Consistent with a protective role of Nrf2-dependent gene expression, Nrf2 -/- mice crossed with mutant APP/PS1 mice showed significant increased intracellular levels of Aβ and AβPP compared to mutant AβPP/PS1 mice, suggesting that inactivation of Nrf2 predisposes to AD pathology [90]. Further evidence of decreased NQO1 at later stages of the disease comes from a study that evaluated the plasma membrane redox system (PMRS) activity in the 3xTg-AD model [91]. The PMRS consists of a number of important components including NQO1, cytochrome b5 reductase, coenzyme Q10, and maintains the overall cellular redox state [92]. In this mouse model, elevated levels of oxidative stress impaired the PMRS that normally protects neurons against Aβ toxicity by reducing oxidative stress [91]. While, NQO1, one of the key enzymes of the PMRS is down-regulated in the hippocampus and cerebral cortex of 3xTg-AD mice , neurons overexpressing PMRS components demonstrate increased resistance to Aβ toxicity [91].

Besides looking at NQO1 enzyme activity and protein expression, several genetic association studies have been carried out in AD patients. In humans, the NQO1 gene is polymorphic [93] with the most frequent and best studied polymorphism called NQO1*2. NQO1*2 (C609T or Pro187Ser) is a single nucleotide polymorphism, a C to T change at position 609 of the NQO1 cDNA, which results in a proline to serine substitution at amino acid 187 of the protein. This polymorphism destabilizes the protein and therefore reduces overall cellular activity [94]. Heterozygote carriers (C/T) only show about 50% NQO1 activity compared to carriers of the C/C genotype and homozygote carriers (T/T) only harbour very low to undetectable residual activity [95, 96]. The NQO1*2 polymorphism has been associated with several pathological conditions and disorders [86, 97-100]. Ethnic variations in the prevalence of NQO1*2 has been extensively studied [93, 101]. In China, nearly 50% of the population are heterozygous and up to 22% are homozygous, whereas among Caucasians only up to 33% of population are heterozygous and up to 5% are homozygous for NQO1*2 (**Table 1**). This means homozygous carriers without NQO1 activity are nearly 400% more prevalent in the Chinese population compared to Caucasians. Consequently, possible associations between NQO1*2 and AD have been mainly studied in the Chinese population. The majority of the studies have shown association between NQO1*2 polymorphism and AD pathology [102-104]. When the presence of NQO1*2 was analysed in 92 sporadic AD cases and 108 normal control individuals in the Chinese population, the frequency of T allele in both C/T and T/T genotype was significantly higher in sporadic cases of AD compared to healthy individuals [102]. A similar result was observed in a study that involved 65 sporadic AD patients, 21 patients with cognitive dysfunction and 110 controls without cognitive dysfunction, suggesting that the NQO1*2 polymorphism may indeed be a risk factor for both cognitive dysfunction and sporadic AD in the Chinese population [103]. Consistent with these results a

higher T-allele frequency and significantly low C/C genotype frequency was found in the AD cases compared to controls in APOE ε4 non-carriers and those over 65 years of age [104], However, no difference in allele frequencies between AD cases and control was reported the late onset AD in Chinese population (104 AD cases and 128 controls) [105]. This outcome was not altered even with stratification of APOE genotype (ε4 allele) which is considered as a significant risk factor in late onset AD [105]. Overall, these results suggest that the C/C genotype may possibly protect against AD progression, while the T allele might be a risk factor for late onset AD [104].

As demonstrated earlier [102, 104] the presence of NQO1*2 polymorphism reduces NQO1 protein levels and therefore its overall activity in AD patients. In 50% of the examined hippocampi from AD patients, NQO1 levels were undetectable, which was associated with the presence of the NQO1*2 polymorphism [98]. These results indicate that the inactivating NQO1*2 polymorphism is a major risk factor for protein levels and therefore activity of NQO1 as well as for the progression of AD. However, it has to be noted that in individuals with normal NQO1 expression, NQO1 activity can be affected by the several other factors such as drugs and toxins exposure.

5. ~~INHIBITORS~~ FACTORS AFFECTING ~~OF~~ NQO1 EXPRESSION OR ACTIVITY

Structural and functional studies of NQO1 have shown that NQO1 contains two active binding sites that work via a "ping-pong" mechanism [106]. One site binds the electron donor NAD(P)H and another is for substrate molecules such as quinones. Once NAD(P)H binds to NQO1, it reduces the tightly bound cofactor flavin adenine dinucleotide (FAD) to $FADH_2$ before $NAD(P)^+$ is released from the active site. This allows the second substrate to bind and subsequently be reduced by $FADH_2$. In addition to genetic factors and age that mainly affect overall NQO1 expression (i.e. C690T NQO1 polymorphism), several drugs and environmental factors (i.e. chronic cigarette smoking) ~~and diet (curcumin)~~ have been reported to inhibit NQO1 activity directly.

One of the best-studied, competitive inhibitors of NQO1 is dicoumarol [3,3′-methylenebis (4-hydroxycoumarin)] **(Table 2)** ~~which is one of the most potent competitive inhibitors of NQO1~~. Dicoumarol competes with NAD(P)H for binding to NQO1 and thus prevents the reduction of FAD to $FADH_2$ [106, 107]. Dicoumarol however lacks specificity and is associated with off-target effects including mitochondrial uncoupling and intracellular ROS generation [108]. To overcome this, a number of dicoumarol-like compounds were synthesised and are claimed to be more potent and have no off-target effects [108]. Warfarin is another coumarine derivative structurally related to dicoumarol, which inhibits NQO1 albeit with less potency compared to dicoumarol. It has been suggested that the mode of inhibition and site of interaction of warfarin is quite similar to dicoumarol [109]. It is important to note that warfarin is currently a mainstay of oral anticoagulant therapy despite the recent emergence of pharmaceutical alternatives with different modes of actions. Millions of people are on warfarin therapy worldwide [110]. Given the widespread use of warfarin and its inhibitory activity of NQO1, together with its ability to cross the blood brain barrier, there is the possibility that warfarin use may contribute to the risk of developing AD in patient populations with normal NQO1 genotype. However there are no studies at present that have addressed this possible connection. In contrast to the reversible inhibition of

dicoumarol, another very potent molecule that acts as an irreversible NQO1 inhibitor is ES936 but is associated with inducing cellular DNA damage [111].

~~Similar to dicoumarol, curcumin, a natural compound found in the spice turmeric inhibits NQO1 activity. Inhibition of NQO1 activity by curcumin leads to the destabilization of the tumor suppressor WT P53, which is normally stabilized by NQO1 after DNA damage to induce apoptosis, thus promoting the dissociation of P53-NQO1 complexes and inhibiting p53-induced apoptosis.~~

The oxidative stress hypothesis of aging postulates that excessive ROS production and a decrease in antioxidant capacity promote the aging process. This results in increased macromolecular oxidation damage and accumulation of such damage gradually leads to cellular dysfunction. It is well known that aging is one of the major risk factors for AD. The levels of both basal and inducible NQO1 during aging in the presence or absence of stressors have been studied in different animal models [86, 112-120]. However, reports of age-related changes in basal expression of NQO1 in animals are conflicting, with evidence of increased or decreased expression, depending on the cell types and tissues investigated (**Table ~~2~~3**). Nevertheless, the majority of studies have shown age-related decline in NQO1 induction in various tissues of aged organisms [113, 114, 117, 121] (**Table ~~2~~3**). Basal NQO1 expression in liver, lung, cerebellum, retinal pigment epithelium, spleen T-lymphocytes of aged mice was higher compared to young mice [112-116]. In contrast, NQO1 expression as well as NQO1 activity in aorta and liver of aged (18 -24 months) rats was lower compared to younger animals (2-12 months) [117, 118]. Similarly, NQO1 levels in the hippocampus, lung and astrocytes of aged mice were lower compared to young mice [86, 119, 120].

In addition to basal NQO1 expression, aging also impairs regulation of Nrf2 [122-124]; as a consequence in aged organisms oxidative stress fails to activate Nrf2, which also impairs the subsequent induction of antioxidant target-genes that are required to detoxify ROS. In fact down regulation of Nrf2 has been reported in aged mouse brain [122]. Increased ROS production, antioxidant depletion and increased oxidative damage to macromolecules due to age-related decrease in Nrf2 target gene expression have been demonstrated in both human and rodents [123, 124]. Furthermore NQO1 induction in response to hydrogen peroxide and glucose was decreased in cultured aorta from old rats (24 months) compared to young rats (3 months) [117]. This age-dependency was also replicated in a non-human primate model, where induction of NQO1 in the carotid artery and vascular smooth muscle cells was blunted in response to hydrogen peroxide and high glucose relative to young animals [121]. Exposure to oxidative stress-inducing agents failed to increase NQO1 levels in the liver, lung and cerebellum of aged mice (21 months) compared to young animals (6 months) [113]. Similarly the impaired induction of NQO1 was also observed in the retinal pigment epithelium of old mice exposed to sodium iodate compared to their younger counterparts [114]. Collectively, these results indicate that basal or inductive NQO1 levels are altered with aging.

Cigarette smoke (CS) is another factor that alters NQO1 expression. CS contains nearly 4700 chemicals and among them free radicals are the most abundant [125]. Each puff of cigarette is reported to contain 10^5 free radicals including O^{2-} and NO [125]. CS contains two phases: the gas phase and the tar or particulate phase.

While the gas phase contains oxygen and carbon centred radicals that are much more reactive such as high concentration of nitric oxide, the tar phase contains several stable radicals including quinone/hydroquinone-pairs [126]. Especially quinones are known to reduce molecular oxygen to generate superoxide, which as a secondary event contributes to the formation of hydrogen peroxide and hydroxyl radicals [126]. The polycyclic aromatic hydrocarbons (benzo (α)-pyrene-3, 6-quinone), heterocyclic aromatic amines and N-nitroso compounds that are found in the tar phase of cigarettes are mostly metabolized by phase II enzymes such as NQO1 [127]. Polycyclic aromatic hydrocarbons are also a known inducer of NQO1. Mice exposed to CS for 5 h/day showed NQO1 induction after one day of exposure and more strongly after 21 days [125]. Exposure of particulate phase of CS for 2h/d for 28 days altered oxidative homeostasis in the lungs in Sprague Dawley rats as a result NQO1/actin ratio as well as NQO1 enzyme activity were increased [128]. Similarly, exposures of primary normal human bronchial epithelial cells to cigarette smoke condensates for 18 h strongly increased NQO1 expression compared to non-treated control cells [129]. However, with chronic exposure to cigarette smoke there is evidence of decreased Nrf2 activation and subsequent decrease in Nrf2-target genes including HO-1 and NQO1 [130, 131]. In a human monocyte/macrophage cell line (THP-1) CS exposure up to 24 hours caused increased nuclear Nrf2 translocation and induction of the Nrf2 target-gene HO-1 [130]. However, prolonged exposure (72h) caused a reduction of HO-1 expression associated with a cytosolic accumulation of Keap 1 and Nrf2 [130]. Failure of Nrf2 translocation in to the nucleus and associated decreased antioxidant levels have also been demonstrated in human lung epithelial cells after 24 h of CS exposure [131]. Consistent with the inhibition of Nrf2-dependent gene expression, chronic cigarette smoking has also been reported as a significant risk factor for inducing AD pathology in preclinical and clinical studies [132, 133]. This observation is supported by reports that suggest smoking-associated cerebral-oxidative stress is potentially contributing to AD pathology [132, 134].

6. CONCLUSION

Mitochondrial dysfunction-mediated oxidative stress has been implicated in the pathogenesis of AD. In line with a causal role of oxidative stress in AD, the inactivation of the physiological detoxifying enzyme NQO1 has also been linked to AD progression. Factors that alter NQO1 activity can include genetic predispositions such as the frequent C690T NQO1 polymorphism, advanced age, ~~diet,~~ cigarette smoke and several drugs (**Figure 2**). In line with their effects on NQO1 expression and activity, these factors may also play a role in the development of AD. To date only a limited numbers of studies have explored the association between NQO1 activity and AD but consistent results indicate a time-dependent connection. Understanding this link may contribute to further insight into the pathogenesis of the disease and may also lead to potential novel therapeutic options for the treatment of AD.

LIST OF ABBREVIATIONS

NADPH = Reduced nicotinamide adenine dinucleotide phosphate

MnSOD = Manganese Superoxide Dismutase

Cu/Zn SOD = Copper/Zinc Superoxide Dismutase

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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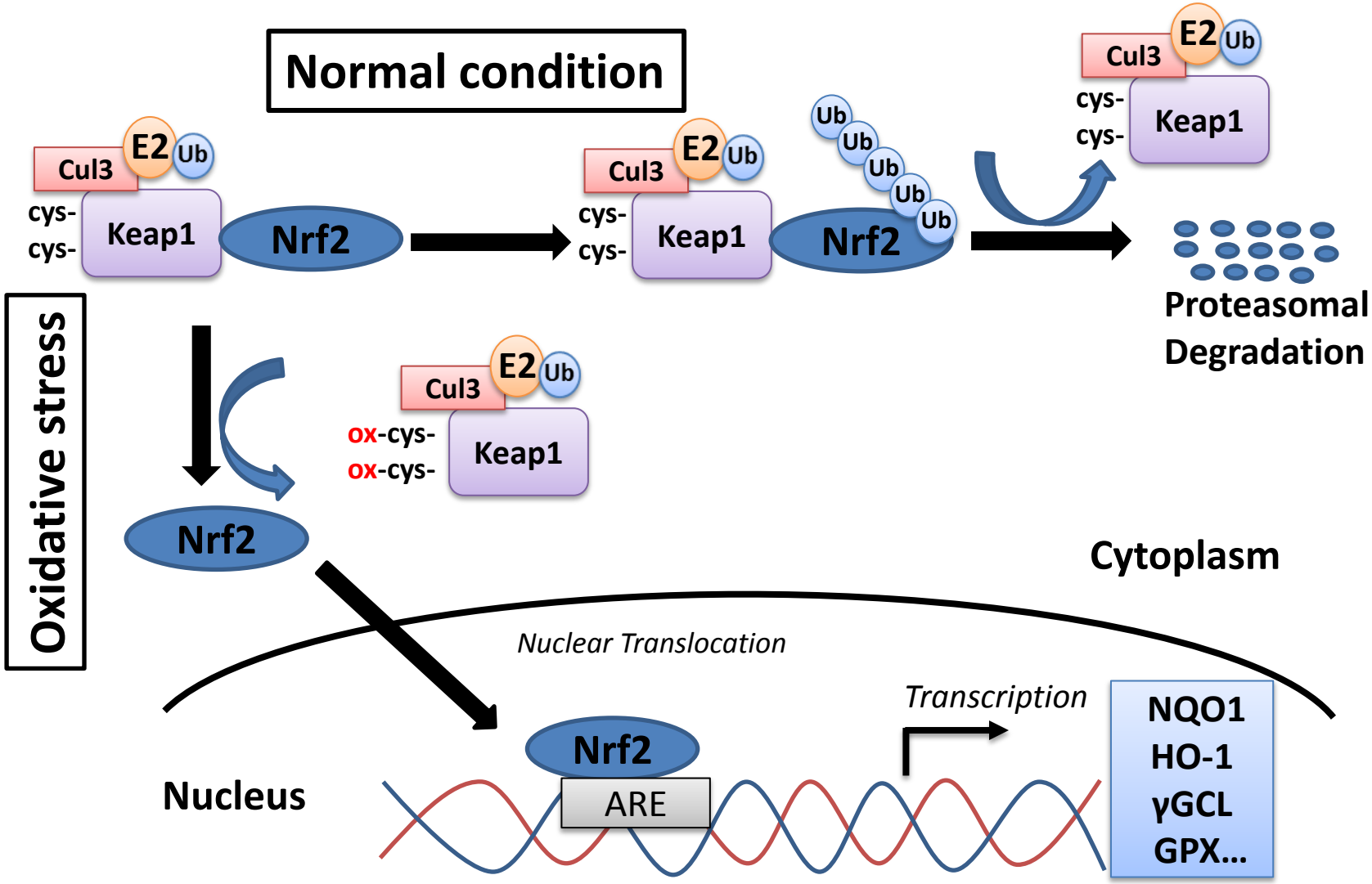

Figure 1: Schematic representation of the Nrf2-dependent induction of cyto-protective genes in response to oxidative stress. Oxidative stress causes oxidation of cysteine residues within Keap1 that lead to the disintegration of the Nrf2-Keap1 complex. This causes stabilization of Nrf2 and consequently induces the transcriptional activation of ARE element-regulated cytoprotective genes, such as NQO1. Cul 3: Cullin 3; E2: Ubiquitin-conjugation enzyme; keap1: Kelch- like ECH-associated protein 1; Nrf2:Nuclear factor-erythroid 2-related factor 2; Cys: cysteine; ox: oxidant; ARE: Antioxidant-response element; NQO1: NADPH quinone oxidoreductase 1; HO-1:hemeoxygenase1; γGCL:γ-glutamyl-cysteine-ligase ; GPX: Glutathione peroxidase.

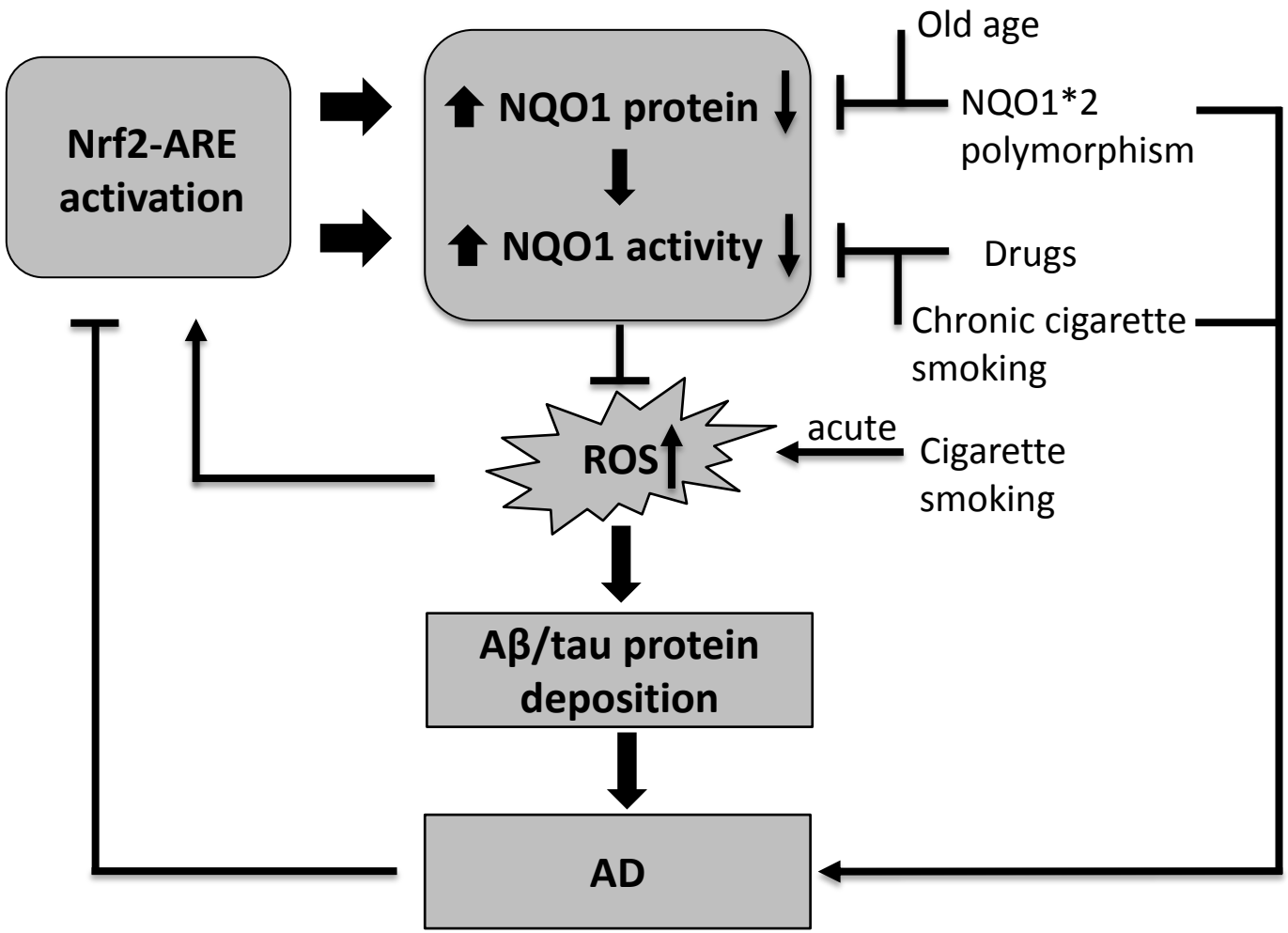

Figure 2: Possible predisposing factors that inhibit NQO1 activity. Predisposition to AD pathology may be linked to inhibition of NQO1 expression or activity caused by NQO1*2 polymorphism, drugs, cigarette smoking and advanced age.

Table 1: NQO1 protein activity in different ethnic groups [93, 101]

Genotype			
Ethnic group	**C/C (%)**	**C/T (%)**	**T/T (%)**
Asia (overall)	31.4	48.3	20.3
Chinese	28.6	50.0	22.4
Caucasians	82	16	2
Africans	61	33.8	5.2

C/C: wild type NQO1; C/T: heterozygote NQO1; T/T: homozygote NQO1.

Table 2: Pharmacological inhibitors of NQO1

Drugs	Mechanism	Inhibition in intact cells*		Advantages	Disadvantages	Ref
		IC95 (nm)	IC50 (nm)			
Dicoumarol	Competitive Inhibitor		404, 1600	Potent	Lack specificity, Off-target effects such as ROS production and mitochondrial uncoupling	[107, 108]
Dicoumarol-like compounds	Competitive Inhibitor		38-3300	Potent , No off-target effects Improved water-solubility compared to dicoumarol		[108]
Warfarin	Competitive Inhibitor		NA	NA	NA	[109]
ES936	Irreversible-Inhibitor	25		Very potent, High specificity	Can lead to DNA damage	[111]
Flavonoids (7, 8 dihydroxyflavone)	Competitive Inhibitor		133000			[107]

*It has to be noted that the given IC50 values for NQO1inhibition are cell-type dependent.
NA: Not Available

Table 3: Age-related *in-vivo* changes of NQO1 levels

Species	Target (cells/tissues)	Stressor	Age	Changes in NQO1		Ref
				Basal	Inducible	
Mouse	Hippocampus	AD AD + age	2 months (3XTg-AD vs control) 6 months vs 2 months (3XTg-AD)	N/A	Increased Impaired	[86]
Mouse	RPE	Age Sodium iodate Sodium iodate + age	15 months vs 2 months 2 months (untreated vs treated) 15 months (untreated vs treated)	Increased	N/A Increased Impaired	[114]
Mouse (C57BL/6)	Cerebellum, liver, lung	Age Nano sized particle Nano sized particle + age	21 months vs 6 months 6 months (untreated vs treated) 21 months (untreated vs treated)	Increased N/A N/A	N/A Increased Impaired	[113]
Rhesus macaques	Carotid-arteries vascular smooth muscle cells	Age High glucose and H_2O_2 + age	20 years vs 10 years 20 years vs 10 years	Decreased N/A	N/A Impaired	[121]
Rat (Fischer 344 x Brown Norway)	Aorta Cultured aorta segments	Age High glucose and H_2O_2 + age	24 months vs 3 months 24 months vs 3 months	Decreased N/A	N/A Impaired	[117]
C57BL/6 mouse	T lymphocytes in spleen	Age	18 months old vs 4 week old	Increased	N/A	[115]
Mouse	Astrocytes	Age	1 or 5 months vs 13 months	Decreased (trend only)	N/A	[120]
Mouse (Female)	Liver	Age	27 months vs 3 months	Increased	N/A	[116]

Rats	Liver	Age	2 or 12 months vs 1.5-2 years	Decreased	N/A	[118]

NA: Not Available; NQO1: NADPH Quinone oxidoreductase 1; AD: Alzheimer's disease.