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Nuclear Factor-Kappa B and Alzheimer Disease, Unifying Genetic and Environmental Risk Factors from Cell to Humans

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Alzheimer’s disease (AD) is the most common form of dementia, an eversible, progressive disease that causes problems with memory, thinking, language, planning, and behavior. There are a number of risk factors associated with developing AD but the exact cause remains unknown. The predominant theory is that excessive build-up of amyloid protein leads to cell death, brain atrophy, and cognitive and functional decline. However, the amyloid hypothesis has not led to a single successful treatment. The recent failure of Solanezumab, a monoclonal antibody to amyloid, in a large phase III trial was emblematic of the repeated failure of anti-amyloid therapeutics. New disease targets are urgently needed. The innate immune system is increasingly being implicated in the pathology of number of chronic diseases. This focused review will summarize the role of transcription factor nuclear factor-kappa B (NF-κB), a key regulator of innate immunity, in the major genetic and environmental risk factors in cellular, invertebrate and vertebrate models of AD. The paper will also explore the relationship between NF-κB and emerging environmental risk factors in an attempt to assess the potential for this transcription factor to be targeted for disease prevention.

Keywords: nuclear factor-kappa B, Alzheimer, dementia, cell lines, invertebrate models, rodents, humans

INTRODUCTION

The global burden of dementia is devastating, with an estimated 35 million people affected and the annual cost estimated to exceed $1 trillion by 2018 (1, 2). Despite greater knowledge of the pathogenic sequelae of disease, repeated failure in drug trials has led to a switch in emphasis from disease treatment to disease prevention (3–5). Alzheimer’s disease (AD) is the most common dementia subtype yet no single theory has been able to account for the multiple risk factors leading to the pathological and clinical features (3). The deposition of excess extracellular beta amyloid (Aβ) protein and subsequent taupathy has long been felt to be a cause of the disease overshadowing alternative hypotheses including microglial dysfunction, vascular disease, mitochondrial insufficiency, and metabolic disease.

However, the recent failure of another promising anti-amyloid treatment in large phase III trials has dealt a significant blow to the credibility of the amyloid hypothesis (6). The pathognomonic role of Aβ is now also being questioned with the discovery that Aβ acts as an antimicrobial peptide (AMP) in cell lines, nematode, and rodent models. Aβ production following exposure to neurotoxic fungi and bacteria provided significant neuroprotection (7). Amyloid over-production may therefore
be a downstream product of immune dysregulation rather than a disease process in itself. In support of this, several genes are involved in innate immunity and are associated with an increase in AD (8) (see Table 1). More work is clearly needed to explore the interaction between amyloid and the innate immune system.

Aging is the most significant risk factor for developing AD and recent findings have shown tissue specific brain inflammation, mediated by NF-κB is associated with aging (12–15). Hypothalamic NF-κB levels are negligible in young mice and Drosophila. Significant activation begins in middle age. The resulting downstream increase in AMPs leads to increased local microglial activity, subsequent decline in gonadotrophins and aging (12, 14). Interestingly, hypothalamic inflammation is relatively higher when compared to neurons and glial cells in other vulnerable brain regions such as the hippocampus (12). In support of this hypothalamic-specific regulation of aging, drugs known to extend lifespan in mice reduce hypothalamic inflammation but have little anti-inflammatory effect on hippocampal neurons (14).

Known inducers of NF-κB activity are highly variable and include reactive oxygen species (ROS), interleukin 1-beta (IL-1β), tumor necrosis factor alpha (TNF-α), bacterial lipopolysaccharides (LPS), isoproterenol, and ionizing radiation (16, 17). In addition to stimuli that activate NF-κB in other tissues, NF-κB in the nervous system can be activated by growth factors and synaptic transmission such as glutamate (18). These activators of NF-κB in the nervous system all converge upon the inhibitor of kinase kinase (IKK) complex (Figure 1).

Nuclear factor-kappa B transcription factors include a collection of proteins with functions conserved from the fruit fly Drosophila melanogaster to rodents and to humans (Figures 1 and 2). They are present in all human and most animal cells and regulate the expression of more than 400 genes, including isoforms of SET, directly implicated in the pathogenesis of AD (22). Conversely, expression of the mammalian family of Sir2A deacetylases, known to attenuate the effects of aging, down regulates NF-κB (23, 24).

Studies of aging populations have enabled the identification of a number of genetic and environmental risk factors that appear to influence susceptibility to developing AD (25, 26). This paper will review the interaction between these risk factors and NF-κB, first looking at the major known genetic risk factors in cell, invertebrate, and vertebrate models. It will then focus on the major known environmental risk factors in these models before reviewing emerging environmental risk factors. Finally, the paper will review protective mechanisms across various experimental models and whether their association with NF-κB.

The repeated failure of disease modifying trials in AD demands that new treatment targets are urgently identified. The recent findings that implicate the innate immune system in AD provides an opportunity to review the evidence for NF-κB as a key immune system regulator in the prodromal stage in the hope of identifying a target for treatment and prevention.

### GENETICS

**Neuronal Cell Lines/Human Autopsy Studies**

Overexpression of the amyloid precursor protein (APP) gene is associated with familial aggregation of late onset AD and dramatically increases susceptibility to early AD in Down’s syndrome. APP is cleaved by the beta-secretase BACE1 into amyloid monomers that form oligomers that eventually become plaques in the brain and vasculature. Both BACE1 and NF-κB are increased in the brains of AD patients, with NF-κB directly upregulating BACE1 and the APP gene (27, 28). Medications such as minocycline, that inhibit NF-κB but not BACE1 or APP, reverse this process (28, 29).

The e4 variant of the APOE gene, which codes for a cholesterol transporting protein, is the largest, single gene risk factor for AD (30). In APOE e4-positive Schwann cell lines, when compared to APOE e3-expressing cells, excess production of IL6, IL10, and nitrous oxide results from a failure to inhibit NF-κB (31). These findings are replicated in neural cells and fibroblasts from AD patients where APOE e4 acts as a transcription factor responsible for regulating NF-κB expression (32). Curiously, cells from the somatosensory cortex of AD patients, an area of the brain that is resistant to disease, display upregulation of NF-κB (33). However, this may reflect the earliest inflammatory hallmark of disease as previous autopsy studies have shown increased NF-κB activation in evolving Aβ deposits with a reduction in areas surrounding more mature plaques (19, 33).

<table>
<thead>
<tr>
<th>Gene implicated in late onset AD</th>
<th>Function</th>
<th>Increased risk of AD</th>
<th>Interaction with NF-κB</th>
<th>Interaction with amyloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREM2</td>
<td>Immunity</td>
<td>Reduced expression increases risk: slight–medium</td>
<td>NF-κB suppresses hippocampal TREM2 expression (9)</td>
<td>TREM2 required for microglial amyloid clearance (9)</td>
</tr>
<tr>
<td>CD33</td>
<td>Immunity</td>
<td>Mild</td>
<td>CD33 activates NF-κB in myeloid cells</td>
<td>CD33 inhibits microglial Aβ uptake and clearance (10)</td>
</tr>
<tr>
<td>CR1</td>
<td>Immunity</td>
<td>Mild–medium</td>
<td>Microglial CR1 activation associated with increase in NF-κB (11)</td>
<td>Uncertain (11)</td>
</tr>
<tr>
<td>INPP5D</td>
<td>Immunity</td>
<td>Mild</td>
<td>Negative regulator of NF-κB expression (12)</td>
<td>Uncertain</td>
</tr>
</tbody>
</table>

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**Table 1**: Emerging genetic risk factors for Alzheimer’s disease (AD) and their associated with nuclear factor-kappa B (NF-κB) and amyloid (8).
Invertebrate Models
A genetic screen for dominant suppressors and enhancers in a Drosophila model of Aβ-driven neurodegeneration revealed that Toll gene (receptor of Drosophila toll pathway) and key downstream components (dif, pelle, cactus), play a central role in mediating the neuropathological activities (34). Conversely, genetic overexpression results in accelerated deterioration of the phenotype suggesting that NF-κB significantly enhances the pathological potential of Aβ (35).

Genetic suppression of the immune deficiency (IMD) NF-κB pathway in glial cells in a Drosophila model of early onset neurodegeneration dramatically rescues brain pathology, reduced activity, and short lifespan (13). Genetic overexpression of NF-κB pathways at neuronal or glial tissue level leads to phenotypes resembling AD models with locomotor disability, accelerated neurodegeneration, and premature mortality (13).

Vertebrate Models
Age is the biggest risk factor for dementia and systemic inflammation increases as animal’s age, a process known as inflammaging (36). Microglia priming in mice induces a highly conserved transcriptional signature with aging characterized by NF-κB expression and neuronal death (37). In rats, NF-κB expression increases in normal aging leading to production of neurodegenerative pro-inflammatory enzymes COX-2 and iNOS (38). These changes are reversed by suppression of brain NF-κB activation using the anti-inflammatory Lactobacillus plantarum var. plantarum C29, restoring brain-derived neurotrophic factor (BDNF) levels and memory (39). In mice, the observed positive correlation between NF-κB activity and neuronal apoptosis suggests a role of NF-κB in hippocampal neuroapoptosis (40).

Supporting this, NF-κB induces pro-apoptotic increases in TNF and iNOS in the hippocampus of rats exposed to neurotoxin (41). Inactivating specific Sirtuin anti-aging genes in mice results in chronic NF-κB overexpression leading to accelerated aging and dramatically reduced lifespan (23). In Sirtuin replete models, overexpression via biofeedback dysregulation results in premature aging through chronic production of excessive ROS, leading to telomere dysfunction, cellular senescence, and premature death (42). Age-related NF-κB activation feeds into a positive
feedback loop in microglial cells causing perpetual inflammation and multiple brain responses including epigenetic suppression of GnRH genes in the hypothalamus (14). Microglial-derived NF-κB-TNF-α axis plays a key role in homeostatic synaptic scaling, a form of synaptic plasticity. However, overexpression results in disrupted neuronal networks and behavior mimicking obsessive–compulsive disorder (OCD) (43, 44). Suppressing this pathway mediates some of the OCD-like behavioral problems in mouse models of frontotemporal dementia (44). Specifically, under-expression of NF-κB in the mouse brain results in delayed onset of age-related pathology across all organ systems via preservation of the hypothalamic–pituitary–adrenal axis and GnRH levels (14).

ENVIRONMENT
Neuronal Cell Lines/Human Autopsy Studies
Type 2 diabetes mellitus (T2DM), a metabolic condition characterized by a decrease in sensitivity to endogenous insulin, is the best established environmental risk factor for the development of AD, increasing relative risk by 50% (45). Diabetes induces Aβ pathology via NF-κB upregulation and independent overexpression of BACE1 (46, 47). Inflammatory mediators are known to contribute to insulin resistance creating a pro-inflammatory feedback loop in diabetes (48). Administering advanced glycation end products that mimic diabetic driven pathology results in elevated BACE1 and consequent NF-κB overexpression in both rat brains in vivo and neuroblastoma cells lines (49). NF-κB suppression using Adiponectin rescues Aβ pathology in human T2DM neuroblastoma cells (50). Similarly, leukotriene D4, an inflammatory signaling molecule elevated in metabolic disorders, induces Aβ synthesis in primary neurons at 24 h with increases in NF-κB seen after just 1 h (51, 52). Treatment of the culture with NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) inhibited Aβ generation with down regulation of Aβ generating beta- and gamma-secretase activity suggesting NF-κB regulates Aβ synthesis in metabolic disease (52).

In human neuroblastoma cells, the metabolic enzyme protein arginine methyltransferase 5 (PRMT5) regulates cellular metabolism, protecting the cell in times of stress. Aβ downregulates this process leading to NF-κB overexpression, metabolic dysfunction, and premature cell death (53). Inhibiting NF-κB reduces
apoptosis and Aβ deposits even in a metabolically dysfunctional organism or human cell lines suggesting a gatekeeper role for NF-κB in maintaining metabolic homeostasis and subsequent neuroprotection independent of PRMT5 activity (53).

**Invertebrate Models**

In *Drosophila* models of chronic diseases including AD, the innate immune system has been identified as a key mediator of neurodegeneration (13, 34, 54, 55). NF-κB overexpression in the fat body cells, analogous to the metabolic syndrome in humans, results in more severe neurodegeneration (56).

Supporting the role of NF-κB in connecting whole-body metabolism with brain health, NF-κB overexpression in the hypothalamus-like pars intercerebralis neurons in *Drosophila* results in overnutrition, impaired metabolic learning, poor memory consolidation, and metabolic disorder characterized by increased lipid levels and shortened lifespan (57). Conversely, genetic knockdown of NF-κB signaling in glial cells leads to elevated adipokinetin and glucagon-like hormone levels, reduced glucose and lipid levels, and extension of “healthspan” (13).

**Vertebrate Models**

In rodent and primate models, diabetes and obesity drive overexpression of NF-κB in the hypothalamus creating a destructive feedback loop where further NF-κB expression promotes hypertension, overnutrition, and decreased insulin sensitivity (58–61). Injecting Aβ into the brains of mice and macaques results in an increase in NF-κB in the cell nuclei of the hypothalamus and subsequent induction of peripheral glucose intolerance (62). In this model, pharmacological inhibition of NF-κB maintained peripheral metabolic homeostasis. Inducing diabetes in rats results in hippocampal NF-κB-dependent neurodegeneration via disruption of CREB phosphorylation, reducing levels of protective downstream proteins including BDNF (63).

The tetracycline derivative Minocycline inhibits NF-κB and prevents further Aβ deposition in a mouse model of diabetes-driven AD. BACE1 activity remained elevated demonstrating an NF-κB-dependent protective mechanism (29). Mice fed on high-fat diets demonstrate elevated brain BACE1 expression as do transgenic diabetic mice. Administration of the anti-inflammatory agent all-trans-retinoic acid reduces BACE1 expression in both WT and mutant but this effect is abolished when the NF-κB-binding site at the promoter region of BACE1 is mutated (64).

**EMERGING RISK FACTORS**

**Alcohol Intake**

In *Drosophila*, alcohol consumption activates Toll-NF-κB signaling increasing ethanol resistance and gene products known to be outputs of innate immune signaling are rapidly induced following ethanol exposure (65). Ethanol treatment of cultured hippocampal rat neurons causes a dose- and time-dependent increase in NF-κB-DNA-binding activity, resulting in significant upregulation of inflammatory markers and increased susceptibility to neurotoxins; reversible by applying NF-κB inhibitors (66, 67).

Opposing, the consumption of moderate amounts of alcohol, particularly red wine, is associated with a reduced risk of AD. Anthocyanin, a polyphenol found in wines, protects rat hippocampal neurons against oxidative stress via NF-κB suppression (41, 68).

**Sleep**

Sleep quality and well-being are symbiotic and reduced sleep quality is increasingly being associated with increased risk of dementia. Sleep–wake cycle homeostasis is important in the processing and removal of Aβ plaques which, in turn, are known to dysregulate this reparative process (69). Sleep disruption and deprivation are known to cause over expression of the NF-κB pathway in hippocampal cell cultures, fruit flies, rodents, and humans (70–73). Improvement in sleep quality in older adults is correlated with a reduction in circulating NF-κB (74).

**Traumatic Brain Injury (TBI)**

Traumatic brain injury activates both microglia and astrocytes and induces self-sustaining inflammatory responses in the brain via NF-κB activation (75, 76). In *Drosophila*, flies with TBI exhibited temporary incapacitation, ataxia, activation of the innate immune response, neurodegeneration, and death similar to humans with TBI (77, 78). Rat models have demonstrated the acute onset and prolonged overexpression of NF-κB in brain regions most commonly associated with post-injury atrophy (79, 80). Recent studies have shown NF-κB to be significantly elevated in the ipsi-lateral cortex of both adult and old TBI mice in a time-dependent manner (81). These interactions started immediately post-injury in the old mice compared to the adult mice suggesting an age-related failure of NF-κB suppression.

**PROTECTIVE FACTORS**

**Exercise**

Under expressing NF-κB in mice results in greater endurance, cognitive performance, and resistance to obesity (82). An aerobic exercise protocol in wild-type rats attenuated age-related memory decline and decreased hippocampal NF-κB levels and atrophy (83). In rats fed a pro-inflammatory diet and subjected to either strength training, aerobic exercise or a combination of both, all protocols reduced liver and muscle NF-κB levels to pre-diet levels (84). These findings are supported by studies demonstrating the indirect suppression of NF-κB via cytokine IL-10, a potent NF-κB inhibitor, induced by exercise (85).

**Diet**

Curcumin, a constituent of turmeric, has gained much attention in recent years for its potential as a neuroprotective compound. In a *Drosophila* model of neurodegeneration, the curcumin analog C150 significantly reduced neuronal cell death, increased healthy lifespan, and reduced DNA mutation in brain tissue by suppressing NF-κB (86).

The neuroprotective effects resulting from the Mediterranean diet or those rich in oily fish may be mediated via the anti-inflammatory properties of key nutrients (87). Aβ induced increases in the translocation NF-κB subunits is attenuated in the presence of...
tyrosol (Tyr) and hydroxytyrosol (OH-Tyr) found abundantly in olive oil (88). Transgenic increases in omega-3 polynsaturated fatty acid in the brain of mice reduces the inflammatory response to LPS challenge via NF-κB pathways (89).

The omega 3 fatty acid eicosapentaenoic acid indirectly downregulates NF-κB expression acting as a ligand at peroxisome proliferator-activated receptor gamma, a regulator of fatty acid storage and glucose metabolism, and reduces symptoms of depression (87, 90). In AD-mouse hippocampal slices, food-derived anti-oxidants provide neuroprotection and reduce Aβ via the anti-inflammatory properties of the polyphenolic compounds within them (91). Specifically, the phenolic compound resveratrol reduces Aβ-induced migrolial activity and neuroinflammation via NF-κB suppression in murine microglial and macrophage cells (92).

**Anti-inflammatory Drugs**

The prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a reduction in the AD risk (93). In primary rat, neurons and human neuronal cell lines NSAIDs strongly inhibit NF-κB-driven expression of BACE1 activity preventing the cleavage of Aβ from APP (28, 94). Long-term administration of potent NSAID indomethacin blocks activation of NF-κB and significantly reduced the amyloid pathology in transgenic AD mice (95).

Aspirin, an NSAID derived from salicylic acid, completely inhibits Aβ activation of the NF-κB pathway, reducing levels of pro-inflammatory cytokines and chemokines, and increasing levels of anti-inflammatory IL-10 in rodent microglia and neurons resulting in recruitment of Aβ phagocytic microglia and improved cognitive and synaptic functioning (28, 96).

**CONCLUSION**

Epidemiological studies are beginning to converge of common risk factors for the development of AD with strong signals also emerging for certain protective factors. The emergence of NF-κB as a regulator of aging and proliferation of studies implicating NF-κB over-activation in a number of neurodegenerative diseases suggests that it may be important in modulating the risk of disease. This review has highlighted the intimate relationship between all known and emerging risk and protective factors for Alzheimer’s and NF-κB activity, implicating over-activation with an increased risk of the disease and suppression being associated with risk reduction. Future work, both in models of disease and trials in man, should focus on therapies that directly target NF-κB overexpression to explore whether early risk identification and targeted anti-inflammatory treatment can significantly increase the time of disease onset and, consequently, reduce the incidence of this devastating disease.

**AUTHOR CONTRIBUTIONS**

SJ and IK wrote the manuscript, and IK designed the figures.

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activation of microglia and reduces Alzheimer disease-like pathology in mice.  

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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