



NEUROINFLAMMATION



in Alzheimer's disease and Down syndrome

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*Al Joan, el meu superheroi preferit,
i al Cristian, per l'amor i la paciència.*

*(To Joan, my favorite superhero,
and to Cristian, for his love and patience)*

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ABSTRACT

Despite the clinical heterogeneity among individuals with Down syndrome (DS), the uniformity with which they acquire Alzheimer's disease (AD) neuropathology as they age makes this population important to study; not only to gain a better understanding of AD, but also because there are currently no effective treatments for DS with AD-like dementia. The pathological links between DS and AD are presumably due to the lifelong overexpression of AD-related genes encoded in chromosome 21, most of them triplicated in DS. The dosage-dependent increase of some of these genes in DS, such as the amyloid precursor protein, leads to plaque formation and further tangle aggregation; changes observed in AD. However, the vast number of chromosome 21 gene products and the complexity of the mechanisms they engender have suggested that they might be giving rise to many diverse neuropathological processes. Recently, the discovery of neuroinflammatory changes in the brains of DS individuals as well as the presence of inflammation-related genes within chromosome 21, prompted the possibility that early events in DS patients might be accelerating AD pathogenesis. Until the moment, DS mouse models have provided a powerful tool for translational research, being the Ts65Dn model the most widely used. The possibility that this model might also prove similarly useful in evaluating AD treatments for DS emphasizes the need to study the aging process in the Ts65Dn model. This review also delves into novel therapeutic insight, propounding the pro-inflammatory mediator GSK3 as a potential target to rescue AD-like neurodegenerative features in the Ts65Dn DS mouse model.

1. INTRODUCTION

Alzheimer's disease

Alzheimer's disease (AD) is the major cause of dementia among elderly (1,2), affecting more than 24 million people worldwide; a number which is predicted to quadruple by the year 2050 (3). Therefore, AD has become a major public health concern because of ever-increasing longevity and the resulting increase in the proportion of the world's population over the age of 60 years (4). AD is characterized by progressive deterioration of cognitive and functional abilities with considerable variability in behavioural manifestation (1,2). Many molecular lesions have been detected in AD, but the two core pathological hallmarks are neuritic plaques composed of extracellular deposits of fibrillar amyloid- β peptides, and intracellular neurofibrillary tangles primarily composed of abnormally hyperphosphorylated tau protein (5,6). These hallmark lesions lead to synaptic dysfunction, neuronal loss and gross brain atrophy (7).

Down syndrome

Down syndrome (DS) is due to triplication of chromosome 21 and is the most common genetic form of intellectual disability (8). It occurs in ~10 in 10,000 and 14 in 10,000 live births in European countries and the United States, respectively (9). The commonly referred to as trisomy 21 can be either the result of the presence of a full extra copy or the major portion of 21 human chromosome (also known as *Homo sapiens* autosome 21 –HSA21–) (1). A ~5.4 Mb region on HSA21q22 containing ~50 genes, commonly referred to as Down Syndrome Critical Region (DSCR), has been proposed to be sufficient to underlie most of the symptoms and signs of the disorder. However, more recent studies have shown that other regions of HSA21 contribute to different disease-related phenotypes. The onset of DS occurs during prenatal development, although some of the manifestations of the disorder may not become apparent until adulthood (9). In addition to mental deficiencies and characteristic physical traits noted at birth (10), triplication of chromosome 21 gene products result in an extremely high incidence of various congenital anomalies (11), as well as a markedly increased risk of AD (5,10,12–14).

The first English description of the link between DS and AD was first reported in 1948 by Jervis (15). Later, neuropathological evidence of the association between DS and AD was provided in 1929 by Struwe, in German (16), and in 1949 by Bertrand and Koffas, in French (17). Because life expectancy for people with DS has more than doubled over the past 30 years, their increased risk for AD has become a concern (1,2,18,19). Virtually, all individuals with DS develop AD-like pathology by their 40s (10,13) and by the age of 55-60, their current lifespan, at least 70% will develop dementia (12).

There is evidence of accelerated biological aging in DS by premature onset of alterations, such as skin changes, osteoporosis, immunological changes and AD-like pathology (2,13). Several studies reveal similarities and differences between DS aging process and AD, which account for the diverse hypotheses surrounding AD's still unclear etiology. The presumed reason for this association is the lifelong overexpression of AD-related genes encoded in chromosome 21, most of them triplicated (1). After the publication of the human genome (20), identification and characterization of the chromosome 21 gene products has become vital to understand the way in which neurodegenerative mechanisms lead to neuropathophysiological progression of sporadic AD in DS (10). However, the mechanisms underlying AD phenotype in DS are not fully understood.

2. PATHOLOGICAL LINKS BETWEEN AD AND DS

Amyloid- β ($A\beta$) peptide is deposited into extracellular plaques and blood vessel walls in brain in both AD and DS (12). Found in normal brain, the biological function of $A\beta$ remains unclear, although several physiological roles have been described (21,22). $A\beta$ peptides are potent neurotoxins, causing membrane permeabilization, increase in intracellular calcium and neuronal death by both necrosis and apoptosis (21). $A\beta$ peptides originate from proteolysis of the amyloid-precursor protein (APP) by the sequential enzymatic actions of β -secretase beta-site APP-cleaving enzyme 1 (BACE1) and γ -secretase, a protein complex with presenilin 1 (PS1) at its catalytic core (Figure 1) (6,7,23). APP is encoded by a gene mapped in chromosome 21 (5,10), therefore triplicated in DS individuals.

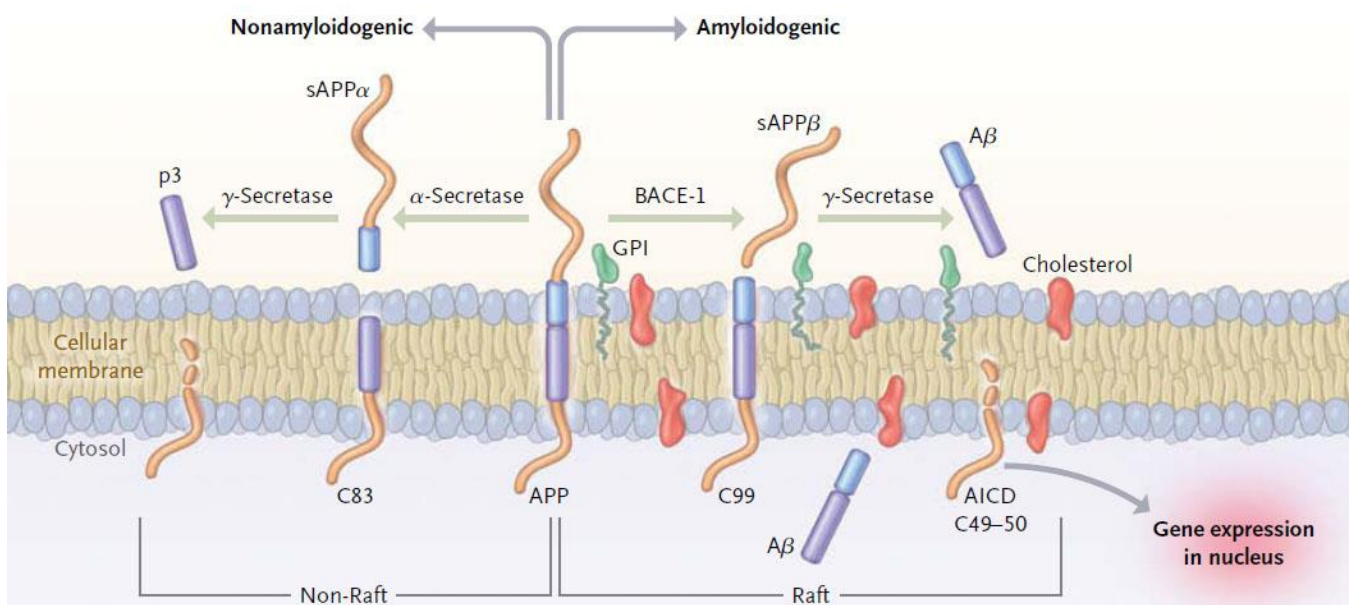


Figure 1 Processing of APP (23).

In addition, while BACE1 maps to chromosome 11, β -secretase BACE2 is located within chromosome 21, providing a logical link between BACE2 and APP processing (24). Both BACE1 and BACE2 cleave APP and lead to the generation of A β . However, BACE2 has been suggested to have a non-amyloidogenic function in the secretory pathway (25), unlike BACE1, which is thought to be the major β -secretase for generation of A β peptides by neurons (26). Due to the high similarity between BACE1 and 2, it has been proposed that the overexpression of BACE2 causes cleavage of APP and promotes A β production, as it is expressed in brain regions where A β accumulates (27).

The products of APP metabolism consist of 36 to 43 amino-acid peptides, in which monomers of A β_{40} are more prevalent than the aggregation-prone and damaging A β_{42} . An imbalance between the production and clearance of these two isoforms causes A β to accumulate (23,28). A β spontaneously self-aggregates into various forms, such as oligomers (2-6 peptides) or fibrils, which arrange into β -pleated sheets to form the insoluble fibers of advanced amyloid plaques (6). Nevertheless, increasingly attention is turning away from the deposits of extracellular insoluble aggregated amyloid in plaques and towards soluble oligomeric and even intracellular A β_{42} peptides (6,28). A β deposition is an early event in AD that precedes neuronal degeneration and cognitive decline by several years or even decades (7). A β deposition has been seen as early as age 8 in individuals with DS, beginning with diffuse A β_{42} deposits and progressing to compacted fibrillar plaques, leading to neurodegeneration (29,30). Similar to AD, A β_{42} is always more abundant than A β_{40} in DS individuals (12).

Imaging studies suggest that DS can exhibit significant amyloid deposition in the cerebral cortex already in their third or fourth decade of life, the earliest pathological changes occurring in the medial temporal lobe. These events, in addition to the cortical distribution of amyloid and neurofibrillary pathology and neuronal loss, resemble those of AD. Neurogenesis impairment in DS individuals makes them more vulnerable to lesions that might contribute to the early onset of dementia (31). Additionally, it has recently been suggested that a pathologic APP-dependent feed-forward process for A β deposition might be taking place in the brain. Binding of aggregated A β to APP would be promoting increased metabolic processing of APP through the amyloidogenic pathway, further contributing to A β deposition, neuritic degeneration, and synapse loss in AD (7,28), and by extension, to DS individuals.

APP overexpression has been shown to be linked to endocytic changes that are seen in both AD and DS brains before significant β -amyloid deposits and neurofibrillary pathology develops (32). Endocytosis is critical for the transport of neurotrophic factors, such as nerve growth factor (NGF) and/or the brain-derived neurotrophic factor (BDNF), in both retrograde and anterograde direction. APP is processed by β - and γ -secretases in endosomes, which are markedly enlarged within neurons in AD brain (12). These endosomal abnormalities are observed in DS fetal brain at 28 weeks of gestation, decades before the onset of AD pathology (33). Endosomal enlargement results in the disruption of many processes essential for neuronal functioning, including protein turnover at synapses, local and retrograde signaling, and subsequent effects on the cytoskeleton and protein synthesis (12,23).

Synaptojanin 1 (SYNJ1) is a lipid phosphatase whose gene is encoded on chromosome 21. SYNJ1 plays an important role in synaptic transmission, as it negatively regulates (dephosphorylating it) the levels of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂), a signaling phospholipid involved in many cellular processes. Perturbation of PtdIns(4,5)P₂ has been associated with cognitive impairment.

Trisomy for SYNJ1 in DS has been reported to be functionally linked to the enlargement of early endosomes. Conversely, SYNJ1 haploinsufficiency appears to be protective against the synaptotoxic action of A β , indicating that SYNJ1 protein levels might be relevant both in DS and AD (12,34–36).

Endocytosis is also critical for apolipoprotein E (ApoE) function, which acts as part of the physiological A β clearance mechanisms (12,37). ApoE has critical functions in redistributing lipids throughout the central nervous system (CNS) cells for normal lipid homeostasis and turnover, repairing injured neurons, maintaining synaptodendritic connections, and scavenging toxins (38). The ApoE ϵ 4 allele (ApoE4) is the strongest genetic risk factor for the age-specific manifestation of AD in people with and without DS (13), as it has been suggested that it inhibits A β clearance and/or stimulates A β deposition (39). Moreover, in patients with sporadic AD, the presence of the ApoE4 allele also leads to predisposition to possess enlarged endosomes (14,33).

The ApoE protein is a lipid carrier, and lipids are required for the assembly of the mitochondria and are oxidized by the mitochondria to generate energy. The ApoE4 gene is closely linked to a short allele of the TOM40 protein gene (40,41), which is in turn the central channel protein for the import of cytosolically synthesized proteins through the mitochondrial outer membrane and thus into the mitochondria (40,42). Sporadic onset AD appears to be associated to mitochondrial function and oxidative stress, being the latter one of the main causes of aging (43). Mitochondrial dysfunction leads to inhibition of the respiratory chain and eventually concludes in an increased reactive oxygen species (ROS). Such facts have been observed in AD and DS patients (38,40,44).

Neurofibrillary tangles (NFTs), which are filamentous inclusions in pyramidal neurons, accumulate later within the brain of individuals with AD (23). In DS brains, the distribution of plaques and tangles is very similar to AD, although the density is greater in DS (12). The major component of the tangles is an abnormally hyperphosphorylated and aggregated form of tau, a microtubule-associated protein (MAP) (6). Normally an abundant soluble protein in axons, tau promotes assembly and stability of microtubules and vesicle transport (45,46). Hyperphosphorylated tau is insoluble, lacks of affinity for microtubules, and self-associates into paired helical filament structures (Figure 2) (23,47).

DS dementia with hyperphosphorylation of tau has been thought to be due to the triplicated gene encoding the dual-specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A). This gene phosphorylates tau protein at several sites (47,48). Phosphorylation by DYRK1A primes further phosphorylation of tau by glycogen synthase kinase 3 β (GSK3 β), cyclin-dependent kinase 5 (CDK5), v-akt murine thymoma viral oncogene homolog (AKT) and other kinases (36,45,48–50). In addition, decreased activity of tau phosphatases has been associated with the development of AD in people with DS (49,51). DYRK1A also affects alternative splicing of tau by phosphorylating several factors that are implied in that process, which results in tau protein containing either 3 repeats (3R-tau) or 4 repeats (4R-tau) of exon 10 of the microtubule-binding domain (MBD) (52). Exclusion of exon 10 results in 3R-tau, whereas inclusion of it results in 4R-tau. Imbalance between 3R and 4R causes several-fold increase of NFTs and consequent neurofibrillary degeneration in the DS brain in comparison to subjects with sporadic AD (12,50,53). Furthermore, the overexpression of DYRK1A in DS has been observed to result in an enhanced phosphorylation of APP that facilitates amyloidogenic APP cleavage, thereby elevating A β ₄₀ and A β ₄₂ levels and contributing to the earlier onset of A β pathology (12,54).

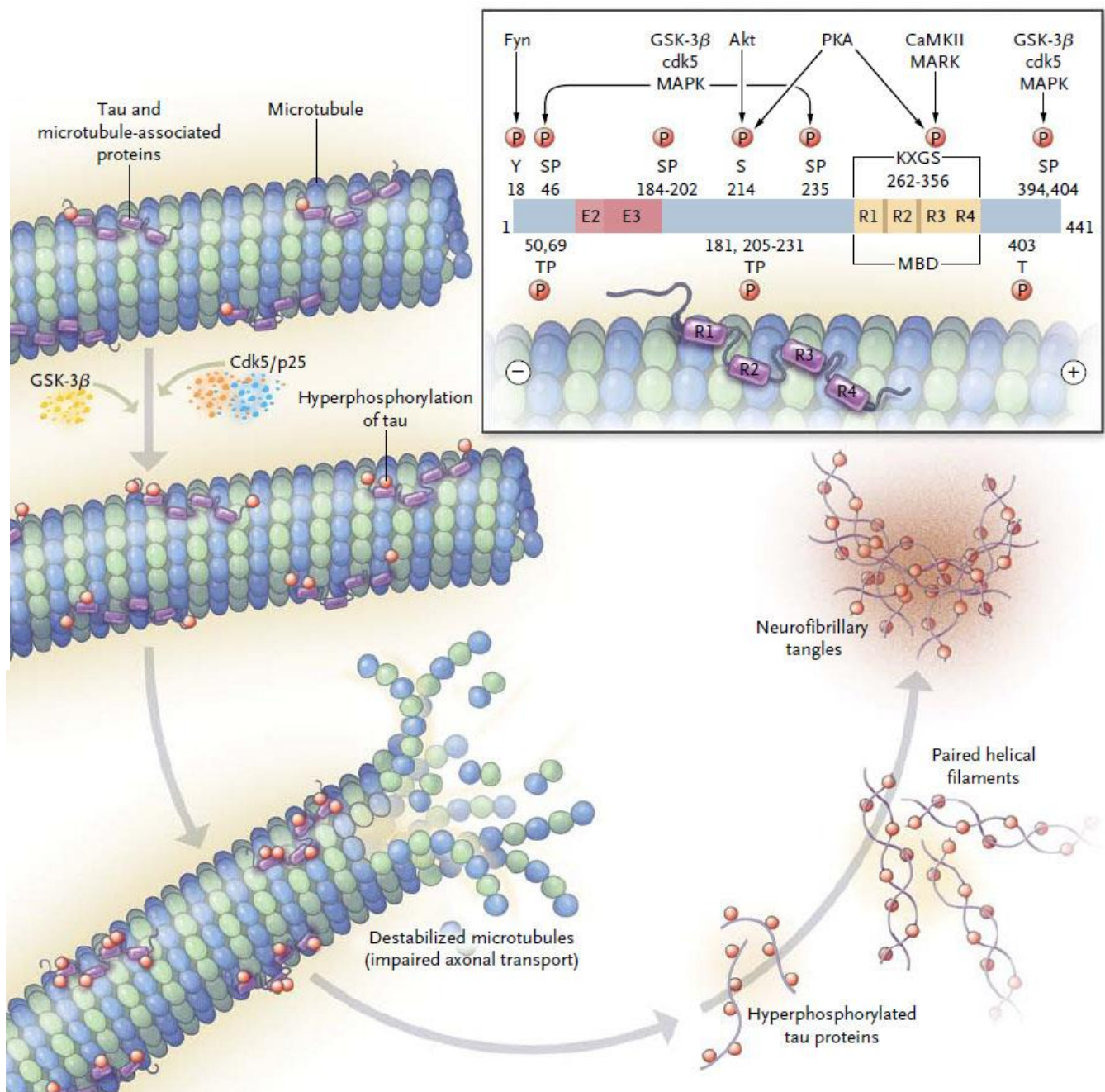


Figure 2 Tau structure and function (23).

The triplicated region of chromosome 21 not only maps for APP, SYNJ1 and DYRK1A genes, but also for important genes associated to mitochondrial function and oxidative stress, such as the regulator of calcineurin 1 (RCAN1) gene, the antioxidant enzyme Cu/Zn superoxide dismutase (SOD1), along with other proteins involved.

RCAN1 was named according to its ability to bind and inhibit the Ca^{2+} /calmodulin-dependent phosphatase calcineurin (55,56). RCAN1 is expressed in many brain regions, being implicated in a great variety of cellular processes, including adaptive responses to oxidative stress, mitochondrial function, immune responses and inflammation (51,57,58). Very transient RCAN1 induction has been proved to be protective against acute oxidative stress (59). However, chronic abnormal expression of RCAN1 has been implicated in the pathogenesis of AD and DS (55), as its overexpression results in

defects in learning and memory, accompanied by abnormal hippocampal long-term potentiation (LTP). Actually, RCAN1 is up-regulated in DS by approximately 1.9-fold in fetal brain tissue and up to 3-fold within the adult hippocampus (58). In addition to the fact that chronic activation of RCAN promotes neurodegeneration, it has been reported that A β peptide stimulates transcription of RCAN1, thus decreasing calcineurin levels, which results in the hyperphosphorylation of tau protein (51).

SOD1 is a potent endogenous neural-relevant copper and zinc binding enzyme which acts as a soluble cytoplasmatic and mitochondrial interspace protein that converts superoxide radicals into oxygen (O₂) and hydrogen peroxide (H₂O₂). SOD1 mutations are commonly associated with genetic susceptibility to anterolateral sclerosis. However, overexpression of non-mutated SOD1 in the absence of a peroxide-detoxifying enzyme promotes oxidative stress. This suggests that the elevation of SOD1 due to trisomy 21, if unmatched with an increase in the levels of peroxide-detoxifying enzyme, would be detrimental (5,10). Interestingly, a proteomics study showed that oxidative stress in fetal DS might not result from overexpression of SOD1 protein but appeared to be the consequence of low levels of antioxidant enzymes involved in removal of the H₂O₂ (60).

Based on these observations, it has been suggested that premature-aging dementia in both AD and DS is the result of mitochondrial dysfunction and impairment of the repair system of oxidatively-damaged mitochondrial DNA. This results in energetic deficiency, increased oxidative stress, altered calcium regulation, and increased cell death (38,40,42,61). Moreover, it has been demonstrated that oxidative stress induced by H₂O₂ results in significant increase in BACE1 promoter activity. Thus, up-regulation of β -secretase transcription by ROS might also contribute to AD pathogenesis (62).

Along with the already-mentioned processes involved in AD pathogenesis, other mechanisms triggering neuronal dysfunction are taking place in the brain of patients with AD and DS. Progressive age-related memory deficits occurring in both AD and in DS have been connected to degeneration of several neuronal populations, such as basal forebrain cholinergic neurons (BFCNs) and/or noradrenergic neurons of the locus coeruleus (LC-NE); although mechanisms are not fully elucidated. While degeneration of BFCNs occurs during normal aging, individuals with AD and DS are defined by rapidly accelerated loss of these neuronal projections, and cholinergic dysfunction correlates strongly with the progression of cognitive decline in both diseases. Likewise, LC-NE degeneration, although less studied than BFCN loss, is another hallmark of AD. LC-NE lesions have been associated with aggravated amyloid accumulation, oxidative stress, and memory loss in AD. Importantly, individuals with DS exhibit early and progressive degeneration of LC-NE neurons (12,57).

Trisomy of chromosome 21 in DS also leads to other cellular changes that precede the appearance of AD neuropathology and related-cognitive deficits. Several genes for inflammatory factors are present on chromosome 21 and thus are overexpressed (20). Inflammation might be a major contributor to the acceleration of AD pathogenesis in DS (13) and, therefore, identification of these factors underlying such exponential increase is essential for the understanding of the etiology of the AD-like neurodegeneration occurring in DS patients.

3. NEUROINFLAMMATION IN AD AND DS

3.1. THE CONCEPT OF NEUROINFLAMMATION

Historically, the strong belief that the blood-brain barrier provided not just anatomical and physiological protection for the CNS but also an “immunological privilege”, brought some results to be dismissed as artifacts about the presence of an inflammatory response within the brain (5). However, it is now clear that the brain may have unique immunologic properties, but it is by no means an immunologically isolated organ (63).

Neuroinflammation is the mechanism of the CNS that occurs in response to trauma, infections, and/or neurodegenerative diseases (64), such as AD. Neuroinflammation is a complex combination of acute and chronic responses orchestrated by the mobilization and interaction of several cell types within the CNS (including neurons, microglia, astrocytes and infiltrating leukocytes) and signaling molecules, producing an effect on both local and systemic extent (21,65).

The effect of neuroinflammation is considered neuroprotective when the inflammatory activity lasts for a short period of time. Acute inflammation comprises the immediate and early response to an insult, being beneficial for the CNS, since it tends to minimize further injuries and contributes to repair damaged tissue. Otherwise, long-lasting and self-perpetuating neuroinflammation results from stimuli that are persistent after the initial insult has passed. Chronic inflammation is associated with harmful consequences for the CNS (46,64–66). This neuroinflammatory response results in synaptic dysfunction, inhibition of neurogenesis, neuronal death, microglial priming inflammation, and an exacerbation of several disease pathologies within the brain (21).

Brain inflammation is characterized by activation of microglia and astrocytes, and expression of key inflammatory mediators and neurotoxic free radicals (67). On the one hand, microglial cells, which act as macrophages in the CNS, are the first and main form of active immune defense in the brain. In resting form, microglia is involved in functions such as neurogenesis, neuroprotection and synaptic pruning. Upon environmental stimulation, microglia becomes activated. Astrocytes, although being conventionally considered to be supporting cells to the neurons, appear to play an active part in the modulation of neural activity, becoming as well activated after an insult to the CNS (64,68).

The programmed response to acute stimuli in the CNS is defined by the activation of a specific gene profile that aims protecting the brain against tissue invaders. The so-called classical activation state (or macrophage activation state 1 –M1–) has a pro-inflammatory effect, characterized by a potent release of multiple cytoactive mediators, such as cytokines, chemokines and ROS. M1 response is mainly orchestrated by interferon γ (IFN γ) and tumor necrosis factor α (TNF α), as well as by traditional inflammatory cytokines interleukin 1 β (IL-1 β), IL-6 and IL-12 (5,21,64,65,69–72). Conversely, it has been reported that the gene profile induced by those insults to the CNS can change, shutting down the production of pro-inflammatory cytokines and increasing the production of factors that participate in tissue repair. This response provides an anti-inflammatory balance to an acute pro-inflammatory response, also referred to as alternative activation state (or macrophage activation state 2 –M2–). M2 response includes three different subtypes of states (M2a, M2b and M2c), which involve distinct factors. IL-4 and/or IL-13 initiate M2a response that is characterized by tissue remodeling

factors found in inflammatory zone 1 (FIZZ1) and chitinase 3-like 3 (YM1), as well as arginase 1 (AG1) and mannose receptor C1 (MRC1). Immune complexes stimulate an M2b response, which has components of both M1 and M2a states. Finally, IL-10 stimulates M2c response, which is sometimes called an acquired deactivation state. The M2c response is characterized by a series of markers that actively antagonize pro-inflammatory M1 signaling pathways (5,21,64,65,69–72).

Among the outcomes of neuroinflammation, neuronal death has been proved to be a direct consequence of signaling molecules produced in excess during the inflammation process, such as TNF α . The synthesis and release of nitric oxide (NO) via the enzyme nitric oxide synthase (NOS), a largely known source of oxidative cell-stress, also causes neuronal apoptosis by inhibiting neuronal respiration (21). Indeed, BFCNs are highly sensitive to inflammation and oxidative stress, since TNF α -induced cortical inflammation at cholinergic terminals leads to retrograde degeneration of these neurons. Moreover, it has been suggested that loss of LC-NE-innervation of BFCNs might trigger inflammation, providing a plausible explanation for the selective vulnerability of these neurons in AD and DS (57). However, neuronal apoptosis as a feature of early progression of dementia has been proved not to be sufficient as a measure of neurodegeneration, as other processes including synaptic impairment can lead to inadequate neurotransmission a long time before cell death. Although some physiological levels of TNF α and IL-1 β appear to be beneficial for synaptic plasticity, it is well known that prolonged neuroinflammation has deleterious effects leading to synaptic dysfunction. The extent to which neurodegeneration affects development of dementia is currently unknown but it is clear that the process is negatively modulated by neuroinflammation. There is evidence that neurogenesis is inhibited by a number of pro-inflammatory cytokines such as IL-6, TNF α , as well as microglia-priming responses, through neuronal death and inhibition of cell differentiation (21).

3.2. NEUROINFLAMMATION IN AD

In AD, A β monomers, oligomers, plaques and NFTs are potent promoters of neuroinflammation and neurodegeneration. Chronic increase of pro-inflammatory cytokines leads to both arise in APP synthesis and tau phosphorylation (21). In AD, many cytokines have been found to be altered, among which the most common are IL-1 β , IL-6, TNF α and TGF β (5).

IL-1 β , when bound to IL-1 receptor, initiates the mitogen-activated protein kinase (MAPK) pathways. p38-MAPK is a stress-activated protein kinase and its stimulation results in many pro-inflammatory responses (21). IL-1 β is found in microglia and astrocytes surrounding amyloid deposits (72). IL-1 β production in response to amyloid deposits initiates a series of events (5) that will be further explained. Moreover, it has also been shown that the IL-1 β -induced p38-MAPK inflammatory pathway increases tau protein phosphorylation and thus leads to increased tangle formation (73).

IL-6 is another cytokine that mediates immune responses and inflammatory reactions. Its main source is microglia, although astrocytes, neurons and endothelial cells are also capable of producing it. In AD brain tissue, IL-6 has been reported to be elevated in pathologically relevant regions (5), leading to the stimulation of CDK5, one of the kinases in charge to phosphorylate tau protein within NFTs (21).

Cytokine TNF α has been described to have both beneficial and detrimental effects in the CNS, as it acts as a potent pro-inflammatory and cytotoxic molecule as well as it provides protection from free-radical neuronal damage. There are two primary receptors for TNF α in the CNS: TNF α receptor 1 (TNFR1) and TNF α receptor 2 (TNFR2). TNFR1 mediates inflammation and neuronal degeneration, via TNF-receptor-1-associated death domain (TRADD) and TNF-receptor-1-associated factor 2 (TRAF2), recruiting enzymes that activate the transcription factor nuclear factor κ B (NF κ B). NF κ B, in turn, induces c-Jun N-terminal kinase (JNK) pathways leading to the expression of various other transcription factors that modulate apoptosis and inflammation. TNFR1 signaling is also directly pro-apoptotic through the Fas-associated protein death domain (FADD), which mediates the production of caspase-8, an enzyme strongly linked to apoptosis and neurodegeneration (21,74). Conversely, TNFR2 appears to mediate the beneficial pro-survival action of TNF α through NF κ B, which has been shown to have both beneficial and detrimental function for neurons during the inflammation process (74,75). In AD, brain expression of TNFR1 is elevated while TNFR2 levels are decreased (5).

In contrast to the cytokines discussed to this point, the transforming growth factor β (TGF β) is associated with repair mechanisms in the CNS. TGF β deficiencies have been reported to occur in AD brain, which might enhance AD pathology (5).

Besides the cytokine-mediated neuroinflammation processes, the immune system complement cascade activation has been associated with the development of amyloid plaques and NFTs. Among the different components of the complement cascade, C1q (the first-activated component within the cascade) has been specifically associated with activated microglia (14,21,76).

Finally, recent evidence has prompted the emergence of GSK3 as a pro-inflammatory mediator implied in neurodegeneration, with particular notability in AD pathology. GSK3 is a constitutively active protein kinase with a great number of biological functions, some of which are directly related to neuroinflammation processes. GSK3 appears to be highly involved in the promotion of microglial migration and inflammation activation, positively regulating the production of TNF α via NF κ B, IL-6 and NO in the brain. Low non-toxic concentrations of GSK3 might also have anti-inflammatory effects (21).

3.3. NEUROINFLAMMATION IN DS: THE ROLE OF CHROMOSOME 21 GENE PRODUCTS

The importance of chromosome 21 genes in AD development owes its evidence to several mutations in these genes linked to familial AD (77) as well as the certainty of development of AD in individuals with DS (78). In order to recognize the importance of an extra copy in the progression of AD pathology in DS, it is vital taking into account chromosome 21 gene products that might regulate expression of neuroinflammatory processes, and/or are regulated by neuroinflammatory cytokines.

Inflammation is known to occur in the brains of both AD and DS patients in response to the presence of neuritic plaques and NFTs. In addition, plaques and tangles can directly influence inflammation (5,76). The presence of aggregated A β and hyperphosphorylated tau can be accelerated by the excessive production of pro-inflammatory mediators, giving rise to the idea that the formation of AD pathology in both AD and DS patients leads to the initiation of several self-propagating cycles (10,21). Such early events have been reported in DS fetuses and are initiated by the overexpression of

the triplicated gene APP. This is followed by a similarly dramatic increase in the levels of the astrocyte-derived cytokine S100 calcium-binding protein β (S100 β), also encoded in chromosome 21, and the cytokine IL-1, encoded by chromosome 2 genes IL-1A and IL-1B. The latter's product are IL-1 α and β , which are up-regulated by both APP and S100 β , whose expression is in turn induced by IL- β (10).

Wilcock and Griffin describe in detail other chromosome 21 gene products that also take part in neuroinflammatory responses, although their influence on neuroinflammation processes and their relation to neuropathogenesis is less studied (5,10). The table below shows these inflammatory-associated genes found in chromosome 21, either the previously mentioned ones and the others still not specified, which are triplicated in most DS patients.

Gene	Protein	Function
<i>βAPP</i>	Amyloid beta precursor protein >5 fold overexpression in DS	Neuronal acute phase protein precursor of fragments A β in Alzheimer plaques and sAPP for induction of IL-1 β
<i>BACE2</i>	β -site APP-cleaving enzymes-2	Cleaves APP for less A β and increases IL-1R2, a decoy protein for excess IL-1 capture
<i>S100B</i>	S100 calcium binding protein astrocyte-derived cytokine	Upregulates IL-1 β and β APP expression, released in response to TNF α
<i>CXADR</i>	Coxsackie virus and adenovirus receptor	Activation of JNK and p38-MAPK pathways leading to production of M1 cytokines.
<i>ADAMTS1</i>	ADAM metalloproteinase with thrombospondin type 1 motif, 1	Secreted protease known to be induced by IL-1 β
<i>ADAMTS5</i>	ADAM metalloproteinase with thrombospondin type 1 motif, 5	Secreted protease known to be induced by IL-1 β and TGF β .
<i>TIAM1</i>	T-cell lymphoma invasion and metastasis 1	Necessary for cytokine-mediated generation of oxidative species through NADPH oxidase.
<i>SOD1</i>	Superoxide dismutase 1	Scavenges superoxide radicals producing H ₂ O ₂ and O ₂ .
<i>IFNAR2</i>	Interferon α , β , and ω receptor 2	Activates JAK/STAT mediated anti-inflammatory pathway
<i>IFNAR1</i>	Interferon α , β , and ω receptor 1	Activates JAK/STAT mediated anti-inflammatory pathway
<i>IFNGR2</i>	Interferon γ receptor 2	Activates JAK/STAT mediated anti-inflammatory pathway
<i>RIPK4</i>	Receptor-interacting serine-threonine kinase 4	Necessary for signaling through TNFR1
<i>CBS</i>	Cystathione- β -synthase	Catalyzes production of hydrogen sulfide (H ₂ S) bimodal regulation of inflammation
<i>PRMT2</i>	Protein arginine methyltransferase 2	Blocks the actions of NF κ B in the nucleus

Table Summary of the inflammation-related genes located in chromosome 21 (10).

The chromosome 21 gene encoding the protein CXADR (coxsackie virus and adenovirus receptor) has a dual function, both as a viral receptor and an adhesion molecule associated with tight junctions. It is highly expressed in brain as well as in systemic secretory organs. In the absence of viral infection, CXADR expression is increased in models of myocardial inflammation. It seems plausible that increased expression of CXADR in DS because of its gene triplication might contribute to overactivated pro-inflammatory response in the brain similar to that in the heart. Moreover, CXADR has been recently shown to induce stress-activated MAPK pathways in the heart, leading to increased production of M1-activation-response-related cytokines, such as IFN γ , IL-12, IL-1 β , TNF α and IL-6; as well as changes in tight junctions in the endothelial cells of the cardiac vasculature. Such altered expression of CXADR on the endothelial cells of the cerebral vasculature in DS patients might lead to altered infiltration of peripheral inflammatory cells into the brain, influencing the inflammatory response. Furthermore, increased neural expression of CXADR and its induction of IL-1 β and MAPK could contribute to the previously reported MAPK-p38-dependent hyperphosphorylation of tau and tangle formation.

IL-1 β induces the expression of two other chromosome 21 genes that encode two secreted proteinases of ADAMTS (a desintegrin and metalloproteinase with thrombospondin motifs) family (ADAMTS1 and ADAMTS5). Both act as inflammation-dependent proteinases that degrade extra

cellular matrix proteoglycans. Given the greater IL-1 β immunoreactivity in DS brain, the interaction between IL-1 β and these proteinases may explain, at least in part, the dramatic overexpression of ADAMTS1 in DS. Moreover, triplication of these proteinases may contribute to exacerbated degradation of extra cellular matrix proteins in response to an inflammatory insult.

The chromosome 21 gene TIAM1 (T-cell lymphoma invasion and metastasis 1) is a critical regulatory factor in IL-1 β -induced synthesis of NADPH oxidase, via induction of Rac1 (guanine nucleotide exchange factor). TIAM1 contributes to the activation of Rac1 for the consequent stimulation of NADPH oxidase in pancreatic β -cells. It has been shown that TIAM1 protein expression is increased in fetal DS brain. By analogy, excess of TIAM1 may contribute to the dramatic oxidative stress in DS brain, in response to an inflammatory insult involving IL-1 β .

The genes encoding for interferon receptors of IFN α 1, IFN α 2 and IFN γ (IFNAR1, IFNAR2 and IFNGR2, respectively) are located on chromosome 21, and are therefore all subject to triplication in DS. IFNAR1 and IFNAR2 both respond to IFN α , β and/or γ , and upon ligand binding activate the JAK/STAT pathway, leading to induction of pro-inflammatory cytokines, including IL-1 β , TNF α and IL-6. IFNGR2 uses the same signaling pathway but uniquely responds to IFN γ . In the trisomy 16 mouse model of DS, both IFNAR2 and IFNGR2 are triplicated, leading to development of significant pathology *in utero* that rarely allows their survival to birth. Anti-IFN IgG treatment of fetuses improves mice phenotype, suggesting that triplication of IFN receptors contributes to the lethal pathology. Furthermore, a partial knockout of IFNAR2 and IFNGR2 improves growth and viability of cultured neurons derived from trisomy 16 mouse fetuses. Therefore, the expected hyper-responsiveness to IFN in individuals with DS may contribute to the elevated inflammatory profile both in the brain and systemically.

RIPK4 (receptor-interacting serine-threonine kinase 4) is a protein kinase involved in multiple cell signaling pathways. One of these pathways is the signaling pathway for the activation of NF κ B, which has already been mentioned to be important in promoting pro-inflammatory responses mediated by cytokines such as TNF α . RIPK4 is also involved in the signaling cascade of the TNF α receptor TNFR1, which is mostly implicated in the toxic effects of TNF α , suggesting that overexpression of RIPK4 increases responsiveness of TNFR1 to TNF α , exacerbating in turn the effects of TNFR1.

The chromosome 21 gene product CBS (cystathionine beta synthase) is a cytosolic enzyme that catalyzes the desulfhydration of cysteine-producing hydrogen sulphide (H₂S). H₂S is a complicated signaling molecule with bimodal action on inflammation, in which low levels appear to be anti-inflammatory, while high levels appear to exacerbate neuroinflammatory processes. Overexpression of CBS in trisomy 21 and its relation to development of AD in DS is, at present, unknown.

The enzyme PRMT2 (protein arginine methyltransferase 2) encoded in chromosome 21 catalyzes methylation of arginine, necessary for regulation of the JAK/STAT pathway, which increases the expression of neuroinflammatory cytokines (IFN γ , IFN α and IL-6) and, via inhibition of NF κ B, might promote apoptosis. Natural degradation of proteins containing methylated arginine results in the production of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS. As in the plasma of DS patients with pulmonary hypertension there is an increase in ADMA levels, it is possible that an increase of ADMA in DS brain may lead to reduced NO production with an increase in the JAK/STAT pathway activity, and changes might occur toward the inflammatory state of microglia.

It can be concluded that an exacerbated glial pro-inflammatory response might occur in DS brain as a direct consequence of trisomy 21. Nevertheless, it must be taken into consideration the relative lack of data regarding the neuroinflammation process in DS in comparison to the significant background on the role of inflammation in AD.

4. DS ANIMAL MODELS TO UNDERSTAND AD-RELATED NEUROINFLAMMATION

The conserved synteny between the genomic segments on HSA21 and mouse chromosome 16 (MMU16), 10 (MMU10) and 17 (MMU17) has led to the generation of many mouse models to understand the phenotypic consequences of gene dosage imbalance and elucidate the role of particular chromosomal regions or specific genes in the pathogenesis of DS (9).

4.1. DS MOUSE MODELS TO UNDERSTAND AD

The most widely used model of DS is the Ts65Dn mouse, which contains a partial trisomy of approximately 104 MMU16 genes orthologous to those found in HSA21, translocated onto a short segment of MMU17 (79). Ts65Dn mice are characterized by progressive loss of learning and memory, reduced hippocampal LTP, cholinergic deficit in the basal forebrain, increased neuronal oxidative stress and neuroinflammation; changes observed in AD. Ts65Dn mice also show age-related elevations in APP and A β peptide levels in the cortex and hippocampus, thought to be responsible for the amyloid deposition and degeneration of the BFCNs found in DS individuals over 40 years of age (12,80,81).

However, two facts regarding the Ts65Dn genetics must be kept in mind in evaluating it as a model for AD and DS. First, the Ts65Dn mouse lacks trisomy of a number of HSA21 genes with functional features that are of compelling relevance to development of AD, such as S100 β . Second, the Ts65Dn model is also trisomic for a small centromeric segment of MMU17 that is not orthologous to HSA21. This segment is more gene rich than previously assumed and indeed includes 50 protein coding genes. Among these, there are paralogs of some HSA21 genes, including SYNJ2 (paralog of SYNJ1) and TIAM2 (paralog of TIAM1), plus several dynein light-chain genes, whose increased dosage could influence endosomal transport (12,36,82).

In addition to the genetic concerns for replicating human trisomy in mice, the lack of plaques and tangles in DS mouse models fails to replicate the age-related hallmarks of AD and DS individuals. This fact might be due to the three-amino-acid difference in the mouse vs. the human A β sequence that might be increasing the tendency of the protein to self-aggregate in humans. There is also evidence suggesting that A β deposition might be a trigger for tau aggregation, which would in turn limit the ability to develop tau abnormalities in mice (12).

Although Ts65Dn mice also fail to develop amyloid plaques, they exhibit elevated levels of APP and associated peptides in the hippocampus, as well as increased phosphorylation of tau protein (57). Likewise, transgenic mice for APP gene, appear to exhibit overexpression of APP protein in neocortex and hippocampal region, mimicking features of both AD and DS. These transgenic models with AD-like pathology also show neuritic plaques and mild learning defects (83). Learning deficits have also been shown in transgenic mice overexpressing SYNJ1, these deficits being however more severe in Ts65Dn

mice (83). The metabolism of PtdIns(4,5)P₂, the substrate of protein SYNJ1, is altered in the brains of Ts65Dn mice, a defect that is normalized by reducing the SYNJ1 gene from three to two copies (36).

In mouse models overexpressing HSA21 genes, hippocampal function is often affected, resulting in reduced LTP due to excessive inhibitory input. Mouse models of DS carrying three copies of large segments of MMU16 syntenic to HSA21 (e.g. Ts65Dn, Ts1Cje and Dp(16)1Yey/+, all of which contain three copies of mouse DYRK1A gene) exhibit decreased hippocampal LTP. In contrast, transgenic mice harboring a single copy of the DYRK1A gene, although showing impaired cognitive behaviors, surprisingly show increased hippocampal LTP. A possible interpretation of this discrepancy could be that the triplication of a single or more HSA21 genes orthologs in Ts65Dn, Ts1Cje and Dp(16)1Yey/+ mice might be responsible for the decrease in hippocampal LTP, and thus triplication of the DYRK1A ortholog might actually help reduce the impact of the causative genes (12,82–84).

Similarly to individuals with DS, the Ts65Dn mice exhibit elevated oxidative stress both systemically and in the brain. Part of the explanation for this might be the triplication and thus overexpression of APP and SOD1 genes, leading to a deregulation of the ROS production and elimination within the brain. ROS accumulation triggers the age-related neuropathology observed in Ts65Dn mice and in DS individuals. However, studies in the Ts1Cje mouse model for DS, which does not include triplication of the SOD1 or APP genes, show that it exhibits neurodegenerative changes in frontal cortex, with neurons showing nuclear and mitochondrial abnormalities. This neurodegenerative phenotype is associated with increased oxidative stress and ROS generation, and suggests that other triplicated genes, but not APP and SOD1, might be involved in mitochondrial abnormalities and increased oxidative stress observed in DS. Moreover, while APP and SOD1 each might contribute to the disease, neither gene is solely responsible for the degenerative changes that occur in DS (57,81).

Similarly to individuals with AD and DS, Ts65Dn mice display signs of neurodegeneration and age-related atrophy and loss of BFCN and LC-NE neuron populations. This feature is not shared by the current AD mouse models. It has been reported that NGF infusion reverses the BFCN deficit, supporting the view that BFCN degeneration might be caused by retrograde transport failure of NGF. Other factors that might lead to neurodegeneration are increased APP production or one of its cleavage products, mitochondrial dysfunction, calcium deregulation, oxidative stress, neuroinflammation or some combination of these factors (12,57,81).

4.2. EVIDENCE OF NEUROINFLAMMATION IN DS MOUSE MODELS

Both AD and DS individuals consistently exhibit chronic brain inflammation characterized by increased microglial and astrocytic activation, coupled with a potent cytokine release (85,86).

Microglial activation typically arises in the entorhinal cortex of the limbic system before developing in the hippocampus and surrounding cortex, as well as in the basal forebrain. BFCNs are highly sensitive to inflammation and oxidative stress, but specific biological mechanisms for their selective loss in AD and DS have not yet been revealed. Inflammation at cholinergic terminals appears to lead to retrograde degeneration of BFCNs. It has also been suggested that loss of noradrenergic fibers from the LC-NE to BFCNs triggers inflammation, providing a plausible explanation for the selective vulnerability of these neurons in AD and DS (13,14,57). Since Ts65Dn mice exhibit significant

degeneration of both BFCN and LC-NE neurons, it is not surprising that they show accelerated and age-related astrocytosis and microgliosis in the hippocampus. Furthermore, depletion of those neuronal terminals in mouse models of AD results in increased inflammatory cytokine production, activated microglia and amyloid deposition. Based on this evidence, it is difficult to determine whether BFCNs and LC-NE degeneration activates the inflammatory pathways, or if the cytokine production by astrocytes and microglia, in turn, causes the neuronal degeneration presented by AD and DS individuals. Most likely, all of these processes have interactive and escalating effects (57).

Similarly to microglia, activated astrocytes produce numerous inflammatory mediators in both AD and DS brain. Though Ts65Dn mice have an extra copy of APP they lack other important AD-related genes, such as S100 β (87). S100 β protein is produced by astrocytes and has a variety of functions, mainly in the brain, during both developmental and adulthood stages. In aged individuals and in pathological states, including AD and conditions of increased inflammation, the protein levels increase. Moreover, S100 β is highly expressed in close vicinity of A β deposits. Transgenic mice overexpressing the S100 β gene exhibit astrocytosis and neurite proliferation in the CNS, such as seen in the early development of AD pathology in DS individuals. However, in contrast to DS brain features, brains of S100 β transgenic mice are normal sized and show no gross pathological hallmarks (88–90). Nevertheless, APP-overexpressing Ts65Dn mice have been reported to increase expression of glial markers, including S100 β , within the neural cells progenitor population along the ventricles. These observations implicate a potential cyclical path of neural progenitor injury whereby overexpression of S100 β and APP leads to mitochondrial impairment, cell death and consequent inflammation (87).

Finally, to our knowledge, the previously mentioned pro- and anti-inflammatory cytokines have not been investigated so far in the Ts65Dn brain. However, it seems plausible that such activation both in microglia and astrocytes might contribute to a potent release of them. This conclusion would, in turn, be in agreement with the already mentioned gene-dosage hypothesis, in which DS brain pathophysiology reflects the interaction between many genes, most of which have not yet been shown to be involved in neuroinflammatory processes.

5. PIVOTAL ROLE OF GSK3 IN AD-LIKE PATHOLOGY: NOVEL INSIGHTS TOWARDS DS THERAPIES

GSK3 is an ubiquitously expressed, constitutively active, proline-directed serine/threonine kinase involved in a variety of cellular processes, including glycogen metabolism, gene transcription, proliferation and growth. There are two GSK3 genes from which GSK3 α and GSK3 β are derived (91,92). Although both GSK3 isoforms are widely expressed in all tissues, insights from mouse models suggest that they exhibit tissue specific physiological functions; being particularly abundant in the brain, where GSK3 β is mainly present (91,93). In the CNS, GSK3 regulates developmental processes, including neurogenesis, migration, axon growth and guidance, and synaptic plasticity (94). The regulation of GSK3 can occur through the phosphorylation of specific amino acid residues and/or by the formation of protein complexes, which result in its subsequent activation or inactivation (95).

The first regulatory mechanism of GSK3 activity involves the phosphorylation of specific residues of GSK3 by other kinases, and auto-phosphorylation (96). Phosphorylation of serine residues at positions 21 in GSK3 α and 9 in GSK3 β correlates with inactivation of its kinase activity (92,94,96).

Many protein kinases are capable of phosphorylating GSK3 at this residue, such as AKT (also termed Protein Kinase B, PKB), PKA and PKC (96). Most of the latter are downstream mediators of pathways that are known to inhibit GSK3 activity through serine phosphorylation, including phosphatidylinositide 3-kinase (PI3K), the mammalian target of rapamycin (mTOR), and indirectly through the mitogen-activated protein kinase / extracellular signal-regulated kinases (MAPK/ERK) pathways (95). These pathways are known to play a critical role in differentiation and survival of neuronal and glial cells (94). Other mechanisms can also mediate the inhibitory phosphorylation of GSK3, such as growth factors, esters, and insulin (95,96). In contrast, protein phosphatases 1 and 2A (PP1 and PP2A) have been reported to dephosphorylate the inhibitory site of GSK3, resulting in its activation (94). Likewise, tyrosine phosphorylation in positions 279 in GSK3 α and 216 in GSK3 β also correlates with an increase of its kinase activity. Different candidate kinases, such as Pyk-2 and Fyn, have been reported to be able to phosphorylate GSK3 on this residue (92,94,96).

In addition to the phosphorylation state, GSK3 activity is also regulated by the formation of protein complexes in the context of several pathways, including Wnt cascade (Figure 3). Although the role of Wnt signaling in mature neurons remains largely unexplored, recent data indicate that Wnt proteins are important mediators of neuronal function and morphology, neurogenesis, and synaptic plasticity; being implied in neurological disorders associated with developmental abnormalities as well as in neurodegenerative diseases (94,95). When extracellular Wnt proteins are absent, frizzled receptor (FzR) and/or the low-density lipoprotein-related protein 5 and 6 (LRP5/6) receptors cannot be activated, making possible the formation of the complex formed by GSK3 and β -catenin, among other proteins. Within this complex, GSK3 phosphorylates β -catenin leading to its degradation through proteasome pathway. Conversely, when Wnt ligands are present, FzR and LRP5/6 receptors are activated, inducing the destabilization of the protein complex. This results in GSK3 inactivation, which favors an increase of unphosphorylated β -catenin levels, allowing its interaction with different transcription factors. As a consequence, the expression of cell survival genes is promoted (92,94–97).

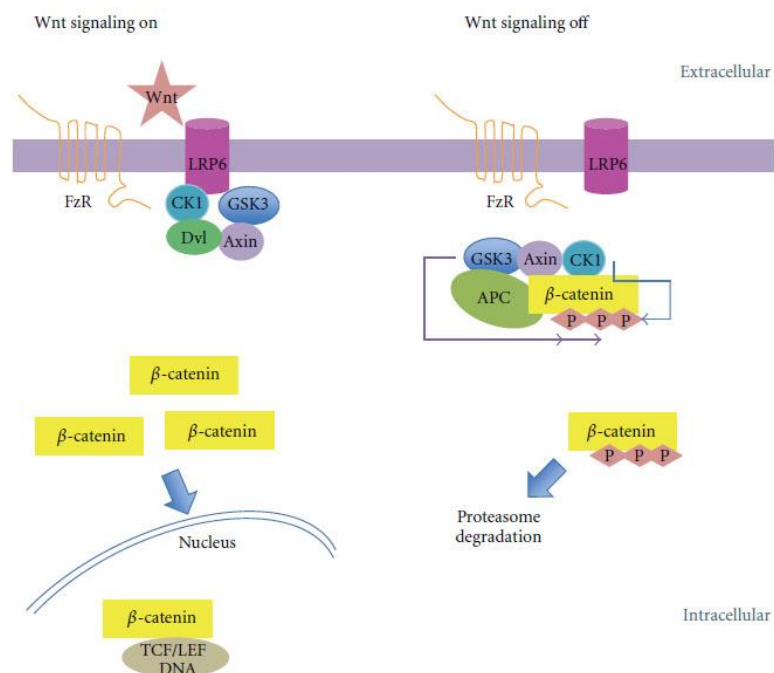


Figure 3 GSK3 regulation by Wnt signaling (94).

5.1. THE ROLE OF GSK3 IN AD

Recent evidence supports a role for GSK3 in producing some of the characteristic pathological hallmarks of AD, such as learning and memory impairment, loss of synaptic plasticity, increased production of A β , hyperphosphorylation of tau, and inflammatory responses. GSK3 also reduces acetylcholine synthesis, a fact in accordance with the cholinergic deficits present in AD. Moreover, GSK3 is a key mediator of apoptosis and thereby might directly contribute to neuronal loss in AD (92). In fact, polymorphisms in the promoter of GSK3 coding sequence have been recently reported to be risk factors for late onset AD (94,96).

GSK3 appears to play a role in LTP and LTD (long-term depression), although the mechanism remains unclear. It has been described either that overactivation of GSK3 in AD leads to inhibition of LTP and induction of LTD (94); as well as that GSK3 is required for LTD induction, but since LTP would inhibit GSK3, therefore LTP might in turn inhibit LTD. However, a direct inhibition of LTD by LTP has not yet been reported (98). In any case, these facts might just partially explain the learning and memory deficits present early in this neurodegenerative disorder, as suppression of Wnt or PI3K signaling has also been described to impair LTP. Since GSK3 is negatively regulated by these pathways, its overexpression might result in such deficiencies observed in AD individuals (92).

Although the role of GSK3 in neurodevelopment remains only partially understood, it is known that changes in neuronal morphology and plasticity are affected by GSK3-induced mechanisms (94). GSK3 promotes microtubule and neurofilament stabilization by phosphorylating cytoskeletal proteins (such as actin and tubulin), a process required for synaptic reorganization during memory formation (92,94). Many downstream substrates of GSK3 are also involved in synaptic remodeling, thus being also implied in the proper establishment of connections during such process (92). In addition, one mechanism related to both synaptic modeling and microtubule dynamics is Wnt signaling. Wnt proteins promote axonal arborization and increase the incorporation of synaptic proteins, having a fundamental role in synapse formation. These effects are achieved through the Wnt pathway in which GSK3 activity is inhibited, and consequently the phosphorylation state of the axon is reduced (94).

Apart from its participation to cognitive impairment in AD, changes in GSK3 activity might be a molecular link between the two main histopathological markers of the disease: A β overproduction and tau hyperphosphorylation (94,96). GSK3 induces the hyperphosphorylation of tau at both primed and non-primed phosphorylation sites, placing the protein as an important tau kinase possibly involved in the formation of further NFTs (96). In brain of AD patients, GSK3 colocalizes with NFTs, appearing in neurons even before pre-tangle changes (94). Additionally, GSK3 also regulates APP cleavage by β - and γ -secretases, leading to increased production of A β (94,97). The neuronal exposure to such increase of A β augments, in turn, GSK3 activity through both Wnt and PI3K pathways.

The proposed model of GSK3 activation by A β peptide in AD is shown in Figure 4. On the one hand, A β induces the expression of the protein dickkopf 1 (DKK1), a negative regulator of Wnt signaling, which internalizes LRP6 receptor, leading to the inhibition of Wnt pathway and thus GSK3 activation. DKK1 has also been reported to promote tau phosphorylation and neurodegeneration. Interestingly, this is up-regulated in AD and has been shown to colocalize with NFTs. A β can also bind

to FzR receptor and inactivate Wnt signaling as well. ApoE protein, which likely binds to the LRP6 receptor, also inhibits this signaling pathway, therefore activating GSK3. Moreover, the LRP6 receptor has been identified as a risk gene for late onset AD in ApoE4 negative individuals. On the other hand, A β oligomers bind to the insulin receptor and inhibit the PI3K pathway, thus preventing AKT to phosphorylate and inactivate GSK3. Proof of this evidence is the reported association of AD with diabetes and insulin resistance, in which insulin genetic studies have found insulin signaling genes to be susceptibility loci for AD (91,92,94,96,97).

A recent study has provided further insights into a potential mechanism by which GSK3 might affect APP turnover by increasing β -secretase BACE1 gene, responsible of cleaving APP (91). This study reports that specific inhibition of the GSK3 β , but not GSK3 α isoform, reduces BACE1-mediated cleavage of APP and the consequent A β production, resulting in an inhibition of neuritic plaque formation and amelioration of memory deficits in AD model mice (99). It has also been suggested that GSK3 might influence γ -secretase activity as well. GSK3 has been shown to bind PS1, the catalytic component of the γ -secretase complex, acting perhaps as a docking protein and regulating phosphorylation of some GSK3 substrates, such as tau and β -catenin. PS1 has been shown to inactivate GSK3 through the PI3K pathway, preventing tau phosphorylation and apoptosis (91).

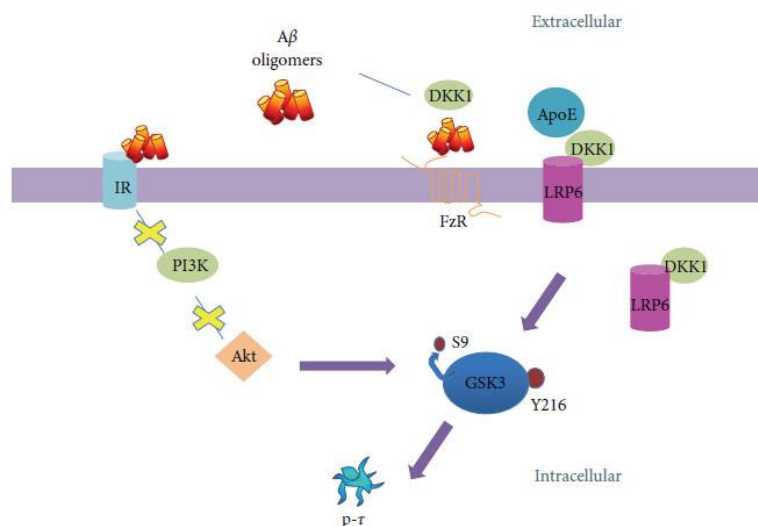


Figure 4 Proposed model of GSK3 activation by A β peptide in AD (94).

In addition, due to the importance of hypoxic abnormalities in AD pathology, interaction between GSK3 activity and oxidative stress has gained interest. It has been proposed that oxidative stress and ROS might promote neurodegeneration and cell death through DNA fragmentation, lipid peroxidation, and mitochondrial pro-apoptotic pathways involving caspases and GSK3. One of the mechanisms by which GSK3 might potentiate apoptosis and cell death is by regulation of transcription factors, including heat shock factor 1 (HSF1), cyclic-AMP-response element-binding protein (CREB) and NF κ B. NF κ B, as already pointed out, is a key mediator of responses to TNF α , and suppresses the signal for cell death. Increased GSK3 activity in AD would lead to decreased expression of these transcription factors, thus promoting neuronal apoptosis. GSK3 has been also shown to regulate cell survival by facilitating various pro-apoptotic pathways, including Wnt cascade. However, the mechanisms underlying this

neuronal loss and if neuronal loss is directly responsible for the progressive dementia in AD are still not clear and should be further studied in depth (100).

In addition to being implicated in the core pathogenic events of AD, GSK3 has been identified as a target for inflammatory-mediated diseases and plays a key role in mediating inflammatory responses all over the body (92). In the brain, GSK3 has recently been shown to be involved in the activation of glial cells, specially microglia and astrocytes. Following stimulation, GSK3 promotes both microglial migration and the production of inflammatory molecules by microglia, thus leading to inflammation-induced neurotoxicity. Migration of microglia plays a critical role in response to an injury, such as it is the amyloid deposition (101). Likewise, GSK3 promotes astrocyte activation, which is characterized by up-regulation of glial fibrillary acidic protein (GFAP) expression and migration (102). Inhibition of GSK3 has been reported to attenuate the production of pro-inflammatory cytokines (IL-1 β and TNF α) and augment the production of anti-inflammatory cytokines (IL-10) by microglia, providing neuroprotection during neuroinflammatory conditions. Moreover, GSK3 inhibitors have also been demonstrated to reduce microglial NO production and migration. In conclusion, GSK3 inhibitors have been shown to provide protection from inflammatory-induced neuronal toxicity (101,102).

5.2. GSK3 INHIBITORS AS A POTENTIAL THERAPEUTIC TOOL FOR NEUROINFLAMMATION IN AD AND DS

In the last two decades, drug discovery and development in the AD field have primarily focused on targets within the amyloid cascade, so far with disappointing results. The unique position of GSK3 as a pivotal player in AD pathogenesis has prompted significant efforts in the last few years to develop selective-potent GSK3 inhibitors, as its inactivation has been reported to reverse some of the pathological effects associated to AD and DS (91,92). Until now, GSK3 inhibition has been carried out using non-selective-isoform pharmacological inhibitors, such as lithium, valproate, as well as small antisense oligonucleotides. However, the exact mechanism by which this occurs remains unclear and, in fact, the isoform specificity of the effect on A β production is still highly controversial (96,103,104). Therefore, at present, the search for new therapies in AD aims to develop specific-isoform GSK3 inhibitors. The multiple functions that GSK3 performs in the brain, also suggest the possibility to treat the GSK3-neuroinflammatory-related effects observed in AD and DS individuals. Such evidence places GSK3 as a potential therapeutic target to attenuate neuroinflammatory effects due to AD pathology. Further understanding of the role of GSK3 in glial activation may provide novel targets for controlling the neuroinflammatory component of neurodegenerative diseases, such as AD and DS (101).

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