

# Hyperphosphorylation of Tau Protein in Down's Dementia and Alzheimer's Disease: Methylation and Implications in Prevention and Therapy

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## Abstract

The changes of insulin signaling, calcium signaling, mitochondrial decline and oxidative stress have been implicated in the hyperphosphorylation of tau protein found in Down's syndrome dementia. Such pathogenic etiologies have clear implications in the prevention and therapy of Down's syndrome (DS) dementia. The occurrence of methylation defects in DS is discussed and though controversial, more recent studies do show significance. Kinases such as DYK1A and GlcNAcylation are discussed as well as a Cdk5 inhibitory peptide (CIP). Even sleep medicine has been demonstrated in that seniors, who have better sleep, suffer less cognitive decline than those with sleep problems with enhanced clearance of  $\beta$  amyloid and tau neurofibrillary tangles. Studies have reported a high incidence of sleep problems in Down's. Environmental toxin arsenite and low dose methyl mercury have been speculated to induce tau phosphorylation. Dietary changes to low glycemic carbohydrate and gluten avoidance should be made. Adding B vitamins may be equally important to prevent brain atrophy especially in those with MTHFR and MTRR gene defects. The therapeutic strategy of reducing insulin resistance by up regulation of PPARs alpha with glitazones and decreasing calcium influx into the mitochondria is mentioned. Protecting mitochondrial decline from oxidative stress with antioxidants, and treatment with CBD, polyphenols, ellagic acid, resveratrol and other grape bioflavonoids and moderate magnetic fields is discussed.

**Keywords:** Hyperphosphorylation; Down's syndrome; Alzheimer's disease

## Introduction

Tau is a group of microtubule-associated proteins found in neurofibrillary tangles (NFT) in Alzheimer's disease (AD) brain. Discovered 39 years ago, as a heat stable protein that facilitates the microtubule assembly by Weingarten [1], Cleveland and associates demonstrated that tau is a phosphoprotein which by phosphorylation, negatively regulates its ability to stimulate microtubule assembly in 1977 [2]. Iqbal and Grundke in 1986 found that tau was hyperphosphorylated in brain extracts from AD cases and that it may cause the defect in microtubule assembly and self-assembly into paired helical filaments, forming neurofibrillary tangles [3].

Tau phosphorylation events are probably sequential and according to Johnson and Stoothoff and the ability for tau to self-associate might be caused by an imbalance in the activity of specific protein kinases or phosphatases (Phases) [4] (Figure 1).

Tau is abnormally phosphorylated in a group of rare autosomal degenerative disease known as front temporal dementia with Parkinson's caused by a mutation of the tau gene located on chromosome 17q21 [5]. Other tauopathies include progressive supranuclear palsy, chronic traumatic encephalopathy, Lytico-Bodig disease (Parkinson-dementia complex of Guam), a tangle-predominant dementia, with NFTs similar to AD, but without plaques. Ganglioglioma and gangliocytoma, meningioangiomas, subacute sclerosing pan encephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, and lipofuscinosis are also listed as tauopathies [6]. Pick complex disorders have been suggested by Kertesz as a spectrum of presentations of the same disorder because of their pathology [7]. In all of these tauopathies, the neurofibrillary changes are made up of abnormally hyperphosphorylated tau, thus highly suggesting that this abnormality causes their dementia and labeled this, the neurotoxic state of tau. The authors suggest that the inhibition of abnormal hyperphosphorylation

of tau offers a promising target for therapy [8].

A recent study by Jellinger's group during the recent 11<sup>th</sup> International Conference on Alzheimer's and Parkinson's Disease in Florence Italy on Tau pathology revealed that neurons surrounded by perineuronal nets containing aggrecan seem to be protected against tau pathology and thus toxicity may be averted by perineuronal nets. These nets consist of negatively charged, sugary proteins called chondroitin sulfate proteoglycans (CSPGs). The nets form a cage around neurons. There is a balance between a fine regulation of axonal and dendritic growth so that adult CNS can preserve important connections while still allowing for structural plasticity and help to stabilize synapses and preserve memories and develops as critical periods end, preventing further plasticity [9].

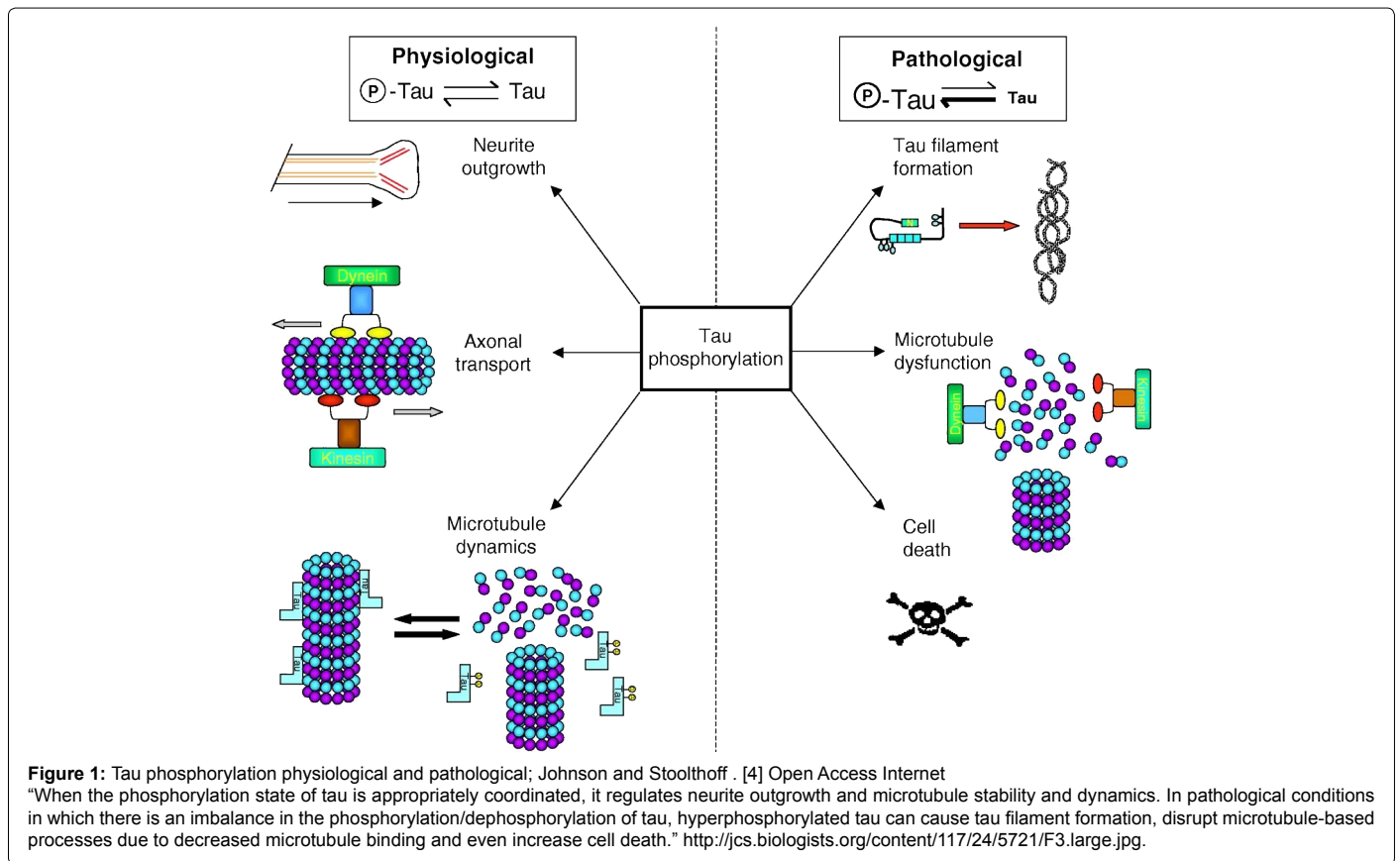
Current dogma is that AD progression begins with amyloid beta deposition in the entorhinal cortex before tau pathology. A recent study by Heiko Braak at Goethe University, Frankfurt, Germany, cast doubt on this sequence by reviving an old debate about whether tau pathology comes earlier. This new work found pre-tangle tau deposits in the brainstem of the majority of people under 30. This has now led some researchers to suggest that the disease starts very early in life, with tau pathology in the brainstem [10]. Therefore early prevention of

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tau hyperphosphorylation may be instrumental than any therapy after dementia is diagnosed.

### Down's Syndrome Dementia

Down's syndrome dementia with hyperphosphorylation of tau has been thought to be due to the gene encoding the minibrain kinase/dual-specificity tyrosine phosphorylated and regulated kinase 1A (DYRK1A). This gene is located in the Down's syndrome (DS) critical region of chromosome 21, which is tripled in Downs. Studies by Weigle and associates show that DYRK1A phosphorylates tau protein, and since this kinase is also involved in tau protein phosphorylation, it contributes to neurofibrillary degeneration, and may be enhanced in patients with DS [11]. A more recent study by Weigle on DYRK1A overexpression in DS brains is that it may contribute to early onset neurofibrillary degeneration directly through hyperphosphorylation of tau and indirectly through phosphorylation of alternative splicing factor, leading to an imbalance between 3R-tau and 4R-tau [12].

Now it isn't just Down's syndrome, where the brain tau is three to four hyperphosphorylated. The abnormally hyperphosphorylated tau binds to normal tau instead of the tubulin and this binding leads to the formation of tau oligomers. According to the Iqbal team, the abnormally hyperphosphorylated tau is capable of sequestering not only normal tau but also MAP1 and MAP2 and causing disruption of the microtubule network promoted by these proteins [13]. Weigle et al. mention the O-GlcNAcylation of tau in their article which brings the concept of this abundant, dynamic and inducible post-translational modification [14].

Similar to phosphorylation, O-GlcNAcylation (or simply

GlcNAcylation) is in some cases, occurring at the same or adjacent sites, modulating each other. GlcNAcylation affects protein-protein interactions, activity, stability, and expression. GlcNAcylation of proteins within the insulin signaling pathway and contributes to insulin resistance and helps explain the increase of Alzheimer's incidence among diabetics. Dias and Hart at Johns Hopkins reported that hypoglycemia within the brain may reduce the normal GlcNAcylation of tau, exposing kinase acceptor sites, thus leading to hyperphosphorylation, which induces tangle formation and neuronal death [15].

### Down's Syndrome and other defects: Methylation

The most common genetic cause of mental retardation is Down's syndrome and the genes involved in homocysteine/folate metabolism plays a role in the etiology of this disorder. The concentrations of the metabolites related to the methylation cycle in the blood of 35 young individuals with DS and 47 controls of comparable age was tested by Obeid and associates in Homburg Germany They applied a mathematical model to learn more about the regulation of the methylation cycle in DS Concentrations of cystathionine, cysteine, betaine, choline, dimethyl glycine, holotran scobalamin ,S-adenosyl homocysteine (SAH), S-adenosyl methionine (SAM), were significantly higher in DS compared to the controls. They concluded that excess of cystathionine beta synthase (CBS) activity, increases in the activities of methionine synthase and betadine homocysteine methyl transferase, and in methionine input [16].

Another methylation gene defect suspected in Down's syndrome is the occurrence of methylene tetra hydrofolate reductase gene (MTHFR). The MTHFR gene encodes an enzyme that plays an important role in processing amino acids, specifically the conversion of homocysteine to

methionine. The most common form of genetic hyperhomocysteinemia results from a 677C>T polymorphism (NM\_005957.4:c.665C>T, rs1801133) in MTHFR. [17].

There has been over 40 point mutations of this gene have been identified. Of these, mutations on the points at C677T and A1298C seem to have the most clinical significance. The C677T polymorphism shows a wide regional and ethnic variation. Homozygosity (TT) among Whites is 6-14%. In African populations and in Blacks living outside of Africa such as in Brazil and in the United States, the frequency falls to less than 2% for the TT variant. The prevalence rises in Mediterranean and Hispanic population. For example, among Hispanics in prevalence ranges as high as 21%. Northern China and Japan show an 18% incidence [18].

An earlier study by Hobbs and associates demonstrated that the results are consistent with the preliminary observation that the MTHFR 677C-->T polymorphism is more prevalent among mothers of children with Down's syndrome than among control mothers, with an odds ratio of 1.91 (95% confidence interval [CI] 1.19-3.05). They also found that the homozygous MTRR 66A -->G polymorphism was independently associated with a 2.57-fold increase in estimated risk (95% CI 1.33-4.99) [19]. The Hobbs and other studies suggesting the connection of MTHFR gene defect with Down's syndrome [20] was considered controversial by other geneticists. Kokotas et al. in a Danish study in 2009 concluded that the common MTHFR 677C>T polymorphism is not likely to be a maternal risk factor for DS in their cohort and that the difference to previous studies could be explained by small sample size or geographic variation in gene polymorphisms involving gene-nutritional or gene-gene or gene-nutritional-environmental factors [21].

Never the less, two more recent studies do suggest a link between Down's syndrome and MTHFR and MTRR. In 2010, folate gene polymorphism and the risk of DS syndrome pregnancies in young Chinese women were published. Genetic polymorphisms in MTHFR, methionine synthase reductase (MTRR), reduced folate carrier1 (RFC-1), methionine synthase (MTR) involved in folate metabolism and the risk of offspring's of young Chinese women with Down's syndrome was examined. They found that homozygous MTHFR 677C>T polymorphism was more prevalent among the mothers of children with DS than among the control mothers, with an odds ratio of 3:51. Reduced (RFC-1) was not associated with MTRR but the homozygous MTRR 66A>G polymorphism was independently associated with a 3.16-fold increase in estimated risk [22]. Functional variant in methionine synthase reductase was described in Down's syndrome. Since methionine synthase reductase (MTRR) is one of the key regulatory enzymes involved in the metabolic pathway of homocysteine, the polymorphism C524T of the MTRR gene association with DS was studied. A total of 104 mothers of children born with DS and 184 healthy mothers were examined. A Significant differences in the distributions of C524T alleles were observed between case and control mothers; a decreased risk of DS was associated with the 524TT genotype (OR=0.34), CT+TT genotype (OR=0.60)[23].

### Calcium channel and tau hyperphosphorylation

S Schuchmann and associates first demonstrated that altered Ca<sup>2+</sup> signaling and mitochondrial deficiencies were found in hippocampus neurons of trisomy 16 mice, a model of Down's syndrome in 1998 [24]. H Jang's group in 2010 described truncated beta-amyloid peptide channels that were found in amyloid plaques of AD and in preamyloid lesions of Down's syndrome, a model system for early-onset AD study [25]. H Zempel et al. went on to publish that Abeta oligomers

cause localized Ca<sup>2+</sup> elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules, mitochondrial and spines depletion in Alzheimer's disease. Incipient and local changes similar to those of Abeta oligomers were evoked by cell. Stressors such as H<sub>2</sub>O<sub>2</sub>, glutamate, serum deprivation) suggesting some common mechanism of signaling [26].

Anekonda and Quinn have published that a specific set of downstream pathways in Alzheimer's included dysregulation of intracellular calcium (Ca<sup>2+</sup>), up-regulation of caspase-cleaved tau and hyperphosphorylation of tau (ptau). They discovered that aging and amyloid beta promote Ca<sup>2+</sup> influx into neurons by way of L-type calcium channels (LTCC). The authors propose that calcium channel blocking as a therapeutic strategy using isradipine which is a FDA approved dihydropyridine that selectively binds to Ca (v) 1.2 in the hippocampus and in their studies in vitro lessened the toxicity of beta amyloid. They discovered that intraneuronal or soluble extracellular Aβ can induce protein kinase A (PKA), which in turn binds to LTCC and promotes increased Ca<sup>2+</sup> influx leading to hyperphosphorylation of tau and suppression of autophagy which is a cleaning system within the cell [27].

**Location of tau hyperphosphorylation, toxicity and CDK5 inhibitory peptide:** Tau which is normally axonal in location in the neuron, shifts to the soma and dendrites of neurons when hyperphosphorylated. Mandelkow reported that when microtubules breakdown or tau becomes hyperphosphorylated and detached from intact microtubules, it becomes free to diffuse into cell bodies and dendrites and therefore toxic. The investigators concluded that there is some barrier of tau-axon microtubule association that inhibits tau from travelling into the cell soma [28]. Since tau hyperphosphorylation is so important in the pathogenesis of Alzheimer's and tauopathies, inhibitors that would block the responsible kinases have become important for therapeutic purposes. Researchers have targeted GSK-3 such as Astra Zenica and Takeda Pharmaceutical, a highly selective GSK3 inhibitor, 2-methyl-5-(3-{4-[(S)-methylsulfinyl]phenyl}-1-benzofuran-5-yl)-1,3,4-oxadiazole (MMBO) that blocks hippocampal tau phosphorylation and tau pathology in mice. Allon Therapeutics is working on davunetide (NAP) which in a mouse model protected tau-hyperphosphorylation and a phase 2/3 human trial of tauopathy, supranuclear palsy, is being tested [29].

Another provocative study involving tau and the mitochondria was the August 23 Neuron 2013 study that tau overexpression indirectly elongates mitochondria, which then malfunction and cause cell death. In fruit flies it binds and stabilizes the cytoskeleton protein F-actin preventing a mitochondrial fission protein from reaching the organelle. According to Mel Feany of Harvard Medical School, this mechanism is one of several sabotaging the mitochondria, leading to cell death. Via a delicate balance of mergers and divisions, the mitochondria are maintained by fusion and fission respectively at just the right length. Mitochondrial abnormality has been found in a number of neurodegenerative diseases including Parkinson's and Alzheimer's. DuBoff and his group further demonstrated that muscle-specific knockdown (Mfn) of the fly or a fission-driving dynamin-related GTPase (DLP1) overexpression rescued tau-induced mitochondrial elongation and toxic effect [30].

Another study on tau looked at the binding for ATP/GTP at the N-terminal region. Farid et al. recently published that tau is able to bind ATP and not GTP and that this binding induces tau self-assembly into filaments. They discovered that ATP concentration was indicative of this. At 1 mM ATP, the filaments are 4-7 nm in width, whereas at 10 mM



ATP, the filaments appeared to establish lateral interaction, bundling and twisting, forming filaments like Paired Helical Filaments (PHF) isolated from Alzheimer disease brain. Interestingly ATP –induced self-assembly was not energy dependent as the nonhydrolysable analogue of the ATP induced the same assembly [31].

In an inflammatory model of AD, APP/PS1/tau triple transgenic mice, Frank Laferla lab's using lipopolysaccharide (LPS) to activate microglia activation produced tau hyperphosphorylation at 6 months in the hippocampus. Normally, the transgenic mice start to show tau hyperphosphorylation at 1 year. The hyperphosphorylation was occurring at selected sites consistent with the action of a specific kinase. They further found the kinase, neither the IL-1-activated p38 MAP kinase nor JNK kinase, but instead was CDK5 [32].

Harish C Pant and his group at NINDS showed that neuronal infections with Cdk5 inhibitory peptide (CIP) selectively inhibit p25/Cdk5 activity and suppress the aberrant tau phosphorylation in cortical neurons [33]. A more recent paper by Pant's team in 2013 looked at the aberrant hyperactivation of Cyclin-dependent kinase 5 (Cdk5), by the production of its truncated activator p25, which results in the formation of hyperphosphorylated tau, neuroinflammation, amyloid deposition, and neuronal death in vitro and in vivo. This occurs as a result of a neurotoxic insult that invokes the intracellular elevation of calcium to activate calpain, which cleaves the Cdk5 activator p35 into p25. They used the selective inhibition of p25/Cdk5 hyperactivation via the overexpression on Cdk5 inhibitory peptide (a central fragment of p35 ;residues 154– 279), of p35; known as CIP which rescues the neurodegenerative pathologies caused by p25/Cdk5 hyperactivation without affecting normal neurodevelopment afforded by normal p35/Cdk5 activity. They were able to demonstrate this by using a transgenic mouse that overexpressed CIP constitutively in the forebrain under the direction of the Camk2a promoter [34].

### **Tau hyperphosphorylation and insulin resistance via PPARS**

There are a number of investigators who believe that AD is fundamentally a metabolic disease with substantial and progressive derangements in brain glucose utilization and responsiveness to insulin and insulin-like growth factor [IGF] stimulation. They state that AD is now recognized to be heterogeneous in nature, and not solely the end-product of aberrantly processed, misfolded, and aggregated oligomeric amyloid-beta peptides and hyperphosphorylated tau. It has even been hypothesized that AD might be 'type 3 diabetes' by the group at Temple University in an extensive review of this hypothesis [35]. Since the regulation of lipid metabolism and glucose utilization is critical for the maintenance of cellular energy homeostasis, cells have developed various mechanisms to respond to internal and external stimuli that can signal imbalances to this. According to Burns and Vanden Heuvel, these mechanisms include rapid responses such as phosphorylation events as well as relatively latent effects on gene transcription. The nuclear receptor superfamily known as Peroxisome Proliferator-Activated Receptors (PPARs) have evolved to become the biological sensors of lipid and glucose metabolism [36].

Anti-diabetic agent rosiglitazone which is a PPAR agonist was used in a Phase III clinical trial because it reduces  $\beta$ -amyloid pathology and inflammation in animal models of AD [37]. In diabetes 2 rats and streptozotocin injected diabetic mice, rosiglitazone (RSG) reduced tau phosphorylation and also is associated with reduction of JNK activity and not of GSK3 $\beta$  activity [38]. However, there was no evidence of efficacy of 2 mg or 8 mg RSG XR monotherapy in cognition or global function in the APOE- $\epsilon$ 4-negative or other analysis populations of

Alzheimer's disease.

The mechanism underlying the A $\beta$ -induced Tau hyperphosphorylation is mediated by the impaired insulin signal transduction as demonstrated by Tokutake et al. His group developed a novel cell coculture system to assess the effect of extracellular A $\beta$  at physiologically relevant levels naturally secreted from donor cells on the phosphorylation of Tau in recipient cells. Treating cells with the insulin-sensitizing drug rosiglitazone attenuated the A $\beta$ -dependent hyperphosphorylation of Tau [39].

### **Prevention of tau hyperphosphorylation in Down's syndrome**

Taking the results of many therapeutic failures of drug therapies to modify cognition in Alzheimer's dementia, the evidence suggest that mono-drug therapy is moot and the most promising approach in Down's syndrome is the arena of the prevention of dementia.

**Sleep medicine needed:** For example, sleep problems in the elderly and the correlation with dementia have recently been published. Bennett and colleagues reported that cognition declined faster in older adults who slept in fits and starts, and that they were more likely to be subsequently diagnosed with AD than those who slept well. They found from 201 autopsies that better sleep consolidation attenuates the effect of APOE genotype on incident AD and development of neurofibrillary tangle pathology [40]. Maiken Nedergaard, University of Rochester Medical Center, New York, found that animals clear A $\beta$  and other metabolites from the brain more effectively while they are sleeping. From real-time analyses using real-time assessments of tetramethyl ammonium diffusion and two-photon imaging in live mice, they showed that natural sleep or anesthesia are associated with a 60% increase in the interstitial space and in turn, convective fluxes of interstitial fluid increased the rate of  $\beta$ -amyloid clearance during sleep. The paper implies that the physiological function of sleep is to essentially wash the brain free of the day's debris [41].

The high prevalence of sleep disorders, particularly obstructive sleep apnea, is well established in children with Down's syndrome. A recent study examined the relationship between sleep and cognition in Down's syndrome. Twenty-nine adolescents and young adults with Down syndrome participated in the study. The authors studied components of executive functions that have been shown to be impaired in previous studies of Down syndrome. They found that participants with Down's syndrome with higher body mass index also had increased caregiver reports of sleep apnea symptoms. Individuals with high ratings of sleep disruption also showed greater difficulties with executive function. They concluded that additional studies are needed to investigate the effect of exercise interventions and weight reduction on sleep disorders in this population [42].

**Clean up the environment:** University of Pennsylvania investigators demonstrated that the environmental toxin arsenite causes a significant increase in the phosphorylation of several amino acid residues (Thr-181, Ser-202, Thr-205, Thr-231, Ser-262, Ser-356, Ser-396, and Ser-404) in tau, which are also hyperphosphorylated under pathological conditions. They discovered that arsenite-induced phosphorylation of some mutant proteins, especially R406W, was altered at several phosphorylation sites, indicating that these mutations can significantly affect the structure of tau in vivo [43].

Investigators at Consumer Reports confirmed Dr. Mehmet Oz's statement that his study found arsenite in apple juice in 10 of three dozen apple-juice samples with total arsenic levels exceeding 10 parts per billion. The Consumer Reports found that roughly 10 percent of our

juice samples, from five brands, had total arsenic levels that exceeded federal drinking-water standards [44].

Low dose methylmercury was studied in neuroblastoma (NB) cells (SH-SY5Y) in the cell culture model to study low-dose effects of MeHg on cell growth, cell survival, reactive oxygen species (ROS), and the phosphorylation of tau protein. When cells were incubated in culture with MeHg (50 and 100 nM), there were significant decreases in cell viability as well as significant increase in ROS generation. Additionally, the level of phosphorylated tau was significantly increased after treatment at both 50 and 100 nM MeHg, compared with controls. Pretreatment of cells with the antioxidant, N-acetylcysteine (1.25 mM) and the calpain inhibitor, MDL-28170 (10  $\mu$ M), significantly attenuated the effects of MeHg (50 and 100 nM) on cell viability as well as on tau phosphorylation [45].

**Novel agents inhibiting tau hyperphosphorylation and modulating microglia:** A marijuana component cannabidiol (CBD) which is totally void of psychoactive effect has been recently proposed as an antioxidant neuroprotective agent in neurodegenerative diseases. A research group in Naples Italy used CBD to rescue PC12 cells from toxicity induced by A $\beta$  peptide aggregates in cell culture. They discovered the effect of cannabidiol is mediated through the Wnt/ $\beta$ -catenin pathway rescue in A $\beta$ -stimulated PC12 cells. The Wnt pathway is disrupted by 6-amyloid and this represents a pivotal event in the neuronal cell death in Alzheimer's disease. Wnt – mediated inhibition of GSK-3 $\beta$  is followed by unphosphorylated  $\beta$ -catenin accumulating in the cytoplasm and translocating to the nucleus. There they bind T cell factor /lymphoid enhancing factor that expressing genes for neuronal survival and homeostasis [46]. It had been found that in the hippocampal and cortical neurons exposed to A $\beta$  peptide, GSK-3 $\beta$  is activated through phosphorylation as Wnt signaling function has been dramatically lost [47].

Another paper examined the effect of Sativex<sup>®</sup>, a mixture of delta 9-tetrahydrocannabinol and cannabidiol acting on both CB1 and CB2 receptors in a parkin-null human tau overproducing mice model of tauopathy. The cannabinoid mixture reduced the deposition of both tau and amyloid in the hippocampus and cerebral cortex of PK- $\beta$ -TauVLW mice and increased autophagy. Sativex<sup>®</sup>, after a short administration in animals with present behavioral and pathological abnormalities, improves the phenotype, the oxidative stress, and the deposition of proteins [48].

In Spain, the effects of CBD with those of other cannabinoids were tested on microglial cell functions in vitro and on learning behavior and cytokine expression after A $\beta$  intraventricular administration to mice. CBD and WIN, a mixed CB(1)/CB(2) agonist, after sub-chronic administration for 3 weeks, were able to prevent non-learning of a spatial navigation task and cytokine gene expression in  $\beta$ -amyloid-injected mice. All the cannabinoids decreased lipopolysaccharide-induced nitrite generation, which was insensitive to cannabinoid antagonism. Also CBD and other cannabinoids promoted microglial cell migration that may serve a beneficial function as a requisite for phagocytosing aggregated A $\beta$ . They concluded that CBD modulated microglial cell function in vitro and induces beneficial effects in an in vivo model of AD [49].

**Changing the diet to low glycemic and avoid gluten:** Previously, the GlcNAcylation of proteins within the insulin signaling pathway and contributing to insulin resistance was mentioned in AD and DS dementia. Therefore the necessity of introducing a low glycemic diet to the patient with Down's is more than apparent.

Besides avoiding insulin resistance, the next important dietary change is gluten. Celiac disease has been found to have a high occurrence in Down's syndrome from 5-10% in some countries. In Turkey, eleven of the 47 patients DS patients (23.40%) were found to be serologically positive, 10 (21.28%) having antigliadin antibody concentrations above normal; and six (12.77%) being positive for antiendomysial antibody [50]. Tissue Transglutaminase (TTG) is now the only antibody available for celiac testing. A group in Naples have published that transglutaminases are ubiquitous enzymes which catalyze post-translational modifications of proteins. This cross-linking of glutamyl residues of a protein/peptide substrate to lysyl residues of a protein/peptide co-substrate may be a factor in neurodegenerative disease such as Alzheimer's disease, Parkinson's disease, supranuclear palsy, Huntington's disease and other polyglutamine diseases. These diseases, are characterized in part by aberrant cerebral transglutaminase activity and by increased cross-linked proteins in affected brains [51].

**Add antioxidants to diet:** Since prevention is the most practical solution in Down's dementia, adding antioxidants to the diet for the prevention of Alzheimer's is gaining traction in the medical literature. A study using cabernet sauvignon and a muscadine wine that are characterized by distinct component composition of polyphenolic compounds, significantly attenuated the development of Alzheimer's disease (AD)-type brain pathology and memory deterioration in a transgenic AD mouse model. The group at Mt Sinai NY found muscadine treatment attenuated A $\beta$  neuropathology and Abeta-related cognitive deterioration in Tg2576 mice by interfering with the oligomerization of A $\beta$  molecules to soluble high-molecular-weight A $\beta$  oligomer species that are responsible for initiating a cascade of cellular events resulting in cognitive decline. [52] Polyphenols have been demonstrated to inhibit amyloid fibril formation regardless of the oxidative properties due to its interaction only when the native confirmation of amyloidogenic proteins transforms to the "assembly" conformation. [53]

Another paper points out the misfolding of tau could trigger microglial activation via MAPK pathways and cause the release of NO, and proinflammatory cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$  and tissue inhibitor of metalloproteinase 1 [54].

Muscadine antioxidants are obtained from a total of 88 phenolic compounds of diverse structures were identified including 17 in the pulp, 28 in the skin, and 43 in the seeds. Seventeen compounds were identified for the first time in muscadine grapes. The compounds identified in seeds included hydrolyzable tannins, flavan-3-ols and condensed tannins, ellagic acid derivatives, and quercetinrhamnoside. The skin contained hydrolyzable tannins, flavonoids, including anthocyanin 3, 5-diglucosides, quercetin, myricetin, resveratrol and kaempferol glycosides [55]. Muscadine seed and skin contain the highest amount of antioxidants are now available in capsule form.

**Moderate magnetic fields in mild to moderate cognitive impairment:** The use of moderate magnetic field therapy in Alzheimer's disease was first published by this author along with L Pearce et al. as an abstract at the Society for Neuroscience in 2006. Research by Alan MacDonald MD [56] and later by Judith Miklossy MD [57] has suggested Borrelia burgdorferi persist in the brain in chronic Lyme neuroborreliosis as the infectious etiology in Alzheimer's disease. Inflammation in the form of reactive astrocytes and microglia is thought to play an important role in Alzheimer's disease pathogenesis. The magnetic therapy of 0.5 Tesla consists of two large and strong non-pulsing DC electromagnet field (EMF) therapy with the patient lying in a focal point between the two electromagnets presented in Figure 2.



**Figure 2.** Patient lying between negative pole of a 0.5 Tesla DC electromagnetic below and positive pole above. In Alzheimer's the poles would be below and above the patient's head.

When the patient was treated with the device there is a temporary increase in the magnetic force on the atoms of the body resulting in a higher velocity and precession of certain orbiting electrons resulting in enhanced electron transfer and chemical reactions. Alzheimer's disease (AD) is a neurodegenerative disease secondary to oxidative stress, associated with genetic and environmental factors such as exposure to pesticides and heavy metals with subsequent depletion of mitochondrial protective enzymes, superoxide dismutase and glutathione via free radical toxicity. Seven AD patients were treated with DC EMF from 12 to 330 hours under an IRB approved protocol. Results: All seven AD patients after magnetic therapy improved in cognition and memory. However, the previous enhanced cognition deteriorated after 6 months of completing the DC EMF therapy in most patients. Discussion: was hypothesized to up-regulate superoxide dismutase and glutathione and eliminate heavy metals. It may even drive *Borrelia Bb* (Lyme disease) due to its strong negative electrostatic charge from the tissues and up-regulate endogenous stem cells. In order to have sustained improvement in Alzheimer patients suspected of having ongoing bacterial infection with *Borrelia*, long term antibiotic therapy (2 months) was proposed for those testing positive for *Borrelia Bd.* instituted along with 200 hours of DC EMF therapy [58].

Later research from Wang along with Kevin Yarema at Johns Hopkins Whitaker Bioengineering Institute that moderate magnetic fields (MMF) can modulate signaling networks was based on reports that lipid bilayers acting as biosensors are capable of responding to magnetic exposure. Specifically, moderate strength magnetic fields can change biophysical properties of membranes that include hyperpolarization, redox potential, and fluidity, thereby altering flux through sodium (Na<sup>+</sup>) and calcium (Ca<sup>2+</sup>) channels. As a result, changes in cytosolic concentrations of the calcium ion – which serves as a second messenger in several signaling pathways – occurs ubiquitously in cells exposed to MMF. Wang and associates delineated known connections between IL-6 and other molecular players (e.g., Ca<sup>2+</sup> and TLR4) as well previously unappreciated links (e.g., ganglioside involvement in IL-6 activation that acts even in the absence of MMF, offering a new controlling mechanism for IL-6. This study concluded by showing that MMF leads towards oligodendrocyte differentiation in human embryonic cells by preferentially stimulating pre-oligodendrocyte markers over the astrocyte markers usually associated with IL-6 exposure [59].

Another paper using published and unpublished research by Wang

and Yarema found up-regulation of genes for insulin factors genes, peroxisome proliferative activity receptor were increased, and calcium channel gene and other genes for mitochondrial ribosomal protein S, and uncoupling protein 2. Down- regulation of tumor necrosis factor alpha and interleukin 6 were demonstrated for this transformation with MMF [60]. Further analysis of changes induced by SMF to date, seems to be particularly potent at modulating WNT5a as well as interleukin 6 (IL-6) via the toll-like receptor 4. Bei Li has published that aberrantly up-regulated Wnt5a signaling is a crucial pathological step that contributes to AD-related neurodegeneration by regulating neuroinflammation [61].

**Add B vitamins for prevention of Down's syndrome and MTHFR and MTRR gene defects:** The previous section on methylation defects caused by MTHFR and MTRR means that by adding B vitamins such as folate in the form of L-5 –MTHF, vitamin B6 in the form of pyridoxal 5 phosphate, vitamin B -12 in the form of methyl cobalamin and a methyl donor trimethylglycine (betaine), that homocysteine, a toxic aminoacid, is inhibited. A recent clinic al study using brain imaging in elderly subjects with increased dementia risk (mild cognitive impairment according to 2004 Petersen criteria), that high-dose B-vitamin treatment (folic acid 0.8 mg, vitamin B6 20 mg, vitamin B12 0.5 mg) slowed shrinkage of the whole brain volume over 2 years by lowering elevated plasma homocysteine [62]. Using methylated forms of these B vitamins, can be conjectured as being more effective since approximately 30% of the population may have the above mentioned gene defects.

A study by Nicolina et al. also demonstrated that B vitamin deficiency promotes tau phosphorylation through regulation of GSK beta and PP2A. They found that GSK3beta and protein phosphatase 2 A (PP2A) were up-regulated by inhibiting methylation reactions through B vitamin deficiency. They concluded that one-carbon metabolism alteration seems to be the cause of deregulation of the equilibrium between GSKbeta and PP2A leading to abnormal hyperphosphorylated tau [61].

There are many questions still not answered in research into hyperphosphorylation of tau in Down's dementia such as why serine and treonine (Ser/Thr) residues are basically phosphorylated and which ones are phosphorylated under pathological conditions. Also why some residues are phosphorylated early versus late in the disease and that distinct residues that are phosphorylated in subjects in Down's dementia are different from Alzheimer's disease.

## Conclusion

Tau hyperphosphorylation found in Down's dementia and Alzheimer's and other tauopathies has been demonstrated with changes of insulin signaling, calcium signaling, mitochondrial decline and oxidative stress. The occurrence of methylation defects in DS and kinases such as DYK1A and GlcNAcylation over expression as well as a Cdk5 inhibitory peptide (CIP) suppression of tau hyperphosphorylation has been documented. Methylation defects in MTHFR and MTRR genes have also been discussed.

Prevention of tau hyperphosphorylation in Down's syndrome with sleep medicine is recommended. Cleaning up the environment especially methyl mercury and arsenate and using foods high in antioxidants may prevent cognitive decline. Cleaning up the diet with avoidance of high glycemic diet and gluten is equally important and eating a diet high in antioxidants and adding B vitamins because of MTHFR and MTRR gene defects. Powerful antioxidants like muscadine grape seed may be especially important in Down's syndrome. Neuroprotectives such as



CBD may prove to be potent therapy in DS dementia and Alzheimer's. Moderate magnetic therapy for moderate cognitive impairment along with antibiotics such as minocycline and doxycycline for spirochetal associated dementia is highlighted as more evidence for the emerging role of pathogens in Alzheimer's disease unfolds. Pharmaceutical and biotec therapy such as BACE1 inhibitors is beyond the scope of this paper but may soon find efficacy as well.

A child born with Down's syndrome should not be doomed to a later life of dementia because they inherited trisomy 21.

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