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## MINIREVIEW

# Next generation tau models in Alzheimer's disease research – virus based gene delivery systems

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**Summary. –** Alzheimer's disease (AD) the most common form of dementia is characterized by cognitive decline and progressive loss of neurons in the central nervous system. Despite huge scientific progress, there are only few animal models that recapitulate at least majority of the AD pathology and related symptomatology. Therefore, alternative methods to develop animal models for neurodegenerative diseases are constantly explored. Recently, recombinant adeno-associated viruses (AAVs) are widely used viral vectors in development of novel models for neurodegenerative diseases. AAV vectors expressing full length, mutant or truncated forms of tau demonstrate early and robust pathology characterized by AT8 positivity, NFT formation, motor and cognitive deficits. Furthermore, AAVs have been used in expression of tau in amyloid rodent models thus developing both lesions of amyloid and tau therefore recapitulating AD like features. Major advantage of AAV as a delivery system is the site specific expression of tau, mostly in hippocampus and cortex, and thus elimination of unwanted ectopic transgene expression. These novel models may help in better understanding of AD etiopathogenesis and provide a platform for development and testing of disease modifying drugs in preclinical efficacy studies.

**Keywords:** adeno-associated viral vector; Alzheimer's disease; transgenic models; tau; tauopathy

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**Abbreviations:** AAV(s) = adeno-associated virus(es); AD = Alzheimer's disease; CBA = cytomegalovirus/chicken β-actin promoter; CNS = central nervous system; EC = entorhinal cortex; NFT = localized neurofibrillary tangles; WPRE = woodchuck hepatitis virus post-translational regulatory element; 4R tau = tau with 4 repeat

## 1. Introduction

Human neurodegenerative diseases represent a group of disorders characterized by progressive dysfunction of the nervous system. Alzheimer's disease (AD) is the most common neurodegenerative disorder demonstrating memory impairment and cognitive decline, progressive impairment of daily activities and various neuropsychiatric symptoms (Cummings, 2004; Selkoe, 2011; Caselli *et al*., 2006). The manifestation of the disease is characterized by loss of neuronal plasticity (Arendt, 2001), synapse loss (Masliah *et al*., 1989, 1992), neuronal loss (Padurariu *et al*., 2012) and neurodegeneration (Masliah *et al*., 1996). The main neuropathological features of AD represent intracellularly localized neurofibrillary tangles (NFT's) composed of tau protein and extracellular accumulation of amyloid β plaques

in certain brain areas. Interestingly, the NFT's and amyloid β plaques pathology are distributed in different brain areas in AD brain (Braak and Braak, 1991; Delacourte *et al.*, 2002) suggesting non-overlapping manifestation of the two lesions. However, evidences suggest a stronger correlation between tau pathology and AD neurodegeneration (Falke *et al.*, 2003; Ingelsson *et al.*, 2004; Nelson *et al.*, 2012). Relationship between regional distribution of phosphorylated tau and clinical signs indicates close relationship between tau and disease manifestation in AD (Braak and Braak, 1991), thus signifying that tau inclusions in AD brain modulate the clinical symptoms of the disease. This suggests that tau protein represents one of the main driving forces in AD neurodegeneration.

The research on molecular mechanisms of AD is especially complicated mainly due to different postmortem delays of collected brain tissues. Nervous tissue acquired from humans is fragile, biochemically unstable and affected by treatment, as well as other eventual neurologic impairments. Moreover, these samples are obtained usually from individuals in terminal stages of the disease, thus disabling investigation of ontogeny. Besides, these individuals may suffer from several other comorbidities, including inflammation-related diseases that might significantly affect postmortem examination. In addition, relatively high inter-individual variability, based on genetic and environmental factors, complicate the elucidation of AD pathogenesis. Another limitation is that the tissue obtained from the patients with neurodegenerative diseases does not allow exploring the progression or monitoring the development of the disease (Mason *et al.*, 2013; Nasrallah and Dubroff, 2013).

Studies based on animal models enable us to evaluate molecular mechanisms of the disease and correlate them with clinical features and behavioral changes. Research utilizing available models of AD facilitates continual progress in our understanding of AD etiopathogenesis (Balmus *et al.*, 2015; Simons, 2008). Currently used tau transgenic models express mostly mutated forms of tau protein. Even though these models develop certain features of AD-like pathology, they only partially mimic the human neurodegenerative disorder (Citron, 2010; Platt *et al.*, 2013). Moreover, it is uncertain as to which extent the pathology in these rodents reflects the pathology in AD patients. Despite above mentioned limitations, animal models allow us to study hypothesis related to AD pathogenesis, and to test properties of new drugs designed for AD treatment (Shineman *et al.*, 2011). Recent advances in development of transgenic animals have enabled the creation of rodent models that reproduce several aspects of human tauopathies (Bugos *et al.*, 2009). Besides, alternate methods to generate animal models for neurodegenerative diseases are being constantly developed and investigated for better understanding of the disease pathogenesis.

## 2. Adeno-associated virus as a delivery system for creation of novel transgenic models

Vector based models have currently been widely used to study human diseases due to their high regional specificity and effectivity. Vector driven models are novel components that imitate slow disease progression (Klein *et al.*, 2005). In particular the recombinant AAV vector driven expression of misfolded proteins has emerged as a promising tool to study the disease pathogenesis of numerous neurodegenerative disorders (Kirik *et al.*, 2002; Lo Bianco *et al.*, 2002; Kirik and Björklund, 2003; Jaworski *et al.*, 2010a; Lathuilière *et al.*, 2012). Wild-type AAVs or adeno satellite viruses are a replication-defective, non-pathogenic single-stranded DNA viruses classified under the family *Parvoviridae* and the genus *Dependoparvovirus*. They require helper virus particles such as adeno virus or herpes simplex virus for infection and replication (Daya and Berns, 2008). However, recombinant AAVs are infectious, but lack virulency and thus provide a platform for sustainable transgene expression in several animal models (Li *et al.*, 2003; Wu *et al.*, 2006). Recombinant AAVs offer broad range of infectious properties that target different cell types and mechanisms in rodents and humans (Bourdenx *et al.*, 2014; Murlidharan *et al.*, 2014; Shevtsova *et al.*, 2005).

Thirteen different serotypes of AAVs and their different tropism have been currently well defined (Handa *et al.*, 2000, Gao *et al.*, 2002, 2005; Cearley and Wolfe, 2006; Zincarelli *et al.*, 2008; Aschauer *et al.*, 2013). The AAV serotypes vary in their origin (Wu *et al.*, 2006), genome sequence, capsid protein (Choi *et al.*, 2005; van Vliet *et al.*, 2008), transduction properties based on proteoglycan binding and glycan modifications (Summerford and Samulski, 1998; Walters *et al.*, 2001; Nonnenmacher and Weber, 2012; Holehonnur *et al.*, 2014; Murlidharan *et al.*, 2014; Table 1) and efficiency of transgene expression (Gao *et al.*, 2002). Mainly in the central nervous system, the AAV serotypes differentially transduce and express proteins, and also vary in their mechanisms of axonal transport (Salegio *et al.*, 2013; Aschauer *et al.*, 2013; Table 1). It is yet unknown if the duration and variable expression in protein levels is attributed to the variable domains of the capsid protein in the AAV serotypes (Zincarelli *et al.*, 2008). Nevertheless, several recombinant AAV vectors are being constantly developed that are better engineered to provide efficient and stronger expression of proteins (Gao *et al.*, 2005; Daya and Berns, 2008).

AAV based neurodegenerative models were initially developed using well characterized AAV2 serotype (Choi *et al.*, 2005; Wu *et al.*, 2006). AAV2 serotype expressing P301L - 4R2N tau (tau with 4 repeat and 2 inserts) under different promoters showed variable expression profile in primary neurons (Klein *et al.*, 2004; Table 2). Tau expression (pTau-W AAV) *in vitro* was observed using AAV2 viral vectors con-

**Table 1. The basic characteristics of various adeno-associated virus serotypes**

| AAV serotype | Source | Glycan receptors | Proteinaceous Co-receptors/ others | Tropism | Efficiency for neuronal transduction | Efficiency for glial transduction | Axonal transport | Spreading | References |
|---|---|---|---|---|---|---|---|---|---|
| **AAV1** | Isolated from human tissues, but also non-human primates | α2,3/α2,6 *N*-linked sialic acid | Unknown | Neuronal and glial cells, skeletal muscle | Moderate levels | Low levels | Anterograde and retrograde direction | Widespread transgene expression | Xiao *et al.*, 1999; Wu *et al.*, 2006; Murlidharan *et al.*, 2014 |
| **AAV2** | Human | HSPG (contain R585 and R588 on capsid) | FGFR1, HGFR, Integrin αVβ5 and α5β1, CD9 | Neuron specific, retina, liver, kidney, muscle | Low levels | No transduction | Anterograde direction | Minimal expression and spreading of transgene to surroundings from site of injection | Summerford and Samulski 1998; Wu *et al.*, 2006; Kaminsky *et al.*, 2012; Murlidharan *et al.*, 2014 |
| **AAV3** | Human | HSPG (lack R585 and R588, requires R594) | FGFR1, HGFR, LamR | Hematopoietic cells | Unknown | Unknown | Unknown | Unknown | Handa *et al.*, 2000; Wu *et al.*, 2006 |
| **AAV4** | Non-human primate | α2,3 *O*-linked sialic acid | Unknown | CNS (ependymal cells) and photoreceptor cells | No transduction | Low levels | Unknown | Minimal expression and spreading of transgene to surroundings from site of injection | Davidson *et al.*, 2000; Wu *et al.*, 2006; Murlidharan *et al.*, 2014 |
| **AAV5** | Human | α2,3 *N*-linked sialic acid | PDGFR | CNS (neuron, ependymal cells and astrocyte) and photoreceptor cells, muscles | Moderate levels | Low levels | Retrograde direction | Widespread transgene expression | Walters *et al.* 2001; Shevtsova *et al.*, 2005; Gao *et al.*, 2005; Wu *et al.*, 2006 |
| **AAV6** | Isolated as a contaminant in laboratory adenovirus stocks/ hybrid between AAV-1 and AAV-2 | α2,3/α2,6 *N*-linked sialic acid<br><br>HSPG (lack R585 and R588, but contain K531) | EGFR for sialic acid binding | Neuron specific, spinal cord, lung, pancreas, muscle | Moderate levels | No transduction | Retrograde direction | Widespread transgene expression | Wu *et al.*, 2006; Salegio *et al.*, 2012; Sebastian *et al.*, 2013; Murlidharan *et al.*, 2014 |
| **AAV7** | Non-human primate | Unknown | Unknown | Skeletal muscle | Unknown | Unknown | Unknown | Widespread transgene expression | Gao *et al.*, 2002; Wu *et al.*, 2006 |
| **AAV8** | Non-human primate | Unknown | Lam R | Brain, liver, heart, pancreas, muscle | Moderate levels | Moderate levels | Anterograde and retrograde direction | Widespread transgene expression | Gao *et al.*, 2002; Akache *et al.*, 2006; Wu *et al.*, 2006; Nam *et al.*, 2007 |
| **AAV9** | Non-human primate | N-terminal galactose | Lam R | Astrocytes, brain endothelial cells, spinal cord, motor neurons, lung, muscle, liver, | High levels | Moderate levels | Anterograde and retrograde direction | Widespread transgene expression | Murlidharan *et al.*, 2014; Wu *et al.*, 2006; Aschauer *et al.*, 2013 |
| **AAV10** | Non-human primate | Unknown | Unknown | Neurons, brain endothelial cells, astrocytes | High levels | Low levels | Unknown | Widespread transgene expression | Murlidharan *et al.*, 2014; Wu *et al.*, 2006 |

HSPG = heparan sulfate proteoglycans; FGFR1 = fibroblast growth factor receptor 1; HGFR = hepatocyte growth factor receptor; Lam R = laminin receptor; PDGFR = platelet-derived growth factor; EGFR = epidermal growth factor receptor.

taining woodchuck hepatitis virus post-translational regulatory element (WPRE) and hybrid cytomegalovirus/chicken β-actin promoter (CBA), but not under CBA promoter alone (pCB-Tau AAV). Although, mild expression of tau was observed *in vivo* using CBA promoter, pTau-W AAV with dual promoter (WPRE and CBA) showed overt expression of P301L tau and abundance in Gallyas and AT100 positivity in neurons around the injected site. The animals showed behavioral deficits as early as 5 days post injection (Klein *et al.*, 2004; Table 2). Furthermore, immune-electron micrographs confirmed the presence of tau protein in intracellular filament deposits in these neurons. Besides, injection of AAV2 tau vectors produced tau-positive dystrophic neurites with amyloid core similar to AD in PS1/APP double transgenic mice suggesting AAV vectors can be used to study multiple lesions in coherence in humanized models.

AAV8 serotype is considered to be one of the most potent serotype in gene delivery to the brain (Klein *et al.*, 2006; Table 2). AAV8 demonstrated stronger transgenic expression in rat neurons, both *in vitro* and *in vivo*. Similar to AAV2, the AAV8 transduced cultured astrocytes but not the astrocytes in the brain (Klein *et al.*, 2006). Injection of two doses of AAV8 vector expressing P301L mutated tau protein resulted in 74–78% of neurodegeneration in substantia nigra. This effect was not observed using AAV5 vector strain. Furthermore, the AAV8 tau was more efficient in inducing neuronal loss when compared to AAV2 tau or AAV5 tau. AAV8 tau vector resulted in significant loss of neurons than AAV2 tau by about 74–78% loss of dopamine neurons (Klein *et al.*, 2006). This profound neuronal loss also caused significant amphetamine stimulated rotational behavior in these animals.

AAV9 and/or AAV10 vectors also show early expression of tau accompanied by robust loss of nigrostriatal system when compared to AAV2 tau vector, mainly in the form of dopaminergic and GABAergic neuron loss (Klein *et al.*, 2008, 2009). This effect was not observed using equal doses of green fluorescent protein (GFP) or alpha-synuclein vectors suggesting that the pathological effect is highly specific to misfolded tau neurotoxicity. Expression of AAV9 P301L tau in hippocampus impaired spatial memory and learning (Mustroph *et al.*, 2012). Interestingly, gliosis was also observed at the sites of injection. Based on these findings, the AAV8, AAV9 and AAV10 serotypes display several advantages for development of novel tau animal models (Klein *et al.*, 2008, 2009).

Tau hyperphosphorylation and somatodendritic mislocalization, a classical hallmark of tauopathy, was observed in AAV6 serotype carrying wildtype tau or P301S tau form. This was accompanied by impairment of motor function in these models. These effects were absent in rodents injected with AAV6 tau harboring aggregation deficient mutation at I277P/I308P (Lathuilière *et al.*, 2013). Dassie *et al.* (2013) generated AAV6 serotype carrying mutant forms of tau (P301L and 3PO tau) to study the interaction of tau and β-amyloid pathology. They observed abnormal tau phosphorylation in dystrophic neurites in close association with amyloid plaques (Dassie *et al.*, 2013). The same was previously observed by Tackenberg and Brandt (2009), and they showed that amyloid β alone was not neurotoxic but can induce toxicity through phosphorylation of tau. AAV 1/2 serotype expressing wildtype 4R tau or P301L tau resulted in near complete loss of pyramidal neurons in CA1/2 region and adjacent cortical layers (Jaworski *et al.*, 2009, 2010b). Temporal progression of the disease was accompanied by marked tau hyperphosphorylation in several AD associated phospho-sites and tau aggregation. Microgliosis was noted during onset and active progression of the disease implying closer association between tau neurodegeneration and microgliosis. Interestingly AAV1/2 expressing truncated 4R tau at aa 255, thereby lacking microtubule domain, failed to induce neurodegeneration or microgliosis when compared to full length counterparts (Jaworski *et al.*, 2009). However, unlike full length tau which showed somatodendritic localization, the tau 255 was localized in the neuronal soma and was partially phosphorylated at AT8 and AT270 epitopes. These findings are in line with our studies showing that microtubule binding domain of tau plays a crucial role in neurodegeneration. Our transgenic models expressing human truncated tau with microtubule binding domain (aa 151–391) shows robust tau pathology in the cortex and brain stem (Zilka *et al.*, 2006; Filipcik *et al.*, 2012). Additionally, this study also establishes the role of truncated tau and the microtubule binding domain in AD disease pathogenesis.

### 3. Recombinant AAV vectors in investigating early stages of Alzheimer's disease

Mimicking early stages of AD disease progression has been the primary goal of many researchers to understand the ontogeny of the disease. In AD, the tau pathology initially manifests in the entorhinal cortex (EC) and spreads to the synaptically connected neural circuits in the hippocampus and cortex (Braak and Braak, 1991). In recent years, AAV animal models have been effectively used to develop and successfully imitate the early stages of disease progression. For example, intra-entorhinal delivery of hybrid AAV2/9 viral vector with synapsin I promoter expressing tau with P301L mutation induced expression in neurons in the ECII layer and associated dendritic processes following the perforant pathway, the hippocampal fissure and the outer molecular layer in dentate gyrus (Siman *et al.*, 2013; Table 2). The model developed several features of AD like tauopathy mimicking early Braak stage I. More importantly, the model was also able to simulate the trans-synaptic spread of tau since injection of AAV particles in the EC showed spread of tau pathology to the dentate gyrus and mossy fibers pathways

**Table 2. List of different serotypes of recombinant adeno-associated viruses expressing various tau isoforms, their sites of injection, promoter used and pathology developed**

| AAV serotype | Promoter | Injection site | Tau form | Mice injected | Pathology | Reference |
|---|---|---|---|---|---|---|
| **AAV1** | CBA with WPRE | Lateral ventricles: (posterior to bregma and L:2 mm lateral to the midline) **bilateral** | hTau P301L | C57Bl/6 mouse pups | NFTs, neuropil threads, dystrophic neurites, gliosis, behavioral changes synaptic abnormalities hyperactivity, anxiety, deficits in a contextual fear conditioning | Cook *et al.*, 2015 |
| **AAV2** | CBA with WPRE | Substantia nigra (AP: 5.4 mm, L: 2.0 mm, DV: 7.6 mm) **unilateral** | hTau P301L | Male Sprague–Dawley rats (3 months old) | tau hyperphosphorylation, loss of dopaminergic neurons in substantia nigra, motor deficit | Klein *et al.*, 2005 |
| **AAV2** | CBA with WPRE | Medial septum (AP: 0,7 mm, L: 0,2 mm, DV: 7,0 mm) | hTau P301L | Male Sprague–Dawley rats (3 months old) | NFTs, Gallyas positive dystrophic neurites | Klein *et al.*, 2004 |
| | | Hippocampus (AP: 2.1 mm, L: 1.2 mm, DV: 2.0 mm) **unilateral** | | PS1/APP mice (2 month old) | tau-immunoreactive neurites | |
| **AAV2** | NA | NA | htau 4R | WT mice (13 months old) | tau fibrillary deposits caspase activation | de Calignon *et al.*, 2010 |
| | | | Δtau aa421 | | | |
| **rAAV2** | CBA with WPRE | Entorhinal cortex (AP: 8.3 mm, L: 3.3 mm, DV: 6.0 or 5.0 mm and AP: 8.8 mm, L: 3.7 mm, DV: 5.0 mm) **bilateral** | hTau P301L | Male Sprague–Dawley rats (Approx 90 days) | AT8 positive neurofibrillary tangles in hippocampus, no overt neuronal or synaptic loss, impaired spatial learning | Ramirez *et al.*, 2011 |
| **AAV6** | PGKP | Entorhinal cortex (AP: 3.0 mm, L: 3.7 mm, DV: 4.0 mm) **unilateral** | hWT 4R0N | TASTPM mice (hAPP695swe and PS-1 M146V) C57BL/6J mice | loss of neurons only after 8 months | Dassie *et al.*, 2013 |
| | | | 3PO-tau | | age dependent loss in neurons | |
| | | | h Tau 4R0N P301S (0N3R) | | AT8, MC1 positive pyramidal neurons and apical dendrites working memory impairment | |
| **AAV6** | NA | Lateral ventricles **bilateral** | hWT 4R | C57Bl/6 mouse pups | neuronal tau pathology progressive motor deficit | Lathuiliere *et al.*, 2013 |
| | | | hTau P301S | | | |
| | | | Double mutated tau I277P/I308P | | no detectable motor phenotype | |
| **AAV8** | CBA with WPRE | Hippocampus (AP: 3.6 mm, L: 2.0 mm, DV: 3.5 or 2.8 mm) Substantia nigra (AP: 5.3 mm, L: 2.1 mm, DV: 7.6 mm) **unilateral** | hTau P301L | Male Sprague–Dawley rats (3 months old) | loss of tyrosine hydroxylase neurons and lesions in substantia nigra | Klein *et al.*, 2006 |

**Table 2. (continue)**

| AAV serotype | Promoter | Injection site | Tau form | Mice injected | Pathology | Reference |
|---|---|---|---|---|---|---|
| **AAV9** | CBA with WPRE | Hippocampus (AP: 2.0 mm, L:1.5 mm, DV: 3.0mm; and AP: 4.2 mm, L:4.5 mm, DV: 5.0 mm) **bilateral** | hTau P301L | Male Sprague–Dawley rats (3 months old) | hyperphosphorylated tau and NFTs, neuronal loss in the hippocampus | Mustroph *et al.*, 2012 |
| **AAV9** | CBA | Hippocampus (AP: 3.6 mm, L: 2.0 mm, DV: 3.5 or 2.8 mm) **bilateral** | htau 4R | Male Sprague–Dawley rats | no behavioral or neuronal deficits | Dayton *et al.*, 2012 |
| **AAV2** | CBA with | Substantia nigra | hTau P301L | Male Sprague–Dawley | loss of dopaminergic neu- | Klein *et al.*, 2008 |
| **AAV8** | WPRE | (DV: 5.3 mm, L: 2.1 | | rats | rons in substatia nigra pars | |
| **AAV9** | | mm, DV: 7,6 mm) | | (3 months old) | compacta | |
| **AAV10** | | **bilateral** | | | degrees of DA loss: AAV9=AAV10>AAV8>AAV2 | |
| **AAV1/2** | HSNP | Hippocampus (AP: 1.94 mm, L: 1.4 mm and DV: 2.2 mm) **unilateral** | htau 4R | Adult WT FVB/N mice | degeneration of pyramidal neurons | Jaworski *et al.*, 2009 |
| | | | hTau P301L | | degenerating and dystrophic neurons with vacuolar structures, neuroinflammation and oxidative stress | |
| | | | Δtau aa255 | | no appreciable neurodegeneration no microgliosis | |
| **AAV1/2** | HSNP | Hippocampus (AP: 1.94 mm, L: 1.4 mm and DV: 2.2 mm) **unilateral** | hTau P301L | WT FVB/N mice (3-4 months old) | degenerating and dystrophic neurons with vacuolar structures dendritic deficits, oxidative stress and neuroinflammation | Jaworski *et al.*, 2011 |
| | | | | YFP- expressing transgenic mice | decrease in dentritic spines and degenerating neurons | |
| **AAV 2/6** | PGKP with WPRE | Perirhinal cortex (AP:1.8 mm, L: 4.2 mm, DV: 4 mm and AP: 3.2 mm, L: 4.1 mm, DV: 3.8 mm) **bilateral** | hTau P301S | Adult C57Bl/6 mice | tau hyperphosphorylation, aggregation and neurodegeneration deficit in synaptic transmission severe object recognition memory deficit | Yang *et al.*, 2015 |
| **AAV 2/9** | HSNP | Hippocampus (AP: 4.0 mm, L: 4.5 mm, DV: 2.9 mm) **unilateral** | hTau P301L | Male CD-1 Mice (3-4 months old) | postsynaptic spreading of tau and loss of perforant pathway synapses and neurons | Siman *et al.*, 2013 |

Htau = human tau; NA = not available; Δtau = truncated tau; WPRE = woodchuck hepatitis virus post-translational regulatory element; HSNP = human synapsin 1 gene promoter; CBA = hybrid cytomegalovirus/chicken β-actin promoter; PGKP = phosphoglycerate kinase 1 promoter; AP = anterior-posterior; L = lateral, DV = dorsal-ventral.

in the striatum lucidum and mossy fibers in the CA3 region. Tau hyperphosphorylation (AT8, pThr231) and aggregation was prominent in the EC. Likewise, Gallyas positive neurofibrillary tangles were observed in these animals in EC at 6 weeks post injection. Tau protein expression induced loss of the cells in the ECII by up to 81% and loss of synapses in the dentate gyrus and lateral perforant layer pathway in the outer molecular layer and molecular layer shank. Delineating the mechanisms of neuronal loss in these cells revealed cell loss by caspase mediated apoptosis. Similarly, caspase positive neurons were detected in Tg4510 mice or htau mice injected with AAV2 vector expressing full length tau 4R (de Calignon *et al.*, 2010; Table 2). These evidences suggest that soluble tau triggers caspase activation in these neurons. Remarkably, caspase cleaved truncated tau at Asp421 was found in caspase positive neurons in these models. Moreover, expression of truncated tau at Asp421 using AAV2 in wild type mice showed Alz50 immuno-reactive neurons which were also positive for PHF1 and AT8 antibodies suggesting misfolded tau conformation in these neurons (de Calignon *et al.*, 2010). Furthermore these results also propose that tau truncation was essential to induce tau misfolding and tau hyperphosphorylation.

Interestingly AAV2/6 serotype with synapsin-1 promoter expressing P301L tau injected in the medial EC developed tau pathology as early as 7 days post injection (Asai *et al.*, 2015). The model also showed rapid progression of tau pathology to dentate granule cells. Notably, the expression of tau reduced the spike amplitude in these animals when compared to AAV-GFP group implying diminished excitability in the dentate gyrus. In addition, pharmacological depletion of microglia reduced AT8 positivity in the dentate gyrus suggesting the role of microglia in progression of tau pathology in AD and other tauopathies (Asai *et al.*, 2015). Similarly, expression of human 4R tau (hWT4R) using AAV2/5 in EC also showed AT8 positivity and induced axonal fragmentation in the perforant pathway axons (Combs *et al.*, 2016). These evidences suggest that precise and selective transduction of AAV serotypes can be used to mimic early AD disease pathogenesis.

Numerous other AAV-tau models have been developed under different genetic background (Table 2). Most of these models build up AT8 positivity (Klein *et al.*, 2005; Ramirez *et al.*, 2011; Dassie *et al.*, 2013; Asai *et al.*, 2015), neurofibrillary tangles (Klein *et al.*, 2006; de Calignon *et al.*, 2010; Ramirez *et al.*, 2011), degenerating and dystropic neurites (Jaworski *et al.*, 2009, 2011), motor impairment (Klein *et al.*, 2008; Cook *et al.*, 2015), synaptic deterioration (Jaworski *et al.*, 2011; Siman *et al.*, 2013; Yang *et al.*, 2015; Cook *et al.*, 2015) and loss of neurons (Klein *et al.*, 2005, 2008; Jaworski *et al.*, 2011; Mustroph *et al.*, 2012; Dassie *et al.*, 2013; Yang *et al.*, 2015) implicating that AAV vectors can be used to generate rodent models in a short span of time which are efficient (transduction and level of expression) and imitate early and progressive pathological changes as in AD and other tauopathies.

## 4. Summary and concluding remarks

Recombinant AAV vectors are currently emerging as the preferred gene delivery vehicles for CNS (Manfredsson 2016). They provide efficient gene transfer, long-term transgene expression, minimal virulence, low immunogenicity (Heilbronn *et al.*, 2010) and scalable manufacture for clinical applications. Despite huge scientific progress achieved in recent years, questions related to AD etiopathogenesis remain unanswered (Ballard *et al.*, 2011). Recombinant AAV systems are currently the most efficient gene delivery vehicles to develop animal models for Alzheimer's disease and Parkinson's disease and are currently used in development for clinical interventions against numerous disorders (Combs *et al.*, 2016). Choice of AAV serotypes along with site specific application allows accurate transduction of extraneous genes to cell type of choice.

There are several advantages of AAV vector models for Alzheimer's disease, including if not limited to: (1) development of pathological lesions that mimic early stages of tau pathology, (2) induction of a disease specific pathology in the different brain regions, (3) induction of tau pathology in specific neuronal subpopulations and in glial cells, (4) the ability to study the effect of multiple pathological lesions simultaneously, (5) the ability to introduce tau lesions in amyloid models, (6) expression can be induced at any stage of nervous system development, and finally, (7) AAV driven transgene expression is reproducible and pathological changes are observed within a shorter period of time.

What's more, the advent of newer recombinant AAV strains offers prospects for generation of animal models which reflect better pathology as in human neurodegenerative diseases. The AAV driven animal tau models may help to understand the mechanism/s of tau pathogenesis and provide opportunities for development of potential therapeutic approaches against Alzheimer's disease and other tauopathies.

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