Role of Phosphorylated Tau and Glucose Synthase Kinase 3 Beta in Huntington’s Disease Progression

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Abstract. The purpose of our article is to critically assess the role of phosphorylated tau in Huntington’s disease (HD) progression and pathogenesis. HD is a fatal and pure genetic disease, characterized by chorea, seizures, involuntary movements, dystonia, cognitive decline, intellectual impairment, and emotional disturbances. HD is caused by expanded polyglutamine (polyQ or CAG) repeats within the exon 1 of the HD gene. HD has an autosomal dominant pattern of inheritance with genetic anticipation. Although the HD gene was discovered 26 years ago, there is no complete understanding of how mutant huntingtin (mHTT) selectively targets medium spiny projection neurons in the basal ganglia of the brain in patients with HD. Several years of intense research revealed that multiple cellular changes are involved in disease process, including transcriptional dysregulation, mitochondrial abnormalities and impaired bioenergetics, defective axonal transport, calcium dyshomeostasis, synaptic damage and caspase, and NMDAR activations. Recent research also revealed that phosphorylated tau and defective GSK-3β signaling are strongly linked to progression of the disease. This article summarizes the recent developments of cellular and pathological changes in disease progression of HD. This article also highlights recent developments in phosphorylated tau and defective GSK-3β signaling and the involvement of calcineurin in HD progression and pathogenesis.

Keywords: Huntington’s disease, hyperphosphorylated tau, medium spiny projection neurons, mitochondrial abnormalities, mutant huntingtin, polyglutamine repeats

INTRODUCTION

Huntington’s disease (HD) is an autosomal-dominant neurodegenerative disorder manifesting motor (chorea, gait abnormalities, and resting tremor) as well as non-motor symptoms (cognitive impairment, dementia, depression, and anxiety) [1–7]. It
has a mean worldwide prevalence of 2.71 individuals per 1,00,000 and is most prominent in people of Caucasian origin (4–10 cases per 100,000) [8]. It is primarily adult onset and fully penetrant, with HD patients surviving for 15–20 years after disease onset [1–3].

Almost a hundred and fifty years ago, George Huntington, an American physician described the disease with reference to ‘Chorea’—dancing propensities of those who are affected by it [9]. Even though the condition was previously reported by other researchers, the precision and comprehensiveness with which he described the symptoms and heredity, earned him the eponym “Huntington’s disease” [2, 10]. In 1983, the HD gene was mapped to the p arm of chromosome 16 [11], followed by its identification in 1993 by collaborations among several laboratories across the world [12]. But in spite of all the intense research over all these years, it is still not clear how the ubiquitously expressed mHTT specifically affects medium spiny projection neurons [13, 14].

The purpose of this article is to highlight recent developments of cellular and pathological changes in disease progression of HD. This article also summarizes recent developments in phosphorylated tau and glycogen synthase kinase-3β (GSK-3β) activation and the involvement of calcineurin in HD progression and pathogenesis.

**Genetics of Huntington’s disease**

HD is a monogenic disorder caused by an expansion of a CAG repeat tract within exon 1 of the HD gene (coding for huntingtin protein). In the normal human population, the CAG repeats range from 6–35 copies whereas 36–75 repeats could lead to adult onset HD and 48–121 repeats to juvenile HD (Fig. 1) [12, 15–17]. As the CAG repeats over 28 are unstable during replication, they exhibit genetic anticipation specially when inherited from the father [2, 18].

**Pathology of Huntington’s disease**

In 1985, Vonsattel et al. established a system of grading HD progression, based on neuropathological variations in 163 postmortem brains of clinically diagnosed HD cases. The severity of neuropathological abnormalities was used to predict clinical severity [19]. This 5-point (0–4 in ascending order of severity) grading system is still considered as a standard reference point in HD pathology.

Although mHTT has a widespread distribution in neuronal and non-neuronal tissues, selective and progressive neuronal loss has been observed in the caudate and putamen of the striatum, cortex (layer 3, 5, and 6), and hypothalamus, and to a lesser extent in hippocampal and subthalamus neurons [19–21]. Neuropathological examinations of HD patients show significant neuronal loss (up to 80%) and reactive astrocytosis in the neostriatum [19, 21, 22]. Reduced volume of frontal and temporal cortical lobes and an atrophy of striatum were found in HD brains [23].

Huntingtin (Htt) is a 350 kDa protein, ubiquitously expressed in the brain and peripheral tissues of HD patients, where the CAG repeats are translated as polyglutamines in the protein product (Fig. 1) [13, 23].

**mHTT localization and protein interactions**

Both wild-type and mutant Htt (mHtt) are reported to localize in the cytoplasm, but small numbers of mHtt are also found in the nucleus, plasma membrane, mitochondria, lysosomes, and endoplasmic reticulum [23–27]. One of the most prominent theories explaining HD pathogenesis is based on formation of the nuclear inclusions by the N-terminal region aggregations of mHtt in cortical and striatal neurons, as seen in HD postmortem brains as well as in HD transgenic mice (expressing exon 1 with a highly expanded CAG repeat domain) [13, 20, 28, 29]. While in the other cell organelles, mHtt forms abnormal interactions with proteins crucial to their function [13, 23–26, 30–34]. The extent of this
interaction is dependent on the lengths of the polyglutamine tracts [31, 34].

**Cellular changes in Huntington’s disease**

As shown in Fig. 2, multiple cellular pathways have been extensively investigated to explain the premature death of medium spiny projection neurons in HD [24, 35, 36], but this neuronal death is not well understood. The following cellular changes have been reported to involve HD pathogenesis: abnormal mitochondrial bioenergetics and axonal trafficking, aggregation of nuclear inclusions, synaptic damage, cytosolic mHTT abnormal protein interactions, caspase and NMDAR activations, calcium dyshomeostasis, phosphorylated tau accumulations, GSK-3β activation, and transcriptional dysregulation [13, 24, 33–38]. The most compelling evidence from these studies implicates mitochondrial abnormalities and selective synaptic degeneration and neuronal damage in HD.

**Mitochondrial abnormalities in Huntington’s disease**

In the past decade, mitochondrial anomalies have emerged as a key player in HD pathogenesis. A progressive grade-dependent reduction in the number of mitochondria and size has been observed in HD [35]. Also, Reddy et al. reviewed that the decrease in glucose utilization and metabolism shown in HD patients could be associated with mitochondrial damage [36]. In addition, various reports indicate a dysregulation of various mitochondrial proteins such as PGC1α, Drp1, Fis1, Mfn1, Mfn2, Opal1, Tomm40, and CypD, indicative of abnormal mitochondrial dynamics in HD [35, 39, 40]. Therefore, probably an upregulation of the mitochondrial fission genes and downregulation of the mitochondrial fusion genes may be responsible for the abnormal mitochondrial dynamics in HD, eventually contributing to neuronal dysfunction [25, 39]. Besides reduced enzymatic activity in the complexes II, III, and IV of the electron transport chain, low mitochondrial ATP, defective calcium-induced mitochondrial permeability, and damage to mtDNA have also been reported in HD [36, 40–45].

Several reports also suggest that mHTT interferes with mitochondrial trafficking in HD neurons [33, 46]. Trushina et al. showed that mitochondria stopped more frequently in HD neurons probably because mHTT selectively binds to mitochondrial components [44]. This is further supported by the fact that mitochondrial trafficking was reduced specifically at sites of mHTT aggregates, compared to those lacking such aggregates [47].

**Synaptic damage in Huntington’s disease**

mHTT has been reported to be involved in formation of oligomers, fibrils, and protofibrils [48–50]. Further, mutant huntingtin is transported together with vesicles into the synapses, segregating with

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**Fig. 2.** Cellular pathways associated with Huntington’s disease (HD).
the synaptic vesicular constituent synaptophysin and is also believed to play a role in retrograde fast axonal transport [30, 51]. Another theory which might explain the synaptic damage and the defective axonal trafficking is the excessive fragmentation of mitochondria and the subsequent mitochondrial dysfunction [35, 36]. In 2012, Shirendeb et al. postulated that mHTT interacts with Drp1 and thus elevates GTPase Drp1 enzymatic activity, eventually leading to abnormal mitochondrial dynamics and finally resulting into defective anterograde mitochondrial movement and synaptic damage [33].

Moreover, studies in mice indicate loss of specific synapses in the basal ganglia and impaired synaptic plasticity due to mHTT [52, 53]. Huntingtin aggregates in synaptic vesicles may also lead to early HD pathogenesis through abnormal neurotransmitter release in mice [54, 55].

### MICROTUBULE ASSOCIATED PROTEIN TAU

Tau is a microtubule-associated neuronal protein abundantly expressed in the axons of the central nervous system [56]. Tau has a variety of microtubule associated functions such as microtubule stability and dynamics as well as promoting microtubule assembly. This in turn is responsible for cellular functions such as promotion of neurite outgrowth, determination of neuronal polarity, membrane interactions, facilitation of enzyme anchoring, and facilitation axonal transport of organelles to nerve terminals [57–60].

Tau is encoded by the MAPT (microtubule associated protein tau) gene, which is located on the long arm of chromosome 17 [61]. For many years now, tau has been known to be associated with neurodegenerative disorders such as Alzheimer’s disease (AD), corticobasal degeneration, progressive supranuclear palsy (PSP), frontotemporal dementia, and more recently in Parkinson’s disease (PD) [62–64].

Tau undergoes many post-translational modifications such as acetylation, deamination, glycation, glycosylation, isomerization, methylation, nitration, phosphorylation, proteolysis, SUMOylation, and ubiquitylation. These modifications can alter the physiological functions of tau such that it may not help microtubule assembly and may form aggregate inclusions by altering its quaternary structure. The tau protein has 45 phosphorylation sites and 79 potential serine and threonine phosphate acceptor residues along its length and excess phosphorylation is the most common source of pathogenic tau. This leads to the formation of tau aggregates known as neurofibrillary tangles (NFTs) [57, 58].

### Tau in Huntington’s disease

The earliest detailed report of the presence of NFTs in a patient suffering from HD was presented in 1978, though this paper does mention an earlier account of presence of a few NFTs in the hippocampal area by Forno and Jose in 1973 [65]. In the following years, many reports have documented the presence of tau in HD postmortem brains and HD mice models as well as a biomarker in the cerebrospinal fluid (CSF) of HD patients [37, 38, 66–81].

### Tau in postmortem Huntington’s disease brains

McIntosh et al. in 1978 first reported the presence of NFTs as well as senile plaques in a 54-year-old man, exhibiting early dementia and marked with pathological alterations characteristic of both HD as well as AD. The man started exhibiting cognitive deficits from his late 20s, had dementia by 35, and chorea by his mid-40s. Apart from atrophy, neuronal loss, and astrocytosis in the caudate nucleus and putamen of the dorsal striatum, they also detected NFTs composed of masses of 200 to 240 A tubules with constrictions every 800 to 1,000 A in the frontal and parietal lobes of the frontal cortex [65].

In the years to follow, various groups of researchers reported the presence of NFTs in HD patients, but they considered this to be a co-existence of AD and HD [66–68]. Myers et al. reported presence of NFTs in virtually all areas of the cerebral cortex in a 77-year-old HD patient, while Moss et al. reported their presence in the parahippocampal gyri, the subiculum, and Sommer’s sector of the hippocampus, in a 70-year-old HD patient [66, 67].

In 1998, Jellinger et al. conducted a study on 27 HD postmortem brains and reported the presence of NFTs but without the presence of significant amyloid deposits [68]. This might have been one of the first reposts to demonstrate that NFTs could be an independent pathological manifestation in HD, and not merely a co-existence of AD and HD in that patient. Following this up in 2009, Caparros-Lefebvre reported a similar neuropathology in a 72-year-old HD patient. By using tau immunostaining, NFTs were detected in the hippocampus, temporal neocortex, caudate nucleus, the putamen, and the substantia
nigra, but with negative amyloid-β immunoreactivity. The NFTs were accompanied by corticobasal degeneration and often colocalized with Huntingtin inclusions [70].

Recently two independent studies demonstrated the presence of neuronal tau nuclear rods and phosphorylated tau inclusions in both striatum and cortex, in each of the 24 and 16 (respectively) analyzed postmortem HD brains, compared to those of control brains where they were virtually absent or very scarce. In both cases, immunostaining was strongly supported by consistent western blot data [71, 73]. In 2016, L’Episcopo and coworkers conducted HD grade-based studies on 22 postmortem brains, where they illustrated progressive neuronal loss with progressive increase in the number of phospho-tau (AT8+) neuronal inclusions in the HD hippocampal sections [38]. Together these studies affirm tau to be a neurotoxic entity in HD.

TAU IN HUNTINGTON’S DISEASE
ANIMAL MODELS

Following the initial reports of presence of tau aggregates in HD postmortem brains, many researchers have recently started using animal models such as mice for the study of tau pathology in HD. Following are some on the commonly used mice models.

R6/1

One of the earliest mouse model studies on HD by Fernández-Nogales et al. featured the R6/1 mouse [71]. This is a well-characterized, transgenic model of HD, with 115 CAG repeats. These exhibit disease symptoms (clasping) at the age of 15–21 and live for 32–40 weeks [82]. These mice show significantly increased total tau at mRNA as well as protein levels in striatum and cortex compared to wild-type mice. To verify this effect when these mice were crossed with Mapt+/– mice (heterozygotes) and Mapt−/− mice (homozygotes), R6/1 Mapt+/– showed partial reduction of tau levels and R6/1 Mapt−/− mice showed absolute reduction of tau levels. Thus, confirming involvement of tau in HD [71].

R6/2

This is the most commonly used transgenic mouse model in HD studies and exhibits very aggressive development of symptoms that is at 5-6 weeks of age and death by 10–13 weeks. It has 145 CAG repeats [82]. The premanifest mice show significantly increased tau phosphorylation at the PHF-1 epitope, while the manifest mice show extensive tau phosphorylation at multiple epitopes such as AT8, CP13, PT205, and PHF-1. The tau phosphorylation was seen to be particularly high in the striatum and cortex [77]. Even after crossing R6/2 male mice with their wild-type female counterparts, the offspring still exhibited increased tau phosphorylation at AT8 [38]. Similarly, a 2015 study by Blum et al., showed an increased tau phosphorylation at Ser396 and Ser404 in the cortex and striatum of R6/2 mice, while unphosphorylated tau was significantly decreased [76].

HD94

These transgenic mice manifest a much slowly progressing phenotype and almost normal lifespan. They have 94 CAG repeats and develop symptoms between 4–20 weeks [82]. But even with the slower progression, these mice showed a similar increase in the 4R Tau, 4R/3R Tau ratio, and total tau levels in cortex and striatum at 20 months (86 weeks) [71].

Q175

The Q175 mice model is a milder knock-in HD model exhibiting HD symptoms around 12 months of age and a life span of around 76 weeks. It was generated by spontaneous expansion of CAG repeats in the KI140 knock-in mouse model and carries 188 CAG repeats [83, 84]. At 12 months of age, Q175 mice showed tau hyperphosphorylation at the CP13 and the PS199 epitope, though the phosphorylation at the PHF-1 was unchanged [77].

KI140

The KI140 murine model is a knock-in HD model with 140 CAG repeats inserted in the exon 1 of the huntingtin gene. These mice display hypoactivity at 4 months and neuropathological anomalies intensify from this point on [84]. Similar to their observation on R6/2 mice Blum and associates found increased tau phosphorylation at Ser396 and Ser404 in the cortex and striatum as well as decreased unphosphorylated tau in these mice [76].
TAU IN HUNTINGTON’S DISEASE CELLS

The STHdh cell lines were generated from an HD knock-in mouse model. These are striatal cells (ST), carrying the endogenous Hdh gene (mouse Huntington disease gene homolog) and are characterized by a mild behavioral phenotype and neuropathological features. The STHdhQ111 cell line carries 111 CAG repeated in exon 1, while its wild type counterpart STHdhQ7 carries 7 CAG repeats in exon 1. Gratuz and co-workers tested the levels of tau phosphorylation in STHdhQ111 cell lines compared these to the STHdhQ7 cells and for unknown reasons could not detect tau protein in both the cell lines. Although calcineurin, a pTau modulator was downregulated in STHdhQ111 compared STHdhQ7 [77].

L’Episcopo et al. generated primary hippocampal cultures from transgenic R6/2 and wild-type mice and found a significantly higher level of AT8 as well as total tau in the former compared to the later. They also demonstrated that increased cell death in R6/2 neuronal cultures and compared wild-type cultures. Based on these observations, it was proposed that increased pTau and pGSK-ß-Tyr216 levels linked to cell death in R6/2 cultures [38].

TAU AS A BIOMARKER IN CSF OF HUNTINGTON’S DISEASE PATIENTS

Tau CSF levels are considered as an important denominator used for disease prognosis as well as to predict disease severity/stage, in many disorders such as AD, dementia, and stroke [85, 86]. Increased tau CSF levels are detected following neuronal and axonal damage, in many tauopathies [86]. As mentioned above, in the past 20–30 years much evidence has been provided that many researchers now consider HD a tauopathy [37, 75]. Consequently, there have been several studies demonstrating elevated tau levels in CSF. Most studies employ ELISA for quantification of tau in CSF [69, 78–80, 87–89].

In 2011, Constantinescu et al. were the earliest to report significantly higher CSF total tau in the HD group compared to the control. The study group consisted of 35 HD patient samples against 35 control samples [69]. However, the study did not take into consideration disease stage or treatment status. Rodrigues et al., in 2016, conducted a study with 76 participants of which 24 were healthy controls, and remaining 52 exhibited various stages of HD. They assessed each of the patients based on UHDRS, and the UHDRS total motor score was significantly correlated with CSF tau after adjustment for age [78, 89]. Based on this data, the authors emphasize that CSF tau could be a good biomarker in HD [78].

Although there have been several studies which indicate CSF tau to be significantly higher in manifest HD patients, no such difference was seen between the premanifest group and healthy control group. Studies by Vinther-Jensen et al. (healthy controls = 24, premanifest = 32, manifest = 48), Rodrigues et al. (healthy controls = 14, premanifest = 3, early stage = 15, moderate stage = 5), Niemelä et al. (healthy controls = 25, premanifest = 13, manifest = 14), and Niemelä et al. (premanifest = 11, manifest = 12) corroborate this [77, 79, 87, 88]. According to Wild et al., the tau concentrations were not significantly associated with cognitive scores after controlling for disease burden (healthy controls = 13, premanifest = 13, early stage = 10, moderate stage = 5, advanced stage = 11) [72]. Therefore, tau may not be used to predict future occurrence of HD.

Tau in Huntington’s disease brains

Giehl et al. in 2016 provided PET images of tau aggregates from three grade II HD patients with a mean age of 64. This was accomplished with the help of a new tracer, [18F]-AV-1451, having binding affinity specific to tau. Patients showed aggregates in the cerebral cortex (occipital, parietal, and temporal lobes), as well as in the putamen and cerebellum [90].

As the demonstration of tau pathology in HD is fairly new, not many researchers have yet attempted to decipher the underlying cellular pathways. The following are a couple of proposed mechanisms but none have been strongly established yet.

Hyperphosphorylation and neurofibrillary tangles

Hyperphosphorylation of tau has two adverse effects on the cell. Tau does not perform its physiological role of promoting microtubule assembly, thus affecting cellular stability as well as leading to the accumulation of insoluble aggregates known as NFTs. Such deposits are a common feature of tauopathies such as AD, fronto-parietal dementia, and other tauopathies [62]. Hyperphosphorylated tau forms paired helical filaments, thus losing its microtubule binding capacity (Fig. 3). This greatly disturbs cellular transport, particularly that of lipids, pro-
tein, cellular organelles, etc., from the cell bodies to the nerve endings, eventually leading to synaptic degeneration and cognitive decline [57]. As discussed earlier, many researchers have reported the presence of NFTs in HD human postmortem brains as well as in HD mice. But the exact pathways by which these can cause neuronal damage in HD may not be clear, though they may be similar to AD.

On the basis of the characteristic distribution pattern of these, Braak et al. classified AD into six stages I–VI with increasing severity [91]. In HD patients, NFTs have been reported in frontal, parietal, and temporal lobes as well as in the entorhinal area of the parahippocampal gyrus, the subiculum, CA1 of the hippocampus, the pyriform cortex, and lateral portion of the amygdaloid nucleus [65, 66, 68]. The electron micrographs of the NFTs revealed masses of 200 to 240 Å tubules with constrictions every 800 to 1,000 Å [65].

Differential tau splicing

The tau gene has 15 exons, of which exons 2, 3, and 10 are alternatively spliced to give the 6 tau isoforms. Due to alternative splicing of exons 2 and 3, tau variants may have either both exons 2 and 3 (2N) or only exon 2 (1N) or neither exon 2 nor 3 (0N). Now exons 9, 10, 11, and 12 each have 4 tandem repeats which also represent the microtubule binding domains of tau. Of these, only exon 10 undergoes alternative splicing such that the tau molecule may have 4 tandem repeats with exon 10 (4R-Tau) or may have 3 tandem repeats without exon 10 (3R-Tau). The combination of the above two splicing events gives rise to a total of 6 tau isoforms: 3R0N, 3R1N, 3R2N, 4R0N, 4R1N, and 4R2N (Fig. 4) [57]. In tauopathies, the ratio of 4R to 3R Tau forms is critical. In a healthy human brain, usually this ratio is 1:1, while in many tauopathies like AD and HD, this ratio has been reported to be more than one [71, 73]. The tandem repeats in exons 9–12 are responsible for the binding and stabilizing the microtubules, hence 4R Tau should ideally stabilize them better [92, 93]. But this does not happen in cellular environments as 4R Tau has a greater tendency to be phosphorylated as compared to 3R. This can be attributed to two factors: 4R tau has more phosphorylation sites than 3R and it is more easily glycated than 3R which further promotes phosphorylation [92, 94]. Another theory attempting to explain this states that the excess 4R Tau in a higher than 1 4R:3R ratio situation is out of binding sites on the microtubules. This unbound 4R Tau is very susceptible to phosphorylation by GSK-3β and CDK5, thus forming NFTs [95]. Therefore an increase in the 4R:3R ratio would destabilized cytoskeleton which would eventually lead to neuropathological damage [96].

Many studies have already reported this phenomenon in tauopathies such as AD, PSP, and Pick’s disease, etc. [97]. In 2014, Fernández-Nogales et al. reported a similar pattern in HD postmortem brains as well as in HD mice models. In the striatum of control postmortem brains, 3R Tau mRNA was slightly predominated over 4R mRNA isoforms, whereas in HD samples, the 4R mRNA was the predominant iso-
form. Western blots as well as immunohistochemistry corroborated this finding such that both demonstrated a significantly higher level of 4R in the HD brains. Also, they reported the presence of rod-like tau deposits that partially or totally spanned the neuronal nuclear space—the TNRs, in HD brains with a relatively higher abundance of 4R Tau forms [71]. Vuono et al. similarly reported generally higher levels of 4R with a lower 3R:4R ratio and specifically 1N3R and 1N4R isoforms were similar with an electrophoretic pattern resembling tauopathy cases, as compared to the healthy controls [73].

The proposed molecular mechanism behind the altered splicing of tau is based on hyperphosphorylation of SRSF6 (serine and arginine rich splicing factor 6) [71, 74]. SRSF6 is responsible for splicing of exon 10 of tau and therefore downregulation of SRSF6 leads to inclusion of exon 10 and thus more 4R isoforms and eventually a disturbed the 3R:4R ratio [74, 98]. Several reports indicating hyperphosphorylation of SRSF6 in the brains of HD patients may help support this molecular mechanism [71, 99].

**Tau and MAP2**

The closest homologue to tau is MAP2. While tau is the usually limited to the axonal microtubules, MAP2 is associated with microtubules of the dendrites and cell bodies [100, 101]. Similar to tau, MAP2 splicing is regulated by SRSF6 [99]. MAP2 levels are found to be lowered in HD patients [38, 99]. Therefore, as reviewed by Fernandez-Nogales et al., altered tau splicing together with lowered levels of MAP2 may contribute to microtubule instability, thus causing neuronal damage in HD patients [74].

**Tau and huntingtin**

Multiple reports in the last 10 years have made it evident that there is co-localization of tau and mHTT [70, 73]. In 2009, Caparros-Lefebvre et al. were the first to provide evidence by staining a HD postmortem brain with tau antibody and with EM48 and 1C2 both for mutant huntingtin. Tau and mHTT were seen to co-localized in the cortex and striatum [70]. In 2016, Vuono et al. followed this up by showing mHTT (EM48) co-localized with pathologically phosphorylated tau (AT8 and pS199) as well as with the two tau isoforms 3R and 4R (RD3 and RD4 antibodies, respectively) of HD brains [73]. These studies could possibly suggest an interaction of the two pathogenic proteins thus leading neurodegeneration associated with HD.

A 2015 study by Blum et al. could help understand the mechanistic part of this interaction better. First, they showed significant tau hyperphosphorylation in HD animals and cultured cells co-expressing mHtt and tau. On the basis of this, the authors implied that the increased expression of mHTT could promote hyperphosphorylation of the of tau. They also conducted in vitro filter trap assays as well as bimolecular fluorescence complementation assays in live cells to study tau and mHTT interactions. The two proteins formed large aggregates with each other and mHtt interacted with tau and hyperphosphorylated tau such that it affected the normal pattern of mHtt aggregation. Also, tau could employ mHTT to the microtubules thus impairing its microtubule-stabilizing properties [76].

Though this has been the only study with a mechanistic approach to analyzed the pathogenesis behind tau and mHTT interactions, there would be more data needed to consider this as a conclusive evidence.
Furthermore, the need for evidence is also demanded by the fact that there are two other contradictory reports which indicate that tau and mHTT do not co-localize or co-immunoprecipitate [71, 77]. They are rather restricted to their respective deposits, that is tau in the TNRs and mHtt in the spheroid inclusion bodies [71].

**Tau genetic variation**

The MAPT gene is a 900 kb fragment located between two extended haplotypes, H1 and H2, which cover the entire MAPT gene [102]. H1 and H2 haplotypes differ in orientation and do not recombine [103]. Most tauopathies like PD, PSP, and AD are known to be associated with a particular haplotype [104]. Considering HD as a tauopathy, Vuono et al. genotyped a large cohort of 473 HD cases of which 60% \( (n = 283) \) were H1 homozygotes \( (H1/H1) \) and 40% \( (n = 190) \) were H2 carriers \( (n = 161 H1/H2 \text{ and } n = 29 H2/H2) \). They observed a significantly higher rate of overall cognitive decline in H2 carriers as compared to H1 homozygotes. Also, the effect of length of CAG repeats on cognitive change was more pronounced in the H2 haplotype cases compared to H1 homozygotes [73].

The exact mechanism behind this may not be known yet but a molecular interaction between the proteins in the H2 haplotype and mHtt could be a contributing factor [37, 73]. Also, it has been shown that in other tauopathies, such as PD and PSP, H1 haplotype is more susceptible to cognitive decline and also that H1 is associated with an increase in the 4R isoform [104, 105]. This contradicts Vuono et al.’s study where they report H2 is the more susceptible haplotype in HD, and, as mentioned before, many studies have already shown that higher levels of 4R are seen in HD patients [73]. This may be explained by the fact that binding of SRSF6 to the CAG repeats in mHtt in a more allele-specific effect on the spliceosome on the H2 as involved in MAPT exon 10 splicing [71, 73].

**CALCINEURIN**

Considering tau hyperphosphorylation is one of the key events in HD pathogenesis, it critical to understand the effects of tau phosphatases that may have a role in this, in particular the serine/threonine protein phosphatases (PPs) which there are of five major groups: PP1, PP2A, PP2B, PP2C, and PP5. All except PP2C are known to dephosphorylate tau [106]. Among these, PP2B or calcineurin showed a strong negative correlation to pTau levels in R6/2 and Q175 mice brains as well as in Q111 cell lines compared to controls. Also, calcineurin inhibition (cyclosporine and CN585 inhibitors) resulted in tau hyperphosphorylation in SH-SY5Y 3R-Tau cells and calcineurin downregulation (siRNA directed) led to tau hyperphosphorylation in N2A cells [77]. Several studies have been already reported that calcineurin is downregulated in HD. This strongly suggests that mHtt could lead to tau hyperphosphorylation through downregulation of PP2B [107–109].

**TAU AND GSK-3β IN HUNTINGTON’S DISEASE PROGRESSION**

GSK-3β is another major tau kinase which phosphorylates tau at many Ser or Thr residues that are followed by proline [110, 111]. In the brain, it has a variety of other functions such as energy metabolism and CNS development [112, 113].

In 2016, L’Episcopo and co-workers provided a strong series of evidence supporting GSK-3β mediated tau hyperphosphorylation in HD. First, they demonstrated an overall upregulation of pTau, GSK-3β, and its active kinase form pGSK-3β-Tyr216 in a HD grade dependent manner. Secondly, they showed changes in GSK-3β cellular localization (cytoplasmic to nuclear in HD) as well as increased neuronal cell death associated with elevated levels of total Tau, AT8, and pGSK-3β-Tyr216. Thirdly, because oxidative stress and mitochondrial dysfunction are critically involved in HD, they showed that a low dose of hydrogen peroxide further increased the upregulation in pGSK-3β-Tyr216 protein levels. This was associated with a sharp decrease in mitochondrial activity and in cell viability accompanied by a significant increase in cell death markers (such as cleaved caspase-3). Finally, they also reported an increased level of proinflammatory cytokines such as TNF-α, IL-6, and NO in transgenic HD cells. Thus overall, they propose that pGSK-3β is causally linked to increased pTau accumulation in HD and this in turn may induce mitochondrial stress leading to cell death (Fig. 5) [38].

This is supported by the fact that several studies suggest elevated pGSK-3β levels in HD brains [114]. But there are many others who report a decrease in the GSK-3β and pGSK-3β activity in HD mice models [115]. This contradiction might be explained by the difference in phosphorylation of GSK-3β, phos-
phorylation at its Tyr residues may increase its kinase activity while phosphorylation at its Ser residues may decrease its kinase activity [38, 114, 115].

**CONCLUSIONS AND FUTURE STUDIES**

As mentioned above, it is only recently that researchers have acknowledged HD to be a tauopathy. Therefore, much research is needed to identify the precise cellular pathways behind its pathogenesis. In spite of all the data available on elevated pTau levels in brain tissues from HD mice as well as human postmortem HD brains and in CSF, studies on pTau levels in HD cell lines seem insufficient. Thus, could be a good unexplored domain to further look into tau pathogenic pathways in HD. Besides, how proteins
of tau H2 haplotype influence HD progression also remains to be explored.

One of the most important characteristics of HD is the colossal neuronal loss as the disease progresses. Emerging evidence does provide for neuronal loss accompanied by elevated tau levels in human post-mortem brain as well as cultured R6/2 cells [38].

Many studies successfully established the presence of NFTs as well as an increase in the 4R:3R Tau ratio in HD, which are distinct characteristics of tauopathies. But a lot of ambiguity exists regarding the exact role of mHTT in inducing the tauopathy. More studies need to demonstrate if tau and mHTT do interact or co-localize. Studies by Blum et al. do indeed provide much insight into the nature of tau and mHTT interactions in vitro, but this needs to be further demonstrated in in vivo conditions too. Even though the proteins may interact in vitro, in the cellular environment they may be restricted to their respective deposits or inclusion bodies, thus preventing their interaction [71, 76]. Extensive immunostaining and co-immunoprecipitation studies could help further clarify the exact cellular nature and extent of this interaction.

Even though Gratuzze and colleagues did successfully demonstrate the downregulation of calcineurin in HD, they could not show any positive tau staining [77]. As lowered calcineurin levels and elevated pTau levels are well established in HD, it sounds as a convincing enterprise that calcineurin causatively hyperphosphorylates tau. But to confidently assert this, both phenomena need to be shown to co-occur on a cellular level.

Hyperphosphorylation of tau by GSK-3β seems to another convincing pathway in tau pathogenesis in HD. Even though L’Episcopo et al. provided much experimental evidence explaining the underlying pathways in this regard, more research needs to be done on the individual steps involved there in [38]. In particular, exactly how pTau or pGSK-3β-Tyr216 affect mitochondrial function remains to be explored. Also, phosphorylation of which epitopes of GSK-3β produces what effect in these terms needs to be looked into.

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