

# Role of oxidative stress, ER stress and ubiquitin proteasome system in neurodegeneration

## Abstract

Neurodegenerative disorders (NDDs) are progressive and chronic disorders characterized by destruction of neurons in sensory, motor and cognitive systems. Free radical's accumulation, oxidative stress, ER stress and a dysfunctional ubiquitin proteasome system can regulate prognosis in NDDs. Oxidative trauma in the brain can result from high rate of oxidative metabolism in contrast to the diminished functional levels of the antioxidant enzymes responsible for detoxification. Endoplasmic Reticulum (ER) advocates a degree of control on cellular parameters such as proper protein folding, posttranslational modification and subsequent protein trafficking in order to maintain normal cellular homeostasis. However, an abnormal ER functioning can lead to loss of integrity of the ER thus resulting in ER stress. In addition to this impairment in the ubiquitin proteasome system (UPS) machinery results in the accumulation of toxic proteins in the brain thus resulting in severe neuronal trauma and subsequent damage. This review explores the disease critical interactions and roles of three critical NDD determinants viz. oxidative stress, ER stress and UPS dysfunction in neurodegenerative conditions.

**Keywords:** ndd, free radicals, ros, oxidative stress, er stress, ups, e3-ligases, mitochondrial dysfunction

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**Abbreviations:** NDD, neurodegenerative disorders; ER, endoplasmic reticulum; UPS, ubiquitin proteasome system; ROS, reactive oxygen species; AD, alzheimer's disease; PD, parkinson's disease; MS, multiple sclerosis; HD, huntington's disease; ALS, amyotrophic lateral sclerosis; PS, presenilin; APP, amyloid beta (a4) precursor protein; MARK1, microtubule affinity-regulating kinase 1; SOD-1, superoxide dismutase 1; VAMP, vesicle-associated membrane protein; ALS2, amyotrophic lateral sclerosis 2; DCTN1, dynactin 1; FUS, fus rna binding protein; TDP-43, tar dna binding protein; PERK, phosphorylation of the er stress kinases; IRE 1, inositol-requiring enzyme 1; SCA, spinocerebellar ataxia; GSK, glycogen synthase Kinase; ASK1, apoptosis signal-regulating kinase 1; JNK, c-jun nh2-terminal kinase; Keap1, kelch-like ech-associated protein 1; MGRN1, mahogunin ring finger 1, E3 ubiquitin protein ligase; MYCBP2, myc binding protein 2, E3 ubiquitin protein ligase; UHRF2, ubiquitin-like with phd and ring finger domains 2; ZNRF1, zinc and ring finger 1, E3 ubiquitin protein ligase; NEDD4, neural precursor cell expressed, developmentally down-regulated 4, E3 ubiquitin protein ligase; NEDD4L, neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein Ligase; HECTD2, hect domain containing e3 ubiquitin protein ligase 2; PJA2, praja ring finger 2, E3, ubiquitin protein ligase; RNF19, ring finger protein 19a, rbr e3 ubiquitin protein ligase; HECTD1, hect domain containing e3 ubiquitin protein ligase 1; MULAN, mitochondrial e3 ubiquitin protein ligase 1; HACE1, hect domain and ankyrin repeat containing e3 ubiquitin protein ligase 1; TRIM13, tripartite motif containing 13; AIMP2, aminoacyl trna synthetase complex-interacting multifunctional protein 2; NRF2, nuclear factor, erythroid 2-like 2; DVL1, dishevelled segment polarity protein 1; MYC, v-myc avian myelocytomatosis viral oncogene homolog; TSC2, tuberous sclerosis 2; FBXO45, f-box protein 45; PCNP, pest proteolytic signal containing nuclear protein; SMAD2, smad family member 2

## Introduction

Neurodegenerative disorders (NDDs) are characterized by the gradual and progressive loss of neurons and neuronal death that ultimately leads to deficient nervous system functioning. It can result due to diverse factors such as oxidative stress, ER stress, mitochondrial dysfunction, impaired ubiquitin proteasomal system and several other determinants such as endocrine conditions, gender, poor education, inflammation, stroke, smoking, hypertension, diabetes, infection, head trauma, depression, tumors, vitamin deficiencies, immune and metabolic conditions, chemical exposure, accumulation of reactive oxygen species (ROS), loss of mitochondrial membrane potential, and ATP depletion. The two hit hypothesis of neurodegeneration states that neuronal cells that have been subjected to a severely stress once, becomes more vulnerable to the negative impact of a second hit and the effect of the toxicity of both the hits of severe stress may be synergistic in nature. Most common neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Schizophrenia, Amyotrophic lateral sclerosis (ALS) and Multiple Sclerosis (MS).<sup>1-5</sup>

Oxidative stress plays a critical role in the progression of several age related brain disorders. Severe oxidative trauma to the neurons can result in neuronal dysfunction and death. However, the neuronal cells are equipped with an arsenal of protective mechanism to prevent the damaging effects of oxidative stress on neuronal integrity and homeostasis. The removal of aberrantly functioning proteins by proteolysis and the synthesis of new and protective counterparts are critical during periods of continuous oxidative trauma.<sup>6</sup> Reactive oxygen species (ROS) can result in oxidative stress and subsequently lead to mitochondrial dysfunction. Moreover, disturbed equilibrium between pro-oxidant/antioxidant homeostasis can generate ROS and free radicals which are detrimental for neurons. ROS in turn modulates

the functionality of antioxidants and biomolecules thus leading to neuronal dysfunction and advances the brain towards progressive neurodegeneration.<sup>7-10</sup>

Endoplasmic reticulum (ER) mediated stress on the other side results from disturbances in the structural integrity and function of the ER, thus leading to the accumulation of misfolded proteins and deviations in the calcium homeostasis. The endoplasmic reticulum (ER) acts as protein quality control in the secretory pathway to prevent protein misfolding and aggregation. Under conditions of stress, the ER mediated machinery can reestablish homeostasis by sophisticatedly regulating various transcriptionally and translationally mediated signaling networks and proteins.<sup>11</sup> The normal ER response is depicted by reduction in damaged proteins levels, caused by translational attenuation, induction of ER chaperones and misfolded proteins proteolysis. However, under prolonged or provoked ER stress ambience, can lead to the activation of apoptotic pathways resulting in neuronal death. Therefore, ER stress situation and remains a subject of curious debate involving the pathogenesis of common NDDs such as Parkinson's disease (PD) and Alzheimer's disease (AD).<sup>12</sup>

Improper functioning of the Ubiquitin proteasome system (UPS) under conditions of severe ER and oxidative trauma leads to the derogatory accumulation of damaged and misfiring stress consistent proteins. The UPS in collaboration with chaperones and co-chaperones constitute the regulatory mechanism responsible for neuronal quality control and survival. In addition, UPS can also operate as machinery for protein quality control and degradation in conjunction with autophagy. Dysfunctional UPS has been held capable in various NDDs and recent reports on *Drosophila* suggested that the role of UPS in protein turnover is essential for maintaining axon guidance, synaptic function and growth, axon pruning, and neuronal maintenance.<sup>13</sup> Under the ambit of this review we have made an attempt to explore the role of oxidative stress, ER stress and UPS dysfunction respectively in neurodegenerative conditions. This interaction shall than be crucial in embellishing the development of potential neurotherapeutics.

## Role of oxidative stress in neurodegeneration

Oxidative stress (OS) condition in the brain results from imbalance between ROS and the body's detoxification mechanism, which results in accumulation of ROS and subsequent neuronal damage. Hence, the outcome of oxidative stress on neuronal cells depends upon the ability of the cell to maintain oxidative homeostasis. High stress levels can cause ATP depletion, necrosis and prevent apoptotic cell death.<sup>14</sup>

Any disproportion in the usual redox state can result in toxicity via the activation of peroxides and free radicals which in turn damages lipids, proteins and cellular DNA. A mammalian cell as a consequence of mitochondrial aerobic respiration generates superoxide radical. Superoxide is sequentially reduced to hydroxyl radicals and hydrogen peroxide that cause severe traumatic injury to the DNA thus leading to mutations, which might be causative factors leading to severe neurodegeneration.<sup>15</sup>

Reactive oxygen species (ROS) also plays a discrete role in cell signaling, by a mechanism known as redox signaling. In order to sustain proper cellular homeostasis, a balance must be reached between ROS production and consumption. Therefore, it is obvious that free radicals need to either be reduced and detoxified by converting them into metabolically nondestructive molecules or be neutralized right after their generation. Any aberration in the cellular antioxidant

defense system, which protects the neurons from free radical assaults, therefore can lead to neurodegenerative conditions and aging.<sup>16</sup>

Brain is the most metabolically active organ of the body that including the spinal cord comprises the central nervous system (CNS), which even in resting condition consumes an estimated 20-22% of the total oxygen uptake. In addition, during active state the brain oxygen demand considerably rises in order to establish normal physiological homeostasis. Blockage or oxygen deprivation can lead to severe and irreversible injuries to the neurons. Oxygen consumption in the brain of oxygen results in production of free radicals and higher oxygen levels in brain leads to even higher concentration of reactive oxygen/nitrogen species. However, in spite of the fact that brain has higher necessity for oxygen, it is relatively deficient in the enzymes capable of metabolizing a number of these toxic oxygen-based reactants to harmless residues. In contrast, CNS is highly enriched with polyunsaturated fatty acids and toxic oxygen derivatives oxidizes these polyunsaturated fatty acids.<sup>17,18</sup> This then makes the neurons more vulnerable to oxidation related damages and the role of the cellular detoxification machinery in these conditions is vital.

Oxidative stress has major impact on several NDDs such as Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) In AD, oxidative stress is associated with a number of critical events which ultimately influence amyloid precursor protein (APP) processing. Tau modification leads to increased brain toxicity and as a result of oxidative stress, APP and Tau processing is altered via activation of different signaling pathways. In general most of the AD cases are late onset and sporadic although there are approximately 10-15% familial AD (FAD) cases. Mutation in three genes namely, APP, Presenilin 1 and 2 (PS1, PS2) can potentially lead to FAD. During normal physiological state proteolytic cleavage of APP is commenced by  $\alpha$ -secretase followed by  $\gamma$ -secretase mediated second cleavage to yield non amyloidogenic fragments.<sup>19</sup> However, mutations in APP results in an altered proteolytic processing where  $\alpha$ -secretase is replaced by  $\beta$ -secretase (BACE1), followed by  $\gamma$ -secretase mediated cleavage in order to yield amyloidogenic  $A\beta_{42}$  which aggregates as insoluble plaques. Widespread cell culture studies have revealed  $A\beta_{42}$  to have toxic effect on brain and can emanate cell death via apoptosis.<sup>20</sup> The hyperphosphorylation of tau protein, by various kinases such as MARK, MAPK and GSK-3 $\alpha$ , results in the formation of paired helical filaments (PHFs), which further combine to form insoluble NFTs.<sup>21</sup> Abnormal hyperphosphorylation of tau is indicative of both an abnormal activation of kinases and decreased phosphatase activity.<sup>22</sup> Experiments on Pin1 knockout mice illustrates a rise in amyloidogenic APP processing thus increasing the levels of  $A\beta_{42}$  and additionally also display tau hyperphosphorylation thus leading to behavioral deficits, motor and neuronal degeneration.<sup>23</sup>

Oxidative can modulate pathogenesis in Parkinson's. PD is the most common neurodegenerative disorder and is clinically demarcated by bradykinesia, progressive rigidity and tremor. Like all other neurodegenerative disorders determinants such as environmental factors, mitochondrial dysfunction, oxidative damage, and genetic predisposition together play a crucial role in both sporadic as well as familial PD.<sup>24</sup> Neurotoxic compounds, such as N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or its active derivative, MPTP and 6-hydroxydopamine (6-OHDA) can provoke oxidative stress, impair mitochondrial respiration and energy metabolism which in turn leads to neurodegeneration. Postmortem tissues from PD patients have revealed a significant insight into the failure of complex I in

the substantia nigra. Complex I is involved in the mitochondrial electron-transport chain, and 30-40% decrease in activity may be the central prognosis of sporadic PD.<sup>25</sup> The decreased activity of complex I could be the result of self-inflicted oxidative damage, under production of certain, complex I subunits, and may be due to complex I disassembly.<sup>26</sup> Immunocytochemical confirmation of protein glycation and nitration in substantia nigra region of human PD brain revealed oxidative damage to DNA and protein resulting from persistent oxidative trauma.<sup>27</sup>

Huntington's disease (HD) is autosomally inherited and is characterized by progressive cognitive impairment, choreiform movements, psychiatric disturbances and loss of long projection neurons, resulting in atrophy of the caudate nucleus, globus pallidus, and putamen.<sup>28</sup> HD mutation is an extension of the CAG trinucleotide repeat inside exon 1 of the huntingtin (*HTT*) gene, the exact role of which is unknown.<sup>29</sup> CAG triplet codes for glutamine expansion and upon mutation, presents a polyglutamine tract at the N-terminus, results in a conformational change of the protein, which eventually results in abnormal protein-protein interaction. Mutant *HTT* presents a dominant "gain of function" to the protein, due to the stretched polyglutamine segment, which finally leads to neurodegeneration. Empirical evidences suggest that the mitochondrial metabolic defect resulting in impaired energy metabolism may be the consequences of *HTT* gene expansion.<sup>30</sup> Altered mitochondrial energy metabolism raises the production of free radicals thus resulting in severe neuronal trauma. Mitochondrial dysfunction is a critical hallmark in the pathogenesis of PD. The activity of mitochondrial complexes I, II, III and IV is significantly altered during HD pathogenesis. Biochemical studies of HD brain tissue have reported defects in the caudate and decreased activities of complex II and III activity. However, no such deviation was observed with complex I or IV.<sup>31</sup>

Amyotrophic lateral sclerosis (ALS) is clinically identified by progressive atrophy, weakness, and spasticity of muscle tissue. ALS is characterized as an adult-onset neurodegenerative disease reflecting the degeneration of upper and lower motor neurons in the spinal cord, cortex, and brainstem.<sup>32</sup> Mutations in the ubiquitous enzyme; Cu/Zn-superoxide dismutase (*SOD-1*), accounts for upto 5-20% all of major genetic defect in ALS. In addition to *SOD-1* gene few other genes such as VAMP-associate protein B (*VAPB*), *Alsin* (*ALS2*), *Dynactin* (*DCTN1*), fused in sarcoma protein (*FUS*), *TAR DNA-binding protein-43* (*TDP-43*), and lipid phosphatase *FIG4* (*FIG4*) can also contribute to ALS pathogenesis. Postmortem tissues from ALS patients have clearly revealed that oxidative stress is the main causative factor that contributes to accumulation of oxidative damage to lipids, proteins, and DNA thus suggesting a direct role in ALS progression.<sup>33,34</sup>

## Role of ER stress in neurodegenerative conditions

Endoplasmic reticulum (ER) is an imperative organelle responsible for the post-translation modification, proper folding, and transport of nascent proteins to target destinations. Loss of ER integrity results in ER stress and may be established due to changes in the calcium homeostasis within the ER and due to the accumulation of unfolded proteins. ER stress plays a crucial role in several signaling cascades including the unfolded protein response (UPR) which counteracts the effects of the original stress.<sup>35,36</sup> The activity of the ubiquitin proteasome system (UPS) is significantly misregulated in these stress conditions and leads to protein aggregates and other toxic

product accumulation thus leading to brain damaging conditions.<sup>37,38</sup> Furthermore, intracellular ER calcium concentration and its release from the ER play a significant role in controlling neuronal death.<sup>39</sup>

As discussed earlier AD is a neurodegenerative disease characterized by the progressive loss of cognitive functions and memory loss. ER stress and an altered calcium homeostasis have major impact on severity of AD pathogenesis.<sup>40,41</sup> Brain tissue from AD patients reports an alteration in calcium metabolism and subsequent neurodegeneration. Neurons containing NFTs shows an increase in the levels of free and protein bound calcium as compared to tangle free neurons. In addition to change in the level of calcium ion due to ER stress, an alteration in APP or PS proteins activity also can define AD prognosis.<sup>42</sup> PS1 and PS2 proteins are the major catalytic components of the  $\gamma$ -secretase complex that facilitates the intramembranous cleavage of APP. ER stress mutations can cause a change in the pattern of APP processing in the affected neurons and as a result increase the amount of the toxic A $\beta$ 1-42 peptide. PS1 and PS2 are ER transmembrane proteins that are richly expressed by the brain neurons and which facilitates a linkage between AD and ER stress. ER stress related alteration in PS1 activity demonstrates altered calcium homeostasis, increased production of A $\beta$  peptides, and enhanced apoptotic sensitivity. Mutant PS1 attaches to and restrains the ER kinase, IRE1 which senses the gathering of misfolded proteins in the ER lumen. IRE also triggers the downstream signals to mediate the transcription of the ER chaperone, BiP.<sup>43</sup>

Cultured neuronal cells, including dopaminergic neurons, reveals that neurotoxic compounds such as N-methyl-4-phenyl-1,2,3,6-tetrahydroxyridine or its active derivative, MPPT and 6-hydroxydopamine (6-OHDA) can elicit ER stress and activate a number of genes such as the ER chaperones and other machinery of the UPR for instance the transcription factor, CHOP/Gadd153. In addition it can also lead to the phosphorylation of the ER stress kinases, PERK and IRE.<sup>44,45</sup> Thus, ER stress in combination with abnormal protein degradation can contribute to the pathophysiology of NDDs.

Human inherited neurodegenerative disorders such as Huntington's disease (HD), dentatorubral-pallidolusian atrophy, spinobulbar muscular atrophy, and six spinocerebellar ataxias (SCA 1, 2, 3, 6, 7 and 17) are caused due to expanded polyglutamine (polyQ) repeats in the brain. In cultured cells, transgenic animals and in human post-mortem brain tissue these disorders are significantly characterized by aggregation of intracellular protein aggregates and selective neuronal death.<sup>46</sup> Additionally, in HD the mutant Huntingtin gene can also have an effect on the calcium metabolism in the cell and sensitize the IP3 receptors in the ER.<sup>47</sup>

Evidence about the role of ER stress in polyQ diseases approaches from studies showing the colocalization of polyQ fragments with various molecular chaperones viz. Hsp70 and Hsp40 that are induced during ER stress. *Drosophila* overexpression of Hsp70 restrains polyQ toxicity. This Hsp mediated effect has also been observed in a few, but not all mouse models of polyQ diseases.<sup>46</sup> SCA3 polyQ fragments also triggers ER stress mediated neuronal cell death, as shown by the activation of PERK, IRE1 and the stimulation of CHOP/Gadd153 and BiP/Grp78. However, this effect is mainly due to impairment in the interaction between the ER and the UPS. Another study reveals that deficient mouse embryonic fibroblasts show activation in the apoptosis signal-regulating kinase 1 (ASK1) which is indispensable for polyQ induced ER-mediated cell death.<sup>48</sup> Moreover, ASK1 forms

a complex with TRAF2 and IRE proteins at the ER and consequently trigger downstream signals, such as the c-Jun NH2-terminal Kinase (JNK).<sup>49</sup>

Transgenic ALS mice and human samples reports intracellular cytoplasmic inclusions in motor neurons. In ALS mice these contain deposits of SOD1 and ubiquitin. The aggregates appear prior to the first appearance of disease symptoms. Although the significance of inclusion bodies in ALS is not clear yet it has been attributed to act as a neurotoxin and inhibits critical cellular functionalities.<sup>50</sup> Dorfin is one such ubiquitin E3 Ligase which play a crucial role in ALS mediated neurodegeneration. In ALS infected neurons, mutant SOD1 is degraded by such ubiquitin E3-ligase through the UPS. Mutant  $\alpha$ -synuclein and aggregated SOD1 in combination with other proteins can alter the function of the UPS and also affects the motor neurons. Although, motor neurons are not the only cell type targeted in ALS. The role of glial neuron and glial cells interactions at some stage in development of the disease suggests that the increase in ROS production combined with ER and oxidative damage to crucial proteins and other cell machinery may play a role in ALS pathogenesis.<sup>51</sup>

Glutamate metabolism is associated with prolonged stimulation of excitatory amino-acid receptors and results in increased intracellular calcium levels, which can easily damage the integrity and functional aspects of mitochondria and the ER. This then result in cleavage of caspase-12 in the spinal cord of transgenic ALS mice. Caspase-12 activity and cleavage in the ALS mice may be due to the activity of the calcium dependent enzyme, Calpain. Caspase-12 acts as substrate for calpain in some cells including neurons. Other biomarkers for ER stress, Bip/ Grp78 function is also altered in the ALS mice.<sup>52</sup>

Furthermore, evidence for ER stress in ALS comes from studies showing an increase in Bip/Grp78 level in spinal motor neurons of transgenic ALS mice proceeding to onset of motor symptoms. It has been also accounted that mutant SOD1 is associated with ER stress, but not wild type SOD1.<sup>53</sup> These findings provide credence to the fact that ER stress is part of the mechanism by which mutant SOD1 contributes to ALS related motor neuron degeneration.

## UPS and E3 ligases in neurodegeneration

Dysfunction of the ubiquitin proteasome system is one of the major events that lead to the progression of neuronal loss. An in vivo report suggests that oxidative stress is caused directly by neuronal proteasome dysfunction in the mammalian brain.<sup>13,54,55</sup> The UPS plays a vital role in regulated degradation of cellular proteins under diverse physiological conditions. Aggregation of misfolded proteins has been attributed in the progression of various neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). Ubiquitin E3 ligases are key regulators involved in mediating the proteasomal degradation of misfolded proteins in the endoplasmic reticulum (ER), as a result protecting neurons against oxidative stress, mitochondrial dysfunction and ER stress.<sup>56</sup> Furthermore, Ubiquitin proteasome system can critically modulate the level of proteins in cells, and robustly control cellular mechanisms. Aberration in UPS function in susceptible neurons results in protein aggregation, increased, oxidative stress, ER stress, and ultimately neuronal death. Conversely, neurons depend on the proper functioning of E3 ligases and UPS to maintain neuronal homeostasis.<sup>57</sup> Table 1 highlights the prospective role E3 ligases in neurodegeneration.<sup>58-80</sup>

**Table 1** E3 ligases in the brain and their functional prospect in neuro degeneration

E3 ligase	Substrates	Functional significance	References
<b>Keap1</b>	Nrf2	Involved in degradation of Nrf2. disruption of protein degradation systems and sustained activation of the Keap1-Nrf2 system occur in the AD brain.	Tanji et al. <sup>58</sup>
<b>PARK2</b>	AIMP2	Parkin is an E3 ubiquitin ligase that has been shown to be a key regulator of the autophagy pathway. Although, mutations in Parkin results into Parkinson's Disease.	Segura-Aguilar et al. & Imam et al. <sup>59,60</sup>
<b>HECW1</b>	DVL1, p53, and mutant SOD1	NEDL1 is another name of HECW1. It mainly interacts with p53 and the Wnt signaling protein DVL1, and may play a critical role in p53-mediated cell death in neurons.	Li et al. <sup>61</sup>
<b>HUWE1</b>	TopBP1, N-Myc, C-Myc, p53, Mcl-1	HUWE1 regulates neuronal differentiation by destabilizing N-Myc, and also modulates p53-dependent and independent tumor suppression via ARF. It is also known as Mule. HUWE1 is a HECT domain E3 ubiquitin ligase which involves in degradation of Mcl-1 and thus regulates DNA damage-induced apoptosis.	Zhong et al. <sup>62</sup>
<b>MGRN1</b>		Involved in melanocortin signaling. Loss of mahogunin function leads to neurodegeneration and loss of pigmentation, and also has mechanism of action in prion disease.	Perez-Oliva et al. <sup>63</sup>
<b>MYCBP2</b>	TSC2, Fbxo45	MYCBP2 associates with Fbxo45 to play a crucial role in neuronal development. MycBP2 is an E3 ubiquitin ligase also known as PAM. MycBP2 also modulates the mTOR pathway through ubiquitination of TSC2.	Han et al. <sup>64</sup>
<b>UHRF2</b>	PCNP	UHRF2 ubiquitinates PCNP and has been shown to play a role in degradation of nuclear aggregates containing polyglutamine repeats mediated Neurodegeneration. UHRF2 is also known as NIRF. UHRF2 is a nuclear protein that may regulate cell cycle progression through association with Chk2.	Mori et al. <sup>65</sup>
<b>ZNRF1</b>		Highly expressed in neuronal cells. ZNRF1 is found in synaptic vesicle helpful in neuronal transmissions and plasticity. It also contains a RING finger motif, which expression is up regulated in the Schwann cells mediated nerve injury.	Araki and Milbrandt <sup>66</sup> & Saitoh and Araki <sup>67</sup>
<b>NEDD4</b>		Highly expressed in the early mouse embryonic central nervous system. It down regulates both neuronal voltage-gated Na <sup>+</sup> channels and epithelial Na <sup>+</sup> channels in response to increased intracellular Na <sup>+</sup> concentrations.	Goulet et al. <sup>68</sup>

Table Continued....

E3 ligase	Substrates	Functional significance	References
<b>NEDD4L</b>	Smad2	It also highly expressed in the early mouse embryonic central nervous system. NEDD4L negatively regulates TGF- $\beta$ signaling by targeting Smad2 for degradation.	Gao et al. <sup>69</sup>
<b>HECTD2</b>		HECTD2 is a likely E3 ubiquitin ligase and may act as a vulnerable gene for neurodegeneration especially in prion disease.	Lloyd et al. <sup>70</sup>
<b>PJA2</b>		Expressed in neuronal synapses. The exact role and substrates of PJA2 are unclear.	Yu et al. <sup>71</sup>
<b>RNF19</b>	SOD1	RNF19 is also known as Dorfin. Accumulation of mutant SOD1 results into ALS disease. RNF19 ubiquitinates mutant SOD1 protein, causing less neurotoxicity in brain.	Sone et al. <sup>72</sup>
<b>HECTD1</b>		HECTD1 is required for normal development of the mesenchyme and neural tube closure.	Zohn et al. <sup>73</sup>
<b>MULAN</b>	mnd2	Involved in degradation of mnd2. mnd2 causes neuromuscular disorder due to loss of Omi/HtrA2's protease activity.	Cilenti et al. <sup>74</sup>
<b>HACE1</b>	NRF2	HACE1 plays a crucial in the NRF2 mediated antioxidative stress response pathway and also involved in HD pathogenesis.	Rotblat et al. <sup>75</sup>
<b>CUL4</b>	TSC2	It promotes proteasomal degradation of TSC2. As a result, Tnfaip8 II/Oxi- $\beta$ competes with TSC2 to bind FBXW5, increasing TSC2 stability through preventing its ubiquitination in PD progression.	Ha et al. <sup>76</sup>
<b>TRIM13</b>		Involved in regulation of ER stress induced cell death. However, the expression of TRIM13 sensitizes cells to ER stress induced neuronal cell death.	Tomar et al. <sup>77</sup>
<b>MGRN1</b>		Over expression of MGRN1 protects against cell death mediated by ER and oxidative stress and also interacts with Cytosolic Hsp70. Lack of MGRN1 functionalities are the hall mark of age dependent spongiform disease in the brain.	Chhangani and Mishra <sup>78</sup>
<b>NEDD4-I</b>	FOXMI B	Up regulated in cultured neurons in response to various neurotoxins, including, hydrogen superoxide, and zinc via transcriptional activation likely mediated by the reactive oxygen species. A level of the insulin-like growth factor receptor (IGF-IR $\beta$ ) is also maintained due to up regulation of NEDD4-I.	Kwak et al. <sup>79</sup>
<b>APC/C</b>		Involved in cell cycle progression in proliferating cells, plays a significant role in post-mitotic neurons. APC/C-activating cofactor, Cdh1, is also helpful for the function of APC/C in neuronal survival.	Almeida <sup>80</sup>

## Conclusion

The future of neurodegenerative disorders depends on the researchers' ability to adjust actions to circumstances and have a clear projection relating to the aberrant mechanisms that ultimately decides the fate of the neurons and henceforth degeneration. Despite tremendous advancement in the field of neurobiology, still the future of such therapies hangs on torrid balance and deceptive hopes Neuronal damage is caused due to free radical's accumulation, oxidative stress and ER stress. In addition, dysfunctional of ubiquitin proteasome systems can also regulate prognosis in NDDs. These factors can cause an imbalance between cellular-antioxidant defence and reactive oxygen species concentration. Further research is therefore needed in order to make bio molecule based neurotherapeutics a blatant reality in conditions of neurodegeneration.

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## Conflict of interest

The author declares no conflict of interest.

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