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# A REVIEW ON NEURODEGENERATIVE DISORDERS WITH SPECIFIC EMPHASIS ON BIOMARKERS, PATHOPHYSIOLOGY, AND PRECLINICAL MODELS.

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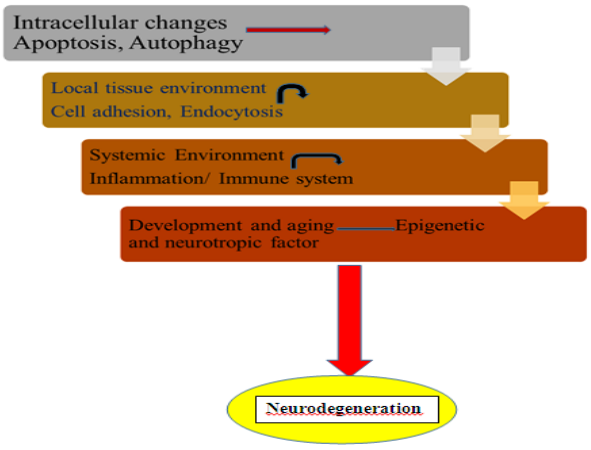
## ABSTRACT
Neurodegenerative disorders (NDD) are described as disorders with selective loss of neurons and distinct involvement of functional systems defining clinical presentation. Studies have shown that alteration in gene coding, proteins with altered physicochemical properties are deposited in the human brain in neurodegenerative disorders (NDD). The present review focuses on the pathogenesis behind these NDD at molecular level and their clinical biomarkers. Accordingly, NDD-related proteins and their biochemical modifications can be used as biomarkers and may be targeted for therapy. We are going to discuss the most common neurodegenerative diseases: Alzheimer disease, Parkinson disease, Huntington disease and prion diseases, with a particular focus on similarities and differences among these syndromes.

**KEYWORDS:** Alzheimer disease; Parkinson's disease; Huntington's disease; Prion's disease, Preclinical models.

## INTRODUCTION
Neurodegenerative diseases (NDDs) are traditionally defined as disorders with selective loss of neurons and distinct involvement of functional systems defining clinical presentation. Comprehensive biochemical, genetic and molecular pathological examinations have expanded this definition. During the last century, many studies have demonstrated that proteins with altered physicochemical properties are deposited in the human brain in NDDs. Furthermore, not only neurons but glial cells also accumulate these proteins. Involvement of proteins has led to the definition of the concept of conformational diseases.[1] Intense research efforts in the past two decades to understand the pathogenesis of neurodegenerative diseases and design effective therapeutics have been focused mainly on neurons. Although this so-called "neuro-centric" view has contributed to our understanding of neuronal dysfunction, death pathways and accumulation of proteinaceous aggregates during chronic neurodegenerative processes, this approach has not resulted in disease-modifying therapeutics. This suggests that the pathogenesis of neurodegenerative disorders is more complex than previously thought and that the lack of success of neuro-centric-based therapies may be due to the participation of non-neuronal cells in the disease process.[2] Mutations in the encoding genes are linked to hereditary forms of disease. Molecular pathological, genetic and biochemical studies have led to reclassification of several disorders and opened completely new avenues for biomarker development or therapeutic strategies.[3] This review aims to summarise the molecular pathological background behind NDD and clinical biomarkers targeted for therapy.




### Alzheimer disease
As the prototype of cortical dementias, Alzheimer's disease (AD) presents with prominent cognitive deficits. Initially, patients display limited forgetfulness with disruption of memory imprinting, which evolves to short-term memory disruption and, eventually, to long-term memory deficits.[4] AD is a chronic, progressive degenerative disease of central nervous system. With the aging of population and prolonging of life expectancy, the incidence of AD is rising. So far, the pathogenesis of AD is not very clear, and there is no specific method to

cure it.[5] The first defined histopathological features of AD were extracellular amyloid plaques and intracellular neurofibrillary tangles. More recently recognized histopathological features include synaptic degeneration, hippocampal neuronal loss and aneuploidy.[6]

**Pathogenesis of Alzheimer's disease**

Two pathogenic AD hypotheses are discussed below, the amyloid cascade hypothesis (which assumes AD is always a primary amyloidosis) and the mitochondrial cascade hypothesis (which assumes most AD is a secondary amyloidosis).

**The amyloid cascade hypothesis**

The cortical plaques of AD brains largely consist of Amyloid β (Aβ) protein. Aβ is produced via processing of its parent protein, Apolipoprotein (APP). The gene encoding APP resides on chromosome 21. Specific APP physiologic roles are not entirely clear, but in a general sense it is felt to contribute to proper neuronal function and perhaps cerebral development.[7] Because plaques contain Aβ and Down's syndrome patients with trisomy 21 manifest both plaque pathology and presenile cognitive decline below their baseline. The amyloid cascade hypothesis proposed altered APP processing drove Aβ production, Aβ gave rise to plaques, plaques induced neurodegeneration and this neuronal loss resulted in the clinical dementia syndrometypical of AD. Even so, the amyloid cascade hypothesis seems most applicable in cases of early onset, autosomal dominant AD. Pursuit of the amyloid cascade hypothesis has yielded in depth insight into how APP processing actually occurs. The APP secretases work in combination. Sequential processing by the α and γ-secretases results in a large N-terminal peptide called soluble APPα (sAPPα) and a smaller 3 kD peptide called P3. Proteolysis by enzymes with α-secretase activity precludes sequential β-γ secretase activity. Aβ degradation is also enzymatically mediated. Enzymes that degrade Aβ include e neprilysin and insulin degrading enzyme (IDE). Interestingly, in the common non-autosomal dominant forms of AD Aβ overproduction is accompanied by IDE downregulation. This suggests amyloidosis in AD is not a toxic accident, but rather part of a coordinated cell response to an upstream event.[8]

**The mitochondrial cascade hypothesis**

The mitochondrial cascade hypothesis attempts a unified explanation for the clinical, biochemical and histologic features of AD.[9] The mitochondrial cascade hypothesis argues non-Mendelian genetic factors contribute to non-autosomal dominant AD. Finally, it posits AD brain mitochondrial dysfunction drives amyloidosis, tau phosphorylation and cell cycle re-entry. Mitochondrial dysfunction is observed in multiple AD tissues. At least brain, platelet and fibroblast mitochondria are involved. Defects of three mitochondrial enzymes are reported.[10] This includes reduced activities of pyruvate dehydrogenase complex, alpha ketoglutarate dehydrogenase complex and cytochrome oxidase.[11] Spectral analysis of cytochrome oxidase indicates AD brains contain normal amounts of cytochrome oxidase, but the enzyme itself is structurally altered.[12] Various mechanisms, such as oxidative stress and proteasome dysfunction, have been postulated to facilitate mitochondrial dysfunction in neurodegenerative diseases such as AD.[13]

Along with these two hypothesis in AD, there are two protein who plays important role in the pathogenesis of the disease.

**Tau protein**

Tau is a microtubule-associated protein that is involved in microtubule assembly and stabilization. In AD, tau is abnormally phosphorylated, aggregates into paired helical filaments and loses its ability to stabilize axonal microtubules.[14] There is a potential link between the two pathological hallmarks of AD Ð senile plaques and nuclear factor (NFT). Aβ fibril induces tau phosphorylation, resulting in the loss of microtubule-binding capacity and somato-dendritic accumulation.[15] Findings suggest that Aβ fibril formation causes an abnormal phosphorylation of tau through the activation of protein kinases such as GSK-3 b and destabilization of microtubules, resulting in neuronal death.

**Apolipoprotein E (ApoE)**

The second gene to be specifically implicated in AD was the ApoE gene located on chromosome 19. Three major isoforms of ApoE (ApoE2, ApoE3 and ApoE4) are products of their respective alleles. The frequency of the ApoE4 allele is significantly higher in lateonset familial AD and sporadic AD patients than in the general population. ApoE4 allele increases the risk and lowers the age of onset distribution of AD, whereas ApoE2 allele lowers the risk and increases the age of onset distribution.[16]

**Alzheimer's disease biomarkers**

Biomarkers can assist in monitoring the progression of AD and inform treatment approaches as the disease advances.

1. One of the principal enzymes responsible for amyloidogenesis, β-site amyloid precursor protein cleaving enzyme 1 (BACE1), has promise as a therapeutic target. Novel techniques for quantifying BACE1 levels from the CSF and plasma are emerging, permitting a primary measure of target engagement. Inhibitors of BACE1 decreased CSF and plasma levels of Aβ.[17]
2. Gamma-secretase modulators and inhibitors have been shown to reduce CSF and plasma Aβ levels reinforcing the utility of investigating peripheral biomarkers in animals and clinical trials. Using stable-isotope-labeling kinetics (SILK) methods, a gamma-secretase inhibitor was shown to reduce CSF Aβ production monkeys without a subsequent rise in Aβ production.[18]

3. Central immunotherapy may produce secondary, undesirable immune responses and it is important to evaluate markers of inflammation as possible indicators of an adverse response. Plasma levels of interleukin (IL)-10, an anti-inflammatory cytokine, have been found to be increased following Aβ immunotherapy. In contrast, T cell infusions specific for Aβ or administration of granulocyte colony stimulating factor (GM-CSF) reduced plasma levels of IL-4, TNF-α and other cytokines. Inflammatory mechanisms and their peripheral markers can be further explored with parallel observations in humans and animals.[19,20]
4. Isoprostanes, which reflect lipid peroxidation and oxidative stress, are elevated in plasma in advance of plaque formation, suggesting isoprostane levels may have utility as a predictive biomarker.[21,22]
5. Microgliasis is an important indicator of drug activity and a common pathological finding in AD. Although microglial activity may be assessed with neuroimaging techniques, important information can be quickly and inexpensively obtained through peripheral measures.[23]
6. The measurement of tau and phosphorylated tau (p-tau) from human or animal fluids is restricted to the CSF.[24]
7. MicroRNAs (miRNAs) have also been implicated in AD pathogenesis and suggested as a putative biomarker.[25]

**Preclinical models for evaluation of Alzheimer disease**

**1. Amyloid-b infusion rodent models**

The amyloid cascade hypothesis of AD states that, regardless of whether the disease is familial or sporadic, cerebral accumulation and aggregation of Ab peptides to form amyloid plaques is the primary culprit driving AD pathogenesis and additional disease processes (NFT formation and inflammation) result from the imbalance between Ab production and clearance. Aspects of AD can be mimicked by intracerebral or intracerebroventricular infusion of Ab peptides in the rodent brain. Ab species can be administered acutely, using a single stereotactic injection, or repetitively, using injections through an implanted cannula. To better mimic the progressive nature of AD, chronic and continuous administration is accomplished by connecting an implanted cannula to an osmotic mini-pump or with microdialysis.[26]

**2. AD models in Drosophila melanogaster**

Drosophila has found major application in the analysis of genetic interaction in neurological disorders, including AD, based on both classical phenotype-based genetic screens and techniques for genetic manipulation, including gene knockdown, deletion and transgenic insertions. A complete APP-processing Drosophila model was achieved by creating transgenic flies that carry constructs encoding both human APP and human b-site APP-cleaving enzyme 1 (BACE1, i.e. b-secretase).[27]

**3. AD models in Caenorhabditis elegans**

Caenorhabditis elegans, a free-living nematode of approximately 1 mm in length, has several characteristics that make it useful as a model organism. The nematodes are transparent, which allows study of embryonic development and gene expression in living animals under the microscope. They also have a very short life cycle (3 days) and a relatively short lifespan (3 weeks), which allow genetic dissection of the mechanisms that affect ageing and lifespan. Several AD-related genes and pathways found in humans have orthologues in C. elegans. The nematode genome encodes three orthologues for PSEN1; (i) sel-12, which has been found in a screen for suppressors of the egg-laying defective phenotype in lin-12 gain-of-function worms and which functions mostly during embryonic development to facilitate Notch/lin-12 signalling; (ii) hop-1, homolog of PSEN1, which is in fact more homologous to human PSEN2; and (iii) spe-4, which has no obvious human counterpart. Three genes, aph-1, pen-2 and aph-2, produce proteins that combined together form a functional g-secretase complex. In addition, an orthologue of Ab (apl-1), has been described in C. elegans.[27]

**4. Cholinergic dysfunction-related animal models**

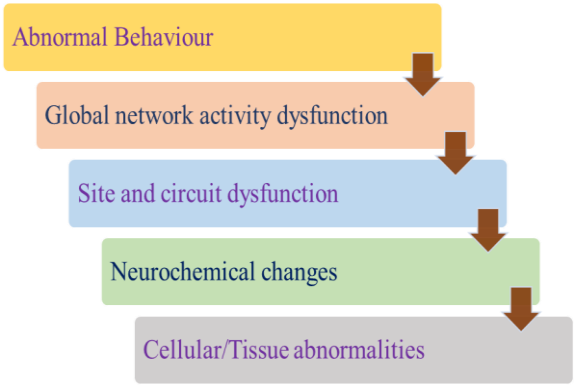
Many types of acute manipulations, including electrocoagulation, use of excitotoxins, transection of the fimbria/fornix, and treatment with a cholinotoxin, AF64A, have been applied to reduce cholinergic activity. A chronic animal model with a continuous intracerebroventricular (i.c.v.) infusion of quinolinic acid was also developed to simulate the slow evolution of the neurodegenerative diseases, including AD. It is suggested that lesions of the medial septal nucleus produce behavioural deficits that are most similar to the cognitive impairments in the earliest stage of AD.[28]

**Parkinson's disease**

Parkinson disease (PD), the second most common neurodegenerative disorder, is characterized by a large number of motor and non-motor features that can have a serious effect on the function and quality of life of the affected individual. Currently, the clinical diagnosis of PD is based on the presence of a combination of cardinal signs, including rest tremor, bradykinesia, rigidity and loss of postural reflexes.[29] The pathological process leading to PD begins decades before the typical motor symptoms and by the time the diagnosis is made, about 70% to 80% of striatal dopamine (DA)[30] and at least one-third of substantia nigra (SN) neurons[31] and striatal dopaminergic fibers[31,32] are already lost.

**Pathogenesis of Parkinson's disease (PD)**

Multiple levels of abnormalities for the pathophysiology of PD behaviour have been studied. These may be depicted as follows:




In this article, we will discuss these levels of dysfunction.

**1. Molecular pathogenesis[33]**

The molecular changes in PD must be ultimately responsible for producing the behavioural changes in PD. Genetic mutations that are associated with PD and which commonly have dementia include PARK1, PARK4 and PARK8. The presence of the abnormal misfolded proteins α-synuclein, amyloid beta (Aβ) peptide and tau in limbic and cortical structures represent logical possibilities for producing behavioural problems in PD. The mechanism by which higher α-synuclein levels could directly cause or be associated with cerebral neuron toxicity is currently unknown. Several mechanisms have been proposed for a-synuclein neuronal toxicity, including membrane disruption; interference with signaling pathways; altering vesicle trafficking; post-translational modification; and others. α-synuclein oligomers have been suggested to cause neurotoxicity through calcium influx and seeding. Membrane disruption of a-synuclein can alter the electrical properties of neurons, causing abnormal neuron firing.[34]

**2. Cellular/tissue abnormalities**

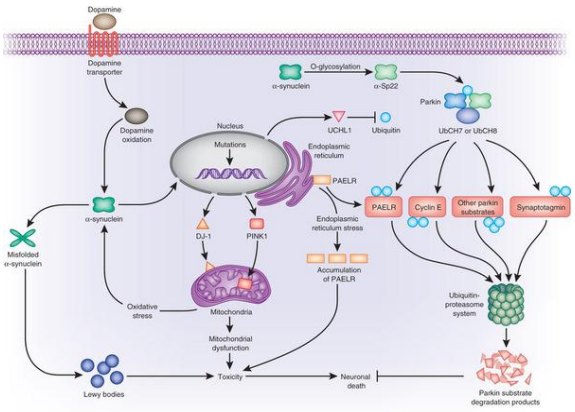
Synaptophysin is a 38 kilodalton Ca2+ binding integral protein of vesicle membranes, and thus detecting its presence provides a presynaptic marker. Synaptic density has been rarely studied in Lewy body disorders (PD), PD dementia, dementia with Lewy bodies [DLB]). Decreases in synaptophysin have been found in the frontal and temporal lobes and hippocampus in Lewy body disorders. In comparative studies, the synaptophysin loss is AD > DLB > PD dementia > PD.[33]

**3. Neurochemical changes**

In PD, the neurons of diffusely projecting acetylcholine, dopamine and serotonin systems that project to cortical areas which are important for behaviour do undergo neurodegeneration.

**4. Site and circuit dysfunction**

The most famous site of dysfunction in PD is the substantia nigra and the most famous circuit is the frontostriate circuit. This comes from the converging evidence that striatal dopamine depletion alters the basal ganglia output to the frontal cortex through the thalamus, frontal cortical areas are commonly affected by Lewy pathology and frontal/executive cognitive deficits are common in PD.[33]




**Neuron loss**

A hallmark pathologic feature of PD and essential for its pathologic diagnosis, is loss of Dopamine (DA) neurons of the substantia nigra pars compacta (SNpc). Neurologic deficits emerge when the availability of DA falls below the level required for rapid compensation or when the system is subjected to certain pharmacologic, environmental, or physiologic challenges.[35]

**Lewy Bodies**

Another pathologic hallmark of PD is the Lewy body, an eosinophilic inclusion identified within neurons. On histologic stains, Lewy bodies have an eosinophilic core and a surrounding pale halo. Lewy bodies are commonly observed in the brain regions showing the most neuron loss in PD, including SN, locus coeruleus, the dorsal motor nucleus of the vagus and the nucleus basalis of Meynert, but they are also observed in neocortex, diencephalon, spinal cord and even peripheral autonomic ganglia. Another major antigenic feature of Lewy bodies is the expression of cellular proteins involved in protein degradation, including ubiquitin and the proteasome.[35]

**Kynurenine pathway**

The kynurenine pathway (KP) is a major degradative pathway of the essential amino acid tryptophan (TRP) that ultimately leads to production of the essential co-factor nicotinamide adenine dinucleotide (NAD+). In terms of KP profiles, neurons and astrocytes are mainly neuroprotective whereas activated microglia and infiltrating macrophages are neurotoxic. The various KP products can have neurotoxic, neuroprotective or immunomodulatory effects. Among them, the excitotoxin quinolinic acid (QUIN) appears to be the most important, leading severely to neuronal cell death and chronically to dysfunction by at least nine separate mechanisms.[36]

**Role of Rho-mediated ROCK-Semaphorin3A signaling pathway**

ROCK belongs to a downstream effector protein of the Rho GTPase, which are the number of a globular, monomeric group of small signalling G-protein molecules. ROCK signaling pathway participates in the pivotal neuronal processes in axonogenesis, stability of synapses, as well as growth cone dynamics. ROCK functions as a potential pharmacologic target for the management of neurotraumatic and neurodegenerative diseases. Semaphorin3A (Sema3A) belongs to class 3 semaphorins which are phylogenetically conserved guidance proteins that are responsible for angiogenesis, branching morphogenesis, axon growth and cell migration. Sema3A is known to result in neuronal apoptosis and functions as a chemorepellent factor for axonal growth.[37]

**Parkinson's disease biomarkers**

In general various biomarkers for PD can be divided into two major categories; (i) premotor (preclinical) biomarkers, and (ii) motor (clinical) biomarkers.

**Genetic biomarkers**

Increasing evidence suggests that both genetic and environmental factors contribute to the etiology of PD. For example, genetic mutations (duplications, triplications or missense mutations) in the a-Syn gene can lead to PD.

1. Autosomal dominant missense mutations in the gene for leucine-rich repeat kinase 2 (LRRK2/PARK8) that have been recognized as the cause of PD.
2. Clinical and pathological findings of autosomal dominant disease linked to mutations in SNCA, LRRK2, ATXN2, ATXN3, MAPT, GCH1, DCTN1 and VPS35 genes. The mutations in PARK2, PARK7, PINK1, ATP13A2, FBXO7, PANK2 and PLA2G6 genes have been identified in addition to the monogenic forms of PD.

**Omics biomarkers**

In general omics biomarkers include proteomic (ii) genomic (iii) lipidomic, metabolomic, glycomic and miscellaneous biomarkers including microRNA. Omics has also reconfirmed the increase in oxidative stress as a common pathway in PD development/progression.[38]

**Increased Rab35 expression is a potential biomarker**

The comparative proteomic study of serum samples demonstrated that protein expression of Rab35 was increased in PD. The serum level of Rab35 was significantly correlated with the age at onset of PD. Overexpression of Rab35 increased the aggregation and secretion of mutant A53T α-synuclein in dopaminergic SH-SY5Y cells.[39]

**Preclinical models for evaluation of Parkinson's disease[40]**

1. **MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)**: Neuropathological data show that MPTP administration causes damage to the nigrostriatal DA pathway that is identical to that seen in PD.
2. **6-OHDA**: Like MPTP, 6-OHDA is a selective catecholaminergic neurotoxin that is used, mainly, to generate lesions in the nigrostriatal DA neurons in rats. Since 6-OHDA cannot cross the blood-brain barrier, systemic administration fails to induce parkinsonism. So, this induction model requires that 6-OHDA be injected (typically as a unilateral injection) into the SNc, medial forebrain bundle or striatum.
3. **ROTENONE**: Chronic systemic exposure to rotenone in rats causes many features of PD, including nigrostriatal DA degeneration. The rotenone-administered animal model also reproduces all of the behavioral features reminiscent of human PD.
4. **PARAQUAT/MANEB**: Regarding animal models, some researchers report that, following the systemic application of paraquat, mice exhibit reduced motor activity and a dose-dependent loss of striatal tyrosine hydroxylase (TH) fibers and SNc neurons with relative sparing of the VTA.

**Huntington's disease**

Huntington's disease (HD) is an autosomal-dominant disorder characterized by irrepressible motor dysfunction, cognitive decline and psychiatric disturbances, which lead to progressive dementia and death approximately 15–20 years after disease onset.[41] Huntington disease (HD) is caused by a CAG repeat expansion in the huntingtin (HTT) gene on chromosome 4, which codes for polyglutamine in the huntingtin protein.

**Pathogenesis of Huntington's disease**

The disease is a genetic disorder of the central nervous system, with symptoms usually consisting of uncontrolled movements, emotional disturbances and mental deterioration. Psychiatric abnormalities, including depression, anxiety, apathy and irritability, are often an early manifestation of HD, appearing before the characteristic neurologic symptoms of overt chorea, or spasmodic movements of the limbs and facial muscles.[42]

**Neuropathology**

Widespread distribution of mutant htt causes selective neurodegeneration, which occurs preferentially and most prominently in the striatum and deeper layers of the cortex in the early stages of HD. In advanced stages, other brain regions, such as the hippocampus, hypothalamus, cerebellum, amygdala and some thalamic nuclei, are also affected. Among these other brain regions, the lateral tuberal nucleus of the hypothalamus exhibits severe atrophy.[43]

Behind the pathology of huntington's disease Htt protein was found to be the main cause in the neurodegeneration but its exact role is still undefined.

**Htt a protein**

It is a largecytoplasmic protein found loosely associated with synaptic vesicles in nerve terminals and with microtubules in dendrites. It has no homology with any other protein and no distinguishing features that predict its biological function. Htt is essential for mammalian development, since deletion of both copies of the HD gene retards the development of the embryo and kills it in mid-gestation. However, when only one copy of HD is knocked out, growth and development appears to be normal.[44]

**Striatal neurons degenerate**

The medium-sized spiny GABAergic neurons of the striatum (caudate nucleus and hallmark of HD. Striatal degeneration is the first and most obvious neoropathology in the early-grade HD brain.[44]

**THE ROLE OF UBIQUITIN, PROTEASOMES AND CHAPERONES**

Cells have a complex array of chaperone proteins that assist in the folding of normal proteins and recognition and handling of abnormally-folded or aggregate-prone proteins. HSP70 and HSP40 family members are associated with huntingtin exon 1 aggregates in cell models.[45] An important intracellular pathway for reducing the levels of misfolded proteins is the ubiquitin-proteasome pathway. Another major route for degradation of proteins with expanded polyglutamine tracts is the autophagy-lysosome pathway. We suggested that autophagy may be particularly relevant to aggregate-prone species.[46] If chaperones cannot refold abnormal proteins correctly, in HD inclusions have been found to be ubiquitinated and also associated with several proteasome subunits, which strongly suggests a failure in the degradative machinery of the cell.[47]

**Excitotoxicity, inflammation and the quinolinic acid pathway**

Excitotoxicity (excessive stimulation of excitatory amino acid receptors, especially NMDA receptors) has long been postulated to be a non-cell-autonomous mechanism with a role in pathogenesis of Huntington's disease. Blockage of extrasynaptic rather than synaptic NMDA receptors (eg, with memantine) might be more effective. Inflammatory proteins such as complement proteins and clusterin are upregulated both peripherally and in the brain in patients with Huntington's disease.[48]

**Huntington's disease Biomarkers**

**1. NEUROTRANSMITTERS**

The earliest studies examining CSF (Cerebrospinal fluid) in HD took a particular interest in neurotransmitters for neuronal function and dysfunction. Homocarnosine, a dipeptide containing GABA, was later found to be significantly lower in HD patient CSF.[49]

**2. TRANSGLUTAMINASE ACTIVITY**

Polyglutamine expansion in HD increases its effectiveness as a substrate for transglutaminases in vitro. Transglutaminases have been implicated in the aggregation of mHTT and cystamine (among other actions, an inhibitor of transglutaminase) ameliorated symptoms in HD, supporting the notion that transglutaminase activity may contribute to the toxic gain of function of Mhtt.[50]

**3. KYNURENINE PATHWAY METABOLITES**

QA is a downstream product of the kynurenine pathway, by which the neurotransmitter amino acid tryptophan is degraded in mammals.[51]

Its possible role in HD neuropathology, the pathway and in particular the enzyme kynurenine mono-oxygenase, is a high-priority target for therapeutic development.[52]

**4. OXIDATIVE STRESS**

Oxidative damage has been associated with many neurodegenerative diseases including HD.[53] F2-isoprostanes are a marker for lipid peroxidation.[54]

**5. NEUROENDOCRINE MARKERS**

Hypothalamic neuroendocrine abnormalities have been suggested to contribute to many symptoms seen in HD such as weight loss, depression and disruption of sleep, supported by evidence of hypothalamic cell loss. Neuroendocrine markers have been targets for several CSF studies in HD.[55] Ghrelin was increased and leptin decreased in plasma of the HD patients but neither was altered in CSF.[56]

**6. MUTANT HUNTINGTIN**

Understanding the function of the huntingtin protein and its mutant counterpart has been a major focus of HD research since the gene was discovered. mHTT is a large, aggregation-prone protein expressed mostly intracellularly and since HD generally progresses slowly, mHTT would be expected to be released very gradually into the CSF from dying neurons.[57]

**Preclinical models for evaluation of Huntington's disease[58]**

**1. Quinolinic Acid (QA)**

In the presence of the enzyme 3-hydroxyanthranilic acid oxygenase, a series of enzymatic reactions converts kynurenine to QA. Normal levels of QA do not cause damage, but only small increases in QA levels cause toxicity. An increase in the enzyme 3-hydroxyanthranilic acid oxygenase, relative to the level in control brains, with the greatest increase in the striatum, the most vulnerable neurons in the HD brain.

**2. 3-Nitropropionic Acid**

3-nitropropionic acid (3-NP) is a toxin that irreversibly inhibits the mitochondrial enzyme succinate dehydrogenase. The 3-NP model is reliable for studying HD because it mimics a downstream process of cell

death seen in the HD brain, namely mitochondrial impairment. Impaired glucose metabolism in brain cells as a result of enzyme deficiencies causes decreased production of ATP.

**3. Transgenic models**

**R6/2**: The R6/2 is the most commonly used transgenic mouse model of HD. This fragment contains only exon 1 of the human htt gene and expresses approximately 144 CAG repeats. The truncated mutant htt gene is randomly inserted into the mouse genome and the mouse expresses three copies of the htt gene—two of its own wild-type copies and one mutant human copy. Expression of the mutant htt gene is driven by the human htt promoter. The mutant gene is expressed in all cells of the mouse.

**N171-82Q**: The N171-82Q transgenic mouse model by inserting the first 171 amino acids from the N-terminal of the human htt gene into the mouse genome. Expression of the N-terminal fragment of htt in this model is driven by the mouse prion promoter and as such, mutant htt is expressed throughout the mouse brain. But its expression is restricted to neurons and is not found in glia.

**R6/1**: The method of creation for R6/1 mice is similar to that for R6/2 mice except that they contain only 116 repeats, making their behavioral phenotype relatively mild. As with the R6/2 mice, mutant huntingtin gene expression is driven by the human htt promoter in all cells of the body, but it is at only 31% of the expression levels seen in the endogenous mouse gene.

**YAC Mice**: YAC transgenic mice are, used a yeast artificial chromosome (YAC) vector system to express the entire human htt gene under control of the human htt promoter. YAC mouse strains contain either 72 or 128 CAG repeats. Both strains show a decrease in the number of neurons, preferentially in the lateral striatum.

**PRION DISEASE**

Prion diseases are a group of fatal neurologic disorders that affect humans and animals and for which there is no available therapy. The basic pathogenic mechanism is linked to posttranslational changes of the host cellular prion protein (PrPc) into a pathologic conformer (PrPTSE) that has a strong tendency to aggregate and form amyloid fibrils. In humans, the most common form of the disease is sporadic.

Creutzfeldt-Jakob disease (CJD), which equally affects females and males of all ages and all ethnic groups.[59]

**Pathogenesis of Prion disease**

**Prion Protein:** The infectious agent consists of PrPSc, that it is devoid of nucleic acid, and that its 'replication' comes about by PrPScmediated, autocatalytic conversion of PrPC to PrPSc. It is not clear that the infectious entity is PrPSc, operationally defined as a protease-resistant, aggregated form of PrP, rather than some other conformer, generically designated as PrP. PrPC is not only produced by neurons: its expression is in fact quite ubiquitous, notably including lymphocytes and stromal cells of lymphoid organs.[60]

**Pathway of prion disease**

The damage brought by prions is mainly evident in the central nervous system, although pathological changes in the spleen of non-human primates have also been noted. Because PrPSc accumulates in the central nervous system and in some instances is deposited as an amyloid, it is has been indicted as the toxic entity causing neuronal apoptosis and eliciting disease. The finding that peptides derived from the PrP region 106–126 form aggregates and are toxic to cultured neuronal cells.[61]

A toxic function gain by a PrP moiety that is different from PrPSc is a distinct possibility.[62]

Prion pathogenesis can be broken down into spatially and temporally distinct phases:
(i) Infection and peripheral replication
(ii) Migration from the periphery to the CNS (neuroinvasion).
(iii) Neurodegeneration.[61]

**Prion disease Biomarkers**

CSF proteome alterations reflect pathological changes in the brain and could possibly be able to provide an early diagnostic tool in prion diseases. There are many protein based biomarkers in cerebrospinal fluid (CSF) that are used for diagnosis of human prion diseases.[62]

1. Biomarker proteins are 14-3-3, tau, phospho-tau/tau ratio, S100, and the neuron-specific enolase.[63]
2. Induced expressional levels of other neurodegenerative-related proteins such as beta-amyloid and alpha-synuclein[64] have also been reported in the CSF of patients with sporadic CJD when compared to control samples.
3. In recent years, total PrP (t-PrP) level in CSF have been described as a new biomarker
4. Exosomes are nanoparticles, secreted into the extracellular environment during the transportation of vesicular factors and are reported as carriers for PrPc and PrPsc. For the diagnosis of prion disease, recently a group showed specific exosomal miRNA signature in prion-infected neuronal cells that can be utilized for specific diagnostic pattern. This signature consists of significant increases in let-7 b, let-7i, miR-128 a, miR-21, miR-222, miR-29 b, miR-342-3 p and miR-424 with decreased miR-146.[62]

**Preclinical models for evaluation of Prion disease[65]**

**1. FFI gene**

FFI (fatal familial insomnia) is one of related brain diseases involving prions. Better known prion maladies include mad cow disease and are also called transmissible spongiform encephalopathies (TSEs). The mutant PRNP gene for FFI was inserted in place of the

normal mouse prion protein. The knock-in mouse also produced infectious prions, the protein-based pathogens that cause TSEs.

2. Genetic prion disease mutations like A117V, D178N and E200K produce prions in animals that are biochemically and phenotypically similar to the corresponding diseases in humans.
3. Neuropathology of ki-3F4-FFI mice: homozygous mice to increase gene dosage, a standard practice for knock-in mice modeling neurodegenerative diseases that require many years to develop in humans and since homozygous carriers of prion mutations develop disease faster than heterozygotes.

## CONCLUSION

Detection of NDD related biomarkers and alterations in them reflects the process of NDD. Finally it is concluded that without the knowledge of pathological way and alterations occurring in them we are not able to find the desired therapy for the disease.

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