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Review Article

Preclinical Screening Models of Huntington's Disease in Drug Discovery: Bridging Mechanistic Insights and Translational Therapeutics

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ABSTRACT

Huntington's disease (HD) is a progressive neurodegenerative autosomal dominant disease characterized by the formation of a mutant huntingtin protein which is a result of the CAG trinucleotide repeat expansion in the HTT gene. This leads to neuronal dysfunction and the gradual loss of striatal and cortical neurons. The main symptoms of HD include motor disorders, progressive dementia, and psychiatric disorders Though currently there is no treatment that can effectively modify the course of the disease. This way, preclinical models of the disease have become very important in uncovering the disease mechanisms and speeding up the development of therapeutics. This article discusses the major preclinical models used in Huntington's disease drug discovery and their translational usefulness. In vitro platforms like immortalized cell lines, primary neuronal cultures, induced pluripotent stem cell (iPSC)-derived neurons, and organoid systems offer great opportunities to understand the molecular basis of the disease and are very helpful for quick screening of therapeutics. Chemical and neurotoxin-based models are capable of inducing excitotoxicity and mitochondrial dysfunction, whereas genetic rodent models show great similarity to human disease for progression and mutant huntingtin pathology. Other models including Drosophila aenorhab ditiselegans zebrafish, and yeast are also widely used for mechanistic studies and high-throughput drug screening. The utilization of these various experimental models in HD has paved the way to the evaluation of promising neuroprotective compounds antioxidants anti-inflammatory drugs, and gene-targeted therapies. Cutting-edge approaches like accurate disease modeling, multi-omics integration, and artificial intelligence (AI)-guided screening are further supporting translational research and giving new hope for Huntington's disease therapeutics.

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Introduction

Huntington's disease is a chronic and hereditary neurodegenerative disorder that leads to the deterioration of physical, mental and emotional health [1]. It was first identified by George Huntington in 1872 as a clinical entity characterized by the presence of involuntary movements, unbalance and incoordination, progressive memory loss, and neuropsychiatric symptoms including depression, anxiety irritability, and changes in behavior [2]. The signs of the disease usually manifest in the middle of adulthood, generally between the ages of 30 and 50, although both juvenile and late-onset forms have been documented. The inexorable advancement of Huntington's disease creates a huge burden not only for the patients, but also for their families and healthcare programs leading to an enormous loss in emotional health, social skills, and economics [3]. Even though Huntington's disease is relatively rare compared to other neurodegenerative diseases, In reality it is passed down from generation to generation and the fact that there is no cure for the disease makes it one of the most heartbreaking neurologic disorders globally.

It is an autosomal dominant mutation that causes the disease by expanding CAG trinucleotide repeats in exon 1 of the huntingtin (HTT) gene on chromosome 4 [4]. Normally, the HTT gene makes huntingtin, a protein with many roles, including intracellular transport, neuron survival, vesicle trafficking, and gene expression regulation. In Huntington's disease, the CAG repeats become excessively elongated which results in a long chain of glutamines, or a polyglutamine tract, in the huntingtin protein. As a consequence, the protein starts to misfold and aggregate, gaining toxic effects which are not normally seen [5]. The number of CAG repeats is a major determinant of the disease, with more repeats usually causing earlier onset and more rapid progression. That is why at an autosomal dominant inheritance, the child of a parent suffering from the disorder has a 50% chance to inherit it [6]. Huntington's disease pathophysiology has several layers and involves numerous mechanisms. The mutant huntingtin protein interferes with various cellular operations like transcription regulation, mitochondrial function, protein degradation systems, neurotransmission, and axonal transport [7].

With these disruptions, oxidative stress, excessive glutamate excitotoxicity, failure of autophagy mechanisms, imbalance of calcium levels, and neuroinflammatory events can take place.The disease's most prominent sign is the gradual loss of medium spiny neurons in the striatum, which is the part of the brain responsible for movement and cognition [8]. Later on, other areas of the brain become affected, resulting in the typical symptoms of the disease. Both the genetic mutation and cellular dysfunction that follow it contribute to a complex therapeutic situation, which indicates a great need for targeted treatment approaches.

Preclinical screening models have gained crucial importance in Huntington's disease research and drug discovery. Numerous model systems, including cellular models, toxin-induced models, transgenic rodentsiPSCs, organoids, and gene-edited models, have vastly expanded our knowledge of the underlying mechanisms of the disease and facilitated the identification of new therapeutic targets [7]. Also, these models make it possible to extensively analyze molecular pathways, behavioral phenotypes, biomarker alterations, and therapeutic efficacy before clinical studies. The current review attempts to cover the pool of existing preclinical screening models for Huntington's disease in detail, highlighting their mechanistic features, translational potential, and their contribution to boosting the therapeutic pipeline [7]. By connecting experimental work with patient care, these models are instrumental in the development of effective therapies for Huntington's disease.

Molecular and Cellular Basis of Huntington's Disease

Understanding the molecular and cellular causes of Huntington's disease (HD) has greatly focused on identifying the initiating genetic mutation and unraveling the sequence of pathological events that ultimately lead to the loss of neuronal function and death [9]. This neurodegenerative disorder is

caused by the abnormal elongation of CAG trinucleotide repeats in exon 1 of the huntingtin (HTT) gene located on chromosome 4 [10]. The CAG repeat length in normal individuals is typically confined to a certain range, but pathological expansions over the disease threshold result in the production of mutant huntingtin protein with an abnormally long polyglutamine tract [11]. The degree of repeat expansion greatly determines not only when the disease will start but also how severe it will be, as longer repeats are linked to earlier appearance of symptoms and quicker disease progression. Generally, the huntingtin protein carries out very important physiological functions that include supporting neuronal survival, intracellular transport, vesicle trafficking, synaptic transmission, and gene expression regulation. Besides that, it plays a role in embryonic development as well as in the maintenance of neuronal integrity [9]. Yet, the structure of huntingtin is A lot changed by the polyglutamine expansion, resulting in a conformational change that makes it highly prone to misfolding and aggregation[12].

Besides going through abnormal cleavage, mutant huntingtin accumulates both in neuronal cytoplasm and nuclei, where it forms toxic oligomers and intracellular inclusion bodies. These aggregates are responsible for the interference with several cellular pathways, disruption of protein-protein interactions, impairment of transcriptional machinery, as well as alteration of mitochondrial and proteasomal function [13]. Striatum, Mostly the medium spiny neurons that are highly susceptible to the mutant huntingtin toxicity, is one of the first and the most severely affected brain areas in Huntington's disease[14]. With time, degeneration goes to cortical regions and other neural structures interconnected to them, which together bring about the usual motor problems, loss of intellect, and mental symptoms of the disease[15]. This kind of selective neurodegeneration happens at the same time as a very significant loss of neuronal connectivity and synaptic plasticity.

Then again, mitochondrial dysfunction is a major aspect of the pathological features of HD as well. Mutant huntingtin disrupts mitochondrial morphology respiration calcium handling, as well as the production of ATP, which overall results in the failure of cellular energy metabolism [16]. Neurons become at risk due to insufficient energy and also get affected by metabolic stress when their energy capacity is reduced. These mitochondrial defects have a close relationship with an overproduction of reactive oxygen species, peroxidation of lipids, protein oxidation, as well as the reduction of antioxidant defenses, which altogether cause a prolonged condition of oxidative stress and redox imbalance[17]. Really, oxidative injury makes neuronal dysfunction even worse and hastens neurodegeneration.

Excitotoxicity also is key in worsening the disease. When glutamatergic neurotransmission is out of control and NMDA receptor activation is abnormal, calcium influx becomes excessive. This overflows calcium inside which the second messengers get activated leading to mitochondrial damage and apoptosis [18]. Meanwhile, mutant huntingtin instigates neuroinflammatory processes, a feature of activated microglia, and an increased liberation of inflammatory factors like tumor necrosis factor-, interleukin-1, and interleukin-6. Long-lasting inflammation causes a toxic microenvironment that aggravates neuronal destruction [19]. Also, defects in autophagy and proteostasis hinder the clearance of mutant proteins and injured cellular components, leading to even greater intracellular build-up and malfunction. A gradual disruption of synaptic transmission and neuronal networks occurs as neurotransmitter levels, receptor signaling, and connections between the cortex and striatum are altered [20]. Altogether, these changes at the molecular and cellular levels make up a complicated pathological network that not only explains the gradual loss of neurons in Huntington's disease but also highlights key targets for therapeutic intervention and preclinical drug discovery **(Figure.1)**.

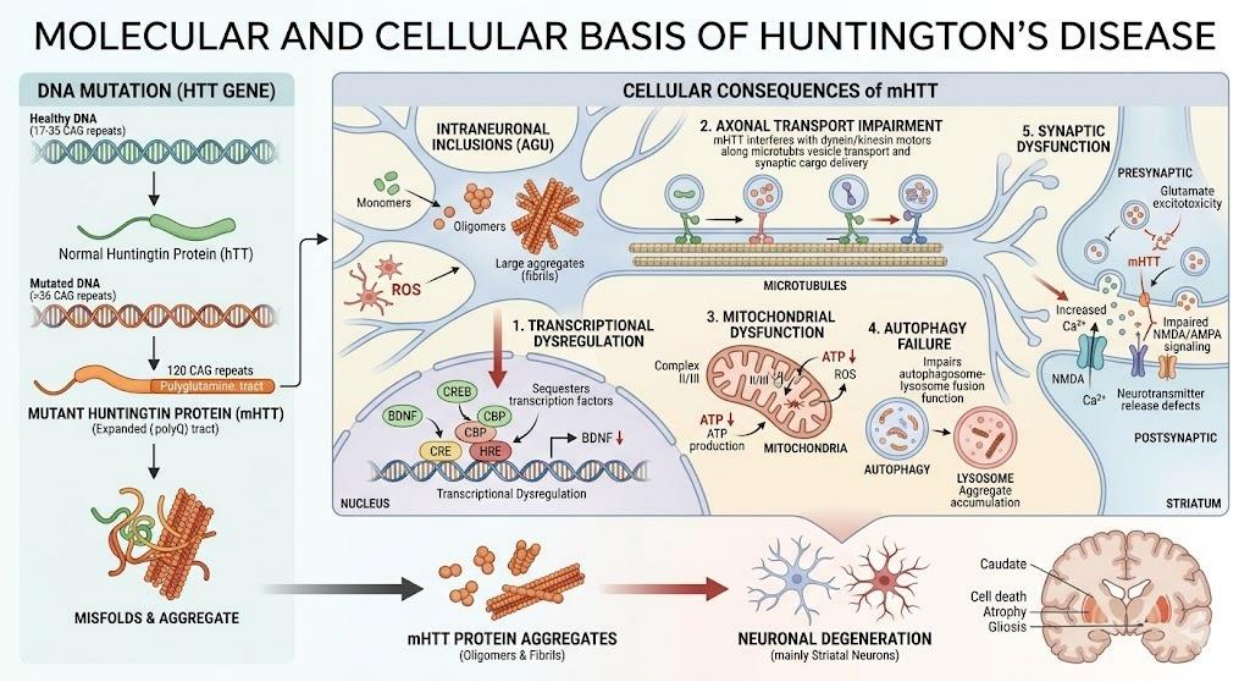

Figure. 1: Huntington's disease is a result of the expansion of CAG trinucleotide repeats in the HTT gene. This mutation leads to the synthesis of mutant huntingtin protein (mHTT) carrying an abnormally long polyglutamine segment. The misfolded mHTT tends to aggregate, forming oligomers, fibrils, and neuronal inclusion bodies. These large aggregates interfere with various cellular mechanisms, like gene regulation, movement of materials along neurons, energy production by mitochondria, the autophagylysosomal pathway, and synaptic signaling. Besides, mHTT triggers oxidative stress and reduces ATP synthesis, which together lead to neuronal failure. Changes at the synaptic level involve glutamatergic neurotransmission alteration and calcium imbalance, which result in excitotoxicity and continuous neuron damage. Altogether, these changes at the molecular and cellular levels cause the targeted death of striatal and cortical neurons, which in turn leads to brain shrinking and the manifestation of motor, cognitive, and psychiatric symptoms typical of Huntington's disease.

Rationale for Preclinical Screening Models in Huntington's Disease Drug Discovery

Preclinical screening models make a difference in Huntington's disease (HD). They offer experimental platforms to better the understanding of disease mechanisms, discovery of therapeutic targets, and preclinical evaluation of candidate drugs [7]. Huntington's disease (HD) is a complicated neurodegenerative disorder resulting from a single genetic mutation but its manifestation is influenced by lots of pathological pathways interacting one another like, protein aggregation, dysfunction of mitochondria, oxidative stress, neuroinflammation, and synaptic degeneration [21]. The complexity of the disease makes it extremely difficult to translate the therapeutic approach directly to humans. So, accurate disease models become a necessity for modeling the major pathological and behavioral features of HD under the conditions of controlled experimentation. Such models provide researchers a powerful tool to investigate the progression of the disease, identify molecular targets, and

evaluate the potential of therapeutics for efficacy, safety, and mechanism of action [7].

Appropriate preclinical systems are very crucial for the development of disease-modifying interventions to continue at one time when the slow progression and biological heterogeneity of patients can be major hurdles are really absent. Generally speaking, a Huntington's disease (HD) model that is capable of reproducing the major clinical and pathological features of the illness is what is needed. Besides, it should still support reproducibility and experimental feasibility [22]. Among the key features are the accurate production of mutant huntingtin protein, gradually evolving degeneration of neurons in the striatum and cortex areas, motor and cognitive problems that resemble those of patients, and chemically detectable occurrences like biochemical abnormalities, mitochondrial dysfunction, and activation of inflammatory signaling [9].

The model should be capable of providing sufficient stability over time for repeated measurements and should also make it feasible to carry out changes in drug treatment. Yet, the choice may also depend on other factors such as the ability to genetically manipulate the model easily, the length of the generation cycle, the cost, and whether or not the model is suitable for keeping track of a marker or for imaging-based methods. Since a single model cannot reproduce all the features of human Huntington's disease, the choice depends largely on the therapeutic aspect you want to explore [23].

The degree of validity of an experimental model greatly determines its usefulness for the purpose it's intended. Face validity is about how well the model imitates the external signs of Huntington's disease like changes in behavior and brain pathology [24]. Construct validity measures the resemblance of the model to the real mechanisms that cause the disease, Mostly the toxicity tied to mutant huntingtin. Predictive validity indicates the extent to which the effects of a treatment in the model correlate with the actual outcomes of that treatment in patients [25]. It is very important to find a balance between these three types of validity when performing drug screening procedures. A model possessing a lot of construct validity may perfectly represent the mutation but may not exhibit clinical features, whereas a model with a high degree of face validity may not replicate the molecular basis of disease fully [26]. Successful translational research depends on the thorough combination of all three.

It is also important to consider disease stage-specific modeling as Huntington's disease progresses in a gradual manner over several years[27]. Models representing early stages are used to identify minor molecular disturbances happening before significant neuronal loss and Because of this, they can be employed to find ways of preventing or modifying the disease [16]. Models of later stages are more accurate in depicting neuronal death, motor problems, and decline in abilities, and So, they are used for assessing drugs that may relieve symptoms[28]. It is highly beneficial to choose the animal or cell model per the disease stage, as this increases the relevance of the study and gives stronger support for targeted drug testing.

Although enormous progress has been made, some translational issues still exist between preclinical and clinical results. The biggest ones are differences among species, lack of a complete disease model, different manifestations of disease, and the time of treatment making it difficult to predict the outcome [29]. A number of substances giving very good preclinical results are unsuccessful in clinical trials since the target is not well engaged, they have limited pharmacokinetics, and they do not adequately represent human disease complexity. Though, accurate preclinical models are something important of Huntington's disease investigation, continuously linking the understanding of the mechanism with therapeutic translation, a process leading to more and better interventions [9].

In-Vitro Screening Models of Huntington's Disease

In vitro screening models are very useful in Huntington's disease (HD) studies because they offer controlled and reproducible systems for dissecting the disease mechanisms and testing therapeutic candidates in a very short time [30]. They provide direct evidence of mHTT toxicity, protein aggregation, mitochondrial dysfunction, oxidative stress, and neurodegenerative signaling and at the same time are cost-effective, scalable, and flexible to experiments [9]. More importantly, since HD is a genetic disease but also involves multiple molecular changes, the in vitro platforms are good tools to separate the different pathological pathways and find therapeutic targets before animal studies [31].

Immortalized cell line-based models are still among the most popular systems in HD research. HEK293 cells are very often used because of their high transfection efficiency and really they can be used for studying mutant huntingtin expression aggregation interactions, and signaling pathways [32]. Despite being non-neuronal, they be a good platform for early mechanistic studies and molecular screening. Neuronal cell lines such as SH-SY5Y and Neuro2a are more related to the disease since they have neurons' properties and can be used for modeling oxidative stress, apoptosis, and synaptic dysfunction after mutant huntingtin exposure [33]. PC12-based Huntington's disease models have also been used quite a lot.

Such cells become neuron-like ones upon exposure to nerve growth factor and provide a good system for studying neuronal morphology, mitochondrial injury, and the effects of neuroprotective drugs [33]. The models derived from striatal cells are of great significance since the striatum is the main area suffering from neuronal loss in HD. These models better replicate disease-specific vulnerability and are a great resource for exploring excitotoxicity and studying the patterns of selective neuronal death [34].

Primary neuronal cultures increase physiological relevance as they maintain the native neuronal structure and signaling functions [35]. Cortical and striatal neurons in culture provide the opportunity to visually observe the effects of mutant huntingtin on toxicity, synaptic changes, calcium imbalance, and early phases of degeneration even outside the body [36]. Transgenic models overexpressing mutant huntingtin allow elucidation of aggregation mechanisms and activation of cellular stress pathways by ICT transfecting huntingtin fragments with expanded CAG repeats into host cells [13]. Also, RNA interference and gene silencing protocols not only contribute to our understanding of the huntingtin gene but also evaluate the therapeutic potential of allele-specific suppression and gene-targeted interventions [37].

One step further in the development of human cell models has been the use of patient-specific induced pluripotent stem cells (iPSC) to model neurons and other relevant cell types of the nervous system such as medium spiny neurons while keeping the patient's genetics intact [38]. These systems open up a way to monitor the course of the disease, the therapeutic reaction of a person, and pathological occasions at early stages, Mainly the brain changes. Also, fibroblast and neuronal cultures from patients provide clinically relevant models of mitochondrial dysfunction, oxidative stress, Huntington's disease pathogenesis, and mutant huntingtin [39]. Brain organoid and three-dimensional neuronal spheroid models are another major development by mimicking the tissue architecture, cellular interactions, and developmental complexity, This way providing deeper insights into disease progression in a more physiologically representative environment [40]. Gene-edited cellular systems based on CRISPR/Cas9 further improve disease modeling by allowing the accurate introduction or correction of HTT mutations and by supporting mechanistic studies with very high genetic accuracy [41].

Such in vitro systems are very important when it comes to high throughput screening of pharmaceutical compounds, as the efficacy, toxicity, and modulation of the target of a very large number of compounds can be evaluated fairly quickly [42]. They shorten the time of early-stage discovery and help in selecting the best candidates for further studies in animals. But, there are still some limitations such as the failure to replicate completely features of systemic disease, simpler tissue structures in the conventional cultures, and instability of the platforms [43]. Though, the in vitro models of screening still remain a major tool in the discovery of Huntington's disease by providing strong mechanistic insights and by acting as an indispensable link between molecular studies and translational therapeutic development (Table.1).

Table.1*: In-Vitro* Screening Models of Huntington's Disease and Their Applications in Drug Discovery

Model type	Representative models	Key pathological features reproduced	Applications in drug screening/research	Advantages
Immortalized cell line-based models	HEK293, SH-SY5Y, Neuro2a	Mutant huntingtin (mHTT) expression, protein aggregation, oxidative stress	Preliminary compound screening, cytotoxicity assays, molecular pathway studies	Easy maintenance, reproducible, cost-effective

PC12-based HD models	NGF-differentiated PC12 cells expressing mHTT	Polyglutamine aggregation, neurite degeneration, mitochondrial dysfunction	Neuroprotective screening and neuronal differentiation studies	Sensitive to toxic insults, neuron-like phenotype
Striatal-derived cellular models	STHdhQ7/Q7 and STHdhQ111/Q111	Selective striatal vulnerability, mitochondrial deficits, transcriptional dysregulation	Target validation and pathway-specific therapeutic screening	Disease-relevant cellular background
Primary neuronal culture models	Cortical and striatal neurons from rodents	Synaptic dysfunction, excitotoxicity, oxidative injury	Mechanistic studies and validation of neuroprotective agents	High physiological relevance
Mutant huntingtin overexpression models	Cells transfected with exon-1 or full-length *HTT*	Aggregate formation, inclusion bodies, apoptosis	Evaluation of aggregation inhibitors and protein clearance enhancers	Rapid disease induction
RNA interference/knockdown models	siRNA/shRNA targeting *HTT*	Reduced huntingtin expression, altered signaling	Gene-silencing therapeutic evaluation	Useful for mechanistic targeting
iPSC-derived neuronal models	HD patient-derived neurons	Endogenous mHTT expression, neuronal dysfunction, altered metabolism	Personalized drug screening and biomarker discovery	Human origin, patient-specific disease modeling
Patient-derived fibroblast and neuronal cultures	Skin fibroblasts and differentiated neurons	Mitochondrial dysfunction, oxidative stress, altered protein homeostasis	Validation of candidate drugs and precision medicine studies	Retains patient-specific genotype
Brain organoid and 3D neuronal spheroid models	Cerebral organoids, striatal spheroids	Cell–cell interaction, tissue architecture, progressive degeneration	Disease modeling and long-term therapeutic screening	Mimics human brain microenvironment
CRISPR/Cas9 gene-edited cellular models	Isogenic HD cell lines	Controlled CAG repeat expansion, endogenous mutation	Mutation-specific drug testing and mechanistic studies	High construct validity
High-throughput screening platforms	Automated imaging and multiwell assays	Aggregation burden, viability, oxidative markers	Rapid identification of therapeutic candidates	Fast and scalable

Chemical and Neurotoxin-Induced Animal Models

Animal models chemically and neurotoxically altered have been extensively used in Huntington's disease (HD) research as they exclude some pathological features of neurodegeneration within a fairly short period and offer practical systems for testing therapeutic interventions [31]. Unlike the genetic models that directly replicate the mutant huntingtin mutation, toxin-induced models mimic downstream pathogenic events such as excitotoxicity, mitochondrial dysfunction, oxidative stress, and selective neuronal degeneration, which are most defining for Huntington's disease progression [44]. These models are extremely helpful in pharmacological screening as they are reproducible, flexible in experimental setup, and able to provide measurable behavioral and biochemical changes over defined periods.

The quinolinic acid-induced excitotoxic model is one of the most accepted toxin-based models. Quinolinic acid is a natural metabolite of the kynurenine pathway and also acts as an agonist at N-methyl-D-aspartate receptors. Injection into the striatum causes excitotoxic neuronal death, Mainly damaging the medium spiny neurons, which are the main neurons lost in Huntington's disease [45]. This model reproduces striatal atrophy, neuronal degeneration, oxidative stress, and motor impairment, So it is very pertinent for studying excitotoxic pathways and testing neuroprotective compounds [45]. Also, quinolinic acid induces inflammatory responses and mitochondrial dysfunctions, This way further resembling several pathological mechanisms related to HD [46].

The 3-nitropropionic acid model is a very popular experimental system However. This mitochondrial toxin through its continuous action, causes irreversible blocking of succinate dehydrogenase resulting in compromised ATP production, mitochondrial failures, oxidative stress, and neuronal energy crisis [47]. When given repeatedly to rodents, it causes preferential striatal degeneration and the behavior showing abnormalities such as motor discoordination, gait disturbance, and cognitive impairment [48]. Since mitochondrial dysfunction and metabolic failure are two major aspects of contributing to Huntington's disease pathology, this model is very helpful actually in the evaluation of drugs directed towards bioenergetics, oxidative damage, and mitochondrial protection [47].

And, excitotoxic models based on kainic acid also make considerable contributions to research on HD. Activation of glutamate receptors is one of the effects of kainic acid, which leads to calcium overload, oxidative stress, and neuronal death. Injection into the necessary brain regions can mimic the excitotoxic damage and behavioral changes of Huntington's disease [49]. Such models help a great deal to study glutamatergic dysregulation and to find potential drugs that could regulate excitatory neurotransmission or protect neurons from calcium toxicity.

Malonate-elicited striatal degeneration is a further noteworthy chemical model. Malonate acts as an inhibitor of mitochondrial complex II which disrupts the processes of cellular respiration and ATP production, culminating in neuronal loss [50]. Since mitochondrial complex II impairment has been highly linked to Huntington's disease, this model simulates very well metabolic stress and selective striatal injury [51]. Behavioral and biochemical measures are used as readouts in these toxin-induced models. Behavioral measures can include measures of locomotor activity, rotarod testing, grip strength, balance and gait and cognitive tests while biochemical measures can include indicators of oxidative stress, mitochondrial enzymes, inflammatory mediators, neurotransmitter levels and histopathological analysis of the striatum and cortex [52]. Such quantifiable markers have contributed to the widespread use of toxin-induced systems in

the evaluation of therapeutic efficacy and the comparison of pharmaceuticals.

The usefulness of these toxins in pharmacological screening should not be underestimated as disease-like changes occur quickly and reproducibly, supporting rapid screening of neuroprotective agents, anti-inflammatory compounds, antioxidants and compounds targeting mitochondria [53]. Such cell models are imperative for early screening before from now on into more sophisticated models.

Yet these represent important limitations. Due to the absence of the genetic mutation involved, these models of toxin injection cannot reproduce the progressive mutant huntingtin aggregation or the hereditary nature of Huntington's disease [54]. The disease process is acute or subacute, rather than chronic, and some of the clinical facets may be underrepresented. Even so, many chemically and neurotoxin-induced animal models are highly useful experimental ways, and continue to play a principal role in preclinical Huntington's disease drug discovery for reproducing the fundamental neurodegenerative pathways in which therapies may eventually be targeted [55]**(Figure.2)**.

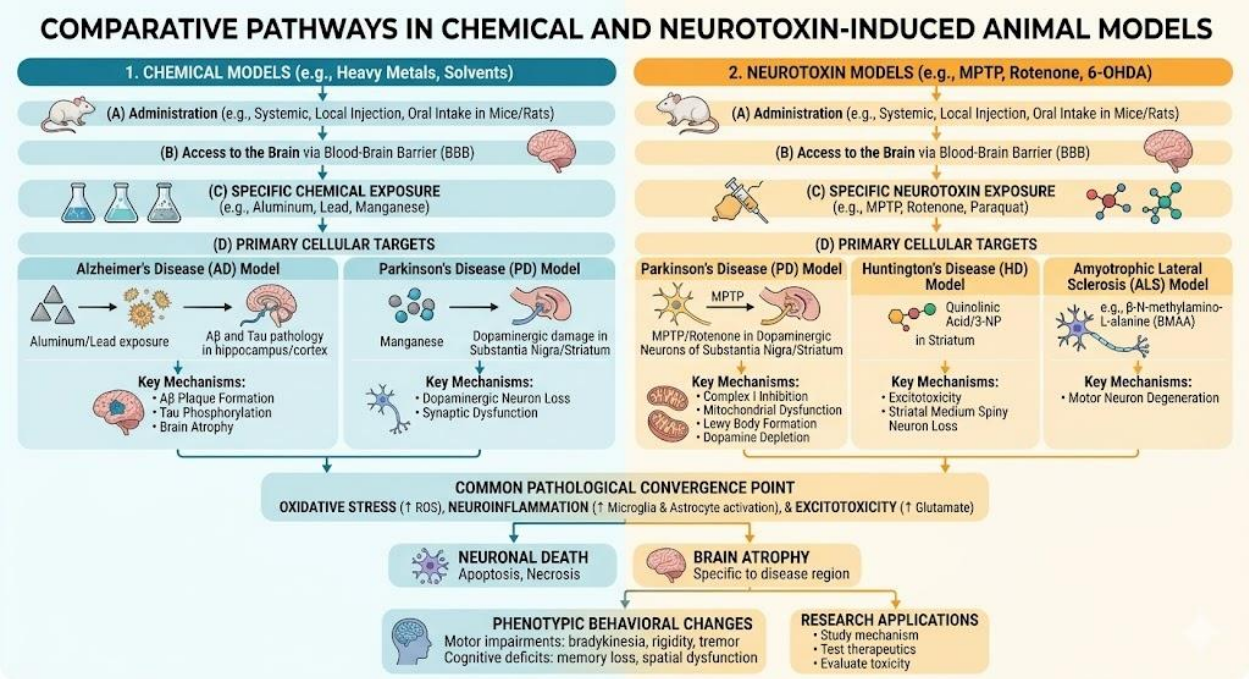

Figure 2: Comparative pathways in chemical and neurotoxin-induced animal models used for neurodegenerative disease research. This illustration shows the main ways chemical and neurotoxin exposures are used experimentally to mimic the pathological features of neurodegenerative diseases in animal models. Chemical models, like those involving heavy metals and compounds related to them, lead to neuronal damage mainly by oxidative stress, mitochondrial failure, and inflammatory signaling, This way inducing pathological alterations typical of Alzheimer's and Parkinson's diseases. Neurotoxin models of animals MPTP rotenone, 6-hydroxydopamine, quinolinic acid, and 3-nitropropionic acid, have a high affinity for neuronal subpopulations and reproduce specific neurodegenerative diseases. In case of Huntington's disease, neuron selectivity, excitotoxicity, and mitochondrial derailment are the main effects induced by quinolinic acid and 3-nitropropionic acid which lead to neuronal striatal loss. Both major types of models despite their differences in the initiation phase and cellular targets eventually point to the main pathological mechanisms including generating more oxidative stress, neuroinflammation, glutamate-induced excitotoxicity, neuronal death, and brain atrophy that is deteriorating over time. These pathological features that linked to the defamation of behavioral have, a lot, been used to provide experimental test beds for the study of mechanisms of the disease, neurotoxic evaluation, and therapeutic intervention screening in preclinical neurodegenerative disease research.

Genetic Rodent Models of Huntington's Disease

Genetic rodent models are the pillar of Huntington's disease (HD) research. They replicate the gene mutation that causes the disorder and, more importantly, allow detailed exploration of disease progression, brain changes, and responses to treatments in conditions similar to those in living organisms [56]. As HD is caused by an expansion of CAG trinucleotide repeats in the huntingtin gene, rodent models carrying mutant huntingtin have been indispensable for understanding how gene mutation leads to brain cell damage [57]. These models effectively emulate the gradual worsening of motor skills, cognitive decline, loss of neurons, and other molecular changes that mark the disease, and are So the mainstay of preclinical drug development studies. Among the transgenic mice models, R6/1 and R6/2 are the two that have been subject to most research. They both harbor an expanded CAG repeat in exon 1 of the human huntingtin gene but differ in the level of disease severity and progression [57]. The R6/1 model initiates symptoms very slowly, showing gradual motor dysfunction, cognitive deficits, and huntingtin aggregates inside the nucleus as time passes. The slow development of the model makes it very appropriate for the long-term evaluation of therapies and research on the pathogenic changes that occur in the early stages of the disease [58]. Then again, the R6/2 model exhibits characteristic signs of the disease very quickly and in a severe manner including heavy motor impairment, weight loss, and behavioral disorders, among others [59]. This model is very valuable for screening therapeutic candidates rapidly as disease symptoms arise early and consistently.

Another major transgenic model is the N171-82Q mouse which produces an N-terminal mutant huntingtin fragment with 82 glutamine repeats. This model shows progressive neurological decline, protein aggregation, and neurodegeneration and comes as a very useful tool in the evaluation of neuroprotective strategies and interventions aimed at the mechanisms of the disease [13]. Models that are full-length huntingtin transgenic are more genetically and pathologically valid since they encode the whole mutant huntingtin gene. The YAC128 model features full-length human mutant huntingtin with extended CAG repeats and it displays the main symptoms of Huntingtons disease including motor dysfunction, cognitive problems, striatal neuron loss, and cortical degeneration, among others [60]. This model is very close to the human condition and it has become a very important translational model. In the same way, the BACHD model, which expresses full-length mutant huntingtin, shows behavioral changes, synaptic and metabolic dysfunction, as well as neurodegeneration [61]. Since these models keep the full protein structure and more faithfully replicate mutant huntingtin biology, they are Most of all good for investigating the long-lasting disease mechanisms and therapeutic approaches that can change the course of the disease [62].

Knock-in mouse models take the genetic accuracy a step further by changing the normal mouse huntingtin gene itself to carry expanded CAG repeats. The HdhQ111 line of mice develops mild molecular and behavioral changes that are indicative of very early stage Huntingtons disease, That means it can be a model of disease initiation and mechanisms operating before symptoms [63]. The zQ175 line however shows major motor symptoms, mental decline, mutant huntingtin aggregation, and striatal damage, and has been the basis for many disease-modifying therapy and biomarker development studies [64]. Since knock-in models maintain physiological gene regulation and protein expression, they are very closely related to the human condition for genetics and show outstanding ability to translate to humans [65]. Rat models of Huntingtons disease are a good supplement to the mouse models by using bigger brain size and higher suitability for operating room procedures, electrophysiological experiments, and behavior testing. Both transgenic and knock-in rat models reveal symptoms of motor disturbances,

dementia, and neurodegeneration while However giving more detailed neuroanatomical analysis and pharmacological testing opportunities [66].

In rodent models, behavioral phenotyping often encompasses evaluating motor skills using rotarod, monitoring exploratory activity in open field, analyzing gait, measuring grip strength, assessing balance, and conduction of cognitive tests wherein, learning and memory are evaluated with maze-based tasks [67]. These methods are instrumental in determining the extent of the disease and the effectiveness of the treatments at different stages. Pathological and biochemical examinations involve checking neuronal loss in striatal and cortical areas, huntingtin protein aggregates, glial responses, levels of synaptic proteins, neurotransmitter alterations, mitochondria abnormalities and oxidative conditions [68].

Different rodent models bring various translational merits. The fragment transgenic models show quick and consistent disease manifestation, full-length transgenic models are more representative of the gradual disease progression, while knock-in models most closely resemble the genetic setting and initial disease processes [69]. Despite In reality no model can perfectly simulate all facets of human Huntington's disease, collectively they offer diverse platforms that have immensely contributed to unraveling disease mechanisms and therapeutic targets, thereby making genetic rodent models a fundamental tool in Huntington's disease research and pharmaceutical development (Table.2).

Table.2: Genetic Rodent Models of Huntington's Disease and Their Translational Relevance

Model	Genetic construct / mutation	Key pathological features reproduced	Behavioral phenotype	Major applications in research	Advantages	Limitations
R6/1 transgenic mouse	Exon 1 of human *HTT* with ~115 CAG repeats	Mutant huntingtin aggregation, striatal and cortical pathology, progressive neurodegeneration	Progressive motor impairment, cognitive decline, anxiety-like behavior	Disease progression studies and preclinical therapeutic screening	Slower disease course, useful for longitudinal studies	Partial gene construct; limited full-length protein expression
R6/2 transgenic mouse	Exon 1 of human *HTT* with ~120–150 CAG repeats	Early aggregate formation, severe neuronal dysfunction, marked striatal degeneration	Rapid motor deficits, tremors, weight loss, reduced survival	Fast screening of neuroprotective compounds	Rapid and robust phenotype	Severe progression may not reflect human disease timeline
N171-82Q mouse	N-terminal fragment of human *HTT* with 82 CAG repeats	Huntingtin inclusions, mitochondrial dysfunction, neuronal loss	Motor incoordination and progressive weakness	Therapeutic efficacy and mitochondrial pathway studies	Moderate disease progression	Fragment-based model lacks full gene context
YAC128 mouse	Full-length human *HTT* with ~128 CAG repeats	Progressive striatal atrophy, cortical dysfunction, synaptic impairment	Motor abnormalities and cognitive deficits	Long-term mechanistic and translational studies	High construct validity and gradual pathology	Longer study duration required
BACHD mouse	Full-length mutant human *HTT* with ~97 CAG repeats	mHTT aggregation, synaptic dysfunction, neuronal degeneration	Motor deficits, anxiety, impaired cognition	Testing disease-modifying therapies	Stable expression of full-length mutant protein	Variable phenotype onset
HdhQ111 knock-in mouse	Expanded CAG repeats inserted into endogenous mouse *Htt* gene	Early molecular abnormalities, subtle neuronal dysfunction	Mild behavioral changes	Early-stage disease and biomarker studies	Strong physiological relevance	Slow progression and subtle phenotype
zQ175 knock-in mouse	Humanized knock-in with expanded CAG repeats	Progressive aggregation, transcriptional dysregulation, striatal degeneration	Motor decline, cognitive dysfunction	Longitudinal therapeutic and biomarker evaluation	Excellent predictive and construct validity	Requires extended experimental period
Transgenic HD rat models	Full-length or truncated mutant *HTT* with expanded CAG repeats	Striatal pathology, synaptic abnormalities, neurochemical changes	Motor dysfunction and cognitive impairment	Behavioral pharmacology and surgical studies	Larger brain facilitates imaging and neurobehavioral analysis	Higher maintenance cost than mice
Conditional rodent models	Tissue-specific or inducible mutant *HTT* expression	Region-specific neuronal degeneration	Variable depending on targeted tissue	Mechanistic exploration of temporal and spatial pathology	Allows controlled gene expression	Complex breeding strategies

Non-Mammalian and Alternative Experimental Models

Non-mammalian and alternative experimental models have become very valuable tools in Huntington's disease (HD) research as these models facilitate rapid genetic modification, low-cost maintenance, and effective screening of disease mechanisms and potential therapies [70]. On one side, these models lack the complexity of the mammalian nervous system; However, they are very useful for studying the molecular pathways involved in mutant huntingtin toxicity, protein aggregation, cellular stress, and neurodegeneration that are conserved among species [71]. On top of that, due to their short life span and compatibility with high-throughput systems, these models are mainly suited for the stages of drug discovery and mechanistic studies that precede validation in rodent models [72].

Drosophila melanogaster has emerged as one of the leading non-mammalian model organisms for Huntington's disease. Mutant huntingtin transgenic flies expressing polyglutamine-expanded repeats display progressive neurodegeneration, protein aggregation, locomotor dysfunction, shortened lifespan, and retinal degeneration [73]. These changes closely parallel key features of Huntington's disease pathology and allow for quick quantification. Since Drosophila has well-defined genetics and a vast array of genetic tools, it enables targeted alteration of signaling pathways related to mitochondrial dysfunction, oxidative stress, autophagy, and neuronal survival [74]. Scientists commonly utilize fly models to pinpoint modifying genes, explore pathways of protein misfolding, and test neuroprotective agents. Besides, flies' short generation time and the capability of producing large experimental groups make them perfect for big screening initiatives [75].

Caenorhabditis elegans is an excellent model organism for Huntington's disease studies because of its simple and transparent neuronal system which allows researchers to directly observe neurodegeneration in living animals [76]. In fact, when mutant huntingtin gene is introduced in C. elegans, it causes protein aggregation, neuronal impairment, locomotor defects, and reduced lifespan [77]. These phenotypic changes provide a basis for research on different aspects of Huntington's disease like proteostasis disturbance, oxidative stress, mitochondrial dysfunction, and cellular signaling [15]. Besides, this model system is very flexible in genetic manipulation as well as apoptosis and stress response mechanisms are conserved across species, So a great resource for elucidating the molecular basis of the disease and also for drug testing aimed at improving longevity and neuronal recovery [78].

The usefulness of zebrafish models has been growing steadily as they preserve the vertebrate brain layout whereas at the same time allow very easy and open experimental interventions. Creating transgenic zebrafish carrying mutant huntingtin leads to various motor dysfunctions, brain damages, physical malformations, and altered behaviors similar to human Huntington's disease symptoms [79]. One of the advantages is that zebrafish embryos are see-through which makes it possible to monitor the brain development, neuron death, and protein buildup as they happen predominantly [80]. Also, zebrafish are extremely effective for chemicals screenings since the drugs can simply be placed in the water in which the fish are living leading to the immediate evaluation of the therapeutic effects [81]. Unlike invertebrates, fish being vertebrates possess specific neural circuits and genes that are very similar to humans making results from fish studies more relevant to human biology while at the same time keeping the advantages of lower costs and quick reproduction [82].

Yeast models have been proven to be an amazing tool to get insights into the cellular toxic effects of mutant huntingtin. Introducing huntingtin proteins with extended polyglutamine tracts to yeast cells is known to cause protein aggregates, faulty mitochondria, oxidative damage, as well as problems with intracellular trafficking and protein homeostasis [83]. Yeast cells do not have neurons even so a lot of fundamental cellular processes implicated in Huntington's disease have been conserved through evolution. Because of this yeast cells are amenable to intensive dissection of mechanisms of protein aggregation, discovery of genes or molecules modifying toxicity, and identifying drugs that can restore proper folding or cell stress response [84].

The main benefit of these alternative experimental systems is that they are very efficient and suitable for scaling. In addition, they allow quick genetic studies, large-scale compound screening, as well as in-depth analysis of the mechanisms of conserved biological pathways at a fraction of the cost and in a shorter time compared to mammalian models [85]. Mainly, they help in finding therapeutic targets and in the process of selection of up to the most potent drug candidates for human testing. Yet, their drawbacks are less anatomical complexity and partial mimicry of human neuropathology [69]. Non-mammalian and other such models Though underlie Huntington's disease research, delivering complementary insights that deepen the understanding of mechanisms and speed up drug discovery for translation [86].

Therapeutic Agents Evaluated Using Preclinical Huntington's Disease Models

The introduction of therapeutic strategies in Huntingtons disease (HD) relies heavily on various preclinical models, which allow for the thorough testing of potential drugs against the multiple neurodegenerative mechanisms that drive the disease [87]. In fact, it is well known that HD pathology is first and foremost triggered by the toxic effects of the mutant huntingtin protein, although it is also characterized by mitochondrial impairment, oxidative stress, neuroinflammation, synaptic dysfunction, and neuronal loss [21]. Because of this, more and more therapeutic approaches aim at not only treating the symptoms but also modifying the course of the disease [88]. Preclinical models for initial drug screening, including cellular cultures and genetically engineered animals, have been pivotal in the identification and proof of concept of such therapies even before embarking on clinical trials [89].

It is mainly small molecules and neuroprotective agents that have become the focus of research with therapeutic options for Huntingtons disease [90]. Numerous compounds have been put through the paces in an effort to find those that can effectively reduce the levels of mutant huntingtin aggregates, promote neuronal survival, reinforce synaptic communication, and ultimately lead to a delay of the pathological features [91]. Experimental findings have Yes shown, for instance, that agents including riluzole, memantine, cysteamine, trehalose, and various histone deacetylase inhibitors, confer positive outcomes mainly by the mechanisms of reducing excitotoxicity, bolstering cellular defenses, or counteracting transcriptional abnormalities [92]. However, neuroprotective compounds with a focus on the brain-derived neurotrophic factor signaling pathway, upregulation of autophagy, and protein degradation control, not only help with neuronal survival but also have a behavioral impact, per studies [93].

In reality oxidative injury and deranged energy metabolism are at the core of HD pathology, there has naturally been a focus on the use of antioxidants and mitochondrial regulators [94]. Among those compounds tested in HD models, both the toxin-induced and genetic ones, are coenzyme Q10 creatine melatonin, N-acetylcysteine, edaravone as well as the mitochondrial-targeted antioxidants [95]. These drugs act at the level of improving mitochondrial function, lessening the production of free radicals, maintaining mitochondrial membrane potential, and safeguarding neurons from energy deficits [96]. Through the use of these antioxidant-related agents, many experimental studies show enhancements in motor skills, reduction of oxidative stress levels, and conservation of striatal neurons. And, there are also agents that affect mitochondrial generation and energy metabolism that are undergoing investigation for continued neuroprotection [97].

Another major reason that anti-inflammatory therapies have been investigated is that chronic neuroinflammation and microglial activation are two main contributors to Huntington's disease progression [98]. Multiple experimental models have shown that there is a rise in inflammatory mediators such as tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6. Because of this, neuroprotective potential of anti-inflammatory compounds like minocycline, non-steroidal anti-inflammatory agents, microglial inhibitors, and natural anti-inflammatory phytochemicals were assessed [99]. Ability of these therapies to diminish inflammatory signaling, prevent neuronal damage, and enhance behavior in animal models has been attested.

Really gene silencing methods can directly go after the biggest reason behind Huntington's disease by shutting down the production of mutant huntingtin is one of the significant therapeutic breakthroughs [100]. Animal experiments with the use of viral vectors, RNA interference technologies, and gene-targeted delivery systems have found out that decrease in mutant huntingtin expression can lessen protein aggregation, enhance neuronal cell survival, and slow down the course of the disease [101]. Because these methods intend not only to alleviate symptoms but also to alter the disease path, they are an Mainly fascinating option for therapy.

One of the most attractive treatment platforms in the area of Huntington's disease research has been the use of antisense oligonucleotides. These are short, chemically synthesized nucleic acid sequences that stick to huntingtin messenger RNA and cut down on the production of mutant protein [102]. Antisense oligonucleotides, in HD models of rodents as well as large animals, have shown potent lowering of huntingtin levels, decreased aggregation, better motor functions, and maintenance of neuronal structure [102]. Besides, their reversibility and allele-selective targeting potential raise their value for clinical use.

Therapeutics based on RNA, such as small interfering RNA, microRNA-based modulation and RNA-targeted editing approaches, greatly broaden the treatment landscape. These therapies can be used to regulate huntingtin expression or to change the pathological signaling downstream and have been very promising in preclinical models [103]. Together, these different therapy categories show how the experimental models of Huntington's disease have been the driving force for the identification of disease-modifying interventions and still act as the main source of proof for the development of therapies that go from bench to bedside [104].

Emerging Technologies and Future Perspectives

Emerging technologies are rapidly changing the focus of Huntington's disease (HD) research and have great potential to greatly increase the accuracy of disease modeling and therapy screening and reduce the failure of translation from animal studies to human clinics in the next few years [105]. Although traditional cellular and animal models have helped explain many aspects of disease biology, the inability of these models to mimic the entire complexity of human Huntington's disease is a key reason why researchers keep developing new and better experimental platforms [30]. These platforms are designed to more accurately reflect genetic variation, disease progression, and patient-specific responses, at the same time as they speed up drug discovery and better translation of laboratory results into clinical practice.

Accurate disease modeling has become a major focus of Huntington's disease research since this approach allows the production of models that quite faithfully reproduce the molecular and cellular changes occurring in the disease [69]. The progress made in genome editing and patient-derived cell technologies opens the door for creating models with specific CAG repeat lengths and genetic backgrounds that are very similar to those causing human disease heterogeneity [106]. Using such systems, researchers could study how the size of the repeat expansion, modifier genes, and environmental factors contribute to disease onset and development [107]. This level of precision not only offers a deeper insight into the mechanisms at work but also paves the way for more focused therapeutic assessments.

Humanized transgenic models add another layer of translational value as they bring human huntingtin gene sequences into the world of experimental animals [108]. By comparison with conventional rodent models, these models have a higher fidelity about reproducing mutant huntingtin expression, processing of the protein, and the neuropathological features of the disease. Humanized models constitute a critical tool for the preclinical evaluation of the varied therapeutic approaches such as gene silencing and allele-selective interventions, that precisely target the human huntingtin gene transcript or protein [109]. They also allow the long-term monitoring of the efficacy and safety of such disease-modifying strategies, which are both essential elements in the transition from experimental therapy to effective clinical treatments.

Artificial intelligence (AI) is a remarkable tool in Huntington's disease (HD) research and therapeutics [110]. AI algorithms with machine learning capabilities quickly sift through enormous datasets from molecular screenings, recognize the characteristics of disease progressions, identify therapeutic targets, and rank drug plant candidates most likely to be successful [111]. AI-driven modeling is a multifunctional support system for virtual screening of molecules, enhancement of lead compounds, discovery of biomarkers, and assessment of treatment response [112]. When the experimental and computational methods are combined, these AI tools lessen the time of development and enhance the identification of novel therapeutic candidates.

Integrating various omics data is also broadening the outlines of disease modeling. Gathering genomic transcriptomic proteomic, metabolomic, and epigenomic information collectively permits a more profound understanding of Huntington's disease [113]. These combined data sets reveal networks of molecular interactions, assist in finding biomarkers, and fine-tune experimental models that are based on disease signatures[114]. The use of multi-omics directed platforms results in an increase in predictive validity and discloses new targets which would not have been identified through single-pathway analysis [115]. Because HD worsens and patients through therapies differently, personal medicine treatment methods are gaining a foothold. The use of patient-induced pluripotent stem cells, patient-specific molecular profiling, and biomarker-directed screening is a new approach in the field of personalized medicine for the development of new drugs targeting the specific phenotypes of the disease [116]. These methods can make a difference in improving the efficiency of treatment and in lessening the variability of clinical outcomes.

One of the biggest technological leaps can be seen in using advanced organoid and microfluidic systems. Brain organoids taken from patient cells show how neurons and other brain cells architect and communicate [117]. Then again, microfluidic devices mimic the natural human microenvironment and give fine control over the supply of nutrients, exposure to drugs, and neural interactions [118]. These methods not only better illustrate how diseases develop but also support continuous testing of therapies. The next step for Huntington's disease (HD) therapeutics will likely be the merge of these groundbreaking tools into unified platforms [119]. The combination of genetically-tailored therapies, humanized models AI multi-omics profiling, and patient-specific drug screening uses a better and more mechanistically based method for drug discovery [120]. These techniques, as their power grows, will shorten the time of introducing drugs that are simultaneously safer and more effective. In the same way, they will push HD research forward to the point where it will finally be possible to tailor and modify treatments as individual patients.

Conclusion

Huntington's disease is still one of the most difficult inherited neurodegenerative disorders to deal with because of its continuous clinical worsening, complex molecular pathology, and absence of effective treatment. Even though the disease is caused by a well-identified CAG repeat expansion in the HTT gene, its downstream changes affect numerous interconnected mechanisms including mutant huntingtin aggregation, mitochondrial dysfunction, oxidative stress excitotoxicityneuroinflammation, impaired proteostasis, and synaptic degeneration. This multifarious characteristic makes the development of therapeutic agents very complicated and at the same time it pinpoints the great necessity of highly reliable preclinical screening platforms that can mimic disease-related biologic events.

Throughout time, preclinical models have not only greatly contributed to the elicitation of Huntington's disease pathogenesis but also have endowed therapeutic evaluations with indispensable tools. In vitro systems like immortalized cell lines, primary neuronal cultures, iPS cell-derived neurons, and organoid-based platforms provide the opportunity to thoroughly dissect the molecular and cellular mechanisms and at the same time facilitate quick and high-throughput screening of therapeutic candidates. Chemical and neurotoxin-induced animal models have been instrumental in mimicking excitotoxic and mitochondrial injury pathways; Yet, genetic rodent models have allowed the investigation of progressive disease pathology, behavioral deficits, and long-term neurodegeneration resulted from mutant huntingtin expression in greater detail. Besides mammalian models, other species like Drosophila melanogaster, Caenorhabditiselegans, zebrafish, and yeast have enhanced the experimental structure by furnishing affordable and effective systems for mechanistic dissection and extensive drug screening.

Taken together, these models have greatly accelerated the testing of various therapeutic approaches like neuroprotective agents antioxidants compounds that affect mitochondria, anti-inflammatory agents, gene silencing approaches, antisense oligonucleotides, and RNA-based drugs. Really, each model offers distinct advantages and at the same time limitations which highlight the importance of integrated and stage-specific model selection during drug discovery. No single experimental model can completely replicate the complexity of human Huntington disease; Still, the planned combination of different models results in better mechanistic understanding and increased translational relevance. The next generation of precision disease modeling, genome editing, humanized transgenic models, artificial intelligence-aided drug discovery, multi-omics integration, and personalized medicine are changing the landscape of Huntington's disease research. These new developments will likely increase the accuracy of prediction and allow for therapeutic interventions to be tailored to the individual patient. The long-standing issues between preclinical successes and clinical testing will finally be addressed through these new approaches. Besides, large scale organoid and microfluidic technologies contribute Really to the progression by providing more physiologically relevant models of the disease and accurately recreating its environment.

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