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REVIEW

An update on mammalian and non-mammalian animal models for biomarker development in neurodegenerative disorders

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Abstract

Neurodegeneration is one of the leading factor for death globally, affecting millions of people. Developing animal models are critical to understand biological processes and comprehend pathological hallmarks of neurodegenerative diseases. For decades, many animal models have served as excellent tools to determine the disease progression, develop diagnostic methods and design novel therapies against distinct pathologies. Here, we provide a comprehensive overview of both, mammalian and non-mammalian animal models, with a focus on three most common and aggressive neurodegenerative disorders: Alzheimer's disease, Parkinson's disease and Spinocerebellar ataxia-1. We highlight various approaches including transgene, gene transfer, and chemically-induced methods used to develop disease models. In particular, we discuss applications of both non-mammalian and mammalian contributions in research on neurodegeneration. It is exciting to learn the roles of animal models in disease pathomechanisms, identifying biomarkers and hence devising novel interventions to treat neuropathological conditions.

Keywords Neurodegeneration · Animal models · Non-human primates · Biomarkers · Alzheimer's disease · Parkinson disease · Spinocerebellar ataxia-1

Introduction

The majority of the studies on pathology of different neurodegenerative disorders (NDD) are validated at the pre-clinical stage in animal-based experiments. In pre-clinical studies, the animal models allow researchers to study multiple alterations at genetic, molecular, and physiological levels to develop effective therapeutic solutions [1]. In case of NDDs, animal models show multiple differences, including genetics, with human patients, for example some hereditary disease causing genes are not found in animal models, such as the gene APOE ε4 allele responsible for AD is not found in mice [2]. It is often debated whether animal experiments are good enough to recapitulate the disease progression. However, with the advances in gene editing methods, such as CRISPR, and gene knock-in techniques, several challenges have been mitigated. Also, animal models provide a tool to understand disease mechanisms, investigate pathological alterations and test new therapeutics. More importantly, they serve as an excellent information tool to study various biomolecules like proteins, miRNAs, metabolites, mRNAs, exosomes etc. to precisely understand the changes occurring in degenerated brains [3–5]. The animal-dependent studies can recapitulate the degenerative changes and progression of NDDs similar to human patient brains and may help elucidate the molecular mechanisms accountable for the condition. In this review, we give an summary of animal models both mammalian and non-mammalian, used widely in studies focusing on NDDs.

The most explored mammalian models to study NDDs are rodents, like mice, and rats, or non-human primates (NHP's), like monkeys, apes, and chimpanzees, etc [1]. Due to the fundamental differences in the brain's structure, complexity and physiology of rodents and humans, it becomes challenging to replicate degenerative changes in rodents.

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Yet, most of the scientific research for investigating neurodegenerative changes in mammalian models focuses on rodent models primarily due to the ease of genetic modulations, maintaining the colony, and handling the animals [6]. On the other hand, NHP's share various genetic, morphological, and physiological characteristics of the brain more closely with humans and can serve as better models to study NDD [7]. However, despite several advantages, NHP's are not as common as rodents in NDD research due to less accessibility, requirement of large breeding areas, ethical considerations, and special breeding programme. All such settings make use of NHP's animals expensive and difficult in biomedical research.

Non-mammalian animals provide additional model tools to study neurodegeneration and pathological progression. Few of these widely used non-mammalian animals are flies (e.g. drosophila), worms (C.elegans), and fish (zebrafish) [8–10]. Non-mammalian models can serve as a simple biological systems to test a hypothesis and investigate a putative biomarker or potential drug candidate before testing in higher animal models. Additionally, non-mammalian animal models provide some advantages over their mammalian counterparts such as they are easy to breed, monitor, and to record changes in the biomarkers or perform therapeutic intervention experiments. But, a major drawback of using non-mammals for neurodegenerative studies is their primitive nervous system that doesn't provide the intricate information and thus are difficult to predict the observations of the human brain. Nonetheless, these systems provide many important and critical insights into molecular pathways and changes caused due to misfolding and aggregation of proteins. In summary, using animal models for studying NDDs, identifying biomarkers, and developing novel therapeutic interventions provides a strong foundation that can subsequently be used for clinical studies in human NDD patients.

In this article, we provide a snapshot of animal models in elucidating the mechanism of neurodegeneration by focusing on three NDDs: Alzheimer's disease (AD), Parkinson's disease (PD), and Spinocerebellar Ataxia-1 (SCA-1). We provide a detailed description of the rodent, NHPs, and some non-mammalian models developed for these neurological diseases. The knowledge gained from such discussion allows us to better understand the mechanism of neurodegeneration, conduct biomarker discovery, and hence subsequently think of possible therapeutic approaches in these NDDs [11, 12]. Our basic logic for selecting AD and PD for our analysis is the high global burden of both these pathologies in the aging population. SCA is another highly aggressive condition with a very low survival rate and thus needs broader scientific attention. We discuss molecular and pathological aspects of alterations in memory, cognition, and movement for these NDDs in these animal models. In the last section, we have comprehensively discussed the limitations of the animal model used in NDDs and have provided possible solutions to overcome such disadvantages. Additionally, in brief, we discussed about the alternative models to study and understand NDDs.

Development of rodent models to understand neurodegeneration

Rodents play a huge part in modeling different types of NDDs. As explained earlier, due to the low cost of maintainence, easy genetic manipulation and breeding, they make an excellent disease modeling choice. Most of the rodent animal models for NDDs are generated by the technique of transgenetics because they don't typically carry genetic mutations observed in NDD patients. This approach allows the generation of rodent animals with genes that are found to be mutated or have a loss of functions in human NDDs. The conventional technique of transgenic animal production involves the injection of male pronucleus into the female egg resulting in its fertilization and formation of embryo. The embryo is then implanted in female mice which would produce transgenic animals [13]. Additionally, retrovirus mediated transfer of foreign gene could be performed into the eight cell embryo [14]. Another process to generate transgenic animal uses in vitro transfer of foreign gene in the embryonic stem cells followed by their injection into the early mouse embryo and subsequent implantation into female mouse [15]. The expression of transgene in animals can also be controlled using methods such as LoxP [16]. In Fig. 1, we have provided a summary of various methods used to develop transgenic animal models.

Alzheimer disease (AD)

AD is the primary reason for dementia in patients around the globe [17]. The disease is identified with progressive loss of memory, which affects patient's decision making, cognitive functions and can finally lead to fear, anxiety, restlessness, and irritation [18]. In AD patient's brain, three pathological symptoms are observed i.e., intracellular neurofibrillary tangles (NFT) generation, formation of extracellular senile plaques, and degeneration in the parts of cortex and hippocampus [19–21]. The NFTs and senile plaques in AD are formed by hyperphosphorylated tau proteins and toxic Aβ peptides respectively [22, 23]. Major genes with mutations affected in AD are APOEε4, presenilin 1 (PSEN1), presenilin 2 (PSEN2), and amyloid precursor protein (APP) [24]. APP gene product – APP protein contributes in the development of toxic Aβ peptides (Aβ42), as it gets cleaved into smaller subunits *via* amyloidogenic and non-amyloidogenic

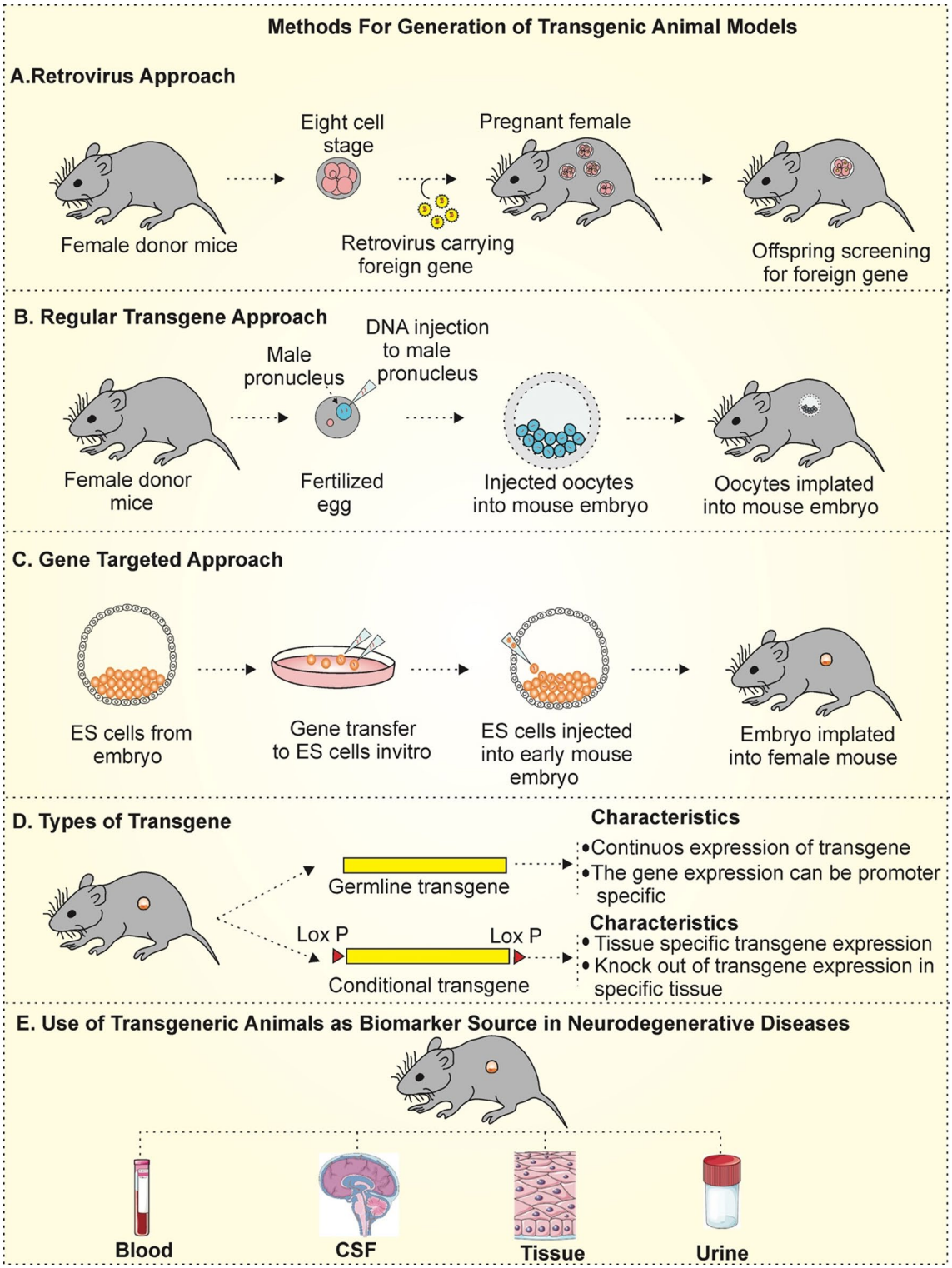


Fig. 1 Techniques to produce transgenic animals and their roles in biomarker development: (**A**) Retroviruses can be used to transfer the gene of interest in the female donor mice egg and then implanted into the female mice for transgene progeny transfer. (**B**) In a different strategy, female mouse-derived egg can be fertilized with male pronucleus and then implanted into mouse embryo. (**C**) Embryonic stem cells derived from female mouse can also be transfected with gene of interest in vitro and then impanted for transgentic progeny generation. (**D**) Continous vs. Lox-P mediated controlled expression of transgene (**E**) Applications of transgenetic animal biofluids in biomarker discovery for translational applications

mechanisms. From these two pathways, amyloidogenic process produces toxic Aβ peptides (Aβ42) using different enzymes i.e., alpha, beta, and gamma secretases [25]. PSEN1 and PSEN2 gene are responsible for production of gamma secretases protein catalytic subunits and hence their mutations are directly linked with the APP processing and generation of toxic Aβ peptides.

Multiple AD transgenic mouse mimicing most of the AD molecular and anatomical features are already been developed. These mice have increased Aβ production and amyloid formation or deposition of hyperphosphorylated tau proteins in NFTs [26, 27]. An AD mouse model with mutations in APP gene has aggregation of Aβ proteins, mimicking the senile plaque accumulation in AD patients [28, 29]. In this context, APP gene mutation carrying platelet- derived growth factor promoter-driven human amyloid precursor protein (PDAPP) was the very first AD mice that showed high Aβ aggregation and had human APP gene with Indiana mutation (V717F) in the regulation of PDGF-β promoter. PDAPP transgenic mice shows human Aβ aggregate formation at 6–9 months and develops gliosis dependent on age [30]. Tg2576 is another AD mice that can overexpress human APP protein containing Swedish mutation (KM670/671NL) in control of promoter hamster prion protein. Tg2576 mice also has formation of amyloid plaques and microglial activation at 10–16 months of age [31].

One more AD mice, APP23 has a Swedish mutation (KM670/671NL) in the APP gene in the regulation of human Thy1 promoter. APP23 mice have Aβ deposition from 6 month of age and these deposits are upregulated both in number and size with time [32]. J20 mice is a separate AD mice with highly expressed human APP gene and also have Swedish (KM670/671NL) and Indiana mutations (V717F) all in the control of PDGF- β [33]. Similarly, TgCRND8 AD mice also have overexpressed human APP gene with Swedish (KM670/671NL) and Indiana (V717F) mutations in the hamster prion promoter control [34]. Likewise, Tg-ArcSwe another AD mice model also contains a highly expressed APP gene of human origin with 2 mutations: Arctic (E693G) and Swedish (KM670/671NL) in control of promoter Thy1 [35]. Furthermore, the A7 AD mice model was also founded on overexpression of APP protein and mutations of Swedish (KM670/671NL) and Austrian (T714I) in the regulation of promoter Thy1.2 [36].

More mouse models for AD are established based on mutations in presenilin (PSEN1 or PSEN2) genes that cause faulty APP protein processing and hence lead to generation of senile plaques with toxic Aβ peptides. One such model is PS2APP mice which is generated by crossing two transgenic mice i.e. APPswe and PS2 (N141I) mice and hence the crossed mice has APP gene of human origin, overeexpressing Swedish mutation (KM670/671NL) and also has PSEN2 human gene with mutations in N141I gene in the control of Thy1.2 and prion promoter of mouse. The mice starts to have amyloid plaques formation from 9 months of age [37]. Another AD mice with the PSEN1 gene mutation is APPswe/PSEN1dE9 (APP/PS1) mice and it has mutations of Swedish (KM670/671NL) and PSEN1 (dE9- which has no exon 9). The mice shows Aβ deposition from 6 months [38]. Likewise, 5xFAD mice was generated by incorporating 5 different mutations in APP/PS1 transgenic mice: Florida (I716V), London (V717I), Swedish (KM670/671NL) in APP gene and L286V, M146L (A>C) mutations in PSEN1 gene and the same causes a high accumulation of Aβ in cerebral regions [39].

In addition to amyloid or Aβ deposition, tau pathology-dependent AD mouse models are also used to comprehend AD [40]. Broadly two types of tau pathology mice model are created i.e., by using genetic modifications or with tau-seed injections to the mice. Genetically modified models are diverse in terms of use of genetic engeineering method (knock-in or tangenic), tau levels in the brain, isoforms of tau expression (0NR, 1N4R) and the brain regions where the genes are introduced. One of the first mice model to mimic tau pathogeneis of AD was JNPL3 mice, which has a human tau gene and inserts of tau (0N4R) with P301L mutation under the influence of mouse prion promoter [41]. JNLP3 mice show tangled tau pathology at 4.5 months in the diencephalon, brain stem, spinal cord, and cerebellar nuclei [41].

Another mouse model used in AD to understand tau pathology is PS19 which expresses 1N4R tau with PS301S mutation under mouse prion promoter control. The mouse develops neurofibrillary tangle from 6 months of age with microgliosis and early synapse loss from 3 months [42]. Similarly, rTg4510 mice developed to comprehend AD tau pathology carries P301L mutation in tetracycline operon-reponsive element and a tetracycline-modulated transactivator in the regulation of calcium/calmodulin-dependent protein kinase II alpha (CaMKIIα) [43]. Additionally, another AD mice with both tau and Aβ deposit i.e., 3xTg mice were generated by knocking in the mutations of APP Swedish (KM670/671NL), PSEN1, MAPT (P301L), and (M146V) in the control of promoter Thy1.2 [44]. Additionally, one tau AD model that uses human MAPT knock-in mice was developed which expresses 6 different isoforms of tau (human) but with a higher amount of 4R than 3R tau [45].

Other than tau knock-in, tau seed injection models are also used to study AD [46]. The tau seed injection model was first developed in 2009 which shows the ability of tau protein to transmit and diffuse across different brain compartments [46]. The tau seed injection model was established by inoculating AD pateints brain/AD mice model brain lysates/

or recombinant tau into the brain of mouse. These injection models show tau dissemination by synaptic connections [47]. However, tau-based AD mouse model also has some disadvantages as these models often focus on replicating the mechanism of tau aggregation but not any changes in tau gene. But in other tau diseases e.g. frontotemporal dementia (FTD), progressive supranuclear palsy, Pick's disease, the tau models replicate tau mutations observed in the patients [48, 49]. Furthermore, tau-based AD mice are not always able to show late human AD related changes in tau protein changes like ubiquitination and acetylation [50]. Apart from the above-described AD mice, many other models are known or are under development. Unfortunately, we could not describe all here due to space constraints. However, we have provided a descriptive account of different AD mouse models in Table 1 that will help researchers gain a good understanding of various AD models. These mice models can serve as good tools for comprehending AD pathomechanisms, finding novel AD interventions, or for early biomarker discovery in the pathology of AD. In one of our upcoming section, we have discussed a few of these AD mouse models for biomarker discovery to explain the clinical significance of AD mice experiments.

Parkinson disease (PD)

PD is characterized by degeneration of dopaminergic neurons (DN) in substantia niagra (SN) with misfolded α-synuclein proteins in Lewy body-like structures [51]. Symptoms of PD include tremor, bradykinesia, and rigidity caused due to the damage to DN in the SN region [52]. At the genetic level, mutations in different genes like α-synuclein, LRRK2, PARK7, Parkin, DJ-1, and PINK1 are accountable for either autosomal dominant or autosomal recessive PD [53]. Mutations in other mouse genes such as SYNJ1, FBX07, DNAJC1, ATP13A2, and PLA2G6 can show PD-like symptoms. Additionally, several less-known genes including VPS35, and eiF4G1 can have PD mutations and leads to different amounts of penetrance [54]. The mouse models of PD are generated by different mechanisms for example neurotoxin injection. Most of the research using this PD model focuses on studying PD symptoms. Two of the frequently used drugs for inducing PD-like symptoms, are 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [55]. Other toxins like rotenone [56], paraquat/maneb [57], amphetamine derivatives (methamphetamine and 3,4-methylenedio

Table 1 Most common mouse models for Alzheimer's disease

	Gene mutations	Pathological changes	References
Transgenic mouse models			
5xFAD	APP- Swedish (K670N/M671L), Florida (I716V), and London (V717I); PSEN1- M146L and L286V	Accumulates high level of Aβ42 in neurons at 1.5 months; severe amyloid pathology, plaque deposition at 2 months.	[230]
APP/PS1	APP- Swedish (K670N/M671L); PSEN1- L166P	Aβ deposition at 1.5 months in cortex; moderate neuron loss	[231]
Tg2576	Overexpression of isoform 695 of APP with Swedish mutation	Formation of parenchymal plaques at approx. 1 year	[232]
APP23	APP- Swedish (K670N/M671L); with murine Thy1 promoter	Plaques appear at 6 months; neuronal loss in CA1 at 14 months	[32]
J20	APP- Swedish (K670N/M671L); Indiana (V717F); with PDGF-β promoter	Aβ puncta in hippocampus in 1month; diffuse plaques at 5–7 months	[33]
TgCRND8	APP- Swedish (K670N/M671L), Indiana (V717F)	Amyloid deposition at 3 months; dense core plaques at 5 months	[34]
rTg4510	MAPT- P301L	Pretangles- 2.5 months, tangle-like inclusions in 4 and 5.5 months in cortex and hippocampus respectively.	[43]
PS19	MAPT- P301L	NFTs in 6 months, neurodegeneration in 9 months	[42]
3x Tg AD	APP -Swedish; MAPT P301L; PSEN1 M146V	Plaque deposition in 6 months; Tau pathology appears by 12 months	[44]
Knock-in and Knock-out mice			
APP NL-F	APP- Swedish (K670N/M671L), Iberian (I716F)	Amyloid plaques at 6 months in homogygotes and 24 months in heterozygotes; no neuronal loss	[233]
APP NL-G-F	APP- Swedish (K670N/M671L), Arctic (E693G); Iberian (I716F)	Amyloid plaques at 2 months in homogygotes and 4 months in heterozygotes; no neuronal loss	[233]
APP SAA	APP- Swedish (K670N/M671L), Arctic (E693G); Austrian (T714I)	High Aβ42/40 ratio in all three: brain, CSF, and plasma; plaques at 4 months	[234]
hAβ-KI	Humanization of Aβ sequence in murine App gene	No plaque formation till 22 months	[235]
Tau KI	Exons 1–14 of mouse MAPT exchanged with human MAPT	Accelerated propagation of Tau	[45]
APOE KI	Humanized APOE4 allele (at exons 2–4)	Lower level of APOE4	[236]
APOE KO	Inactivated mouse APOE	Higher plasma cholesterol; artherosclerotic lesions	[237]
TREM2 KO	Trem2 gene replaced with LacZ gene	Not available	[238]

xymethamphetamine) can also be used to generate PD-like features in mice [58, 59]. Notably, such drug treated mouse are not able to repeat many of the human PD symptoms, such as lack of LB formation (in MPTP and 6-OHDA mice) [60, 61]), have ambiguous nigrostriatal DA effects (in paraquet/maneb) [62]. Moreover, toxins can also cause significant death in the mice population (for example rotenone) and hence alternate PD mice models, e.g., transgenic PD were developed [63].

Additional PD mouse models are dependent on overexpression of human α-synuclein mutations and develop PD symptoms like formation of Lewy bodies [64, 65]. Few of these human α-synuclein variants used for overexpression have mutations in A53T, E46K, A30P genes or they express truncated human α-synuclein (from 1 to 120) in control of several promoters [67–68]. Furthermore, lentivirus-based delivery of α-synuclein in mice brain regions (striatum, substantia nigra, amygdala) also leads to Lewy body formation and PD pathology [69]. Despite aggregation of α-synuclein, these mice don't show significant loss in DA neurons, which are a typical symbol of human PD patients [65]. Another limitation of such PD mice is the disease phenotypes essentially depends upon the promoters and hence may not reflect some of the spatial and temporal human PD pathologies distribution.

Many other PD animal models involve injection of synthetic or pathological α-synuclein extracts (from postmortem PD patient brain) into the mice brain [70, 71]. These injection-based models show the capability of α-synuclein to propogate from one cell to another and hence induce PD pathology symptoms. Another transgenic mouse model of PD is formed by inducing LRRK2 mutations, like G2019S or R1441C/G, in animal models [72, 73]. Such LRRK2 based models replicate a few symptoms of DA neurons anomalies, but they lack other PD symptoms like DA neurons degeneration [74]. Similarly, more PD mouse models are generated based on deletion of crucial genes participating in PD pathologies such as parkin, PINK1 which leads to DA neurons loss [75, 76]. DJ-1 KO mice also show some features of PD, including reduced locomotion and decreased evoked dopamine secretion. However, it has no changes in DA neuronal population in SNc regions and has no effect on overall DA content [77, 78]. We have tried to cover most important PD mouse models in our discussion, but there is more work going on to develop new PD mice models, which we may have not discussed here due to lack of space. We provide an overview of most commonly used PD mouse models in Table 2. Moreover, many of the above-discussed PD models have also found applications in understanding the clinical progression of PD which we have covered in our upcoming sections.

Spinocerebellar ataxia 1 (SCA1)

Spinocerebellar-ataxias are a family of disorders with a typical dominant pattern of inheritance, wherein the cerebellum is the commonly degenerated brain region, followed sometimes by the brain stem and other areas of CNS, but never in the peripheral nervous system [79]. Till now, more than 40 different types of autosomal dominant SCAs have been identified based on the gene involved [80]. In the majority of the SCAs, the primary mutated gene is one of the many

Table 2 Most common mouse models for Parkinson's disease

Model Name	Gene mutations	Pathological changes	References
Transgenic mouse models			
PDGFβ-hαSyn	Human α-syn Tg	Progressive accumulation of synuclein	[239]
Thy1-SNCA	Human α-syn Tg	Deposition of α-syn at synapses and neurons	[240]
Thy1-hSYN-A53T	Human α-syn with A53T mutation	Aggregates in brainstem, hippocampus and cerebellum in 6 months	[241]
$LRRK2^{R1441G}$	Point mutation in mouse LRRK2 gene	Slow movement in response to levodopa; low dopamine release	[242]
Knock-in and Knock-out mice			
LRRK2 G2019S	Two nucleotides (GGG to AGC) mutation in exon 41 of LRRK2 gene	Impaired dopamine release at 12 months; mitochondrial abnormalities in striatum	[243]
Parkin KO	Skipping exon 3 resulting in frame-shift and premature stop codon	Higher extracellular dopamine concentration	[244]
PINK1 Null mouse	Germ-line deletion of exons 4–7	No change in dopamine at 9 months; reduction in LTP is observed	[245]
MitoPark	Tfam1 conditional KO	Decreased locomotion in 12 months; tremors, twitching at 20 months	[246]
DJ1- KO	Exon 2 deletion	Reduced body weight at 1 year; nigral neurons were less sensitive	[77]
Neurotoxin-induced PD mice			
6-OHDA	-	Inhibition of respiratory complex I in mitochondria	[247]
MPTP	-	Complex I inhibition in mitochondria	[248]
Rotenone	-	Mitochondrial complex I inhibition	[56]
Paraquat	-	Glutathione and thioredoxin's redox cycling in altered	[249]

ataxin (ATXN) genes. For example, in SCA1, the gene that shows an abnormal functioning is ATXN1 [81]. The disease of SCA1 is characterized by the presence of CAG repeat expansion in the coding region of exon 8 and exon 9 [82]. Since CAG encodes for the glutamine, the disease of SCA1, due to the presence of several glutamine residues, is also categorized under polyglutamine disorder [81]. In healthy individuals, the number of CAG repeats are 6–35; however, sometimes, these repeats can also range from 36 to 44, which is due to CAT (which codes Histidine) interruptions in the CAG expansion [83]. It is generally observed that in mutant ATXN1 protein, the number of glutamine residues is > 39, with no CAT interruptions [84].

To better understand the pathology of SCA1, different types of mouse models are produced. The very first knock-in mouse for SCA1 was developed to drive the expression of ATXN1 protein with 82 glutamine amino acids and was called BO5 SCA1 [85, 86]. To make the expression of ATXN1 with 82 glutamine residues specific to Purkinje cells, Purkinje cell protein 2 (Pcp2) promoter was used. This Pcp2 promoter was under the control of tetracycline stimulation [87]. The conditional mouse model showed that the pathology of SCA1 is reversible if the expression of mutant ATXN1 is halted which allows cells to clear the load of aggregated ATXN1 protein. Another knock-in mouse model to replicate SCA1 pathology shows the production of a mutant ATXN1 protein with either 78Q or 154Q using the approach of knocking-in 78 or 154 polyglutamine expressing human CAG repeats under mouse SCA1 locus [88, 89]. Among the two lines, the 154Q expressing line resembled characteristics very similar to human SCA1 patients in terms of cognitive impairment [88]. The animal model of SCA1 is useful in characterizing biomarkers for predicting the progression of SCA1 in human patients. Table 3 provides a summarized description of most wellknown SCA1 models. All such information and a detailed discussion on how to use such knowledge in describing biomarkers is needed to get a good understanding of the diagnosis and progression of SCA1 pathology.

Table 3 Most common mouse models for SCA1 disease

Model Name	Gene mutations	Pathological changes	References
Knock-in mice			
BO5SCA1(or $SCA1^{82Q/2Q}$)	ATXN1mRNA with 82 CAG repeat inserted in SCA1 gene	Ataxia phenotype-3 month, loss of Purkinje cell, Bergman glia proliferation in cerebellum	[86]
Conditional $SCA1^{82Q/2Q}$	ATXN1mRNA with 82 CAG repeat inserted in SCA1 gene under Pcp2 promoter and tetracyclin stimulation	6 week-Purkinje cell pathology with pruning, cytoplasmic vacuoles 12 week-increased Purkinje cell pathology	[87]
$SCA1^{78Q/2Q}$	ATXN1 mRNA with 78 CAG repeat inserted in SCA1 gene	Weak histopathological brain changes at 18 months old mice compared to other knock in SCA1 mouse	[89]
$SCA1^{154Q/2Q}$	ATXN1 mRNA with 154 CAG repeat inserted in SCA1 gene	16 week mice had reduced brain weight but Purkinje cell loss was noted in 34 week mutant mice	[88]

Identification and characterization of potential biomarker candidates from rodent models of NDDs

Biomarkers are biomolecules that are used to detect or perform prognosis of a pathology. Identifying the disease at an early stage helps a clinician to intervene and prevent the advance symptoms. An ideal biomarker has several necessary features, including specificity, cost-effective, easy to isolate and should be highly sensitive for detection. Most importantly, the biomarker should be able to distinguish various neurological disorders from one another. Below we have explained how crucial animal models can be used to identify biomarkers in AD, PD, and SCA1 diseases.

Identifying AD biomarkers from AD mouse models

AD transgenic models have seen a significant clinical application for use in AD prognosis. One of the crucial clinical biomarkers identified for AD patients using the AD mouse model is Aβ and its different isoforms [90]. In the transgenic Tg2576 AD mice model, a reduced load of CSF and plasma Aβ42 was observed [91, 92]. Similarly in PDAPP, another transgenic AD mouse, an increased Aβ deposition is observed in the amyloid plaques [93]. The same Aβ42 from AD mouse is also validated as a biomarker for early detection and therapeutic interventions in AD patients indicating the application of AD mouse-derived biomarkers in AD patients [94, 95]. Also, in a different work, Aβ42 was established as a surrogate biomarker in the mice aqueous humor, and during this study, Aβ42 monomers were injected intracerebroventricular in a healthy and diseased (5XFAD) AD mouse and Aβ42 levels were measured in CSF, blood plasma and aqueous humor of the healthy controls and old-age 5XFAD mice. The study identified Aβ42 in the aqueous humor of the mice in both healthy and old 5XFAD mice; however, the concentration of Aβ42 in CSF decreases with age, while it increases in the eye [96]. The findings of the presence of Aβ42 in mice aqueous humor were also

corroborated with an observed increase in Aβ42 in AD patients' eyes hence demonstrating the importance of rodent models in identifying novel clinical biomarkers for AD [97].

Additional biomarkers, isoprostane, which is specific for oxidative stress and lipid peroxidation, is found to be elevated in Tg2576 mice of AD [98]. Later findings in humans indicate that isoprostane can be used as an AD biomarker where the level of isoprostane goes up with aging and latent AD [99]. Another study indicates that the phosphorylated tau is present and elevated with age in the CSF of Tg AD mice and hence can be a crucial AD biomarker [100]. The potential of p-tau as an AD biomarker is confirmed with a separate work where it is showed that in AD patients p-tau is increased with aging and can help in predicting disease progression [101]. In a separate report, the importance of insulin-like growth factor-1 (IGF-1) as a biomarker in AD pathology was established. Authors quantified IGF-1 in two AD mice models i.e., APPswe and PS1(dE9 i.e., without exon 9), and observed a decrease in the level of CSF/plasma ratio of IGF-1 in both. The study also corroborated its findings using AD patients and found similar results in AD patient samples compared to their age match control indicating the efficacy of the AD mice model in predicting the development of AD [102]. In a separate work, researchers used APP/PS1 transgenic mice to identify novel AD biomarkers where mice brain tissues were evaluated for the changes in protein expression. The study showed a total of 23 differentially expressed proteins in mutant mice by LC-MS/MS and 2D-gel electrophoresis. Out of 23 proteins, 11 were successfully validated using western blot and from 11, 5 proteins showed changes in AD patients' serum sample. Among these proteins, cathepsin D, cofilin-2, and VDAC-1 were increased, whereas Alix and ACAP1 decreased [103]. There are other studies also covering the clinical practicality of AD mice models to understand AD and we have not covered each one of them here but we acknowledge these studie's contribution to developing AD diagnosis and therapy.

Identifying PD biomarkers from PD mouse models

Different PD mouse models have shown application in developing biomarkers for PD patients. PD animal models established using neurotoxins like paraquat, 6-OHDA, MPTP, rotenone, amphetamine lead to the pathology of PD with symptoms, such as reduction in dopaminergic neuron numbers in the substania niagra, occurrence of tremors etc [104]. In one study, 6-ODHA treatment in rats caused an increase in the amount of 8-OHdG (a oxidative product), produced as DNA damage marker [105]. The same 8-OHdG is also found to be elevated in PD patients different body fluids i.e. serum, CSF, and urine showing the impact of using PD mouse model identified marker to predict disease prognosis in PD patients [106, 107]. Similarly, PD MPTP mice has elevated amount of oxidized DJ-1 in the erythrocytes and mice brain, and the same oxidized DJ-1 is proposed to be a good PD biomarker [108]. The increase in the DJ-1 level is recorded in the PD patients suggesting that the MPTP based model can serve as a useful tool to study biomarker in PD patients [109].

Genetic models of PD can also be used to investigate and characterize novel biomarkers and one such model is α-synuclein model, where the aggregation of α-synuclein is observed [110]. The genetic animal model of PD shows phenotypes very similar to the human PD patients [110]. For example, GSH, an antioxidant, is found to be significantly reduced in the substantia niagra of PD patients [111, 112]. The same finding is replicated in the *PARK2* null mice [113]. Another study indicated that in the PARK7 null mice astrocytes culture, when there is an overexpression of PARK7 gene product i.e. DJ-1, it rescues the cells from the proinflammatory responses [114]. This indicates the importance of using DJ-1 as a biomarker in PD patients and successively there are many reports which point to the reduction of DJ-1 in the PD patients biofluids i.e., CSF, blood, etc [115, 116]. Other protein like Ras-related protein 35 (Rab35) was found to be increased in the serum of PD patients, which is also observed in different PD models, like MPTP, rotenone, LRRK2 PD mice. The study showed that increased Rab35 level predicts PD progression and its level also changes with age [117]. Furthermore, Valosin-containing human protein (VCP), a protein with importance in protein homeostasis, was found to be decreased in the early symptomatic and presymptomatic MPTP PD mice models and the result of this experiment was also replicated in samples obtained from 52 PD patients [118]. More research can help in elucidating the role of genetic PD models in finding suitable biomarkers to study PD pathology.

Inflammation is a key pathological mechanism observed to be dysregulated in PD, and some inflammatory cytokines are observed to be highly upregulated in the pathology of PD. Few of those, like IL-1β and IL-6 were observed to be induced in the PD LRRK (p.R1441G) mice model [119]. Interestingly an increased level of both IL-1β and IL-6 are also found to be present in the PD patients [120, 121]. Similarly, PINK1 knockout mice have shown high levels of IL-1β, IL-10, and IL-12 in the striatum after treatment with LPS [122]. In one more work, TNF-α level was found to be upregulated in the PINK1 knockout mice [123]. Similarly, other work has found TNF-α to be induced in PD patients [124, 125]. More research can help in elucidating the role of genetic PD models in finding suitable biomarkers to study PD pathology. In addition to our literature, there are more findings on the use of animal models to find novel biomarkers for PD which we have not explained here due to space

limits but we recognize those studie's importance and their role in PD prognosis and therapy development.

Identifying SCA1 biomarker from SCA1 mouse models

Interestingly in the pathology of SCA1, using an animal model to develop a suitable biomarker candidate needs more studies and analysis. However, we have learned from other SCAs that mouse models are very helpful in predicting the prognosis and exploring suitable care for SCA1. In one such SCA i.e., SCA3 mouse model MJD84.2 there are transcriptional alterations in the level of different types of genes viz. *Tmc3*, TnSFS14, *Car2*, *Zfp488*, and *Chdh* in brain regions of cerebellum, cortex, brainstem, cortex, and striatum [126]. The changes in the transcriptional level of some of these genes were also verified in the SCA3 patients [127]. Since SCA3 and SCA1 share some genetic features and hence the changes at the transcriptional level of these genes can also be tested in SCA1 mice $SCA1^{154Q/2Q}$ and in SCA1 patients.

Other biomarkers, which is found to be succesfully validated in SCA mice, are neurofilament (Nf) proteins. Nf proteins are a family of proteins that can be categorized as neurofilament light (NfL), neurofilament medium (NfM), and neurofilament heavy (NfH) chain. These are cytoskeletal proteins in neurons and are found to be damaged and then released by neurons during neurodegeneration. Nfs can serve as an excellent biomarkers in different NDDs and is found to be useful biomarker in SCAs too. In one study done in SCA3, serum levels of NfL and phosphorylated NfH were found to be increased in two independent mutlicentric SCA3 patient cohorts and the results of the study was also replicated in SCA3 mice [128]. High NfL levels were present in patient even before disease i.e., in preataxic condition indicating the early-disease prognosis application of this biomarker [128]. The impact of NfL as a biomarker validated in SCA3 mice models is also validated in SCA1 patients where in one study, NfL levels were found to be high in SCA1 both in pre-disease and in disease conditions [129]. NfL is also used to differentiate between different neurodegenerative disorders like from mutliple system atrophy-cerebellar showing the effectiveness of biomarkers for clinicians to correctly diagnose disease [130]. Therefore, the study denotes that NfL can be a good biomarker for predicting the SCA1 progression, and identification and similar NfL biomarker based therapeutic intervention studies in SCA1 mice model can be done to discover a therapeutic cure for SCA1.

Another useful strategy involving SCA1 transgenic mice based biomarker identification involve quantifying proteins which are known to be direct interacting partners of ATXN1. One of such ATXN1 interacting protein is Capicua (Cic) [131]. ATXN1 forms a complex with protein Cic and affects its activity and stability. SCA1 null mouse has shown to have a reduce level of Cic and those genes which are repressed in presence of Cic are upregulated when Cic is homozgously deleted in $SCA1^{154Q/2Q}$ mice [131]. Another interesting protein to explore as biomarker in SCA1 patient can be vascular endothelial growth factor (VEGF) as its level on protein as well as at RNA is found to be decreased in the cerebellum of the $SCA1^{154Q/2Q}$ [132]. Similarly, one more biomarker of interest in SCA1 is glial fibrillary acidic protein (GFAP) as its level is increased in the $SCA1^{154Q/2Q}$ transgenic mice cerebellum, and in brainstem. GFAP can also be explored in SCA1 patients as a biomarker of interest [133].

Non-human primates in comprehending neurological pathologies

Non-human primates (NHP) hold an important position in identifying the mechanisms and progression of neurodegenerative diseases. NHPs are crucial clinical translational model of human diseases as they have preserved genomic sequence, highly developed cerebral cortex, good cognitive abilities, motor skills and similartiy to human anatomy and physiology [7]. Research in NHP is also eased by genome mapping performed in rhesus (*Macaca mulatta*) and common marmosets's (*Callithrix jacchus*) which was effectively achieved in 2007 and 2014 respectively and the same proved to be beneficial in identifying species similarities, or differences and improve NHP models to study NDDs [134, 135]. Here, we discuss the application of NHPs in NDDs.

Alzheimer's disease

NHPs cognitive skills and brain anatomy are well understood and the same has significantly helped in elucidating the neuroanatomical origin of AD development. Common methods to generate AD symptoms in monkey involves lesioning of cholinergic brain areas [136]. New NHP models which are dependent upon amyloid β and tau pathology are also in developmental phase. Like aged humans, aged monkeys can also develop phenotypes that mimc AD [137]. In general, monkeys can serve as excellent age-linked brain Aβ deposition model because of their phylogenetic closeness to humans as the monkey APP sequence is very similar to humans [138, 139]. These shared features allow us to assess amyloid burden in NHP model based on criteria for human study and hence permitting their application to model AD brain amyloidosis. Studies have also found that NHPs don't develop neurofibrillary tangles like human AD patients do but aged NHPs have shown tau hyperphosphorylation and

an intracerebroventricular administration of Aβ oligomers can induce neurofibrillary degeneration in NHP model mimicking AD development [140, 141].

Identifying biomarkers using NHP based model to characterize AD and hence subsequently identify therapeutic interventions in AD has good promise. Few studies have observed age-related changes similar to AD patients like a decrease in memory, amyloid plaque deposition, atrophy and loss of cholinergic and monoaminergic neurons in NHPs [142]. While aging is not AD, but aged animals provide a great platform to comprehend aging risk factor in AD development. These studies show that deviations in the subcellular localization of many of these proteins is species-dependent, and not brain region-dependent and larger animal models can closely mimic the disease symptoms. Hence, studying the NHP model in AD can give a good advantage over other small animal model of AD. However, due to different limitations, such as maintaining NHP, special expertise necessity, prerequisite of specific breeding, high funding requirements, use of NHP models are highly restrictive in AD and other NDDs.

Parkinson's disease

The application of using NHP in PD study dates back to 1983 when for the first time, PD was discovered in the humans. Burns et al. for the very first time used MPTP and administered it intravenously into the monkeys and observed that this led to postural defects, eyelid instability, rigidity, and also different pathological symptoms which resemble PD [143]. The same PD-based symptoms can be alleviated using L-Dopa based therapy in the PD monkey models [143]. The use of MPTP lead to the development of an ideal NHP PD model e.g., in marmoset, macquae monkeys which have all the clinical features usually present in the idiopathic PD patients [144, 145]. Dopaminergic neuron numbers reduction in the substantia niagra pars compacta can be succefully replicated in these MPTP-injected animal model and this model also has several non-motor symptoms of PD like cognitive loss, gastrointestinal defects and sleep/wake problem [146]. Another neurotoxin used for generation of NHP PD model is 6-OHDA as this toxin stimulates cell death *via* oxidative stress and after the injection, 6-OHDA is transported by the catecholamine system of DA and nor-epinephrine. As an example of the 6-OHDA model, in one study, 9 injections of 6-OHDA in common marmoset monkey striatum led to PD symptoms development [147]. In a different work, same model was generated following 9 injections of 6-OHDA, which was sucessfully treated for PD symptoms using 10 weeks of cell therapy but the model was later shunned and a new model with 18 unilateral injections of 6-OHDA was used [148]. The new model of 18 injections recapitulates several of PD symptoms like nigrostriatal lesion which was found to retrograde and progressive and hence can be used to screen different drugs against PD [148].

Similar to neurotoxins, there are other NHP based models developed for PD like with the help of gene transfection. In one work, a wild-type α-synuclein gene was inserted in the brain substantia niagra region of mormoset monkey with the help of a r-AVV1 vector, and the euthanized monkey after 16-weeks of injection showed a upregulation in the expression of α-synuclein, have dinsintegrated neurites, decreased tyrosine hydroxylase amount, all features indicating PD [149]. However, a later study also showed that a injection of α-synuclein gene with A53T mutations in marmoset monkey has worst effects of PD symptoms [150]. Age-based NHP model of monkeys similar to human patients also shows degeneration of the nigrostriatal system, striatal dopamine decrease [151], dopamine transmission loss [152], and reduced number of tyroxine hydoxylase and dopamine transporter containing nigral neurons [153]. Interestingly, in PD, CRISPR/Cas9 mediated disruption of PINK1 and Parkin in monkeys showed degenerative phenotypes, while they did not make significant differences in transgenic pigs. In one study, in PD, CRISPR/Cas9 mediated disruption of PINK1 and Parkin in monkeys showed degenerative phenotypes, while they did not make significant differences in transgenic pigs [154]. In another work, neuronal cell loss was observed when AAV mediated co-disruption of DJ-1 and exon-3 of PINK1 was done in cynomolgus monkeys [155–157]. Similarly, lentivirus-mediated mutant α-syn (A53T) expressing transgenic rhesus monkeys exhibited age-dependent motor loss and cognitive deficits [158].

NHP model with PD symptoms has also found its application in development of biomarker for PD study. In one work, MPTP-treated monkey serum was used to find out the metabolic changes associated with PD. The authors compared the serum metabolic profile of the MPTP-treated NHP with other animal PD models and also with PD patient's biobank data available from NIH and from an Italian based study. Authors found interesting metabolite similarity (such as decreased betaine and valine) between NHP model, other PD animal model and also PD patients [159]. Further study in another MPTP PD model, analysis of the putamen of the animal using proteomics-based technique has identified different glyco and phosphoproteins which are observed to be dysregulated in the pathology of PD. Some of these glycoproteins are ubiquitin carboxyl-terminal hydrolase isozyme L1, neurofascin, GFAP, gamma-synuclein, contactin-1 and few of these phosphoproteins were ankyrin-2, syntaxin-1 A were observed to be dysregulated both in NHP putamen and also in PD patients [160]. All of these above-described

studies show the broad impact of using the NHP model to predict and explore therapeutic cures for PD pathology but due to restrictions in modeling many of the genetic changes of PD patients in NHPs and also other limitations as mentioned earlier for AD, use of NHP model for PD is difficult.

Spinocerebellar ataxia 1

Generating a transgenic NHP model is difficult for SCAs due to the complexity of delivery of the transgene, maintaining expression of the transgene, taking care of NHP colony after transgene transduction and mimicking all SCA symptoms. One type of SCA that has seen effective generation of transgenetic SCA NHP model is SCA3 where a transgenic marmoset was generated by using a lentiviral based induction of human ataxin 3 gene containing 120 CAG repeats [161]. The NHP model for SCA1 doesn't exist but NHP in SCA1 has been used to analyze the effectiveness of the SCA1 gene silencing method. In one case, 9 adult male and 3 adult female rhesus monkeys were transduced with SCA1 miRNA carrying rAAV1.miS1eGFP in the deep cerebellar nuclei causing a loss of SCA1 expression [162]. The explained NHP SCA1 expression silencing study was performed to confirm earlier pre-clinical mouse results where the authors achieved SCA1 gene silencing in deep cerebellar nuclei [163, 164]. Thus, such NHP models can confirm previous pre-clinical findings of lower animals and due to a higher similarity to humans, NHPs can be tested for finding cures for human SCA1 patients.

In Fig. 2, we have explained how animal models are developed succesively for all these different NDDs that we have explained in our manuscript. Interstingly, we have observed that the major of biomarker-based research are performed on rodents since they are easy to maintain, breed, and manipulate, and are not difficult to analyze. However, the sucess for translation to human patients needs closer analysis. In the next section of our review, we have explored facets of using non-mammalian models as a valuable resource to understand the possible pathomechanism of these NDDs.

Non-mammalians models in NDDs

Non-mammalian models, especially flies, fish and worms provide several advantages in studying NDDs. and one such benefit is that these animals have shorter life cycles which reduces the time to obtain transgene-expressing progeny,

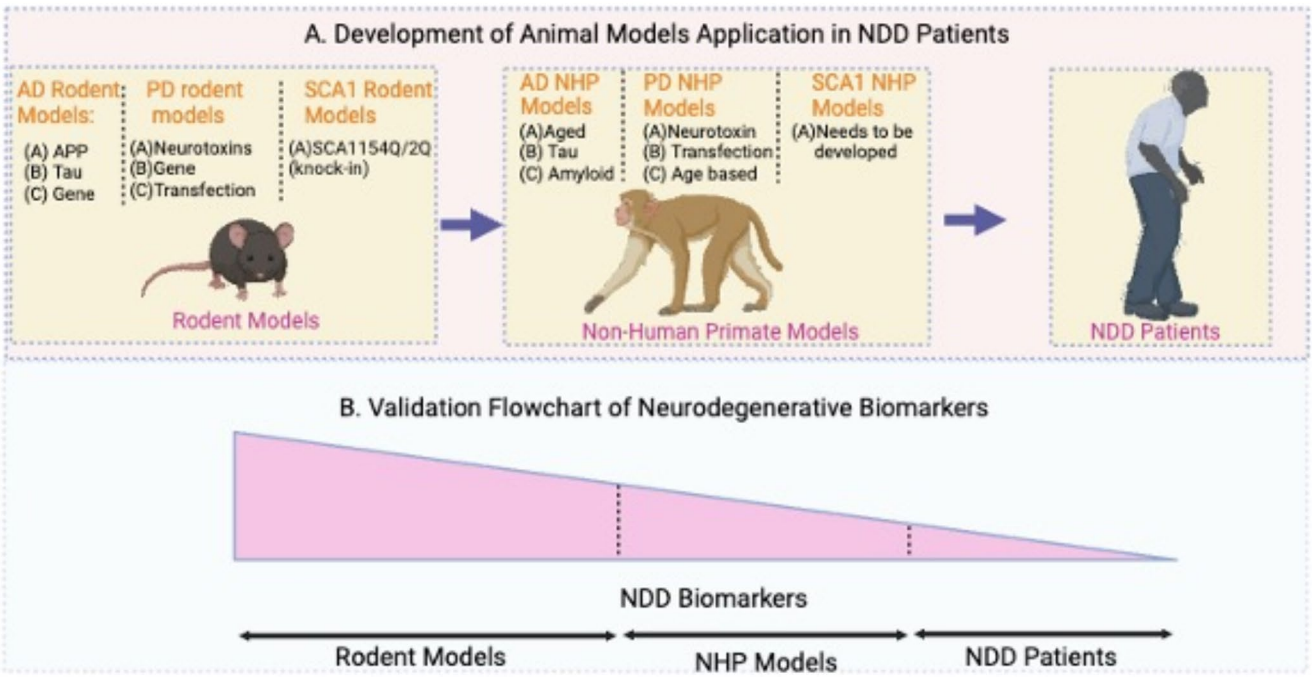


Fig. 2 Translational application of mammalian models to study human neurodegenerative disorders: (A) Commonly used rodent models for NDD studies are generated using different approaches such as transgenetic manipulations, gene delivery or by using neurotoxins. Rodent models derived findings or discoveries for NDDs can be validated using higher or anatomicaly human comparable non-human primate animals. Due to a very high physiological and genomic similarity between NHPs and human NDD patients, several of experimental results derived from NHPs can also be tested in NDD patients. (B) Decades of work have contributed in finding novel biomarkers from animal models to be validated succesively in human NDD patients. Many of these results suggests that the rodent are a rich source of biomarker for later validation in NHP and in human NDD patients.

which allows the researchers to screen multiple NDD- susceptible genes in a very short duration [165]. In the text below, we have assembled and investigated the contribution made by different non-mammalian animal models in NDDs of AD, PD, and SCA1.

Non-mammalian models in AD

In AD pathology, non-mammalian models, like *Drosophila*, zebrafish and *Caenorhabditis elegans* contain AD genes, that are homologous for APP, MAPT, BACE1 and PSEN1 [166]. However, these models lack various regions in these genes that can cause AD pathology. Hence, these non-mammalian models, may require external sources for the expression of AD associated proteins [166]. For example, in *Saccharomyces cervisiae*, which doesn't contain tau ortholog, human tau 4R overexpression causes its aggregation and hyperphosphorylation [167]. However, modeling AD in non-mammalian models using orthologous genes is limited due to the inherent genetic dissimilarity of genome between non-mammalian animals and humans.

A well-known non-mammalian model to mimic AD is zebrafish. AD non-mammalian model of zebrafish has different patients AD gene analogs e.g. for MAPT [168], PSEN1 [169], PSEN2 [170], APP [171], APOE [172], and BACE2 [173]. Exposure of zebrafish to AD- inducing drug, like scopolamine can also lead to phenotypes which resemble AD, for examplecognitive impairment and memory dysfunctions [174]. Another well-developed and understood non-mammalian AD model is *Drosophila*. The overexpression of mutant tau in *Drosophila* is linked with a loss in functions of sensory axons, and retinal cell degeneration and leads to neurofibrillary pathology in *Drosophila* [175]. Similar tau overexpression phenotypes from *Drosophila* are also observed in *C. elegans*, with overexpression of wild-type and mutant tau protein causing disruptions in locomotion, cholinergic transmission by neuron, and progressive ageing [176]. The non-mammalian models in AD are found to have crucial applications in understanding disease pathology or mechanism of AD progression.

The similarity of many such AD phenotypes such as formation of tau tangles, toxic Aβ accumulation, loss of cholinergic/glutamergic neurons, etc., between AD patients and non-mammalian AD animal models can be taken as advantage for the development of biomarkers for detection and prognosis of AD. For example, in one study, GSK3β which is found to be hyperactive in the patients of AD was also observed to be induced in the zebrafish Aβ injected mice model [177, 178]. Additionally, in a proteomic study on in *Drosophila*, expression of Aβ42 peptides in *Drosophila* showed alterations in >500 proteins where >200 proteins were found to be crucial for AD pathology and hence providing a reference for exploring novel therapeutic targets for AD in the future [179].

Non-mammalian models in PD

In Parkinson, non-mammalian animals, like *C. elegans* is found to be very useful because they show an increased sensitivity towards toxins such as MPTP [180]. Multiple valuable PD mimicking non-mammalian models have been produced e.g. leech, flatworm (a planarian), *C. elegans*, zebrafish and *Drosophila* [181–184]. These models are produced either by using specific neurotoxins, like MPTP, 6-OHDA or by changing the genetic make-up of these animals by manipulating genes like LRRK2, Parkin, PINK1, etc [181, 182, 184]. In neurotoxin-induced PD non-mammalian animal model, one such example is *Hirudo medicinalis* (a leech) where after treatment with MPTP, the animal has shown PD symptoms like a reduction in the dopaminergic neuron numbers and dysfunction of motor neurons [181]. Similarly in a different work, a planarian flatworm i.e., *Dugesia japonica*, was developed as an MPTP model and it was used for investigating the efficacy of the anti-parkinsonian drug talipexole [185]. The zebrafish PD model is also produced by using toxins MPTP [186], 6-OHDA [187], rotenone [186], and paraquat [188]. Such toxins-developed PD non-mammalian animals shows phenotypes, like dopaminergic neurons decline in substantia niagra, decrease in dopamine, reduced tyrosine hydroxylase expression, swimming or locomotion defects, increased apoptosis and reactive oxygen species, etc.

As mentioned earlier, other than neuro-toxin generated PD animals, different transgenic PD non-mammalian animals like *Drosophila* with transgenes e.g. DJ-1, Parkin, PINK1, and LRKK2 are generated to replicate the PD symptoms, such as dopoamingeric neurons loss, motor functions dysfunctions [184]. The genetically modified models like LRRK2 of *Drosophila* and *C. elegans* has also been used in testing LRRK2 kinase inhibitor GW5074 and sorafenib to rescue the dopaminergic neurons in PD pathology [189]. Additionally, zebrafish PD models that have mutations or loss of functions in PD-associated genes like LRRK2, alpha-synuclein, DJ-1 [190], PINK-1 [191], and PARKIN [192] are also being created.

Non-mammalian models in SCA1

Non-mammalian models have also been developed for SCA1 pathology. SCA1 *Drosophila* model has shown that overexpression of ataxin-1 mutant protein containing 82 polyglutamine repeats in the photoreceptor axons leads to ommatidia disorganization and bristle loss in the interommatidia [193]. Further, these flies also show

eye phenotype, like retina shortening, loss of tissue, and abnormality in the photoreceptors neurons [193]. The study indicates how in *Drosophila* SCA1^{82Q} mutant, various genes related to mechanisms of protein folding, protein degradation, RNA metabolism, nuclear transport, oxidative stress, heat shock response are affected [193]. One study linked the importance of DNA repair gene RpA1 in the SCA1 *Drosophila* model [194]. The finding showed that the RpA1 gene is present at the hub position in the DNA repair gene network and a wildtype ataxin-1 protein interacted with RpA1 and its co-partner BRCA1/2. However, the same interaction was disturbed when the mutant ataxin-1 protein is expressed in the *Drosophila* [194]. In one study, a transgenic zebrafish SCA1 model carrying human ataxin-1 protein with 82 polyglutamine (82Q) sequence was developed. The model revealed an age-dependent purkinje cell degeneration, which later progresses in the juvenile and adult stage of the zebrafish [195]. The non-mammalian model of nematode *C. elegans* also proves to be an excellent source to study SCAs. Separate reports are available for *C. elegans* role in various types of SCAs, but no such finding is available to investigate the pathology of SCA1.

Various available reports on non-mammalian models to study NDDs indicate that most of the findings though useful to characterize such diseases in human patients, but are very restrictive in nature. Many of these non-mammalian models doesn't represent very high similarity in terms of gene sequence to human SCAs proteins; hence most of the pathways which are disturbed in these non-mammalian models not always correspond to the same in higher animals. Additionally, the non-mammalian models doesn't inherently express the NDD responsible mutant proteins, and the same needs to be expressed ectopically, making it difficult to replicate all phenotypes observed in higher mammals from the endogenously affected mutant proteins. Figure 3 shows these different observations on the application of non-mammalian model in NDD research.

Limitations, latest development and suggested improvements in NDD modeling

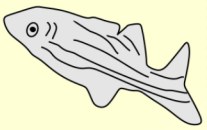
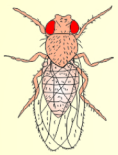
The animal models both mammalian and non-mammalian for different NDDs serve as excellent tool to look at the disease pathology, and possible therapeutic strategies available. These models are used to predict disease progression and help in early disease identification by the help of biomarkers. A successful animal model in human NDD should have many similarities to the disease pathomechanism, and behavioural changes. Here in our review, we have provided a close account of mammalian and non-mammalian animal models of three common NDDs. From our analysis, we observed that mice and primate NDD models are most succesfull in understanding the disease pathology of different human NDDs and furthermore, we also noted that non-mammalian animal models can help in exploring NDD pathomechanism but their results need to be confirmed in higher animals and humans.

Despite the advances in creating animal models of neuronal disorders, challenges remain. One significant challenge that always stands true is the ethical concern regarding the use of animals in research. Ethical guidelines such as the "3Rs" (Replacement, Reduction, Refinement) have been developed to address these issues, promoting methods that minimalize animal suffering and consider alternatives to using animals whenever possible [196]. Moreover, researchers must also be cautious in extrapolating results from animal models to human conditions due to inherent genetic and anatomical differences between species [1]. For example, the substantial differences in brain development and anatomy between rodents and higher mammals underscore the necessity of utilizing large animal models for the study of neurodegenerative diseases in patients. One example of these differences is the absence of gyrification in rodent brains compared to large mammals [197].

As mentioned earlier, inherent genetic differences between animal models and human NDD patients also make it difficult to recapitulate NDD phenotypes in animals. A close analysis of different animal models suggests that C. elegans (40%), fruit fly (75%), zebrafish (70%), non-human primates (93%), and rodents (80–90%) has the indicated percentage similarity to human brains [198]. This suggests that different animal models genome are dissimilar to each other and to human NDD patients that makes replication of human pathology difficult. For example, the APOE ε4 allele, an allele of the APOE gene (an AD-causing gene) found in human AD patients, is absent in most AD animal models [199].

In PD patients, an important gene DJ-1 lack-of-function is found to be crucial for disease pathogenesis and results in loss of the nigrostriatal system, but a DJ-1 null mutant mice fails to show such pathogenesis [77, 200]. This indicates that possibly DJ-1 has a more critical role in human PD patients and due to genetic differences between human and animal models, this gene cannot be reliably used in animals for PD modeling. Nevertheless, such inherent genetic differences between animal species and human NDD patients can be solved with alternative disease modeling approaches or by generating transgenic animals.

Inherent genetic dissimilarity between animal models and human NDD patients can be tackled by producing transgenic animals with modern genetic approaches.

Non-Mammals	Involved in Neurodegenerative Diseases	Orthologous Proteins
 Danio rerio	• D.rerio shows AD symptoms like cereberovascular defects, angiogenic sprouting and plaque formation. • MPTP treatment in D.rerio PD models has lead to PD symptoms loss of dopaminergic neurons, decrease of dopamine, norepinephrine. • SCA1 and SCA3 orthologs exist in D.rerio with SCA3 model shows severity in swimming and a reduced survival.	•PSEN1 •PSEN2 •APPA •APPB •Th1 •Th2 •Atxn1a/1b
 Drosophila melanogaster	• D.melanogaster shares common genes responsible for causing AD • The MPTP toxins are used for producing PD specific D.melanogaster models. The model showed susceptibility in homologous genes responsible for causing PD. • In polyglutamine diseases like SCA1 and SCA3, mutants of disease protein leads to neuronal damage, ommatidia disorganization in D.melanogaster.	•dAPP1 •GSK3β •dLRRK •Appl •Datx2
 Caenorhabditis elegans	• C.elegans AD model has shown disruption in movement, ageing and neuronal signal transmission loss after mutant tau expression. • Transgenic PD worms with loss in functions of important PD genes have shown dopamine neurons loss, stress sensitivity. • Transgenic PD worms with loss in functions of important PD genes have shown dopamine neurons loss, stress sensitivity, age-related aggregation.	•APL-1 •SEL-12 •DAF-16 •lrk-1 •pdr-1 •catp-6 •ATX-1

Non-mammals as Biomarker Source in Neurodegenerative Diseases

Benefits	Limitations
•Short life-cycle in non-mammals makes monitoring of changes in biomarkers easy. •Due to less complex nervous system of non-mammals, effect of different therapies on biomarkers levels in nervous system can be observed. •Some biomarkers such as related with neuronal development, neuronal differentiation are easier to study in non-mammals .	•Due to complexity of brain in mammalian system, the biomarker changes of mammalian brain are not easy to observe in lower mammal. •Correlation between phenotype and biomarker levels are of non-mammals are not same as mammals. • Several genes responsible for NDD in higher mammals are not present in lower mammals.

Fig. 3 Different non-mammals are also excellent source to investigate the progression of neurodegenerative disorders. Few of such non-mammals which have found good use in NDDs are Zebrafish (*Danio rerio*), Fruit fly (*Drosophila melanogaster*), Round worms (*Caenorhabditis elegans*). Such non-mammals may not have same endogenous genetic make-up to show the pathology of human neurological complications but most of the time they have orthologous protiens similar to human NDDs. Hence, such non-mammals can be used to explore human NDDs by observing NDDs based phenotype in them either by ecotopic expression of pathological genes or by inducing mutations in the orthologous genes present in their genome.

CRISPR-Cas9 is a great genetic engineering tool that can be used to produce a transgenic animal model, which will have similar genetic ablations as present in NDD patients. Humanized AD mice which carries the point mutations (M139T) of the human APP gene is a great example of the use of CRISPR-Cas9 application to produce animal models with close resemblance to human NDD conditions [201]. Another AD mice model which has a deletion of exon1 of MAPT gene and results in tau-based AD pathology is also produced by using CRISPR-Cas9 deletion [202]. Several other NDD animal models are generated using the CRISPR-Cas9 method. Although discussing every model here would be out of the scope of this review. Generating animal models using CRISPR-Cas9 is associated with different challenges, like cost, availability, and long time to generate models.

Also, there are issues like mosaicism in offspring generated with CRISPR-Cas9 poses additional challenges.

To further address concerns of animal models in NDDs, alternative disease modeling approaches like NDD patients-derived human induced pluripotent stem cells (hiPSC) cultures, 3D cell-based cultures (e.g., brain organoids), microfluidic systems (e.g., organ on chip), are also considered [203–205]. Some of these systems like hiPSC and brain organoids are successful in answering the disease mechanistic questions and in developing therapies (e.g., stem cell-based therapies). iPSC was initially developed using patients-derived cells and hence was found to be genetically more closer to human NDD patients [203]. But the cells in hiPSCs are usually cultured in 2D layer and have multiple limitations to better understand disease mechanisms, like cell to cell interactions present in the in vivo conditions.

More sophisticated iPSC-derived modeling methods like 3D cell culture with the help of extracellular matrix scaffolds (e.g. hydrogels) were produced [206]. Such 3D culture systems are commonly called organoids (or spheroids) e.g., brain organoids. These 3D systems are becoming valuable in understanding the pathomechanisms of NDDs, cell-cell interactions, exploring drug testing in NDD, and identifying novel drugs [207, 208]. All these alternative models for NDDs are reducing the usage of animals in NDD studies as they offer advantages like genetic closeness to patients, no need for large breeding space, non- maintenance of animal colonies etc. Moreover, stem cell techniques and 3D cultures also have limitations. Both methods are expensive due to the use of high-end chemicals, needing special isogenic controls, and purchase of differentiation inducing small molecules [209]. In addition, both iPSC and 3D cultures show tendency for genetic instability, tumorigenicity, and immunogenicity making them difficult to use in complex human NDDs [210]. Furthermore, iPSC and 3D cell cultures are found to have a large variability in drug response from batch-to-batch in drug screening for NDDs, which makes it difficult for scientists to consider their results in different drug discovery projects [211]. Additionally, throughout NDD research, scientists have observed the constant interaction of the brain with other organs (such as the gut) which become crucial for the pathogenesis and hence treatment of different NDDs [212]. In these alternative models, imitating such interactions is difficult, while the same can be studied in animal models of NDDs making them a good option for NDD study.

The application of alternative disease modeling approach in understanding the pathogenesis of NDDs and its usage in drug development and drug discovery is also emphasized in a recent FDA Modernization Act 2.0 and 3.0 [213, 214]. The act states that to reduce the animal numbers in drug testing, sponsors (or pharma industries) can use alternative approaches like hiPSCs, organoids and organs on chips based methods [215]. However, FDA has not yet released guidelines on how to integrate such alternative models with preclinical and clinical trials and hence making it difficult for pharma industries to completely implement such models in their drug development programs [216, 217]. Additionally to completely replace the in vivo models in drug development in NDDs with alternative approach is debatable due to lack of sufficient evidence on alternative models on comprehending drug-organ interactions, drug toxicity, drug dosing, drug pharmacokinetics etc [218]. Hence a good pre-clinical approach in drug development for NDDs may include the use of both animal and alternative models and with sufficient evidence the alternative models may gradually replace the animal models. Overall, a better model to investigate the different types of NDDs depends upon the questions that researchers want to answer.

An interesting application of animal models is their suitability to test the therapeutic efficacy of different drugs and then successively confirming the findings in humans. In this context, some rodent transgenic AD animal models e.g. PDAPP, APP23 were found to have shown positive effects against anti-Abeta antibody with improved cognitive phenotypes and reduction in Aβ accumulation. A similar effect was recapitulated in AD patients in clinical trials indicating the application of animal models [219–221]. Other studies also suggest that acetylcholinesterase inhibitors (e.g. Donepezil, rivastigmine, etc.) mediated improvements in AD mice (e.g. Tg2576, APP23) are recapitulated in AD subjects [222, 223]. Similarly, a different compound i.e. scyllo-Inositol shows therapeutic benefits e.g. increased cognitive and decreased Aβ42 deposition in multiple AD animal models (TgCRND8, PS1XAPP, 5XFAD) [39, 224]. The compound was later tested in clinical trials for AD patients and indicated a safe toxicity profile; however the efficacy of the compound was questioned in later trials [225].

Drug development using animal models has also seen progress in PD. The A53T α-synuclein PD mouse model was used for testing the efficacy of resveratrol against PD. The study concluded that resveratrol can reduce the accumulation and aggregation of α-synuclein and hence lower the resulting neuroinflammation and oxidative stress due to α-synuclein, although the same needs to be tested in PD patients [226]. In another study, Levodopa (L-DOPA) efficacy was tested in a PD A53T mice model and the study demonstrated the beneficial effects of L-DOPA [227]. Notably, L-DOPA is an FDA-approved drug for PD suggesting that the beneficial effects of PD therapies can be successfully recapitulated in both PD patients and animal models [228].

Drug responses observed in NDD animal models are not always successfully repeated in human NDD patients. There

are many reasons for non-translation of such therapies from animal models to humans such as biased observation, a smaller number of animals, no repetition of the experiment, and high toxicity of many drug candidates in NDD subjects. One particular study has reported that in >4400 datasets which analyzed 160 meta-data (for different treatment conditions) from stroke to AD concluded that there are too many animal studies with very high statically significant findings (>50%) than expected (<25%) [229]. The report suggests that these animal studies suffer from the problem of too small sample size, poor blinding, and urgency to publish only positive results [229]. Moreover, it suggests that conducting animal modeling-based studies for different NDD conditions needs an observer non-bias approach, more repetitions, a higher number of animals, and rigorous monitoring of the experiments.

Animal models in NDDs are a crucial tool to better understand the progression and pathogenesis of complex neurological diseases. Identifying an ideal animal model to be used in a specific study depends upon many factors including animal handling facility, equipment's, availability of expertise, funding, translational application of work etc. Choosing a suitable animal model to mimic the NDD conditions needs various considerations before planning and execution of the proposed work.

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