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Review

Animal models for Alzheimer's disease: a focused review of transgenic rodent models and behavioral assessment methods

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Abstract

With the increased morbidity and unclear etiology of Alzheimer's disease (AD), there is an urgent need to put more effort to investigate the causes of the disease and develop novel drug to stop or reverse the disease progression. Transgenic rodent models mimicking different types of AD-like pathologies are essential resources to discover potential drug targets and study the mechanisms of drug actions. The common symptom of AD is the cognitive deficits. The ultimate readout for any interventions should be evaluated by the test of learning and memory. Although a multiply number of rodent models and behavioural assessment methods have been widely utilized in mechanism studies and screening of novel drug candidates, large variability still exists in the methodologies, especially in terms of how the rodent models are being utilized. To select suitable and valid models for supporting AD research, it is important to understand the characteristics and applicability of the rodent models and behavioural assessment methods. This review seeks to summarize and discuss the pathological feature of some transgenic rodent models that are commonly used in AD research (e.g. APP, PS1/2 and tau gene mutations). Moreover, the characteristics and applicability of some behavioural assessment methods (e.g. Morris water maze and radial arm water maze) will be summarized. Finally, we will discuss the applicability of these models and methods in AD research.

Keywords

Neurodegeneration; cognitive impairment; transgenic mice; behavioural alteration; Morris water maze; Radial arm water maze.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative dysfunction with the most common early symptom as memory loss. Two histopathological hallmarks of AD are usually observed, which include: (1) extracellular amyloid plaques primary comprised 39 to 42 amino acids peptides or polymers that produced by proteolytic cleavage of amyloid precursor protein (APP) with the beta and gamma secretases, and (2) intracellular neurofibrillary tangles (NFTs) comprised of hyperphosphorylated tau protein aggregated in oligomeric structures. The presence and/or accumulation of these misfolded proteins are thought to be the reasons for neuronal apoptosis and synaptic deficits, which eventually lead to systemic cognitive impairments [1].

The incidence of AD exhibits a continuous growth in recent decades, partly due to the aging population

as the incidence may increases as the percentage of people older than 65 increases [2]. It was predicted that there will be 13.2 million people suffer from this neurodegenerative dysfunction by 2050 [3]. Accordingly, the worldwide societal cost of dementia especially AD was estimated to break through $422 billion in 2009 [4]. As incidence and cost are forecasted to rise at an alarming speed in the next decades, intensive and meticulous research on AD are urgently needed.

Despite decades of pathological studies on AD, the etiology and mechanism of AD are still unknown, and many basic questions also remain unanswered. Continuing research into the underlying mechanism of AD as well as renewed effort in seeking for disease-modifying drugs are essential to address this problem. Rodents have been extensively applied in AD research on account of the relatively high similarity in physical structure and cognitive system, as well as the availability and relatively low cost in comparison with primate system. Drugs targeting aspects related to the pathophysiological mechanisms and disease-modifying therapies are usually tested in rodent models before being advanced to clinical trials in humans. Indeed, the most striking characteristic of AD is the cognitive impairment. As a consequence, the crucial aim for therapy is to prevent and/or ameliorate the cognitive dysfunction. In particular, the cognitive assays provide the advantage of targeting cognitive functions without the requirement of pathological hypothesis. So far, a multiply number of rodent models and behavioural assessment methods have been widely utilized in mechanistic studies and screening of novel drug candidates. However, large variability still exists in the methodologies and how the rodent models are utilized. Hence, it is necessary to understand the characteristics and applicability of the rodent models and behavioural assessment methods for choosing suitable and valid models.

This review summarized the pathological characteristics and the applicability of transgenic rodent models with APP, presenilin 1/2 (PS1/2) and tau mutations mimicking the familial AD (FAD) models. Given the close relationship between rodent models and behavioural assessment methods, this review discussed the features, influential factors and applicability of two classical behavioural assessment methods including Morris water maze (MWM) and radial arm water maze (RAWM) which may be used to study the learning and spatial working memory as well as to assess damage on cerebral cortex and hippocampus. Since hippocampus is closely related to cognitive function where AD is thought to initiate and develop, the hippocampus-dependent cognitive tests are ideally-suited for AD search to maximize the possibility of selecting a target or drug that is relevant to cognitive function *in vivo*.

In brief, this review will summarize some transgenic rodent models and behavioural assessment methods that are commonly used in AD research. Then we will discuss the applicability of these rodent models and behavioural assessment methods in AD research.

2. Alzheimer's disease pathology and the relevant transgenic mice

The pathology of AD still remains unclear so far, although there are several hypotheses about AD's pathology. Among these hypotheses, Aβ hypothesis and tau hypothesis appeared to be widely accepted [5]. Amyloid plaques and NFTs are the two striking hallmarks of AD. Aβ and tau protein are the primary constituents of amyloid plaques and NFTs, respectively. More importantly, mutations in APP result in AD with 100 % penetration, and FAD-related mutations of APP bring about an enhancive formation or aggregation of Aβ [6,7]. Alternatively, the illumination of mutated tau protein in FTDP-17 definitively proved that the dysregulation of tau can bring about neurodegeneration and eventually leading to AD [8]. Evidence of the abnormal aggregation of the Aβ peptides and highly phosphorylated tau protein exemplified a crucial pathogenic characteristic of this disease [9,10]. All these findings support the Aβ

hypothesis and tau hypothesis.

Given the importance of having a validated animal model for drug discovery and mechanism research, a multiple number of mutations in the genes of APP, PS1/2 and tau had been discovered in familial AD (FAD) accordingly. Although more than 95 % AD cases belong to late-onset AD (LOAD), and FAD only account for less than 5 % of the total AD cases. There exists very high phenotypic similarity between FAD and LOAD, suggesting that the knowledge about mechanism obtained from FAD will also be directly relevant for LOAD. Therefore, deeper insight into the investigation about AD using transgenic rodent models is critically important. Original transgenic mouse strain were developed to study AD carrying familial mutations of APP [11,12], while subsequent models were relied on PS mutations [13], tau mutations [14], or a combination of 2 or 3 mutations [15].

2.1. Aβ hypothesis

In AD's process, amyloid plaques are the striking hallmark presenting in the autopsy of AD patient brain tissues. In many rodent models including transgenic and non-transgenic rodents, high levels of Aβ peptides (> nanomolar) can dramatically weaken neuronal physiology and synaptic density, which were also distinctive feature of AD [12]. More importantly, excess generation of Aβ peptides induced cognitive dysregulation impairing the animals' performance in learning and memory tasks [16,17]. These findings supported the importance of Aβ hypothesis in AD pathology.

2.1.1 Transgenic rodent models with APP or/and PS mutations

Serving as the fundamental principles for many genetically modified AD rodent models as well as responsible for the Aβ hypothesis, a large amount of rodent models with APP and PS1/2 gene mutations had been developed. All of these transgenic models were manifested to increase the ratio of Aβ 42 to Aβ 40 and enhance the Aβ aggregation that resulted in higher levels of amyloid plaques [18]. So far, more than 30 APP mutations and nearly 200 PS1/2 mutations had been authenticated and linked with AD [19]. As shown in Table 1, mice from the strains with APP gene mutations all display amyloid plaques at 2-9 months old, and the cognitive impairments are observed at 2-6 months old. Unfortunately, NFTs are not detected in these mice. It is worth noting that the strains with PS gene mutation lack of most of the AD-like phenotype except synaptic deficits.

Since APP and PS1/2 mutations are important in the development of AD, combining APP and PS mutations lead to an accelerated AD-like phenotype. Prominent amyloid plaques depositions are observed as early as at 2-3 months along with a striking increased production of Aβ 42 levels at 1-2 months old in these mice, which is developed before cognitive impairment appearing at 6 months old.

2.1.2 Application in Alzheimer's disease

These transgenic rodent models with APP and PS mutations have been utilized in mechanism investigation and drug candidate discovery successfully. Some therapeutic vaccine and antibodies had been developed using the PDAPP mice model [29-31]. The Tg2576 mice were used to investigate the role of reactive oxygen species on the cerebral amyloid angiopathy, which was an important cause of cognitive dysfunction in elderly patients with and without AD [32]. Surprisingly, some anti-ulcer and anti-tumor drugs were found to be effective to ameliorate the amyloid-like pathology in APP23 mice. Since the safeties of these drugs have been confirmed in human, these drugs can be considered as candidates for the prevention of AD [33, 34]. In addition, some transgenic mice models were used to explore the novel pathway or therapeutic target for the treatment of AD pathogenesis. For example, an extracellular matrix

protein called reelin, could delay delays amyloid plaque and rescue the recognition memory deficits in J20 mice [35]. So the reelin pathway deserved consideration as a novel therapeutic target for AD pathogenesis [35].

Table 1. Transgenic rodent models with APP or/and PS mutations

`	*PDAPP*	*Tg2576*	*APP23*	*J20*	*PS1*	*PSAPP*	*5XFAD*
Mutation(s)/transgene	*APP (Indiana)*	*APP (Swedish)*	*APP (Swedish)*	*APP (Swedish and Indiana)*	*PS1 (M146L)*	*PS1 (M146L) and APP (Swedish)*	*PS1 and APP*
Outcome	*Enhanced cleavage by gamma-secretase; increased Aβ 42:40 ratio; tau hyperphosphorylation*	*Enhanced cleavage by beta-secretase; expansive amyloid plaques deposition*	*Obvious amyloid plaques and cerebral amyloid angiopathy*	*Combination of effects on APP processing/Aβ*	*Enhanced Aβ 42:40 ratio; dysfunction of intracellular calcium; without cognitive deficits*	*Accelerated phenotype and pathology; without formation of NFTs*	*Accelerated amyloid plaques; increased production of Aβ 42 levels; without formation of NFTs*
Amyloid plaques (age)	*Yes (6–9)*	*Yes (9)*	*Yes (6)*	*Yes (6)*	*None*	*Yes (3)*	*Yes (2)*
NFTs (age)	*No*	*No*	*No*	*No*	*No*	*No*	*No*
Neuronal loss (age)	*No*	*No*	*Yes (14-18)*	*No*	*No*	*No*	*Yes*
Synaptic deficits (age)	*Yes*	*Yes*	*Yes*	*Yes (2-4)*	*Yes*	*Yes*	*Yes*
Memory deficits (age)	*Yes (6)*	*Yes (4-6)*	*Yes (3)*	*Yes (2-4)*	*NA*	*Yes*	*Yes*
References	*[20]*	*[21, 22]*	*[23, 24]*	*[24]*	*[25]*	*[26]*	*[27, 28]*

2.1.3 Brief summary of transgenic mice with APP or/and PS mutations

Transgenic rodent strains with APP and /or PS mutations have obtained important information about Aβ pathology in AD and strongly support the Aβ hypothesis. However, these transgenic rodent strains do not display NFTs or prominent neurological alterations that commonly observed in AD, making them incomplete AD models. Both pathological changes and behavioral impairments are crucial judgment criteria of AD. Because there exists a free clearance capacity for amyloid plaques in vivo, only a small amount of amyloid plaques deposition do not mimic the complete AD model. Even so, these rodent models with APP and/or PS mutations are considered as ideal models of Aβ pathology and have their vital part in reflecting the effects of anti-Aβ therapies on Aβ dynamics.

2.2. Tau hypothesis

Despite considerable attention had been paid to the Aβ in AD research and drug discovery, more and more evidences demonstrated that the abnormal aggregation of tau protein also played a vital mediating role in the development of AD. This is referred to as the tau hypothesis [36]. Recent study revealed that the reduced expression of tau lessened the neurotoxicity in Aβ-treated cells and animal models [37, 38]. These results indicated that tau hypothesis was as important as Aβ hypothesis in the rationalization of the neuropathology of AD.

2.2.1 Transgenic rodent model with tau mutation

Tauopathy is a pathological change in AD with the pathological aggregation of tau protein named NFTs in hippocampus of brain. The rate of neuronal loss was much higher than that of NFTs, indicating that there might be a relevant mechanism between NFTs formation and neuronal apoptosis [39]. The tau hypothesis was conclusively supported by the discovery of a tau gene mutation in chromosome 17 (FTDP-17) in frontotemporal dementia (FTD), elucidating that tau dysfunction or abnormality alone independently

induced cognitive impairments and neurodegeneration [14]. As shown in Table 2, mice from the strains with tau gene mutation all display NFTs at 4.5-6.5 months old as well as neuronal loss and synaptic deficits.

Table 2. Transgenic rodent model with tau mutation.

Characterization	*JNPL3*	*rTg4510*	*hTau*
Mutation(s)/transgene	*Tau (P301L)*	*Tau (P301L inducible)*	*Human tau*
Outcome	*NFTs in several regions of the brain and spinal cord; without amyloid plaques and cognitive deficits*	*NFTs and neuronal apoptosis in the CA1 zone of the hippocampus; without amyloid plaques*	*NFTs and neuronal loss in the neocortex and hippocampus similar with human; without amyloid plaques*
Amyloid plaques (age)	*None*	*None*	*None*
NFTs (age)	*Yes (4.5-6.5)*	*Yes (4.5-5.5)*	*Yes (6)*
Neuronal loss (age)	*Yes*	*Yes*	*Yes (15)*
Synaptic deficits (age)	*Yes*	*Yes*	*Yes*
Memory deficits (age)	*NA*	*Yes*	*Yes (12)*
References	*[40]*	*[41]*	*[42, 43]*

2.2.2 Application in Alzheimer's disease

Many transgenic rodent models with tau mutations have been developed and used in the pathogenesis research and drug discoveries, for example, P301L, rTg4510 and hTau mice. The P301L mouse model was utilized to investigate the distribution of tau protein, finally to found that regulating the synaptosomal tau level might be a potential target for a therapeutic intervention directed at preventing neurodegeneration [44]. In addition, calpastatin was found to inhibit the activity of calpain to alleviate the taupathy in AD using P301L mice [45]. Interestingly, it was reported that methylene blue could ameliorate tau-related neurodegeneration prevented behavioural deficits and reduce soluble tau levels in the brain rTg4510 mice [46]. However, it could not dissolve existing neurofibrillary tangles in rTg4510 mice [46]. Furthermore, the rTg4510 mice were used to conduct the longitudinal evaluation of blood-brain barrier (BBB), finally to found that BBB was damage with progressive IgG, T cell and red blood cell infiltration as the mice grow older [47].

2.2.3 Brief summary of transgenic mice with APP and PS mutations

The transgenic mice with tau mutation can display NFTs which is one of the important hallmarks of AD. Unfortunately, amyloid plaques pathology has not been detected, making them as limited models of AD. Yet, these transgenic rodent models are ideally-suited for the investigation of tau dysregulation and neuronal apoptosis in AD.

2.3. Transgenic rodent model with APP, PS1/2 and tau mutations

As shown in Table 3, the multiple transgenic rodent models display accelerated AD-like phenotype with both amyloid plaques and NFTs, which do not exist in other transgenic rodent models simultaneously. The first transgenic rodent strain with mutations both in APP and tau is the TAPP mouse, which is developed by the crossing with the Tg2576 strain and the JNOL3 strain [15]. These mice display amyloid plaques at 9 months old, which is similar to Tg2576 mice in their distribution, development and severity.

Another transgenic rodent strain is the 3xTg-AD mouse, which is developed by simultaneously inserting Swedish APP mutation and the P301L tau mutation into PS1 mice [48]. These mice display relatively complete pathological changes and behavioural impairments including amyloid plaques, NFTs, neuronal apoptosis, reduced synaptic density and cognitive deficit. In the first instance, soluble Aβ is observed within neurons at 3–4 months old in the neocortex and at 6 months old in the hippocampus. Amyloid plaques are developed at about 6 months old, and learning deficits occur prior to the formation of amyloid plaques at

about 4.5 months old [49]. Finally, NFTs are developed in the hippocampus and amygdale regions at about 12 months old.

Table 3. Transgenic rodent model with APP, PS1/2 and tau mutations.

Characterization	*TAPP*	*3xTg-AD*
Mutation(s)/transgene	*APP (Swedish) and tau (P301L)*	*PS1, APP, and tau*
Outcome	*Accelerated phenotype and pathology; without cognitive deficits*	*Accelerated phenotype and pathology*
Amyloid plaques (age)	*Yes (9)*	*Yes (6)*
NFTs (age)	*Yes*	*Yes (12)*
Neuronal loss (age)	*Yes*	*Yes*
Synaptic deficits (age)	*Yes*	*Yes*
Memory deficits (age)	*NA*	*Yes (4.5)*
References	*[50, 51]*	*[52, 53]*

2.3.1 Application in Alzheimer's disease

These multiple transgenic mice models also have been utilized in the Alzheimer's disease research widely, since they can display both Aβ plaques and NFTs simultaneously. Firstly, the hAPP mice, were used to evaluate the effect of O-linked N-acetyl glucosamine on AD [54]. The result showed that O-linked N-acetyl glucosamine could improve the performance in the Morris water maze, decrease amyloid plaques levels, which provided good support for O-linked N-acetyl glucosamine as a promising therapeutic target to alter disease progression in AD [54].

The 3xTg-AD mouse model is a more popular transgenic model in the research of AD. The old 3xTg-AD mice (21-24 months old) were used to evaluate the neuroprotective effect of quercetin (a flavonoid generally found in fruits and vegetables, such as onions and apples, and red wine) on AD [55]. The results showed that quercetin could decrease the extracellular β-amyloidosis, tauopathy, astrogliosis and microgliosis in the hippocampus and the amygdala, as well as improving the performances both in Morris water maze and elevated plus maze [55]. In addition, this mouse model was used to investigate the effect of the endogenous tau on NFTs and cognitive deficits by crossing 3xTg-AD with mtauKO mice to obtain a novel transgenic strain, named 3xTg-AD/mtauKO [11]. The data showed that the endogenous tau was contributed to the generation of NFTs, but it could not affect the change of cognitive capacity [11]. Moreover, some publications reported the age-dependent differences (reference and working memory deficits [56], amyloid plaques levels and NFTs [57], synaptic dysfunction [58], mitochondrial dysfunction [59] and neurogenesis damage [60]) of this transgenic mouse model to illuminate the pathology development process.

3. Behavioural assessment methods

The hippocampus and the surrounding regions belong to limbic system, which is responsible for a variety of activities such as learning, memory and emotional behavior. In particular, hippocampus had been demonstrated to be critical for spatial memory and emotional behavior which were closely related to cognitive deficit in AD [15]. Moreover, hippocampus was easy to damage at the earliest stages of AD. Therefore, it was considered as a key region to understand the disease pathophysiology comprehensively [61]. Indeed, most of the behavioral tasks are designed to assess the hippocampal-dependent memory. So far, many behavioral tasks have been developed to evaluate the behavioral alterations in AD.

3.1. Morris water maze (MWM)

The MWM [62] is a behavioural task that depends on hippocampus for assessing spatial learning and long-term memory (LTM) in rodents [63,64]. This task has been extensively utilized in behavioural

assessment on account of its simple handling, high repeatability and reliability. MWM has been successfully utilized in testing the learning and memory capacities reflecting the therapeutic effect of novel drugs [64]. Considering the widespread use of MWM in the neuroscience and psychology for studying spatial learning and memory, a mathematical model was established to understand the exact mnemonic and navigational demands of the task [65]. This model could identify different parameter values and suggest the activation of different neuronal pathways [65].

3.1.1 MWM in the development of AD model

A multiple of animal model have been developed to mimic the pathology of AD for the drug screening and mechanism research. Considering that the importance of spatial learning and memory deficit in the criterion of AD, MWM has been applied in the judgement of many potential AD animal model. MWM was used to test the cognitive deficits of a novel animal model of AD, the hemizygous transgenic McGill-R-Thy1-APP (Tg+/−) rat [66]. The data revealed that this transgenic rat exhibited spatial memory deficit in the MWM as early as 3 months old, which persisted at 6 and 12 months old when compared to wild-type rats [66]. In addition, MWM was used to test different ages of B6C3-Tg (APPswe/PSEN1dE9) double-transgenic mice to provide the evidence as an AD model [67].

3.1.2 MWM in novel drug candidate discoveries

Spatial memory deficit is a prominent feature of AD, and MWM is a standard task to test the spatial memory deficit [65]. So MWM has been applied in the novel drug candidate discoveries widely. For example, MWM was used to evaluate the effect of silibinin on AD, and the result showed that silibinin could improve the performance of APP/PS1 transgenic mice after dosing with silibinin [68]. And the mechanism revealed that silibinin might act as a dual-target drug for the treatment of AD by inhibiting amyloid β peptide aggregation and the acetylcholinesterase activity [68]. For another example, lycopene was found to abrogate neuroinflammatory cascade in intra cerebroventricular injection of β-amyloid1–42-induced learning and memory impairment mice using MWM [69]. Similarly, a lipid amide named palmitoylethanolamide, was proved to decrease the escape latency in MWM task, which suggested to be a potential drug candidate not just to alleviate the symptoms but also to modify disease progression [70].

3.1.3 MWM in the pathological mechanism research of AD

Although a lot of endeavours were made to the pathological research of AD, the mechanism is still unclear so far. MWM has been applied in the investigation of AD pathogenesis widely. It was reported that MWM was used to test the cognitive function in an estrogen receptor alpha (ERα) knockout mice with Aβ intracerebroventricular injection to understand the effect of estrogen in the development of AD [54]. Furthermore, in the investigation of the relation between hippocampal long-term depression and spatial learning, MWM was utilized to test the spatial learning capacity [71].

3.2. Radial arm maze (RAM)

RAM, is also a behavioural task depends on hippocampus for assessing spatial learning in rodents [72]. Compared with MWM, RAM is suitable for the assessment of STM concluding working memory and reference memory. The RAM is a hippocampal-dependent task, where food-deprived mice must learn to locate maze arms baited with food rewards [73]. During this task, mice have to enter the arms to find food. Then, each visit (entry of the full body, excluding the tail, into an arm) is scored as a correct visit or a wrong visit [73]. A correct visit was defined as an entry into a baited arm that had not been visited earlier in the same trial [73]. Finally, the “wrong visits” were analysed as working memory errors and reference memory

errors to determinate the basis of learning. A working memory error was determined as re-entering the baited arm that the rat already had visited. And a reference memory error was scored as entries into an arm that was not baited at first [74].

3.2.1 RAM in novel drug candidate discoveries

Similar to MWM, RAM also has been applied in the novel drug candidate discoveries broadly. In the effect evaluation of a tumor necrosis factor-α inhibitor on AD, RAM was used to test the learning and memory function of the triple transgenic mice after 10 weeks of treatment of this inhibitor [75]. For another example, curcumin was prepared into nanoparticles to alleviate the AD pathology of Tg2576 mice, and RAM was used to test cognitive performance of these mice after orally administered with curcumin nanoparticles for 3 months [76].

3.2.2 RAM in the pathological mechanism research of AD

RAM is also utilized in the pathological mechanism research of AD as WMW. For instance, RAM was used to test the memory function of endothelial nitric oxide synthase deficient mice to find out the relation between endothelial nitric oxide and amyloid precursor protein [77]. And the results suggested that chronic loss of endothelial nitric oxide might be a crucial contributor to both Aβ related pathology and cognitive deficit [77]. In addition, in the investigation of the effect of GABAA α5 positive allosteric modulators on AD, RAM was used to evaluate the spatial learning and memory capacities change after dosing with these modulators [78]. Furthermore, in the study of the relation between toll-like receptor 9 signalling and AD pathology, RAM was used to test the spatial cognitive function in Tg2576 AD model transgenic mice [79].

4. Future direction

A multiple number of transgenic rodent models and behavioural assessment methods have been established to investigate the underlying mechanisms and screen novel drug candidates in AD. The evaluation criterion of therapeutic effects is based on the pathological and behavioural alterations. However, the high-profile failures of novel drugs in clinical trials strongly call for the need of choosing suitable and correct transgenic rodent models and behavioural assessment methods.

Generally speaking, a good model means that it can truly mimic the relevant aspects of AD including aetiology, symptomatology, treatment and physiological basis. Unfortunately, none of the models satisfies all the aspects of the AD pathologies. The models discussed above represent some specific aspects of AD pathologies that may play a crucial role in the assessment of therapeutic effects of novel drugs. These models can also attribute to the research of underlying pathophysiological mechanisms of AD. Indeed, it is difficult to conduct the evaluation process clinically on account of the morality, ethics and law. Accordingly, rodent models may help to establish the relationship between drug candidates and human.

Moreover, behavioural assessment methods are also important in drug discoveries. Behavioural assessment may serves as readout without the requirement of the disease pathogenesis that might be disproved by future studies. Undoubtedly, behavioural assessment can offer useful information on the efficacy and validity of the drug candidates. However, it is insuperable to break out the species barrier between animal and human. Nevertheless, rodent models are still essential for accessing AD-like pathology in vivo. In spite of many deficiencies in rodent model and behavioural assessment method, it is acceptable that rodent model is irreplaceable. Ideally, at least three or more rodent models and three or more behavioural assessment methods are needed to evaluate novel drugs. For instance, if a compound is

designed for inhibiting the aggregation of Aβ, then the transgenic rodent models displaying increased Aβ levels (e.g. APP mice, PS1 mice and Tg2576 mice, etc.) might be selected for studies. On the other hands, if a compound is designed for inhibiting the NFTs, then the transgenic mice models displaying tau dysfunction (e.g. JNPL3 mice, TAPP mice and 3xTg-AD mice, etc.) might be selected for studies. Obviously some deficits still exist in the current transgenic rodent models. It is beneficial to apply a rodent model expressing relatively complete pathological changes and behavioural impairments (e.g. the 3xTg-AD model, see Table 1) for AD study.

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