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BASIC ASPECTS IN SELECTING A SUITABLE TRANSGENIC RODENT MODEL FOR ALZHEIMER'S DISEASE

Ioana Miruna Balmus[1], Alin Ciobica[1,2], Anca Negura[1,3], Lucian Negura[3] & Emil Anton[3]

[1]*Department of Biology, "Alexandru Ioan Cuza" University, Iasi, Romania*
[2]*Center of Biomedical Research of the Romanian Academy, Iasi Branch, Romania*
[3]*"Gr. T. Popa" University of Medicine and Pharmacy, Iasi, Romania*

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SUMMARY

Due to Alzheimer's disease (AD) great aggressiveness, many worldwide health associations began to globalize research efforts in order to find a suitable treatment and to clarify once and for all its controversial aetiology. Moreover, the animal modelling research is one of the best tools to evaluate molecular mechanisms and to correlate them with clinical features and behaviours. However, in order to provide valuable scientific data correlated to low error sources, a rigorous algorithm of selecting the proper animal model for testing is required. An ideal animal model for AD research has probably not yet been developed, but by a careful selection of the existent models or even by developing new models suitable to research conditions, consistent progress in this area of research can be achieved. This paper aims to show and centralize some of the valuable information gathered along the past years of failure and success in Alzheimer's disease animal modelling, in order to provide a theoretical ground for new and innovative aspects in this rather new area of research.

Key words: *animal models - chemical versus transgenic - selection criteria - Alzheimer's disease*

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Introduction

Alzheimer's disease (AD) represents the main cause of dementia and affects more than 10 million individuals worldwide (Citron 2004). Due to AD high destructive effects, many worldwide health organisations begun to increase global awareness in order to find suitable treatment that not only can provide symptomatic modelling, but also can provide clues and explanation at a molecular level such as regarding cell signalling or modern cell biology paradigms (Strac et al. 2015). Interestingly enough is the fact that the mentality in research has radically changed from symptomatic alleviation to finding the origin of the symptom. Because most of the clinical AD trials that have been conducted over the past years have failed, almost 98% of the drugs failing in Phase 3 trials (Cummings et al. 2014) and the only approved drug being actually a cognitive enhancer not a targeted-drug (memantine) (Cummings et al. 2014), the research community focussed on finding the true although controversial aetiology of AD and on testing more advanced drugs targeting molecular symptomatology.

In this way, it is the animal modelling the best tool to evaluate molecular paths and to correlate them one by one with the associated clinical features and behaviours (Simmons 2008). In AD research, there are hundreds of animal models available mainly categorized by species or higher taxonomic groups, modelling technology, and research purpose (Figure 1).

The perfect animal model would be a natural organism effectively selected so that the disease which is studied could be expressed as closely as it can be observed in the natural model – in our case the patient – in other words, a miniature replica of the patient with all his clinical and molecular pathological features. Therefore, in essence, any of the pathological features should be identical in both animal model and human disease (Laurijssens et al. 2013). As it is known, in AD animal modelling, none of the available models seems to satisfy this requirement mainly due to the fact that a small number of animal species exhibits Alzheimer's disease or other forms of dementia including pets - dogs and cats (Berns 2013), but also wild life specimens - the Tsushima leopard cat (Chambers et al. 2012). In this way, the importance and relevance of animal modelling in AD could be highly argued.

Despite these, there are several aspects that could provide positive arguments for this very useful tool. For an instance, many biologists think that in order to understand complex mechanisms, these should be break down in parts or to be studied as less complex mechanisms in simpler organisms. As the simpler mechanisms get understood, the complexity of the organisms used in animal studies can grow until a sufficiently complex animal can be studied in order to understand the whole process initially investigated. More than that the comprehension of the animal selected in study should be as high as possible in order to rule out any of the false positive or negative results possibilities. Therefore many of the theorized steps in animal modelling always refer to the general design steps (Figure 2) that follow the research purpose (exploratory physiology or biochemistry/drug testing/impairment pathway), but no complex thinking in animal modelling design have been properly theorized yet.

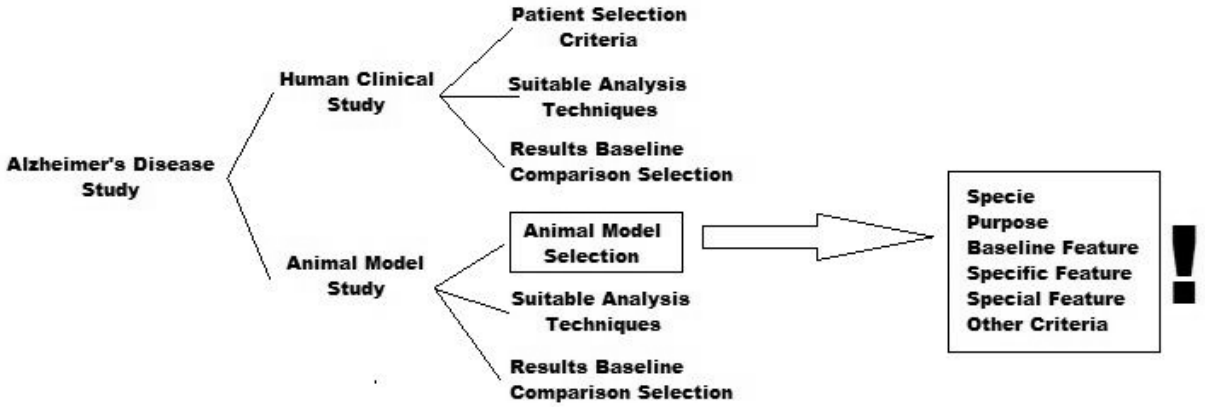

Figure 1. Steps in research study composition

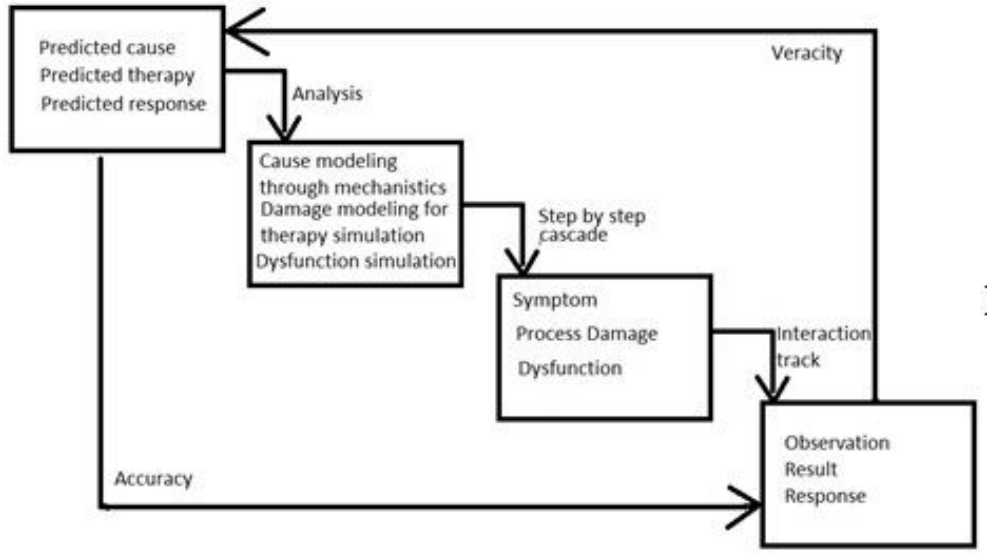

Figure 2. General theoretical framework in animal modelling

In spite that no perfect animal model has been designed, it seems that animal modelling still remains a key tool in AD research. In the last years, many studies have been carried out, primarily in transgenic rodents, in order to characterize the onset and course of AD or even for candidate therapeutics with great promise. Many of the information regarding the disease pathology, such as amyloid-beta disposition pattern, taupathy characteristics or even the correlation between visible features and physiological brain changes, have been characterized through animal modelling. Based on these findings, drug development research studies have been conducted but unfortunately, a very few of these studies reached clinical trial level due to many inconveniences also occurred due to the questionable scientific results and modifiable lifestyle factors uncounted in error standardization (Cavanaugh et al. 2014). More than that, it seems that the Romanian medicine system difficultly manages the diagnosis and treatment of dement patients making AD one of the mostly incurable and hardly manageable mental disorder (Sorbi et al. 2012).

In order to design a solid research study based on animal modelling, alongside the commonly known steps, several key details should be considered. First of all, in order to correctly reach the feature or mechanism the study should observe, it is important to correlate all the steps from general to particular. Therefore selecting an animal group, then a certain species, then a way to induce the symptomatology and, in the end, the additional features necessary for the requirements of the study, should be several key steps followed in designing. As the newest trend in molecular biology and behavioural and clinical research is the transgenic animal modelling, one should keep in mind the fact that most of the transgenic AD models rely on the familial AD forms which represent as few as 5% of all the AD cases. This 5% although almost insignificant turned to be extremely important by the fact that provides a solid and known cause of AD which is the mutation of several genes which encodes protein factors involved in amyloid cerebral metabolism or neuronal cytoskeletal integrity (Brickell et al. 2006). More than that, it seems that these familial cases can be predicted by genetic testing due to their strong known genetic component (Binetti 2012). Besides all of these remain more than 90% cases which are sporadic AD and for which a model has not been developed yet.

Selecting animal model group

In this way, over time, many species had been used in order to study AD features. Thus, less complex studies were conducted on invertebrates such as insects (Drosophila melanogaster) (Gunawardena & Goldstein 2001), fish (Petromyzon marinus) (Hall et al. 2002), or common round worms (Coenorhabditis elaegans) (Goutte et al. 2002). These were actually of great use due to their biological, physiological and biochemical organization. For example, the sea lamprey was considered suitable for modelling due to its simple organization of central nervous system, a one big well individualized nervous ganglion. On the other hand, the round worm was an excellent genetic model due to its simple genetic material organization and great similitude with higher species. Surprisingly, Drosophilae amyloid precursor protein (APP) showed many common features to human APP, so the fruit fly served as a great molecular model. All these may serve as proof that it is not imperative to study high complexity organisms to find answers to complex questions. In fact, over time it was proved that studying simpler animal models may help to find the origin of the troubling mechanisms. As the complex to simple way of thinking is sometimes productive, it seems that, in animal research, breaking down complex mechanisms in the simpler ones observed in less complex organisms would be a rather adequate research approach.

As studies begun to evolve and require more and more complex models, researchers developed mammalian models that can effectively mimic human AD features – mice, rats, octodons (Inestrosa et al. 2005), dogs (Cotman & Head 2008) or non-human primates (Darusman et al. 2014). Whereas octodons and dogs showed some APP similitude to human AD model, they have been proven inefficient due to delicate life conditions (Inestrosa et al. 2005) or due to no notable differences observed in clinical trials (Cotman & Head 2008).

Also, non-human primates (NHP) are extremely valuable assets but due to ethical and life condition reasons their number is restricted in research. It was the NHP studies that yielded connections between age and poor memory (Heuer et al. 2012), but then, a human/ non-human primate comparison showed uniquely human predisposition to Alzheimer's disease (Darusman et al. 2014).

However, in contrast, mice and rats modelling seem a more legit compromise due to great life conditions, short periods of development, ease of breeding and, more importantly, perfectly studied behaviour. Nevertheless mice modelling showed several drawbacks, such as own APP overexpression in elders that gives rise to effects not seen in human AD, neuroprotective APP fragments alongside with toxic APP fragments and artefacts (Takashi et al. 2014).

Still, rat modelling showed drawbacks as well as mice modelling, but they are insignificant compared to numerous advantages. In this way, animals display a well characterized behaviour, live complex life environment, have post-natal brain development, that can be used in drug/therapies testing and also the rat/human genome high similarity are just few of the advantages of using rats as AD models (Gibbs et al. 2004). However it has not been found an ideal animal model that can mimic the entire AD features, in order to closely resemble the human AD. For example, the hallmark of AD, massive or selective neuron loss has not been observed in any mice models, with one exception (Santa Cruz et al. 2003). Massive neuron loss may be human-specific due prolonged life or different brain tissue vulnerability. In addition, the models which exhibit amyloid-beta and NFT formations do not exhibit the same behavioural features due to the murine specific amyloid distribution which is rather different from humans. Also, an entire tau human gene expressing model which could express all the six isoforms of tau protein has not been developed yet and no increase in tau expression could lead to AD-like features in absence of a specific mutation (Duff et al. 2000).

Selecting between classic and transgenic

The classic AD animal models should be the ones which are already well known and studied, such as the chemical-induced dementia models or inhibitors treatment models. These models may be currently outdated due to the advance in molecular biology and the infinite possibilities brought by the genetic engineering techniques and more than that due to the research necessities. But in other circumstances, both classic and transgenic rodent models of AD provide an excellent tool for investigating pathogenic mechanisms and treatments. Still there are several differences that must be discussed. Behavioural features of AD and neuropsychiatric symptoms alleviation can be studied on both animal models types, but genetic and molecular features can be studied only on transgenic closely resembling models. There are several features such as amyloid accumulation and tau specific phosphorylation that can only be observed in transgenic, and more than that, humanized rodent models. As it was mentioned before, several features in human AD cannot be observed into animals which naturally can't exhibit the disease. More than that, several current transgenic rodent models are highly questionable due to the exogenous promoter hypothesis. As it seems that AD may be a problem of genetic regulation too, and the recombinant DNA used in transgenic mice is always under an exogenous promoter, no regulating native sequences were needed in the transgenic construct this being a great disadvantage in the observation of the humanized constructs working in sites (Nuber et al. 2009).

Thus, neuropsychiatric symptoms resembling AD behaviour in human can be induced in mice using different chemicals administered via different ways – scopolamine, atropine (Buccafusco 2009), okadaic acid (Kamat et al. 2013), streptozotocin (Yassin et al. 2013), aluminium chloride (Mehla et al. 2013), saporines (Hunter et al. 2004) and the more complex ferrous sulphate heptahydrate, LButhionine-(S,R)-sulphoximine, and amyloid peptide mixt treatment (as described by the TACONIC group).

Table 1. Several transgenic animal models used in Alzheimer's disease research

Mice Model	Species	Genotype/Phenotype	Purpose	Special features	Disadvantages
APPSWE - Model 1349 (Hsaio et al. 1996)	Mouse	Genotype: Human APP transgene + Swe mutation. Phenotype: High concentration of APP and amyloid, amyloid plaques development, memory impairment.	APP expression studies, amyloid plaques formation, neuronal decline, loss of memory. Therapeutic potential compounds testing.	rd1 mutation	rd1 models can loss sight – no behaviour tests can be conducted. At 7 to 12 weeks of life, males become aggressive and begin to fight.
APPSWE – Model 2789 (Hsaio et al. 1996)	Mouse	Genotype: Human APP transgene + Swe mutation. Phenotype: High concentration of APP and amyloid, amyloid plaques development, memory impairment at 9 to 10 months.	APP expression studies, neuronal decline, loss of memory. Therapeutic potential compounds testing.	disc1 mutation	20% of males can suffer from premature death. At 7 to 12 weeks of life, males become aggressive and begin to fight.
Tg2576 Model (Hsaio et al. 1996)	Mouse	Genotype: Overexpression of human APP transgene + APPK670/671L mutations. Phenotype: Development of amyloid plaques and progressive cognitive deficits.	Gene expression studies, neuronal decline, loss of memory.		Individuals can suffer from premature death. At 7 to 12 weeks of life, males become aggressive and begin to fight.
McGill-R-Thy1-APP Model (Leon et al. 2010)	Rat	Genotype: human APP transgene + Swedish and Indiana mutations. Phenotype: Amyloid deposits, cognitive impairment.	Gene expression studies, neuronal decline, loss of memory.		Only homozygous double mutants can be used.
CVN Model (Davis et al. 2004; Colton et al. 2008)	Mouse	Genotype: Triple mutant human APP gene (Swe, Dutch and Iowa) + NOS2 gene knockout. Phenotype: Abundant plaques aggregated, hyper phosphorylated tau tangles, Neuronal loss.	Gene expression studies, neuronal decline, loss of memory.		Behaviour AD specific at 12 months. No synaptic loss reported.
APPSWE-Tau (Lewis et al. 2000)	Mouse	Genotype: Human APP transgene+ Swe mutation+ Human MAPT gene+P301L mutation. Phenotype: High concentration of APP and amyloid, amyloid plaques development, memory impairment. Motor disturbance and NFT morphology similar to Tau models.	Study of AD with both amyloid plaques and NFTs. Therapeutic potential compounds testing.	rd1 mutation	Complex control models system. rd1 models can loss sight – no behaviour tests can be conducted.
TgF344-AD (Cohen et al. 2008)	Rat	Genotype: Mutant human amyloid precursor protein (APPsw) and presenilin 1 (PS1ΔE9) genes. Phenotype: Amyloid plaques, apoptotic loss of neurons, and cognitive disturbance.	Gene expression studies, neuronal decline, loss of memory. Therapeutic potential compounds testing.		Closely resembling human AD, but with more special needs.
Tau - Model 2508 (Lewis et al. 2000)	Mouse	Genotype: Human MAPT gene + P301L mutation. Phenotype: NFT development associated with behaviour and motor disturbances.	AD, Pick syndrome and other neurologic syndromes (tauopathies) studies.		AD non-specific organisms.
Tau – Model 1638 (Lewis et al. 2000)	Mouse	Genotype: Human MAPT gene + P301L mutation. Phenotype: Behaviour and motor disturbances associated with NFTs.	AD, Pick syndrome and other neurologic syndromes (tauopathies) studies.	rd1 mutation	rd1 models can loss sight – no behaviour tests can be conducted. AD non-specific organisms.
3xTgAD (Oddo et al. 2003)	Mouse	Genotype: Human MAPT, APP and PSEN1 genes + P301L, Swe and M146V, respectively, mutations. Phenotype: Both plaque and tangle pathology.	Alzheimer's disease		For maintaining a live colony, mice that are homozygous for the Psen1 mutation and homozygous for the co-injected APPSwe and tauP301L transgenes must be bred together.

Table 1. Continous

Mice Model	Species	Genotype/Phenotype	Purpose	Special features	Disadvantages
APPSwe/ PSEN1(A246E) (Borchelt et al. 1997)	Mouse	Genotype: Chimeric APP + Swe mutation and PSEN1 + A246E mutation. Phenotype: Amyloid plaques develop at 9 months, in hippocampus and later in cortex.	Dystrophic neuritis, gliosis, Alzheimer's disease		Impairments clearly associated to amyloid accumulation, but with severe damages making behavioural testing rather problematic.
TgCRND8 (Christi et al. 2001)	Mouse	Genotype: Mutant human APP + Swedish and Indiana mutations. Phenotype: Early plaque formation at three months.	Alzheimer's disease		The mice had increased mortality with 25% and 17% respectively reaching 365 days.
hAPPJ20 (Mucke et al. 2000)		Genotype: Mutant human APP + Swedish and Indiana mutations + PDGF promoter. Phenotype: Age-dependent increase in neuronal Aβ throughout the hippocampus.	Alzheimer's disease		Show severe learning deficits making behavioural testing rather problematic.
hAPP/Sod2+/- (Lee et al. 2012)	Mouse	Genotype: Human APP gene+ SOD2 Phenotype: Behaviour disturbance and modified oxidant balance	AD, oxidative stress, antioxidant therapies		Not available on the market (by biotechnological means), custom model
SOD2/Tg2576 (Bitner et al. 2012)	Mouse	Genotype: Human APP gene+ Swe + mitochondrial SOD Phenotype: amyloidosis, but decreased oxidative stress	AD, oxidative stress		Not available on the market (by biotechnological means), custom model

Also, as many other chemical induced models have been proven to be inefficient and the existing one highly questioned, it has been obvious that a transgenic model can be more useful. Due to human gene transgenesis, single gene knock-outs and knock-ins and conditional gene modifications, a transgenic model can mimic more than one feature at a time more precisely and accurately to human features. More than that, drug testing studies require humanized animal models so that drug effect could be estimated closely to human. Several drawbacks can be noted: often the transgene works under a murine promoter sequence making an expression regulatory study almost impossible.

Due to transgenesis, many offsprings in a homozygous mutant state are not compatible with life and several (almost 20%) of the hemizygous die at young age. There is also a financial reason too – the classic models are easier to obtain, breed and use with lower costs than transgenics. On the other side, usually transgenics cannot be breed due to their specific genetic features, often in hemizygous state.

Selecting between transgenics

This criterion generally refers to transgenic modelling of baseline, specific and special features of the animal model according to study requirements. Baseline features generally refer to common laboratory species features (laboratory breed races, fur colour, and body size). For example (Table 1), some transgenic mice come with brown or black fur. Coat colour may be associated with several behaviour features, such as sensitivity to noise and odours, but researchers can use coat colour as a simple way of distinguishing between different featured breeds in laboratories. Baseline features do not interfere in transgenic studies by being neutral features. Specific features generally refer to study specific requirements. In our case, a transgene or a mutation that can resemble human AD specific features can be a specific genetic feature that leads to a specific phenotype useful in research. Because AD is a polygenic and multifactorial disease, sometimes a transgenic animal model can exhibit more than one specific feature due to combined transgenesis. It has been also shown that combined APP and Tau humanized mice closely resemble human AD (Lewis et al. 2001). Special features can be closely or distantly interacting with specific features; therefore they can be considered additional features to the "standard" study model.

Also, several of these special features refer to eye colour and retinal degeneration (possessing a rd1 gene mutation), or a protective modification for a specific feature (a mutation in disc1 gene that prevents cross-symptoms of AD and schizophrenia or a mutation of nnt gene closely related to robust weight gain on a high fat diet). These special features can be considered only in research context. For example, a transgenic APP model possessing rd1 mutation cannot be used in behaviour test because it develops blindness. As well as an nnt wild type model cannot be used in apolipoprotein E polymorphic influence on AD study because it easily gain weight developing other metabolic issues. Considering all of these, selecting between transgenic models can be a long dialog especially considering the purpose of the study.

Other selection criteria

There are many other selection criteria that generally refer to individual conditions such as geographic position of developer and beneficial, shipping distances,

laboratory conditions, animal diets, or breeding licenses which are only important to the study developer at the moment of the study design and depending on the facilities and location.

Other important criteria are research laboratory conditions. Some of the mice models develop aggressive or social behaviour. In order to create proper study conditions, the research laboratory must be able to provide appropriate shelter for the animal models. Some other mice models require specific diets prescribed by the developer, but this is not necessarily a vital condition in AD research mice models. If the research requires model breeding in generation observation or cross-reproduction, the selected mice models must be accompanied by breeding licenses. These refer most of the time to financial condition, but they often refer to uncertain breeding results.

In this way, by breeding hemizygote mutant mice it can be possible to obtain homozygous mutants often incompatible with life, which can cause a low breeding yield. Also, hemizygous mutants and wild type individuals could be troublesome in differentiating.

Potential drawbacks in Alzheimer's disease animal modelling

Although animal modelling via transgenesis is extremely useful, it also can involve high risks. Biotechnological immixture in animal natural biochemistry and genetics could generate several issues. Firstly, it is possible that due to laboratory conditions or human presence, a certain level of stress to occurs (Balcombe et al. 2004, Sorge et al. 2014) and it can be applied to all animal research experiments. In addition, as AD does not naturally occur in other organisms except human and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and only theorized in pets (especially dogs), the induction of the pathological features which impairs the natural order in the host organisms could bring molecular or biochemical changes in metabolism or even in genetic regulation patterns. In these conditions, "recognition of distress in laboratory animals requires knowledge of what is normal for the species and strain used. Genetically modified animals should be evaluated in reference to the normality of their genotype", claims the NRCC of USA (CRADLA 2008). This observation can also be linked to the environmental housing conditions mentioned before.

More than that, stress can also occur due to diet, water, and behaviour to mates. It is the case of the APPSWE transgenic male mice that become aggressive (Van Loo et al. 2003), therefore they begin to fight if sheltered together. In this way, a modified social behaviour will occur therefore an unusual change in behaviour due to external factors independent of the study requirements. In the case of behavioural testing, this should be a real problem, the aggressiveness of the transgenic subjects modifying the statistical data. Not only are the transgenic models reported to be aggressive, but also the non-transgenic male mice, as the aggressions between male mice has been reported to be a serious issue in experimental animal housing and testing (Garcia-Allonza et al. 2006).

Also, due to the genome changes that occur during transgenesis, some of the model types exhibit short life periods and early or premature death. More than that, in double or triple transgenic models in order to recapitulate the desired pathological features only the homozygous mutant individuals can be used. Therefore an extra effort needs to be made as the breeding rules imply not only homozygous offsprings but also hemizygous and wild type ones. Other models can exhibit neuronal loss or AD features only late in life when results are highly questioned as implicating aging or transgenesis.

Last but not least, some of the more complex models that would possess a closer resemblance to human AD features are extremely sensible and possess complex molecular regulation system. For example, it can be observed that despite the fact that oxidative stress is a major influence factor in AD pathology, there aren't many options in transgenic models to study on. As it can be seen in Table 1, few models address this feature of AD and they are not available in the market being custom designed. The reason why this happens is that these changes are extremely delicate and the animals require additional attention and care. More than that, serious questions have been asked regarding the methodological limitations of detecting oxidative stress meaning that the current findings could be hampered by the scarcity of experiments dedicated to demonstrate which of the parameters of oxidative status are relevant according to the specific features of the study (Meitern et al. 2003). Therefore as the oxidative stress is extremely relevant in the AD context it is crucial to find ways of excluding animal modelling and experiment design errors.

Another important progress has been made on the line of humanized models. It seems that transgenesis may not be the key tool in generating models enough suitable to respond to the complex questions of the AD pathology. Therefore the AD research moved into the Petri dish. The latest researches show that induced pluripotent stem cells (iPSC) can easily differentiate in neurons and, when harvested from Alzheimer's patients, could lead to demented AD-like neurons (Choi et al. 2014). The research based on iPSC is also focused on frontotemporal dementia and spinocerebellar ataxia, alongside familial forms of AD. The fibroblasts obtained from simple skin biopsies are reprogrammed to dedifferentiate and to be capable to redifferentiate in other mammalian cell types. In this way, reprogrammed differentiated fibroblast-originating neurons can be cultivated on Petri dishes and then be used as an innovative model for neurodegeneration.

Conclusions

Many Alzheimer's disease animal models have been proven to be appropriate for mechanisms of action or aetiology elucidation and therapeutic compounds testing. By definition, an animal model is not exactly the disease, but a close resemblance of it in an animal organism. Therefore the question that rises is what the idealistic model would be like, considering all the complex features of AD and all the drawbacks which the imperfections of the available animal species and research conditions provide. This would be the actual discrepancy between the natural disease and modelling – the understanding that modelling is not a replication process but a representation one. Therefore, an ideal animal model will never be developed except for that species not yet discovered that naturally exhibits AD pathology similar to human. However, AD research using animal modelling is a valuable tool that can provide key information in further research, by a rigorous selection of the existent models or even by developing new models suitable to research conditions, consistent progress in this area of research can be achieved.

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Correspondence:
Alin Ciobica, MD
Department of Biology, "Alexandru Ioan Cuza" University
B dul Carol I, 11, 700506 Iasi, Romania
E-mail: alin.ciobica@uaic.ro