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

Ioana Miruna Balmus1, Alin Ciobica1,2, Anca Negura1,3, Lucian Negura3 & Emil Anton3

1Department of Biology, "Alexandru Ioan Cuza" University, Iasi, Romania
2Center 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

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 began to increase global awareness in order to find suitable treatment that not only can provide symptomatic alleviation 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 focused 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 broken 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.
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, tauopathy 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 difficulty manages the diagnosis and treatment of dementia 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 (Drosophilia melanogaster) (Gunawardena & Goldstein 2001), fish (Petromyzon marinus) (Hall et al. 2002), or common round worms (Coenorhabditis elaeagns) (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 promotor 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 (Mehta 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>
<thead>
<tr>
<th>Mice Model</th>
<th>Species</th>
<th>Genotype/Phenotype</th>
<th>Purpose</th>
<th>Special features</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPSWE - Model 1349 (Hsaio et al. 1996)</td>
<td>Mouse</td>
<td>Genotype: Human APP transgene + Swe mutation. Phenotype: High concentration of APP and amyloid, amyloid plaques development, memory impairment.</td>
<td>APP expression studies, rd1 mutation. amyloid plaques formation, neuronal decline, loss of memory. Therapeutic potential compounds testing.</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>APPSWE – Model 2789 (Hsaio et al. 1996)</td>
<td>Mouse</td>
<td>Genotype: Human APP transgene + Swe mutation. Phenotype: High concentration of APP and amyloid, amyloid plaques development, memory impairment at 9 to 10 months.</td>
<td>APP expression studies, disc1 mutation. neuronal decline, loss of memory. Therapeutic potential compounds testing.</td>
<td>20% of males can suffer from premature death. At 7 to 12 weeks of life, males become aggressive and begin to fight.</td>
<td></td>
</tr>
<tr>
<td>APPSWE-Tau (Lewis et al. 2000)</td>
<td>Mouse</td>
<td>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.</td>
<td>Study of AD with both amyloid plaques and NFTs. Therapeutic potential compounds testing.</td>
<td>Complex control models system. rd1 models can loss sight – no behaviour tests can be conducted.</td>
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<tr>
<td>TgF344-AD (Cohen et al. 2008)</td>
<td>Rat</td>
<td>Genotype: Mutant human amyloid precursor protein (APPsw) and presenilin 1 (PS1AE9) genes. Phenotype: Amyloid plaques, apoptotic loss of neurons, and cognitive disturbance.</td>
<td>Gene expression studies, neuronal decline, loss of memory. Therapeutic potential compounds testing.</td>
<td>Closely resembling human AD, but with more special needs.</td>
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<tr>
<td>Tau – Model 1638 (Lewis et al. 2000)</td>
<td>Mouse</td>
<td>Genotype: Human MAPT gene + P301L mutation. Phenotype: Behaviour and motor disturbances associated with NFTs.</td>
<td>AD, Pick syndrome and rd1 mutation. other neurologic syndromes (tauopathies) studies.</td>
<td>rd1 models can loss sight – no behaviour tests can be conducted. AD non-specific organisms.</td>
<td></td>
</tr>
<tr>
<td>3xTgAD (Oddo et al. 2003)</td>
<td>Mouse</td>
<td>Genotype: Human MAPT, APP and PSEN1 genes + P301L, Swe and M146V, respectively, mutations. Phenotype: Both plaque and tangle pathology.</td>
<td>Alzheimer’s disease</td>
<td>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.</td>
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</table>
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 than one feature at a time more precisely and accurately. Due to transgenesis, many offsprings in a homozygous mutant state are not compatible with life and several (almost 20%) of the he imizygous 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 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 modeling 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.

**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 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 certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebus murinus, the mouse lemur) (Bons et al. 2006), a wild cat species and certain monkey species (Microcebu
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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