Modelling Alzheimer's disease: good intentions, poor choices?

More than 400 therapeutic approaches with preclinical success have failed to reach market over the years; this lack of success may be explained by use of the wrong behavioural tests in animal models.

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In England and Wales, the leading cause of death was dementia (prior to the COVID-19 pandemic) with Alzheimer's disease being the most common cause behind it[1,2]. This disorder has devastating consequences on both a personal and an economic level without clear routes of prevention or treatment[1] – motivating countless research groups to develop a solution. More than 400 therapeutic approaches to Alzheimer's Disease with promising preclinical results have failed to reach market over the years; this lack of success may be explained by common pitfalls related to study design choices in animal research. When modelling neurodegenerative disease this includes the choice of a relevant research model, choice of behavioural test to evaluate cognitive improvement and the study design. Here I review the translational viability of different mouse models used in Alzheimer's research from 2010 – 2020. I assessed the relevance of the different mouse models used against pathology and symptoms specific to Alzheimer's, evaluating the translational potential of each strain. I also assess the reporting and design of the studies reviewed and discuss the relevance of behavioural test choice in this type of research.

What is Alzheimer's disease?

Alzheimer's disease is a progressive neurodegenerative disorder meaning that the brain cell loss ("neurodegeneration") observed gets worse over time. This loss leads to a wide range of symptoms, dementia or memory loss being one of the most well-known – however, this disorder is much more than that (Table 1). Patients can present with language difficulties, depression, hallucinations among others – eventually disrupting both simple and complex daily life tasks (for example, getting dressed and grocery shopping, respectively)[4].

Most people get diagnosed when their loved ones notice the changes in behaviour these symptoms come with – however, scientists have long been aware that the disease-causing changes start decades before the first symptoms appear[1]. These molecular and cellular characteristics are well-

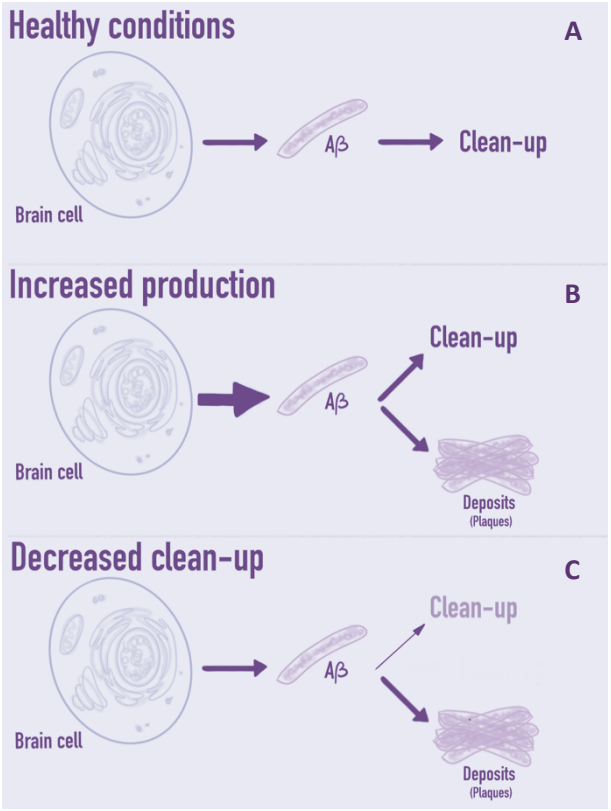


Figure 1. The pathological mechanisms behind Alzheimer's disease. Changes in production or in clean-up of Aβ can lead to deposits which then disrupt essential functions eventually leading to the onset of symptoms.

Table 1. Alzheimer's disease is much more than memory loss; the symptoms affect all aspects of life of the patient and their loved ones.

Cognitive Dysfunction	Psychiatric & behavioural disturbances	Difficulties with daily activities such as:
Memory loss	Depression	Shopping
Language difficulties	Hallucinations & delusions	Driving
Executive dysfunction	Agitations	Dressing & eating unaided

described and always include an accumulation of so called amyloid β deposits (Figure 1), neurodegeneration in certain brain areas and the formation of "neurofibrillary tangles" that are thought to be destructive[1,2]. Outside of this, minor behavioural changes and the inflammation of brain cells is also a commonly accepted feature of Alzheimer's disease[1,2].

The exact cause of these events is yet to be discovered; however, most scientists believe that the formation of amyloid deposits is one of the key components[1] (although some suggest that they might have at least partial protective properties[5]).

Most of us are familiar with the late-onset Alzheimer's disease that is responsible for more than 99% of all cases, however, there is another type called early-onset Alzheimer's disease which can run in the family. Unlike the common form, multiple genes were identified that put an individual at risk of developing the early-onset version of the disorder. As not nearly as much is known about the late-onset Alzheimer's, and their courses appear to be similar, many scientists justify using these genes as a starting point of their research tackling questions about its development[1,6].

The heritable genetic alterations mainly affect one or more of three key proteins: amyloid precursor protein (APP), presenilin 1 (PSEN1 or PS1) and presenilin 2 (PS2). In health, the cutting up of APP forms the amyloid β (Aβ) protein which is then cleaned up via various mechanisms[1] (Figure 1A). In disease, however, the Aβ is cut longer than it should be more often than in healthy individuals[1,6,7] and it starts to accumulate leading to deposits. This accumulation could be due to increased production of Aβ overwhelming the cleaning processes (Figure 1B), less efficient cleaning (Figure 1C) or a combination of the two[6].

1.2. Why study it?

Understanding the disease-causing mechanisms of Alzheimer's disease could lead to earlier detection which, especially in the case of a progressive disorder, means providing the best chances for the patient. The preclinical phase (when no symptoms are present) is thought to be the ideal therapeutic target, however, most patients are not aware of their condition until the onset of dementia[1].

To find answers about how to detect it early and potentially stop or even reverse the damage, scientists have been keen to use rodent models once genetic engineering became more accessible in the 1990s[2]. This technological advancement was necessary for this type of work as rodents do not develop an Alzheimer's-like disorder naturally[1].

There are other techniques available (see discussion), however, as often in research, animal models are more readily used, the focus of my review was on the mouse models most used when studying preclinical features and potential treatment options.

1.3. Animal models under the scope

The amyloid deposit formation (Figure 1) is a widely accepted and evidenced characteristic of Alzheimer's disease, therefore it is no surprise that their presence is a key component of many scientific approaches.

These APP mouse models can broadly be grouped into two main generations based on the techniques they used to create the genetically altered animals.

First generation mouse models

The first generation uses a "transgenic" technique which means that one or more tweaked human gene called transgene (here, in all cases *APP*, sometimes in combination with others) is delivered to the mice where the human gene is inserted into their genetic database. This technique, albeit often used, has multiple caveats that all models in this generation suffer from: for example, there is a risk that the transgene is inserted into an unwanted place that in turn alters other mechanisms leading to undesirable and unpredictable consequences[1]. These complications cannot be predicted nor controlled for.

Based on the number of different transgenes delivered, the first generation can be further broken down into three subgroups: single, double, and triple transgenic mice.

Single transgenic: The first ever mouse lines that were produced with the intention of modelling AD. The mice receive copies of one transgene (*APP*) only. In my review, I have included five: PDAPP[8], Tg2576[9], APP23[10], J20[11] and TgCRND8[12].

Double transgenic: These mouse lines (APP/PS1[13] and 5XFAD[14]) received transgenic copies of both APP and PSEN1.

Triple transgenic: 3xTg-AD mouse lines harbour three different transgenes (PSEN1, APP and tau). Tau is linked to another key Alzheimer's-characteristic, neurofibrillary tangles.

Second generation mouse models

These models are relatively recent – NL-F and NL-G-F were developed by Saito and colleagues in 2014 using a different technique called "knock-in" strategy[15]. Instead of overproducing APP (leading to overall increased Aβ levels), they "humanised" the mouse's own while introducing previously explored mutations. Due to the technique, they could create their own negative control when using non-mutated humanised gene carrying mice.

1.4. Aims of the project

My project had two main goals: to assess translational viability and potential reproducibility. For translational validity, I was curious to explore how well this selection of often-used mouse models reproduce the characteristics of Alzheimer's' disease. On top of this, I also explored the potential reproducibility of published studies involving the strains identified by using 'The ARRIVE Essential 10: Compliance Questionnaire".

2. Methods of Assessment

Translational Validity

The 25 binary questions shown by Table 2 can be broken down into five key groups, with the middle three being of special importance: molecular, cellular, and behavioural characteristics. I created this scoring sheet based on the clinical presentation of Alzheimer's disease in humans[1,2] where the maximum score achievable for each model was 25 (with a hypothetically "ideal" scoring 25). When deciding whether a model was an appropriate model for the disorder, I found it important to appreciate the limitations of them outside of the species difference – this was the fifth group of questions.

The default score for each strain was 0 unless proven otherwise (except for Q5.3.2. concerning sudden death), the points then were added up and divided by 25 to present as a percentage score: results above

70% were considered as appropriate models of preclinical Alzheimer's disease; from 80%, they were good, and above 90%, were great models.

Table 2. Marking criteria for mouse model strains. Ideal model as an example can be seen on the right side with a maximum score of 25. All other strains were compared to this score to assess reliability for modelling Alzheimer's disease.

	Criteria	Scoring	Ideal model
1	Overview		
1	Strains		
2	Does it model AD?	Yes = 1, No = 0	1
2	Molecular characteristics		
1	Senile plaques		
1	Present?	Yes = 1, No = 0	1
1	Before/without cognitive impairment?	Yes = 1, No = 0	1
2	Is the appearance progressive?	Yes = 1, No = 0	1
2	Location		
1	Cerebral cortex?	Yes = 1, No = 0	1
2	Hippocampus?	Yes = 1, No = 0	1
3	Is it the right size?	Yes = 1, No = 0	1
4	Is it the Ab1-42/Ab1-40 ratio increased?	Yes = 1, No = 0	1
2	Nurofibrillary tangles		
1	Present?	Yes = 1, No = 0	1
1	Is the appearance progressive?	Yes = 1, No = 0	1
3	Cellular characteristics		
1	Neural loss?	Yes = 1, No = 0	1
2	Is neurodegeneration present?	Yes = 1, No = 0	1
3	Are there any signs of innate immune activation?	Yes = 1, No = 0	1
1	Is astrocytosis activated?	Yes = 1, No = 0	1
2	Is microgliosis activated?	Yes = 1, No = 0	1
4	Synaptic disruptions?	Yes = 1, No = 0	1
4	Behavioural characteristics		
1	Are there any behavioural abnormalities observed?	Yes = 1, No = 0	1
2	Is the cognitive function affected?	Yes = 1, No = 0	1
3	Is it progressive?	Yes = 1, No = 0	1
5	Potential complications		
1	Genetic level		
1	Is the issue of integration of trans gene solved?	Yes = 1, No = 0	1
2	Is there a lack of overexpression related artifacts?	Yes = 1, No = 0	1
3	One or less mutations introduced?	Yes = 1, No = 0	1
4	Are non-coding regions present?	Yes = 1, No = 0	1
3	Other		
1	Is there a suitable negative control?	Yes = 1, No = 0	1
2	Is there no sudden death linked to it?	Yes = 1, No = 0	1
			25

Potential Reproducibility

The ARRIVE Essential 10: Compliance Questionnaire was adapted to score 5-10 original research articles for each strain (Table 3). The ARRIVE Guidelines were created by NC3Rs with the intention to aid both authors and editors to publish their results in a clear, easy to understand manner in a way that could potentially be reproduced by other gr oups (2.0 version explained in more detail by Percie du Sert and colleagues[16]).

Table 3. The ARRIVE Essential 10: Compliance Questionnaire

Section		Questions	Comments
1	Study Design	1 Are all experimental and control groups clearly identified?	e.g., if strains are identified
		2 Is the experimental unit (e.g. an animal, litter or cage of animals) clearly identified?	e.g., if strains are identified
2	Sample Size	1 Is the exact number of experimental units in each group at the start of the study provided?	e.g., in the format of 'n='
		2 Is the method by which the sample size was chosen explained?	
3	Inclusion & Exclusion Criteria	1 Are the criteria used for including and excluding animals, experimental units or data points provided?	Exclusion of females was not accepted unless justified to not favour sexism in neuroscience
		2 Are any exclusions of animals, experimental units, or data points reported, or is there a statement indicating that there were no exclusions?	
4	Randomisation	1 Is the method by which experimental units were allocated to control and treatment groups described?	If genotypes were randomly assigned due to mating and wild type animals were used as controls, yes
5	Blinding	1 Is it clear whether researchers were aware of, or blinded to, the group allocation at any stage of the experiment or data analysis?	
6	Outcome Measures	1 For all experimental outcomes presented, are details provided of exactly what parameter was measured?	
7	Statistical Methods	1 Is the statistical approach used to analyse each outcome detailed?	
		2 Is there a description of any methods used to assess whether data met statistical assumptions?	Optional
8	Experimental Animals	1 Are all species of animal used specified?	
		2 Is the sex of the animals specified?	
		3 Is at least one of age, weight or developmental stage of the animals specified?	
9	Experimental Procedures	1 Are both the timing and frequency with which procedures took place specified?	Optional
		2 Are details of acclimatisation periods to experimental locations provided?	Optional
10	Results	1 Are descriptive statistics for each experimental group with a measure of variability?	e.g. mean and SD, or mean and range
		2 Is the effect size and confidence interval provided?	Optional

The scoring sheet consists of 10 points with 1-3 binary questions each (Table 3). For every 'Yes', 1 point was given, and the maximum score varied based on the inclusion of optional questions which were not relevant to the type of research conducted (e.g., Section 9 was only relevant to behavioural experiments, not purely molecular ones).

Due to the variability of the maximum scores, individual percentage scores were given for each article and the averages of those were taken for each strain. For easing interpretation, the scores were categorised into groups of 'poor' ($x \leq 60\%$), 'acceptable' ($60\% < x < 80\%$) and 'good' ($80\% \leq x$) potential reproducibility. *The scores do not represent the quality of the research undertaken.*

The selection of eligible papers was through the Classic Web of Science (v5.35) using the search code "ALL FIELDS: ("gene- name") AND ALL FIELDS: (Alzheimer)" within the 'Web of Science Core Collection' database. To achieve consistency of national regulations, only research in the UK published in or after 2010 were included (unless the technique was developed during that period, in which case, articles were limited to post-technique years only).

Reviews were excluded from analysis and, if more than ten articles came up meeting the criteria, the results were sorted by relevance and the top 10 hits were selected for assessment. In some cases, irrelevant articles were included in the results where the gene name was mentioned but was not in focus. If this happened and there were more papers relevant, they replaced them; otherwise the irrelevant article(s) were excluded.

1. Results & Discussion

Prior to diving in, I would like to again highlight the fact that rodents do not develop Alzheimer's naturally[1], therefore, all readers and undertakers of animal studies must bear this in mind along with the innate between-species differences.

All models from both generations present with progressive Aβ deposits in the relevant brain regions[17,18,19] which is one of the major marks of preclinical Alzheimer's disease – alongside which they also meet the criteria for disruptions in brain cell connectivity to varying levels[1,17,20,21,22], and immune activation as well[1,18,23]. However, this was not enough to be a suitable Alzheimer's model: the molecular basis of these observed features and the limitations left us with the two second generation models as the best for the job (Table 4). But are baseline characteristics enough to make a good model?

Table 4. Summary of some of the mouse models commonly used for Alzheimer's disease research. First generation models were found not be a suitable model for AD using this study's parameters and even if the transgenic technique's limitations were ignored, only the 5XFAD strain meets molecular, cellular, and behavioural criteria. The second generation, on the other hand, appears to be a good pre-clinical model for the neurodegenerative disorder.

	1st generation								2nd generation	
	Single transgenic					Double transgenic		Triple transgenic	Single App KI	
	PDAPP	Tg2576	APP23	J20	TgCRND8	APP/PS1	5XFAD	3xTg-AD	NL-F	NL-G-F
Molecular	X	X	X	X	X	X	✓	X	✓	✓
Cellular	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Behavioural	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Limitations	X	X	X	X	X	X	X	X	✓	✓
Overall match (%)	64	48	52	52	52	60	68	64	88	84

Generational differences

1st generation – Is it the past?

It is not that straightforward as this generation encompasses three groups with eight strains combined. The main obstacle here, however, is innate to the technique used: the unpredictability of transgene insertion site, the overexpression-related by-products (that could theoretically cause the desired cellular and behavioural consequences) and the lack of suitable negative control is an issue yet to be tackled.

If we put the limitations to the side, however, one strain rises above the others in the generation: 5XFAD. This is the only one out of the eight that presents with all the essential molecular characteristics: the progressive Aβ deposits prior to cognitive impairment[1,24] (unlike all five single transgenic strains that presents with behavioural deficiencies before deposit accumulation[1]); the deposits are the right size[1] (which is only the case in PDAPP of the 1st generation); and the result of the ratio shift in long:short Aβ products[25].

Despite this fundamental molecular mismatch of 7/8 strains, many articles using them claim they were studying models of Alzheimer's disease[20,26,27,28]. Regardless, the outcomes of these existing studies can still be useful when observed in the right context and with precaution.

2nd generation – New hope?

Saito and colleagues' technique has only been around since 2014[15], so their profile is not as deeply explored as the previous generation's. Their molecular[1,15,18,29,30,31], cellular[1,15,18,21,29], and behavioural[1,18,21] characteristics, however, appear as impressive as of 5XFAD's without the worries related to random localisation[1], overexpression-linked artifacts[1], while important non-coding regions are also included[18] to aid a more controlled function.

Translatability of Mouse Behaviour

During pre-clinical Alzheimer's disease, only minor behavioural changes can be observed, mostly when shifting to mild cognitive impairment[1]. Behavioural experiments are regularly undertaken by researchers – somewhat surprisingly, the findings even within a certain strain are not always clear or consistent.

In their systematic review[32], Stewart and colleagues explored the wide range of spatial memory tests Tg2576 mice were used in. Their results highlighted that the frequency of use of a technique does not relate to sensitivity: even though Morris Water Maze was used in over half the studies explored by them, the largest cohorts either did not show impairment or had only moderate sensitivity. On the other hand, the less frequently used T-maze was found to detect genotype differences in a more sensitive manner. Their report also suggested that procedural differences might play a more important role in studying behaviour than sample size or the age of the cohort.

Interestingly, when looking at the papers published since 2010 in the UK for all mouse strains explored, most researchers opt for the same behavioural memory tests (most often the non-efficient Morris Water Maze[32]) with mice, however, one group equipped non-conventional behavioural paradigm. Romberg and colleagues[33] opted for a touch screen-automated cognitive test battery in acknowledgement of the fact that results from traditional memory tests on mice do not translate well to the presentation in humans. Their approach was inspired by the use of this technique in patient diagnosis, thereby increasing the relevance to human results with recording attention deficits and executive function alongside memory impairment. The change in paradigm was successful with their mice yielding results matching that of early-stage Alzheimer's disease patients.

Both research groups[10,11] shine light on how even the best model could be useless if the wrong tests are used. If using animals to study behavioural deficits in the future, the behavioural tests should be carefully chosen with maximising translatability.

This revelation mainly implicates the behavioural scores of my translatability assessment: I found all animal models to be presenting with the desirable behaviour – but what if these are false positives (with the exception of Romberg and colleagues' study[33]) due to the poor translatability of behavioural paradigms to human pathology?

How do researchers decide which strain to use - if any?

When choosing any research model or method, reading prior studies to explore the potentials is one of the first major steps. Reviews of pre-existing rodent models can inform scientists deciding whether to go ahead with animal models in the first place and if so, which one to use. It should not be forgotten that review articles published prior to 2014 do not include the second-generation models, but nonetheless, provide valuable information.

To decide whether an article replicates the pathology of Alzheimer's disease, having as much information as possible about the study is crucial to allow readers to judge the validity of the results themselves, and provide details of the methodology so that the study to be replicated and reproducibility assessed. The quality of the experimental design and the detail in the reporting of the studies for this reason, was assessed in my project to establish 'potential replicability' of each research paper[16].

Overall, papers using either generation scored above 65% for each strain on average, however the ratios of papers in 'poor', 'acceptable' and 'good' categories varied between groups (Figure 2). While it is reassuring to see that the 'good' and 'acceptable' articles were more than 2/3rds of each group of strains, there is still room to grow.

For my project, this means that the results found when scoring the mouse models should be interpreted carefully as, if generalised, more than 30% of the original studies were not considered potentially replicable. Research that is poorly designed and/or reported will likely have poor reproducibility which means the outcomes cannot be replicated putting a question mark over the validity of the original research. Authors and journal editors have a responsibility to ensure this is not the case by including specific details and information in published papers.

One of the most surprising outcomes of the assessment was that, despite the repeated description of the importance of the genetic background of mice[34], many papers reviewed did not include this crucial information.

Is there a future for mouse models in Alzheimer-research?

Animal (especially rodent) models are often the first choice, but that does not mean that they are the best option available. As presented above, despite the low-quality match for Alzheimer's disease pathology, first generation of transgenic mice are still equipped and presented as Alzheimer's disease-models.

The emergence of more efficient rodent models[1,15] might open a new door for further animal research, however, the impact of producing such models cannot be ignored: creating and maintaining knock-in lines like the second generation is not just time consuming but requires a high number of animals to create, around four units of rodents to create one unit of rodents for experiments[35]. The numbers are not the only

issue: the impacts of genetic modification of other circuits and animal welfare are not clear either (See more here).

If going ahead with animal experiments, following a set of guidelines like the ARRIVE 2.0 introduced last year, increases the transparency and accuracy of the published results[16]. Although hundreds of journals have endorsed this and the earlier version of the guidelines, only a few made meeting a set of ARRIVE-based criteria mandatory for in vivo experiments (Further information can be found here). One way the quality of ongoing research could be raised is by using clinically more translatable models as described by Romberg and colleagues[33].

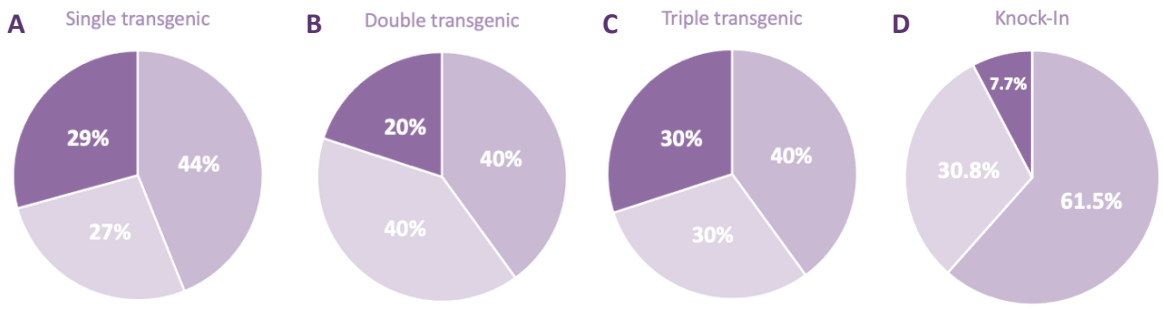
Opportunities like the F1000Research gateway and the UnTangle CRACK IT Challenge motivate researchers at all career stages to develop and equip methods that reduce or replace animal use by overcoming challenges of traditional approaches. It gives hope that even outside of these high-funded programs, computer models for real-time diagnosis of early Alzheimer's disease are being developed[36] as well as biophysical computer models of pathology guiding drug development[37].

By carefully considering options available based on more informative and replicable articles scientists can choose the best model for their purposes (animal or not), develop more sensitive and accurate computer models, and make the progress of research more efficient – consequently providing better chances for patients, their families along with reducing the number of animals used in the field[16].

Approaching Alzheimer's disease from another angle

As mentioned in the introduction, the animal models use mutations associated with early-onset Alzheimer's disease – which accounts for less than 1% of all cases. The remaining are late-onset Alzheimer's – of which the exact cause is yet to be discovered[1,4,6], although numerous common variants associated with this type have already been identified[6]. In contrast to the early-onset mutations which increase production, these appear to be linked to abnormal cleaning-up which leads to the Aβ deposits[6].

Figure 2. The proportion of papers complying with the 'ARRIVE Essential 10' guidelines. The ARRIVE Essential 10: Compliance Questionnaire was adapted for scoring 5-10 research papers for each mouse model strain. Due to the variability of the maximum scores, individual percentage scores were given for each article and the averages of those were taken for each strain. The colour code represents which category the articles were put in (medium shade = good replicability (reaching 80% or higher), lightest shade = acceptable replicability (scoring between 60-80%), darkest shade = poor replicability (achieving 60% or less)).
A. Single transgenic strains: papers from PDAPP, APP23, Tg2576, J20 and TgCRND8 research were used. (First generation)
B. Double transgenic strains: APP/PS1 and 5XFAD articles were combined. (First generation)
C. Triple transgenic strain: 3xTg-AD papers were used. (First generation)
D. Knock-in strains: the scores of NL-F and NL-G-F strains were pooled. (Second generation)



On a cellular level, "microglia" (immune cells within the central nervous system) are responsible for plaque-clearance[6], in patients with late-onset disease, however, the network of immune reactions appear to have alterations disrupting this clearing process[38]. For instance, changes in the number of copies a cell has of a gene that produces a molecule assisting Aβ-clearance[4], limits the capability to clear Aβ leading to increased inflammation in the brain[6].

Analysis of the patterns in genetic characteristics of patients with late-onset Alzheimer's disease may reveal potential targets for preclinical diagnosis as well as give us a better understanding of the underlying mechanism behind more than 99% cases.

2. Conclusions

In the review I have carried out here it is clear choosing the right model is not enough, especially when it comes to the use of animal models. The design of the experiment, memory test type and reporting of the methods and results all influence the outcomes of the research and how they can be used. Using rodent models as discussed above, and following the ARRIVE 2.0 guidelines or similar, improves the transparency and replicability of the outcomes leading to a bigger potential impact on science in the future.

It must also be understood that the use of animals is not the only route for Alzheimer-research – genome wide association studies ("approach that involves rapidly scanning markers across the complete sets of DNA, or genomes, of many people to find genetic variations associated with a particular disease" as defined by the National Human Genome Research Institute) can provide information about the late-onset Alzheimer's disease and other nonanimal, human relevant research methods may hold the key to improving our understanding of the underlying mechanisms causing Alzheimer's disease and improve treatment options.

The continuing focus on improving the characteristics of genetically modified mouse strain for this type of research may produce a living model that is closer to the human condition but will still have limitations and require large numbers of animals to produce – making it even more prominent that the appropriate behavioural tasks are used (if used) and the results are appropriately reported.

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