Why have transgenic rodent models failed to successfully mimic Alzheimer's Disease. How can we develop effective drugs without them?

Rafael Franco[1,2], Eva Martínez Pinilla[3,4,5] and Gemma Navarro[2,6]

[1]Dept. of Biochemistry and Molecular Biomedicine. Faculty of Biology. Universitat de Barcelona. Barcelona. Spain.

[2]CiberNed: Centro de Investigación en Red, enfermedades Neurodegenerativas. Instituto de Salud Carlos III. Madrid.

[3]Departamento de Morfología y Biología Celular. Facultad de Medicina. Universidad de Oviedo. Asturias. Spain.

[4]Instituto de Neurociencias del Principado de Asturias (INEUROPA). Asturias. Spain.

[5]Instituto de Salud del Principado de Asturias (ISPA). Asturias. Spain.

[6]Dept. of Biochemistry and Physiology. Faculty Pharmacy and Food Sciences. Universitat de Barcelona. Barcelona. Spain.

Corresponding authors:

Rafael Franco
Dept. of Biochemistry and Molecular Biomedicine. Faculty of Biology.
Universitat de Barcelona.
Diagonal 643. Prevosti Building. 08028 Barcelona. Spain.
Tel +34934021208.
rfranco@ub.edu
rfranco123@gmail.com

Eva Martínez-Pinilla
Departamento de Morfología y Biología Celular.
Universidad de Oviedo.
Julián Clavería s/n. 33006. Asturias. Spain.
martinezpinillaeva@gmail.com

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1. Introduction

Alzheimer's disease (AD) does not yet have an adequate animal model and, in addition, the mechanisms explored so far have not led to efficacious therapies. Some of the issues concerning animal models of AD are covered elsewhere [1]. AD animal models are mainly transgenic and developed using tools constructed taking into account research in heritable early-onset (EOAD) cases. Unfortunately, these cases only account for a 10% of AD since the majority of cases are "sporadic"; in those the main risk factor is age and causes/mechanisms are multifactorial. Good expectation was due to the fact that transgenic models, upon aging, presented to so-called "pathological AD hallmarks": amyloid plaques and neurofibrillary tangles. During last decades, advances using these models have detected potential interventions for either EOAD or sporadic AD. In this sense, pharmaceutical companies have bet for mechanisms of disaggregation of amyloid plaques using a variety of approaches among them the use of antibodies has become fashionable. However, the results have not yet provided the desired outcomes and there is even the doubt about the real impact of amyloid aggregates in AD pathogenesis. It is known that individuals without the disease may have significant number of amyloid plaques. In fact, decades ago there was even a controversy about the beneficial or detrimental role of beta amyloid deposition.

2. Transgenic AD models

AD models are mainly transgenic and expressing mutant forms of human proteins that are related to EOAD (amyloid precursor protein (APP), presenilins and tau). Their development has been mainly guided by the need to have early AD-like cognitive impairments to speed-up the discovery of therapeutic targets. The transgenic Tg2576 animal carries the double ("Swedish") mutation of human APP ($K^{670}N$, $M^{671}L$) in the C57BI/SJL background begins to show cortical and hippocampal amyloid plaques and to express cognition deficits at 10-16 months of age [2,3]. Noteworthy, the AD-like phenotype may be accelerated by mixing two mutation in the transgene, e.g. the Swedish and Indiana ($V^{717}F$) mutations of the human APP ($APP_{Sw,Ind}$ transgenic line) [4]. The phenotype may also be accelerated by crossing the Tg2576 animal with another carrying the transgene of human presenilin 1 $M^{146}L$ mutation [5]. Interestingly, the mouse that only has the presenilin-1 transgene does not display any AD-like pathology although the brain levels of amyloidogenic APP-related peptides are increased [6]. Apart from doubts on real usefulness of these models, it is important to highlight that each experiment involving behaviour requires the use of aged animals. In

addition, it is a risk to put the focus only in one of the two major pathological hallmarks of AD. Sporadic AD is a multifactorial disorder that involves different cytotoxic events acting in conjunction; those aspects should be considered when exploring therapeutic strategies. On the one hand, the role of amyloid beta deposition, even today, is not entirely clear. In fact, some studies proposed that amyloid beta peptide is toxic for neurons, however, other authors suggest that it may part of a cellular defense system in AD brains. Added to this, the accumulation of this protein is not restricted to diseased brains but occurs across all ages, from infants to older individuals without a specific symptomatology. On the other hand, animals have incorporated transgenes for tau in order to obtain animals displaying aberrant tau phosphorylation and intracellular tangles [7]. Nowadays, one of the most used, is the so-called "triple" 3xTg-AD mouse that harbors transgenes of mutants of APP_{Sw}, of presenilin 1 ($M^{146}V$) and of tau ($P^{301}L$) [8]. The main advantage the significant shortage in the time for cognitive deficit expression.

Failures to find appropriate therapies for patients using these models, which were at first very promising, are multifactorial and we will here highlight two of them. One is the clear inability of current animal models to represent the full range of events identified in the human disease, for instance, the neuronal loss. It should be noted that a recent report using a *Drosophila* model suggests that neuronal loss may be protective in AD [9]. This opens the door to novel hypothesis that if proven would be quite atypical as in other neurodegenerative conditions, e.g. Parkinson's and Huntington's diseases, where neuronal loss is the main neuro pathological feature [10–13]. Another factor is recent evidence suggesting that $APP_{Sw,Ind}$ mice are already born with M2-skewed activated microglia, which displays a neuroprotective phenotype [14] and may lead to long-lasting prevention of neuronal death. This finding may constitute the basis to target microglia to afford neuroprotection.

3. Approaches to address symptoms

There are already enough pharmacological targets and tools to propose novel clinical trials. The failed attempts to demonstrate that antibodies can remove amyloid deposits have led to a shortage of patients willing to participate in clinical trials to test all the compounds that have provided benefit in transgenic animals. A paper has suggested that "*Careful analysis of why different approaches reduce symptoms and/or degeneration in animals will surely help in accelerating translational research and provide human-successful medications to fight the two sides of AD: neurodegeneration and cognitive dysfunction"* [1]. Apart from the neuroprotection issue that is addressed below, we really think that objective tools may be used by people not directly involved in AD research to select a few molecules that in a supranational effort could be tested for safety and efficacy in enhancing patient's cognition and life quality. Once the clinical trials reach phase II with public money/resources, it is expected that the pharmaceutical industry will take over to arrive to phases III and IV with those selected compounds. We would like to highlight again that a good stratification of patients (also requiring objective tools and experts not directly involved in the disease) is instrumental for proper analysis to select promising drug candidates.

4. The difficulties in demonstrating neuroprotection

The main problem with any neuroprotective approach, especially if it is pharmacological or based in gene or cell replacement therapies, is the impossibility to demonstrate neuroprotective effects in humans. Although, FDA rules are well worked out and expert

committees take the most convenient decisions, those rules should be revisited to be able to demonstrate neuroprotection. This fact is relevant in this Editorial for two reasons. One is related to the lack of neuronal loss in current transgenic models; therefore, drug discovery using those models will address symptoms. Importantly, these animals may be instrumental to study mechanisms involved in preventing neuronal loss (see [14] for the potential involvement of protective microglia) and to ascertain why, despite young animals already overexpress "toxic" proteins, the onset of cognitive symptoms occurs upon aging. Interestingly, extensive preclinical research has provided already available candidates for neuroprotection. Some are even approved; this is the case of an adenosine A_{2A} receptor antagonist, available for prescription in Japan as coadjuvant in the therapy of Parkinson's disease (PD) (see [15]), that is reported as neuroprotective for AD [16].

A seemingly alternative approach would consist of preventing cognition loss by improving life styles and by the use of "memory enhancers". Due to space limits, it is impossible to enter into some of the details covered in the literature. In brief, the underlying idea is to establish a program of long-term consumption of already available safe supplements, and check cognitive status in the long run. Obviously, such interventions should be incorporated into global (national or multinational) epidemiological studies to assess for both adherence and potential benefits of this "chronic" treatment.

5. Conclusion

It is unlikely that established or alternative animal AD models will, in the near future, provide novel candidates for combating the human condition. Some of the drugs with proved usefulness in AD animal models could be already being selected to be tested in patients. In this sense, a holistic approach with a marked epidemiological component and centered in humans seems a very good option to advance in finding solutions to combat dementia and/or afford neuroprotection.

6. Expert opinion

Cell engineering to make iPS pluripotent cells as a model of AD [17] is, in our opinion, not very promising. There are not yet appropriate tools/biomarkers to validate stem cells themselves as a model for AD. Moreover, the successful model likely requires a whole brain; hence, cultured cells would not be enough to mimic the different aspects of the pathology. Even the use of flies may not be appropriate as model of neurodegenerative diseases. Despite compelling evidence about nicotine and caffeine being beneficial (neuroprotective and/or decreasing the risk of suffering the disease) in PD [18,19], it has been reported that in "*Drosophila* PD models" coffee without caffeine and tobacco without nicotine afford neuroprotection [20]. Instead, non-transgenic models may be considered; one of the most promising carries no transgene in nuclear DNA but mitochondria coming from transgenic mothers. The model suggests that maternal imprinting confers higher risk for AD-like phenotype display [21]. Apart from usefulness to study maternal transmission it may be helpful to detect targets for early preventive interventions. Research using animal models of AD should likely decrease and the best choice would be to focus in humans.

Analysis of necropsies and *post-mortem* imaging of affected brain regions from patients are still necessary but less than decades ago, when A. Alzheimer described the pathological findings for the first time. Once neglected but gaining momentum now is the work with alive

humans, i.e. with patients and close relatives. A big issue when working in the disease/human interface is variability but another is to put the risk factors related with the disease into the focus, i.e. into the probabilities of suffering the disease by having such or such genetic polymorphism or elevated concentration of a compound in body fluids. Biomarkers are needed but, unfortunately, they are mainly implemented when the disease has started and not in prodromal stages. In other words, a good biomarker for the course of disease may not be such in a prodromal stage (take glycosylated haemoglobin and diabetes as example). The search for biomarkers should include both a good stratification of patients (surely the actual score is only possible after death) and also collaboration from age-matched controls. Indeed, a difficult year-consuming task but very beneficial if appropriately designed and performed. As in other human interventions, an extensive and global collaboration is desirable to obtain data from thousands of patients and controls.

Research on prevention in AD is more relevant than in any other disease. While difficult to implement, there are advantages in the neurodegenerative diseases whose prevalence correlates with age (AD but also PD, etc.). If clear evidence is shown, people can take preventive approaches with enthusiasm. This issue brings up a need, which is to a drift/shift in the type of AD-related research, from being molecular using animal models, to be more epidemiological (but with the right focus). Two examples will illustrate our opinion. A general belief, recently covered in the mass media, is the potential benefit of music/dancing. This tactic in nursing homes surely provides smiles but the impact in AD pathology seems negligible. Also, the level of studies and of general culture have been taken into account since the first findings from the longitudinal "nuns study" [22]. What it would be very relevant is getting a graphic of the progression of behaviour-based (mini-mental state exam –MMSE- etc.) cognitive tests with age in as many individuals as possible besides making multiple correlations with life style, education, hobbies, etc. We take profit of this forum to propose a study on whether professional music players have more or less risk to develop AD. On the one hand, music learning (in a professional or semi-professional manner) likely increases the central nervous system (CNS) neuronal connections which (theoretically) prevents neuronal death. On the other hand, this hypothesis may be complemented with data on music playing schedules (every day rehearsal, every week playing in a bar/club etc.). Proven or disproven, this hypothesis may lead to very relevant information.

Surely, nuclear magnetic resonance (NMR) studies of all kinds (i.e. also functional magnetic resonance imaging (fMRI)) are still needed and probably provides better information before the appearance of clinical symptoms. Sophisticated NMR equipment will be becoming cheaper and more potent to reduce the scanning time. In parallel, the health-related information of smart watches may be included in the epidemiological data. Especially important in our mind is the heart rhythm, the oxygen saturation (smart watches may hopefully be designed to measure it) and quality of the sleep. In that sense, we wonder whether there is any study correlating severe snoring, i.e. the one causing <90% oxygen saturation, and onset age of dementia.

Potential AD biomarkers include but are not limited to the level of metabolites, neurotransmitters and exosomes in body fluids. Surely, the discovery of reliable biomarkers would lead to more possibilities of study the disease in humans, but also to find more appropriate animal models. Even one may speculate that reliable biomarkers obtained by research in humans (controls and patients) may help in advancing towards successful therapies using the already available transgenic AD mice models.

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