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Misfolded amyloid-β strains and their potential roles in the clinical and pathological variability of Alzheimer's disease

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Potential causes for the clinical and pathological variability observed in Alzheimer's disease (AD): AD is an age-related neurodegenerative disorder characterized by the impairment of cognitive functions such as memory, learning, and reasoning. These commonly described clinical symptoms are due to particular pathological changes in the brain, including inflammation, synaptic loss, and neuronal death. These changes are a consequence of the accumulation of abnormally folded amyloid-β (Aβ) and tau proteins in specific areas of the central nervous system. Considering the progressive aging of the world's population, the number of people affected by AD is expected to substantially and consistently increase in the coming years. This positions AD as one of the main public health challenges in the near future.

It is important to note that AD is clinically and pathologically diverse. For example, some of the clinical manifestations of the disease, such as the age of disease onset, the degree and rate of cognitive decline, and the duration of the disease can vary among patients. Additionally, variability in AD pathology can be found in the degree of atrophy in the patients' brains as evaluated by imaging techniques (Zhang et al., 2016), dissimilarities in plaque morphology (Thal et al., 2015; Duran-Aniotz et al., 2021), the tropism of amyloid pathology to brain or vascular structures, and the variable clinical manifestations associated with Aβ plaques (e.g., similar Aβ plaque load in clinically advanced AD patients *vs*. cognitively normal individuals (Zolochevska and Taglialatela, 2016)).

These clinical and pathological variabilities resemble pathological events observed in prion diseases, which are caused by the misfolding and abnormal accumulation of the disease-associated prion protein (PrP^{Sc}). In prion diseases, several conformations or "strains" of PrP^{Sc} trigger clinically and pathologically diverse diseases (Morales, 2017). Considering the accumulation of the misfolded Aβ peptide as an early event in AD pathogenesis, changes in its conformation may be at the root of the clinical and pathological diversity observed at late stages. This mini-review will introduce the concept of "Aβ strains" and discuss relevant literature concerning this intriguing subject.

Prion-like transmission of misfolded proteins and prion strains: Due to the typical deposition of misfolded Aβ in the AD brain, this disease can be classified as a protein misfolding disorder (PMD). PMDs are associated with a multitude of proteins that are prone-to-misfold including, but not limited to, Aβ and tau in AD, α-synuclein (α-syn) in Parkinson's disease, and PrP^{Sc} in prion diseases. Conformational changes in these proteins provide them with common biochemical properties and intrinsic toxicity (Morales, 2017). These deleterious conformations self-propagate at the expenses of physiologically synthesized/normally folded proteins. These events, first described for infectious prions, are now widely accepted to occur in all proteins associated with PMDs. This suggests that prion-like mechanisms are at the core of all these diseases. Whether all PMDs-associated proteins act as infectious agents, similar to prions, is still a matter of intense research and debate.

The features defining prions as infectious agents have been tested in several PMDs-associated proteins. Among them, misfolded Aβ aggregates have been shown to accelerate brain amyloidosis in susceptible mice through either intra-cerebral or peripheral routes of exposure (Morales et al., 2021) and induce pathology in mice that do not typically express amyloidosis (Morales et al., 2012). Interestingly, misfolded Aβ structures seem to be polymorphic, and arrange in different conformations or "strains". As mentioned above, this resembles the case of prion strains, which are associated with different clinical and pathological manifestations (Morales, 2017). In addition to the clinical and pathological divergence they induce, prion strains can be differentiated by their specific biochemical properties, such as resistance to proteolysis, conformational stability, seeding activity, and size distribution of aggregates, and many other features (Morales, 2017). Analogous properties have been identified for disease-associated Aβ in *in vitro* and *in vivo* systems (Petkova et al., 2005; Eisenberg and Jucker, 2012; Makowski, 2020; Lau et al., 2021). However, the biological significance of these "Aβ strains" has not yet been elucidated. Current evidence supporting the existence of pathological Aβ strains is summarized in the following section.

Conformational variation in misfolded Aβ and links with AD pathological and clinical diversity: Brain Aβ deposition is a distinctive feature of AD and it is attributed as an early change triggering tau accumulation, brain inflammation, synaptic loss, and neuronal death. As discussed above, Aβ aggregates in AD brains can be found in a variety of arrangements, including intra-cellular aggregates, diffuse plaques, vascular deposits, soluble Aβ oligomers, dense-core senile plaques, and many others (Thal et al., 2015). These different morphological deposits are reminiscent of tau inclusions seen in different tauopathies (Clavaguera et al., 2013) or PrP^{Sc} deposits induced by different prion strains (Morales, 2017). Considering this, it is plausible that the different Aβ deposits observed within and across AD patients could be composed by conformationally different misfolded Aβ aggregates.

Aβ strains can self-propagate in *in vitro* and *in vivo* systems, similar to how *bona fide* prion strains do (Castilla et al., 2008). Early experiments by Petkova et al. (2005) that used purified/synthetic Aβ peptides were able to produce two different misfolded fibrillar polymorphs through different aggregation protocols. These misfolded Aβ aggregates, which were extensively characterized for their conformational motifs at the atomic resolution level, were the first evidence demonstrating that Aβ is able to self-propagate different disease-associated conformations. This fact was later confirmed by several other experiments using susceptible animal models overexpressing mutated forms of the human Aβ precursor protein. Specifically, brain-derived or synthetically generated Aβ aggregates were shown to differentially propagate in susceptible animals (reviewed in Lau et al., 2021). Although these experiments confirmed the initial report using synthetic peptides (Petkova et al., 2005), they largely failed in exploring the biological significance of Aβ strains in a disease context. More recently, some studies suggest that the conformational stability and size distribution of Aβ aggregates are responsible for rapidly progressive or slowly progressive disease phenotypes (Cohen et al., 2015). Follow-up studies by the Tycko's group have demonstrated that amyloid deposits derived from different AD patients are associated with conformationally different Aβ fibrils (Qiang et al., 2017). Future research is needed to clarify whether conformational variants of Aβ activate specific disease pathways, and thus lead to pathologically and clinically variable AD.

A recent report from our group used AD patient brains with differing amyloid pathology to demonstrate that their misfolded Aβ seeds were able to induce diverse pathological traits in susceptible mice (**Figure 1**; Duran-Aniotz et al., 2021). In summary, this study included *APP*/*PS1* transgenic mice inoculated with brain homogenates from patients diagnosed with AD dementia. However, the patients displayed a diverse array of pathological features. Specifically, clear differences in the Aβ deposits, such as the shape, distribution, reactivity to amyloid-binding dyes, and tropism to blood vessels were observed. Interestingly, these differences were reminiscent of the strain-specific pathological features observed in the brains of people affected by different strains of infectious prions. Most of the AD-brain treated *APP*/*PS1* mice displayed significant amyloid burdens compared to control groups, however, the induced pathology was dependent on the specific inoculum used. Notably, differences seen in the same parameters listed above were also recorded in treated mice. This strongly suggests that the Aβ seeds present in the diseased individuals are responsible for the variable pathological outcomes observed between them. In turn, this may explain the clinical differences largely reported across individuals afflicted by this specific type of dementia.

Tauopathies involve conformational variants of misfolded proteins: Hyperphosphorylated-misfolded tau is an important contributing factor in AD. It is widely accepted that cognitive decline correlates more directly with tau rather than amyloid pathology. In that sense, differences at the tau level need to be considered when trying to explain clinical variation in AD.

Tau is involved in several clinically diverse diseases that are known as tauopathies. Importantly, tauopathies are associated with unique tau arrangements that propagate disease-specific features in susceptible hosts (Clavaguera et al., 2013). These pathological differences observed across tauopathies may be due to the different tau isoforms preferentially recruited in each disease, among other factors. Recent reports show that these clinically variable tauopathies are actually linked with different structures of misfolded tau (Shi et al., 2021). These experiments demonstrate the conformational plasticity of misfolded tau

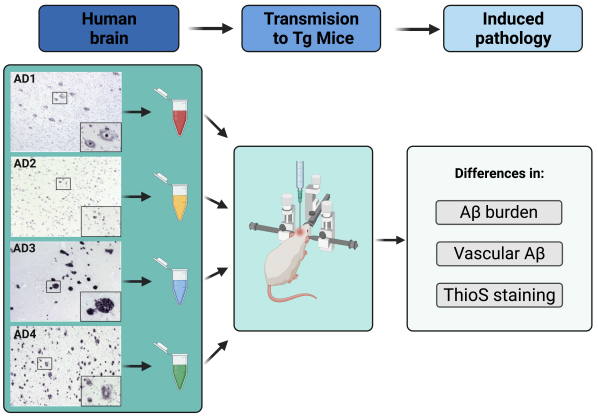

Figure 1 | Amyloid-β (Aβ) pathology arrangements in Alzheimer's disease (AD) brains promote dissimilar pathology in susceptible mice. Brains from AD patients displaying variations in their Aβ pathology were homogenized and intra-cerebrally inoculated into susceptible transgenic (Tg) mice (Aβ over-expressors). Mice were allowed to incubate Aβ seeds for five months and sacrificed to assess for induced pathology. Experimental mice presented differences in their brain pathology as assessed by histological means (amyloid burden, vascular amyloidosis, and reactivity of amyloid deposits to thioflavin S (ThioS)). Modified from Duran-Aniotz et al. (2021).

proteins, and strongly suggest an active role of tau polymorphs across tauopathies. Whether tau conformational differences contribute to AD features needs to be evaluated in future studies.

Conclusions and perspectives: It is now widely accepted that misfolded Aβ and tau proteins spread in a manner akin to that of prions. In that sense, several properties of infectious prions have been attributed to these proteins. Among them, both disease-associated Aβ and tau aggregates appear to adopt different conformations, reminiscent of prion strains in prion diseases (Morales, 2017). Considering the variable clinical manifestation observed among AD individuals, it is plausible that differences in the conformation of misfolded proteins may explain why this is occurring. There is compelling evidence suggesting that different tauopathies are associated with different conformations of the tau protein (Shi et al., 2021). Changes in Aβ conformation have also been shown between AD patients (Qiang et al., 2017). Whether clinical variation in AD is due to misfolded protein strains, and whether these differences are encoded in Aβ, tau, or both proteins, is still unknown.

Considering Aβ misfolding as one of the earliest events in AD, it is logical to think that conformational variants of this protein may differentially activate pathways leading to different clinical outcomes years later. In the same line, putative tau conformations in AD may be a result of the interaction with different Aβ strains. An intriguing case involving brain Aβ amyloidosis involves the so-called "non-demented Alzheimer's neuropathology" individuals (Zolochevska and Taglialatela, 2016). These cognitively normal patients, displaying substantial accumulation of Aβ in their brains without clinical manifestations, are an enigma and several groups are actively working to identify what makes these patients' brains more resilient. On one hand, several researchers support the idea that Aβ deposition is a normal event in aging with questionable relevance in pathophysiological events. This is supported by several failed clinical trials showing that amyloid reduction provides no significant improvements in cognition (Ackley et al., 2021). However, the failure of these trials may be due to several factors, including starting the treatments when extensive brain damage has already occurred. In that sense, early treatments may be key to preventing or substantially delaying brain pathology and subsequent clinical outcomes as suggested in preclinical models (Uhlmann et al., 2020). On the other hand, decades of research (not discussed here for space constraints) have strongly established a relevant (or possibly a major) role of misfolded Aβ in AD (Hardy and Higgins, 1992; Bloom, 2014). Considering the information presented above, it is plausible that specific conformations of misfolded Aβ are responsible for non-demented Alzheimer's neuropathology. If proven, this will perfectly fit with the Aβ strain hypothesis explaining the pathological and clinical variability observed in AD.

The identification of Aβ strains may be beneficial on several fronts. Mechanistically, the identification of the most deleterious particles will provide us with better tools to pharmacologically modify this disease. In terms of diagnosis, the early identification of the Aβ strain type may allow us to provide a more accurate prognosis and better treatment plans on an individual basis. In fact, personalized medicine, targeting the patient's most relevant Aβ strains, is envisioned if the existence and relevance of Aβ strains are finally confirmed.

We apologize for the many missing references that should also be quoted. Several review articles have been listed for further reading.

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