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COMMENTARY LETTER

Calling α-synuclein a prion is scientifically justifiable

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For more than a decade, the "prion-like" hypothesis for Parkinson's disease (PD) has remained one of the most controversial topics in neurodegenerative disease research [21, 34]. Seminal studies revealed that α-synuclein pathology in the brains of PD patients follows a stereotypical progression pattern and that α-synuclein aggregates can spread between cells [3, 9], leading to the theory that a cell-to-cell propagation of protein misfolding may drive disease progression in PD and related synucleinopathies such as dementia with Lewy bodies and multiple system atrophy (MSA). At the core of the prion-like hypothesis is the notion that self-propagating α-synuclein aggregates are able to escape from a cell, enter a neighboring cell, and then act as a seed to induce the aggregation of α-synuclein in the recipient cell. This non-cell autonomous mechanism is similar to what occurs in prion diseases such as Creutzfeldt-Jakob disease (CJD), where misfolded prion protein (PrP) catalyzes the conformational conversion of normal PrP into additional copies of the misfolded form (PrP^{Sc}). The ability of prions to self-propagate allows them to spread within host tissues and underlies the transmissible nature of the prion disorders [29]. Given the similarities between α-synuclein aggregates and prions, it is not surprising that there has been considerable debate over whether they should be referred to as "prions", "prion-like", or something else entirely [33].

A key piece of evidence supporting the idea that α-synuclein can become a prion is that pre-formed α-synuclein aggregates induce a progressive synucleinopathy when intracerebrally injected into susceptible transgenic mice [15, 20, 27, 35]. Non-transgenic animals also develop cerebral α-synuclein pathology following inoculation with α-synuclein fibrils, albeit in the absence of a lethal neurological disease [14, 17]. The ability of α-synuclein pathology to be transmitted to laboratory rodents with the concomitant development of neurological illness and neurodegeneration in some circumstances is essentially identical to the transmission of prion disease to mice following inoculation with a prion-containing sample. While direct inoculation into the brain is the most efficient way of transmitting experimental prion disease, it is also possible to initiate disease via multiple different routes of peripheral inoculation [16]. The neuroinvasive properties of prions allow prion disease to be transmitted between individuals under rare circumstances, such as the cannibalism of infected brain tissue in the case of kuru [18], iatrogenic CJD deriving from the use of prion-contaminated growth hormone extracts [6], and the transmission of variant CJD via blood transfusion [22].

A recent article from the Tamgüney lab has demonstrated that oral or intravenous challenge of M83 transgenic mice overexpressing mutant human α-synuclein with recombinant α-synuclein fibrils initiates a progressive synucleinopathy characterized by overt motor dysfunction as well as pathological deposition of α-synuclein aggregates within the brain [13]. Importantly, the authors used hemizygous M83 mice in their studies, which do not develop a synucleinopathy spontaneously, allowing them to detect disease transmission following extended incubation periods of more than 1 year following inoculation. These results clearly indicate that α-synuclein aggregates, like prions [16], are transmissible by the oral and intravenous routes, which is in agreement with other studies demonstrating the emergence of a cerebral synucleinopathy in transgenic mice following peripheral inoculation with α-synuclein aggregates [1, 5, 28, 32]. While one could argue that these transmissions were aided by the use of a mouse model that overexpresses a mutant form of α-synuclein under the control of a non-native promoter [7], the very recent demonstration that peripheral application of α-synuclein fibrils in non-transgenic mice can also induce cerebral α-synuclein pathology and behavioral deficits leaves little doubt that α-synuclein aggregates

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possess an innate ability to propagate from the periphery to the brain [12]. This may have important implications for understanding the genesis of PD, as it has been speculated that the disease may originate in the gut before propagating to the brain in a prion-like fashion [4].

Given that α-synuclein aggregates exhibit several other key characteristics of prions, such as the ability to exist as distinct strains and resistance to inactivation by formaldehyde [2, 23, 30], why has there been such reluctance to label them as prions? One distinction that is brought up frequently is the issue of transmissibility, as there is currently no evidence that any of the human synucleinopathies can manifest via an infectious etiology [10]. The original definition of a prion, as coined by Stanley Prusiner, is a "small proteinaceous infectious particle which is resistant to inactivation by most procedures that modify nucleic acids" [26]. In an attempt to encompass prion phenomena occurring across many areas of biology, Prusiner has since updated the definition of a prion to "proteins that acquire alternative conformations that become self-propagating", leaving out the requirement for infectivity [25]. Using the modern definition, α-synuclein aggregates should unequivocally be classified as prions, as there is strong evidence that α-synuclein can polymerize into aggregates that are able to self-multiply during disease, leading to "cellular transmission" of protein aggregation [8]. However, for many, infectivity remains the defining feature of a prion. As described above, there is ironclad evidence that α-synuclein aggregates can transmit disease to mice, including by natural routes of infection as demonstrated in the Tamgüney paper [13]. Should the lack of evidence for disease transmission between humans prevent α-synuclein from becoming a member of the prion club?

There is no question that most, if not all, prion diseases involving PrP^{Sc} have the potential to be transmissible under experimental conditions. However, while prion diseases such as scrapie in sheep and chronic wasting disease in deer are clearly transmissible between animals by natural routes [19], the vast majority of human prion diseases are not naturally transmissible. Cases where interindividual transmission has occurred have arisen accidentally due to medical intervention or via non-natural practices such as cannibalism [6]. As far as we know, the most common human prion disorder, sporadic CJD, is not transmissible via any natural route of infection. Thus, person-to-person transmissibility is not an obligate feature of the prion diseases, and neither is the ability to be transmitted between species. Most of the time, interspecies prion transmission is severely restricted by the "species barrier". The most famous example of interspecies prion transmission occurred with bovine spongiform encephalopathy (BSE), or "mad cow disease", which caused variant CJD in a subset of exposed individuals [37]. However, it could be argued that even this was not a "natural" transmission, as it is probable that the repeated passaging of BSE prions in cattle, via the inclusion of contaminated bovine tissue in animal feed, led to the selection of a prion variant with an artificially enhanced ability to transmit between species. Therefore, interspecies prion transmission is more of an exception rather than a rule for prions.

While there is sufficient scientific justification for placing the prion label on α-synuclein aggregates, it would be disingenuous to imply that PrP^{Sc} and "prions" composed of aggregated α-synuclein are fully alike. First, it is likely that α-synuclein aggregates are considerably less infectious than PrP^{Sc}. In the Tamgüney paper, large quantities of α-synuclein fibrils (50 or 500 µg) were used in the peripheral inoculation studies, and only a subset of the orally-exposed mice developed disease [13]. The specific infectivity of α-synuclein aggregates, that is the level of infectivity per amount of protein, is not known but might be orders of magnitude lower than that of prions. Picogram quantities of purified PrP^{Sc} are infectious by the intracerebral route [36] whereas most α-synuclein transmission studies use microgram quantities of pre-formed α-synuclein fibrils to initiate disease transmission. Second, prions are notoriously difficult to destroy and therefore require harsh conditions for complete inactivation. The extreme resilience of prions likely contributes to the high level of infectivity they possess. While certain types of α-synuclein aggregates may also exhibit enhanced resistance to inactivation [38], matching the resistance displayed by PrP^{Sc} seems improbable. Third, even within the spectrum of α-synuclein aggregates there is likely to be considerable variability in pathogenicity. For instance, several studies have illustrated that α-synuclein aggregates from MSA patients are much better at seeding α-synuclein pathology than aggregates from PD patients [24, 39, 40]. Thus, if α-synuclein is indeed a prion, it is probably a less pathogenic prion than PrP^{Sc}.

Setting the science aside for a moment, there are other considerations when deciding whether to call α-synuclein a prion. Admittedly, the term "prion" has a lot of baggage associated with it. For non-specialists, the first thing that likely comes to mind when the word prion is used is the BSE epidemic that occurred in Europe and the associated transmission of the disease to humans in the form of variant CJD. Hence, concern has been expressed that attaching the prion label to α-synuclein aggregates in PD or MSA may lead health practitioners and caregivers to assume that these disorders are communicable from person-to-person which, as mentioned above, is highly unlikely to be true. Calling α-synuclein a prion may also have implications for biosafety since prions, especially those that are potentially infectious to humans, necessitate enhanced biosafety measures. Stricter biosafety requirements have the potential to restrict brain bank donations or stunt research progress on these disorders since not all investigators may have access to appropriate

facilities. While it may be prudent to exercise additional caution when studying certain types of α-synuclein aggregates, perhaps tissues from MSA patients, the relative risk posed by investigating α-synuclein aggregates under routine laboratory settings is likely to be vanishingly small. To combat unintended consequences of utilizing the prion nomenclature, it would be critical to be proactive in preventing the spread of misinformation, which in itself seems capable of rapidly proliferating in a prion-like fashion.

Had the term prion been utilized to describe the self-propagating properties of α-synuclein aggregates in PD and MSA prior to the discovery of PrP and prion diseases, it is conceivable that there would have been no issue with labelling PrP^{Sc} aggregates in CJD as a particularly aggressive type of prion. There is no written law that all prions need to be of equal pathogenicity. The term "virus" when used in isolation means only a small, acellular infectious agent that requires a host cell to replicate. It could refer to dangerous viruses such as Ebola or smallpox that pose a high risk to humans and require extensive biosafety precautions for study, or it could refer to more benign viruses, such as rhinovirus, which causes the common cold. Likewise, the term "bacteria" could refer to anything from non-pathogenic laboratory strains of *Escherichia coli* to *Bacillus anthracis*, which causes anthrax in humans. The same subclassification of relative risk could equally be applied to different types of prions. For instance, PrP^{Sc}, particularly that of human or bovine origin, would represent the highest risk whereas prions composed of α-synuclein or other non-PrP entities would be classified as lower risk commensurate with their known properties.

Despite the ongoing nomenclature debate, the underlying science of the prion-like propagation hypothesis for PD seems to have gained widespread acceptance within the field, and the paper from the Tamgüney group has added an important piece to the puzzle [13]. Research into how the cell-to-cell transmission of α-synuclein aggregates contributes to the pathogenesis of PD and related disorders will continue even in the absence of consensus terminology. Indeed, several novel therapeutic and diagnostic strategies motivated by the similarities between prions and α-synuclein aggregates have emerged, such as using antibodies to intercept the cell-to-cell propagation of α-synuclein and detecting minute quantities of α-synuclein aggregates in cerebrospinal fluid from synucleinopathy patients by seeded amplification [11, 31]. In the end, it is the science, not the nomenclature, that matters most. It may ultimately be decided that α-synuclein should not be called a prion to avoid unwanted side effects, which is certainly not an irrational point of view. However, this should not be misconstrued as a lack of evidence, as there is overwhelming scientific justification for calling α-synuclein aggregates prions.

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