Prion-like intracerebral and peripheral transmission of amyloid-β and infectivity of Alzheimer's disease

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Abstract:
Due to the ageing population, the amount of Alzheimer's disease patients is rapidly increasing. Prion-like properties of one of the main factors in Alzheimer's, amyloid-β, raised concerns whether it also shared prion-like infectivity. Amyloid-β forms seeds in Alzheimer's patients which cause misfolding of more amyloid-β and leads to aggregation and senile plaques. These seeds are transferrable through intracerebral, intraperitoneal and intravenous injection of amyloid-β rich brain extracts. They are also transferrable through injections with cadaveric human pituitary glands infected with Creutzfeld-Jakob disease, contaminated neurosurgical equipment and blood transfusions from donors with high amyloid-β levels. When transferred to a new host, these seeds cause amyloid-β deposits in the arteries of the brain and in the brain parenchyma. This leads to cerebral amyloid angiopathy increasing the risk of strokes, but not to the full pathology of Alzheimer's disease. The most worrying routes of transportation are through neurosurgical equipment and through blood transfusions, since these procedures happen in the medical world. Injection with amyloid-β rich brain extracts or cadaveric human pituitary glands never happen to patients, and are not a risk. The two routes of transmission that do pose a risk are not fully understood, so the full extent of the danger is not known. These routes of transmission require further experimental and epidemiological investigation.

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Introduction:

Due to advances in medical knowledge and application, the worldwide population is on average getting much older. According to the World Health Organization by 2050 the percentage of total population that is over 60 years old will rise from the 12% it currently is to 22% [1]. This comes with a lot of complications, one of which is an increase in number of patients suffering from Alzheimer's disease (AD). The worldwide number of AD patients is projected to increase from 46.8 million patients in 2015, to 131.5 million patients in 2050 [2]. AD is already the most common cause of cognitive decline in the population above 60 years of age [3], with a large effect on quality of life. There is still much that is not understood about AD, and there are no effective treatments yet.

AD is a chronic neurodegenerative disease that is characterized by a large loss of neurons, leading to a reduction in brain matter. One of the key elements of AD is the presence of senile plaques in the brain. The main component of these plaques is amyloid-beta (Aβ), which is formed by the cleavage of human amyloid precursor protein (APP) into monomers. APP is a membrane protein found mostly in synaptic membranes of neurons. These cleaved monomers aggregate to form neurotoxic Aβ oligomers. Further aggregation of the Aβ oligomers lead to the formation of extracellular fibrils. Once this happens the fibrils become resistant to proteolytic cleavage, and can no longer be removed by the body [4].

The main neurotoxic part of Aβ are thought to be the soluble oligomers [5]. These oligomers cause neuronal cell-death through mitochondrial dysfunction, loss of synapse function leading to cognitive decline and inhibit hippocampal long-term potentiation which affects learning and memory [4]. There is currently little evidence for the toxicity of Aβ monomers, and the toxicity of the fibrils is under discussion.

What causes the aggregation has been a mystery for a long time. It has been hypothesized that the accumulation of Aβ is caused by overproduction of Aβ or by failure of the clearance mechanism. These mechanisms would cause there to be an abundance of Aβ monomers which could bind to each other, creating misfolded and toxic Aβ oligomers [6]. A different explanation for the aggregation of Aβ has been proven however, where Aβ forms prion-like "seeds" [7]. These seeds cause other Aβ monomers to form aggregates, thus leading to the formation of the senile plaques common in AD patients. This is known as the "prion-hypothesis of neurodegenerative disease" [8]. It is named this because the mechanism of a misfolded protein causing other proteins to mis-fold is very similar to the spreading mechanism of prion diseases such as Creutzfeldt-Jakob disease (CJD), which is a neurodegenerative disease caused by the prion protein. These diseases are characterized by the transfer of proteins that cause host proteins to also start misfolding [9].

It was thought that, unlike prions, Aβ seeds could not infect other organisms. This belief was challenged after evidence was found that patients with dura mater grafts and/or injections with human growth hormone derived from cadaveric pituitary glands infected with CJD also displayed disproportionally large amounts of Aβ in their brains. This was shown to not be caused by the CJD, supplying circumstantial evidence for the transference of Aβ pathology from person to person [10]. The debate is still out on the actual infectivity of Aβ, and through which routes this infection could take place. If

human transmission of Aβ seeds is an actual risk, this would have large implications on the medical world.

In this report the prion hypothesis will be discussed in further detail and the current knowledge about Aβ seeds and their transmission and pathology will be explored.

Prion hypothesis:

The prion hypothesis is named after the pathogenic protein responsible for a group of fatal neurodegenerative diseases known as "transmissible spongiform encephalopathy" (TSE). Human TSEs include CJD, Gerstmann-Straussler-Scheinker disease, fatal familial insomnia and kuru. These diseases all have a very similar disease progression and are caused by the same pathogen [11]. What sets TSEs apart from other diseases is that the pathogen is not a virus or bacterium, but an aberrant form of a highly conserved normal protein found in most mammals. This aberrant form was called a *proteinaceous infectious particle,* or prion [12].

All prion diseases have the same pathogenic mechanism involving the normal cellular prion (PrP^{C}), which is a glycoprotein bound to the cell surface that is expressed in many tissues, including the central nervous system [13]. This pathogenic mechanism relies on the conversion of PrP^{C} into a pathogenic isoform known as PrP^{Sc}. PrP, the name for the generic prion protein, can take on different special structures. When multiple PrP monomers form β-sheet rich aggregates they become the pathogenic PrP^{Sc}. This has a very low chance of happening in healthy individuals due to a large energy barrier based on thermodynamic and kinetic principles. PrP^{C} is a thermodynamically stable monomer and does not want to change to PrP^{Sc}. Pathogenic mutations however can decrease this energy barrier and allow the conversion into PrP^{Sc} more easily [14]. In this case the carrier of this pathogenic mutation will develop a familial prion disease, where there is a genetic component to the disease. Other ways of developing TSEs are spontaneous, non-genetic misfolding of the protein, or coming into contact with exogenous PrP^{Sc} and it causing the misfolding. The spontaneous, non-genetic misfolding is very rare due to the high kinetic barrier. What factors cause this misfolding to be able to take place are not currently known [15].

Once the PrP^{Sc} has been formed, the prion disease enters a state known as the "lag phase" or as an initial slow nucleation phase. During this time the small amount of PrP^{Sc} behaves as a seed, also known as a nucleus, that recruits other PrP molecules to grow itself. While the PrP^{Sc} seed is recruiting PrP monomers to grow, the body is trying to fight of the infection [16]. This causes the growing of the seed to slow down, and this causes a relatively long incubation time for TSEs. Once the seed has grown large enough, and thus has recruited many PrP monomers into its misfolded oligomer, the initial seed will fracture into smaller PrP^{Sc} seeds known as progeny seeds and start to spread throughout the central nervous system. At this point the progression of the disease will start exponentially increasing in speed, since the amount of seeds recruiting and misfolding PrP continues yo increase due to further fragmentation. This process is known as nucleated growth, or nucleation dependent aggregation.

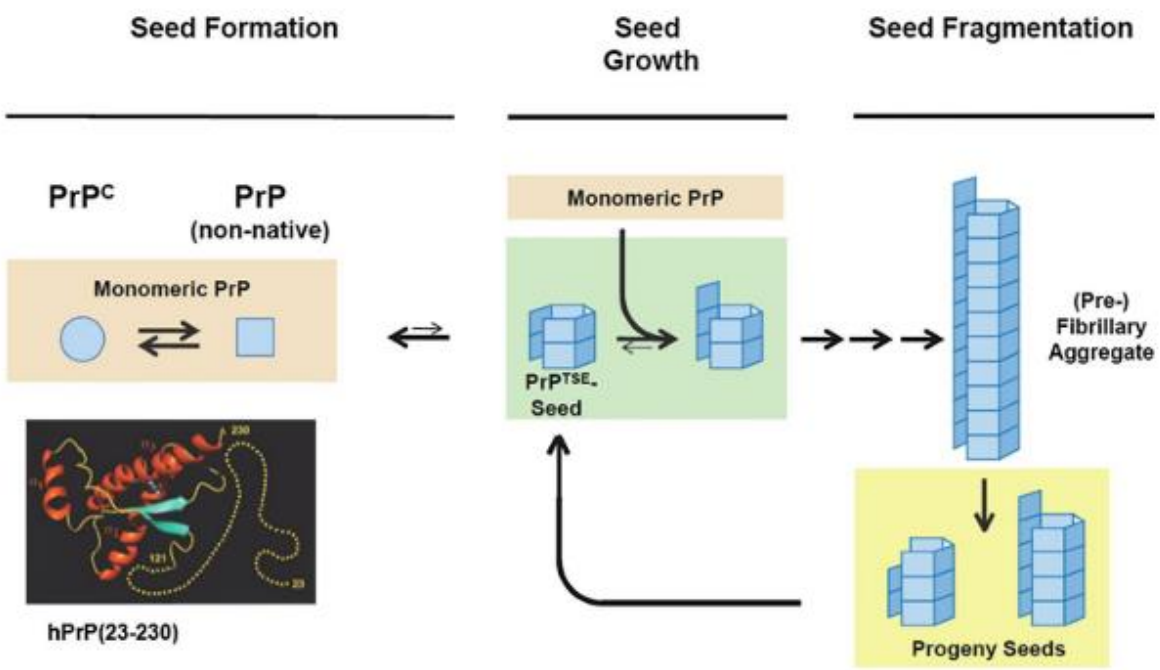

Figure 1: The mechanism of prion formation and nucleation dependent aggregation [17].

In Alzheimer's disease, the progressive spread of Aβ through the brain is an indication of prion-like mechanism of propagation [18]. Another indicator that Aβ functions like a prion is the lack of AD pathology before the age of 50 in patients with mutations in APP that cause early onset AD, despite the mutations being present since birth. This is an indication that despite the mutations, the formation of the Aβ seed is required for the disease to progress, which takes time. This leads to an assumption that Aβ in the brain also accumulates through a nucleation dependent aggregation system, and that the late development of AD is because of a long lag-phase where the Aβ seed is formed. A place where Aβ differs from prions however, is that there is not one single ordered configuration: monomers exist as unstructured monomers, α-helical monomers and monomers in a primarily β-sheet configuration [19]. PrP^{C} is found in only one ordered configuration. The large variation in Aβ monomers is in contrast to the structure of the oligomers, fibrils and plaques, which mostly consist of β-sheet structures. This indicates a prion-like mechanism where an Aβ seed causes the many variations in structures to fold into the β-sheet structure of the oligomers [20]. The Aβ seed is in this case more versatile than PrP^{Sc}, being able to recruit many monomer confirmations. PrP^{Sc} in contrast is only able to recruit the one PrP monomer.

After the production of the Aβ seed, it starts recruiting Aβ monomers and thus leading to aggregation of oligomers, which in turn leads to plaques. The Aβ seed is the main cause of the formation of the clinical picture of AD according to this hypothesis, not the over-abundance of Aβ monomers. Prion like propagation of Aβ also raised concerns over whether or not Aβ was not infectious, because exogenous seeds could be harmful if they also shared the infectivity of prions. To test if the infectious properties of exogenous prions are also shared by Aβ, *in vivo* experiments had to be done.

Intracerebral Aβ transmission:

Early *in vivo* experiments were inconclusive and sometimes seemed to give evidence both for and against their own hypothesis. Research done by Goudsmit et al. in 1980 was one of these early experiments [21]. In their experiment they inoculated multiple species of non-human primates with brain tissue derived from human AD patients. 61 primates were infected with brain matter of 19 different AD patients, after which the primates were observed for a period of at least 50 months to account for the incubation time. Only 3 of the 61 primates developed AD-like cerebral lesions, the other 58 animals showed no signs of infection from the inoculation. This result was inconclusive since only a very small portion of the primates showed signs of infection, but there had never been cases of AD-like brain lesions in uninoculated animals. This supported the infectivity hypothesis, so further research was necessary to find a more conclusive answer.

A study done by Baker et al. in 1993 provided additional evidence [22]. They inoculated 3 marmosets, also a species of non-human primates, intracerebrally with brain tissue of a patient with early onset AD. After an incubation time of 6-7 years the brains of the injected primates were compared to a control group, and the injected brains showed significant amounts of Aβ plaques and cerebral amyloid angiopathy (CAA), which is a build-up of Aβ in the arteries of the brain increasing the chance of strokes. This research proved the seeding ability of Aβ in primate brains, but because of the long incubation time it became difficult to do meaningful research into the implications for humans. The animal model that is used most often for Aβ seeding research more recently is a mutant form of mice that is transgenic for the human β-amyloid precursor protein (APP23 transgenic mice). These mice overexpress human APP in the brain, leading to increasing Aβ deposits as the mice get older. From the age of approximately 9 months the transgenic mice begin to develop senile plaques [23]. Research done in these mice showed that injection with brain tissue from AD patients caused the senile plaques to form at an earlier age, further supporting the infectious ability of Aβ when injected intracerebrally [24]. The short incubation time and use of human APP make these transgenic mice the perfect animal model for research in AD and the infectivity of Aβ.

Using these mice, the susceptibility of different brain regions to Aβ seeding was tested. After injection with Aβ containing brain extract from APP transgenic mice evidence of induction was seen in all injected brain regions, namely the olfactory bulb, the parietal cortex, the entorhinal cortex, the striatum and the hippocampus [25]. Although all injected regions show exogenous induction of Aβ deposits, the induction of Aβ deposits is far stronger in the hippocampus than in the striatum. The Aβ in the hippocampus is also less diffuse than in the striatum, meaning it has been recruited into fibrils more. This follows the normal age-related Aβ deposits in the brains of APP transgenic mice [26].

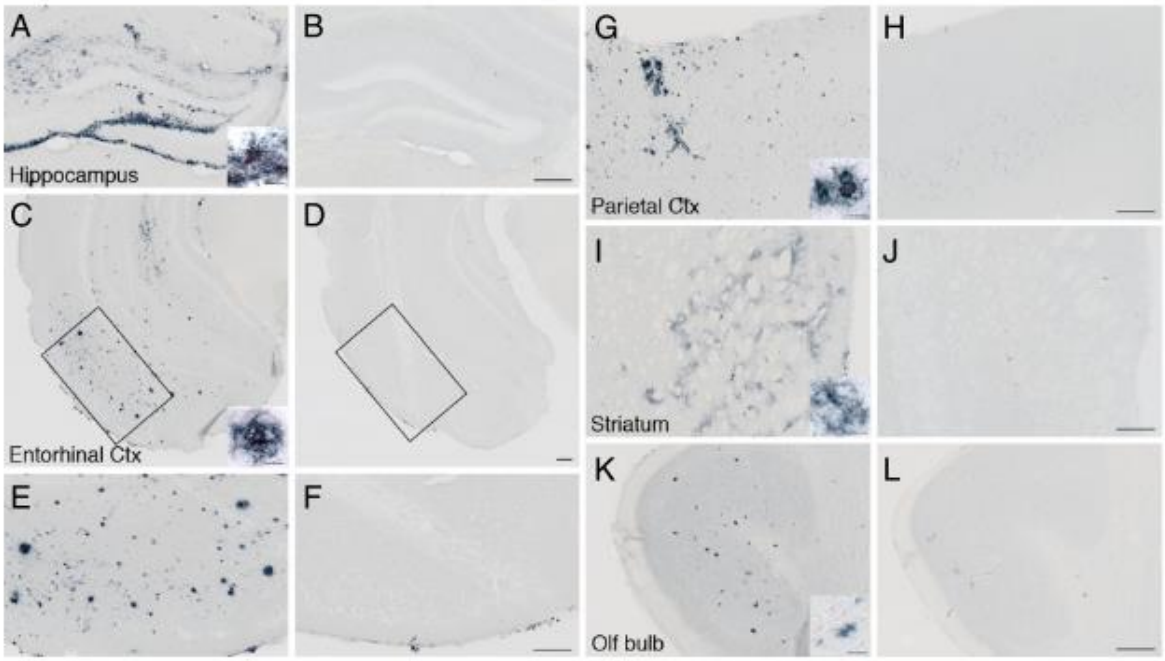

Figure 2: Induction of Aβ deposition in different brain regions of young APP mice. Shown are brain regions six months after inoculation. A, C, E, G, I and K were injected with Aβ containing brain extract while B, D, F, H, J and L were injected with control brain extract that did not contain Aβ.[25]

Aβ deposits are not only found in the inoculated brain regions, but after a while also spread throughout the brain. Further evidence for the seeding capability is that 1 week after the injection with Aβ containing brain matter, no Aβ deposits are present [25]. This indicates that the formed deposits are not the same Aβ as the injected material.

A key characteristic that makes prions so dangerous is their ability to infect new hosts without relying on external cofactors. This helps them infect new hosts without relying on their susceptibility as much. Aβ shares this characteristic when injected intracerebrally. Injecting APP transgenic mice with synthetic Aβ produced the same results as Aβ containing brain extracts [18]. This rules the chance out that enzymes responsible for Aβ aggregation were copurified with the Aβ, and thus proving that Aβ itself is responsible for the further aggregation and development of senile plaques.

Intracerebral injections with Aβ containing brain matter causing cerebral β-amyloidosis, while interesting, is no cause for concern for humans because this route of transmission should never happen. Prion diseases are also able to be transmitted by surgical equipment that is contaminated with very small amounts of prion proteins [27]. If the same is true for Aβ this could be a dangerous route of transmission for humans. To test if this was the case, Eisele et al. immersed stainless steel wires in Aβ-rich brain extract, dried them and implanted the wires permanently in in the hippocampus and neocortex of APP transgenic mice [25]. Analysis 4 months later showed that this is indeed a possible route of transmission, as there were large deposits of Aβ in the brain around the extract-coated wire. The cause of the Aβ aggregation was ruled out to be caused by a reaction to just the metal wires, because wires immersed in Wild-type brain extracts did not cause the same Aβ deposits. Preventing the transmission through stainless steel wires requires plasma-sterilization, cleaning them through normal means did not diminish the Aβ deposits after implanting [28]. This could have implications for cleaning surgical equipment and

transmission of Aβ through neurosurgery, since Aβ seeds could infect patients this way even after cleaning.

Evidence for transmission through neurosurgery has been shown by Jaunmuktane et al. [29]. Patient histories of the United Kingdom's National Hospital for Neurology and Neurosurgery in London were searched for patients with young onset CAA after neurosurgery as a child. 4 such cases were found where the CAA could not be explained by mutations linked to early onset CAA, such as mutations in APP, PSEN1 and PSEN2 genes. These patients also all died before the age of 55, at which age CAA occurs sporadically [30]. Explanations for the presence of CAA other than infection with Aβ seeds were all less likely. Head trauma has been theorized to be a risk factor for AD, and all 4 patients had severe head trauma that required surgery. Head trauma related neuropathological changes are characterized by aggregation of hyper-phosphorylated tau however, which was absent in these patients. The neurological abnormalities seen in these patients was very similar to that of mice inoculated with Aβ seeds. Similar results have been found by Hamaguchi et al. and Giaccone et al. [31], [32], bringing the total number of patients to 11. This evidence, while small in sample size, indicates possible transmission through neurosurgery. Increasing frequency of neurosurgical interventions on elderly people with cerebral Aβ might increase the chance of Aβ spreading through neurosurgery. A redeeming fact however is that the surgeries performed on the 11 patients happened over 50 years ago, and since then hygiene and safety standards have improved. The chances of trace amounts of Aβ being on surgical equipment is therefore much lower currently.

Intracerebral transmission of Aβ seeds has thus been proven, but this does not mean that AD is transmissible as well. The model animals and the humans which are thought to be infected through neurosurgery showed a different neurological picture than AD patients. The Aβ plaques and Aβ deposits in arteries are present in both groups, but the tau tangles and loss of synapses is only found in AD patients. So while the intracerebral transmission of Aβ has been proven, the transmission of AD has not.

Peripheral Aβ transmission:

While Aβ seeds looking to be transmissible through neurosurgery is worrying, there are not that many people undergoing neurosurgery at a young age. If Aβ is also capable of inducing amyloidosis when it enters the body in the periphery, for example through blood transfusions, this is a larger problem. First evidence for the peripheral inoculation of cerebral amyloidosis was found in combination with CJD. In a study done by Jaunmuktane et al. [10], 4 patients who had received cadaveric human pituitary derived growth hormone injections to stimulate growth which were infected with CJD also showed substantial Aβ deposits in their brain. 2 other patients showed focused Aβ deposits in only one brain area, and 1 other patient had Aβ deposits within the prion protein plaques. 4 of these 7 also showed extensive CAA pathology. The age of these patients ranged from 36-50 years, at which age this kind of Aβ pathology is extremely rare [33]. To exclude the possibility that the Aβ deposits were caused or accelerated by CJD, Aβ pathology in a control group of 116 other patients with prion diseases who had undergone autopsy was compared to the 7 patients. None of the patients in the control group showed any comparable Aβ pathology. After this the presence of Aβ in human pituitary glands was confirmed, leading to the conclusion

that Aβ seeds might have been injected along with the human growth hormone and the prion protein. This would mean that Aβ is able to be transmissible through the periphery, even though in this study it was only shown to be in combination with CJD. Other ways of peripheral transmission might also be possible.

To test other peripheral transmission routes, the main routes of peripheral prion infection were also tested for Aβ by Eisele et al. [28] APP transgenic mice were given Aβ containing brain extracts through the intravenous, oral, intranasal and intraocular routes. The amount of brain extract injected was much higher than what was injected intracerebrally, since in prion transmission studies the amount of prion protein also needed to be higher to cause infection. Analysis after 4-8 months showed no induction of β-amyloidosis in the brain for all of the peripheral routes, indicating that either Aβ seeds are not conveyed from the peripheral sites to the brain or the seeds require a longer incubation time when administered peripherally. One route of administration that was not checked in this study was intraperitoneal injection. The intraperitoneal route in transmission of prion disease is a more efficient route of transmission than the oral route [34], so this could also be the case for Aβ and thus be a possible route of inoculation. Another study done by Eisele et al. [35] investigated the possible inoculation through this route. 2-month-old APP transgenic mice were administered two intraperitoneal injections with Aβ rich brain extract from aged APP transgenic mice. The injections were administered one week apart. After 7 months the brains of the inoculated mice were compared to the brains of littermates that received no injections. In the brains of the intraperitoneally injected mice a significant induction of cerebral β-amyloidosis was present. Control groups of mice that were intraperitoneally injected with either a phosphate-buffered saline or brain extract from non-APP transgenic Wild-Type mice not containing Aβ did not show β-amyloidosis (figure 3).
Compared to intracerebral inoculation, intraperitoneal inoculation takes more Aβ rich brain extract and takes a longer time to develop cerebral β-amyloidosis [35]. Eisele et al. estimate it takes 1000 times as much Aβ and 2 to 5 months longer to get the same Aβ deposits in the brain. The pathology in the brains after intraperitoneal injection is very similar to that of intracerebral inoculation where there are Aβ plaques and Aβ deposits in the arteries, but no tau pathology is observed.

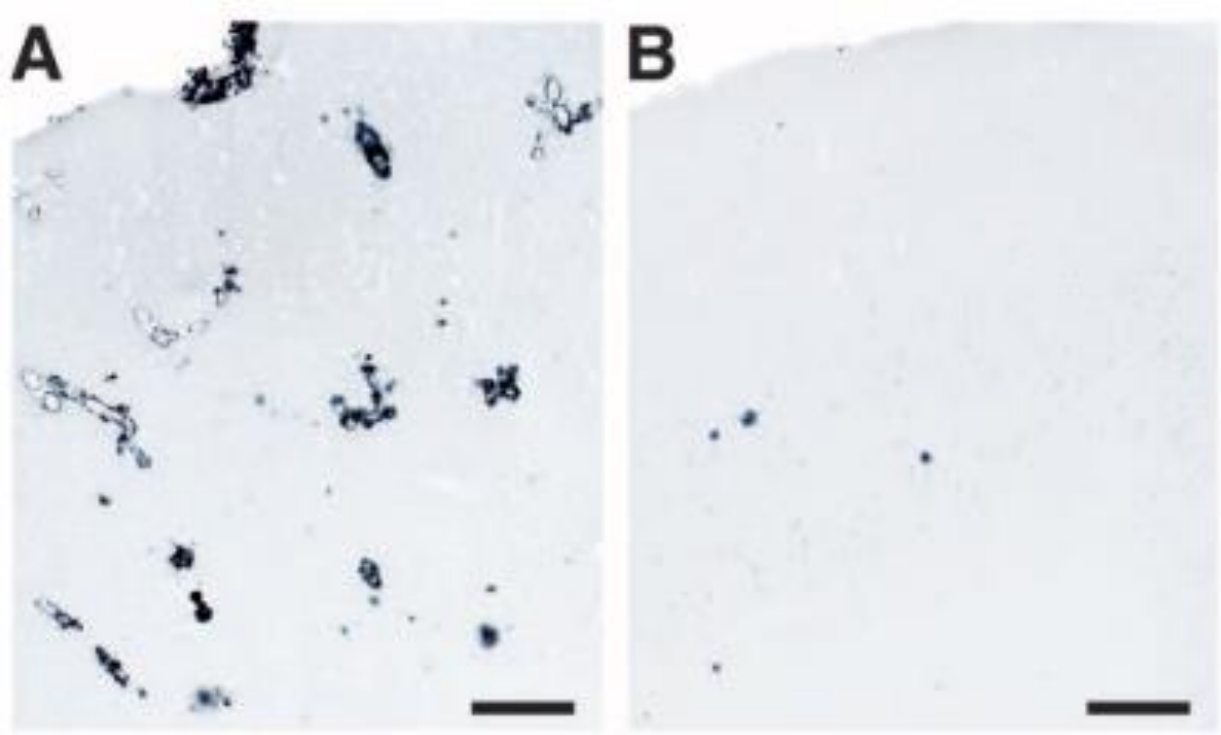

Fig. 3. Aβ-immunostaining in the frontal cortex of APP mice injected intraperitoneally with Aβ-containing brain extract (A) or non Aβ-containing brain extract (B). [35]

Since in APP23 transgenic mice human APP is only present in the nervous system [36], it is likely that the seed causing the amyloidosis was the injected seed itself, not peripherally formed Aβ aggregates. A later study using a different line of APP transgenic mice which does not express murine APP showed that even in the absence of both human and murine peripheral APP, peritoneal inoculation with Aβ seeds still leads to Aβ deposits in the brain [37], lending further evidence to the theory that the original injected Aβ seeds reach the brain and start the Aβ aggregation.

The lack of human APP in the periphery of APP23 transgenic mice could be a factor in the lack of peripheral transmission of human Aβ pathology by routes other than intraperitoneal inoculation. A study by Burwinkel et al. [38] used APP/PS1 mice to investigate this. These mice also express human APP in the periphery, so they can be used to investigate the peripheral inoculation more accurately. When injected with Aβ containing brain extracts intracerebrally these mice have the same CAA as APP23 mice. To test the effect of intravenous injections with Aβ seeds in these mice, diluted brain matter of 2 human AD patients and diluted brain matter of a non-demented person as a control was injected into the tail veins of multiple 6-8 weeks old APP/PS1 mice. 180 days after the injection a significantly larger deposit of Aβ in the arteries of the thalamus was seen in the mouse injected with brain extract from AD patients compared to the control group. At 270 days after injection the same observation was made: there was significantly more thalamic CAA in the Aβ rich brain matter injected mice. CAA was also increased at both days after injection in the cortices, but there was no plaque formation. The presence of human APP in the periphery of these mice may support the intravenous inoculation of CAA by aiding transport of the Aβ seed to the brain.

Just like intracerebral injections with Aβ rich brain matter, intravenous or intraperitoneal inject with Aβ rich brain matter should also never happen in humans. The peripheral inoculation of Aβ seeds would be much more worrying if blood-derived Aβ could also cause Aβ aggregation in the brain, since in theory blood transfusions could then lead to the development of CAA. An experiment done by Bu et al. [39] investigated if blood-derived Aβ could induce amyloidosis by a model of parabiosis, linking the blood circulation of a 10 month old APP/PS1 mouse to that of an age matched Wild-type mouse. This was done to test whether human Aβ in the blood of the mutant mouse can enter the brain of the wild type mouse. Analysis was done after 2, 4, 8 and 12 months after the parabiosis surgery. After 12 months significant Aβ deposits were observed in the cerebral arteries and Aβ plaques were found in the brain of the wild type mouse, providing evidence for Aβ inoculation through blood derived Aβ. Other AD pathologies were also found in the brains of these parabiotic mice, such as long-term potentiation inhibition, tau hyperphosphorylation and neurodegeneration. This is the first route of inoculation that caused not only CAA but also the cognitive decline associated with AD. This could perhaps be because of the long term connection instead of a single injection, but could also be caused by transfer of tau pathology through blood, which was already shown to be possible by Clavaguera et al. [40].

The experiment by Bu et al. showed that Aβ pathology is transmissible through blood, but inoculation through more often occurring procedures like blood infusions containing Aβ seeds was still a mystery. Morales et al. [41] did an experiment to see if single or multiple blood transfusions would also induce Aβ pathology, or even Alzheimer's disease. For most of their experiments they used 50 days old APP23 transgenic mice, which they injected with blood from 12-14 months old APP23 mice. Dose dependency was tested by giving mice either one injection or two injections at 30 days apart. When the brains of the mice were analyzed at 250 days old, the brains of the mice that were injected once showed no difference to non-injected mice, nor to mice that were injected with blood from wild type mice. The mice that underwent two injections however showed significantly larger Aβ deposits in their brain. The amount of insoluble Aβ found in their brain was fourfold higher than the aforementioned 3 groups, indicating that while Aβ seeds are transmissible through blood transfusions, it requires a either a high quantity of Aβ in the donor blood and/or repeated exposure to the donor blood. A difference between Aβ seeds and prions is seen in the percentage of animals that get infected after blood transfusion. Prions have a low infectivity through blood through blood transfusion [42], but the mice that were injected twice in this experiment showed an infection rate between 80 and 100 percent. Almost all mice developed severe CAA after 2 blood transfusions. The difference in infectivity between blood plasma and blood cells of aged APP23 mice was also tested. While the blood plasma showed the same results as non-isolated blood, the blood cells did not transfer Aβ pathology to recipient mice. This is to be expected, since the Aβ seeds circulate in the blood and are not part of cells. So, this extensive study on Aβ seeding through blood transfusions provides evidence that this route of transmission, which is a very common medical procedure, is possible in APP transgenic mice. But can the same be said for transmission in humans? A possible mitigating factor in the risk of Aβ pathology transmission through blood transfusion is that in the experiment of Morales et. APP transgenic mice were used. These mice overexpress human APP at a high level, so they could be much more susceptible to Aβ seeds than humans might be. However, even if this is the case humans with mutations that make them develop early-

onset AD, and thus higher Aβ production, would still be susceptible to Aβ pathology after a blood transfusion.

Discussion:

The studies discussed in this paper raise potential red flags for the medical field, in particular the risk of Aβ seeds being transmitted through neurosurgery in humans and blood transfusions in mice. These two procedures are common enough to raise concerns about iatrogenic CAA developing in patients. Most of the other discussed routes of transmission should never happen under non-experimental condition, except for the cases where Aβ pathology was transferred alongside CJD through cadaveric pituitary glands. This procedure has already been phased out however, so this is also no longer a risk factor. In the case of the infections through neurosurgery, proper cleaning of neurosurgical equipment is necessary to reduce risk of Aβ seed transfer. When this is done the risk of infection through this route is very low, but it does require all hospitals to implement proper cleaning procedures. Because of the considerable latency of Aβ seed transmission through neurosurgery, usually over 20 years, the risk of infection might be underestimated currently since most research into this route of transmission is very recent. Further research is required to see if it is necessary to monitor CAA development in patients who underwent neurosurgery at a young age.

Aβ seeds being transferable through blood transfusions is the most concerning finding in this paper, since blood transfusions are very common. A previous study using the Scandinavian Donations and Transfusions Electronic Database looked into the risk of infection of neurodegenerative diseases [43]. Data of almost 1.5 million people was analyzed, and no transmission of AD was found. What this study did not look at, and what has been proven by Morales et al. [41], was transmission of CAA through blood transfusions. Patients who have received multiple blood transfusions from a patient with AD might be at a high risk of developing CAA later in life, requiring more monitoring of the receiving patients and checks in blood donors for signs of amyloidosis.

The implications for humans are not yet clear, as there is not enough research done into the transmission of Aβ pathology in humans. Once this is done there could be more checks needed for blood donors and better hygiene standards for neurosurgical equipment. Currently however, there is no clinical evidence to suggest action needs to be taken. There has been no correlation found between patients receiving blood transfusions and early signs of Aβ deposits or CAA. In the study conducted using the Scandinavian Donations and Transfusions Electronic Database [43] the transmission of neurodegenerative diseases, including AD, through blood transfusions was already ruled out. So while further research is required, there is currently no cause for alarm and widespread changing of donor screening procedures.

Conclusion:

To summarize, Aβ aggregation follows a prion like system of seed formation. These seeds cause the misfolding of Aβ monomers into isomers which cause neurodegeneration and aggregate into senile plaques. These seeds are transmissible between individuals through multiple routes, and when entering a new host induce amyloid pathology. All known routes of transmission only transfer Aβ pathology and cause Aβ deposits in the brain parenchyma and arteries in the brain, leading to CAA,

but not the rest of Alzheimer's disease associated pathologies. The most potent inoculation is intracerebral injection with Aβ rich brain matter, but this poses no risk to humans since this should never happen. Neither should intravenous and intraperitoneal injection with Aβ rich brain matter. Previously, evidence has been found that cadaveric pituitary glands infected with CJD are also a possible route of transmission, but this practice has already been phased out. The most worrying routes of transmission are contaminated neurosurgical equipment and blood transfusions. The risk of transmission through these routes is not well understood and requires further research, both experimental in animals other than transgenic mice and large epidemiological studies that look at the development of CAA. I believe blood transfusions might be a risk factor in development of CAA, even if it might be only in the case of people that are at risk of developing early-onset AD. The amount of people that could be at risk for CAA after blood transfusions from patient with Aβ pathology might be higher than previously thought. There is currently no clinical evidence for this supposed higher risk however, so changing blood donor screening procedures is at this time not necessary as it would only increase costs. If this evidence is ever found, then changes can be made. It would not make sense at this time, since all evidence is circumstantial and in animal models.

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