Amyloid-β transmission or unexamined bias?

ARISING FROM Z. Jaunmuktane *et al. Nature* **525**, 247–250 (2015); doi:10.1038/nature15369

Jaunmuktane and colleagues reported on eight persons of short stature who had been treated with preparations of human-derived growth hormone and subsequently developed iatrogenic Creutzfeldt–Jakob disease (CJD)[1]. On autopsy, the authors found marked deposition of parenchymal and vascular amyloid-β (Aβ), which was unexpected given the relatively young age (36–51 years) of the patients. The selected comparator group included patients with sporadic CJD, who were not of short stature and did not receive any growth hormone treatment. These sporadic cases did not show marked Aβ pathology. Although the authors make an interesting case for iatrogenic transmission of Aβ pathology, their findings could also be explained by two notable differences between the eight growth-hormone-treated patients and the comparator group: the indication for growth hormone treatment and the treatment itself. There is a Reply to this Brief Communication Arising by Collinge, J. *et al. Nature* **537**, http://dx.doi.org/10.1038/nature19087 (2016).

The eight patients at the centre of this study received growth hormone treatment for various reasons, including panhypopituitarism (numbers 1, 2, 7), mental retardation (number 2), microcephaly (number 2), craniopharyngioma (number 5), and idiopathic short stature (numbers 3, 4, 6, 8). The patients with marked Aβ deposition (numbers 4, 5, 6, 8) were generally of short stature owing to unknown causes. Common to this heterogeneous group of patients is the lack of endogenous growth hormone, a hormone that plays an important role in learning and memory, synaptic plasticity and neurogenesis, and is considered as a treatment for patients with cognitive impairment resulting from its deficiency[2]. Insulin-like growth factor-1 (IGF-1) is one of the main downstream targets regulated by growth hormone and supports cell survival and growth at multiple levels, with IGF-1 being important in the brain. It has a well-documented role in many aspects of neurodegeneration, including Alzheimer's disease[3,4], and IGF-1 promotes Aβ production[5,6]. Lack of IGF-1 has been proposed to cause neurodegenerative disorders such as Alzheimer's disease owing to the disturbed trophic support to neurons (for a review, see ref. 7). Absence or reduction in IGF-1 can thus promote neurodegeneration and, particularly relevant for the conclusion of Jaunmuktane *et al.*[1], increase Aβ depositions. This mechanism is similar to the lack of insulin seen in type 1 diabetes. In other words, the underlying disease state, which was the indication to start growth hormone treatment, can act as a shared cause of Aβ deposition and—through treatment with human-derived growth hormone—CJD. This should therefore be considered a confounder of the effect under study (that is, confounding by the indication of growth hormone treatment). The comparator group presented by Jaunmuktane *et al.*[1] does not allow for confounding adjustment, as all exposed (that is, growth-hormone-treated) individuals are growth-hormone deficient (and of short stature) and all unexposed individuals are not, which means that any differences between the comparator groups could also be explained by growth hormone deficiency.

Furthermore, growth hormone treatment itself should have been considered as an important alternative cause of the Aβ deposition. Although this might seem counterintuitive, as increased IGF-1 levels should increase Aβ clearance, this alternative explanation is not without support. Both low and high levels of IGF-1 have been observed in neurodegenerative diseases, and this is also the case for Alzheimer's disease[8,9]. It has been proposed that this is due to an abundance of IGF-1 that reduces the sensitivity of the cells[7]. Given the long-term treatment of patients with growth hormone, in which the complex circadian and age-dependent rhythm of growth hormone secretion is not taken into account, it is plausible that this led to cell resistance to IGF-1. This mechanism is similar to the increased levels of insulin (to which patients are resistant) seen in type 2 diabetes. Therefore, growth hormone treatment could possibly lead to the development of Aβ depositions in individuals at an earlier age than if untreated. Similar to the previous point, this would also confound the authors' interpretation, in this case confounding by growth hormone treatment.

The authors mention prion disease as an improbable cause for their findings, for example, through protein cross-seeding or clearance overload, which they attempt to rule out by comparing the iatrogenic CJD patients to sporadic cases. On the basis of the lack of marked Aβ depositions in the sporadic cases, the authors concluded that prion disease does not predispose to Aβ depositions and thus another factor must cause these deposits. This conclusion does not appear to be fully warranted, as the excess of Aβ in iatrogenic CJD compared to sporadic CJD does not indicate whether prion disease causes Aβ depositions (independently of this other factor). In other words, by restricting the study to patients who all have prion disease, the effect of prion disease compared to no prion disease cannot be examined. A comparison of persons with CJD to those without CJD but who are similar with regard to important confounders (for example, age, sex, other medical conditions and treatments) would better inform such an effect, as has been done in a previous study[10]. Although the greater deposition of Aβ in the iatrogenic CJD cases compared to the sporadic CJD therefore does not prove or disprove prion disease as a cause for this, we agree with the authors that this points to a factor other than prion disease causing the additional Aβ deposits. We have already mentioned the indication for growth hormone treatment and the growth hormone

Table 1 | Four potential causes of marked Aβ deposition in persons of short stature treated with human growth hormone and subsequently developing iatrogenic CJD

Potential cause of Aβ deposition*	Consistency of the purported causal effect with findings	Stated conclusions from the authors
Short stature (vs normal stature)	Yes	Not discussed
GH treatment (vs no GH treatment)	Yes	Not discussed
Human-derived GH (vs synthetic GH)	Inconclusive†	Causal
Prion disease (vs no prion disease)	Inconclusive‡	Not causal

The first column shows four potential causes for the observed Aβ deposition in persons of short stature who were treated with human-derived growth hormone and developed CJD as a result of prion transmission (and their causal contrasts in parentheses). The second column indicates whether the causes are consistent with the findings of ref. 1, whereas the third column contains the conclusions of the authors.
*The unexposed comparator group should otherwise be exchangeable with the exposed group. For example, to assess the possible effect of growth hormone treatment versus no growth hormone treatment, subjects should ideally have similar stature and be otherwise comparable, perhaps conditional on measured covariates.
†Inconclusive given that this was not compared to the causal contrast, that is, persons treated with synthetic growth hormone. The authors' suggestion of causality for human-derived growth hormone versus no human-derived growth hormone is consistent with the findings, but is intractably confounded by growth hormone treatment and indication for growth hormone treatment.
‡Inconclusive given that this was not compared to the causal contrast, that is, persons without prion disease.
Abbreviations: Aβ, amyloid-β; CJD, Creutzfeldt–Jakob disease; GH, growth hormone; IGF-1, insulin-like growth factor-1.

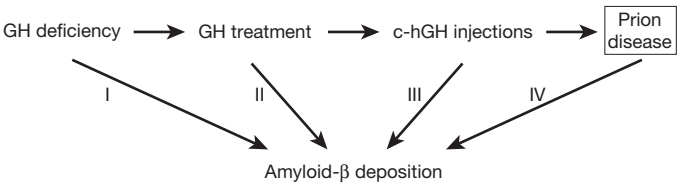

Figure 1 | Causal diagram depicting the proposed causal pathways, the authors' interpretation, the two confounding biases and the inconclusive conditioning on presence of prion disease. Horizontal arrows depict the known causal pathway of growth hormone deficiency being the indication for growth hormone treatment (administered using human-derived growth hormone injections), which in turn can cause CJD if contaminated with prions. The numbered arrows indicate possible effects on Aβ deposition. From their data, the authors of ref. 1 conclude (see below) that CJD could not have a direct effect, that is, that the arrow IV is not present. They then conclude that the shared cause must lie in the human-derived growth hormone injections (arrow III), which they infer contained infectious prions as well as infectious amyloid. However, there are two alternative shared causes for the co-occurrence of prion disease and amyloid, namely the indication of growth hormone treatment (arrow I) and the treatment itself (arrow II). In the current study design, these alternative explanations are therefore confounders of the proposed arrow III if arrows I and/or II are present. The authors sought to rule out arrow IV by comparing iatrogenic CJD patients with sporadic CJD patients. However, all patients included in this comparison had prion disease, which thus entails conditioning on the exposure (indicated with the square box around prion disease). While this comparison may suggest that another factor is causing additional Aβ (e.g, arrows I–III), it does not inform about prion disease causing Aβ deposition; that is, arrow IV cannot be proven or ruled out. Finally, for simplicity, the causal diagram above does not include additional unmeasured shared causes; in particular, if prion disease and Aβ deposition shared other causes beyond those described here, then additional confounding and/or selection biases invalidating the authors' interpretation may also be present. Aβ, amyloid-β; CJD, Creutzfeldt–Jakob disease; GH, growth hormone; hdGH, human-derived growth hormone.

treatment itself as two plausible causes for Aβ deposits; the authors focus on the potential human transmissibility. On the basis of the data presented by the authors alone, it is not possible to determine which factor or factors are actually causal, as their findings are consistent with multiple explanations.

Given that the results of Jaunmuktane *et al.* are inconclusive in this respect, what data would help to disentangle the hypothesized transmissibility from these competing explanations? One study design option would involve comparing persons of short stature who received synthetic versus human-derived growth hormone, as the synthetically produced treatment could not transmit any infectious agent from another person. We understand the difficulty concerning persons treated with synthetic hormone as a comparator group, as they have longer expected lifespans than those with (iatrogenic) CJD. Although neuropathology in a comparable age group will therefore be relatively difficult to obtain, non-invasive methods to quantify Aβ depositions in the brain, for example using positron emission tomography (PET) imaging, may be useful. Four possible causes of the marked deposition of Aβ are summarized in Table 1 along with, for each of these factors, consistency with the findings of Jaunmuktane *et al.*, and the emphasized conclusion of the authors. Figure 1 depicts the suspected confounding biases in the current study design[11].

In conclusion, the study presented by Jaunmuktane *et al.* is consistent with multiple explanations for the marked deposition of Aβ. However, the authors emphasize one hypothesis that is indirectly supported by the data over other hypotheses, although a considerable body of previous empirical evidence argues in favour of these alternative explanations. Furthermore, the improbable explanation of CJD cross-seeding was disregarded on the basis of experiments that provide no such evidence, and was subsequently discussed at length whereas the plausible alternatives have not been mentioned. For these reasons, and in particular given the public health implications incited by the publicity of the Jaunmuktane *et al.* study[12], it is imperative to carefully consider confounders and study design[13,14] when weighing the possibility of human transmissibility of Aβ.

Hieab H. H. Adams[1,2], Sonja A. Swanson[1,3], Albert Hofman[1,3] & M. Arfan Ikram[1,2,4]

[1]Department of Epidemiology, Erasmus MC, Rotterdam, 3015 CE, The Netherlands.
email: m.a.ikram@erasmusmc.nl
[2]Department of Radiology and Nuclear Medicine, Erasmus MC, Rotterdam, 3015 CE, The Netherlands.
[3]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts 02115, USA.
[4]Department of Neurology, Erasmus MC, Rotterdam, 3015 CE, The Netherlands.

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Collinge *et al.* reply

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In the accompanying Comment[1], Adams *et al.* argue that we have not excluded two alternative explanations for our finding of extensive amyloid-β (Aβ) deposition in relatively young individuals who received extracts of human pituitary glands: that this pathology may be a consequence of either the underlying diagnosis for which the treatment was given or of the treatment with growth hormone itself, irrespective of whether it was contaminated. As we made clear in our letter[2], our study was observational rather than an epidemiological or experimental one. Although, by its nature, it cannot exclude these hypotheses (or other possible explanations for which there is no supportive evidence), we considered them unlikely. These patients received cadaveric pituitary-derived human growth hormone (c-hGH) for various reasons and the individuals in our cohort that developed Aβ deposition were treated for pathogenetically unrelated conditions, or because of short stature of no obvious cause, making a common mechanism unlikely. We also identified no publications that report a causal relationship between panhypopituitarism, short stature or craniopharyngioma and Alzheimer's disease or increased Aβ deposition. We also consider the proposal that growth hormone itself (acting through the growth-hormone–IGF (insulin-like growth factor) axis) administered through adolescence is a possible trigger of Aβ deposition to be unlikely. IGF-1 production is stimulated by growth hormone, and several reports describe increased IGF levels to be associated with increased Aβ clearance[3,4] and decreased risk of Alzheimer's disease[5], although the authors also cite a single report stating the opposite[6].

Adams *et al.* refer to our hypothesis (that Aβ seeds in batches of c-hGH triggered Aβ amyloidosis in recipients) as being untested. Clearly this cannot be experimentally tested in humans but there is a substantial body of experimental data *in vitro* and *in vivo* demonstrating Aβ seeding, including, as we cited in our letter, that peripheral inoculation of laboratory mice with Alzheimer's disease brain extracts leads to cerebral amyloid angiopathy[7]. Investigating the role of seeded protein aggregation (often referred to as 'prion-like' mechanisms) is one of the most active current areas of neurodegeneration research[8]. As we note, it will be important to examine archived batches of hGH for presence of Aβ seeds by animal inoculation studies and this work is planned.

To suggest that our comparison with patients with other forms of prion disease unrelated to hGH treatment was futile is inappropriate. Were we to have found that patients with sporadic CJD or other forms of prion disease had a similar frequency and severity of Aβ pathology at comparable ages, or an increased frequency compared with controls, this would have argued strongly against our hypothesis, and indeed the two alternative hypotheses suggested by Adams *et al.* By comparison, with a large series of controls we noted that the frequency of Aβ pathology in sporadic CJD was in keeping with chance coincidence[9], as did other studies[10]. These analyses in our paper argue strongly against an effect of prion disease *per se* on Aβ pathology, as suggested by Adams *et al.* An important question that we posed in our letter was whether similar pathology suggestive of transmission of Aβ seeds is seen in iatrogenic CJD caused by other medical procedures (unrelated to c-hGH treatment or the underlying conditions for which it was given). The other most common cause of iatrogenic CJD is use of dura mater grafts. Importantly, following our study, a recent Austrian and Swiss series of dura mater graft recipients has now been reported, which also found frequent vascular and parenchymal Aβ pathology consistent with our hypothesis[11,12].

However, we agree with Adams *et al.* that carefully controlled epidemiological studies are valuable and indeed we hoped that our study would stimulate such discussion and encourage precisely such work. With respect to comparing patients treated with hGH and synthetic hormone, this will be difficult as these necessarily represent different age cohorts.

The authors of this Reply represent the authors of the original paper.

John Collinge[1,2,3], Zane Jaunmuktane[4], Simon Mead[1,2,3], Peter Rudge[1,3] & Sebastian Brandner[2,4]

[1]Medical Research Council Prion Unit, Queen Square, London WC1N 3BG, UK.
[2]Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK.
email: s.brandner@ucl.ac.uk
[3]National Prion Clinic, The National Hospital for Neurology and Neurosurgery, University College London Hospitals NHS Foundation Trust, Queen Square, London WC1N 3BG, UK.
[4]Division of Neuropathology, The National Hospital for Neurology and Neurosurgery, University College London Hospitals NHS Foundation Trust, Queen Square, London WC1N 3BG, UK.

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