Transmission of amyloid-β protein pathology from cadaveric pituitary growth hormone

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We previously reported[1] the presence of amyloid-β protein (Aβ) deposits in individuals with Creutzfeldt-Jakob disease (CJD) who had been treated during childhood with human cadaveric pituitary-derived growth hormone (c-hGH) contaminated with prions. The marked deposition of parenchymal and vascular Aβ in these relatively young individuals with treatment-induced (iatrogenic) CJD (iCJD), in contrast to other prion disease patients and population controls, allied with the ability of Alzheimer's disease (AD) brain homogenates to seed Aβ deposition in laboratory animals, led us to argue that implicated c-hGH batches might have been contaminated with Aβ seeds as well as with prions. However, this was necessarily an association, and not an experimental, study in humans and causality could not be concluded. Given the public health importance of this hypothesis, we proceeded to identify and biochemically analyse archived vials of c-hGH. Here we show that certain c-hGH batches to which patients with iCJD and Aβ pathology were exposed had significant levels of Aβ40, Aβ42 and tau proteins, and this material can seed the formation of Aβ plaques and cerebral Aβ–amyloid angiopathy in intracerebrally inoculated mice expressing a mutant, humanized amyloid precursor protein. These results confirm the presence of Aβ seeds in archived c-hGH vials and are consistent with the hypothesized iatrogenic human transmission of Aβ pathology. This experimental confirmation has implications both for the prevention and treatment of Alzheimer's disease, and should prompt a review of the risk of iatrogenic transmission of Aβ seeds by medical and surgical procedures long recognised to pose a risk of accidental prion transmission[2, 3].

Human prion diseases occur most commonly as sporadic or inherited conditions but, critically, are also experimentally transmissible and rare cases are acquired by environmental exposure to infectious prions via diet or medical procedures[2]. This aetiological triad in prion disorders was thought to be unique amongst neurodegenerative diseases but growing evidence from experimental cellular and animal models has implicated propagation and spread of multimeric assemblies of

misfolded host proteins in the pathogenesis of Alzheimer's, Parkinson's and other neurodegenerative conditions[2, 4].

Iatrogenic transmission of CJD, an invariably lethal neurodegenerative disease, can result following a range of medical and surgical procedures[2, 5]. The range of incubation periods of acquired prion diseases are known to span over five decades[6]. Prior to 1985, when the risk of causing iCJD was not appreciated, children with short stature were treated with growth hormone extracted from large pools of cadaver-derived pituitary glands, some of which will have been infected with prions[7]. More than 200 individuals treated with c-hGH worldwide have died of iCJD. We previously reported moderate to severe grey matter and vascular Aβ pathology in four of eight relatively young adults who had died of iCJD following childhood treatment with c-hGH[1]. A further two had focal Aβ pathology and only one was entirely negative for Aβ. All eight lacked genetic risk factors for Alzheimer's disease or cerebral Aβ-amyloid angiopathy (CAA). These findings stood in marked contrast to other prion disease and population controls and suggested that some of the c-hGH with which they were treated was contaminated with Aβ seeds as well as human prions. While these individuals did not have the full diagnostic neuropathological features of Alzheimer's disease - which also requires the presence of intracellular neurofibrillary tangles - some did have undoubted CAA disease with circumferential vessel-wall degeneration. Had they not died of iCJD at a relatively young age[1], these individuals would have been expected to develop cerebral haemorrhage.

CAA can occur independently from Alzheimer's disease, but at autopsy CAA is detected in the large majority of Alzheimer's cases[8, 9]. That CAA, a pathology that leads to cerebral haemorrhage and dementia, is most often caused by Aβ deposition in blood vessels is undoubted[10]. Indeed, as with Alzheimer's disease, autosomal dominant mutations in, or triplication of, the amyloid precursor protein (APP) gene can cause CAA[11, 12 13]. The transmissibility of CAA, and potentially Alzheimer's disease, by iatrogenic routes raises important public health issues and would also indicate a clear shift in understanding their aetiology and suggest new approaches to prevention and treatment[2 3].

Alternative interpretations of our findings have been proposed[14, 15], although we have not consider these to be as plausible as the human transmission hypothesis[16, 17]. Given the potential public health importance of our findings, we proceeded to examine experimentally whether c-hGH batches to which these patients were exposed contained viable Aβ seeding activity, albeit after storage for over 30 years.

In the UK, 1,883 patients were treated with c-hGH over the period 1958-1985 and 80 have so far developed iCJD (to July 2018) with recent incubation periods exceeding 40 years[7, 18]. During this period of treatment, multiple preparations using several different extraction methods were used, and patients generally received multiple batches from different preparations. However, one preparation, produced by the Hartree-modified Wilhelmi Procedure (HWP) was received by all individuals who went on to develop iCJD[7, 18]. It is thought that size-exclusion chromatography, used in non-Wilhelmi preparation methods, may have reduced prion contamination[7]. Fortuitously, Public Health England has maintained an archive of vials of c-hGH batches used to treat patients and we were able to obtain vials from a range of batches and production methods to which the eight patients we described[1] were exposed, plus additional vials from two further HWP batches (table 1 and Extended data table 1).

We analysed vial contents biochemically for presence of Aβ peptides (x-40 and x-42) and tau protein (table 1 and methods). All HWP vials analysed were clearly positive for Aβ x-40 and tau, and all but one were also positive for Aβ x-42 peptides. Aβ and tau levels in vials from all other c-hGH purification methods examined - FL (Lowry preparation), LJ (Roos method) and TPL (Centre for Applied Microbiological Research)[19] - were below limits of detection. There was therefore unequivocal biochemical evidence for presence of Aβ peptides and tau protein in some of the batches (produced by the HWP method) to which iCJD patients with Aβ pathology were exposed. However, to determine whether seeding activity is present in this material requires a biological

rather than biochemical assay, as the composition and structure of seed-competent Aβ entities is unknown. Indeed, total Aβ peptide concentrations may be misleading in this regard.

For seeding studies we used homozygous APP NL-F knock-in mice[20] which express APP bearing Swedish (KM670/671NL) and Beyreuther/Iberian (I716F) mutations, with a humanised Aβ domain; these mice produce the first signs of Aβ deposition at ~6 months of age[21]. We conducted extensive in-house time-course studies of uninoculated *App*$^{NL-F/NL-F}$ mice (C57BL/6J background) and confirmed a similar evolution of pathology as described previously[21]. Inoculating these mice with brain homogenates (1% w/v) prepared from three autopsy-confirmed typical Alzheimer's patients (designated AD 1-3) or a normal control individual, or with vehicle alone (phosphate-buffered saline, PBS), intracerebrally injected into groups of female *App*$^{NL-F/NL-F}$ mice at 6-8 weeks of age - showed clear seeding of Aβ pathology from the Alzheimer's disease cases (Extended data figure 1). Mice were culled at serial time points: 2, 7, 15, 30, 45, 60, 90, 120, 240, 360 and 480 days post-inoculation (dpi). Representative images of Aβ immunohistochemistry at selected time points are shown in Extended data figure 1. No parenchymal or vascular Aβ deposits were observed in any mice (n=5 per group) at 2 d.p.i., demonstrating that Aβ deposition at later time points could not be attributed to persistence of the original inoculum. Meningeal CAA (mainly at the dorsal brain surface) and parenchymal deposition (mainly in the corpus callosum, but also in cerebellum, hippocampus and cerebral cortex) was detected at 120 d.p.i. in groups inoculated with AD 1-3, but not in those inoculated with PBS or normal brain homogenate (n=15 mice per group; Extended data figure 1). At 240 d.p.i., while PBS- and normal-brain-inoculated mice had almost no amyloid deposits in blood vessels (with minimal deposits in 1 out of 25 and 2 out of 15 mice, respectively), AD-brain-inoculated animals had consistent CAA with ventral meningeal blood vessels (surrounding the olfactory bulb) and many dorsal meningeal blood vessels affected in all mice (n=14-15 mice per group; figures 1 and 3).

The CAA count was significantly higher in AD brain-inoculated *App*$^{NL-F/NL-F}$ mice than in PBS-inoculated controls (P<0.0001; figure 1). Consistent with previous descriptions of spontaneous pathology in this mouse line[21], at 240 d.p.i., *App*$^{NL-F/NL-F}$ mice inoculated with PBS or normal brain had only occasional parenchymal plaques in cerebral cortex and hippocampus, while cerebellum, olfactory bulb and other areas entirely lacked plaques. However at this time point widespread parenchymal plaques were evident in cerebral cortex, hippocampus, corpus callosum, cerebellum and olfactory bulb in all AD-brain-inoculated animals (figure 3). The mean percentage area covered by parenchymal plaques was significantly higher in AD-brain-inoculated mice than in PBS-inoculated mice (PBS control versus AD1 p = 0.017; AD2 p < 0.0001; AD3 p = 0.0005) (data not shown). The difference in parenchymal Aβ deposition between AD- and control-inoculated mice was most pronounced in the cerebellum where deposition was almost completely absent in PBS- or normal brain-inoculated mice but marked in AD-inoculated animals (figure 2a).

At 360 d.p.i., the localisation of plaques observed in AD brain-inoculated mice was similar but more severe than at 240 d.p.i. Notably, at this time point in PBS- and normal brain-inoculated mice CAA was evident only in some dorsal meningeal blood vessels over the cerebral cortex, in marked contrast, in AD brain-inoculated mice Aβ deposition was seen in almost all meningeal blood vessels (n=15 mice per group) (Extended data figure 1).

To investigate possible toxicity of intracerebrally administered human growth hormone in mice before we used the scarce c-hGH samples, we inoculated groups of three female C57BL/6J mice with 30 µl of recombinant human growth hormone (rec-hGH) at 1.2, 3.6 or 11 mg/ml, corresponding to doses of 0.1, 0.3 and 1 international units (IU) respectively. There was no evidence of toxicity in any of the mice which were culled at 240 d.p.i. When mice (n=2) were injected with a higher concentration of recombinant growth hormone (20 mg/ml; 1.8 IU), they died immediately after the injection.

To establish whether seeding activity was present in Aβ–positive c-hGH batches, we used vials from c-hGH batches HWP 42 and 51, for which sufficient material was available for inoculation into groups of mice -for similar intracerebral injection into female congenic *App*$^{NL-F/NL-F}$ mice at 6-8 weeks of age. We expected these seeds, if present, to be at a very low titre by comparison with AD- brain homogenate and therefore used the much more efficient route of intracerebral injection of c-hGH, rather than the peripheral injection that patients with iCJD underwent, in order to optimize the chance of detecting seeds in this scarce material[22]. We also inoculated rec-hGH as a further control, in case growth hormone itself might induce Aβ deposition. Mice received doses of either 0.3 IU of HWP 42, 0.75 IU HWP 51 or 1 IU rec-hGH.

As additional experimental controls, AD- or normal-brain 1% w/v homogenate, vehicle alone, HWP 42 and HWP 51 were also injected in wild type C57BL/6J mice at 6-8 weeks of age. All mice were analysed at 240 d.p.i. As expected, none of the wild-type C57BL/6J mice groups, expressing only murine APP, developed Aβ deposition (n = 8-10 mice per group) (data not shown). Similarly, no cerebellar Aβ deposits were detected in rec-hGH inoculated *App*$^{NL-F/NL-F}$ mice (n=5 mice per group) and CAA score was not statistically different from that of PBS- or normal brain-inoculated groups (figures 1, 2b and 4).

By contrast, CAA and cerebellar Aβ deposits were clearly evident in HWP 42- and HWP 51-injected *App*$^{NL-F/NL-F}$ mice (figures 1, 2b and 4 and Extended data figure 2) demonstrating the presence of seeding activity in archived HWP c-hGH vials. That the degree of CAA and cerebellar Aβ deposition in c-hGH-inoculated *App*$^{NL-F/NL-F}$ mice was less pronounced than in those inoculated with AD brain is consistent with expected much higher seed titre in AD brain than in archived c-hGH samples. Indeed, it is remarkable that detectable seeding activity has persisted at all after decades of storage.

Our proposal that human transmission of Aβ pathology had occurred as a result of intramuscular injection of c-hGH is now firmly supported by experimental evidence. While the individuals we described in our earlier report[1] did not meet the full neuropathological criteria for Alzheimer's

disease, they might have done so if they had not died of iCJD at a relatively young age. Although tau pathology was not detected, it is interesting that HWP c-hGH batches to which these individuals were exposed also contained biochemically measurable levels of tau. In future studies it will be important to determine whether the tau in c-hGH vials can seed aggregation in mice expressing human tau. However, it is important to emphasise that the seeded Aβ deposition is not benign: several of these patients had an undoubted disease caused by Aβ deposition - CAA. This can now be described as iatrogenic CAA (iCAA) and CAA can be considered a transmissible disorder with attendant public health implications.

After the publication of our original report suggesting human transmission of Aβ via c-hGH therapy[1] - which raised the possibility of it can also be transmitted by other routes known to be a risk for the transmission of CJD prions - there have been several published reports of Aβ deposition in young individuals following neurosurgical procedures (notably involving dura mater grafting), as well as following c-hGH inoculation[23-28]. Although we reiterate that there is no suggestion that Alzheimer's disease is contagious, and no supportive evidence from epidemiological studies that it is transmissible (notably by blood transfusion[29, 30]), we consider it important to evaluate the risks of iatrogenic transmission of CAA, and potentially Alzheimer's disease. Given the lack of disease-modifying therapeutics for Alzheimer's disease and other distressing and fatal neurodegenerative conditions, it will be important to consider introducing improved methods for removing proteopathic seeds from surgical instruments on a precautionary basis.

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Supplementary information is available in the online version of the paper

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Author contributions

SAP and MAF coordinated animal experiments and performed data analysis. JL, TN and SB performed neuropathological analysis. DXT, ZC, DM and DMW performed and analysed biochemical

assays. TS and TS provided NL-F mice. PR coordinated identification and sourcing of relevant archival c-hGH batches. JC oversaw the study and drafted the manuscript with contributions from all authors.

Author information

Reprints and permissions information are available at www.nature.com/reprints. Competing interest statement: J.C. is a shareholder and Director of D-Gen Limited, an academic spin-out company working in the field of prion disease diagnosis, decontamination and therapeutics. Correspondence and requests for materials should be addressed to J.C. (jc@prion.ucl.ac.uk).

Preparation method	Batch number	Aβx-40 (pg/vial)	Aβx-42 (pg/vial)	Tau (pg/vial)
HWP	40	582	116	12411
HWP	42	288	NQ	13631
HWP	43	575	112	14581
HWP	47	772	108	14569
HWP	51	991	136	18155
FL	4	NQ	NQ	NQ
FL	5	NQ	NQ	NQ
FL	6	NQ	NQ	NQ
LJ	7	NQ	NQ	NQ
LJ	9	NQ	NQ	NQ
LJ	10	NQ	NQ	NQ
TPL	3	NQ	NQ	NQ
TPL	6	NQ	NQ	NQ
TPL	14	NQ	NQ	NQ
TPL	18	NQ	NQ	NQ
TPL	25	NQ	NQ	NQ

Table: Quantification of Aβ species and tau in c-hGH preparations. With the exception of HWP 42 and HWP 43, all batches were administered to patients with Aβ pathology described in Jaunmuktane et al[1]: Each patient received multiple injections from a number of different batches and preparations, although the table lists only the batches and preparations where vials were available for us to test. All patients received HWP-prepared c-hGH; only batches 40, 42, 43, 47 and 51 were available. A full list of the number of injections, preparations and batches which each patient received is provided in Extended data Table 1. NQ indicates that samples did not have quantifiable amounts of analyte. The lowest amount of analyte measurable in a vial is calculated based on the lower limit of quantification (LLoQ) for the assay plus a mathematical adjustment to account for sample dilution. The predicted lowest measurable amount of Aβ40, Aβ42, and tau per vial were 148.3 pg, 71.2 pg and 11.4 ng, respectively.

Figures

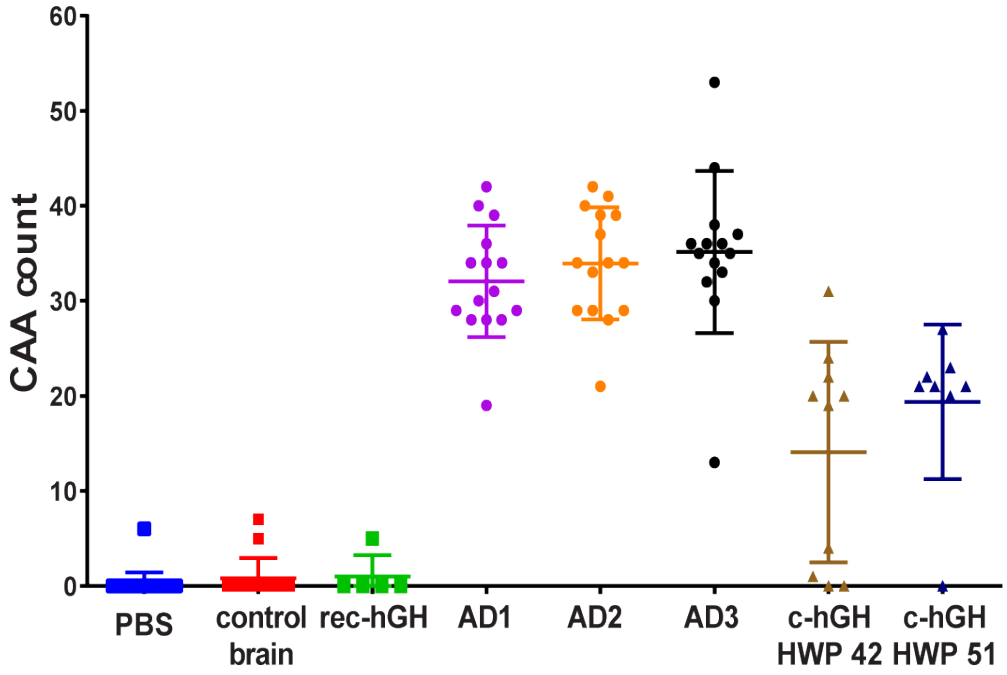

Figure 1 Quantification of vessels with CAA in *App*$^{NL-F/NL-F}$ mice following inoculation with Alzheimer's disease or control human brain, vehicle alone (PBS), or recombinant or cadaveric human growth hormone. There were highly significant differences between vehicle (PBS)-inoculated mice and those inoculated with either AD brain homogenates or cadaveric growth hormone (c-hGH) preparations (PBS n=25; AD1 n=15, PBS vs AD1 P<0.0001; AD2 n=15, PBS vs AD2 P<0.0001; AD3 n=14, PBS vs AD3 P<0.0001; c-hGH HWP 42 n=10, PBS vs HWP 42 P<0.0001; c-hGH HWP 51 n=8, PBS vs HWP 51 P<0.0001) (One way ANOVA followed by Dunnett's multiple comparison test). There were no significant differences between PBS-inoculated mice and those inoculated with control human brain homogenate or recombinant growth hormone (rec-hGH) (PBS n=25; control brain n=15, PBS vs control brain P=0.99; rec-hGH n=5, PBS vs rec-hGH P=0.99). Data are expressed as mean ± standard deviation, n= number of mice per group.

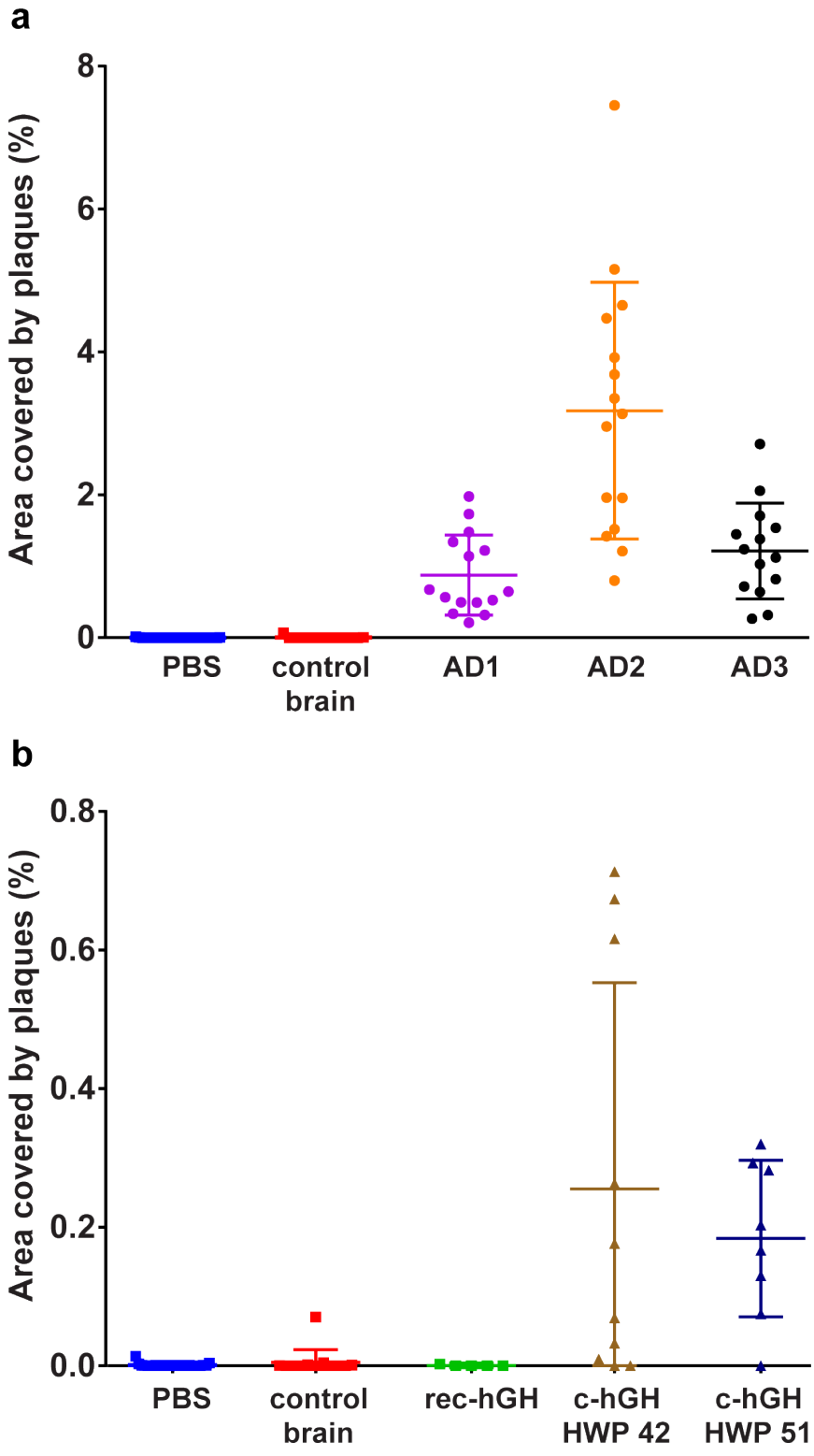

Figure 2 Quantification of cerebellar plaque area in *App*$^{NL-F/NL-F}$ mice inoculated with Alzheimer's or control human brain, vehicle alone, or recombinant or cadaveric human growth hormone. The area covered by plaques is expressed as a percentage of the total area: (a) following inoculation with

PBS, control normal brain or AD brain; (b) following inoculation with PBS, control human brain or rec-hGH or c-hGH. There was no significant difference between PBS- and control brain- or rec-hGH-inoculated mice (PBS n=25; control brain n=15, PBS vs control brain P=0.99; rec-hGH n=5, PBS vs rec-hGH P>0.99). However, there were significant differences between PBS-inoculated and AD- or c-hGH-inoculated mice (PBS n=25; AD1 n=15, PBS vs AD1 P=0.007; AD2 n=15, PBS vs AD2 P<0.0001; AD3 n=14, PBS vs AD3 P=0.0002; c-hGH HWP 42 n=10, PBS vs HWP 42 P<0.0001; c-hGH HWP 51 n=8, PBS vs HWP 51 P=0.002) (One way ANOVA tests followed by Dunnett's multiple comparison test). Mean values ± standard deviation, n= number of mice per group.

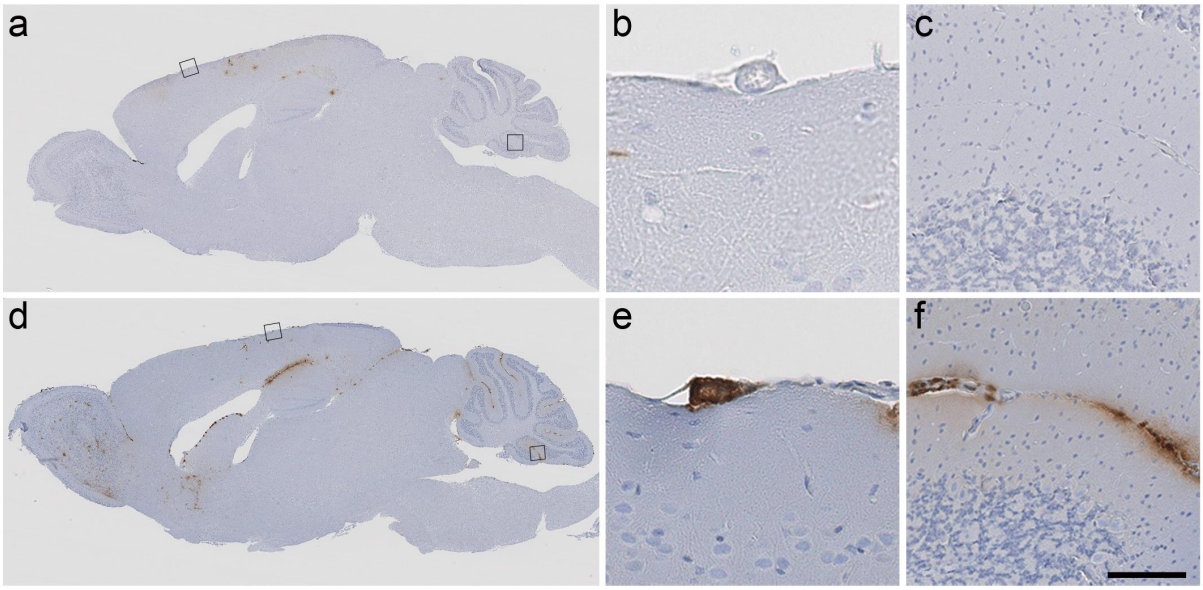

Figure 3 Aβ plaque deposition and CAA in *App*$^{NL-F/NL-F}$ mice following inoculation with AD or control brain. *App*$^{NL-F/NL-F}$ mice were inoculated with either human control brain (a-c) or AD brain (d-f) homogenates and culled after 240 days (control human brain n=15 mice; AD brain n=44 mice). Aβ deposition was assessed on sagittal sections (a, d). CAA (b, e) and cerebellar deposition (c, f) were evident only on AD brain-inoculated animals. Boxes denote areas magnified in middle and right panels. Scale bar: 1.5 mm for whole section (a, d), 25 µm for CAA (b, e), 50 µm for cerebellar region (c, f).

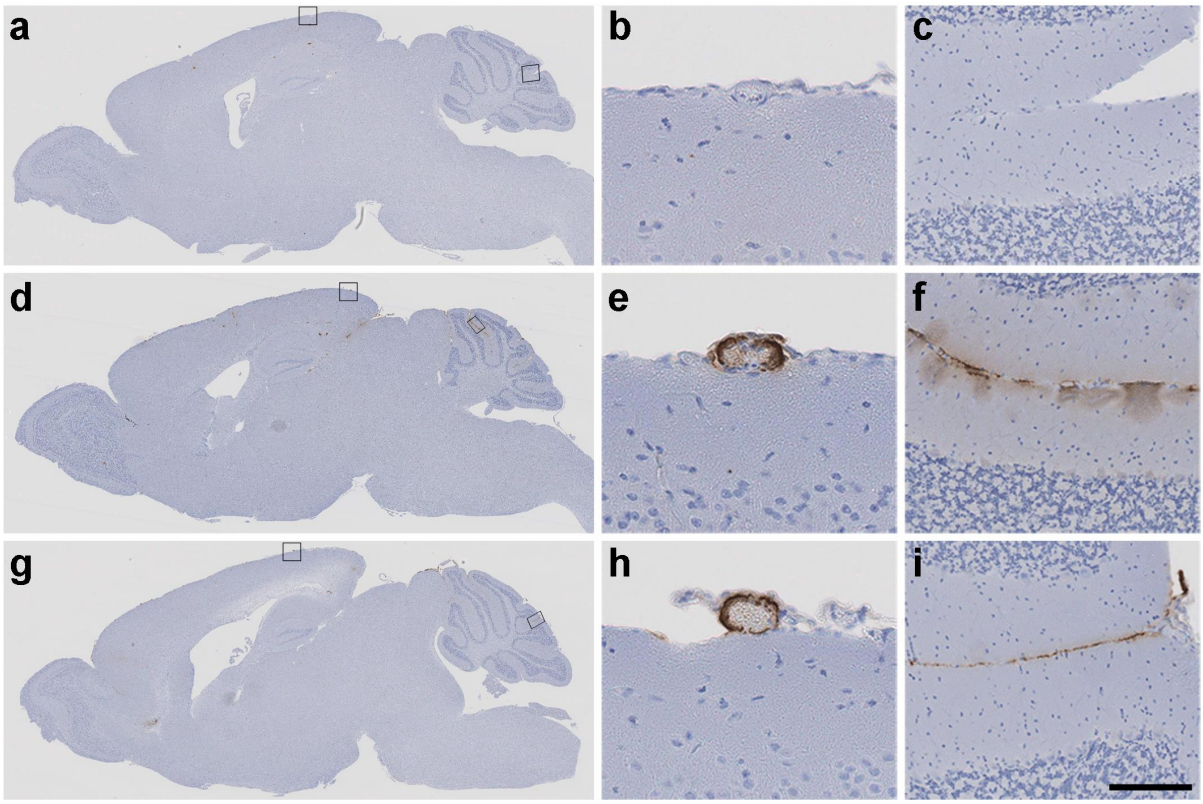

Figure 4 Aβ plaque deposition and CAA in *App*$^{NL-F/NL-F}$ mice following inoculation with cadaveric or recombinant growth hormone preparations. *App*$^{NL-F/NL-F}$ mice were inoculated with rec-hGH (a-c), c-hGH batch HWP 42 (d-f) or c-hGH batch HWP 51 (g-i) and culled after 240 days (rec-hGH n=5 mice; c-hGH HWP 42 n=10 mice; c-hGH HWP 51 n=8 mice). Aβ deposition was assessed on sagittal sections (a, d, g). CAA (b, e, h) and cerebellar deposition (c, f, i) were evident in c-hGH- but not rec-hGH-inoculated animals. Boxes denote areas magnified in middle and right column panels. Scale bar: 1.7 mm for whole section (a, d, g), 25 µm for CAA (b, e, h), 50 µm for cerebellar region (c, f, i).

Methods

Use of human tissues and research ethics

This study was carried out following ethics approval from the North East - Newcastle & North Tyneside 2 Research Ethics Committee; REC reference: 11/NE/0348 and London Queen Square Research Ethics Committee REC reference: 03/N038. The storage, biochemical analysis of human tissue samples and transmission studies to mice were performed in accordance with informed consent from all patients or a person in a qualifying relationship to the deceased, or a legal representative in accordance with applicable UK legislation and Regulatory Codes of Practice. Anonymised post-mortem brain samples (three neuropathologically confirmed cases of Alzheimer's disease and one control with no signs of neurodegenerative disease) were kindly provided under a material transfer agreement from the Oxford Brain Bank, Oxford University Hospitals NHS Trust and the Queen Square Brain Bank for Neurological Disorders, UCL Institute of Neurology. Samples were obtained and used in accordance with the requirements of each providing tissue bank.

Sourcing of archived c-hGH material

Human cadaveric pituitary-derived growth hormone material, from batches manufactured in the mid-1980s, is stored at Public Health England (PHE) under contract from the Department of Health and Social Care, with accompanying batch manufacturing records where available. Material from specific manufactured batches, was supplied from this archive for the purposes of the study, on request of the MRC Prion Unit at UCL and with the approval of the Department of Health and Social Care. All material has been stored at ambient temperature in sealed vials since the date of transfer to PHE.

Biochemical analysis of c-hGH vials

The contents of each c-hGH vial were re-suspended directly in 316 µl 6M guanidine hydrochloride and all analyses were conducted with the investigator blind to sample identity. For determination of Aβx-40 concentrations samples were diluted 12-fold prior to analysis on a Meso Scale Diagnostics platform using anti-Aβ antibody 266 for capture and biotinylated 2G3 antibody for detection[31]. Aβx-42 concentrations were determined following 72-fold dilution on an Erenna instrument (Quanterix Lexington, MA) using anti-Aβ antibody 266 for capture and fluorescently-labelled 21F12 for detection[31]. Tau levels were determined by ELISA following 144-fold dilution, with anti-tau BT2

(Thermo Fisher Scientific) used for capture and alkaline phosphatase conjugated Tau5 antibody (Thermo Fisher Scientific) used for detection[32]. Lower limits of quantification (LLoQ) for the determination of Aβx-40 and tau levels were defined as the lowest standard with a signal higher than the average signal for the blank plus 9 standard deviations (SDs) and allows a percent recovery ≥100±20% and a coefficient of variance (CV) ≤20%. The LLoQ for Aβx-42 was defined as the lowest interpolated standard that provided a signal two-fold the background with a percentage coefficient of variance (CV) ≤20% and allows a percent recovery ≥100±20%.

Mouse transmission studies

Mouse studies were performed under approval and licence granted by the UK Home Office (Animals (Scientific Procedures) Act 1986); Project Licence number 70/9022 and conformed to University College London institutional and ARRIVE guidelines (http://www.nc3rs.org.uk/ARRIVE/).

We utilised homozygous APP NL-F knock-in mice[20] that express APP bearing Swedish (KM670/671NL) and Beyreuther/Iberian (I716F) mutations, and in which the Aβ domain has been humanised.. These were speed backcrossed (Jackson Laboratories, USA) and maintained on an inbred C57BL/6J background and used as homozygotes (designated *App*$^{NL-F/NL-F}$). Wild-type C57BL/6J mice were purchased from Jackson Laboratories via Charles River Laboratories. Mouse genotype was confirmed by PCR of ear punch DNA and mice were uniquely identified by sub-cutaneous transponders. Mice used were all female as is our standard practice for long term prion transmission experiments for consistency, animal welfare and logistical grounds because of increased fighting amongst groups of males which then need to be housed separately. Mice (female, aged 6–8 weeks) were randomly assigned to experimental groups, anaesthetised with a mixture of halothane and O_2, and intracerebrally inoculated into the right hemisphere in the parietal region with 30 µl of a 1% (w/v) human brain homogenate prepared in Dulbecco's phosphate buffered saline lacking Ca^{++} or Mg^{++} ions (D-PBS), vehicle (D-PBS) alone, 11 mg/ml recombinant human growth hormone (rec-hGH) (Humatrope Eli Lilly, UK) or c-hGH material prepared in D-PBS. For preparation of human brain homogenate, grey matter was dissected from frontal cortex samples of one healthy control brain and three AD cases (AD1-3), homogenised using glass grinders with D-PBS at 10% (w/v) and subsequently diluted at 1% in D-PBS to inoculate the mice. Levels of $Aβ_{40}$ and $Aβ_{42}$ in the 10% homogenates were quantified using V-Plex Aβ Peptide Panel 1 6E10 kit (Meso Scale Diagnostics platform) using anti-$Aβ_{40}$ and -$Aβ_{42}$ monoclonal antibodies for capture and anti-Aβ antibody 6E10 for detection. The healthy control brain had 2 ± 0.6 ng/ml $Aβ_{40}$ and 6.7 ± 2.8 ng/ml $Aβ_{42}$. AD1 had 13.7 ± 0.6 ng/ml $Aβ_{40}$ and 30.5 ± 1.4 ng/ml $Aβ_{42}$. AD2 had 160.1 ± 10.7 ng/ml $Aβ_{40}$ and 43.4 ± 10.6 ng/ml

$A\beta_{42}$. Finally, AD3 had 14.2 ± 0.3 ng/ml $A\beta_{40}$ and 57.7 ± 2.1 ng/ml $A\beta_{42}$ (mean ± standard deviation). It is important to note that total Aβ peptide concentrations determined by biochemical assay may not relate to Aβ seeding activity. For transmission studies each c-hGH vial was re-suspended in 200 µl D-PBS and the contents of six vials from each batch were pooled prior to inoculation. This corresponds to each mouse receiving 0.75 IU of HWP51, 0.3 IU of HWP42 or 1 IU of rec-hGH. HWP42 was labelled as containing 2 IU per vial whereas the HWP51 was labelled as containing 5 IU per vial, hence although we used the same number of vials from each batch for inoculation the dose of GH was different. Amounts of Aβ peptides in each 30µl inoculum: HWP 42 - Aβx-40 43 pg, Aβx-42 below limit of quantitation; HWP 51 - Aβx-40 149 pg, Aβx-42 20 pg. Inocula were prepared following strict biosafety protocols in a microbiological containment level III laboratory and inoculations performed within a class 1 microbiological safety cabinet, using disposable equipment for preparation of each inoculum. Safety cabinets were decontaminated prior to the preparation of inocula to avoid cross contamination. Mice were culled at 8 months post-inoculation by exposure to CO_2, brains were removed and prepared for immunohistochemistry.

Antibodies and immunohistochemistry

Tissue was fixed in 10% buffered formal saline followed by incubation in 98% formic acid for 1 hour. Following further washing in 10% buffered formal saline, tissue samples were processed and paraffin wax embedded. Serial sections of 5 µm nominal thickness were taken. Aβ deposition was visualized using biotinylated 82E1 (cat n.10326, IBL, Japan) as the primary antibody, using a Ventana Discovery automated immunohistochemical staining machine (Roche, Burgess Hill, UK) and proprietary solutions. Visualization was accomplished with development of 3'3 diaminobenzidine tetrahydrochloride as the chromogen (DAB Map Kit, Ventana Medical Systems). Haematoxylin was used as the counterstain.

Image capture

Histological slides were digitised on a LEICA SCN400F scanner (LEICA Milton Keynes, UK) at 40x magnification and 65% image compression setting during export. Slides were archived and managed on LEICA Slidepath (LEICA Milton Keynes, UK).

Quantification of cerebral amyloid angiopathy (CAA) and parenchymal amyloid β-protein

All immunohistochemical quantification was performed blind to experimental group. CAA was present in small meningeal vessels and occasionally in small superficial cortical vessels. Because of the small size of meningeal vessels, reliable automated image analysis of CAA was not possible, and negative and positive vessels were quantified by visual inspection. One paramedian (approximately 200 µm) sagittal section per mouse was analysed. The extent of the CAA was determined by counting the number of Aβ negative and positive blood vessels in six anatomical areas of the meninges covering the dorsal part of the brain, including the olfactory bulb and the cerebellum.

Parenchymal Aβ, present in the form of diffuse deposits or as plaques are amenable to image quantification on whole slide images as described previously [1]. All Aβ immunostained sagittal sections (approximately 200 µm para sagittal) of whole mouse brains were digitised as described above. Digital image analysis on whole slides was performed using Definiens Developer XD 2.6 (Definiens, Munich, Germany). Initial tissue identification was performed using resolution equivalent to x10 magnification and stain detection was performed at x20 magnification. Regions of interest (ROI) were manually selected to separate cortex, hippocampus, and cerebellum; larger artefacts were also manually selected for exclusion from analysis. Tissue detection and initial segmentation was done to identify all tissue within the image, separating the sample from background and non-tissue regions for further analysis. This separation was based on identification of the highly homogeneous relatively bright/white region of background present at the perimeter of each image. A composite raster image produced by selecting the lowest pixel value from the three comprising colour layers (RGB colour model) provided a greyscale representation of brightness. The mean brightness of this background region was used to exclude all background regions from further analysis.

Stain detection (brown) is based on the transformation of the RGB colour model to a Hue-Saturation-Density (HSD) representation[33]. This provides a raster image of the intensity of each colour of interest (brown and blue). A number of fixed thresholds (T_x) are then used to identify areas of interest (A_x). The thresholds used are in arbitrary units (au) with a scale of 0 au to 3 au in HSD images. The threshold $T_{brown\ stain}$ was allocated the value 0.15 au, with all pixels above this threshold classed as "stain" (A_{stain}) and those below as "unstained" ($A_{unstained}$). A_{stain} was excluded if the intensity of blue staining was not significantly lower than the level of brown stain (difference less than 0.1 au) to remove generically dark areas. The remaining A_{stain} were further categorised using threshold $T_{dark\ brown}$ of 0.5 au to give A_{light} and A_{dark}.

Plaques were then constructed from these A_{light} and A_{dark} objects. Each A_{dark} was classified as a plaque seed; these were then grown into all surrounding A_{light} to give $A_{potential\ plaque}$ (constructed of

A_{light} and A_{dark}) and $A_{non\text{-}plaque}$ (constructed of only A_{light}). A number of exclusions were then applied: Any $A_{potential\ plaque}$ with area less than 20 μm^2 (see below) or containing less than 3 pixels previously classified as A_{dark} were reclassified as $A_{non\text{-}plaque}$. $A_{potential\ plaque}$ with a relatively high stain intensity (mean brown ($\bar{B}$) intensity higher than 0.35 au) and low variation in brown stain level (standard deviation in brown stain ($B\delta$) below 0.1 au), were removed into $A_{unstained}$ as artefacts; $A_{potential\ plaque}$ with a $\bar{B}$ higher than 0.5 au and $B\delta$ below 0.25 au were reclassified as $A_{non\text{-}plaque}$, as they display an uncharacteristically dark and varied stain character for plaques; $A_{potential\ plaque}$ with area less than 40 μm^2 and elliptic character less than 0.2 (scale of 0 (random shape) to 1 (perfect circle)) were reclassified as $A_{non\text{-}plaque}$; and $A_{potential\ plaque}$ with area greater than 40 μm^2 and relative proportion of A_{dark} greater than 70% were reclassified as $A_{non\text{-}plaque}$. The remaining $A_{potential\ plaque}$ give our final A_{plaque}.

For each ROI the total area analysed ($A_{unstained}$+A_{plaque}+$A_{non\text{-}plaque}$), A_{plaque}, $A_{non\text{-}plaque}$ and #A_{plaque} were exported and the percentage area covered by A_{plaque} determined.

To establish the optimal minimum area for $A_{potential\ plaque}$, analyses using 10, 20, 30, 40 and 50 μm^2 were performed with a minimum area of 20 μm^2 being selected.

Statistical Analysis and Reproducibility

All statistical analysis and graphs were generated using the package GraphPad PRISM v6 (GraphPad Software, Inc., La Jolla, USA). Error bars on graphs denote standard deviation, with statistical significance determined by one way ANOVA followed by Dunnett's multiple comparison test (two-tailed). Statistical significance was set to $P < 0.05$. Experiments with mice were performed only once to avoid unnecessary use of animals and biochemical assays were not replicated due to scarcity of cadaveric human growth hormone.

Reference List

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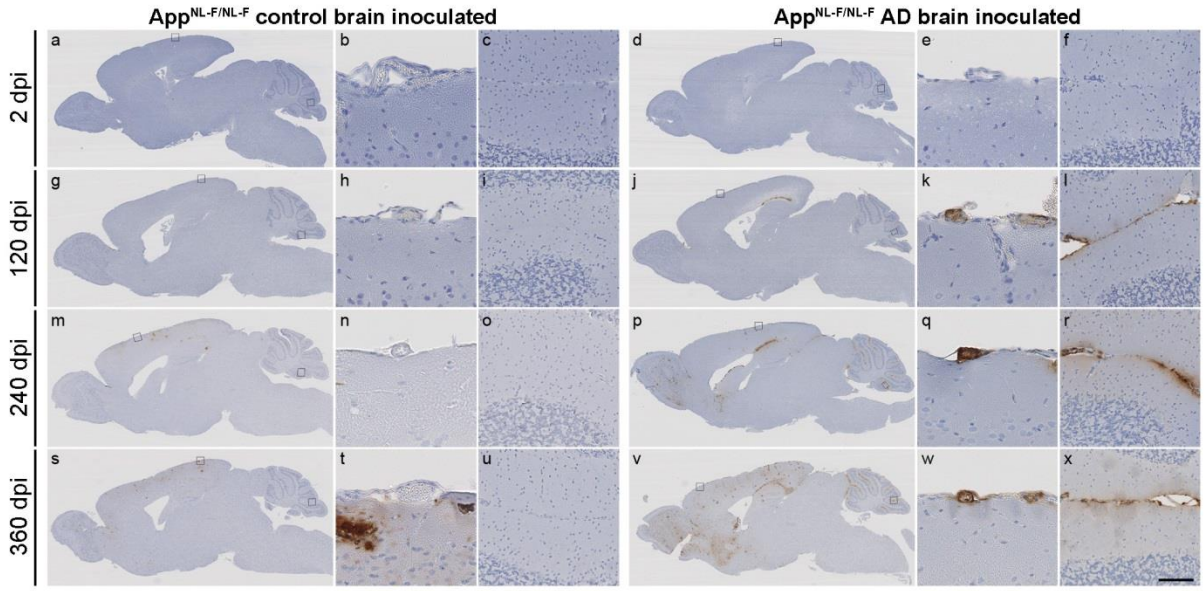
32. Kanmert,D. *et al.* C-Terminally Truncated Forms of Tau, But Not Full-Length Tau or Its C-Terminal Fragments, Are Released from Neurons Independently of Cell Death. *J Neurosci* **35**, 10851-10865 (2015).

33. Der Laak,J.A., Pahlplatz,M.M., Hanselaar,A.G., & de Wilde,P.C. Hue-saturation-density (HSD) model for stain recognition in digital images from transmitted light microscopy. *Cytometry* **39**, 275-284 (2000).

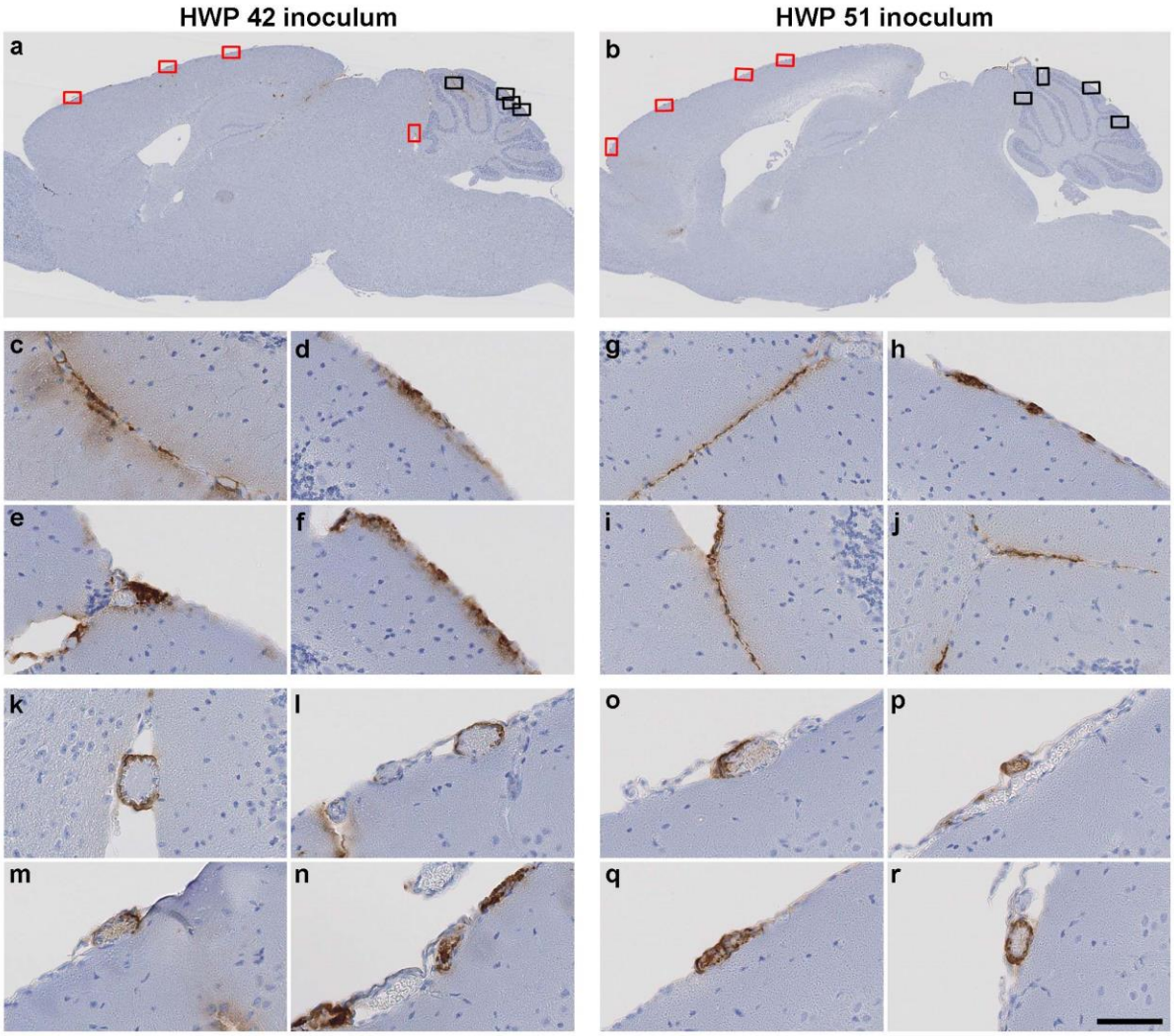
Data availability statement

Source data for figures 1 and 2 are available from the corresponding author upon reasonable request.

Extended Data:



Extended data figure 1: Time course of CAA and Aβ deposition in control and AD brain inoculated *App*$^{NL-F/NL-F}$ mice. Mice were inoculated with either control (a-c, g-i, m-o, s-u) or AD brain (d-f, j-l, p-r, v-x) and culled at stated times. Aβ deposition was assessed on sagittal sections (a, d, g, j, m, p, s, v). CAA (b, e, h, k, n, q, t, w) and cerebellar deposition (c, f, i, l, o, r, u, x) was evident only on AD brain inoculated animals. Boxes denote areas magnified to the right. Scale bar: 1.4 mm for whole section (a, d, g, j, m, p, s, v), 25 µm for CAA (b, e, h, k, n, q, t, w), 50 µm for cerebellar region (c, f, i, l, o, r, u, x). dpi = days post-inoculation.



Extended data figure 2: Aβ plaques and CAA in *App*$^{NL-F/NL-F}$ mice following inoculation with c-hGH preparations. *App*$^{NL-F/NL-F}$ mice were inoculated with either c-hGH batch HWP 42 (a, c-f, k-n) or HWP 51 (b, g-j, o-r) and culled after 240 days. Aβ deposition was assessed on sagittal sections (a, b). Black and red boxes denote areas magnified to better show cerebellar Aβ deposits (c-j) and CAA (k-r), respectively, in middle and lower panels. Scale bar: 1.1 mm for whole section (a, b), 50 µm for cerebellar region and CAA (c-r).

	Cadaveric Human Growth Hormone Preparations						
Patient number*	**HWP**	**K**	**FL**	**LJ**	**TPL**	**R**	
1	HWP 00, 44, 45, 51	K 79972	FL 6	LJ 4, 7, 9, 10	TPL 18		1 batch hGH unspecified
2	HWP 13 batches unspecified		FL 1, 4, 8, 10		TPL 4 and 2 batches unspecified	R 2 batches unspecified	
3	HWP 9, 10, 15, 19, 20, 28, 29, 31, 40	K 79250	FL 4, 8, 9	LJ 5, 6, 8	TPL 14, 15 1 batch unspecified	R 15, 16	
4	HWP 9, 10, 15, 44, 51	K 79972	FL 5, 6, 7 and 4 batches unspecified	LJ 4, 7, 8, 10	TPL 3, 6, 12, 18, 21, 22, 25	R 15, 16	10 batches hGH unspecified
5	HWP 11, 21, 23, 28, 29, 38, 40, 51 and 1 batch unspecified	K 79250	FL 1, 4, 8, 9, 10 and 1 batch unspecified	LJ 4, 5	TPL 6	R 18, 19	
6	HWP 00, 44, 51 and 4 batches unspecified	K 79972		LJ 3, 7			1 batch hGH unspecified
7	HWP 00, 45, 47 and 1 batch unspecified		FL 5, 6				
8	HWP 00, 38, 44, 45		FL 5, 6				2 batches hGH unspecified

Extended data table: c-hGH preparations and batches received by each patient

*'Patient number' refers to the patients described in ref.[1].

c-hGH preparations were as follows: HWP - Hartree-modified Wilhelmi preparation; K - Kabi commercial preparation; FL - St Bartholomew's Hospital preparation using Roos-Lowry method; LJ - Commercial preparation using Roos method; TPL - Centre for Applied Microbiology and Research, Porton Down preparation; R - Raben preparation. The final column shows where c-hGH was given but the type of preparation was not specified on medical records.