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

Silvia A. Purro[1], **Mark A. Farrow**[1], **Jacqueline Linehan**[1], **Tamsin Nazari**[1], **David X. Thomas**[1], **Zhicheng Chen**[2], **David Mengel**[2], **Takashi Saito**[3], **Takaomi Saido**[3], **Peter Rudge**[1], **Sebastian Brandner**[1,4], **Dominic M. Walsh**[1,2], **John Collinge**[1,*]

[1]MRC Prion Unit at UCL, UCL Institute of Prion Diseases, London, UK

[2]Laboratory for Neurodegenerative Research, Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.

[3]Laboratory for Proteolytic Neuroscience, RIKEN Center for Brain Science, 2-1 Hirosawa, Wako-shi, Saitama, Japan.

[4]Division of Neuropathology, National Hospital for Neurology and Neurosurgery, London, UK.

Abstract

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. These relatively young individuals with such treatment-induced (iatrogenic) CJD (iCJD), unlike other prion-disease patients and population controls, showed marked deposition of grey-matter and vascular Aβ; this finding, allied with the ability of Alzheimer's disease brain homogenates to seed Aβ deposition in laboratory animals, led us to argue that the 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 our 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 have substantial levels of Aβ1–40, Aβ1–42 and tau proteins, and that 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

* jc@prion.ucl.ac.uk.

Correspondence and requests for materials should be addressed to J.C.

Author contributions S.A.P. and M.A.F. coordinated animal experiments and performed data analysis. J.L., T.N. and S.B. performed neuropathological analysis. D.X.T., Z.C., D.M. and D.M.W. performed and analysed biochemical assays. T.Saito and T.Saido provided NL-F mice. P.R. coordinated the identification and sourcing of relevant archival c-hGH batches. J.C. oversaw the study and drafted the manuscript with contributions from all authors

Competing interests: 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.

Extended data is available for this paper at

Supplementary information is available for this paper at

implications for both the prevention and the 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 recognized 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 the 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 is known to span more than five decades[6]. Before 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 would 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 of 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 [10]. Indeed, autosomal dominant mutations in, or triplication of, the amyloid precursor protein (APP) gene can cause both CAA and Alzheimer's disease[11–13]. 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]. Alternative interpretations of our findings have been proposed[14,15], although we have not considered 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 more than 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 the 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. 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 the limits of detection. There was therefore unequivocal biochemical evidence for the 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 a 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 the Swedish (KM670/671NL) and Beyreuther/Iberian (I716F) mutations, with a humanized Aβ domain; these mice produce the first signs of Aβ deposition at around six months of age[21]. We conducted extensive in-house time-course studies of uninoculated $App^{NL\text{-}F/NL\text{-}F}$ mice (C57BL/6J background) and confirmed a similar evolution of pathology to that 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 $App^{NL\text{-}F/NL\text{-}F}$ mice at 6−8 weeks of age—showed clear seeding of Aβ pathology from the Alzheimer's cases (Extended Data Fig. 1). Mice were culled at serial time points, namely 2, 7, 15, 30, 45, 60, 90, 120, 240, 360 and 480 days post-inoculation (d.p.i.). Representative images of Aβ immunohistochemistry at selected time points are shown in Extended Data Fig. 1. No parenchymal or vascular Aβ deposits were observed in any mice ($n = 5$ per group) at 2 days post-inoculation, 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 the 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 Fig. 1). At 240 d.p.i., while PBS- and normal-brain-inoculated mice had almost no amyloid deposits in blood vessels (with minimal deposits seen 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 vessels affected in all mice (n = 15 mice per group; Figs 1 and 3).

The CAA count was significantly higher in AD-brain-inoculated $App^{NL\text{-}F/NL\text{-}F}$ mice than in PBS-inoculated controls ($P < 0.0001$; Fig. 1). Consistent with previous descriptions of spontaneous pathology in this mouse line[21], at 240 d.p.i. $App^{NL\text{-}F/NL\text{-}F}$ mice inoculated with PBS or normal brain had only occasional parenchymal plaques in the cerebral cortex and hippocampus, while the cerebellum, olfactory bulb and other areas entirely lacked plaques. However, at this time point widespread parenchymal plaques were evident in the cerebral cortex, hippocampus, corpus callosum, cerebellum and olfactory bulb in all AD-brain-inoculated animals (Fig. 3). The mean percentage area covered by parenchymal plaques was significantly higher in AD-brain-inoculated mice than in PBS-inoculated mice (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 (Fig. 2a).

At 360 d.p.i., the localization of plaques observed in AD-brain-inoculated mice was similar but more severe than at 240 d.p.i. Interestingly, 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 Fig. 1).

To investigate the 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^{-1}, 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 $App^{NL\text{-}F/NL\text{-}F}$ mice at 6−8 weeks of age. We used 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; we expected these seeds, if present, to be at a very low titre by comparison with AD-brain homogenate, which is a much more efficient transmission route[22]. We also inoculated rec-hGH as a further control, in case growth hormone itself might induce Aβ deposition. Mice received doses of 0.3 IU of HWP 42, 0.75 IU of HWP 51 or 1 IU of rec-hGH.

As additional experimental controls, AD- and normal-brain 1% w/v homogenate, vehicle alone, HWP 42 and HWP 51 were also injected into 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 the CAA score was not statistically different from that of PBS- or normal-brain-inoculated groups (Figs 1, 2b and 4).

By contrast, CAA and cerebellar Aβ deposits were clearly evident in *App*$^{NL-F/NL-F}$ mice injected with HWP 42 or HWP 51 (Figs 1, 2b, 4 and Extended Data Fig. 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 the expected much higher seed titre in AD brain than in the 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 the 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 emphasize 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 that 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 (notably involving blood transfusion[29,30]) that it is transmissible, we consider it important to evaluate the risks of iatrogenic transmission of CAA, and potentially of 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.

METHODS

Use of human tissues and research ethics

This study was carried out following ethics approval from the North East–Newcastle and North Tyneside 2 Research Ethics Committee (REC), reference 11/NE/0348, and the London Queen Square REC, reference 03/N038. The storage and biochemical analysis of

human tissue samples and the transmission studies involving mice were performed in accordance with informed consent from all patients, from a person in a qualifying relationship to the deceased, or from a legal representative, in accordance with applicable UK legislation and regulatory codes of practice.

Anonymized post-mortem brain samples (three neuropathologically confirmed cases of Alzheimer's disease and one control with no signs of neurodegenerative disease) were 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 our study, on the 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 resuspended directly in 316 μl 6M guanidine hydrochloride and all analyses were conducted with the investigator blinded to sample identity. For determination of Aβx-40 concentrations, samples were diluted 12-fold before 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, USA) using anti-Aβ antibody 266 for capture and fluorescently labelled 21F12 for detection[31]. Tau levels were determined by enzyme-linked immunosorbent assay (ELISA) following 144-fold dilution, with anti-tau antibody BT2 (Thermo Fisher Scientific) used for capture and alkaline-phosphatase-conjugated Tau5 antibody (Thermo Fisher Scientific) for detection [32]. We defined lower limits of quantification (LLoQs) for the determination of Aβx-40 and tau levels as the lowest standards with a signal higher than the average signal for the blank plus 9 standard deviations (s.d.), allowing a percentage recovery of 100 ± 20% or more, and a coefficient of variance (CV) of 20% or less. The LLoQ for Aβx-42 was defined as the lowest interpolated standard that provided a signal twofold that of the background with a percentage CV of 20% or less, and allows a percentage recovery of 100 ± 20% or more.

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 UCL institutional and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (http://www.nc3rs.org.uk/ARRIVE/).

We used homozygous APP NL-F knock-in mice[20] that express APP bearing the Swedish (KM670/671NL) and Beyreuther/Iberian (I716F) mutations, and in which the Aβ domain has been humanized. These were speed-backcrossed (Jackson Laboratories, USA) and maintained on an inbred C57BL/6J background, and used as homozygotes (designated AppNL-F/NL-F). Wild-type C57BL/6J mice were purchased from Jackson Laboratories via Charles River Laboratories. Mouse genotype was confirmed by polymerase chain reaction (PCR) of ear-punch DNA, and mice were uniquely identified by subcutaneous transponders. All of the mice used were female, as is our standard practice for long-term prion-transmission experiments, for reasons of consistency, animal welfare and logistics: increased fighting amongst groups of males requires them to be housed separately.

Mice (female, aged 6–8 weeks) were randomly assigned to experimental groups, anaesthetized with a mixture of halothane and O2, 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 PBS lacking Ca2+ or Mg2+ ions (D-PBS), vehicle (D-PBS) alone, 11 mg ml−1 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 Alzheimer's cases (AD 1–3), homogenized using glass grinders with D-PBS at 10% (w/v), and subsequently diluted at 1% in D-PBS to inoculate the mice. Levels of Aβ40 and Aβ42 in the 10% homogenates were quantified using a V-Plex Aβ Peptide Panel 1 6E10 kit (Meso Scale Diagnostics platform), using anti-Aβ40 and anti-Aβ42 monoclonal antibodies for capture and anti-Aβ antibody 6E10 for detection. The healthy control brain had 2 ± 0.6 ng ml−1 Aβ40 and 6.7 ± 2.8 ng ml−1 Aβ42. AD1 had 13.7 ± 0.6 ng ml−1 Aβ40 and 30.5 ± 1.4 ng ml−1 Aβ42. AD2 had 160.1 ± 10.7 ng ml−1 Aβ40 and 43.4 ± 10.6 ng ml−1 Aβ42. Finally, AD3 had 14.2 ± 0.3 ng ml−1 Aβ40 and 57.7 ± 2.1 ng ml−1 Aβ42 (mean ± s.d.). Note that the total Aβ peptide concentrations determined by biochemical assay may not relate to Aβ seeding activity.

For transmission studies, each c-hGH vial was resuspended in 200 μl D-PBS and the contents of six vials from each batch were pooled before 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 growth hormone was different. Amounts of Aβ peptides in each 30-βl inoculum were as follows: 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 to prepare each inoculum. Safety cabinets were decontaminated before preparing inocula to avoid cross-contamination. Mice were culled at 8 months post-inoculation by exposure to CO2; brains were then removed and prepared for immunohistochemistry.

Antibodies and immunohistochemistry

Tissue was fixed in 10% buffered formal saline and incubated in 98% formic acid for 1 h. Following further washing in 10% buffered formal saline, tissue samples were processed and

paraffin wax embedded. Serial sections of 5 βm nominal thickness were taken. Aβ deposition was visualized using biotinylated 82E1 (catalogue number 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 digitized on a LEICA SCN400F scanner (LEICA Milton Keynes, UK) at ×40 magnification and 65% image-compression setting during export. Slides were archived and managed on LEICA Slidepath (LEICA Milton Keynes, UK).

Quantification of CAA and parenchymal Aβ 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, is amenable to image quantification on whole-slide images as described previously[1]. All Aβ-immunostained sagittal sections (approximately 200 μm parasagittal) of whole mouse brains were digitized 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 a resolution equivalent to ×10 magnification, and stain detection was performed at ×20 magnification. Regions of interest (ROIs) were manually selected to separate the 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 constituent 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) was based on 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) was then used to identify areas of interest (A_x). The thresholds used were in arbitrary units (AU), with a scale of 0 AU to 3 AU in HSD images. The threshold $T_{\text{brown stain}}$ 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 intensity of brown stain (that is, if the difference was less than 0.1 AU), in order to remove generically dark areas. The remaining A_{stain} areas were further categorized using a 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} area was classified as a plaque seed; these were then grown into all surrounding A_{light} areas to give $A_{potential\ plaque}$ (constructed of A_{light} and A_{dark}) and $A_{non\text{-}plaque}$ (constructed of only A_{light}). Several exclusions were then applied, as follows. Any $A_{potential\ plaque}$ regions with an area less than 20 μm^2 (see below) or containing fewer than three pixels previously classified as A_{dark} were reclassified as $A_{non\text{-}plaque}$. $A_{potential\ plaque}$ areas 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. $A_{potential\ plaque}$ regions 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 displayed an uncharacteristically dark and varied stain character for plaques. $A_{potential\ plaque}$ regions with an area of less than 40 μm^2 and elliptic character of less than 0.2 (scale of 0 (random shape) to 1 (perfect circle)) were reclassified as $A_{non\text{-}plaque}$. $A_{potential\ plaque}$ regions with an area greater than 40 μm^2 and relative proportion of A_{dark} greater than 70% were reclassified as $A_{non\text{-}plaque}$. The remaining $A_{potential\ plaque}$ regions give our final A_{plaque}.

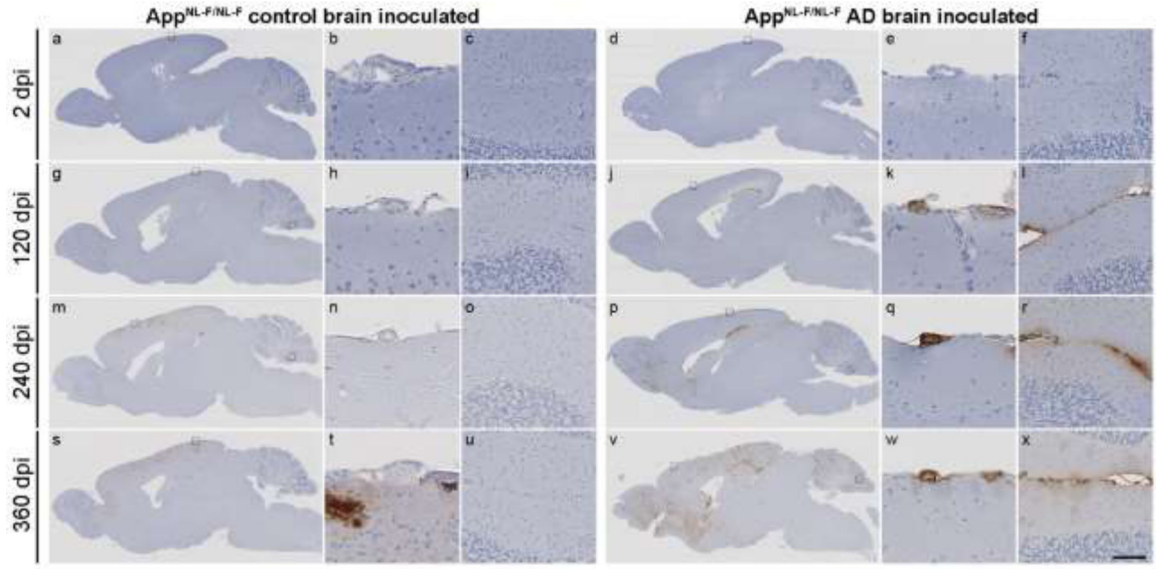
For each ROI, the total area analysed ($A_{unstained} + A_{plaque} + A_{non\text{-}plaque}$), A_{plaque}, $A_{non\text{-}plaque}$ and number of A_{plaque} were exported and the percentage area covered by A_{plaque} was determined.

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

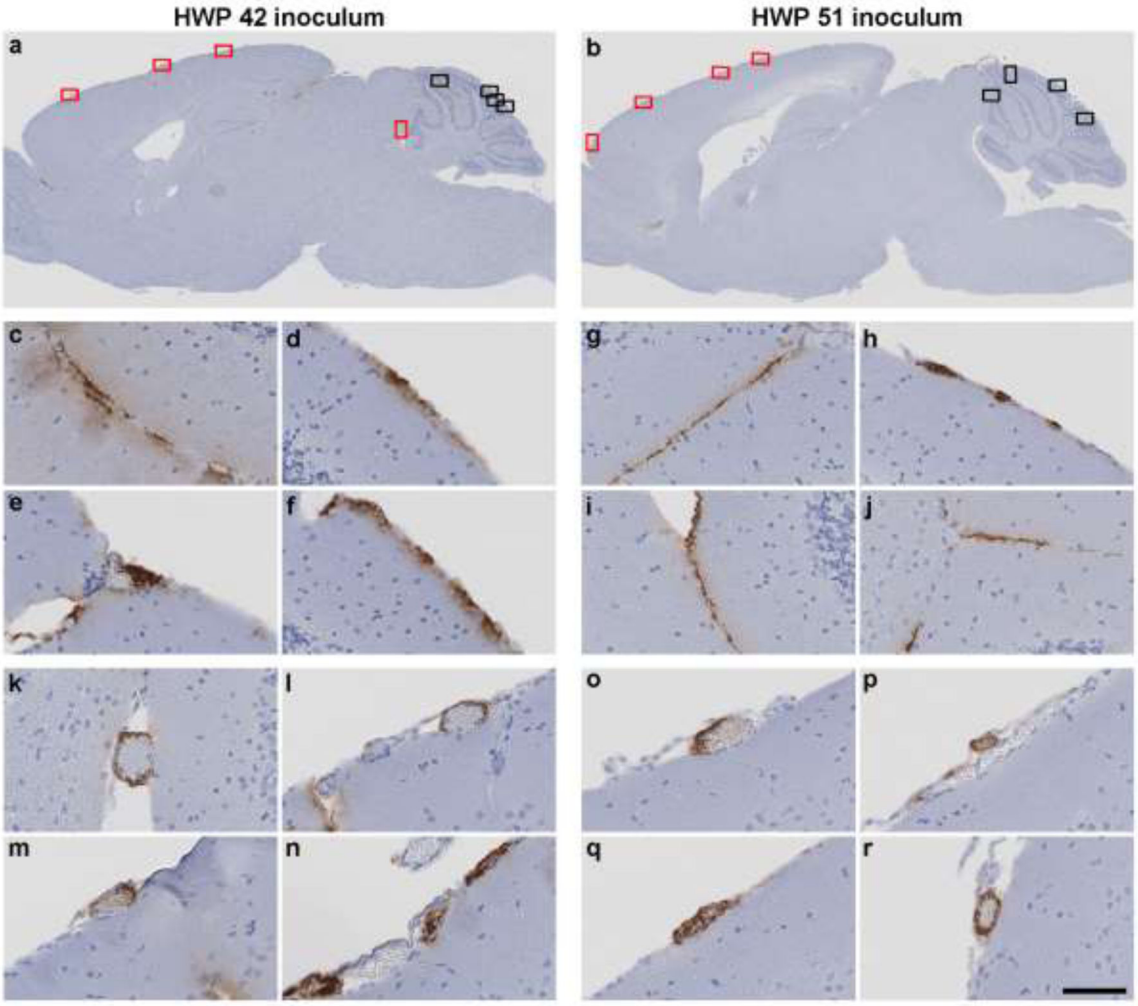
All statistical analysis and graphs were generated using the package GraphPad PRISM v6 (GraphPad Software, La Jolla, USA). Error bars on graphs denote standard deviations, with statistical significance determined by one-way ANOVA followed by Dunnett’s multiple comparison test (two-tailed). Statistical 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 because of the scarcity of cadaveric human growth hormone.

Extended Data



Extended Data Fig. 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-brain homogenates (**a**–**c**, **g**–**i**, **m**–**o**, **s**–**u**) or AD-brain homogenates (**d**–**f**, **j**–**l**, **p**–**r**, **v**–**x**) and culled at the 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**) were evident only in AD-brain-inoculated animals. Boxes denote areas magnified to the right. Scale bars represent 1.4 mm for whole sections (**a**, **d**, **g**, **j**, **m**, **p**, **s**, **v**), 25 μm for CAA (**b**, **e**, **h**, **k**, **n**, **q**, **t**, **w**), and 50 μm for the cerebellar region (**c**, **f**, **i**, **l**, **o**, **r**, **u**, **x**).



Extended Data Fig. 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 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 the middle and lower panels. Scale bars represent 1.1 mm for whole sections (**a**, **b**) and 50 μm for the cerebellar region and CAA (**c**–**r**).

Extended Table 1 |

c-hGH preparations and batches received by each patient

	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

	Cadaveric Human Growth Hormone Preparations						
Patient number*	**HWP**	**K**	**FL**	**LJ**	**TPL**	**R**	
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

*'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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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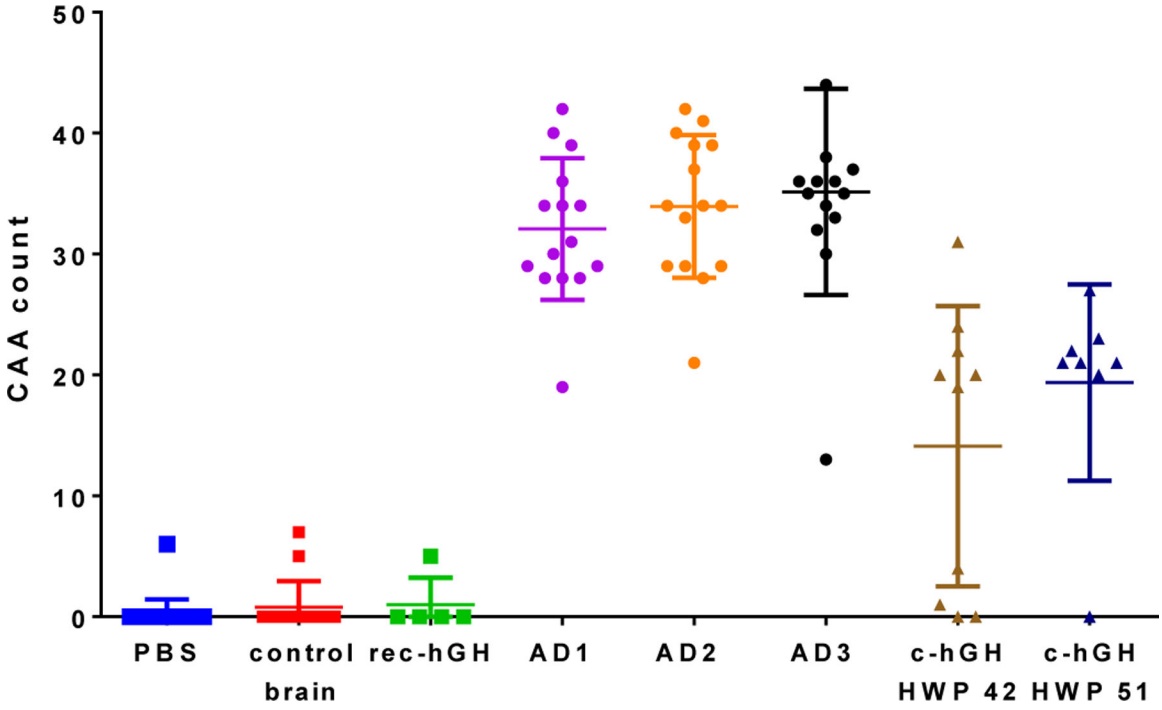

Fig. 1 |. Quantification of vessels with CAA in *App*$^{NL-F/NL-F}$ mice following inoculation with Alzheimer's or control human brain, vehicle alone, or recombinant or cadaveric human growth hormone.

There were highly significant differences between vehicle (PBS)-inoculated mice and those inoculated with either AD brain homogenates or c-hGH preparations (PBS, $n = 25$; AD1, $n = 15$; PBS versus AD1, $P < 0.0001$; AD2, $n = 15$; PBS versus AD2, $P < 0.0001$; AD3, $n = 14$; PBS versus AD3, $P < 0.0001$; c-hGH HWP 42, $n = 10$; PBS versus HWP 42, $P < 0.0001$; c-hGH HWP 51, $n = 8$; PBS versus HWP 51, $P < 0.0001$; one-way analysis of variance (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 rec-hGH (PBS, $n = 25$; control brain, $n = 15$, PBS versus control brain, $P = 0.99$; rec-hGH, $n = 5$, PBS versus rec-hGH, $P = 0.99$). Data are expressed as means ± standard deviation; n = number of mice per group.

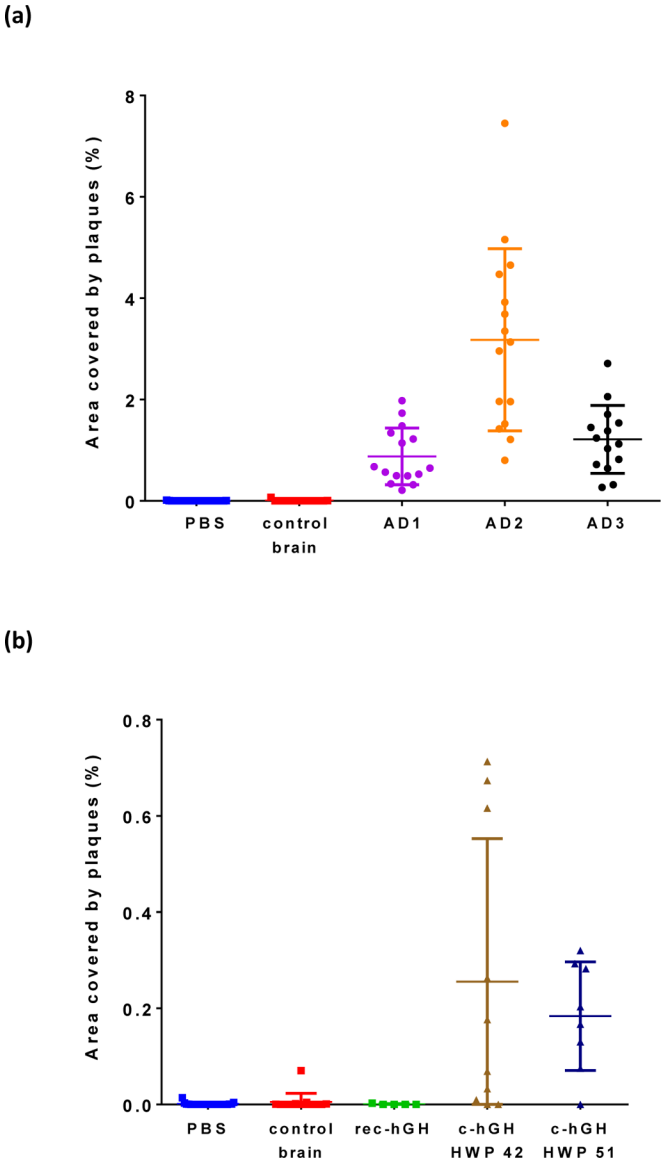

Fig. 2 |. Quantification of cerebellar plaque area in *App*$^{NL-F/NL-F}$ mice inoculated with Alzheimer's or control human brain, vehicle alone, or recombinant or cadaveric human growth hormone.

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, rec-hGH or c-hGH. There was no significant difference between PBS-inoculated, control-brain-inoculated or rec-hGH-inoculated mice (PBS, $n = 25$; control brain, $n = 15$; PBS versus control brain, $P = 0.99$; rec-hGH, $n = 5$; PBS versus rec-hGH, $P > 0.99$). However, there are significant differences between PBS-inoculated and AD- or c-hGH-inoculated mice (PBS, $n = 25$; AD1, $n = 15$; PBS versus AD1, $P = 0.007$; AD2, $n = 15$; PBS versus AD2, $P < 0.0001$; AD3, $n = 14$; PBS versus AD3, $P = 0.0002$; c-hGH HWP 42, $n = 10$; PBS versus HWP 42, $P < 0.0001$; c-hGH HWP 51, $n = 8$; PBS versus HWP 51, $P = 0.002$; one-way ANOVA followed by Dunnett's multiple comparison test). Data are expressed as means ± standard deviation.

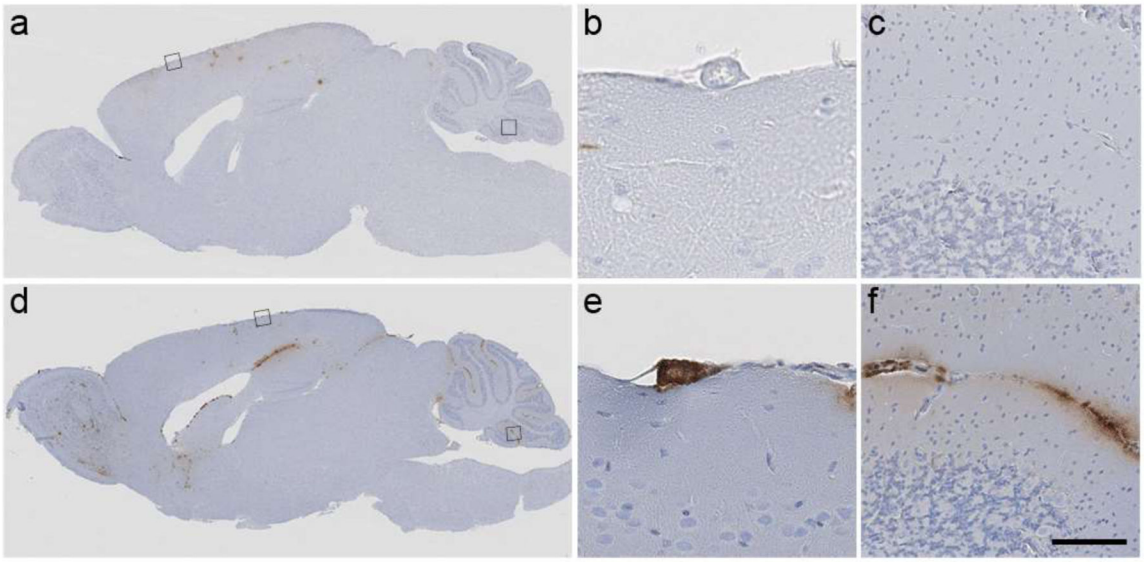

Fig. 3 |. Aβ plaque deposition and CAA in *App*$^{NL-F/NL-F}$ mice following inoculation with AD or control brain.

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

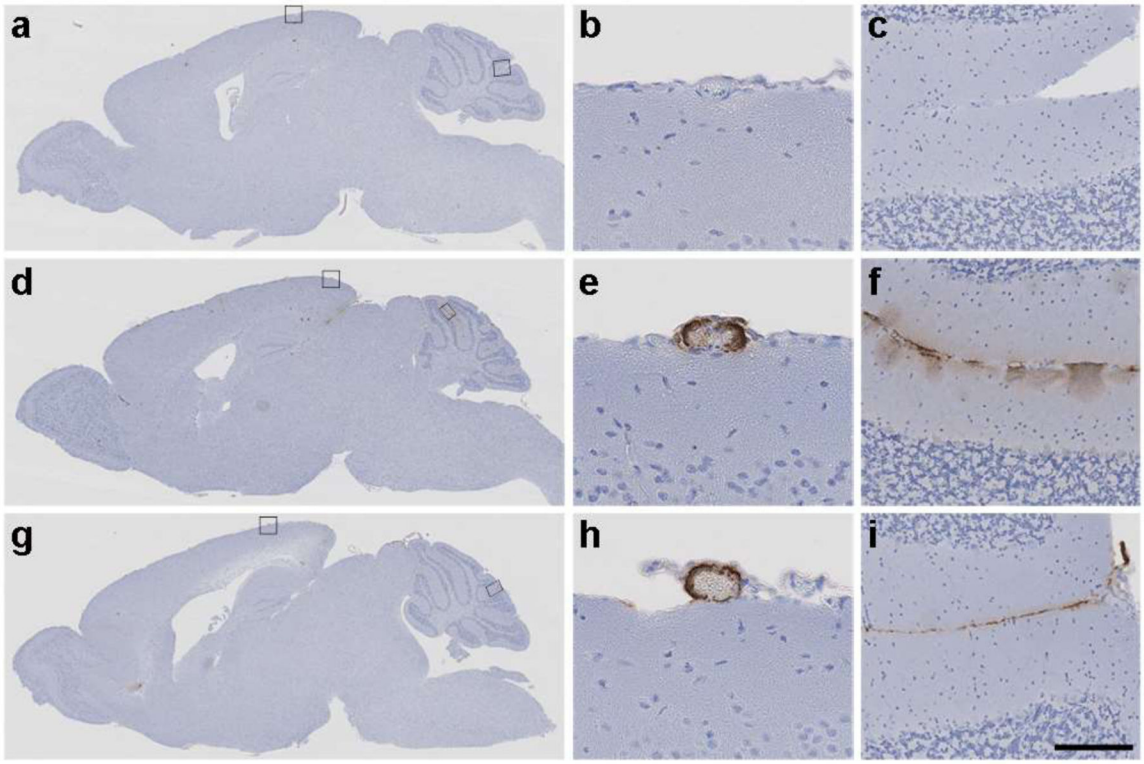

Fig. 4 |. Aβ plaque deposition and CAA in *App*$^{NL-F/NL-F}$ mice following inoculation with cadaveric or recombinant growth hormone preparations.

a–**i**, *App*$^{NL-F/NL-F}$ mice were inoculated with rec-hGH (**a**–**c**; *n* = 5 mice), c-hGH batch HWP 42 (**d**–**f**; *n* = 10 mice) or c-hGH batch HWP 51 (**g**–**i**; *n* = 8 mice) and culled after 240 days. 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 the middle and right columns. Scale bars represent 1.7 mm for whole sections (**a**, **d**, **g**), 25 μm for CAA (**b**, **e**, **h**), and 50 μm for cerebellar regions (**c**, **f**, **i**).

Table 1 |

Quantification of Aβ species and tau in c-hGH preparations

Preparation method	Batch number	Aβx-40 (pg per vial)	Aβx-42 (pg per vial)	Tau (pg per vial)
HWP	40	582	116	12,411
HWP	42	288	NQ	13,631
HWP	43	575	112	14,581
HWP	47	772	108	14,569
HWP	51	991	136	18,155
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

With the exception of HWP 42 and HWP 43, all batches were administered to the patients with Aβ pathology described in ref[1]. each patient received multiple injections from a number of different batches and preparations, although the table lists only the batches and preparations for which 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 that 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 on the basis of the lower limit of quantification for the assay, plus a mathematical adjustment to account for sample dilution. The predicted lowest measurable amounts of Aβ40, Aβ42 and tau per vial were 148.3 pg, 71.2 pg and 11.4 ng, respectively.