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Perspective

The Unfolding Story of Protein Misfolding Causing Alzheimer Disease in Recipients of Human Pituitary Growth Hormone

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Abstract

Human growth hormone (hGH) has been in clinical use for children with GH deficiency (GHD) since the late 1950s. The original formulations were considered very safe with few adverse events reported. That changed remarkably in 1985 when the first patients with GHD, who had been treated with cadaveric hGH, were diagnosed with Creutzfeldt-Jakob disease (CJD).

Fortunately, that same year a robust supply of recombinant hGH was released to the market whose adverse event profile did not include CJD. Patients who had received National Hormone and Pituitary Program hGH have been continuously followed since 1985.

It is clear that prions are causative for CJD. Within the last 10 years there have been reports that similar preparations of cadaveric hGH may have been contaminated with amyloid β (Aβ) protein, a material that is related to Alzheimer disease. Eight patients in the United Kingdom, who had received cadaveric hGH extracted in an analogous manner to that in the United States, had conditions compatible with Alzheimer disease, although they did not fulfill all of the requirements for that diagnosis. In this report we discuss the findings of both CJD and Alzheimer disease, especially as they relate to a possible transmission of the diseases by prions and Aβ protein.

Key Words: growth, human growth hormone, pituitary, Creutzfeldt-Jakob disease, Alzheimer disease

Abbreviations: Aβ, amyloid β; APP, amyloid precursor protein; CJD, Creutzfeldt-Jakob disease; FDA, US Food and Drug Administration; GHD, growth hormone deficiency; hGH, human growth hormone; HWP, Hartree-modified Wilhelmi procedure; NFTs, neurofibrillary τ tangles; NHPP, National Hormone and Pituitary Program; NPA, National Pituitary Agency; PrPc, misfolded protein; rhGH, recombinant human growth hormone.

Human growth hormone (hGH) has been in clinical use for children with GH deficiency (GHD) since the late 1950s. The original formulations were considered very safe with few adverse events reported. These included antibody formation (some growth-attenuating) and some growth-related orthopedic side effects—advancing scoliosis and slipped capital femoral epiphysis [1]. This comfort zone changed remarkably first in 1985 with the causative linking of a form of Creutzfeldt-Jakob disease (CJD) to human pituitary growth hormone (GH) preparations ([2] and discussed later) and later to the possible linking of a transmissible form of Alzheimer disease ([3], and discussed later).

In this perspective article we shall trace some of the history of the connections between the early preparations of GH extracted from human pituitaries to the detection of these two neurodegenerative diseases in treated patients.

GH was first extracted, purified, and tested in a bioassay, the weight gain in weight-plateaued female rats, by Evans and Long in 1921 [4]. After Marie associated the clinical signs and symptoms of acromegaly with an enlarged sella turcica and pituitary tumors, many attempted to extract the growth-promoting activity from the pituitary (reviewed in Graber, 2021 [5]). Over the next 3 decades a number of investigators produced more purified preparations that could be quantified by increasingly sensitive bioassays (reviewed in Graber, 2021 [5]) until C. H. Li produced virtually pure bovine GH [6, 7]).

Unlike insulin and the steroid hormones, GH is species specific. Only the primate hormone is biologically active in humans [8]. This problem, the inactivity of animal GHs in humans, led several investigators to enzymatically hydrolyze bovine GH to find "active cores" that might have biological activity in humans and might not be immunogenic to form neutralizing antibodies [9]. Although some weakly active peptides were derived, none could replace the GH missing in GHD children. In 1958 hGH was finally purified in quantities that could treat a small number of severely GHD children for the long term [10-12]. It was considered virtually side effect free, and with the formation of the National Pituitary Agency (NPA) in 1961 [13], more than 7700 children in the United States were treated, leading to an estimated 8 miles

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(~13 km) of additional height in GHD. Initial studies of recombinant GH from Genentech in adults in the early 1980s revealed contamination with endotoxin and *E coli* proteins, delaying its clinical introduction until 1985 [14, 15].

Proteinopathies, Prion Diseases, and Creutzfeldt-Jakob Disease

Proteinopathies are conformational disorders in which proteins misfold and disrupt the function of cells, tissues, or organs. The usual physiological form is the monomer; however, in some neurodegenerative diseases conformational changes lead to oligomer formation and perhaps aggregates of higher-order structures. Mammalian prions are protein-only infectious agents in which higher-order aggregates may cause neurodegenerative disease [3]. They lead to misfolded protein (PrPc)-forming amyloid fibrils that may propagate [3, 16]. PrPc is a platelet and cellular prion protein that self-aggregates and propagates as it becomes infectious. Prion-based diseases manifest as late-onset, sporadic conditions, such as sporadic CJD. Acquired CJD is quite rare and has been identified after the administration of human cadaveric pituitary hormones (discussed later).

In May, 1984 the first pituitary-derived GH-treated children were diagnosed with CJD [2, 17-19]. The first patient, an 11-year-old boy, was identified following a short prodrome of dizziness and mild speech impairment followed by cerebellar signs. His condition worsened quickly and within 6 months he died. Postmortem evaluation of the brain revealed CJD, a very rare diagnosis in a person so young. Although concern was raised at a meeting attended by scientists at the National Institute of Diabetes, Digestive and Kidney Disease; the National Institutes of Health; the Food and Drug Administration (FDA); and the National Hormone and Pituitary Program (NHPP), no immediate action was taken ([2]). However, over the next few months, concern was raised over several other patients who had odd and troubling neurological signs that quickly led to death. These patients had received pituitary GH from the NHPP, but CJD had not been considered as a cause of the rapidly progressive neurological disease. This additional information led on April 19, 1985, to the immediate discontinuation of the distribution of hGH ([20]).

Several other patients with rather acute onset of neurological findings were described, but a diagnosis of CJD was not made. In retrospect, all had been treated with cadaveric hGH from the National Pituitary Agency.

The clarion moment came in March, 1985, when Dr Raymond Hintz at Stanford University filed a letter with the FDA noting that one of his patients who contracted CJD had been treated with cadaveric GH [17]:

> "[T]he patient was treated for 14 years with growth hormone and I feel that the possibility that this was a factor in his getting CJD should be considered," Hintz R, 1995, p. 2299

This was a shock both to families receiving NPA GH and to the pediatric endocrine community, for no one had considered CJD as a potential serious side effect of pituitary-derived hormones. Interestingly, many families after being informed of the serious nature of the neurologic consequences of CJD, including death, were reluctant to stop the therapy. Fortunately, at this same time a purified version of recombinant hGH (rhGH) that had salutary benefits (the natural hormone had clinical efficacy in children with GHD with a relatively small demand to diminish manufacturing demands) became commercially available [21]. It is important to state that current preparations of rhGH do not carry any risk of Aβ protein or prion contamination (discussed later). rhGH is currently produced by recombinant DNA technology first developed in the early 1980s and commercialized in 1985 [15]. There is no use of actual human tissue in the production of any of the currently commercially available rhGH preparations, whether they be the daily forms or the newer weekly preparations.

Alzheimer Disease

Sporadic Alzheimer disease is the most common type of dementia, accounting for 60% to 80% of all dementia cases. The pathological hallmarks of Alzheimer disease include the buildup of extracellular amyloid β (Aβ) plaques and the aggregation of intracellular neurofibrillary tau tangles (NFTs). Aβ protein is formed through the breakdown of amyloid precursor protein (APP). Abnormal processing of APP leads to the formation of Aβ42, which is thought to play a key role in the development of amyloid plaques. Although the etiology of the disease remains unknown, the most widely recognized model of Alzheimer disease development suggests that upstream aggregation and deposition of Aβ peptides in the neocortex creates a permissive environment that eventually promotes downstream hyperphosphorylation and polymerization of τ into NFTs in the medial temporal lobe and other temporal and limbic regions. It subsequently leads to neurodegeneration and memory decline decades later [22, 23].

Although some of the dementia symptoms of CJD and Alzheimer disease are similar, the progression of disease in CJD is typically much more rapid than that in Alzheimer disease [24, 25]. Additionally, the etiology of the conditions is different. Whereas CJD is caused by prions (transmissible misfolded proteins that result in misfolding of similar normal proteins in the host), Alzheimer disease is due to an accumulation of Aβ peptide in the brain [3]. This story represents iatrogenic transmission, for there are no specific data to implicate transmission as an acquired infection between individuals. The etiology of why Aβ accumulates in some individuals and not others remains unclear.

Additionally, up until the last 25 years, Alzheimer disease was not considered a transmissible disease. Rather, cases were considered sporadic or due to genetic mutations that increase the risk of adult-onset neurodegenerative diseases. Although there are more than 100 genes known to be associated with adult-onset neurodegenerative disease, mutations in the amyloid precursor protein gene (*APP*), genes that alter its enzymatic cleavage (*PSEN1* and *PSEN2*), and certain allelic mutations in the apolipoprotein E gene (*APOE* ϵ4) are associated with Alzheimer disease, in particular [3]. Data gathered over the last quarter century have challenged the idea that Alzheimer disease is not transmissible. Interestingly, contaminated hGH, a cause of CJD, may also be a culprit in the transmission of Alzheimer disease [3].

The idea that Aβ protein could be transmitted from host to recipient was first demonstrated in 2000 [26]. β-Amyloid precursor protein transgenic mice were inoculated with brain homogenates of deceased Alzheimer disease patients. The homogenates were injected directly into one hemisphere of the brains of these animals. These mice, who are at increased risk of developing Aβ plaques as they accumulate Aβ over

time, developed unilateral amyloid plaques within 5 months of inoculation. There was no plaque deposition in transgenic mice inoculated with control subject homogenates. This study provided a proof of concept for transmission of Aβ and development of amyloid plaques in at-risk animals. A later study showed that the same inoculation did not result in plaque formation in those mice that did not carry the susceptibility gene, showing that although Aβ can be transmitted, disease may also be determined based on whether the host animal was at increased risk of plaque formation [27].

Dénouement

Prions and Alzheimer disease pathological hallmarks including Aβ and τ share the property that they both represent misfolded proteins that can deposit in the central nervous system and cause neurological disease. Studies indicate that in several neurodegenerative diseases, specific misfolded proteins seed and proliferate in the brain similarly to the way that prions cause CJD [28-30]. In primates, intracerebral injection of brain homogenate containing Aβ resulted in development of Aβ plaques with an incubation period greater than 3.5 years [30]. Other studies have suggested similar prion-like activity for τ [31-33]. τ Prions from patients with tauopathies, including Alzheimer disease, can infect mammalian cells [31]. More recently, an inverse association has been shown between longevity in patients with Alzheimer disease and the prion-like τ activity, despite the presence of increasing NFTs [32].

Prions are transmitted even when there is not direct inoculation into the central nervous system. In 2010, Aβ was found to enter the brain when injected into the peritoneum of genetically susceptible mice [34]. The continued similarities between Aβ, τ, and prions raised the question whether Alzheimer disease could be transmitted in humans as it seemed in the transgenic mice. In the United Kingdom, most patients with idiopathic CJD have been recruited to the National Prion Monitoring Cohort study, which seeks to increase information about prion disease, including incubation periods and disease etiology [3]. In 2015, a report of the autopsy of 8 patients with CJD, who had previously been treated with hGH, revealed that half also had Aβ deposits in their brains in a pattern similar to that noted in patients with Alzheimer disease [35]. This finding raised the question of whether Alzheimer disease, in some cases, may be a transmissible disease associated with a prion disease.

When the 4 patients with CJD and Aβ deposits were discovered, it was thought that perhaps the hGH they had received as children had been contaminated with Aβ in the same way that it had been contaminated with prions. Archived batches of hGH were tested for Aβ. Those prepared by the Hartree-modified Wilhelmi procedure (HWP), which had previously been shown to be most at risk of carrying prions, were confirmed to have high Aβ concentrations. The batches prepared with other purification methods did not have any detectable Aβ. Again, transgenic mice at increased risk for Aβ plaque formation were injected with the affected hGH preparations and developed Alzheimer-like plaques as opposed to wild-type mice and those injected with rhGH [36].

More recently, transmission of Aβ protein and subsequent development of Alzheimer disease has been suspected in humans who had previously received contaminated hGH [3]. In addition to the 4 previously described patients, 4 additional patients, who had previously received hGH and had concerns for prion exposure, were described. All 8 had received hGH prepared using the HWP method. The oldest patient described with symptoms of Alzheimer disease was age 57 years, younger than typically noted in those with idiopathic Alzheimer disease. All had biomarker and/or imaging findings consistent with Alzheimer disease, even if they were not showing Alzheimer disease symptoms (as opposed to CJD symptoms). Genetic testing for other causes of neurodegenerative disease was negative for 5 of 8 of the patients. Other patients referred for concern of prion exposure who had not received hGH purified by the HWP method did not have any cognitive symptoms consistent with Alzheimer disease at the time of their visit. Additionally, referring physicians did not have any data about the preparation of hGH the patients had received, only that they had been treated in the past. This eliminated the possibility of referral bias. The only factor common to all the patients in the cohort with symptoms of Alzheimer disease was that they had been treated with hGH purified by the HWP method. Given the previous evidence of the transmissibility of Aβ protein from HWP-purified hGH in animal models, it seems plausible that in the cohort reported, their Alzheimer disease was transmitted iatrogenically.

Not all investigators agree with the conclusions of the report from Banerjee et al [3]. Dr Ellen Leschek and colleagues at the National Institutes of Health in Bethesda, Maryland, have continued surveillance of the NHPP that since 1985 has followed the prevalence and long-term outcome of those at risk for CJD from cadaveric hGH. They have carefully and thoughtfully reviewed the 8 patients with purported Alzheimer disease noted in the work by Banerjee and colleagues [3]. The group stated that the original report did not provide enough evidence to conclude that these patients had Alzheimer disease because there were possible alternative explanations, including comorbidities for some of the described cognitive decline [37]. Thus, more information is required to be certain that Alzheimer disease is transmissible under these conditions.

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Data Availability

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References

1. Souza FM, Collett-Solberg PF. Adverse effects of growth hormone replacement therapy in children. *Arq Bras Endocrinol Metab*. 2011;55(8):559-565.

2. Koch TK, Berg BO, De Armand SJ, Gravina RF. Creutzfeldt-Jakob disease in a young adult with idiopathic hypopituitarism: possible relationship to administration of cadaveric human growth hormone. *N Engl J Med*. 1985;313(12):731-733.
3. Banerjee G, Farmer SF, Hyare H, *et al.* Iatrogenic Alzheimer's disease in recipients of cadaveric pituitary-derived growth hormone. *Nat Med*. 2024;30(2):394-402.
4. Evans HM, Long JA. The effect of feeding the anterior lobe of the hypophysis on the oestrous cycle of the rat. *Anat Rec*. 1921;21:62.
5. Graber E, Reiter EO, Rogol A. Human growth and growth hormone: from antiquity, to the recombinant age to the future. *Front Endocrinol*. 2021;12:709936.
6. Li CH, Evans HM, Simpson ME. Isolation and properties of the anterior hypophyseal growth hormone. *J Biol Chem*. 1945;159(2): 353-366.
7. Li CH. A simplified procedure for the isolation of hypophyseal growth hormone. *J Biol Chem*. 1954;211(2):555-558.
8. Knobil E, Greep RO. Physiology of growth hormone with particular reference to its action in the rhesus monkey and the "Species specificity" problem. *Recent Prog Horm Res*. 1969;15:1-69.
9. Kostyo JL. The search for the active core of pituitary growth hormone. *Metabolism*. 1974;23(9):885-899.
10. Raben MS. Preparation of growth hormone from pituitaries of man and monkey. *Science*. 1957;125(3253):883-884.
11. Raben MS. Growth hormone 2. Clinical use of human growth hormone. *N Engl J Med*. 1962;266(2):82-86.
12. Beck JC, McGarry EE, Dyrenfurth I, Venning EH. Metabolic effects of human and monkey growth hormone in man. *Science*. 1957; 125(3253):884-885.
13. Raiti S, Tolman RA. Isolation and purity of human pituitary hormones. *J Clin Endocrinol Metab*. 1978;46(5):853.
14. Dinarello CA, O'Connor JV, LoPreste G, Swift RL. Human leukocytic pyrogen test for detection of pyrogenic material in growth hormone produced by recombinant Escherichia coli. *J Clin Microbiol*. 1984;20(3):323-329.
15. Hsiung H, Becker GW. Secretion and folding of human growth hormone in Escherichia coli. *Biotechnol Genet Eng Rev*. 1988;6(1): 43-66.
16. Manka SW, Wenborn A, Collinge J, Wadsworth JDF. Prion strains viewed through the lens of cryo-EM. *Cell Tissue Res*. 2023;392(1): 167-178.
17. Hintz R. The prismatic case of Creutzfeldt–Jakob disease associated with pituitary growth hormone treatment. *J Clin Endocrinol Metab*. 1995;80(8):2298-2301.
18. Blizzard RM. Growth hormone as a therapeutic agent. *Growth Genet Horm*. 2005;21:49-54.
19. Brown P. Human growth hormone therapy and Creutzfeldt-Jakob disease: a drama in three acts. *Pediatrics*. 1988;81:85-92.
20. Underwood LE, Fisher DA, Frasier SD, *et al.* Degenerative neurologic disease in patients formerly treated with human growth hormone. Report of the committee on growth hormone use of the Lawson Wilkins pediatric endocrine society, may 1985. *J Pediatr*. 1985;107(1):10-12.
21. Cronin MJ. Pioneering recombinant growth hormone manufacturing: pounds produced per mile of height. *J Pediatr*. 1997;131(1): S5-S7.
22. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science*. 1992;256(5054):184-185.
23. Hampel H, Hardy J, Blennow K, *et al.* 2021. the amyloid-β pathway in Alzheimer's disease. *Mol Psychiatry*. 2021;26(10):5481-5503.
24. Rudge P, Hyare H, Green A, Collinge J, Mead S. Imaging and CSF analyses effectively distinguish CJD from its mimics. *J Neurol Neurosurg Psychiatry*. 2018;89(5):461-466.
25. Dorey A, Tholance Y, Vighetto A, *et al.* Association of cerebrospinal fluid prion protein levels and thedistinction between Alzheimer disease and Creutzfeldt-Jakob disease. *JAMA Neurol*. 2015;72(3): 267-275.
26. Kane MD, Lipinski WJ, Callahan MJ, *et al.* Evidence for seeding of beta -amyloid by intracerebral infusion of Alzheimer brain extracts in beta -amyloid precursor protein-transgenic mice. *J Neurosci*. 2000;20(10):3606-3611.
27. Meyer-Luehmann M, Coomaraswamy J, Bolmont T, *et al.* Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science*. 2006;313(5794):1781-1784.
28. Jucker M, Walker LC. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature*. 2013;501(7465): 45-51.
29. Jucker M, Walker LC. Propagation and spread of pathogenic protein assemblies in neurodegenerative diseases. *Nat Neurosci*. 2018;21(10):1341-1349.
30. Ridley RM, Baker HF, Windle CP, Cummings RM. Very long term studies of the seeding of Beta-amyloidosis in primates. *J Neural Transm (Vienna)*. 2006;113(9):1243-1251.
31. Woerman AL, Aoyagi A, Patel S, *et al.* Tau prions from Alzheimer's disease and chronic traumatic encephalopathy patients propagate in cultured cells. *Proc Natl Acad Sci U S A*. 2006;113: E8187-E8196.
32. Aoyagi A, Condello C, Stöhr J, *et al.* Aβ and tau prion-like activities decline with longevity in the Alzheimer's disease human brain. *Sci Transl Med*. 2019;11(490):eaat8462.
33. Condello C, Merz G, Aoyagi A, DeGrado WF. Prusiner SB.Aβ and tau prions causing Alzheimer's disease. *Methods Mol Biol*. 2023;2561: 293-337.
34. Eisele YS, Obermüller U, Heilbronner G, *et al.* Peripherally applied A beta-containing inoculates induce cerebral beta-amyloidosis. *Science*. 2010;330(6006):980-982.
35. Jaunmuktane Z, Mead S, Ellis M, *et al.* Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy. *Nature*. 2015;525(7568):247-250.
36. Purro SA, Farrow MA, Linehan J, *et al.* Transmission of amyloid-β protein pathology from cadaveric pituitary growth hormone. *Nature*. 2018;564(7736):415-419.
37. Nath A, Holtzman DM, Miller BL, Grinberg LT, Leschek EW. Insufficient evidence for an association between iatrogenic Alzheimer's disease and cadaveric pituitary-derived growth hormone. *Alzheimers Dement*. 2024;20(10):7399-7402.