Cite this article as: Gorbunova V. & Seluanov A. (2024). Hiding in Plain Sight: FDA-Approved Cholesterol Drug Ezetimibe as a Treatment for Alzheimer's, *AgingBio*, *2*, e20240037. doi: 10.59368/agingbio.20240037.

Editorial

Hiding in Plain Sight: FDA-Approved Cholesterol Drug Ezetimibe as a Treatment for Alzheimer's

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https://doi.org/10.59368/agingbio.20240037
Accepted: 9/23/2024, Published: 10/23/2024

Comment on:
Ganne A., et al. (2024). Ezetimibe Lowers Risk of Alzheimer's and Related Dementias over Sevenfold, Reducing Aggregation in Model Systems by Inhibiting 14-3-3G::Hexokinase Interaction, AgingBio, 2, e20240028. doi: 10.59368/agingbio.20240028.

Age is the most prominent nongenetic risk factor for Alzheimer's disease (AD), with incidence doubling every 5 years beyond age 65. Protein aggregation is one of the most consistent features of age-progressive diseases, as well as normal aging. Many age-associated diseases add disease-specific aggregates on top of those observed in the healthy aged—including essentially all neurodegenerative diseases, diverse myopathies, lung disease, and chronic kidney disease. Such data are consistent with, but do not prove, the hypothesis that aggregation may be a common mechanism driving both aging and the numerous pathologies that accompany it.

A new study by Ganne et al.[1] reports that ezetimibe, a drug they had selected for its disruption of aggregates *in silico* and in diverse preclinical models of age- and Alzheimer's-associated pathology, reduced the prospective risk of Alzheimer's Disease and Related Dementias (ADRD) by sevenfold in the general population and eightfold among heart-disease patients for whom the risk of subsequent ADRD was roughly doubled. Ezetimibe was approved by the FDA in 2002 for reduction of circulating cholesterol levels. The authors note that, while the controls (nearly 1 million subjects not taking ezetimibe) were well matched to ezetimibe recipients for age, gender, and established ADRD risk factors such as hypertension, diabetes, and kidney disease, the groups were ***not*** matched for cholesterol levels. It would not be unreasonable to assume that the patients prescribed ezetimibe had initiated it due to relatively high serum cholesterol levels, so these researchers point out that double-blind randomized trials of newly enrolled patients would have to be conducted to establish a causal connection. There is ample evidence that high LDL-cholesterol increases AD risk[2,3], suggesting that statins could achieve the greatest risk reduction among such patients.

A recent meta-analysis across 21 studies, comprising 1.2M subjects, calculated a 32% reduction in relative risk of AD for patients on statins versus untreated subjects, that is, OR = 0.68 with a 95% confidence interval of 0.56–0.81[4]. The OR for all dementias, derived from 36 studies and over 5M subjects, was lower (although not significantly so) at 0.80 with a 95% CI of 0.75–0.86[4]. Reduction in cholesterol could thus account for only a fraction (certainly less than half) of the 86% ADRD risk reduction (95% CI 0.06–0.34) observed by Ganne et al., implying that other factors are also involved. There have been several previous studies implying that ezetimibe has targets unrelated to hyperlipidemia: it decreases neuronal apoptosis by activating autophagy in a rat model of arterial occlusion[5]; improves diabetic nephropathy and glucose tolerance by protecting pancreatic beta cells in a mouse model of diabetes[6,7]; and prevents muscle wasting in a mouse model of muscular dystrophy[8]. Induction of autophagy is a contributory mechanism supported by the studies of Ganne et al., in that ezetimibe restored autophagy in human neuroblastoma cells exposed to rapamycin[1].

This putative mechanism is consistent with the protein–protein interaction implicated as a "lynchpin" in AD aggregate cohesion[1,9], for which ezetimibe was predicted (and confirmed) to target the 14-3-3G::hexokinase 1 interface[1]. Both of these proteins are markedly elevated in human hippocampi from AD patients[9] and also in those from heart-disease patients[1]—for which ADRD risk is elevated[1,10]. Their co-occurrence in brain aggregates was found *only* in AD and heart disease subjects[1]. Evidence supporting ezetimibe disruption of 14-3-3 interaction with hexokinase *in vivo* was convincing, but of necessity limited to the observation that their co-immuno-pulldown from human neuroblastoma cell cultures was reduced 2.5-fold by this drug[1].

Autophagy is the principal route by which larger aggregates can be cleared[11], and it is stimulated by 14-3-3 paralogs[12,13], so the sparing of 14-3-3 proteins from aggregation would ameliorate their conspicuous deficiency in AD[14]. We are left with the conclusion that three mechanisms of opposing Alzheimer's are possible and that any or all may apply: (a) reduced intestinal uptake of cholesterol and thus of its plasma levels, the mechanism shared with statins; (b) reduction in aggregate accumulation, lowering inflammation via a vicious cycle (dubbed the "aggregation-inflammation cycle"); and (c) rescue of autophagy, the loss of which either precedes or accompanies AD and other neurodegenerative diseases.

There is one minor caveat to this argument: mammals possess a small family of seven 14-3-3 proteins, and it is left in doubt whether 14-3-3G (gamma) was the sole paralog implicated. Most antibodies to 14-3-3 target the central, conserved "cradle," so it may be conjectured that 14-3-3Z (zeta) is also a candidate partner to hexokinase, given that this paralog has been previously

implicated in AD through its affinity for hyperphosphorylated tau[15,16]. That uncertainty does not detract from the importance of this remarkably thorough and compelling paper.

The route taken by Ganne et al. to discovery of this unheralded property of ezetimibe is an interesting and instructive one. They began with data from proteomic analyses of AD aggregates and their internal cross-links and then used bioinformatic procedures to prioritize them by their "influence" over aggregate cohesiveness (i.e., the total degree). This led them to the highly influential 14-3-3(G?)::HK1 interface, which was next screened *in silico* for affinity of binding FDA-approved drugs. From among the top drugs, ezetimibe was selected based on its clinical record of very rare and minor side effects over the 22-year history of its clinical availability. There may well be other drug candidates, not yet approved by the FDA, that will eventually supersede or augment ezetimibe to ultimately eliminate the threat of AD entirely. That process and the goal it suggests are still many years away. However, the recent discovery by this group of a small molecule that negates the elevated AD risk to carriers of the APOE4 gene (four to fivefold per allele copy)[17] further enhances the prospect that one day, Alzheimer's will no longer be the scourge that it is currently.

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