REVIEW

# Impact of Apolipoprotein E Epsilon 4 in Brain Injuries of Boxers

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Through research done on causes of late-onset Alzheimer's disease (AD), a link has been made between the amyloid-beta protein and the APOE ε4 allele. Not only do they play a major role in leading to AD, they also result in an increased risk of brain injury. Looking at studies done on patients with traumatic brain injuries, with an emphasis on injuries received while boxing, the possibility exists that having the APOE ε4 allele can lead to being more at risk for having a traumatic brain injury than non-carriers. Currently, there is no screening for the APOE ε4 allele in prospective athletes. The topic of potential screening should be further discussed as well as additional research done to be better equipped for educating the public on possible risks.

Boxers have long been known to be susceptible to extensive injuries, especially to the brain. Knowledge of brain injuries in boxing date back to the 1920s with the discovery of *dementia pugilistica*, or punch-drunk syndrome, which is now called chronic traumatic encephalopathy (CTE) and is known to affect players of all contact sports, such as American football, ice hockey, and rugby (*1*). However, trauma to the brain is not the only factor that affects the increase in brain injuries. Genetics can also play a role in a boxer's susceptibility (*2*).

How the brain reacts to trauma immediately and long term can give insight to how parts of the brain, from the genetic level to plaque build-up, interact with each other leading to permanent brain damage. The Apolipoprotein E epsilon 4 allele has been shown to increase the amount of amyloid plaques in the brain, which leads to nerve cell and synapses death (*3*). The presence of the APOE ε4 allele in boxers could be a factor in whether the athlete will develop a brain injury in the future. Options for reducing risk include possible gene therapy as well as screening as a preventative measure, allowing boxers to know their predisposition to brain injury.

## Punch Trauma

A study done by Zhang et al. used diffusion-weighted imaging and magnetic resonance to assess the microscopic alterations that occur in professional boxers' brains due to chronic traumatic brain injury (*4*). Diffusion-weighted imaging is a technique used to measure the Brownian motion of water molecules in the tissues of the body. White matter diseases such as Alzheimer's are able to be detected early on due to the diffusion-weighted imaging being able to spot the microstructural abnormalities that are caused by diffusion (*5*). The researchers found that the measured diffusion values in boxers were much higher than those of the non-boxer controls. The magnetic resonance most commonly showed that the boxers had brain volume loss that was inappropriate for their ages, followed by cavum septum pellucidum, subcortical white matter disease, and periventricular white matter disease. Matser et al. did a study that was aimed at discovering the short-term effects on the brain in amateur boxing, which requires headgear unlike professional boxing (*6*). They performed neuropsychological examinations aimed at measuring memory, mental and fine-motor speed, planning and attention. These tests were done before and shortly after a boxing match. The competing boxers showed an acute traumatic brain injury pattern of impaired performance in planning, attention, and memory capacity, despite the use of headgear.

A similar study analyzing pre-bout and one-month post-bout cognitive function shows that though there was decreased performance after the bout, after the one-month recovery time cognitive abilities improved to above the baseline functioning. The researchers suggested that this is likely due to the baseline not being accurate because of pre-bout anxiety, rapid weight loss, and the intense training that comes before a fight. It was also found that professional boxers who had more bouts had poorer cognitive ability during the one-month recovery period (*7*). Not all research points toward a negative outcome, however. A 1992-2001 study done on twenty amateur boxers shows no neuropsychological deterioration over the nine years despite participating in competitions and sparring (*8*).

## Amyloid-β

When the brain receives an injury, such as a punch, it triggers production of the amyloid plaques in the cortex (*3*). These plaques are found in the brains of up to a third of patients who have received a traumatic brain injury (*9,10*). Amyloid plaques, most commonly known for their negative role in the onset of Alzheimer's disease (AD), are accumulations of beta-amyloid protein, which gathers in the brain, disrupting cell communication and causing inflammation that kills the brain cells (*11*). Patients who have died from head injuries had more plaques than those without head injuries, with the plaques being found more often in those with the APOE ε4 allele (*12,3*). Those with AD are more likely to have inherited the APOE e4, compared to the other isoforms (*13*). This connection of AD, amyloid-beta, and APOE ε4 has been a large stepping stone in research related to traumatic brain injuries like those sustained in boxing.

Roberts et al. studied beta amyloid protein (βAP) deposits in 152 deceased patients who received a head injury and survived between four hours and five years and between the ages of eight weeks and 81 years. Their findings were

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compared to a control group of 44 neurologically normal people aged 51 to 80 years. By immunostaining the samples with antibody specific for βAP, the researchers found that 30% of the head injury cases had deposits of βAP and the older the patient, the greater the extent of the deposits. They also looked at the expression of beta amyloid precursor protein (βAPP) and discovered that brain injury induced overexpression of βAPP which leads to the subsequent deposition of βAP. It is believed that the deposition of βAP is an acute phase response to the nerve cell stress of brain injury and that these plaques might indicate a long-term consequence of head injury (*14,15,16*).

**APOE ε4**

The APOE ε4 allele genotype has been associated with poor outcome after a traumatic brain injury (TBI) (*17,18,19*). Apolipoprotein E is a protein, encoded by the APOE gene, that forms lipoproteins that are responsible for packaging fats such as cholesterol and moving them through the bloodstream (*20*). After a traumatic brain injury, these lipoproteins promote neuronal survival, provide antioxidant effects, and mediate synaptic repair, among other helpful properties. There are three isoforms of the gene, ε2, ε3, and ε4, made different by how they alter the charge and structural properties of the protein (*21,22,23*). Everyone has two copies of the allele, and therefore have a combination of the two. The ε2 allele is the rarest and having this isoform seems to reduce the risk of developing AD. E3 is the most common and does not seem to affect a person's risk. However, having one copy of the ε4 allele increases risk by 2 to 3 times, while having two copies increases risk by 12 times (*24*). Epsilon 4 is the one focused on in this review and is the allele that provides the most harmful effects. APOE ε4 is known to increase an individual's susceptibility to develop late-onset AD, which is due to the allele's association with increased amyloid plaques. Late-onset is the most common form of the disease, early-onset is rare, occurring in up to 5% of those with AD. The plaque buildup has been shown to cause neurodegeneration and dementia (*25*). The APOE ε4 allele was shown to influence memory performance in patients who had sustained a TBI, which suggests and APOE isoform-specific effect on neuronal repair processes (*26*).

A study done by Teasdale et al. shows that patients with the APOE ε4 polymorphism are more than twice as likely to have some unfavorable outcome six months after a head injury (*12*). Similarly, a study by Farlow et al. was done on carriers of the APOE ε4 allele, and it shows that those with the genotype and mild cognitive impairment had greater impairments in memory and daily activities than those without the ε4 genotype (*27*). In 1997, a study was done on 30 professional boxers by measuring the severity of traumatic encephalopathy in conjunction with their APOE ε4 genotype. The researchers created a 10-point clinical scoring matrix, the Chronic Brain Injury (CBI) scale, to evaluate the severity of brain injury associated with boxing. The results show that those boxers with high exposure (number of bouts) had higher CBI scores than those with low exposure. High exposure boxers who also had the APOE ε4 genotype had a considerably higher CBI score than those with high exposure without the allele (*28*).

A study done by Nesellus et al. looks at cerebrospinal fluid (CSF) to see if there were damages or biomarkers that indicated TBI (*29*). CSF and blood were collected from amateur boxers after a bout and various proteins, including APOE ε4, were analyzed. Their results show that boxers carrying the APOE ε4 allele had similar biomarker concentrations as non-carriers. This does not mean that possession of the allele does not affect predisposition towards a chronic brain injury. However, it does show that it will not help clinicians diagnose and monitor TBI, because it does not influence biomarker levels. Teasdale et al. also show that the possession of the allele alone does not affect outcome after head injury. However, they did find that the APOE ε4 genotype in association with age, particularly children and young adults, did reduce the prospect of a favorable outcome (*30*).

**Amyloid-β and APOE ε4**

The connection between the APOE ε4 genotype and the accumulation of amyloid-β plaques is the main factor in associating the genotype with decreased brain functioning after brain injury. Researchers compared the amount of amyloid-β deposits produced by APOE ε4 and ε3 in human brains with AD. They found that the ε4 isoform enhanced the synaptic toxicity of oligomeric amyloid-β much more than the ε3 isoform. It was also discovered through single synapse analysis that carriers of the APOE ε4 allele were over three times as likely to have co-localization of the APOE protein with oligomeric amyloid-β than ε3 carriers (*31*).

The APOE ε4 has also been very recently shown to not only be connected to amyloid-β deposits but also tau-mediated neurodegeneration. Tau protein is known for the plaques it develops into in AD by producing intracellular neurofibrillary tangles by antibody reactivity (*32*). Shi et al. show that mice containing the ε4 isotype had a greater extent of somatodendritic tau redistribution at three months of age than the other isotypes. By nine months, the mice had developed a significantly higher amount of brain atrophy and neuroinflammation. Their results showed that those with tauopathy (neurodegenerative diseases associated with aggregation of tau protein) had higher regional neurodegeneration with the presence of the APOE ε4 allele (*33*).

**Evidence Against the Connection**

The connection between the APOE ε4 allele and degeneration of the brain in fighters has received increasing attention in the last 20 years. However, as can be seen in the dates of the primary sources obtained for this article, most of the interest in researching that connection has fizzled out. More recent papers have surfaced that disprove the connection between the allele and predisposition towards brain injury. The Professional Fighters' Brain Health Study (PFBHS) was a study done in 2015 by Bernick et al. that found an association between repetitive head trauma in professional fighters and smaller thalamic and caudate volumes in the brain as well as slower

processing speed (*34*). A 2017 study done by Banks et al. (a researcher from the previous study and including another scientist from that study) furthered the PFBHS by collecting genetic data of the participants of the study to test the hypothesis that the APOE ε4 allele would have an impact on the relationship. They compared the relationships between age, fight history, APOE status cognitive abilities, and brain volumes, specifically the caudate and thalamic regions. Using real-time PCR to genotype the APOE alleles and processing MRI images to detect volume and cortical thickness were the two main methods of statistical analyses used to determine cross-sectional relationships. Their results showed that the ε4 allele had no impact on volumes in the thalamus and caudate or in aspects of cognition despite having replicated their earlier findings of fight exposure negatively associated with these aspects (*35*).

One study aimed to determine if carriers of the apolipoprotein E ε4 allele were more likely to sustain a concussion and if preseason genetic testing for athletes was necessary. They took blood samples from 318 collegiate student athletes from University of Toronto over the course of September 2002 through April 2006 to determine possession of the allele. After identifying the amount of concussions, the researchers' results showed no important association between carriers of the ε4 allele and susceptibility to a concussion (*36*).

Millar et al. did a 2003 study on whether a head injury would lead to late-onset cognitive decline years later. They assessed head injured patients both with and without the APOE ε4 genotype at time of injury, six months later, and a mean of 18 years later. Using the Glasgow outcome scale, which determines the level of disability and "overall" mental outcome, the team discovered that twice as many patients had deteriorated cognitive abilities as improved between the 6-month assessment and the late assessment. However, when they compared APOE ε4 carriers and noncarriers, they found that possession of the allele had no significant effect on neuropsychological function after the head injury. In fact, those with the allele were less likely to have a negative outcome. One short fall of this study is that despite the follow up interval of 15-25 years, the participants in the study are still too young, with the mean age being 42.1 years, to assess the head injury and ε4 genotype affect the risk of Alzheimer's disease (*37*).

**Screening**

The question of banning boxing has presented itself many times throughout the years. The general consensus is that the rules of boxing may need some work, but that the choice of participating in the sport is still up to the individual. In knowing that athletes who carry the APOE ε4 allele could be more susceptible to a negative outcome after receiving an injury, the prospect of screening for the genotype would be beneficial in allowing the athlete to decide if they want to participate in the sport, despite the dangers. There are multiple ways of detecting the genotype. Polymerase chain reaction-single strand conformation polymorphism was used by some researchers to detect the isotype present in the APOE gene, which is located on chromosome 19, in AD patients, non-AD dementia patients, as well as age-matched controls. This was done by amplifying DNA from the APOE target sequence using PCR, which was then electrophoresed in a non-denaturing polyacrylamide gel and stained to visualize (*38*). Other detection methods include capillary electrophoresis and matrix-assisted laser desorption/ ionization TOF mass spectrometry (*39,40*).

**Conclusion**

The studies addressed in this review have almost all pointed towards the APOE ε4 genotype having a positive correlation with long-term head injury, suggesting a genetic susceptibility to the effects of a brain injury. However, there has not been much research on the subject in the past five years which suggests that either the results were not promising enough, or people are hesitant and unsure of how to put bans or restrictions on a sport when the participants know that there is a risk and may still choose to compete. More current, and possibly promising, research has been done on the brain proteins neurofilament light chain and tau, which are detectible in the blood and would help determine which fighters are more at risk of developing long-term brain injuries (*41,42*). Another question is what to do if the APOE ε4 genotype is found to be indicative of long-term brain injury susceptibility. One option is screening for the allele and informing the athlete of their chances of a negative outcome. There is also the option of banning those who are more susceptible to it from contact sports, though this has ethical concerns attached to it. With diagnosing will come research into treatments, which may include gene therapy.

## REFERENCES AND NOTES


1. R. J. Castellani, G. Perry, Dementia pugilistic revisited. *J Alzheimers Dis.* 60, 1209-1221 (2017).
2. B. D. Jordan, Genetic susceptibility to brain injury in sports: a role for genetic testing in athletes? *The Physician and Sportsmedicine*. 26, 25-26 (1998).
3. G. W. Roberts, S. M. Gentleman, A. Lynch, D. I. Graham, βA4 amyloid protein deposition in brain after head trauma. *The Lancet*. 338, 1422-1423 (1991).
4. L. Zhang, L. Ravdin, N. Relkin, A. M. Ulug, Increased diffusion in the brain of professional boxers: a preclinical sign of traumatic brain injury? *American Journal of Neurology*. 24, 52-57 (2003).
5. V. Baliyan, C. J. Das, R. Sharma, Diffusion weighted imaging: technique and applications. *World J Radiol*. 8, 785-798 (2016).
6. E. J. T. Matser, A. G. H. Kessels, M. D. Lezak, J. Troost, B. D. Jordan, Acute traumatic brain injury in amateur boxing. *The Physician and Sportsmedicine*. 28, 87-92 (2015).
7. L. Ravdin, W. B. Barr, B. Jordan, N. Relkin, Assessment of cognitive recovery following sports related head trauma in boxers. *Clin J Sport Med*. 13, 21-27 (2003).
8. M. D. Porter, A 9-year controlled prospective neuropsychologic assessment of amateur boxing. *Clin J Sport Med*. 13, 339-352 (2003).
9. G. Scott, A. F. Ramlackhansingh, P. Edison, P. Hellyer, J. Cole, M. Veronese, R. Leech, R. J. Greenwood, F. T. Turkheimer, S. M. Gentleman, R. A. Heckemann, P. M. Matthews, D. J.

Brooks, D. J. Sharp, Amyloid pathology and axonal injury after brain trauma. *Neurology*. 86, 821-828 (2016).
10. J. A. Nicoll, G. W. Roberts, D. I. Graham, Apolipoprotein E ε4 allele is associated with deposition of amyloid beta-protein following head injury. *Nat Med*. 1, 135-137 (1995).
11. "Beta-amyloid and the amyloid hypothesis," (Alzheimer's Association, 2017; https://www.alz.org/national/documents/topicsheet_betaamyloid.pdf).
12. G. M. Teasdale, J. A. R. Nicoll, G. Murray, M. Fiddes, Association of apolipoprotein E polymorphism with outcome after head injury. *The Lancet*. 350, 1069-1071 (1997).
13. D. B. Carter, The interaction of amyloid-beta with ApoE. *Alzheimer's Disease*. 38, 255-272 (2005).
14. G. W. Roberts, S. M. Gentleman, A. Lynch, L. Murray, M. Landon, D. I. Graham, Beta Amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*. 57, 419-425 (1994).
15. D. I. Graham, S. M. Gentleman, A.Lynch, G. W. Roberts, Distribution of beta-amyloid protein in the brain following severe head injury. *Neuropathol Appl Neurobiol*. 21, 27-34 (1995).
16. A. Iwata, X. H. Chen, T. K. McIntosh, K. D. Browne, D. H. Smith, Long-term accumulation of amyloid-beta in axons following brain trauma without persistent upregulation of amyloid precursor protein genes. *J Neuropathol Exp Neurol*. 61, 1056-1068 (2002).
17. K. C. Kutner, D. M. Erlanger, J. Tsai, B. Jordan, N. R. Relkin, Lower cognitive performance of older football players possessing apolipoprotein E ε4. *Neurosurgery*. 47, 651-657 (2000).
18. I. Llaquat, L. T. Dunn, J. A. Nicoll, G. M. Teasdale, J. D. Norrie, Effect of apolipoprotein E genotype on hematoma volume after trauma. *J Nerosurg*. 96, 90-96 (2002).
19. G. Friedman, P. Froom, L. Sazbon, I. Grinblatt, M. Shochina, J. Tsenter, S. Babaey, A. Ben Yehuda, Z. Groswasser, Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury. *Neurology*. 52, 244 (1999).
20. U. S. National Library of Medicine, "APOE gene" (2018; https://ghr.nlm.nih. gov/gene/APOE#).
21. J. K. Yue, C. K. Robinson, J. F. Burke, et al. Apolipoprotein E epsilon 4 (APOE-ε4) genotype is associated with decreased 6-month verbal memory performance after mild traumatic brain injury. *Brain Behav*. 7(9):e00791 (2017).
22. D. I. Graham, K. Horsburgh, J. A. R. Nicoll, G. M. Teasdale, Apolipoprotein E and the response of the brain to injury. *Current Progress in the Understanding of Secondary Brain Damage from Trauma and Ischemia*. 73, 89-92 (1999).
23. P. B. Verghese, J. M. Castellano, D. M. Holtzman, Roles of apolipoprotein E in alzheimer's disease and other neurological disorders. *Lancet Neurol*. 10, 241-252 (2011).
24. D. M. Michaelson, APOE ε4: the most prevalent yet understudied risk factor for Alzheimer's disease. *Alzheimers Dement*. 10, 861-868 (2014).
25. C. C. Liu, T. Kanekiyo, H. Xu, G. Bu, Apolipoprotein e and Alzheimer disease: risk, mechanisms, and therapy. *Nat Rev Neurol*. 9, 106-118 (2013).
26. F. C. Crawford, R. D. Vanderploeg, M. J. Freeman, S. Singh, M. Waisman, L. Michaels, L. Abdullah, D. Warden, R. Lipsky, A. Salazar, M. J. Mullan, APOE genotype influences acquisition and recall following traumatic brain injury. *Neurology*. 58, 1115-1118 (2002).
27. M. R. Farlow, Y. He, S. Tekin, J. Xu, R. Lane, H. C. Charles, Impact of APOE in mild cognitive impairment. *Neurology*. 63, 1898-1901 (2004).
28. B. D. Jordan, N. R. Relkin, L. D. Ravdin, et al, Apolipoprotein E ∈4 associated with chronic traumatic brain injury in boxing. *JAMA*. 278, 136-140 (1997).
29. S. Nesellus, H. Zetterberg, K. Blennow, J. Marcusson, H. Brisby, Increased CSF levels of phosphorylated neurofilament heavy protein following bout in amateur boxers. *PLOS*. (2013).
30. G. M. Teasdale, G. D. Murray, J. A. Nicoll, The association between APOE ε4, age and outcome after head injury: a prospective cohort study. *Brain*. 128, 2556-2561 (2005).
31. R. M. Koffie, T. Hashimoto, H. C. Tai, K. R. Kay, A. Serrano-Pozo, D. Joyner, S. Hou, K. J. Kopeikina, M. P. Frosch, V. M. Lee, D. M. Holtman, B. T. Hyman, T. L. Spires-Jones, Apolipoprotein e4 effects in alzheimer's disease are mediated by synaptotoxic oligomeric amyloid-beta. *Brain*. 135, 2155-2168 (2012).
32. E. M. Mandelkow, E. Mandelkow, Biochemistry and cell biology of tau protein in neurofibrillary degeneration. *Cold Spring Harb Perspect Med*. 2, (2012).
33. Y. Shi, K. Yamada, S. A. Liddelow, S. T. Smith, L. Zhao, W. Luo, R. M. Tsai, S. Spina, L. T. Grinberg, J. C. Rojas, G. Gallardo, K. Wang, J. Roh, G. Robinson, M. B. Finn, H. Jiang, P. M. Sullivan, C. Baufeld, M. W. Wood, C. Sutphen, L. McCue, C. Xiong, J. L. Del-Aguila, J. C. Morris, C. Cruchaga, Alzheimer's Disease Neuroimaging Initiative, A. M. Fagan, B. L. Miller, A. L. Boxer, W. w. Selley, O. Butovsky, B. A. Barres, S. M. Paul, D. M. Holtzman, ApoE4 markedley exacerbates tau-mediated neuro-degeneration in a mouse model of tauopathy. *Nature*. 549, 523–527 (2017).
34. C. Bernick, S. J. Banks, W. Shin, N. Obuchowski, S. Butler, M. Noback, M. Phillips, M. Lowe, S. Jones, M. Modic, Repeated head trauma is associated with smaller thalamic volumes and slower processing speed: the professional fighters' brain health study. *Br J Sports Med*. 15, 1007-1011 (2015).
35. S. J. Banks, J. B. Miller, R. A. Rissman, C. B. Bernick, Lack of influence of apolipoprotein E status on cognition or brain structure in professional fighters. *J Neurotrauma*. 34, 380-384 (2017).
36. V. L. Kristman, C. H. Tator, N. Kreiger, D. Richards, L. Mainwaring, S. Jaqlal, G. Tomlinson, P. Comper, Dpes the apolipoprotein epsilon 4 allele predispose varsity athletes to concussion? a prospective cohort study. *Clin J Sport Med*. 18, 322-328 (2008).
37. K. Millar, J. Nicoll, S. Thornhill, G. Murray, G. Teasdale, Long term neuropsychological outcome after head injury: relation to APOE genotype. *J Neurol Neurosurg Psychiatry*. 74, 1047-1052 (2003).
38. W. Kamruecha, S. Chansirikarnjana, E. Nimkulrat, C. Udommongkol, W. Wongmek, W. Thangnipon, Rapid detection of apolipoprotein E genotypes in Alzheimer's disease using polymerase chain reaction-single strand conformation polymorphism. *Southeast Asian J Trop Med Public Health*. 37, 793-797 (2006).
39. E. S. Koay, M. Zhu, T. Wehr, M. L. Choong, M. C. Khaw, S. K. Sethi, T. C. Aw, Detection of apolipoprotein E genotype by capillary electrophoresis. *Talanta*. 45, 673-681 (1998).
40. N. Ghebranious, L. Ivacic, J. Mallum, C. Dokken, Detection of ApoE E2, E3, and E4 alleles using MAALDI-TOF mass spectrometry and the homogeneous mass-extend technology. *Nucleic Acids Res*. 33, (2005).
41. H. Zetterberg, M. A. Hietala, M. Jonsson, et al., Neurochemical aftermath of amateur boxing. *JAMA*. 63, 1277-1280 (2006).

42. S. Nesellus, H. Bisby, A. Theodorsson, K. Blennow, H. Kitterberg, J. Marcusson, CSF-biomarkers in Olympic boxing: diagnosis and effects of repetitive head trauma. *PLOS*. (2012).

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