REVIEW

Genetic vulnerability following traumatic brain injury: the role of apolipoprotein E

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Apolipoprotein E (APOE) is thought to be responsible for the transportation of lipids within the brain, maintaining structural integrity of the microtubule within the neurone, and assisting with neural transmission. Possession of the APOE ϵ4 allele has also been shown to influence neuropathological findings in patients who die from traumatic brain injury, including the accumulation of amyloid β protein. Previous clinical studies reporting varying outcome severities of traumatic brain injury, including cognitive and functional recovery, all support the notion that APOE ϵ4 allele possession is associated with an unfavourable outcome. Evidence from experimental and clinical brain injury studies confirms that APOE plays an important role in the response of the brain to injury.

Genetic differences in the ability of the brain to form new connections and undergo neuroplasticity may explain variation in outcome after traumatic brain injury. Recently, an increasing appreciation of the role of apolipoprotein E (APOE) in modifying neurological outcome after traumatic brain injury has been reported, although the mechanisms by which this occurs remain poorly defined. In this brief review, we will discuss the current status of APOE polymorphism and its role in the outcome of patients following traumatic brain injury.

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APOLIPOPROTEIN E

Site of synthesis

APOE is mainly synthesised by astrocytes packed together with cholesterol and phospholipid to form lipid–protein complexes, which are then released into the extracellular space. These complexes bind to APOE receptors on the surfaces of nerve cells, which are internalised into the cell, thereby providing a mechanism for the maintenance and repair of cell membranes, the growth of neurites, and synaptogenesis.[1]

> "Apolipoprotein E assumes the major role in lipid transport in the cerebrospinal fluid"

APOE is the major apolipoprotein in human cerebrospinal fluid, existing as small spherical, discoidal lipoproteins that transport cholesterol and phospholipid. Unlike plasma, in which apolipoprotein B (APOB) containing low density lipoprotein (LDL) is the major lipoprotein involved in lipid transport, the cerebrospinal fluid lacks APOB and LDL, so that APOE assumes the major role in lipid transport in this medium.

Functions

APOE is thought to be responsible for the transportation of lipids within the brain and maintaining the structural integrity of the microtubule within the neurone; it may also assist with neural transmission.[2–4] Recent reports from transgenic closed traumatic brain injury models also support the role of APOE in the inflammatory response and neuronal repair mechanisms following traumatic brain injury.[5 6]

Apart from being involved in lipid redistribution, both among the cells of different organs and among the cells within an organ or tissue, APOE has other functions unrelated to lipid transport, as listed below.

- The extremely high concentrations of APOE produced by macrophages in the distal stump of the rat sciatic nerve and the expression of LDL receptors on the growing tips of neurites and Schwann cells strongly suggest a role for APOE in nerve regeneration. Other speculative roles include the suggestion that APOE could be a neurotrophic factor involved in one of several events required for nerve survival and repair.[7]
- APOE is postulated to be involved in smooth muscle cell proliferation, differentiation, or both.
- APOE modulates the immune response and further investigations may provide an insight into the effects of lipoproteins on tumorigenesis.

Genetic polymorphism

Mature APOE is a 299 amino acid protein, with a relative molecular mass of 34 000 Da, which is the product of a single gene. The APOE gene spans 3.7 kilobases, has four exons, and is located on chromosome 19.[8] APOE polymorphism was established using isoelectric focusing and confirmed by two dimensional electrophoresis.[9] Three major isoforms of APOE—referred to as APOE 2, APOE 3, and APOE 4—are products of three alleles (ϵ2, ϵ3, and ϵ4) at a single gene locus, and occur with a frequency of 7%, 78%, and 15%, respectively, in white populations.[10] Three homozygous phenotypes (APOE 2/2, APOE 3/3, and APOE 4/4) and three heterozygous phenotypes (APOE 2/3, APOE

Abbreviations: APOE, apolipoprotein E; APP, amyloid precursor protein; LDL, low density lipoprotein

3/4, and APOE 2/4) arise from the expression of any two of the three alleles. APOE 4 displays normal binding but is associated with raised plasma cholesterol and LDL concentrations.[8 9]

APOE polymorphism is differentiated by analysis of the amino acid sequences of the three isoforms. Amino acid substitutions account for the differences.

Variation in prevalence rates of the APOE ε4 allele

In Western population groups, APOE ε4 has been shown to influence the risk of development of Alzheimer's disease. However the influence of APOE ε4 on the development of Alzheimer's disease in African Americans is still not clear, with conflicting reports showing either little or no increase[11–13] or conversely that Alzheimer's disease in African Americans is associated with the ε4 allele.[14] Human APOE exhibits genetic polymorphism with varying prevalence rates in all populations examined to date.[15–19] Numerous reports document the higher frequency of APOE ε4 in people of African heritage,[17–19] even in population groups as far south as Southern Africa.[20 21]

Surprisingly, the association of APOE ε4 and Alzheimer's disease has not been found in sub-Saharan populations of African heritage.[20–26] The Indianapolis-Ibadan dementia project—a longitudinal population based study—clearly showed the striking difference in the incidence of Alzheimer's disease and dementia between African Americans in a developed community and black Africans in a developing community.[23 25] This led Corbo and Scacchi[15] to propose that the exposure of the APOE ε4 gene to contemporary environmental conditions (for example, Western diets, longer life spans) may have rendered APOE ε4 a susceptible allele to influence coronary artery disease and Alzheimer's disease. The absence of the association of APOE ε4 with coronary heart disease[20] and Alzheimer's disease in sub-Saharan Africans, and its presence in African Americans, seems to confirm this hypothesis.

TRAUMATIC BRAIN INJURY OUTCOME AND APOLIPOPROTEIN ε4 ALLELE

The link between Alzheimer's disease, traumatic brain injury outcome, and the APOE ε4 allele

APOE ε4 has important direct effects on the nervous system. Possession of the APOE ε4 allele has been shown to result in a greater propensity to develop age related cognitive impairment,[27 28] a decrease in the synapse–neurone ratio,[29] and increased susceptibility to exogenous neurotoxins,[30] and hippocampal atrophy.[31] It has now been shown that the APOE ε4 isoform is associated with an increased risk of late onset familial and sporadic Alzheimer's disease in Western populations, which results in the standard molecular and cellular neuropathology of Alzheimer's disease.[32 33] In addition, many epidemiological studies have identified a history of a previous head injury as an important environmental risk factor for the development of Alzheimer's disease.[34 35] Mayeux and colleagues[36] showed that a history of a previous head injury and APOE ε4 interact synergistically; there was a 10 fold increase in the risk of Alzheimer's disease when both APOE ε4 and a history of traumatic head injury were present compared with a twofold increase in risk with APOE ε4 alone. Additional evidence has been provided by a study on dementia pugilistica, which is a progressive dementia disorder similar to Alzheimer's disease, found in boxers and in patients who have been subjected to repeated head injury. A worse outcome was found in a group of boxers with the ε4 allele compared with those without.[37]

> "The APOE ε4 isoform is associated with an increased risk of late onset familial and sporadic Alzheimer's disease in Western populations, which results in the standard molecular and cellular neuropathology of Alzheimer's disease"

Although the mechanisms underlying these effects are unclear, evidence suggests that both APOE ε4 and traumatic brain injury may influence the risk of Alzheimer's disease via interactions with amyloid β protein. Deposition of the amyloid β protein, a molecule proteolytically cleaved from the precursor molecule, amyloid precursor protein (APP), plays a key role in the pathogenesis of Alzheimer's disease. There is now in vivo evidence linking APOE ε4 with amyloid β protein deposition.[38] APOE is associated with reduced growth and the branching of neurites in cell culture, an effect that is mediated by the LDL receptor related protein, which mediates the entry of APOE into neurones, and is also increased after injury. APOE ε4 also binds less aggressively to cytoskeletal proteins and amyloid β protein, compared with the other isoforms of APOE, reducing any potential protective effect. APOE ε4 also promotes more rapid aggregation of amyloid β protein into amyloid fibrils in vitro.[39 40]

The APOE ε4 allele and human traumatic brain injury outcome

It has been reported that head injury triggers amyloid β protein deposition in those with genetic susceptibility conferred by APOE ε4, and that amyloid β protein deposition is recorded in one third of severe head injured patients at necropsy.[38] It has been postulated that head injury related deposition of amyloid β protein in those who survive may be followed by the development of the full spectrum of Alzheimer's disease pathology later in life.[38]

The Glasgow group were the first to report in a clinical setting the influence of APOE ε4 and a poor outcome following traumatic brain injury.[41] Since then, numerous clinical studies in all types of traumatic brain injury have supported the association of APOE ε4 allele possession with an unfavourable outcome.[41–44] It was reported recently that the possession of the APOE ε4 allele predisposes a patient to a larger sized intracerebral haematoma.[45]

Similarly, possession of the APOE ε4 allele has also been shown to be associated with a poor outcome following spontaneous non-aneurysmal intracerebral haemorrhage,[46] haemorrhage associated with amyloid angiopathy,[47–49] subarachnoid haemorrhage,[50] and, more recently, an increased risk of developing cerebral amyloid angiopathy in patients recovering from traumatic brain injury.[51]

> "It has been reported that head injury triggers amyloid β protein deposition in those with genetic susceptibility conferred by APOE ε4"

Presently, very few reports are available documenting the effect of APOE ε4 status and human traumatic brain injury. Previous reported studies were on small cohorts and are generally institutionally based, with very few studies looking at moderate to severe traumatic brain injury. Furthermore, most if not all, studies reported to date were conducted in white population groups, with a predominance of males.

FUTURE DIRECTIONS

Unfortunately, traumatic brain injury is ubiquitous and remains a major cause of considerable morbidity, neuropsychological sequelae, and death. Despite immense advances in the management of clinical traumatic brain injury, no treatment exists to date that can reverse the sequelae of the molecular and cellular mechanisms that lead to post-traumatic death. Understanding the pathobiology of traumatic brain injury is pivotal to halting and reversing the devastating effects of secondary brain injury. Currently, research is primarily focused on the cellular and subcellular mechanisms that are believed to hold the key to understanding the complex networks or cascades unleashed at the time of insult. A brief discussion on potential avenues for future investigation is provided.

Genetically engineered animal models of traumatic brain injury

This form of technology has the potential to recreate many clinical and pathological aspects of traumatic brain injury. Typically, it involves the artificial expression or targeted deletion (knockout) of a specific gene. Thus, genetically engineered animals offer us a unique opportunity to evaluate mechanistic links in specific defined disease entities and provide a basis for the evaluation of potentially effective pharmacological treatment paradigms.

To add to the complexity of this field, certain strains of transgenic mice will accumulate APP following traumatic brain injury, but this has not been associated with amyloid β protein plaque formation, probably because of differences in amino acid composition between different species.[52] Following cortical contusion injury in APP transgenic yeast artificial chromosome mice, Murai *et al* found no post-traumatic differences in cognition or motor deficits.[53] However, when a second strain of mice was used, which expressed a mutant APP minigene driven by a platelet derived growth factor promoter, the mice overexpressed mutant APP 10-fold compared with control mice, and plaques were found at 6 months.[54] When exposed to cortical contusion injury, these mice showed exacerbation of their cognitive impairments and produced an increase in hippocampal amyloid β protein 1–40 and amyloid β protein 1–42 (two principal forms of β amyloid peptide generated from APP degradation), with 80% cell loss in the CA3 region in the injured hemisphere, leading the investigators to postulate the "two hit hypothesis". The first insult or hit is the genetic vulnerability (high concentration of amyloid β protein, which is influenced by the individual's E4 allele status), which is only manifested after the a second independent epigenetic event or insult, such as traumatic brain injury.[55]

However, Uryu *et al* were the first to provide the mechanistic link between Alzheimer's disease and traumatic brain injury.[56] Using transgenic mouse models, they were able to prove that repetitive traumatic brain injury accelerates brain amyloid β protein accumulation and oxidative stress, which they suggested could act synergistically to drive the process of Alzheimer's disease. These studies also confirmed previous epidemiological reports suggesting that the more severe the brain injury, the greater the possibility of developing Alzheimer's disease,[57] and that lipid peroxidation is enhanced by traumatic brain injury and is linked to increased amyloid accumulation and deposition. Other transgenic mouse model studies have also confirmed the role of APOE ϵ4 in influencing the neurodegenerative cascade following traumatic brain injury via the effect of amyloid β protein.[58 59] Many studies have suggested that oxidative stress promotes amyloid deposition and fibril formation.[56 60 61]

Ethnic and regional differences: influence on APOE polymorphism

As previously mentioned, numerous reports have clearly documented the differences in the prevalence rate of the APOE ϵ4 allele. Furthermore, all reports documenting the effect of the APOE ϵ4 allele on traumatic brain injury outcome have been performed almost exclusively in white populations or those derived from white individuals. Contrary to reports from developed regions, it has been reported recently that the APOE ϵ4 allele has no significant effect on traumatic brain injury outcome in an exclusive black African cohort with homogenous traumatic brain injury.[62]

This racial difference in the effect of the expression of the APOE ϵ4 allele on traumatic brain injury outcome may be related to: (1) interpopulation differences in the sequence variation underlying the three protein isoforms of APOE; (2) the presence of other modifier gene(s); or (3) more importantly, gene–environment interactions that play an important role in modifying the response to traumatic brain injury. Despite the higher frequency of the APOE ϵ4 allele in sub-Saharan population groups (pygmies, Khoi-San (bushmen), and black Africans),[15 19–21 24 63 64] the low prevalence of Alzheimer's disease and the non-significant effect of the APOE ϵ4 allele on closed traumatic brain injury in black Africans supports the susceptibility of the APOE ϵ4 allele to varying contemporary environmental conditions.

Sex based differences and traumatic brain injury outcome: role of APOE polymorphism

Current knowledge of the central nervous system response to traumatic brain injury, in addition to potential treatments, are limited primarily to male subjects. Recent attention to sex based variation following experimental traumatic brain injury has revealed striking differences between the sexes.[65 66] It has been postulated that the reduced vulnerability of the female brain may result from the neuroprotective effects of oestrogen[67–69] and progesterone.[70 71]

Currently, it is not clear whether the effects of oestrogen are receptor or non-genomic based. Recently, it has been shown that Premarin, which is widely used for hormonal replacement therapy, has a greater effect on APOE gene expression than glial fibrillary acid protein expression in mixed glial cell cultures.[72] This not only adds further support for the neuroprotective effect of oestrogens, but also serves as evidence that APOE induction supports neurite outgrowth as compared with glial fibrillary acid protein induction, which inhibits neurite outgrowth, thereby promoting glial scarring. To date, no clinical report exists documenting the influence of APOE on sex differences.

LINK BETWEEN APOE AND TRAUMATIC BRAIN INJURY OUTCOME: CURRENT SHORTFALLS

Evidence from experimental and clinical traumatic brain injury studies has confirmed the important role that APOE plays in the inflammatory response and neuronal repair mechanisms following traumatic brain injury. Sporadic clinical reports have supported the association between APOE e4 status and human traumatic brain injury outcome. Currently, there are many unanswered questions. Is the effect of APOE ϵ4 on traumatic brain injury outcome confined to white or white derived population groups only? It is presently unknown whether APOE polymorphism is consistent in modifying the genetic response to traumatic brain injury in all major racial groups and in different geographical regions. Given the high interpopulation and regional variation, together with the genetic susceptibility of the APOE ϵ4 allele to contemporary environmental factors, further clinical studies are warranted. In addition, it is unknown presently how observations recorded in transgenic animal models will translate to human conditions.

How does the APOE ϵ4 allele influence traumatic brain injury severity, or the type of injury—diffuse versus focal? What is the effect of APOE ϵ4 on sex based human traumatic brain injury outcome? Is this effect confined to adults only or does it apply to a developing brain also, and what is the influence of APOE ϵ4 status on injury to other parts of the neuraxis, such as the spinal cord?

> "It is presently unknown whether APOE polymorphism is consistent in modifying the genetic response to traumatic brain injury in all major racial groups and in different geographical regions"

Another alarming facet of APOE status is that the prognostication of traumatic brain injury outcome may have serious medicolegal and financial ramifications for patients and their families. It may be possible in the future that patients are biased by insurance companies according to their expression

Take home messages

- Apolipoprotein E (APOE) ϵ4 has been shown to contribute to the development of Alzheimer's disease, although there appear to be racial and geographical variations, and this may not be true for African populations
- This variation may the result of Western environmental conditions, such as diet and longer life spans
- APOE ϵ4 and traumatic brain injury appear to act synergistically in the development of Alzheimer's disease
- APOE ϵ4 is associated with a poor outcome in traumatic brain injury, but again there may be racial and geographical variations
- The mechanisms underlying these effects are unclear but the amyloid β protein is thought to be involved
- More research into APOE ϵ4 and traumatic brain injury is needed

of genetic and molecular markers. In addition, insurance companies may preclude an individual on the basis of his or her APOE ϵ4 status from pursuing certain high risk sports or occupations where the potential for traumatic brain injury is greater. The ramifications of APOE ϵ4 status may assume a whole new dimension with "genetic stereotyping" taking on greater importance in the future.

CONCLUSION

As a result of recent reports, the role of APOE ϵ4 in contributing to the development of Alzheimer's disease is gaining increasing importance. However, APOE genotyping is not yet part of current medical practice. Undoubtedly, despite the extensive investigation of APOE in lipid metabolism, ischaemic cardiovascular disease, and Alzheimer's disease, more investigations are needed to determine a clear cut effect of APOE ϵ4 status on human traumatic brain injury in all its facets. A greater research effort needs to be undertaken and the interest in APOE status and traumatic brain injury should be cultivated more extensively and not confined to a few selected groups. Neurosurgeons, in particular, are uniquely placed to play a more meaningful role in this quest to determine the effect of APOE status on traumatic brain injury outcome.

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