Lack of Influence of Apolipoprotein E Status on Cognition or Brain Structure in Professional Fighters

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Abstract

The role of the apolipoprotein e4 allele in moderating cognitive and neuroanatomical degeneration following repeated traumatic brain injury is controversial. Here we sought to establish the presence or absence of such a moderating relationship in a prospective study of active and retired boxers and mixed martial arts fighters. Fighters ($n=193$) underwent cognitive evaluations, interviews regarding fight history, MRI of the brain, and genetic testing. We used a series of moderator analyses to test for any relationship of apolipoprotein genotype on structural volumes of brain regions previously established to be smaller in those with the most fight exposure, and on cognitive abilities also established to be sensitive to fight exposure. No moderating relationship was detected in any of the analyses. The results of this study suggest that there is no impact of apolipoprotein genotype on the apparent negative association between exposure to professional fighting and brain structure volume or aspects of cognition.

Keywords: apolipoprotein E; cognition; hippocampus; thalamus; trauma

Introduction

AMONG PROFESSIONAL BOXERS, apolipoprotein E (APoE) status may be related to clinical impairment. Jordan and colleagues assessed 30 boxers, and found that boxers who had high levels of exposure to head trauma and an APoE e4 allele were more likely to have a high impairment score.¹ The same group found that e4-positive older football players with greater exposure to football were more likely to score lower on cognitive tests than either younger players or players of a similar age with less experience.² However, other research suggests that a link between outcome from sports-related brain injury and APoE status may be tenuous: a study of 318 athletes failed to find any evidence to suggest a link between APoE status and disposition to concussion.³ Repetitive traumatic brain injury, especially within the context of sports, has been linked to a neuropathological diagnosis, chronic traumatic encephalopathy (CTE). Omalu and colleagues documented seven CTE patients with known APoE genotypes, of whom two had an e4 allele, consistent with population norms.⁴ In 70 CTE brains, Boston University has not established a correlation with APoE status.⁵

The Professional Fighters' Brain Health Study (PFBHS) is a large, prospective, longitudinal study assessing the fight exposure, cognition, and brain health of professional fighters (boxers, mixed martial arts, and martial arts). Earlier results show relationships between exposure to fighting and cognition and also volume of the thalamus, hippocampus, and regions of the basal ganglia. Further,

correlations existed between these regions and cognitive ability, suggesting a functional impact of the anatomic–fight exposure relationship.^{6,7} More recently, we started to collect genetic data on participants. We tested the hypothesis that previously revealed that relationships would be moderated by e4 status. Given the association with Alzheimer's, we further expected there to be an association between APoE status and hippocampal volume, but not thalamic volume.

Methods

Ethics, consent, and permissions

All methodology was approved by the Cleveland Clinic Foundation Internal Review Board and all participants gave written informed consent for their participation.

Procedure

Detailed methods of the PFBHS have been listed elsewhere.⁷ Briefly, professional fighters with a history of either boxing or mixed martial arts fighting were recruited in two cohorts, active and retired. Once screened for their appropriateness for the study, including ability and willingness to undergo an MRI, they were consented and underwent a visit including a structured interview on demographics and fight exposure, cognitive testing using central nervous system (CNS) vital signs, blood draw for genetic analysis, and MRI scan including anatomical and functional images.

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Cognitive tests are presented as composite scores. Specifically, verbal memory is a combination of immediate recognition and delayed recognition of a 15 item word list, processing speed is a total of number correct on a symbol digit coding task, psychomotor speed combines number correct on coding with the finger tapping score, and reaction time is derived from the Stroop task.

Here, we address cross-sectional relationships among APoE status, demographic factors (age, education), fight history, cognitive data, and hippocampal caudate and thalamic volumes as assessed using FreeSurfer (<http://surfer.nmr.mgh.harvard.edu/>).

Real-time polymerase chain reaction (PCR) for APoE genotyping

Genotyping of APoE alleles was performed using real time PCR restriction fragment length polymorphism analysis by the Alzheimer's Disease Cooperative Study (ADCS) Biomarker Core according to standard operating procedures.⁸⁻¹⁰ Briefly, genomic DNA was collected from blood DNA extracted using Qiamp DNA blood maxi kit (Qiagen) and APoE genotyping was performed using Applied Biosystems TaqMan SNP Genotyping Assay. The amplification reaction contained 5 μ L genomic DNA, 2.5 U of Taq DNA Polymerase (New England Biolabs, Inc, Ipswich, MA), 1 $\times$ ThermoPol Reaction Buffer (New England Biolabs), 0.3 mmol/L deoxynucleotide (dNTPs), 10% dimethyl sulfoxide (DMSO), and 0.3 μ mol/L of each primer (forward primer: 5'-ACGCGGGCACGGCTGTCCAAGGA-3'; reverse primer: 5'-GCGGGCCCCGGCCTGGTACAC-3'). The assay was run on a Bio-Rad CFX96. Five positive controls for each genotype and one negative control were included in each plate to confirm accurate determination.

Statistical analysis

MRI images were processed using FreeSurfer. This program results in volumes and cortical thickness of pre-set regions of the entire brain. We were particularly interested in the thalamus and caudate, because these regions had been shown in our earlier research to be related to exposure and cognition,⁶ and in the hippocampus, given its role in Alzheimer's disease and in older adults with an APoE e4 allele.

After genotype coding, participants were grouped into those with and without APoE e4. Active and retired fighters were studied collectively. One way analysis of variance was used to compare APoE e4-positive and negative groups on key demographic variables including age and education, as well as the number of years fighting at the professional level, which served as a proxy for exposure. Chi-square tests were used to ensure comparability among groups on categorical demographic variables, including race, gender, and type of fighter (e.g., boxer, mixed martial artist, martial artist). One-way analysis of covariance was used to evaluate for group differences on cognitive measures using raw scores as primary dependent variables and group membership as the dependent variable, with any demographic differences entered as covariates. We used raw scores, because different age groups are compared against different normative values for standardized scores, thus confounding our results. Similar analyses were repeated using structural volumes as the dependent variables and total intracranial volume as the only covariate. Alpha levels were adjusted to $p < 0.01$ to account for multiple comparisons.

To evaluate the effect of genotype status on the relationships between fighting exposure and cognitive functioning, and exposure and structural volume, several moderated regression models were tested. A total of 10 independent regression models were fit, using the six individual brain structures and the four cognitive domain scores as the primary dependent variable and exposure and genotype status as predictors. Structural models also included intracranial volume and cognitive models included years of education as predictors. To evaluate the moderating effect of genotype status, an

TABLE 1. GROUP CHARACTERISTICS

	APoE e4 Positive		APoE e4 Negative	
	(n = 54)		(n = 139)	
Demographics	Mean	SD	Mean	SD
Age (years)	30.1	9.3	31.1	9.4
Education (years)	12.8	2.7	13.8	2.8
Years of professional fighting	5.5	5.5	5.1	5.3
Cognitive scores	(n = 50)		(n = 132)	
Verbal memory	93.55	17.89	95.01	17.29
Processing speed	85.16	17.81	87.73	16.10
Psychomotor speed	91.98	16.50	93.93	16.56
Reaction time	79.33	18.27	82.56	18.59
Regional volumes	(n = 48)		(n = 129)	
Left thalamus	6564.9	780.3	6726.2	983.9
Right thalamus	6849.3	892.5	6900.6	975.9
Left hippocampus	3836.2	394.7	3884.3	491.5
Right hippocampus	3960.3	433.1	3976.4	487.2
Left caudate	3594.0	444.6	3748.8	587.4
Right caudate	3707.2	459.6	3833.0	578.0

Cognitive scores reflect standardized subject index scores from central nervous system (CNS)-vital signs (standard; i.e., mean of 100 and standard deviation of 15). Regional imaging volumes generated by FreeSurfer. There were some missing data points.

APoE, apolipoprotein E.

interaction term was calculated between the genotype status and the years of fighting, which was then added to each of the models via forward entry.

Results

A total of 193 professional fighters with genetic data were included in the present analyses. Demographic, cognitive test data, and regional volumes for each group are presented in Table 1. Fighter classifications are presented in Table 2. The APoE e4-negative group (4NEG) was 90% male, 17.3% African American, 46.8% Caucasian, 6.5% multiracial, 23.7% other (Asian, Pacific-Islander, American Indian/Alaskan Native, or unspecified), and 5.7% unreported. The APoE e4-positive group (4POS) was 94% male, 42.6% African American, 27.8% Caucasian, 5.6% multiracial, 20.4% other (Asian, Pacific-Islander, American Indian/Alaskan Native, or unspecified), and 3.6% unreported. Chi-square tests

TABLE 2. TYPES OF FIGHTERS

	APoE e4 Positive (n = 54)	APoE e4 Negative (n = 139)
Active boxer	27	50
Active MMA	16	56
Active MA	0	7
Mixed fighter	5	13
Retired boxer	6	12
Retired MMA	0	1

MMA, mixed martial arts; MA, martial arts.

TABLE 3. MODERATED REGRESSION MODEL SUMMARY STATISTICS FOR REGIONAL BRAIN VOLUMES

Structure	Predictors	Unstandardized coefficients		Standardized coefficients		p	R ² change
		B	SE	Beta			
<i>Left hippocampus</i>							
Model 1	Constant	4089.93	103.47	—	—	0.143***	
	e4 status	−35.67	73.69	−0.04	0.63		
	Exposure	−32.44	6.26	−0.37	<0.001		
Model 2	Constant	4085.04	139.63	—	—	0.000	
	APoE e4 status	−31.88	103.42	−0.03	0.76		
	Years of professional fighting	−31.51	18.85	−0.36	0.10		
	e4 status × exposure	−0.70	13.44	−0.01	0.96		
<i>Right hippocampus</i>							
Model 1	Constant	4149.62	106.88	—	—	0.131***	
	e4 Status	−4.18	76.12	−0.004	0.96		
	Exposure	−32.18	6.47	−0.36	<0.001		
Model 2	Constant	4228.43	143.94	—	—	0.004	
	APoE e4 status	−65.21	106.61	−0.06	0.54		
	Years of professional fighting	−47.18	19.43	−0.53	0.02		
	e4 status × exposure	11.34	13.86	0.19	0.41		
<i>Left thalamus</i>							
Model 1	Constant	7253.39	202.16	—	—	0.212***	
	e4 status	−119.27	143.98	−0.06	0.41		
	Exposure	−79.99	12.24	−0.45	<0.001		
Model 2	Constant	7551.86	270.57	—	—	0.013	
	APoE e4 status	−350.38	200.4	−0.17	0.08		
	Years of professional fighting	−136.79	36.53	−0.77	<0.001		
	e4 status × exposure	42.95	26.05	0.37	0.10		
<i>Right thalamus</i>							
Model 1	Constant	7343.55	204.29	—	—	0.226***	
	e4 status	−6.67	145.5	0.00	0.97		
	Exposure	−85.5	12.37	−0.48	<0.001		
Model 2	Constant	7629.9	273.65	—	—	0.011	
	APoE e4 status	−228.4	202.68	−0.11	0.26		
	Years of professional fighting	−139.99	36.95	−0.78	<0.001		
	e4 status × exposure	41.21	26.34	0.34	0.12		
<i>Left caudate</i>							
Model 1	Constant	4115.58	120.19	—	—	0.208***	
	e4 status	−131.96	85.61	−0.11	0.13		
	Exposure	−45.80	7.28	−0.44	<0.001		
Model 2	Constant	4224.43	161.70	—	—	0.005	
	APoE e4 status	−216.25	119.77	−0.18	0.07		
	Years of professional fighting	−66.51	21.83	−0.64	0.003		
	e4 status × exposure	15.66	15.57	0.22	0.32		
<i>Right caudate</i>							
Model 1	Constant	4147.44	119.29	—	—	0.204***	
	e4 status	−91.39	84.96	−0.08	0.28		
	Exposure	−45.77	7.22	−0.44	<0.001		
Model 2	Constant	4238.45	160.63	—	—	0.003	
	APoE e4 status	−161.86	118.97	−0.13	0.18		
	Years of professional fighting	−63.08	21.69	−0.61	0.004		
	e4 status × exposure	13.10	15.46	0.19	0.398		

*** $p < 0.001$.

APoE, apolipoprotein E.

did not reveal significant differences between groups on gender or type of fighter, although the racial distribution did significantly differ ($\chi^2 [5, n = 183] = 19.23, p = 0.002$). One-way ANOVA did not reveal significant differences with regard to age or exposure; however, groups did differ significantly in number of years of education ($F[1, 184] = 5.78; p = 0.017$). Years of education and race were, therefore, used as covariates in subsequent analysis of cognitive data.

After accounting for intracranial volume, there were no significant regional volume differences between groups for any of the studied brain structures (all p values >0.25). Groups also did not differ with regard to any of the cognitive variables after accounting for years of education and race (all p values >0.37).

Using an adjusted alpha level of $p < 0.01$, moderated regression analyses (Table 3) revealed that fighting exposure, but not genotype status, was significantly associated with the volume of the left and right thalamus, and the left and right caudate, but neither the left nor the right hippocampus. In none of the models was the interaction between genotype status and exposure significant, nor did its inclusion significantly improve model fit.

For verbal memory, age was a significant predictor, but race, education, fighting exposure, and genotype status were not. Processing speed and reaction time were predicted by education, but not age, race, genotype status, or exposure. Psychomotor speed was predicted by both age and education, but not race, exposure, or genotype status. The interaction between genotype status and exposure was not significant in any of the cognitive models and did not improve model fit.

Discussion

Although we replicated our earlier findings that fight exposure is negatively associated with volumes in the thalamus and caudate and aspects of cognition, there was no moderating effect of APoE status on any of the relationships between brain volume and cognition. Although this goes against some early findings in small samples of older fighters, it is consistent with studies of larger groups although none has had a well-characterized, prospective cohort exposed to repetitive head trauma with cognitive and imaging data.

Whereas there have been calls to test for APoE in athletes who are at risk for head trauma, the current findings would suggest that even in sports in which head trauma is a common occurrence, APoE genotype does not appear to add to the risk of cognitive impairment, at least in younger athletes. Further, in this sport the regions impacted are quite different from those seen early in Alzheimer's disease, and, therefore, many cellular-level pathology underlying change in brain structure volume or cognition may be distinct from the beta amyloid/tau changes seen in Alzheimer's disease, which are likely mediated by APoE genotype.

Although the PFBHS is both prospective and longitudinal, we do not yet have genetic data on fighters who have been seen over several years. The current data set utilized cross-sectional data to assess for relationships between exposure and outcome, with APoE status as a moderating variable. Assessing changes within subjects over a period of time will be necessary to ensure that APoE genotype does not moderate decline in brain health for individual athletes. Further, understanding the impact of APoE on older, retired athletes, will be important in determining whether or not there is a relationship between genotype and later development of neurodegenerative conditions, including Alzheimer's disease.

The percentage of APoE $\epsilon 4$ carriers in our cohort was slightly higher than what has been reported in population studies. Further,

there was a notable overrepresentation of the $\epsilon 4$ allele in black participants. An increased frequency compared with whites has been previously reported,¹¹ with the $\epsilon 4$ allele also appearing to be less of a risk factor for Alzheimer's disease in black patients in Evans and colleagues' study.

Limitations of the current study include the relative brevity of the cognitive testing completed. It could be that more thorough testing, especially of memory, could elucidate a mediating relationship of APoE on cognition and exposure. We will address this in currently recruiting substudies involving more thorough neuropsychological batteries. However, the number of participants in the main PFBHS remains large and, therefore, the current studies were well powered to find a moderating relationship with the tests that were given.

Conclusion

In conclusion, the current study supports the notion that APoE genotype does not moderate the apparently deleterious impact of multiple brain injuries on the volume of the thalamus, caudate or hippocampus, or on cognition.

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Author Disclosure Statement

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