

Apolipoprotein E Genotype and Concussion in College Athletes

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Objective: To evaluate the association between apolipoprotein E (APOE) polymorphisms (E2, C/T Arg158Cys; E4, T/C Cys112Arg; and promoter, g-219t) and the history of concussion in college athletes. We hypothesized that carrying 1 or more APOE rare (or minor) allele assessed in this study would be associated with having a history of 1 or more concussions.

Design: Multicenter cross-sectional study.

Setting: University athletic facilities.

Participants: One hundred ninety-six male football (n = 163) and female soccer (n = 33) college athletes volunteered.

Interventions: Written concussion history questionnaire and saliva samples for genotyping.

Main Outcome Measures: Self-reported history of a documented concussion and rare APOE genotype (E2, E4, promoter).

Results: There was a significant association (Wald $\chi^2 = 3.82$; $P = 0.05$; odds ratio = 9.8) between carrying all APOE rare alleles and the history of a previous concussion. There was also a significant association (Wald $\chi^2 = 3.96$, $P = 0.04$, odds ratio = 8.4) between carrying the APOE promoter minor allele and experiencing 2 or more concussions.

Conclusions: Carriers of all 3 APOE rare (or minor) alleles assessed in this study were nearly 10 times more likely to report a previous concussion and may be at a greater risk of concussion versus noncarriers. Promoter minor allele carriers were 8.4 times more likely to report multiple concussions and may be at a greater risk of multiple concussions versus noncarriers. Research involving

larger samples of individuals with multiple concussions and carriers of multiple APOE rare alleles is warranted.

Key Words: polymorphism, MTBI, APOE E2, APOE E4, APOE promoter g-219t

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INTRODUCTION

Sport-related concussions account for up to 1.6 to 3.8 million injuries annually,¹ with many occurring in football and soccer at the collegiate level.² A concussion is caused by acceleration forces,³ occurring directly via a blow to the head, face, or neck or indirectly through an impulsive force transmitted to the head.⁴ Concussions can result in poor short-term⁵ and long-term outcomes, including depression,⁶ emotional disturbances,⁷ and alterations in motor function,⁸ as well as an increased risk of chronic traumatic encephalopathy.⁹ The influence of apolipoprotein E (APOE) genotype on concussion risk has garnered attention from the international community,¹⁰ and some authors have studied this effect in relation to athletic participation.^{11,12}

Apolipoprotein (ApoE) is a plasma lipoprotein, which plays a role in nervous tissue healing.^{13,14} It aids in neuron protection and repair via the transportation of lipids as demonstrated by its upregulation after neuronal stress.¹⁵ Apolipoprotein E gene variations (eg, polymorphism) occur in the promoter (eg, g-219t)¹⁶ and coding (eg, E4)¹⁷ regions, which can affect ApoE expression and quality and quantity, respectively.¹⁸ The altered neural environment created by APOE polymorphisms may influence the ability of neurons to function and recover properly from mechanical stress and therefore be particularly hazardous if carried by athletes with a high number of head impacts. For example, there may be an increased likelihood of concussion from one impact or multiple subconcussive impacts in athletes who possess one or more APOE rare alleles.

There has been a paucity of research examining the association between sports-related concussion occurrence and APOE genotype. Terrell et al¹² reported that carrying the APOE promoter rare (or minor) allele (g-219t) T was associated with self-reported concussion history and greater concussion severity in collegiate athletes. In a prospective cohort study, Kristman et al¹¹ reported no association between college athletes carrying the E4 allele and sustaining their first

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concussion. In the current study, we examine the association between APOE genotype (E2, E4, and promoter) and self-reported concussion occurrence. Based on the current literature, we hypothesized that carrying 1 or more of the APOE rare alleles assessed in this study would be associated with having a history of 1 or more concussions.

METHODS

A multicenter cross-sectional design was used to examine the association between APOE genotype and concussion occurrence. Of approximately 292 possible athletes (response rate = 67%) from 3 area universities, 196 collegiate male football ($n = 163$) and female soccer ($n = 33$) athletes (age = 19.68 ± 1.48 years; height = 180.51 ± 14.67 cm; body mass = 92.26 ± 22.86 kg) volunteered to participate in this study. Collegiate football and women's soccer players were chosen because of their relatively high risk of concussion and the concussion rates are similar for each sport.² Data were collected during the 2008-2009 preseason in the university athletic training facilities. All participants completed an institutional review board approved informed consent and Health Insurance Portability and Accountability Act compliance forms before data collection. There were no exclusionary factors. Athletes who were cleared for activity at the time of the study were able to participate in the study.

Concussion History Questionnaire

All participants completed a researcher-assisted paper and pencil assessment, which was used to indicate the athlete's concussion history. A concussion was operationally defined as an injury that was documented by a physician or certified athletic trainer. Athletes also reported their sex, ethnicity, years of sport experience, and the number of documented concussions.

DNA Collection and Genotyping

All participants provided a saliva sample for genotyping using Oragene DNA Self-Collection Kits according to the manufacturer's guidelines (OG-300 Tube Format, Oragene DNA/saliva; DNA Genotek, Ottawa, Canada). The saliva was stored at room temperature according to manufacturer's guidelines. DNA was extracted according to standard methodology and sequenced at the university laboratory. Genotyping was performed using pre-made TaqMan single nucleotide polymorphism genotyping assays C_905013_10 (rs405509), C_3084793_20 (rs429358), and C_904973_10 (rs7412; Applied Biosystems, Foster City, California). The genotyping was performed using an ABI 7300 Real-Time PCR instrument (Applied Biosystems) according to the manufacturer's instructions with modifications. Briefly, 5 to 20 ng (11.2 μ L) of DNA was added to each well of the 96-well reaction plate containing 13.8 μ L of reaction mix (12.5 μ L 2 \times TaqMan Universal PCR Master Mix, No AmpErase UNG, and 1.25 μ L 20 \times single nucleotide polymorphism genotyping assay mix), along with negative (no template control) and positive (DNA samples with known genotype) controls in a final volume of 25 μ L. An allelic discrimination assay was performed under the following parameters. Step 1: incubation

at 92°C for 10 minutes; step 2: 40 cycles (incubation at 95°C for 15 seconds followed by incubation at 60°C for 1 minute). Control DNA samples with known genotypes (Coriell Institute for Medical Research, Camden, New Jersey) were used for assay validation. Analysis of DNA sequence variation was performed using Statistical Analysis Software Genetics Package (SAS Institute, Cary, North Carolina). We presented the genotype data using a dominant model [group 1, carriers of normal homozygous genotype vs group 2, carriers of heterozygous or variant (or minor) homozygous genotypes].

Statistical Analysis

Demographic characteristics and candidate alleles were analyzed as possible predictors of the occurrence of concussion. Single and multiple concussions were used as outcomes in the analysis. Each predictor was analyzed using univariate logistic regression appropriate for binary outcomes. All statistical analyses were carried out using SAS version 9.2 software (SAS Institute, Cary, North Carolina).

RESULTS

Selected participant characteristics relative to concussion history are presented in Tables 1 and 2. Forty-eight athletes (24.5%) reported 1 documented concussion, whereas 9 (of the 48) athletes (4.6%) reported 2 or more documented concussions. The APOE E2 and E4 rare alleles and promoter minor allele were present in 35 (17.7%), 62 (31.9%), and 99 (50.0%) athletes, respectively. Chi square test data are presented in Tables 3 and 4. There was no significant association between carrying the individual APOE E2 and E4 rare alleles and having a history of concussion. Four athletes (2.0%) carried all APOE rare alleles and 3 of the 4 (75%) experienced a previous concussion ($P = 0.05$; odds ratio = 9.8; 95% confidence interval, 1.00-96.55) (Table 3). There was also

TABLE 1. Selected Participant Characteristics and Their Association With Concussion History

Factor	No History of Concussion n = 148 (75.5)	History of Concussion n = 48 (24.5)	P*
Sex, n (%)			0.20
Male	126 (85)	37 (77)	
Female	22 (15)	11 (23)	
Years of experience, mean (SD)	9.27 (4.4)	10.47 (4.0)	0.10
Genetic factors, n (%)			
E2	24 (16)	11 (23)	0.29
E4	47 (32)	15 (31)	0.95
Promoter	73 (49)	26 (54)	0.56
E2/E4	2 (1.4)	3 (6.2)	0.09
E2/Promoter	7 (4.7)	4 (8.3)	0.35
E4/Promoter	30 (20)	11 (23)	0.70
E2/E4/Promoter	1 (0.7)	3 (6.2)	0.05†

Sport was not presented because it was the same as the sex data; E2, CT, or TT; E4, TC, or CC; Promoter, GT, or TT.

* P value of χ^2 test.

† $P \leq 0.05$.

TABLE 2. Selected Participant Characteristics and Their Association With >2 Concussion History

Factor	No History of >2 Concussions n = 187 (95.4)	History of >2 Concussions n = 9 (4.6)	P*
Sex, n (%)			0.19
Male	157 (84)	6 (67)	
Female	30 (16)	3 (33)	
Years of experience, mean (SD)	9.42 (4.4)	12.55 (3.3)	0.04†
Genetic factors, n (%)			
E2	33 (18)	2 (22)	0.72
E4	59 (32)	3 (33)	0.91
Promoter	91 (49)	8 (89)	0.04†
E2/E4	4 (2.1)	1 (11)	0.13
E2/Promoter	10 (5.3)	1 (11)	0.47
E4/Promoter	38 (20)	3 (33)	0.35
E2/E4/Promoter	3 (1.6)	1 (11)	0.09

Sport was not presented as it was the same as the sex data; E2, CT or TT; E4, TC or CC; Promoter, GT or TT.

*P value of χ^2 test.

†P ≤ 0.05.

a significant association ($P = 0.05$; odds ratio = 8.4; 95% confidence interval, 1.03-68.79) between carrying the APOE promoter minor allele and experiencing 2 or more concussions (Table 4). Specifically, 8 of the 9 athletes (89%) who reported experiencing 2 or more concussions carried the APOE promoter minor allele.

DISCUSSION

We studied the association between having a previously documented concussion and carrying 1 or more APOE rare alleles. We hypothesized that carrying 1 or more of the APOE rare alleles assessed in this study would be associated with having a history of 1 or more concussions. Although there is no association with the most commonly studied APOE variant (E4), the results did indicate an association with carrying the

TABLE 3. Association of Risk Factors and History of One Concussion

Factor	Wald χ^2	P	OR	95% CI
Sex	1.66	0.20	0.59	0.26-1.32
Sport	1.66	0.20	0.59	0.26-1.32
YREXP	2.75	0.10	1.07	0.99-1.15
E2	1.10	0.29	1.54	0.69-3.43
E4	0.00	0.95	0.98	0.48-1.97
Promoter	0.34	0.56	8.43	0.63-2.33
E2/E4	2.90	0.09	4.87	0.79-30.04
E2/promoter	0.87	0.35	1.83	0.51-6.54
E4/promoter	0.15	0.70	1.17	0.53-2.56
ALL	3.82	0.05*	9.80	1.00-96.55

*P ≤ 0.05.

YREXP, years of experience in sport; OR, odds ratio; ALL, presence of all rare (or minor) alleles assessed.

TABLE 4. Association of Risk Factors and History of Multiple Concussions

Factor	Wald χ^2	P	OR	95% CI
Sex	1.71	0.19	0.38	0.09-1.61
Sport	1.71	0.19	0.38	0.09-1.61
YREXP	4.13	0.04*	1.20	1.01-1.44
E2	0.12	0.72	1.33	0.26-6.71
E4	0.01	0.91	1.08	0.26-4.48
Promoter	3.96	0.04*	8.43	1.03-68.79
E2/E4	2.20	0.13	5.71	0.57-57.20
E2/promoter	0.51	0.47	2.21	0.25-19.46
E4/promoter	0.85	0.35	1.96	0.46-8.20
ALL	2.83	0.09	7.66	0.71-82.11

*P ≤ 0.05.

YREXP, years of experience in sport; OR, odds ratio; ALL, presence of all rare (or minor) alleles assessed.

promoter-219G/T rare allele and a history of 2 or more concussions. In addition, carrying the combination of the E4, E2, and promoter-219 G/T rare alleles was associated with history of concussion. This indicates that research focusing on the promoter in combination with other rare alleles, rather than E4 allele alone, may be most prudent in the area of sports-related concussion research.

We found no significant association between carrying the E4 allele and history of concussion. This is consistent with recent previous findings^{11,12} and indicates that carrying the E4 allele alone may not influence concussion occurrence. The E4 variation is believed to change ApoE's affinity for beta-amyloid, causing increased neural plaque formation¹⁹ and altered cerebrovascular integrity resulting in worse outcome after brain injury.²⁰⁻²² Theoretically, if carrying the E4 rare allele results in altered cerebral blood flow, and mechanical stress upregulates this altered protein, then it is plausible that E4 carriers subjected to repetitive concussive and subconcussive mechanical stress could have an increased risk of brain damage. At present, our results do not confirm this hypothesis.

The T allele of the -219G/T APOE promoter polymorphism alters transcriptional activity, influencing the amount of ApoE expressed.²³ In the current study, there was a significant association between carrying the g-291t APOE promoter rare T allele and experiencing 2 or more concussions. This finding is similar to the previous research¹² and seems to indicate that a polymorphism that influences APOE expression could alter an individual's susceptibility of sustaining a concussion and concussion severity. Terrell et al¹² reported that carrying the APOE promoter rare allele was associated with self-reported concussion history and greater concussion severity in collegiate athletes. Although the former and current studies resulted in different associations with the promoter allele (ie, more severe concussion and ≥2 concussions, respectively), each outcome indicates greater brain injury susceptibility. In our sample, 89% (8 of 9) of athletes with multiple concussions carried the promoter rare allele versus 48% (91 of 189) of athletes with zero or 1 concussion. Our findings indicate that instead of focusing on the E4 allele as the

most clinically relevant brain injury susceptibility genotype, the APOE promoter allele and the levels of APOE gene products (eg, mRNA and polypeptide) should be further studied alone and in combination with other rare alleles.

Years of sport experience may have played a role in the promoter polymorphism's significant association with the history of multiple concussions. We computed an ancillary analysis exploring significant differences in years of experience (the other significant factor) between participants with and without the promoter rare allele. There was a trend ($P = 0.054$) indicating that promoter carriers (10.2 ± 4.1 years) played longer than noncarriers (9.0 ± 4.5 years). Therefore, years of experience could certainly have an influence on promoter carriers' concussion history. This is interesting as variation in the promoter region can affect APOE expression and, in this case, may influence concussion susceptibility in those with longer exposure to head impacts.

Most complex phenotypes (eg, sports-related concussion) will be dependent on an interaction between 1 or more genetic and environmental factors.¹⁸ For example, healing response to brain injury has recently been reported to be influenced by APOE allele-dependent gene expression.¹³ Specifically, E3 contributes to gene expression resulting in a favorable neurogenic response to injury versus E4 (eg, glial fibrillary acidic protein upregulated more with E3).¹³ Jordan et al²⁴ reported that high-exposure professional boxers (>12 bouts) with at least one E4 rare allele (maternal or paternal) had significantly higher chronic brain injury scores versus high-exposure boxers with no E4 allele. Similarly, Kutner et al²⁵ reported that older (>27 years old) professional football players carrying the E4 allele exhibited lower cognitive performance scores versus their non-E4 carrying counterparts. These cognitive deficits in E4 carriers with high head impact exposures could be due to a gene \times environment interaction.

Our study is the first to report a significant association between carrying multiple APOE rare alleles and history of concussion in collegiate athletes. We found that 75% of the individuals who carried all 3 rare alleles had a history of concussion. This is an interesting finding, even though only 4 subjects (2%) in our population carried all rare alleles, because it indicates a possible APOE polymorphism \times polymorphism interaction. In the case of sport-related concussion, the interaction of multiple APOE rare alleles and repetitive impacts of varying magnitudes and time elapsed between impacts may yield a vulnerable nervous system in some individuals. The reported detrimental effect caused by carrying the E4 allele^{19,24–28} could be further influenced by carrying an APOE promoter rare allele, which results in greater levels of ApoE,²³ even without mechanical stress induced by sports participation. Perhaps, instead of focusing on the E4 allele as the most clinically relevant brain injury susceptibility genotype, a combination of APOE rare alleles should be further studied.

There are several limitations in the current study that are common to retrospective research designs and studies requiring concussion self-report, including selection and recall bias. Our response rate was 67%, indicating that 33% of potential subjects did not participate, possibly leading to selection bias. Also, athletes with a history of concussion may have discontinued participation in sports requiring head

impacts before college. The concussion data were collected retrospectively and are subject to recall bias. Subjects were asked to recall the number of their documented concussions and may have done so incorrectly. Concussion incidence may also be inaccurate due to an underreporting of symptoms. Underreporting of sports-related concussion has been identified previously^{29–31}; individuals in the current study may have been misclassified due to either lack of reliable recall or lack of awareness. Our assessment method and definition of concussion provided to athletes may have helped mitigate recall issues and ensure proper group assignment. Specifically, 1) the researchers were present at assessment time to answer questions, 2) a concussion was defined as an injury documented by a physician or certified athletic trainer, and 3) the subjects were aware that their data were confidential and would not influence playing time or the like.

CONCLUSIONS

In this study population, carriers of all 3 APOE rare alleles assessed in this study were nearly 10 times more likely to report a previous concussion and may be at greater risk of concussion versus noncarriers. Athletes carrying the promoter rare allele were 8.4 times more likely to report multiple concussions and may be at greater risk of multiple concussions versus noncarriers. Research involving larger samples of individuals with multiple concussions and carriers of multiple APOE rare alleles is warranted.

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