Apolipoprotein E ε4 allele associated with poor recovery from mild traumatic brain injury is not associated with elite rugby status but is present in 30% of athletes

S. M. Heffernan[1, 9], L. P. Kilduff[2], R. M. Erskine[3,4], S. H. Day[1], G. K. Stebbings[1], A. Grainger[9], M. R. Antrobus[1], J. Brazier[1], C. J. Cook[2,5], S. M. Raleigh[6], M. A. Bennett[2], G. Wang[7], M. Collins[8], Y. P. Pitsiladis[7] & A. G. Williams[1,4]

[1]MMU Sports Genomics Laboratory, Manchester Metropolitan University, Crewe, UK, [2]A-STEM, College of Engineering, Swansea University, Swansea, UK, [3]Research Institute for Sport & Exercise Sciences, Liverpool John Moores University, Liverpool, UK, [4]Institute of Sport, Exercise and Health, University College London, London, UK, [5]School of Sport, Health and Exercise Sciences, Bangor University, Bangor, UK, [6]Centre for Physical Activity and Chronic Disease, Institute of Health and Wellbeing, University of Northampton, Northampton, UK, [7]FIMS Reference Collaborating Centre of Sports Medicine for Anti-Doping Research, University of Brighton, Brighton, UK, [8]Devision of Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town (UCT), Cape Town, South Africa, [9]School of Public Health, Physiotherapy, and Sports Science, University College Dublin, Dublin 4, Ireland

Introduction

The *APOE* gene encodes apolipoprotein E-based peptide and is a candidate marker for risk and severity of mild-traumatic brain injury (mTBI). ApoE is a 299 amino acid protein and has three common isoforms (Apo ε2, ε3, and ε4). Multiple meta-analyses have shown those possessing the ε4 allele (ε4+) have an increased risk of poor outcome >6 months after mTBI in[1,2]. Recently, Merritt and Arnett[3] showed that ε4+ athletes (as apposed to those without (ε4-)) suffered more severely, with a medium effect size for cognitive (d = 0.60) and a large effect size for physical symptoms (d = 0.87) within 3 months post-mTBI.

Current mTBI (concussion) incidence in elite rugby union (RU) ranges between 4.6 - 8.9 per 1000 playing hours[4] with higher rates in elite rugby league (RL), ranging between 14.8 - 28.3[4,5]. As *APOE* ε4/ε4 genotype and ε4+ individuals are at greater risk of experiencing more severe symptoms after mTBI, they are probably more likely to miss important training, selection and competitive events critical for their career progression. Thus, ε4/ε4 genotype and ε4+ individuals are potentially at a disadvantage compared to ε4- individuals in terms of their ability to achieve success in elite competitive rugby.

Therefore, the aims of the present study were to establish the proportion of elite rugby athletes expected to be at higher risk of mTBI (ε4+) and to investigate if *APOE* genotype differed between elite rugby athletes and a control population.

Table 1 *Genotype distribution of controls and rugby athletes sub-divided by code and international status, presented as genotype counts and percentage in parentheses.*

Genotype	Controls	All athletes	RL athletes	RU athletes	RU Internationals	RU Non-Internationals
APOE						
ε2/ε2	1 (0.2)	1 (0.2)	0	1 (0.2)	1 (0.4)	0
ε2/ε3	69 (12.0)	71 (12.2)	14 (13.3)	57 (11.8)	19 (7.5)	38 (16.3)
ε2/ε4	14 (2.4)	8 (1.4)	1 (0.9)	7 (1.4)	1 (0.4)	6 (2.6)
ε3/ε3	350 (60.9)	338 (58.2)	53 (50.5)	290 (59.8)	156 (61.9)	134 (57.5)
ε3/ε4	127 (22.1)	144(24.7)	34 (32.4)	113 (23.5)	68 (27.4)	45 (19.3)
ε4/ε4	14 (2.4)	19 (3.3)	3 (2.9)	16 (3.3)	6 (2.4)†	10 (4.3)
Total	575	581	105	484	251	233

RU, rugby union, RL rugby league. Eight athletes competed in both elite RL and RU and were included in both groups that were analysed separately. †Different from RU non-international (P = 0.01)

Method

Participants

Ethical approval was granted by the Manchester Metropolitan University, Glasgow University and the University of Cape Town ethics committees. Elite Caucasian male rugby players (League and Union; n = 581; stature 1.85 ± 0.07 m, mass 101.4 ± 12.5 kg) and controls (n = 575; stature 1.74 ± 0.10 m, mass 75.1 ± 13.5 kg) were recruited for the present study having given informed consent. The athletes comprised of players competing in the top league from Tier 1 rugby nations (51% international standard).

Sample collection, DNA isolation & Genotyping

Biological samples were obtained via venous blood, saliva (DNA Genotek Inc., Ontario, Canada) or buccal swab (Omni swab, Whatman, Springfield Mill, UK) and stored at -20°C until subsequent analysis. DNA was isolated using automated spin column protocol (Qiagen, West Sussex, UK). Genotyping was performed using real-time PCR on either a StepOnePlus real-time detector (Applied Biosystems) or Chromo4 (Bio-Rad).

Data analysis

Genotypic data was assessed for Hardy-Weinberg Equilibrium. Pearson's Chi square (χ^2) was used to investigate genotypic and allelic frequencies using SPSS (SPSS Inc., Chicago, IL). Benjamini-Hochberg corrections were applied to correct for multiple testing. Odds ratios (OR) were calculated for estimations of the probability of an interaction between genes and elite rugby status. The alpha level was set at P = 0.05.

Results & Discussion

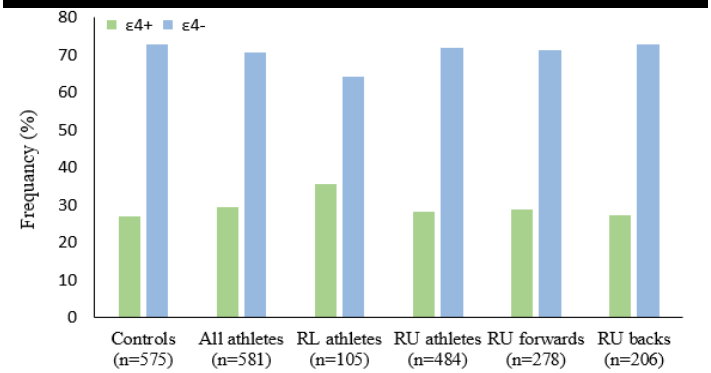

Fig 1 *Presence or absence of ε4 allele in controls and athletes. Green bars represent ε4 carriers and blue bars represent those possessing no ε4 alleles.*

No data violated H-W equilibrium. There were no differences in *APOE* genotype or ε4+ frequency when comparing all (P = 0.27), RU (P = 0.15) or RL athletes (P = 0.54) with controls (additive values presented; Table 1). However, prior to BH correction RL was different from controls (P = 0.048; OR = 0.67 95%CI 0.43-1.04). No genotype frequency or ε4+ differences were observed between RU backs and forwards (P = 0.86, P = 0.70, respectively, Fig 1).

Here we present the first large data set of *APOE* genotype and elite rugby athlete status. In contrast to our hypothesis, there were no differences in ε4/ε4 genotype or ε4 allele frequency between elite rugby athletes and non-athlete controls (Fig 1). Furthermore, there were no differences in ε4/ε4 genotype or ε4 allele frequency between RU playing positions (Fig 1).

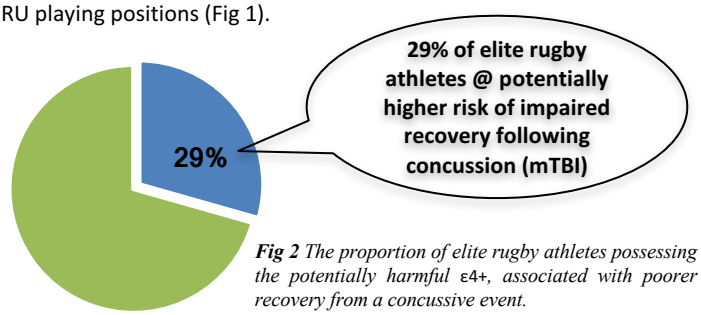

Fig 2 *The proportion of elite rugby athletes possessing the potentially harmful ε4+, associated with poorer recovery from a concussive event.*

It is noteworthy that there are considerable numbers of ε4/ε4 (3.3%) and ε4+ (29.4%) rugby athletes who may be at greater risk of cognitive and physical impairments following mTBI, compared to non-carriers[3] (Fig 2). World Rugby (the international governing body of rugby union) estimates that there are 7.23 million rugby union players worldwide of all competitive standards (http://www.worldrugby.org/development/player-numbers). Assuming similar allele frequencies to the Caucasians we have studied amongst all players worldwide for the purpose of this discussion (i.e. ignoring geographic ancestry), over two million ε4+ rugby players may be at relatively greater risk of poorer outcome following mTBI than their ε4- counterparts.

References

1. Li L, Bao Y, He S, Wang G, Guan Y, Ma D, et al. The association between Apolipoprotein E and functional outcome after traumatic brain injury: A Meta-Analysis. Medicine. 2015;94(46):e2028. 2. Zeng S, Jiang J-X, Xu M-H, Xu L-S, Shen G-J, Zhang A-Q, et al. Prognostic value of apolipoprotein E epsilon4 allele in patients with traumatic brain injury: a meta-analysis and meta-regression. Genetic Testing and Molecular Biomarkers. 2014;18(3):202-10. 3. Merritt VC, Arnett PA. Apolipoprotein E (APOE) ε4 allele is associated with increased symptom reporting following sports concussion. Journal of the International Neuropsychological Society. 2016;22(1):89-94. 4. Gardner AJ, Iverson GL, Williams WH, Baker S, Stanwell P. A systematic review and meta-analysis of concussion in rugby union. Sports Medicine. 2014;44(12):1717-31. 5. Savage J, Hooke C, Orchard J, Parkinson R. The incidence of concussion in a professional Australian rugby league team, 1998–2012. Journal of Sports Medicine. 2013;doi10.1155/2013/304576.