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Identification of Genetic Markers for Skill and Athleticism in Sub-elite Australia Rules Football Players: A Pilot Study

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Abstract

Natural genetic variation contributes towards athletic performance in various strength/power and endurance based sports. To date, no studies have explored the genetic predisposition towards skill and athletic performance in Australian Football (AF) players. The present pilot study recruited 30 sub-elite AF players who completed tests of endurance, power and technical skill. Specific polymorphisms in nine genes were screened, and assessed for a possible influence on athletic and skill traits. Statistical analysis using generalised linear models identified a number of polymorphisms predictive of endurance and technical skill. The angiotensin-converting enzyme (ACE), normally responsible for regulation of body fluid volume, was a significant factor in predicting ‘all round’ athletic performance and skill. Specifically, the deletion allele (DD) of ACE was identified as a predictor for AF power ($p \leq 0.008$), endurance ($p = 0.001$) and skill assessments ($p \leq 0.003$). In addition, polymorphisms in the brain-derived neurotrophic factor, D2 dopamine receptor, and catechol-O-methyltransferase genes were also shown to contribute to kicking skill outcomes ($p \leq 0.044$). This is the first study to implicate the ACE deletion allele for a multi-dimensional sport in such a way. Further, the results from this study have identified several new candidate genes in predicting athletic and technical skill outcomes.
Introduction

Athletic performance has commonly been identified through a number of discipline-specific physical and technical skill assessments. In recent times, a focus on the genetic contribution towards athleticism has revolutionised the field of talent identification (TID) and selection (Breitbach, Tug, & Simon, 2014). The advent of genetic markers potentiates identification of athletic prowess and performance from a young age without the sole dependence on physical assessments. As many sports involve apparatus manipulation or equipment use, genetic markers implicated in motor skill acquisition and coordination are pertinent in TID. Australian Football (AF) is a dynamic team sport involving a unique combination of physical capabilities such as endurance, strength, and power. Similar to other football codes, selection into elite AF squads can be associated with a player’s performance in physical and technical skill assessments (Robertson, Woods, & Gastin, 2015; Woods, Raynor, Bruce, & McDonald, 2015). In contrast to other sporting codes, to date, there have been no probing studies exploring a potential genetic predisposition towards AF performance.

Owing to the diverse array of desirable physical attributes in sport, several polymorphisms within the human genome have previously been highlighted. For example, the angiotensin-converting enzyme (ACE) and alpha-actin-3 (ACTN3) genes have both been linked with elite levels of athletic performance in endurance (Eynon et al., 2012; Myerson et al., 1999; Tsianos et al., 2004) and/or strength/power dominant sports (Cieszczyk et al., 2011; Eynon et al., 2012; Myerson et al., 1999; Papadimitriou, Papadopoulos, Kouvatsi, & Triantaphyllidis, 2009). In addition, the peroxisome proliferators-activated receptor g co-activator 1a (PPARGC1A) gene and the beta-adrenergic receptors 1/2/3 (ADRB 1/2/3) have been linked with maximal oxygen uptake
(VO$_{2\text{max}}$), endurance performance and body mass index (Eynon et al., 2010; Maciejewska, Sawczuk, Cieszczyk, Mozhayskaya, & Ahmetov, 2012; Santiago et al., 2011). Despite the intriguing nature of these studies, there has been no strong candidate genes associated with performance in the skill component of sport. Polymorphisms within the brain-derived neurotropic factor (BDNF), dopamine D2 receptor (DRD2) and catechol-O-methyltransferase (COMT) genes have all been associated with motor control and/or learning (Fritsch et al., 2010; Huertas, Buhler, Echeverry-Alzate, Gimenez, & Lopez-Moreno, 2012; Morin-Moncet, Beaumont, de Beaumont, Lepage, & Theoret, 2014; Noohi et al., 2014). However, none of these genes have previously been investigated in the context of professional sport.

The present study aims to elucidate the role of previously identified genes in the context of AF, and examine the potential relationship between genes, and sporting performance and skill. Polymorphisms of the ACE, ACTN3, ADRB 1/2/3, PPARGC1A, BDNF, DRD2 and COMT genes will be examined in a group of sub-elite AF players. The aims of this study are to be the first to assess the link between candidate genes and skill assessments in sport, and the first to identify the ACE deletion polymorphism as an ‘all round’ predictor for performance in Australian Football.

**Methods**

**Participants**

The proposed exploratory pilot study targeted a cohort of sub-elite AF players from a single Western Australian Football League team. A total of 30 players ($n = 30$) from a playing squad were recruited from within the selected Football Club. The players were male, aged between 17 and 19 years of age, and were active in the 2015 season. Consent
was obtained directly from participants, and in the case of those under 18 years of age, parental consent was obtained in addition to that of the participant. In accordance with the University’s ethics committee requirements, signed consent forms were collected prior to the commencement of data collection. To ensure anonymity, the players were assigned a randomised, non-identifiable code. The University of Notre Dame Australia granted approval for this study (Human Research and Ethics Committee approval number: 014175F).

20-metre sprint assessment

Participants performed three 20 m sprints on a hard timber floor in an indoor environment. They were instructed to continue sprinting beyond the end of the 20 m track to maximise results. Electronic timing gates (Smartspeed, Fusion Sport Pty. Ltd., Queensland, Australia) were used to measure the 20m sprint, eliminating human error in reaction time. The times for three attempts were recorded and the best time was used.

Time trial endurance test

Assessment of endurance was conducted using a 3x 1000 m time trial. The time trial was conducted on grass around a 500 m oval. Participants were required to complete each of the three 1000 m time trials as quickly as possible, with each subsequent run beginning 7 minutes and 30 seconds from the start of the preceding run. This method meant that those who ran the trail quicker had a longer rest period.

Vertical jump assessment

The vertical jump data was collected using a Vertec vertical jump system (Swift Performance Equipment, Lismore, Australia). Both standing and running vertical jump data was collected. For the standing and running vertical jump tests, participants were
asked to stand flat footed next to the Vertec, reach up with their dominant hand, and push away as many of the vanes as they could reach. A countermovement jump was performed for each standing vertical jump. The running vertical jump data used both left and right foot take-offs, with the participant starting their run-up five-metres from the Vertec. Three attempts were made for each jump and the best attempt was used for analysis.

**Australian Football skills assessments**

A fundamental component of AF involves the skills associated with ball handling and disposal. The Nathan Buckley kicking test and the Matthew Lloyd clean hands test (measurement of handball ability) were used to test the individuals on critical skills associated with AF. The reliable skill tests were performed as instructed by the AFL National draft combine and as previously described (Cripps, Hopper, & Joyce, 2015).

**Blood collection and genotyping**

Screening for genetic markers required the collection of a single vial of player’s blood during the competitive season. Briefly, 3ml of whole blood was taken via median cubital vein venepuncture, and stored in a standard BD EDTA vacutainer® (Becton Dickinson and Company, Franklin Lakes, N.J.). DNA extraction and genotyping was undertaken by the Australian Genome Research Facility, Queensland, Australia (AGRF). The different genes, specific polymorphisms and frequency are shown in Table 1.

**Statistical Analysis**
Statistical data was analysed using IBM-SPSS V.22 (Armonk, NY). One-way analysis of variance (ANOVA) tests were used to compare the difference between participants of different genotypes. Pearson correlation coefficient was calculated to examine the relationship between examined variables. The correlation criteria adopted were: $r < .1$ trivial, $.1 - .3$ small, $.3 - .5$ moderate, $.5 - .7$ large and $>.7$ very large. A significant nominal $p$-value of $<.05$ was employed.

A general linear model (GLM) was created to analyse the relationship between homo- or heterozygosity of genetic polymorphisms, and independent physical and skill variables. The genetic polymorphisms included as independent variables in the GLM were rs4343, rs1815739, rs1801253, rs1042714, rs4994, rs6265, rs4680, rs1076560 and rs8192678. Dependent variables included 20m sprint, 3x 1000m time trial, standing vertical jump, AF kicking and handball assessments. Non-significant factors were removed singularly until the final model was determined. A significant nominal $p$-value of $<.05$ was employed.

RESULTS

Genetic polymorphisms contribution towards power

The 20 m sprint and vertical jump were used to determine if polymorphisms within the candidate genes contributed towards measures of player power. Two loci, ACTN3 and ACE, were consistently associated with performance in both assessments (Figure 1; Table 2 and Table 3). The deletion ACE DD genotype conferred an advantage over the ID genotype in 20m sprint assessments ($-0.085s/3.045s; p<0.008$), and standing vertical jump ($-7.48cm; p=0.007$). Similarly, the player’s heterozygous for the ACTN3
polymorphism (CT) achieved significantly faster (-0.049s, p=0.049) 20m sprint times and vertical jump scores than CC individuals (-7.126cm, p=0.047).

**INSERT TABLE 2 ABOUT HERE**

**INSERT TABLE 3 ABOUT HERE**

**Genetic polymorphisms contribution towards endurance**

A time trial assessment is frequently undertaken for the evaluation of endurance, and the 3x 1000 m time trials significantly correlated with five of the candidate genes (Figure 1; Table 4). In three of the genes, particular genotypes showed to be detrimental in performance (p<0.05), the heterozygous ACE (46.029s, p=0.001) and BDNF (-5.776, p<0.001) genotypes, and the homozygous AA COMT genotype (-78.326s, p<0.001). The dopamine D2 receptor showed an allelic dose effect whereby AA genotype (72.437s, p=0.004) conferred a greater disadvantage than heterozygosity (30.738s, p=0.020), when compared to players with a CC genotype. Lastly, the PPGARGC1A CC genotype showed an advantage in the time trial relative to AA (-69.674s, p=0.004) and AC genotypes.

**INSERT TABLE 4 ABOUT HERE**

**INSERT FIGURE 1 ABOUT HERE**

**Genetic polymorphisms contribution towards skill**

Of the candidate genes assayed, six were found to be predictors of dominant foot kicking skill assessments (Figure 1). As in endurance results, the ACE ID (p<0.001) and II (p=0.007) genotypes were associated with poorer performances, when compared to the
positive results of individuals with the DD genotype (Table 5). Players homozygous for the ADRB3 C allele also performed significantly better at the kicking assessments than individuals with a TT genotype (6.703, $p=0.001$). Further, players with a C allele in the ADRB2 gene (rs1042714) showed a significant advantage in the football kicking assessment, compared to the homozygous GG genotype (GC: 7.510, $p<0.001$; CC 8.629, $p<0.001$).

**INSERT TABLE 5 ABOUT HERE**

Interestingly, the A allele of both the DRD2 (rs1076560) and BDNF (rs6265) polymorphisms were significant predictors of performance in the dominant foot kicking assessment. When incorporated into a GLM, the AA genotype of DRD2 (9.729, $p<0.001$) and the AA genotype of BDNF (4.955, $p=0.006$) improved player scores compared to CC and TT genotypes respectively. The polymorphism in the COMT gene exhibited a smaller effect size, but still provided significant advantage in individuals with an AG genotype (1.791, $p=0.012$).

When assessing handball skill performance for the dominant hand, the II genotype of the ACE gene had significantly poorer results (-2.661, $p=0.003$), with the D allele showing to be more beneficial to the skill. There was also a trend towards the C allele of the ADRB3 gene being more advantageous in handballing assessments, but the results did not reach statistical significance (Table 6).

**INSERT TABLE 6 ABOUT HERE**

Discussion
The present study sought to elucidate what influence different genetic polymorphisms have on performance in physical and skill assessments associated with AF. This study is the first of its kind in AF, and the first to examine genetic variability in the context of sporting skill. The AF sporting code is multi-dimensional, allowing players with a variety of physical and athletic abilities to succeed at all levels of the competition (Robertson et al., 2015). Within the scope of this study, we identified significant predictors for AF endurance, power and skill assessments. The previously identified insertion/deletion ACE polymorphism significantly contributed to all aspects of AF, while polymorphisms in BDNF, COMT and DRD2 revealed a novel role in predicting endurance and skill in the kicking assessment (Figure 2).

**INSERT FIGURE 2 ABOUT HERE**

The most notable findings from this study involve the ACE polymorphism, which was implicated \( p \leq 0.008 \) in every testing parameter (Figure 2). A component of the renin-angiotensin-aldosterone system, ACE regulates cardiovascular function (Gineviciene, Jakaitiene, Tubelis, & Kucinskas, 2014), skeletal muscle growth (Mustafina et al., 2014) and circulatory homeostasis (Puthucheary et al., 2011). The deletion allele is associated with higher plasma levels of ACE (Rigat et al., 1990), increasing blood pressure by constricting blood vessels (Bernstein et al., 2013). The ACE deletion allele has previously been linked with power and strength based sports (Myerson et al., 1999; Tsianos et al., 2004). In fitting with this, players in this study with the ACE DD genotype yielded faster 20m sprint times and higher vertical jumps. Unexpectedly, the ACE ID genotype showed the poorest endurance performance results. These results are in contrast to previous...
studies, where the I allele has been associated with endurance performance (Gayagay et al., 1998; Myerson et al., 1999).

The role of the ACE polymorphism in sporting skill assessments has previously not been shown. Here, the DD genotype was positively related to dominant foot kicking in AF above the ID (−7.649, p<0.001) and the II (−1.742, p=0.007) genotypes. In addition, the results from the dominant handballing skills test also show that the DD genotype performed significantly better than the II genotype. Taken together, this suggests that the DD genotype is preferable for skill tasks while the I allele is detrimental to skill performance. Interestingly, recent evidence has linked the I allele with the development of Alzheimer’s disease (Hassanin, Moustafa, & El Masry, 2014), supporting the benefit of the DD genotype in learning.

A second major finding arose from the skills assessment results and the novel markers BDNF, COMT and DRD2; which are classically prominent in motor control and learning (Fritsch et al., 2010; Huertas et al., 2012; Noohi et al., 2014). The binding and internalisation of BDNF can affect axonal path finding (Zhang & Poo, 2002), striatal neuronal survival (Baydyuk & Xu, 2014), and formation and conservation of late-phase potentiation (Cunha, Brambilla, & Thomas, 2010). The BDNF Val66Met polymorphism is known to reduce the ability for humans and mice to development motor skills (Fritsch et al., 2010). Further, the Val66Met polymorphism is also related with short-term motor learning, with Val66Met carriers having poorer motor learning in simple motor tasks compared to Val66Val genotypes (Morin-Moncet et al., 2014). As different forms of exercise can induce different levels of neuroplasticity, and therefore different effects on learning and memory (Liu et al., 2009), perhaps the BDNF genotypes may predispose individuals to different levels of aerobic fitness and thus have an effect on learning.
Dopamine is a key regulator of the “motivation and reward” functional cortical circuits, whereby repeated muscle actions are potentiated by positive stimuli (Bromberg-Martin, Matsumoto, & Hikosaka, 2010). Dopaminergic signalling and the D2 receptor are important in skill acquisition, suggesting a role in optimising a novel motor skill (Molina-Luna et al., 2009). A combination of the AA COMT genotype and the C allele of the DRD2 polymorphism had been purportedly associated with higher motor learning rates (Noohi et al., 2014). However, this is at odds with the results presented herein where the AG and AA genotypes of COMT and DRD2 respectively, were found to be advantageous. Despite contrary results, this study is the first to suggest SNP-mediated changes in dopamine levels may contribute to sport-specific skill acquisition.

Recent research has focused attention onto the ACTN3 polymorphism (rs1815739), after certain alleles correlated with strength/power or endurance sports. The ACTN3 gene functions to facilitate coordinated muscles fibre contractions, especially in the fast twitch muscle fibres (Vancini et al., 2014). Previous studies have found that the CC genotype is associated with power and strength athletes (Cieszczyk et al., 2011; Kim, Song, & Kim, 2014; Yang et al., 2003). In contrast, the T allele has been associated with elite endurance due to the higher proportions of slow twitch muscle fibres (Eynon et al., 2012). Contrary to previous research the CC genotype of the ACTN3 gene showed the poorest performance in standing vertical jump height, and did not contribute significantly to 20m sprint time. It has been previously suggested that the ACTN3 gene does not impact athletic performance in team sports (Massidda et al., 2015), and this current study presents supportive evidence.
The PPARGC1A gene is a transcriptional co-activator with a role in skeletal muscle oxidative phosphorylation and mitochondrial biogenesis (Puigserver & Spiegelman, 2003). Previous literature has suggested that the polymorphisms of the PPARGC1A gene could play a role in aerobic capacity as it is strongly related to the function of the skeletal muscle mitochondria (Egan & Zierath, 2013). The AA genotype of the PPARGC1A gene had a significantly better endurance time trial test result (70 seconds), while the AG genotype had a significantly poorer result (30 seconds). This supports previous research that has shown the PPARGC1A AA genotype to be favourable to endurance status as it has a strong relationship with high VO2max (Maciejewska et al., 2012), and in general higher aerobic capacities in sedentary adults (Nishida et al., 2015). Additionally, ADRB2 and ADRB3 have never been associated with motor skill, yet the CC ADRB3 (6.703, p=0.001), and both the CC (8.629, p<0.001) and CG (7.510, p<0.001) ADRB2 genotypes performance significantly better in the dominant foot kicking AF skills test. This suggests ADRB2 and ADRB3 may also play a role in motor development although the mechanism of this remains to be seen.

While this exploratory pilot study has a significant limitation in sample size, integrating these results into the GLM consistently implicates polymorphisms associated with performance, as well those involved in motor skill acquisition and reward circuitry. For example, using our GLM, a player with a COMT (A/A), ACE (D/D), BDNF (A/G) and PPARGC1A (A/A) genotype is predicted to perform 4 minutes and 27 seconds faster in a time trial, than a player with a COMT (G/G), ACE (I/D), BDNF (G/G) and PPARGC1A (G/G) genotype. Although there are some inconsistencies with previous reports, there is immediate applicability in athletic assessments. The prospect of identifying a genetic component that contributes to player motor skill and athletic development opens up an array of possibilities for future experiments in neuromuscular physiology.
Conclusions

To date, several hundred polymorphisms relating to athletic performance in elite sporting groups have been described. While the majority of studies have focussed on individual sports, such as distance running and swimming (Ben-Zaken et al., 2015), few studies have examined multi-dimensional team-based sports. Australian Football is one such team sport, whereby new talent is assessed through a number of athletic and skill-dependent assessments. With this in mind, the rationale behind the current pilot study was to explore if several polymorphisms could predict player performance in current AF talent identification assessments. We found that the ACE DD genotype, associated with higher plasma ACE levels (Rigat et al., 1990), had the greatest positive impact on AF players in traditional power and aerobic athletic assessments, as well as in sport-specific skill assessments. In addition, this study presents novel findings on the BDNF, DRD2 and COMT genes and their relationship with skill assessments. The identification of potential markers of motor skill acquisition in AF is intriguing, and presents a new prospect for identifying future genetic links to sporting skill. The encouraging results from this pilot study supports further research into the effects of polymorphisms on AF athletic assessments, and presents new evidence for the genetic contribution towards sporting skill.

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Table and Figure legends

Table 1 Summary of genes and specific polymorphisms screened in this study

Table 2 Final Model Parameter Estimates: Predictors of standing vertical jump.

Table 3 Final Model Parameter Estimates: Predictors of 20-metre sprint.

Table 4 Final Model Parameter Estimates: Predictors of player performance in the time trial assessment.

Table 5 Final Model Parameter Estimates: Predictors of dominant foot kicking assessment.

Table 6 Final Model Parameter Estimates: Predictors of dominant hand handballing assessment.

Figure 1 Genetic polymorphisms showing significant involvement in (A) 20m sprint, (B) vertical jump, (C) Handball, (D) Time trial and (E) football kicking assessments (*p<0.05 **p<0.01 ***p<0.001).

Figure 2 A Venn diagram summarizing genotypes screened in this study, and identified to be significant predictors of performance in AF athletic and skill assessments.