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Genetic predictors of match performance in sub-elite Australian football players: A pilot study

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ABSTRACT

The current study aimed to determine whether previously identified candidate polymorphisms were associated with match performance in sub-elite Australian Rules Football (ARF) players. The genotypes of thirty players were analysed along with 3x1-kilometre time trial results, ARF-specific skill assessments (handball and kicking), and match performance (direct game involvements) per minute (DGIs/min) to investigate if there was a relationship between any of the variables. Results support previous findings that aerobic time trials are a significant predictor of DGIs/min in sub-elite ARF players. Significant associations were found for genotypes *ADRB2* CC ($p = .001$), *PPARGC1A* AA ($p = .001$), *PPARGC1A* AG ($p < .001$), *ACE* ID ($p < .001$), *COMT* AA ($p = .003$), *BDNF* AG ($p = .008$), *ADRB1* CC ($p = .018$) and *ADRB3* CC ($p = .010$) and the 3x1-kilometre time trials ($p < .001$). In the current study, a variant in the *DRD2* gene was a strong predictor of handball possessions during a match. Significance was seen for variants in the *BDNF* and *COMT* genes when the kicking and handball skill test results were combined and used in a linear mixed model to predict DGIs/min, suggesting a potential relationship with motor learning. The confirmation of genetic predictors of player performance in a team sport, such as ARF, suggests a portion of the physiological mechanisms of skill and ARF-specific talent may be explained by the expression of a specific number of genes.

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Introduction

Australian Rules Football (ARF) is a team sport that involves short bursts of high-intensity efforts dispersed between walking and jogging movement patterns.¹ Such short bursts include acceleration, deceleration, change of direction, and sprinting efforts.² The physical demands of ARF require players to have physical characteristics in the areas of endurance, strength, and power. Tests of endurance, strength and power often act as an indicator of an individual's physical capacity, playing an important role in team selection. However, these tests do not necessarily associate directly to successful player match performance.^{3,4}

Currently, there are several methods of determining individual

player achievement within a sport: team selection, career success, coach perception, and match performance within a season.⁵ Match performance is a measurement of contributions to a game (e.g. effective handballs, kicks) and is reported as game day statistics.^{5,6} Player results in the 3-km time trial have been previously shown to significantly predict match performance in semi-professional ARF players.⁵ Similarly in American Football, players that were ultimately drafted performed better sport-specific tests than those not drafted.⁷ While physical performance tests have been shown to have relationships with match performance, and ultimately athlete success across various sports, match statistics may not necessarily reflect the player's overall influence on the game.⁸

Individuals can be influenced by maturation,⁹ as well as many external factors on the day of testing, such as previous training history, testing order, injury, and fatigue.^{10,11} Therefore, the true potential of an individual may be overlooked, or go unacknowledged. As such, one mechanism for identifying athletic potential may involve the assessment of genetic variants. A vast array of

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candidate genetic variants have been studied within the human genome, with over 200 polymorphisms associated with physical performance, and at least 20 associated with elite levels of athleticism.¹² Polymorphisms within candidate genes have been linked with athletic performance in various single-disciplinary and team sports.^{13–17}

The two homozygous polymorphisms of the *angiotensin-converting enzyme (ACE)* and *alpha-actin-3 (ACTN3)* genes are associated with increased endurance and/or increased strength and power.^{18,19} respectively. In theory, possessing either gene predisposes an individual to excel at a related sport, for example marathon running or weightlifting. Further, the *peroxisome proliferators-activated receptor g co-activator 1a (PPARGC1A)* and the *beta-adrenergic receptors 1/2/3 (ADRB 1/2/3)* genes all have polymorphisms associated with either increased or reduced endurance capacity,^{20–22} which could potentially influence an individual's aerobic fitness. More recently, we have shown polymorphisms in the *brain-derived neurotrophic factor (BDNF)*, *dopamine D2 receptor (DRD2)* and *catechol-O-methyltransferase (COMT)* genes can predispose athletes to better performance in ARF-specific talent identification assessments.²³ While these candidate genes have been previously linked with motor coordination and/or learning,^{24,25} a role in sport-specific performance is a possibility.

Presently, no research has been conducted into the relationship between genetics and match performance in ARF. While associations between endurance and match performances in ARF have been identified,⁵ discrepancies between pre-season and in-season fitness levels are known to exist, potentially due to factors such as injury and accumulative fatigue. We have previously identified candidate genes as predictors of ARF specific endurance and skill tests,²³ however it remains unclear if the same results exist during competitive season performances. To address this, the current study aims to determine whether previously identified candidate polymorphisms can associate with match performance in a population of sub-elite, semi-professional ARF players. The confirmation of genetic predictors of player performance in a team sport, such as ARF, may present a new understanding of physiological mechanisms of skill and talent identification.

Methods

Participants

The current study involved a cohort of sub-elite, semi-professional ARF participants from a single Western Australian Football League (WAFL) team. A total of thirty male participants from a playing squad, aged between 16 and 18 years ($M = 17.00$, $SD = 0.76$ years), were recruited from within the selected football club. Written informed consent was obtained from participants, as well as parental or guardian consent for those who were under the age

of 18 years. Randomised, non-identifiable codes were assigned to each participant for anonymity. The University of Notre Dame Australia Human Research Ethics Committee granted approval for this study (014175F).

Blood collection and genotyping

Blood collection was undertaken as previously described,²³ prior to the commencement of the 2015 competitive season. Three millilitres of whole blood was taken via the median cubital vein venepuncture by a trained phlebotomist in the medical room at the WAFL club, and stored in a standard BD EDTA vacutainer® (Becton Dickinson and Company, N.J., USA). DNA extraction and polymorphism screening was conducted by the Australian Genome Research Facility (AGRF), Queensland, Australia. The AGRF utilised the Sequenom® MassArray (Sequenom, CA, USA) for the polymorphism analysis. The Custom Genotyping service used by the AGRF is accredited, and a certified service provider for Mass Array genotyping. A summary of the participant genetic data is shown in Table 1.

Australian football skills assessments

ARF kicking and handballing assessments used in this study have previously been developed for the Australian Football League (AFL) national draft combine, and were undertaken as previously described.²⁶ In the current study, both the Nathan Buckley kicking test and the Matthew Lloyd clean hands test²⁷ (measurement of handball ability) were used for the assessment of ARF-specific skills. Participants completed assessments during the pre-season, prior to the commencement of the competitive season, under the supervision of a researcher with protocol training and ARF coaching experience.

Direct game involvements (DGIs)

Game day statistics were recorded via Champion Data (Victoria, Australia), during the 2015 WAFL competitive season. The game day statistics collected included the total number of handballs, kicks, marks, and tackles per competitive round. From these statistics, a measure of direct game involvement (DGI) was calculated by summing the four game day statistics for each participant. Participant DGI was then converted into a game time relative number by dividing the DGI by game time (in minutes) for each match (DGIs/min).⁵ The conversion into a relative number was because match day statistics from three WAFL levels (colts, development league, and league) was used; all which have different total playing times.⁵ A total of 320 match samples from the 2015 WAFL home-and-away and finals series were used.

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

Gene and polymorphism	Genotype		
	Homozygous(n)	Heterozygous(n)	Homozygous(n)
Angiotensin converting enzyme I/D (rs4343)	10 (D/D)	11 (I/D)	9 (I/I)
Alpha-actinin-3 C/T (rs1815739)	12 (T/T)	12 (C/T)	6 (C/C)
Beta 1 adrenergic receptor C/G (rs1801253)	18 (C/C)	12 (C/G)	0 (G/G)
Beta 2 adrenergic receptor C/G (rs1042714)	12 (C/C)	17 (C/G)	1 (G/G)
Beta 3 adrenergic receptor C/T (rs4994)	25 (T/T)	4 (C/T)	1 (C/C)
Brain-derived neurotrophic factor A/G (rs6265)	22 (G/G)	7 (A/G)	1 (A/A)
Catechol-O-methyltransferase A/G (rs4680)	10 (A/A)	14 (A/G)	6 (G/G)
Dopamine D2 receptor A/C (rs1076560)	16 (C/C)	9 (A/C)	5 (A/A)
Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha A/G (rs8192678)	11 (C/C)	17 (C/T)	2 (T/T)

3x1-kilometre time trial as a predictor of match performance

The 3-km time trial has previously been shown to be a significant predictor of direct game involvement at a senior WAFL level.⁵ To determine if a 3x1-kilometre pre-season assessment could act as a similar predictor, we recruited a population of sub-elite junior football participants prior to the competitive season. Participants were asked to complete each kilometre within seven and a half minutes. Those who competed the kilometre faster had a greater rest time. Time trials were conducted on a 500-m grass AF oval at the beginning of the training session after a relevant, dynamic warm-up. Three-time trials were conducted during the three-month pre-season, with the best of these three-time trial attempts analysed.

Statistical analysis

Data was analysed using SPSS (version 22, IBM SPSS Statistics for Windows. IBM Corp., Armonk, NY, USA). Participant skill and endurance pre-season assessment scores, along with match performance data were described (mean, standard deviation). For rare participant alleles (rs1042714, rs4994 and rs6265) minor allele and heterozygous participants were combined. A Chi-squared test was conducted to confirm that the genotype frequencies were in Hardy-Weinberg equilibrium.

Generalized linear mixed models (GLMM) were used to investigate the longitudinal data, as they are able to account for repeated measures for individuals. A Bonferroni correction factor was applied with the GLMM and adjusted *p*-values are reported. A significant nominal *p*-value of <.05 was employed. The linear mixed models (LMM) reported by Piggott and colleagues⁵ were replicated, with the subsequent incorporation of all nine genetic polymorphisms as independent variables. The nine polymorphisms were included as factors for each of the LMM models, with dependent variable outcomes being DGI/min, total kicks per match, and total handballs per match. Participants were included as a random effect in the model to account for individual differences. Possible confounders to the outcome measures were also included (3x1-kilometre time trial, game time in minutes, kicking results from an AFL skills test, and handballing results from an AFL skills test). A sum of the kicking and handballing skill assessments were also calculated for the final GLMM, as disposals in a match of ARF are described as the accumulation of kicks and handballs.

Models were investigated with all variables included, removing non-significant variables one at a time until only significant variables remained using the SPSS step-wise procedure; when multiple variables were non-significant, the least significant variable was removed and the model was re-run using the remaining variables. A residuals check for normality as per the GLMM assumption was conducted on the final models. For determining the presence of multicollinearity, the variance inflation factor (VIF) was calculated for all independent variables. For all reported models, VIF values were less than 2.

Results

Pre-season and match performance statistics

Participant skill and endurance pre-season assessment scores, along with match performance data are displayed in Table 2. The mean direct game involvement relative to time was 0.23 ± 0.11 , with the higher the measurement equating to greater impact per game.

Table 2

Mean \pm SD for in-season, match related variables used in models.

	Assessment/Statistic	Mean \pm SD
Pre-season assessments	Kicking assessment results	22.16 \pm 3.50
	Handballing assessment results	16.89 \pm 2.24
	3 x 1-km time (min:sec)	10.30 \pm 0.42
Overall Match day statistics	Game time (min)	80.07 \pm 13.49
	Disposals	13.02 \pm 6.50
	Kicks	7.94 \pm 4.30
	Handballs	5.08 \pm 3.29
	Tackles	2.20 \pm 1.78

Kicking and handballing skills tests as a predictor of match performance

A GLMM was used to analyse if kicking and handballing skill tests could predict match performance. No significance was found between the sum of kicking and handballing results and DGIs/min ($p = .411$), handballing skill test results and total number of handballs during the season ($p = .296$) or kicking skill test results and total number of kicks during the season ($p = .616$).

Genetic predictors of match performance (as measured by DGI/min)

To determine if there was an association between a participant genetic variation and match performance, a GLMM was used to examine whether genotype could in part predict DGI/min. Replication of the Piggott and colleagues⁵ LMM found the 3x1-kilometre pre-season assessment used in this study could act as a similar predictor to the 3-km time trial. It was found that polymorphisms within certain genes have an impact on match performance, as measured by DGIs/min. Significant associations were found for genotypes *PPARGC1A* AG ($p = .002$), *ACE* ID ($p = .003$), *COMT* AA ($p = .004$), *COMT* AG ($p = .034$) and 3x1-kilometre time trials ($p < .001$; Table 3).

Genetic predictors of match kicking and handballing performance

Next, the genotypes were used to examine if they associate with kicking performance in a match setting. The GLMM examined the effect of certain polymorphisms in match performance skill, as measured by total kicks. Only the *BDNF* AG ($p = .038$) genotype had a significant association with total kicks (Table 4).

To follow up the previous analysis, the genotypes of players were used to investigate if there was an association between genetics and handballing in match performance. The *ADRB1* CC ($p = .004$) and *DRD2* AA ($p = .044$) genotypes had significant association with total handballs with the GLMM. Interestingly, the handball skill test results were not significant to the final model, which is reported in Table 5.

To further examine the effect of genetics on match performance, the total number of kicks and handballs was investigated along with player genotypes to determine if they could predict DGIs/min. It was found that the sum of kicking and handballing pre-season skill test ($p = .005$), *PPARGC1A* AG ($p = .014$), *ADRB1* CC ($p = .043$), and *DRD2* AA ($p = .003$) genotypes were all significant. These results are summarised in Table 6.

Discussion

This study is the first to report a relationship between participant genetics and match performance in ARF. Coaches and sport science professionals are constantly striving to achieve the greatest outcome and performance from their athletes. In previous

Table 3
Linear mixed model parameter estimates explaining performance as measured by direct game involvement per minute with player genotypes and 3x1-kilometre time trial as variables.

Variable		β	SE	t	p
Intercept		0.956	0.187	5.103	<.001
3 × 1 km time		−0.001	0.000	−4.000	<.001
PPARGC1A	A/A	−0.078	0.042	−0.333	.064
	A/G	0.074	0.023	2.968	.002
	G/G	0*	0	0	0
ACE	I/I	−0.009	0.026	−2.890	.739
	I/D	0.083	0.028	−2.135	.003
	D/D	0*	0	0	0
COMT	A/A	−0.101	0.035	−1.858	.004
	A/G	−0.061	0.029	3.183	.034
	G/G	0*	0	0	0

Note: Significant effects **bolded**. * indicates comparison group.

Table 4
Linear mixed model parameter estimates explaining performance as measured by total kicks with player genotypes as variables.

Variable		Estimate	SE	t	p
Intercept		8.052	0.596	13.500	<.001
BDNF	AA, A/G	−2.489	1.122	−2.219	.027
	G/G	0*	0	0	0

Note: Significant effects **bolded**. * indicates comparison group.

Table 5
Linear mixed model parameter estimates explaining performance as measured by total handballs with player genotypes and handball skill test results as variables.

Variable		Estimate	SE	t	p
Intercept		3.943	0.610	6.466	<.001
Dopamine D2 receptor	A/A	−2.745	1.057	−2.597	.010
	A/C	−0.275	0.720	−0.82	.703
	C/C	0*	0	0	0
ADRB1	C/C	1.971	0.662	2.978	.003
	C/G	0*	0	0	0
BDNF	AA, A/G	1.591	0.746	−2.132	.034
	G/G	0*	0	0	0

Note: Significant effects **bolded**. * indicates comparison group.

Table 6
Linear mixed model parameter estimates explaining performance as measured by direct game involvement per minute, with player genotypes and the sum of kicking and handballing skill test results as variables.

Variable		Estimate	SE	t	p
Intercept		−0.087	0.107	−0.812	.418
Sum of kicking and handballing results		0.007	0.003	2.827	.005
PPARGC1A	A/A	−0.075	0.042	−1.797	.074
	A/G	0.067	0.027	2.466	.014
	G/G	0*	0	0	0
ADRB1	C/C	0.050	0.025	2.033	.043
	C/G	0*	0	0	0
Dopamine D2 receptor	A/A	−0.102	0.034	−2.974	.003
	A/C	−0.053	0.028	−1.892	.060
	C/C	0*	0	0	0

Note: Significant effects **bolded**. * indicates comparison group.

studies,^{6,28} the relationship between physical assessments of fitness and match performance in ARF has been established. Young and Pryor⁶ measured match performance using the number of disposals (kicks and handballs) and found it related to levels of endurance and maximal aerobic capacity. Moreover, the findings from the current study support those from Piggott et al.⁵ with results from both studies showing that aerobic time trials are a significant predictor of DGIs/min in sub-elite ARF players.

When predisposing genetic polymorphisms and endurance performance have been previously studied, the DD genotype of the ACE gene was found to be a significant predictor for performance in

a pre-season 3x1-kilometre time trial.²³ The results from the present study show the DD genotype of the ACE gene produced a significantly poorer DGIs/min compared to the heterozygous ID genotype, when the 3x1-kilometre time trial was also used as a variable. In both the current study and the previous study by Jacob et al.,²³ the significant differences within the ACE gene were between the ID and DD genotypes, rather than the two homozygous genotypes, as has been reported in various sporting codes.²⁹

To date, there have been few studies investigating the influence of genetic polymorphisms on athletic skill performance, primarily due to the complexity of motor coordination. Previously, the ACE

DD genotype has been significantly associated with better performances in ARF-specific kicking and handballing assessments.²³ However, the results from the current study show that the *ACE* gene was not strongly associated with DGIs/min as a measurement of match performance (Table 6). This could be explained by the measurement of skill in each study. Previous findings²³ used sport-specific skill tests on a single testing day as the measurement of skill performance, while the current study collated match statistics over a 23 week season. For example, the skill tests performed were graded on accuracy, while the match day statistics used did not take into consideration the effectiveness of each disposal. However, match statistics are shown to be an accurate depiction of game day impact at sub-elite levels of ARF⁵ and team success at elite levels of basketball.³⁰

The *PPARGC1A* gene has been previously linked to endurance performance due to its role in glucose and lipid metabolism control and muscle fibre formation.^{31,32} In relation to DGIs/min, the current study showed an AG genotype (rs8192678) in the *PPARGC1A* gene proved to be a significant predictor when compared to the GG genotype. Previous studies show that European men homozygous for the A allele (AA genotype) have an extremely strong correlation with higher VO₂ max and endurance.²² However, the results of the current study indicate that the AA genotype is not significantly associated with DGI/min. One possible reason for this could be the nature of ARF, with the multi-faceted game involving elements of sprinting and endurance running, unlike sporting disciplines previously studied.

In the current study, variations in both the *DRD2* (rs1076560) and *COMT* genes (rs4680) were shown to associate with DGI/min per minute. The *DRD2* AA genotype was a significant negative predictor of handball frequency (Table 5) and match performance (Table 6), when compared to the beneficial CC genotype. While there are limited studies relating to sport, C allele carriers have been shown to continue learning more when presented with a visual-motor task that requires procedural learning.³³ Moreover, the C allele has been shown to provide increased reward responsiveness after stress induction.³⁴ A related gene, *COMT*, encodes an enzyme that promotes the breakdown of dopamine, a process essential for cognitive function.³⁵ A *COMT* polymorphism at position 158 (Val158Met), has been associated with motor control, and linked with the expression of *DRD2*.²⁴ Participants with the *COMT* AA and AG genotypes performed significantly poorer in the current study in relation to DGIs/min (Table 4), supporting previous findings.²³ The results present an interesting scenario in game performance, whereby participants with the GG genotype, and subsequent reduced dopamine activity, show greater DGI/min. Such results require further exploration, particularly as dopamine is known to be influential in motor function and physical performance.³⁶

The *BDNF* Val66Met polymorphism (rs6265) has previously been associated with motor learning,^{25,37} and sport-specific skill assessments.²³ The current results demonstrate the AA and AG genotypes as predictors for both total kicks and handballs within an ARF game. This polymorphism is related with short-term motor learning and changed motor cortex plasticity,²⁵ making it an ideal variant when investigating sporting performance. Interestingly, the corticospinal *BDNF* protein output in participants with the AA genotype increases after motor training, with the opposite effect occurring in participants with the GG genotype.³⁸ Such reports are in agreement with the findings from this study, and suggest the possibility of *BDNF*-induced cortical plasticity.

In the *ADRB1* gene, individuals with a CC genotype (rs1801253) can show a drastically increased VO₂ max, exercise time and endurance in comparison to those with the GG genotype.³⁹ However, the *ADRB1* gene has not been previously linked with motor

control or skill acquisition. Findings from the present study identify the CC genotype as a significant positive predictor of total handballs during a match (Table 6). When the kicking and handball skill test results were combined and incorporated into a predictive LMM for DGIs/min, the *ADRB1*, *PPARGC1A* and *DRD2* gene polymorphisms remained as significant predictors. Previous literature has not found an association between *PPARGC1A* and *ADRB1* in motor skill development or performance. Alongside the combination of handball and kicking assessments, such a combination of genetic and pre-season variables could be used to predict DGI/min in ARF.

In light of the findings presented in this study, ethical implications surrounding the identification and screening of athletes need to be acknowledged. The rationale behind using a genotype assessment in a sporting context is to employ an additional predictive measure of an athlete's potential success,²⁹ alongside traditional measures. However, it should not solely be used as a definitive method of selection as other environmental and external factors can influence an individual's phenotype, particularly during puberty and adolescence.⁴⁰

In conclusion, the main finding from this study is that genetic markers can be used as a method to determine player match performance. These results suggest that genotype assessments can be used alongside physical and skill assessments to determine the performance potential of an individual player. In addition, the present study confirms previous research⁵ that endurance performance in pre-season testing can predict match performance in ARF. While the results from this study are novel, there are a number of variables that contribute to complex athletic traits, such as skill. Subsequent studies investigating elite sporting populations should be undertaken to further validate the findings of this study.

Limitations

While the current study consisted of a relatively small pilot sample of sub-elite athletes, the results provide valuable data towards ARF specific assessments. A main limitation of this study was the sub-elite nature of the cohort. Participants are not full-time professional athletes, however they represent junior athletes that have been selected on the basis of talent, into a structured ARF program, training multiple times a week in an ARF specific training schedule. For analysis of rare alleles using the LMM approach, heterozygous and homozygous genotypes were combined, not allowing differentiation between genotypes. Moreover, given the sample size, the current study has not explored the possibility of gene-by-gene interactions confounding the results.

Practical applications

Using a 3x1-kilometre time trial, the results of the current study reiterate the importance of pre-season aerobic fitness in influencing match performance. Strength and conditioning trainers should explain to players the relevance, and highlight the importance of training to develop physical capacity to improve individual match performance. Specifically players should be made aware that better performance in aerobic fitness tests such as the 3x1-kilometre time trials are associated with greater number of DGIs per minute, and are an important game performance predictor. In light of this, coaches should be allocating sufficient time for players to develop physical capacity within the pre-season program. Another finding of the present study was that ARF-specific skill test performance might not be an accurate depiction of match performance. This finding shows that coaches should make match day selections based around match play and open-environment drills, rather than closed-environment drills and scenarios. It also highlights that player skill development would benefit from dynamic,

game-related drills and play rather than static drills, as it relates to on-field decision making, skill execution and ultimately match performance. Finally, for sport scientists, the genetic evidence from the current study demonstrates a novel method of determining player potential. However, the selection and/or exclusion based on genotype data alone is unethical, but rather presents an additional informative variable which may assist in conveying valuable information to coaching staff on player responses to training regimes. This finding could allow for a diversified selection process to join sub-elite and elite competition, and potentially allow for the development of an individualized training program to efficiently target predisposed player attributes.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jesf.2018.10.007>.

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