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Gene Expression: Analysis of Corresponding Polymorphisms of the Ace and Actn3 Genes in Adolescent Rugby Union and Association Football Players

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Abstract

Aim: The purpose of the present study was to analyse and quantify the magnitude of the corresponding polymorphisms of the ACE and ACTN3 genes in Rugby Union (RU) and Association Football (AF) players.

Materials and Methods: A total of 71 players were involved, 33 RU players and 38 AF players. Players were analysed on the basis of genotype, polymorphism and playing position. Estimated maximal oxygen uptake was obtained from the 20 metre shuttle-run test. Stature and body mas were measured to the nearest 0.1 cm and 0.1 kg respectively. Statistical analyses were carried out using Pearson's X^2, ANOVA, independent t-tests, coefficients of correlation, effect sizes and 95% confident intervals.

Results: Mean values were significantly different between RU and AF players in body mass (87.34 vs 72.27 kg, $p = 0.0001$) and estimated maximal oxygen uptake (60.92 vs 53.57 ml. /kg/min^{-1}, $p = 0.015$). Genotype frequencies between ACE and ACTN3 genes in both RU and AF players were not significant ($p > 0.05$), although allele frequencies were evident in both genes ($p = 0.05$ and $p = 0.01$ respectively). RU forwards were heavier than backs (92.79 vs 79.04 kg, $p = 0.01$), but estimated maximal oxygen uptake was greater in backs than forwards (52.76 vs 49.55 ml/kg/min $p = 0.039$). In AF players goal-keepers and defenders had greater stature than midfield players (181.03 vs 175.76 cm, $p = 0.016$). The effect size of AF players was relatively low in all polymorphisms: II vs XX ($r = 0.03$), ID vs RX ($r = 0.13$), DD vs RR ($r = 0.13$) respectively. In RU players II vs XX (endurance) and DD vs RR (power) had medium effect sizes of 0.29 and 0.24 respectively.

Conclusion: The analyses did not identify exceptional differences between corresponding polymorphisms of the ACE and ACTN3 genes. Given these circumstances a dominant solution would be for a functional adaptation of 'gene expression' to be facilitated where appropriate. Training sessions should be designed to identify the physiological and metabolic characteristics of match-play and the demands of training and conditioning.

Keywords: *ACE; ACTN3; Gene expression; Association football; Rugby Union Football*

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Introduction

In the United Kingdom there are two major professional team sports, Association Football (AF) and Rugby Union Football (RUF). Association football originated from the formation of the Football Association in London, England, in 1863. The game was based on models to enable players to compete against each other without dispute and which specifically prohibited them from handling the ball and deliberately kicking opponents in open-play. The modern game is played by two teams, each comprising 10 players and one goalkeeper. Replacements are allowed depending upon the status of the game.

The origins of RUF can be traced back to the playing regulations of 1845 and the resolution of one of the teams to leave the FA. This resulted in the formation of the Rugby Football Association in 1871. RUF was a strictly amateur game and payment to players was restricted, thus a separate code was inevitably accredited and identified as Rugby League. RUF was declared an 'open' game in 1995 and accordingly professionalism was sanctioned. In Wales RUF is engrossed at a high level of national concentration and ambition, particularly when played at international level. This is the consequence of the cultural heritage associated between Wales and RUF. Both team games are played comprehensively at a variety of ages and levels with an elevated degree of stimulation and enthusiasm. There is a diversity of functional requirement between the two games. RUF, unlike many other team games, deliberately encourages whole-body, vigorous, dynamic and sustained physical contact in the application and execution of its playing skills and techniques, at both a high and a low level of intensity [40,10]. However, playing irregularities which are considered dangerous or contravene regulations are penalized. Association football, technically, is a non-contact sport, although occasionally this is disregarded by players, who are disciplined by officials when the offence has been observed. Goal-keepers are the only players allowed to control the ball with their hands or arms within their own penalty area. Outfield players use their feet to pass or strike the ball but can also use their head or body advantageously to assist skilled performance.

The fundamental differences between these two sports require considerable understanding, appreciation and biological enlightenment. In the present study, for example, to what extent is the interpretation of the meaningfulness of the polymorphisms used satisfactorily? With respect to the ACE gene, what precisely does the term 'endurance' mean; does it reflect intermittent or prolonged duration of physical activity, or perhaps both? [8,47]. Similarly, for the ACTN3 gene; while variables such as power, speed and strength are all phenotypes required for an elite level of anaerobic performance, can the terms be applied indiscriminately, or is there a degree of uniqueness in each term? Power, is defined as the quantity of work per unit time; the product of force and velocity [24] which is markedly influenced by muscle fibre type. Running speed is determined by the length of stride and the number of strides per unit time [1]. The maximal force that a muscle can generate is termed strength. However, it is not necessarily assured that greater absolute strength will enhance improved anaerobic performance, since this will depend, to some extent, upon factors such as age, gender, maturity and body size. Furthermore, the nature of the movement of the muscle fibres themselves is crucial; are they executed in a concentric, eccentric, or isometric manner in relation to the movements involved? [44). The essence of all these basic concepts is vital to the eventual expression and capability of the polymorphism.

The polymorphisms of the human gene encoding ACE (17q 23.3) are characterised by the presence (insertion, I allele) rather than the absence (deletion, D allele) of a 250 bp Alu sequence fragment found within intron 16 [39]. All individuals carry two versions of the ACE allele, therefore three ACE genotypes are identified; those who are homozygous for the insertion (II) and deletion (DD) alleles, and those heterozygous for the ID allele. The I allele is associated with low levels of ACE activity in serum and tissues and the D allele with increased levels of ACE activity, thus ACE is consistently highest in DD subjects, intermediate in ID and lowest in II subjects [13]. In sporting performance, the ACE insertion allele and the II genotype, are considered to be associated positively with enhanced endurance performance in a variety of sporting events [47,35].

The α-actinins which belong to a group of α-actinin-binding proteins are functionally important in the configuration and regulation of the cytoskeleton (Blanchard 7, 33). The skeletal muscle isoforms ACTN2 (1 q42-q43) and ACTN3 (11 q13-q14) are the essential

central structural elements of the sarcomeric Z line, where they cross-link α-actinin-containing thin filaments to assist the stabilization and architecture of muscle contraction. Inclusively, α-actinins interact with other proteins to participate in wide-ranging signal transduction complexes which facilitate adaptive physiological change to exercise [14,46].

In humans, α-actinins -2 and α-actinins -3 are encoded by their genes ACTN2 and ACTN3 respectively. The expression of the two sarcomeric α-actinins diverged during the period of mammalian evolution, such that α-actinin -2 provided the heart and oxidative skeletal muscle fibres, while α-actinin -3 was restricted largely to type II fast-twitch fibres, where they initiated force-generating glycolytic energy production [27]. A variation of the ACTN3 gene [28] has been described which has resulted in the replacement of an arginine (R) with a stop codon (X) at amino acid 577 (R577X). This departure creates two contrasting alleles; the 577R version, which is the normal functional variation of the gene, and the 577X allele which is accountable for extensive α-actinin -3 deficiency in the general population at large. While the phenotypic characteristics of the RR genotype, such as speed, power and strength, are held responsible for enhanced anaerobic performance of elite athletes [50], the XX genotype is considered to influence elite aerobic performance [29]. Two copies of the ACTN3 gene are inherited from parents, thus three genotypes are prescribed: RR, RX, and XX. The population of the ACTN3 gene for athletic performance has been comprehensibly published; [48,32,49]. For young adult amateur RU players, genotypes for forward positions have been identified as RR = 28%, RX = 53%, XX = 19%, and for back positions RR = 31%, RX = 55%, XX = 14% [3]. In 37 professional AF players, Pimenta. [34] Classified individuals as RR = 41%, RX =35 %, and XX = 24%, while Santiago [41] recorded elite players (n=60) to be RR = 48%, RX = 37%), and XX = 15%. A recent study [22] identified an association between ACTN3 R577X and elite RU playing status and playing position, but there was no associated relationship with the ACE I/D variants.

Materials and Methods

A total of 71 players were involved in the investigation, 33 RU players and 38 AF players. A cross-sectional case-control study was employed using a candidate-gene approach. The groups as a whole were homogeneous in terms of age, gender, ability and ethnicity; individuals were competitive at their respective performance levels on the basis of their preferred playing position. For RU players these positions were props/hookers (P/H), locks/back-row (L/BR), scrum-half/outside-halves (SH/OH) and centres/wings/full-backs (C/W/FB). Association football players were classified accordingly as goal-keepers/ defenders (GK/D), mid-field players (MF) and strikers (S).

Polymorphisms of the ACE gene in RU players (Table 2, n = 33) were II (n = 9), ID (n = 15), DD (n = 9), and for the ACTN3 gene XX (n = 5), RX (n = 17), RR (n = 11). The polymorphisms of the ACE gene in AF players (Table 3, n = 38) were II (n = 8) ID (n = 14), DD (n = 16) and in the ACTN3 gene XX (n = 5), RX (n = 19) and RR (n = 14). Sample sizes for phenotypes were determined using a standardised difference of 0.9, an α-level of 0.05, and a power value of 0.8; the estimated total sample size for each of the two groups was found to be n = 38 [20].

Genotype Analyses

All players provided a 5 ml sample of saliva into a conspicuously labelled sterile container which was placed in storage at 4°C until buccal cell DNA extraction was required (Qiagen QiaAamp DNA Micro kit). Three-primer polymerase chain reaction (PCR) was used to assay the region of interests for the I and D variants of the ACE gene. Three primers were used; Forward, Reverse and Internal Forward.

Cycling conditions were carried out at 94°C for 12 minutes (heat activation step), followed by 35 cycles of 94°C denaturing for 30s, annealing at 60°C for 30s, extension at 72°C for 30s, concluded by a final extension period for 10 mins at 72°C. A 5-µl sample of the PCR product was mixed with 5-µl of gel loading buffer and placed into the well containing agarose gel, which was then completed by electrophoresis at 100 volts. Allele identification was observed using ethidium bromide staining.

The ACTN3 gene was similarly analysed using two primers. This generated a PCR product 628 bp in length which extended the R577X Single Nucleotide Polymorphism (SNP) and contained a ubiquitious Ddel digestion site upstream of R577X. Aliquots of the digested products were subject to electrophoresis for size discrimination, and bromide staining for allele identification, as previously described. Each individual player was genotyped for both the ACE and the ACTN3 gene.

Anthropometry

A Harpenden stadiometer was used to measure standing height to the nearest 0.1 cm using the stretching-up technique; body mass was assessed on a digital scale to the closest 0.1 kg.

Estimated maximal oxygen uptake

The time frame available for testing players precluded the use of standardised physiological procedures such as the treadmill, bi-cycle, and gas analysis systems to directly quantify the maximal oxygen uptake (ml. kg^{-1}. min^{-1}), thus estimated maximal oxygen uptake was determined using a shuttle-run test.

The progressive 20m shuttle run test [36] consisted of running between two markers placed 20m apart at increasingly faster speeds. The test was taken indoors, individuals running on an artificial track surface. Timing of the running speed increased by 0.14 $m.s^{-1}$ each minute and was controlled by a micro-computer. The change in speed was referred to as a change in level. To stimulate and encourage maximal performance individuals ran in small groups, with adjudicators placed at each marker to ensure subjects completed the full 20m distance. The test itself was originally validated against 74 volunteers (36 males and 38 females) on an uphill treadmill test to determine VO2 max directly (ml. kg^{-1}. min^{-1}), a 5 km time trial, and the results of the 20m shuttle-run tests. The validity of the test indicated that VO2 max (ml. kg^{-1}. min^{-1}) could be predicted from the level attained on the 20m shuttle-run test, with an estimated standard deviation from the regression line of 3.5 ml. kg^{-1}. min^{-1} ($r = 0.92$, $p < 0.01$). A variety of similar tests are available which can be used for this purpose depending upon the prevailing circumstances [25,16,30,31].

Statistical Analyses

The null-hypotheses of the study was that there were non-significant quantification differences between the dependent variable, estimated maximal oxygen uptake, in corresponding polymorphisms of the ACE and ACTN3 genes in adolescent RU and AF players.

Pearson's chi-square (X^2) was used to analyse the goodness-of-fit in a comparison between the ACE and ACTN3 genotypes of Association and RU football players. The assumptions for the χ^2 test were fulfilled according to the recommendations of Field [15]. One-way ANOVA, independent t-tests (two-tailed), and coefficients of correlation were used to identify supporting information. Homogeneity of variances were declared by Levene's test. Data were analysed using SPSS versions 20/22. The alpha-level for significance was set at $p \leq 0.05$. Raw values are reported as means ± SD, unless otherwise stated. Effect sizes [15] were calculated as coefficients of correlation using the value of 't^2' and the appropriate degrees of freedom, to contrast estimated maximal oxygen uptake between corresponding polymorphisms of the ACE and ACTN3 genes.

For AF players, the genotype comparisons of estimated oxygen uptake were evaluated as follows: II vs XX, which was considered to represent enhanced endurance capacity; ID vs RX, values regarded as intermediate in status and related to both endurance and power; and DD vs RR, which reflects an esteemed category of power. The same assumptions were applicable in analysing the data of RU players.

Polymorphic data were listed independently of the procedures employed. Each polymorphism was given a code to classify individuals belonging to that particular polymorphism. This practice was checked and authenticated before analysis. Subsequent independent *t*-tests were then carried out to contrast genotype comparisons. Similar deliberations were employed when investigating playing positions. All participants were volunteers and gave their informed consent. The study was approved by the School of Sport Ethics Committee.

Results

Table 1 displays the mean values for raw descriptive characteristics for age, height, body mass, and estimated maximal oxygen uptake between RU and AF players. There were significant differences between groups in body mass (87.34 ± 12.0 vs 72.80 ± 8.7 kg, t

= -5.92, p = 0.0001) and estimated maximal oxygen uptake (50.92 ± 4.5 vs 53.57 ± 4.4 ml.$^{-1}$ kg^{-1}, min^{-1}, t = 2.51, p = 0.015). Age and height were similar and not significantly different.

	Age (yr)	Height (cm)	Body mass (kg)	Oxygen (ml. kg^{-1} min^{-1})
Rugby Union	18.79 ± 0.8	180.43 ± 6.3	87.34 ± 12.0	50.92 ± 4.5
Association	18.32 ± 0.93	178.80 ± 5.4	72.80 ± 8.7	53.57 ± 4.4
	p = 0.770	*p = 0.261*	*p = 0.0001*	*p = 0.015*
	t = 0.294	*t = 1.134*	*t = -5.92*	*t = 2.51*

p = probability value t = t-test value

Table 1: *Mean values of age, height, body mass, and estimated maximal oxygen uptake for Association (n = 38) and Rugby Union (n = 33) football players.*

Genotypes and allele frequencies between ACE and ACTN3 genes in RU and AF are given in Tables 2 and 3. The χ^2 value of 3.8 with 2 df in RU players (Table 2) was not significantly different between the two genes (p > 0.05); the major component in the ACE gene was the ID genotype (n = 15, 46%) and in the ACTN3 gene the RX genotype (n = 17, 52%). Allele frequencies were identical in the ACE gene (I = 33, 50% and D = 33, 50%) although marginally greater in the ACTN3 gene (R = 39, 59%) and (X = 27, 41%); the allele frequency χ^2 of 5.3 was significantly different at p = 0.05.

	Genotype			Allele frequency	
	II	ID	DD	I	D
ACE	9 (0.27)	15 (0.46)	9 (0.27)	33 (0.50)	33 (0.50)
	XX	RX	RR	R	X
ACTN3	5 (0.15)	17 (0.52)	11 (0.33)	39 (0.59)	27 (0.41)

Relative frequency of genotype and allele frequency is given in brackets. X^2 between genotypes (2df) = 3.8 (p > 0.05). Between allele frequencies χ^2 (1df) = 5.3 (p = 0.05).

Table 2: *Genotype and allele frequency between ACE and ACTN3 genes in Rugby Union football players (n = 33).*

	Genotype			Allele frequency	
	II	ID	DD	I	D
ACE	8 (0.21)	14 (0.37)	16 (0.42)	30 (0.39)	46 (0.61)
	XX	RX	RR	R	X
ACTN3	5 (0.13)	19 (0.50)	14 (0.37)	47 (0.62)	29 (0.38)

Relative frequency of genotype and allele frequency is given in brackets. X^2 between genotypes (2df) = 3.41 (p > 0.05). Between allele frequencies χ^2 (1df) = 16.7 (p = 0.01).

Table 3: *Genotype and allele frequency between ACE and ACTN3 genes in Association football players (n = 38).*

In AF players (Table 3) genotype χ^2 was 3.41 and statistically non-significant (p > 0.05). Allele frequencies in the D allele were *n* = 46, (61%) and in the X allele, *n* = 29, (38%). The ACE gene I allele accounted for 39% (*n* = 30) and the ACTN3 gene R allele 62% (*n* = 47); allele frequency χ^2 with 1df was 16.7 and significant (p = 0.01).

Estimated maximal oxygen uptake (ml. kg^{-1}. min^{-1}) in AF players (n = 38) was related negatively with body mass at *r* = -0.45 (*p* = 0.01) and with stature at r = 0.31 (*p* > 0.05); there was a positive relationship between stature and body mass at r = 0.51 (*p* = 0.01). In RU players (*n* = 33) the relationship between estimated maximal oxygen uptake and body mass was *r* = -0.48 (*p* = 0.01), with stature r = -0.06, and between body mass and stature, positively at r = 0.57 (p = 0.01).

Table 4 provides the analysis of corresponding polymorphisms of the ACE and ACTN3 genes in Association and RU football players. The data utilized for comparisons included, 't^2' values, degrees of freedom (df) and coefficients of correlation (r); this concept transformed 't^2' values and df to measure the strength of an exploratory effect. The effect size is constrained to lie between 0 (no effect) and 1.0 (perfect effect). Effect sizes used were as follows: *r* = 0.1 small effect; *r* = 0.3 medium effect: *r* = 0.5 large effect [11]. Effect sizes are informative since they provide an objective measure of the importance of an effect. The oxygen uptake values (ml. kg^{-1}. min^{-1}) were similar between polymorphic comparisons and no probability values were found to be significant. In AF players the effect size was low for all polymorphisms (*r* = 0.03, 0.13 and 0.13 respectively) which would account for about 1% of the total variance. In RU players, II vs XX and DD vs RR have medium effect sizes of 0.29 and 0.24 respectively which would clarify approximately 9% of the total variance [15]. The 95% confidence intervals [19] were determined for mean differences between estimated maximal oxygen uptake values in corresponding polymorphisms of the ACE and ACTN3 genes and are provided in Table 4.

Association Football (n = 38)

II vs XX	ID vs RX	DD vs RR
II 53.21 ± 6.44 (n = 8)	ID 52.54 ± 3.61 (n = 14)	DD 54.64 ± 3.94 (n= 16)
XX 53.47 ± 2.74 (n= 5)	RX 53.64 ± 4.59 (n= 19)	RR 53.50 ± 4.94 (n = 14)
t = - 0.09, df = 11, p = 0.93	*t* = 0.74, df = 31, p = 0.47	*t* = -0.71, df = 28, p = 0.49
r = 0.03, CI = -7.0 to 6.5	*r* = 0.13, CI = -1.9 to 4.1	*r* = 0.13, CI = -2.1 to 4.4

Rugby Union Football (n = 33)

II vs XX	ID vs RX	DD vs RR
II 48.63 ± 4.25 (n = 9)	ID 51.57 ± 4.92 (n = 15)	DD 52.11 ± 3.31 (n = 9)
XX 51.10 ± 4.11 (n = 5)	RX 50.85 ± 5.01 (n = 17)	RR 50.54 ± 3.45 (n = 11)
t = 1.05, df =12, p = 0.31	*t* = 0.40, df = 30, p = 0.69	*t* = 1.03, df = 18, p = 0.32
r = 0.29, CI = -2.6 to 7.5	*r* = 0.07, CI = -3.0 to 4.4	*r* = 0.24, CI = -1.5 to 4.8

Polymorphic values are expressed as ml. kg^{-1}, min^{-1}.

t = t-test value, df = degrees of freedom, p = probability value

r = effect size CI = confidence intervals

Table 4: *Analysis of corresponding polymorphisms of the ACE and ACTN3 genes in Association and Rugby Union Football Players.*

Discussion

An epigenetic trait is a hereditary phenotype resulting from changes in a chromosome without alteration to the DNA sequence [5]. It is the study of trait variations which are caused by external or environmental factors that diversify genes to interchange and affect how cells interpret genetic information [45] rather than facilitating changes to the DNA sequence itself. DNA methylation allows for the attachment of a methyl group (CH3) to the outside of the DNA strand. The resulting change is normally permanent and unidirectional, but can be removed passively or actively by hydroxylation of the methyl groups [6]. It is an epigenetic mechanism used by cells to control

gene expression and is commonly used as a signalling tool to position genes to the 'inoperative' position; that is, 'off' in preference to 'on' [37-38].

Biological convention declares that the transfer of genes within DNA is the only method which can transmit biological information between generations. However, there is some recent epigenetic evidence to suggest that cytosine methylation in the gene encoding for FKBP5 (binding protein) was observed in parents surviving the Holocaust (n = 32), as well as their adult children (n = 22). This suggests the possibility of site-specificity to environmental influences and corresponding inter-generational effects [51].

The present human genome was fashioned and cultivated during the late Palaeolithic period (50,000-10,000 BC) when obligatory physical activity was essential for survival and our ancestors were, occupationally, simply 'hunters and gatherers'. A sedentary lifestyle in such conditions would lead ultimately to capitulation. The phenotype of modern *Homo sapiens* is distinctly unlike that of our ancestors, largely as a consequence of expressing evolutionary programmed late Palaeolithic genes in an environment that was, for the most part, sedentary. For this reason Booth., *et al.* [9] suggests that the current genome is maladapted, and results in abnormal gene expression. In sedentary cultures regular physical activity would normalize gene expression according to those behavioural patterns required to maintain genetic polymorphisms which would be the most advantageous in the relevant circumstances. Estimates of physical activity in the late Palaeolithic era, and in present day physical activity societies, are much larger than those undertaken in contemporary sedentary lifestyles [12].

There are natural demographic characteristics between competitors in the two games. The main playing re-starts in RU are the line-out and the scrummage. In general, players are selected for the various positions based largely on their morphological size, shape and body composition [4]. The hooker, who is metaphorically one of the smallest of forwards, is the individual who usually propels the ball into the line-out. The line-out requires individuals who are particularly tall in stature and who are able to compete robustly, together with the provisional support of the remaining forwards, to obtain possession of the ball. The line-out is a well-rehearsed strategic technique used advantageously depending upon the immediate circumstances as well as the current status of the game. In Table 1 no significant differences were apparent in stature between RU and AF players (t = 1.134, p = 0.261).

Body mass, however, is crucially important for success in obtaining the ball during the scrummage. Despite its technical difficulties, which initially need to be controlled by officials, there are many illegal transactions which are undertaken by players resulting in infringements of play. Differences in body mass between the two groups of players (Table 1) are quite substantial and statistically significant (t = -5.92, p = 0.0001); this, no doubt, is due to the varying nature of the two games. The position is also evident when comparing the body mass of forwards (92.79 ± 12.25 kg) with that of backs (79.94 ± 6.49 kg) (p = 0.01).

A key feature of Table 1 is the larger estimate of maximal oxygen uptake in AF players compared to RU players (53.57 ± 4.4 vs 50.92 ± 4.5, ml. kg^{-1}. min^{-1}), and indicates, perhaps, one of the major physiological performance differences between the two games. (t = 2.51, p = 0.015). Association football has more fluidity and latent directness than RU, and in addition to playing consequent games for a greater period of time, the availability of additional oxygen uptake, is likely to be beneficial. In the modern game of AF there are fewer nominal positions adopted. Players are accommodated to a position or area on the field of play which best explores their individual capability and co-operation with other team players; this relationship exists in both attacking and defensive situations. A team could therefore play with four defenders, four midfield players, and two strikers, or any other appropriate combination of these players depending upon the attacking or defensive requirements of the team. Despite this flexibility there would still be necessary tactical exchanges required between playing positions. In general terms, it is more likely that midfield players, who had the largest oxygen uptake values (54.27 ± 5.02, ml. kg^{-1}. min^{-1}) would be the most creatively influential players for decisive periods of the game. This spontaneity is one of the characteristics that midfield players have cultivated over the years. From a defensive point of view, it is natural for goal-keepers and defenders to be the tallest of playing groups (181.03 ± 5.43 cm, p = 0.016). This may be compensated for by defenders initiating attacking plays from

defence, mid-field, or at set-pieces of play in the attacking third of the field. Conventionally, players play to feet, but more penetrating approaches will be achieved if space in the desired attacking channel is threatened. This frequently creates confusion for defenders and permits attacking players to exploit more decisive scoring positions.

The major configuration of the ACE genotypes in RU players (Table 2, n = 33) was the ID component (n = 15, 46%); I and D allele frequencies were identical at 50%, the dominant constituent of the ACTN3 gene was the RX genotype (n = 17, 52%). The X allele frequency contributed 41% and the R allele frequency 59%. There was no significant association between the ACE and ACTN3 genotypes in RUF players: (χ^2) = 3.8, d/f = 2, p > 0.05). In principle, therefore, the ACE I and D allele frequencies contributed nominal amounts of endurance and power, whereas the ACTN3 R and X allele frequencies provided intermediate amounts of power and endurance.

In AF players (Table 3, n = 38) the ACE gene contributed 21%, 37% and 42% for the II, ID and DD genotypes respectively. These values compare with 13%, 50% and 37% for the corresponding XX, RX, and RR genotypes. The DD genotype was the dominant component in the ACE gene (42%), as the RX genotype was in the ACTN3 gene (50%). The D allele frequency (61%) and the R allele frequency (62%) were conspicuously superior to the I and X allele frequencies, and therefore clearly associated with the esteemed nature of power.

Elite professional RU players (n = 15 backs and 13 forwards) undertook a maximal oxygen uptake test, a lung capacity test, a 3km timed run and the measurement of body fat [42]. Peak oxygen uptake was greater in backs than forwards (48.3 ± 2.1 vs 41.2 ± 2.7 ml. kg^{-1}. min^{-1}, p < 0.05). Forwards were taller, heavier, and had greater amounts of body fat than backs. There was a significant negative correlation between peak oxygen uptake, the 3 km run time (*r* = -0.45, p < 0.05), and body mass (*r* = -0.54, p < 0.003). These relative peak oxygen uptake values are generally smaller than would normally be expected for elite RU players.

A study on the polymorphisms of the ACTN3 gene in individuals participating in different sporting activities has been carried out by Holdys., *et al*. [23]. The objective was to identify the relationship between aerobic fitness as measured by VO2 max (ml. kg^{-1}. min^{-1}) and the polymorphisms of the ACTN3 gene (RR, RX and XX). The analysis was performed on 239 individuals (154 males and 85 females). In males, values for the descriptive statistics were as follows: RR = (54.90 ± 6.8, n = 63), RX = (54.96 ± 7.0, *n* = 70), XX (56.38 ± 8.2, *n* = 21). There were no significant differences between the genotypes. When individuals were separated into non-training and training groups, values of the polymorphisms in the training group were highest with RR > RX > XX, although VO2 max values were not statistically significant between genotypes.

Maximal oxygen uptake is an important physiological and clinical variable as a consequence of its association with cardiovascular disease. Hagberg., *et al.* [21] assessed the relationship between the ACE genotypes in postmenopausal women with different habitual physical activity levels, and maximal O2 uptake derived from a treadmill test. Age, body composition and habitual physical activity did not differ between genotypes. The ACE II genotype had a 6.3 ml. kg^{-1}. min^{-1}, greater VO2 max than the DD genotype group after taking into account physical activity status (p < 0.05). The II genotype group also had a greater VO2 max than the ID genotype (3.3 ml. kg^{-1}. min^{-1}, p < 0.05).

The association between α-actinin 3 deficiency and human athletic performance has been examined and discussed in detail by MacArthur., *et al*. [29] using α-actinin 3 knock-out (KO) mice. In general terms KO mice exhibited normal morphological characteristics, behavioural activity, and fibre type proportions compared with wild-type (WT) mice. KO mice performed as well as WT mice in simple motor performance tasks indicating that α-actinin 3 deficiency was within normal limits. Isolated KO muscles recorded a decrease in maximal force but demonstrated accelerated recovery from fatigue. KO muscles also displayed a variety of phenotypic changes which included a reduction in the diameter of fast-twitch fibres and changes in enzymatic activities related to aerobic metabolism. MacArthur., et al. [29] concluded that their findings were compatible with a transformation of the properties of fast-twitch fibres towards those conventionally related to slow-twitch fibres. Thus the R577X allele was affiliated with reduced performance in power but increased capacity in endurance.

The molecular basis for the mechanisms of ACTN3 gene in fast-twitch muscle fibres has been implicated in the transduction of signals that control the hypertrophy of cardiac muscle and slow-twitch fibre gene expression in skeletal muscle. A group of calcineurin-interacting proteins (calsarcins) have been identified with the responsibility for the affiliation of calcineurin and a-actinin at the Z line of the sarcomere of cardiac and skeletal muscle cells. Calsarcin 1 and calsarcin 2 are specific for the development of adult cardiac and skeletal muscle during embryogenesis, although calsarcin 2 is restricted to fast-twitch skeletal muscle fibres [17]. The final member of the calsarcin family, calsarcin 3, is specifically enhanced in fast-twitch skeletal muscle fibres and acts in conjunction with calcineurin and the Z disc proteins. The function of calsarcins operate to identify specific areas for the interactions of associated proteins in the Z disc architecture, in addition to facilitating signal transduction in striated muscle fibres [18].

During prolonged periods of physical activity the metabolic characteristics of skeletal muscle respond to increased physiological demand, and occurs as a result of the metamorphosis of substrate utilization and gene expression. Calcium-regulated calcineurin is further associated with the transduction of motor neurone signals which transform gene expression programs in skeletal muscle [26]. In transgenic mice, activation of calcineurin increases glucose incorporation into glycogen and lipid oxidation in skeletal muscle. Activated calcineurin diminishes skeletal muscle glucose oxidation and increases lactate release. Increased lipid oxidation was sustained by increased expression of a number of genes for lipid metabolism and mitochondrial oxidative phosphorylation. As a consequence, the genes relevant to glycolysis were down-regulated, while those of pyruvate dehydrogenase kinase were restrained. Changes in the pattern of gene expression were thus associated with decreased glucose utilization and enhanced glycogen storage. These physiological demands and effects are discussed extensively in relation to training and match-play performance in elite Association football players [2], although technical strategies of the modern game are likely to have changed in recent years and may well have modified gene expression. The totality of physical performance status of players will ultimately be determined by the specificity of individual training programmes to facilitate the appropriateness of functional gene expression.

As is now well identified about 16% of the global population are α-actinin 3 deficient as a result of the homozygosity for a common nonsense polymorphism in the ACTN3 gene [43], thus sporting performance at elite, competitive and recreational levels will, consequently, be diminished. The apparent reduction in power output and the improvement of endurance capacity, will therefore be facilitated by the prolonged metabolic, physiological and signalling processes of fast-twitch fibres. The deficiency of α-actinin 3 results in increased calcineurin activity in mice and human skeletal muscle, thus an improved adaptive response to endurance training will occur. Additionally, α-actinin 2 competes with calcineurin for binding to calsarcin-2, which encourages improved calcineurin signalling and reprogramming of the metabolic phenotype of fast-twitch muscle fibres.

Conclusions

In the present study there were significant differences between the mean body mass of RU players (87.34 kg) and AF players (72.80 kg) (Table 1). χ^2 values (Table 2) revealed no significant differences between ACE and ACTN3 genotypes in RU players although differences in allele frequencies were evident. The position was much the same in AF players (Table 3). The only significant difference between forwards vs backs in RU players was the greater body mass in RU forwards (92.79 kg vs 79.94 kg) and the greater estimated maximal oxygen uptake in backs (52.76 vs 49.55 ml. kg^{-1}. min^{-1}). Body height in AF midfield players was significantly smaller (175.76 cm) than either goal-keepers, defenders or strikers. At the present level of sporting performance there were no significant differences identified for estimated maximal oxygen uptake between corresponding polymorphisms of ACE and ACTN3 genes (II vs XX), (ID vs RX) and (DD vs RR) in either AF or RU players (Table 4). Values are reported for effect size (r) and confidence intervals (CI). Confidence intervals are relevant whenever an inference needs to be made from the results of a particular study to the larger population group in general. Confidence intervals provide a range of values on the basis of the actual sample data in which the total population value for a given difference is likely to be accessible. For example, in AF players (Table 4) the width of the 95% confidence intervals for the polymorphisms II vs XX are larger than those of the ID vs RX and the DD vs RR. Therefore, in general, increasing the sample size will reduce the width of the confidence intervals. At a developmental level of the game the analyses, results and discussions undertaken will provide an improved

understanding of the essential biological, physiological and metabolic characteristics of individual players in an attempt to quantify the potentiality of 'gene expression' and performance in adolescent RU and AF players.

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