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The ACTN3 Gene and Differences between Playing Positions in Bone Mineral Content, Fat Mass and Lean Tissue Mass in the Arms, Legs and Trunk Of Rugby Union Football Players

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Abstract

Aim: The function of the present study was to identify differences between individual playing positions in bone mineral content, fat mass, and lean tissue mass, in the arms, trunk and legs of young adult Rugby Union football players who carried the ACTN3 gene.

Subjects and methods: A cross-sectional case control study was carried out using a candidate gene approach (n=55). Individuals belonged to a homogeneous group of players relative to age, gender, ability, and ethnicity. Players were allocated to their preferred playing position. These were the front row (n=14), second and back rows (n=16), scrum and outside-half (n=11), and centres, wings and full-backs (n=14). A 5 ml sample of saliva was obtained from each player and specimens stored at 4°C until buccal cell DNA extraction was carried out. Height was measured to the nearest 0.1 cm and body mass to the closest 0.1 kg. Dual-energy X-ray absorptiometry was measured using a Hologic QDR Discovery fan beam model. Statistical analyses were undertaken using ANOVA, ANCOVA and MANOVA.

Results: The study sample comprised 22% RR, 60% RX, and 18% XX genotypes of the ACTN3 gene respectively. Players in the second and back rows were significantly taller than other positions. Body mass differences, were significantly greater in forwards than backs. There were non-significant differences between positions in adjusted bone mineral content or adjusted lean tissue mass. Adjusted fat mass reflected differences between left and right arms, but not left and right legs.

Conclusion: At a developmental level of performance, an understanding and practical application of the structural, physiological and body composition characteristics of individual players, will facilitate personal and team accomplishment, efficiency of training and conditioning, and nurture the potential of young adult players.

Keywords: Playing position; ACTN3 gene; Body composition

Introduction

The regional diversity of body tissues has important implications for sport and physical activity; for example, in their relationship with training, playing performance and rehabilitation. Nindl et al. [1] using Dual-Energy X-Ray Absorptiometry (DXA), found that increasing fatness in young, lean, fit, soldiers was unrelated to increased deposition of fat at the trunk, but occurred preferentially at the arm. The basic conjecture was that individuals who were fat had different regional placement of tissues than those who were lean. This concept will apply equally to differences within and between different sports.

There is considerable evidence suggesting that the response of activity to Bone Mineral Content (BMC) and Bone Mineral Density (BMD) is site-specific [2,3]. This being the case, bone remodelling will depend, in part, upon the nature of the sport, the age at which it commenced, together with the length and type of training undertaken. The intensity of exercise is an important factor in bone stimulation and adaptation, and moderate to high impact weight bearing activities are likely to be more effective than low impact ones. Differences in bone constitution exist within and between sporting groups, consequently similar training programmes are unlikely have the same effect on bone mass [4]. Factors such as age, gender, genetics and ethnicity, are inherent determinants of BMD which are controlled by a variety of genes [5]; thus the design of effective functional training regimes need to specify as many of these relevant variables as is possible [6].

The α-actinins, which belong to a group of actinin-binding proteins, are affiliated to dystrophin and spectrins, and are functionally important in the configuration and regulation of the cytoskeleton [7]. In skeletal muscles, α-actinin-2 and α-actinin-3 are the essential structural elements of the sarcomeric Z line, where they cross-link actin-containing thin filaments to assist the stabilization and architecture of muscle contraction [8]. Additionally, α-actinins, interact with other proteins to participate in wide-ranging signal transduction complexes which precipitate physiological change [9].

In humans α-actinin-2 and α-actinin-3 are encoded by their separate genes ACTN2 and ACTN3, respectively. ACTN2 is

expressed in all muscle fibres, including cardiac muscle and the brain, whereas ACTN3 is restricted mainly to type II fast-twitch fibres where they initiate force-generating glycolytic energy production at high velocity, and are therefore responsible for the development of factors such as speed, power and strength, the major determinants of superior athletic performance [10]. A variation of the ACTN3 gene [11] has been described which resulted in the replacement of an arginine (R) with a stop codon (X) at amino acid 577 (R577X). As a result, there are two contrasting alleles which constitute the ACTN3 gene; the 577R allele, which is the normal functional variation of the gene, and the 577X allele, which results in extensive α-actinin-3 protein deficiency in the population at large (null X allele). Whilst the RR genotype formulates speed, power and strength [12], the XX genotype is thought to influence endurance performance [13], although the results of studies are not exclusively compatible between these two points of view [14].

Two copies of the ACTN3 gene are inherited thus three genotypes are expressed: RR, RX and XX. The population distribution of the ACTN3 gene has been widely reported; for example, Greek male and female adolescents presented as RR=34%, RX=48%, and XX=18% [15]. Similar findings have been published for other studies [16]. The lowest frequency of the X allele has been found in Kenyan, Nigerian and South African groups (8 -11%) [17]. On the basis of playing position, Rugby Union forwards have presented as RR=28%, RX=53% and XX=19%, and backs RR=31%, RX=55%, and XX=14% (P=0.822) [18].

Rugby Union football is a team game comprised of 15 players and 7 substitutes; it is played for two periods of 40 minutes in each half. Eight players are classified as forwards and seven as backs. Although these two groups have varying playing responsibilities, they also have common basic playing techniques and patterns of play. The game, physiologically, involves a variety of skills and techniques, performed at both high and low intensity effort [19-22]. One of the desirable characteristics of the game is the controlled dynamic and assertive physical contact permitted between opposing teams.

At a senior level of performance, there is serious consideration given to the identification of intuitive characteristics of the ACTN3 gene phenotype which will maximise playing performance. The primary aim of the present study, therefore, was to identify the magnitude of differences between playing positions in the components of body composition; these being BMC, fat mass and lean tissue mass, in the arms, trunk and legs of players who carried the ACTN3 gene.

Methods

Study design and participants

A case-control study was carried out using a candidate gene approach. Individuals belonged to a homogeneous group (age, gender, ability and ethnicity) of developing young adult players who were members of a University Centre of Excellence for Rugby Union in the UK. All players were engaged systematically in skill and conditioning training programmes relevant to their natural playing position. Players (n=55) were assigned according to playing groups as follows; props/hookers (P/H) (n=14), locks/back row (L/BR) (n=16), scrum/outside-halves (SH/OH) (n=11) and centres/wings/full-backs (C/W/FB) (n=14).

Genotype analysis

In short, players provided a 5-ml saliva sample into a sterile container. Specimens were stored at 4°C until buccal cell DNA extraction was carried out (Qiagen, QIAamp DNA Micro Kit). For the ACTN3 gene the following two primers were used:

5'-AGGCTTCTGACCCACTACG-3´ and

5'-CGAGATTTCAGGGTGGTCACA-3´

Polymerase Chain Reaction (PCR) was used to amplify the resulting fragment. The primers generated a PCR product of 628 bp in length which spanned the R577X Single Nucleotide Polymorphism (SNP) and contained a ubiquitous DdeI digestion site upstream of R577X. Aliquots of these digested products were subject to electrophoresis using a 2% agarose gel for size discrimination. Allele identification was visualized using ethidium bromide staining.

Anthropometry and body composition

Height was measured on a Harpenden stadiometer to the closest 0.1 cm using the stretching-up technique, and body mass to the nearest 0.1 kg on a digital weighing scale. DXA was measured using a Hologic QDR Discovery fan beam model [23,24]. Subjects wore light clothing and were asked to remove metal or other dense materials from the body. Each subject was aligned with the long axis of the scanning couch and requested to remain perfectly still during the scan. The same observer positioned the subjects, identified the measurements and carried out the digital analysis. The digital image of each subject was partitioned into the head (not used in the analyses), left and right arms, trunk, and left and right legs, using the algorithm provided by the DXA manufacturer (version 12.4 : 3). The quality of images and analyses was confirmed by a second independent observer. Calibration was carried out and checked on a daily basis.

Statistical analyses

There were two null hypotheses. The first was that there were non-significant differences between DXA tissue masses (BMC, fat mass and lean tissue mass) in the arms, trunk, and legs, according to playing position. The second was that there were non-significant differences in BMC, fat mass, and lean tissue mass, between the left and right arms and the left and right legs. ANCOVA was carried out using, height, body mass, and age as potential covariates. The covariate/s used in each case was/were confirmed by the relationship between the covariate and the dependent variable/s using the coefficient of correlation. Normality of saved residuals was confirmed using the Anderson-Darling technique and homogeneity of variances with Levene's test. The remaining assumptions of ANCOVA were found to be satisfactory [25].

The analysis of DXA tissues relative to left and right arms and left and right legs was carried out with MANOVA. In the multivariate tests Pillai's trace was the statistic used on the grounds of its acceptable power and robusticity against violations of assumption. Univariate tests used a Bonferroni-type adjustment (alpha/number of tests) in identifying differences between dependent variables, and Partial Eta Squared reported the proportion of variance accounted for by the dependent variable [25]. The alpha level for significance was set at $p<0.05$ unless otherwise stated. Raw values are reported as means ± SD; adjusted data as means ± Std error. The study was approved by the School of Sport Ethics Committee: all participants volunteered and gave their written informed consent.

Results

The descriptive characteristics of age, height and body mass are presented in Table 1 as raw, rather than adjusted data. This was deliberate in order to quantify and demonstrate the absolute size of players; these measurements therefore were analysed by ANOVA. Age between groups was fairly homogeneous and not statistically significant ($p=0.476$).

The L/BR were the tallest of all players (187.9 ± 5.6 cm), and significantly taller than P/H (180.5 ± 4.1cm, $p=0.002$), SH/OH (175.6 ± 6.2 cm, $p=0.001$) and C/W/FB (181.9 ± 5.9 cm, $p=0.018$). Differences occurred also between SH/OH and C/W/FB (175.6 ± 6.2 vs. 181.9 ± 5.9 cm, $p=0.027$).

Body mass was greatest in P/H (103.2 ± 7.2 kg) and significantly heavier than SH/OH (79.9 ± 7.0 kg, $p=0.001$) and C/W/FB (84.2 ± 5.2 kg, $p=0.001$). The L/BR (99.1 ± 9.9 kg) were also significantly heavier than S/H/OH (79.9 ± 7.0 kg, $p=0.001$) and C/W/FB (84.2 ± 5.2 kg, $p=0.001$).

ANCOVA adjusted BMC was comparable for all playing positions. The left and right arms were similar in size (~0.23 kg; $p=0.987$ and $p=0.462$ for left and right arms respectively). The trunk had the largest values of all positions (0.89 to 0.97 kg, $p=0.433$), succeeded by the left and right legs respectively, which themselves were reasonably comparable (~ 0.54 to 0.60 kg, $p=0.585–0.611$). The sum of values for individual positions was consistent with subtotal values, although none were statistically significant ($p=0.594$).

Figure 1 illustrates the pattern of adjusted fat mass for arms, trunk and legs. There were significant differences between P/H and L/BR in both the left (1.02 ± 0.05 vs. 0.82 ± 0.05 kg, p=0.014) and right (1.07 ± 0.06 vs. 0.87 ± 0.05 kg, $p=0.014$) arms respectively, but no corresponding differences between positions in the left ($p=0.267$) and right ($p=0.320$) legs.

Fat mass is usually very variable, particularly at the trunk, which had the greatest amount of fat of all segments (Figure 1). There were significant differences between P/H and L/BR (9.68 ± 0.59 vs. 6.63 ± 0.50 kg, $p=0.0001$) and between P/H and C/W/FB (9.68 ± 0.59 vs. 6.73 ± 0.55 kg, $p=0.013$). Subtotal values were of the order of ~19 kg for P/H, ~ 17 kg for SH/OH, ~15 kg for L/BR and C/W/FB respectively ($p=0.002$).

There were non-significant differences in adjusted lean tissue mass between playing positions in any of the segments. The hierarchy of values were the trunk (~35 kg, $p=0.907$), followed by the legs (~12 kg , $p=0.115–0.383$) and arms (~ 5 kg, $p=0.203–0.073$). Positional variability within segments provided a high degree of consistency. Subtotal values for playing positions were ~70.0 kg ($p=0.303$).

A one-way MANOVA was carried out between left and right arms and left and right legs to identify whether differences existed between BMC, fat mass and lean tissue mass. A statistically significant difference was identified in fat mass between the left and right arms (Pillai's trace=0.244, F=2.320, $P=0.039$, partial eta squared=0.122). When using a Bonferroni adjusted alpha level of 0.025, the significant difference was confirmed in fat mass between left ($p=0.006$) and right ($p=0.008$) arms. Non-significant differences were evident between left and right legs in either BMC ($p=0.585$ *vs.* 0.611), fat mass ($p=0.267$ *vs.* 0.320) or lean tissue mass ($p=0.115$ *vs.* 0.383).

Discussion

The present generation of rugby players, seek to identify and take advantage of even the smallest characteristic related to their playing position, physical structure, and body composition in the pursuit of improved individual and team performance, irrespective of the subtlety and variety of playing ability that may be lost to the game.

Raw descriptive characteristics of players are given in Table 1. As a homogeneous group there were non-significant differences in age between positions, although differences did exist in height and body mass respectively, particularly between forwards (P/H and L/BR) and backs (SH/OH and C/W/FB).

Table 1: Descriptive characteristics of age, height and body mass according to playing position.

	P/H	L/BR	SH/OH	C/W/FB	*P*
Age (yr)	21.0 ± 3.8	20.3 ± 1.3	19.9 ± 2.0	19.7 ± 0.9	*0.476*
Height (cm)	180.5 ± 4.1a	187.9 ± 5.6abd	175.6 ± 6.2bc	181.9 ± 5.9cd	*0.001*
Body mass (kg)	103.2 ± 7.2ab	99.1 ± 9.9cd	79.9 ± 7.0ac	84.2 ± 5.2bd	*0.001*

P/H = props/hookers; L/BR = locks/ back row; SH/OH = scrum half/outside half ; C/W/FB = centre/wing/ full back

Height
a: P/H vs L/BR, *P = 0.002*
b: L/BR vs SH/OH, *P = 0.001*
c: SH/OH vs C/W/FB *P = 0.027*
d: L/BR vs C/W/FB *P = 0.018*

Body Mass
a: P/H vs SH/OH, *P = 0.001*
b: P/H vs C/W/FB, *P = 0.001*
c: L/BR vs SH/OH *P = 0.001*
d: L/BR vs CWFB, *P = 0.001*

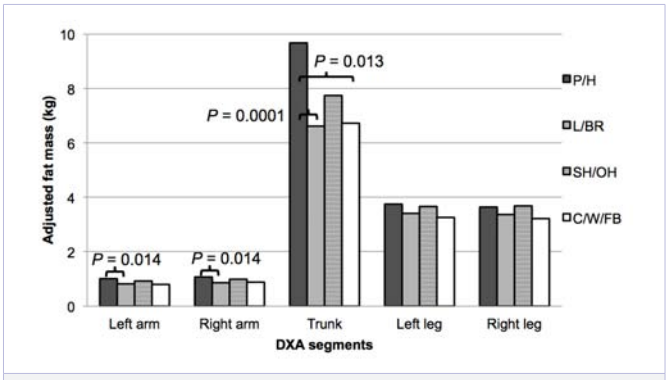

Figure 1: Adjusted fat mass for arms, trunk and legs according to playing position.

The positions of loose-head prop, hooker, tight-head prop (front row) and the two locks (second row), are primarily ball-winners at set-pieces of play such as the scrummage and line-out respectively; this is exemplified by the greater mass of the props, and the height of the locks. It seems reasonably clear that the front five players are selected for their position based partly on their body size and shape.

The scrummage, in particular, is a fundamental and dynamic part of the game. Technically, it is a complicated and dangerous manoeuvre, since each front row, and particularly the hooker, seeks to gain an advantage to win the ball. To minimise the danger of injury which can occur during the scrummage, the International Rugby Board has introduced a strict engagement sequence of 'crouch, bind and set'. Both front rows must now maintain the bind position until the referee calls 'set'. At this point the two packs engage physically and the scrummage becomes part of play.

At the line-out the aim is to support the jumper, usually one of the tallest players, and to close any gaps that occur in defence. Back-row players (flankers and No 8) should provide an aggressive contribution to the scrummage, as well as being able to defend, support and initiate structured attacking plays from the scrum. There is, therefore, a considerable requirement for strength, power, speed *and endurance* in all forward positions, which will enable players to complete the game in an attacking and positive manner; this characteristic is especially important.

Developmental and philosophical changes will occur with time, and may alter the structural configuration and style of individual players. A recent study of professional English Premiership Rugby Union players [26] found that during the years 2002–2011, selected players were taller, heavier, and younger, than in previous years. Outside-halves were significantly taller and heavier; props taller and younger; and back-row forwards heavier.

Bone mineral content

Bone mineral content refers to the total amount of bone mineral (g) within a measured bone area. The main bone mineral is *calcium hydroxyapatite* [27]. Areal BMD is obtained by dividing BMC by the area of bone scanned, and is expressed in gm/cm^2. There were non-significant differences between playing positions in any of the variables in BMC. Right and left arms were very similar in size (~ 0.23 kg) as were right and left legs (~ 0.60 kg). The largest dimension was the trunk (~ 0.93 kg). This lack of difference between positions is due in some measure to the statistical adjustment of body size, since absolute size is related to total body mass. Thus there would appear to be no strategic advantage in adjusted BMC differences between playing positions, although the value of BMD itself is one of the best predictors for the risk of osteoporotic fractures. Bones are dynamic tissues which provide the architecture for individual size and shape. They are replaced with newly deposited bone as they age or become damaged, and provide important essential functions, such as mechanical protection for internal organs, and locomotion of the limbs.

Yang et al. [28] demonstrated that α-actinin-3 deficiency was associated with a reduction in bone mass in both humans and mice. α-actinin-3 is known to be expressed in bone precursor cells, thus a loss of α-actinin-3 will be associated with a significant reduction in BMD. Femurs isolated from 6-month old female mice were scanned using quantitative computed tomography (Q-CT) analysis. Total BMC was significantly reduced (6-9%) in knock-out (KO) mice compared with wild-type (WT) mice (*p<0.01-0.001*). Femurs isolated from 2 month old male mice demonstrated larger reductions in total BMC (14-20%) compared with WT mice (*p<0.01–0.001*), confirming initial observations that KO mice have a reduced bone mass. Decreases in long bone structural mass are inclined to reduce the resistance of these bones to fracture or injury. The conclusion suggested that ACTN3 contributed to the regulation of bone mass as a result of its management of bone turnover.

The BMC and BMD of Tunisian Rugby Union players who had played competitive rugby for 13 years were analysed by Elloumi et al. [29]. There were three groups; forwards (n=10), backs (n=10) and non-active controls (n=29). Total body mass, lean mass, and body fat mass were significantly greater in players than controls. There were also larger BMC and BMD values in forwards than backs at the legs, arms and pelvis. It was concluded that long term participation in Rugby Union players commencing at puberty, were associated with definitive increases in BMC, BMD and bone size at all skeletal sites. Some of these increases will have developed during the increased growth period during adolescence.

Fat

The amount of fat (g) in soft tissue is estimated from the assumed constant attenuation of pure fat and bone mineral-free lean tissue. Since DXA provides the proportion of fat and lean in each pixel, it is fat, rather than adipose tissue, which is being measured [23].

Adjusted fat mass for the DXA segments relative to position for ACTN3 players are illustrated in Figure 1. Differences in adjusted fat mass in the left arm were significantly dissimilar between P/H and L/BR, as they were in the right arm (*p=0.014*). The arms are functionally very important for skills such as passing and

receiving, handing-off, turning opponents in tackles, rucks and mauls, and attacking and defensive tackling, but are probably more important in the binding which occurs between the props and the hooker at the scrummage. This process is fundamental in maintaining a technically correct scrummaging position.

The trunk, with players having a physique conditioned for upper-body strength, particularly back, shoulder and neck strength, is important during the scrummage, since a great deal of the force is directed through the props [30]. The principal site of fat deposition in males is usually the abdomen. Conventionally, props are the heaviest of players, largely because of their size, morphological shape and playing requirement. In the present study, despite adjustment for body size, P/H still had a greater amount of fat mass at the trunk (9.7 kg) compared with either L/BR (6.3 kg, *p=0.0001*) or C/W/FB (6.73 kg, *p=0.013* (Figure 1). This increase in superficial body mass is absolutely crucial during attacking or defending against loose-play incidents adjacent to the try line.

Nindl et al. [1] emphasised the point that abdominal fat had a higher concentration of beta-adrenergic receptors and a greater degree of lipoprotein lipase activity than fat at other anatomical locations, which inferred that regular and consistent training could lead to preferential mobilization of abdominal fat as a source of fuel. However, the largest decline in fat mass occurred at the suprailiac skinfold. When body fat was examined by quintiles, the fatter subjects had lost a significantly greater proportion of arm fat than lean subjects. Even for the leanest subjects, there appeared to be restricted fat loss from the arm, despite continued fat losses at the trunk and legs. As a consequence, it was insinuated that the mobilization of arm fat might occur early during the process of initial or continuous fat metabolism, which would be advantageous whenever circumstances were appropriate.

In the present study the proportional amount of fat in left and right legs was virtually identical (Figure 1). The mean fat value of all playing positions for the left (20.9%) and right (20.7%) legs was not significant. Clearly, the legs participate in a wide repertoire of skills in both forwards and backs. In backs where running is pre-eminent, unnecessary amounts of fat mass in the legs will simply increase inertia, adding to the energy requirement when running at maximum speed, or alternatively, when providing the necessary leg power during attacking and defensive strategies of forwards.

If speed was the superior factor required, then the two wing players, and possibly the full-back might be expected to carry the RR genotype. In the present group of ACTN3 players there were just 22% of players with the RR genotype; 60% had the RX genotype, and 18% the XX genotype. Similar genotype proportions have been presented elsewhere by Bell et al. [18]. There seems no logical reason why players with the RX genotype (which are in the majority), suitably conditioned physically for their particular skills, should not be able to compete for speed or power with a player carrying the RR genotype. The decisive influences in these circumstances would be the net effect of the nature and length of the training programmes undertaken.

Lean tissue mass

Lean Tissue Mass (LTM) consists of lipids (triglycerides), extracellular and intracellular water, total body protein, carbohydrates, and soft tissue minerals.

Muscle fibre types are physiologically distinct from each other. Originally, they were classified simply as type I (slow twitch), and type II (fast twitch) fibres, but because of their different structural and functional characteristics type II fibres were divided into two sub-groups; types IIa (fast red) and IIx (fast white). On average, most muscles are composed of approximately 50% type I fibres, 25% type IIa fibres and 25% IIx fibres. Adjusted LTM of the arms, trunk and legs were not significantly different between playing positions in any of the segments (*p=0.073–0.907*). The left and right arms had mean values of 4.49 ± 0.2 and 4.68 ± 0.2 kg, respectively, with larger mean values found in the left and right legs (12.47 ± 0.4 and 12.5 ± 0.4 kg, respectively). The trunk had the greatest amount of LTM with a mean value of 35.1 ± 0.4 kg.

From a total sample size of 90 young adults, biopsy samples from the right vastus lateralis of two sub-groups of ACTN3 subjects (22 XX and 22 RR genotypes) were taken by Vincent et al. [31] to identify the relationship between the polymorphism, distribution of muscle fibre type, and knee extension strength. The average relative fraction of type I, IIa and IIx, was 52%, 36% and 12% respectively for the total group. Genotype specific differences for type IIx were ~5% higher in the RR than the XX group (*p<0.05*); the relative muscle surface area covered by the type IIx fibres was also significantly higher in the RR group (*p< 0.05*). The RR allele showed significantly higher relative dynamic quadriceps torques at 300^0/s and confirmed that the R allele enhanced high velocity muscle tasks in healthy young adult males. There was also a proposal arising from this study that ACTN3 variation could be one of the genes contributing to the heritability of fibre type distribution as a consequence of its relationship with the protein phosphatase, calcineurin.

The association between α-actinin-3 deficiency and human athletic performance has been examined by MacArthur et al. [13] using the α-actinin-3 KO mouse. KO mice displayed normal morphological characteristics, behavioural activity and fibre type proportions compared with WT mice. KO mice performed as well as WT mice in a simple motor performance test for weakness and fatigue sensitivity, indicating that α-actinin-3 deficiency was within normal limits. However, KO mice were found to have significantly lower average grip strength values than WT mice in males (7.4%) compared with females (6.0%).

Isolated KO muscles generated lower maximal force, but demonstrated accelerated recovery from fatigue. Further analyses of KO muscle displayed a number of phenotypic changes which included a reduction in the diameter of fast-twitch fibres and alterations in enzymatic activities associated with aerobic metabolism. Physiological evaluation of isolated muscles demonstrated that α-actinin-3 deficiency was associated with slower twitch half-relaxation times. MacArthur et al. [13] concluded that their findings were compatible with a

transformation in the characteristics of fast-twitch fibres towards those conventionally represented by slow-twitch fibres. That is, the R577X allele was associated with a reduced performance in power but an increased capacity in endurance.

It is questionable whether a squad of Rugby Union players, at any level of performance, would consist wholly of ACTN3 gene carriers. Nontheless, appreciable physiological adjustments would need to be made using unambiguous training programmes, to provide characteristic aerobic and anaerobic requirements for individual players, whatever their genotype; this in part, is the nature of the physiological mechanisms underlining the ACTN3 gene [13].

The molecular basis for the mechanisms of the ACTN3 gene in fast-twitch muscle fibres is not yet fully understood; one possibility is the calcium-and calmodulin-dependent protein phosphatase, *calcineurin,* which is indirectly associated with sarcomeric α-actinins at the Z-disc through their reciprocal binding to *calsarcins*. Calsarcin-1 is specific to the development of adult cardiac and slow-twitch muscle fibres, while calsarcin-2 is limited to fast-twitch muscle fibres; calsarcin-3, likewise, is engaged developmentally in skeletal muscle and enhanced in fast-twitch muscle fibres [32,33].

Calsarcin-2, which specifically accommodates fast-twitch muscle fibres, inhibits calcineurin signalling *in vivo,* therefore a deficiency of calsarcin-2 in mice demonstrate a marked improvement in endurance performance, and adaptation of those muscle characteristics, which are a feature of slow-twitch fibres. In summary, the outcome of the above [32,33] together with related studies [34-37] advocate molecular mechanisms which outline pathways of motor nerve activity, to adjudicate selective changes in gene expression and substrate utilization responsible for promotion of the specialized requirements of slow-and fast-twitch myofibres respectively. Thus, it seems likely that as a result of their training regimes the players themselves will ultimately be responsible for their ability to participate at a specific level of performance.

Conclusion

The main findings of the present study of the ACTN3 gene in Rugby Union players adjusted for body size comprised 22% RR, 60% RX, 18% XX genotypes. Players in the second and back rows were significantly taller than other positions and body mass significantly greater in forwards than backs. Non-significant differences occurred between playing positions in adjusted bone mineral content or adjusted lean tissue mass. Adjusted fat mass reflected differences between left and right arms, but not between left and right legs. Differences between components of body composition were discussed in relation to the ACTN3 gene. At a developmental level of the game an understanding and practical application of the structural, physiological and body composition characteristics of individual players will facilitate individual and team accomplishment, efficiency of individual training and conditioning, and nurture the potential of young adult players.

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