The Genetics of Athletic Performance

DNA Sport

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DNALYSIS BIOTECHNOLOGY

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1. Genetic variants associated with injury susceptibility

Soft tissue injuries are a common occurrence in competitive and recreational sports and activities. These injuries can be due to spontaneous ruptures (acute onset injuries) or can be chronic overuse injuries due to repetitive strain. Injury to any soft tissue is considered a multifactorial condition that is caused by a complex interaction between intrinsic risk factors, including genetic risk factors, and extrinsic, non-genetic, risk factors.

By the identification of genetic risk factors and the various biological processes that contribute to injury development, therapeutic strategies for injury prevention and treatment can be established.

1.1. Collagen 1 Alpha 1 (COL1A1): Sp1 G>T

Type I collagen is the most abundant protein in the body and is a major structural component of bones, tendons, ligaments and joint cartilage. Type I collagen is a heterodimer that consists of two α1 chains and one α2 chain, encoded by the *COL1A1* and *COL1A2* genes respectively. Along with small amounts of type III and type V collagen, type I collagen molecules assemble into a highly organised array that results in a mature connective tissue structure, essential for tensile strength.[1]

Variations within the *COL1A1* gene have been linked to risk of soft tissue injury as well as alterations in bone mineral density, osteoarthritis and fracture risk.

The rare TT genotype of the functional *COL1A1* Sp1 G>T SNP has been linked to a substantially reduced risk of cruciate ligament and shoulder dislocation injuries in comparison to the GG genotype.[2] In agreement, Posthumus *et al.* (2009) showed that the TT genotype was significantly under-represented in an injured group (with anterior cruciate ligament ruptures) in comparison to matched active controls,[3] demonstrating the possible protective role of this genotype. Combined analysis of the *COL1A1* Sp1 G>T SNP from independent studies further advocates the protective role of the TT genotype in acute soft tissue ruptures,[2–5] indicating the importance of this SNP being included in a model to determine injury risk.

Functional analysis shows that when the *COL1A1* Sp1 T allele is present, an increase in the affinity for the transcription factor Sp1 results in increased *COL1A1* gene expression.[6] The consequence of this is not yet fully understood, however it is hypothesized that an increased production of COL1A1 in TT individuals increases the tensile strength of tendons and ligaments, while the resulting imbalance between the α1 and α2 chains conversely impairs bone strength.[2,6]

1.2. Collagen 5 Alpha 1 (COL5A1): BstUI C>T

COL5A1 encodes the α1 chain of type V collagen, a minor constituent of soft tissues such as tendons and ligaments that is essential for life. Type V collagen is a fibrillary collagen that intercalates with type I collagen to form heterotypic fibres. Along with other proteins, type V collagen plays an important structural role in regulating fibrillogenesis (fibril formation) and fibril diameter within tendons, ligaments and other connective tissues.[7]

The COL5A1 BstUI C>T polymorphism has been associated with injuries such as Achilles tendinopathy and anterior cruciate ligament ruptures as well as range of motion and endurance performance. [8]

The CC genotype of the COL5A1 BstUI polymorphism has been associated with protection from soft tissue injuries. In 2006 Mokone *et al.* found that the CC genotype was associated with protection from chronic Achilles tendinopathy, with these individuals being less likely to develop the symptoms associated with the injury.[9] This study was replicated in South African and Australian populations yielding the same results.[10] When this polymorphism was investigated as a risk factor for anterior cruciate ligament ruptures, the CC genotype was significantly over-represented in active female controls when compared to the ACL injured counterparts.[11] More recently the COL5A1 BstUI T allele has been linked to an increased risk of tennis elbow.[12] In 2017, Brown *et al.* further verified the role of COL5A1 as a genetic determinant of injury risk.[13]

The function of the COL5A1 polymorphism has been associated with altered mRNA stability, affecting COL5A1 gene expression. The T allele is linked to increased COL5A1 mRNA stability and the C allele to lower synthesis of the alpha 1 chain of type V collagen. [14] It is suggested that the relative content of type V collagen in soft tissues alters the mechanical properties and injury susceptibility.[8] Animal models have reported an association with the increase in content of type V collagen and a decrease in fibril diameter and biomechanical properties of tendons.[15]

COL5A1 BstUI C>T has also been associated with range of motion.[16] A 2010 study by Brown *et al.* showed that older individuals with the CC genotype had a greater range of motion and protection from injury.[17] Due to the association between increased stiffness (decreased range of motion) and improved running economy, the COL5A1 gene was investigated for an association with athletic performance. COL5A1 Sp1 TT individuals were found to have a faster marathon completion in an Ironman triathlon in comparison to CT and CC individuals.[18] An additional study showed that TT individuals were less flexible and completed a 56km ultramarathon significantly faster than participants with TC and CC genotypes.[19]

From the collective studies, the hypothesis is that the TT genotype results increased production of COL5A1, altered mechanical properties of musculoskeletal soft tissues, increased risk of injury, reduced joint ROM (flexibility), and increased endurance running ability.[8]

1.3. Growth Differentiation Factor 5 (GDF5): 5' UTR C>T

GDF5 encodes the Growth Differentiation Factor 5 protein, part of the bone morphogenetic protein (BMP) family and transforming growth factor β (TGF-β) superfamily. GDF5 is involved in tissue growth, development, homeostasis and healing, including that of bones, cartilage and various musculoskeletal soft tissues.[20] This gene has been linked to mechanical strength, with animal models showing significantly weaker tendons with lowered collagen content in GDF5 deficient mice.[20] Conversely, the addition of exogenous GDF5 showed improved ligament and tendon repair, tensile strength and biomechanical testing in rat and canine models.[21,22]

The functional polymorphism within the 5'UTR of GDF5 has been shown to influence susceptibility to soft tissue injuries as well other multifactorial disorders.

In 2010 Posthumus *et al.* investigated the association of GDF5 with risk of developing Achilles tendinopathy. Individuals with the TT genotypes were identified as having a 2-fold increased risk of injury.[23] The T allele of this GDF5 polymorphism is correlated with reduced expression of the GDF5 gene within soft tissues. In line with this, the collective studies suggest that the reduced expression of this gene with the GDF5 T allele leads to an increased risk of exercise related soft tissue injuries.[24]

The GDF5 T allele has also been strongly linked to an increased risk of osteoarthritis, critically of the knee.[24–27]

2. Genetic variants associated with recovery from exercise

The recovery genes included in DNA Sport are the best example within this genetic panel of the need to integrate training and nutritional advice when supporting an athlete's health and fitness. Two important biological areas are considered with regards to recovery: inflammation and oxidative stress.

Although not generally considered within sports science or sports nutrition, systemic inflammation and oxidative stress that results from heavy training and other lifestyle stresses, can greatly affect an athlete's health and rate of recovery between training bouts and sessions. Knowledge of the genes that code for these variables can provide athletes with an enormous opportunity to improve their specific health and training responses via nutrition, stress management and nutraceuticals. Six genetic polymorphisms for recovery are included in the DNA Sport test: they have been identified as those with strong scientific credibility and those which could make a difference to the training, nutrition and lifestyle interventions of an athlete, based on knowledge of their genes.

2.1. Interleukin-6 (IL-6): -174 G>C

IL-6 is a cytokine secreted by T cells and macrophages, which stimulates an immune response to trauma such as strenuous exercise, leading to inflammation in the muscle and fatty tissue. IL-6 stimulates energy mobilisation, causing an increase in body temperature. IL-6 is also a myokine (muscle cytokine) and regulates the gene encoding C-reactive protein (CRP). The immune system of athletes is affected by the intensity and duration of exercise training - the inevitable muscle damage results in an inflammatory repair process, which is mediated by inflammatory cytokines such as IL-6. Overtraining syndrome (OTS) has been hypothesised to be caused by excess cytokine release during exercise, resulting in a chronic inflammatory state.

The C allele has been linked to an increased IL-6 and CRP inflammatory response to exercise, which may induce more pronounced fatigue and prolong recovery times. Perhaps because IL-6 levels are hard to measure in the plasma, there was some discrepancy in the literature about whether the C or G allele is associated with higher IL-6 levels, but the majority and best evidence points towards the C allele.

In a study of 54 military recruits during their 8-week basic training, it was noted that acute exercise increased plasma IL-6 levels more in subjects with the CG genotype compared to GG homozygotes.[28] Interestingly, subjects with the CG genotype also made the greatest gains in VO2max. In support of this, Yamin *et al.* (2008) found that subjects with the C allele experienced greater creatine kinase (CK) levels than GG homozygotes (3-times the risk of a massive CK response) after eccentric training, showing that the C allele may be a risk factor for exercise-induced muscle injury.[29] The IL-6 gene has been shown to be amongst a number of genes that were predictive of sports performance,[30] and the G allele seems to be the most common in power athletes compared to control and endurance athletes,[31] which may be due to improved muscle repair after eccentric exercise.[29]

Under-performance syndrome (UPS), another name for overtraining syndrome, is thought to be influenced by cytokine sickness, an over-production and/or intolerance to IL-6 and other cytokines.[32]

This suggestion was supported by Robson-Ansley *et al.* (2007) who noted that an acute period of intensified training can suppress the innate immune system and chronically increase IL-6 levels.[33] These elevated cytokines can in-turn increase fatigue and malaise, which are related to the cytokine theories of UPS. The IL-6 genotype can also influence glucose levels: McKenzie *et al* (2004) found that baseline fasting glucose levels were higher in the CC genotype compared to carriers of the G allele: 6 months of aerobic training successfully decreased the glucose area under the curve during an oral glucose tolerance test, but a significant decrease only occurred in the GG genotype.[34]

2.2. Interleukin 6 Receptor (IL-6R): Asp358Ala (A>C)

Interleukin 6 receptor (IL-6R) is a type I cytokine receptor, made up of a protein complex consisting of an IL-6 receptor subunit (IL-6R) and a signal transducer (gp130). The IL6R gene specifically encodes this IL-6R subunit, which in-turn influences IL-6 cytokine action. Proteolytic cleavage of the membrane-bound IL-6R protein leads to the generation of a soluble form of IL-6R (sIL-6R), which is able to bind to IL-6. The resulting IL-6/sIL-6R complex is also capable of binding to gp130 and inducing intracellular signalling,[35] increasing the biological activity and half-life of IL-6.[36] The resulting IL-6-induced inflammatory response has been linked to fatigue during exercise, the ability to recover from training sessions and potentially the risk of overtraining. Regular exercise reduces baseline long-term inflammatory biomarkers, but single high-intensity exercise sessions will acutely increase local inflammation.

An A to C SNP on the IL-6R gene has been shown to have an effect on sIL-6R and consequently IL-6 levels. Subjects with the CC or AC genotypes may have higher levels of sIL-6R and IL-6, thereby increasing the acute inflammatory effects of exercise. Individuals with these genotypes are generally advised to increase their recovery time between training bouts and training sessions, regularly check their inflammatory biomarkers and increase anti-inflammatory nutrition support.

It has been demonstrated in a study of 70 subjects that C allele carriers for the IL-6R gene have significantly higher baseline sIL-6R levels.[37] In African- and European-American subjects, one copy of the C allele has been shown to cause a 1.06 to 1.15-fold increase, and two copies of the C allele caused a 1.2 to 1.43-fold increase in IL-6 levels.[38] It has been clearly shown that IL-6 levels are increased during acute exercise bouts: during one hour of cycling at 90% of lactate threshold, Gray *et al* (2008, 2009) found a 5-fold increase in IL-6 and 1.2-fold increase in sIL-6R immediately afterwards. These levels returned to baseline 1.5 hours after exercise.[36,39] Robson-Ansley *et al* (2011) also showed that the plasma concentrations of IL-6 increased post-exercise when athletes ran at 60% of their vVO2max for 2 hours, followed by a 5km time trial. In this study, when a carbohydrate drink was ingested during the exercise, post-exercise IL-6 levels were significantly reduced.[33] The same investigators tracked 13 cyclists who rode 468 km over a six-day period: IL-6 levels were increased post-exercise on day 1, but remained unchanged for the following five days. sIL-6R, CRP and creatine kinase where unchanged post-exercise on day 1, but were elevated at baseline for the rest of the trial. It was demonstrated that sIL-6R was significantly correlated with CRP and that these inflammatory markers may affect subjective sensations of post-exercise fatigue.[33] The investigators concluded that strenuous,

prolonged exercise stimulated an acute-phase inflammatory response, which was maintained throughout the 6-day event.

2.3. C-Reactive Protein (CRP): 219 G>A

CRP is an acute-phase protein, which rises in response to inflammation in the body: IL-6 and other inflammatory cytokines trigger the synthesis of CRP and fibrinogen by the liver. Its physiological role is to bind to phosphocholine, which is expressed on the surface of dead or dying cells in order to activate the complement system, which is part of the innate immune system. CRP is used as a biomarker for acute or chronic inflammation. Regular exercise and favourable dietary habits can reduce baseline long-term inflammatory biomarkers (such as CRP), whereas single high-intensity exercise sessions will acutely increase local inflammation.

The CRP AA genotype, which is only found in 12% of the population, has been associated with lower levels of CRP compared to carriers of the G allele, who make up the majority of the population (88%).

Obisesan *et al* (2004) have shown that AA homozygotes have significantly lower CRP levels (40%) than G allele carriers and that CRP levels decrease significantly with 24 weeks of exercise training and a low-fat diet (although GG genotypes still had the highest CRP levels).[40] This has been confirmed by Eiriksdottir *et al* (2009), who found that carriers of the G allele had significantly higher CRP levels than non-carriers.[41] Kullo *et al* (2007) have shown that higher circulating levels of IL-6, CRP and fibronectin are associated with lower VO2max levels after controlling for other variables.[42] This observation was confirmed by Kuo *et al* (2007) in a huge study of 1438 healthy adults.[43] When monitoring the recovery from an Ironman triathlon event, Neubauer *et al* (2008) found that CRP levels were raised post-exercise, along with several other immune and inflammatory markers. By 19 days post-event, most markers were back to normal except for CRP and myoglobin, which were still slightly raised, indicating low-grade inflammation for several days after such a large exercise bout.[44]

It has been shown by a number of studies that the inflammation induced by eccentric exercise is greater during recovery when subjects eat a high-carbohydrate compared to a low-carbohydrate diet.[45,46] For example, Depner *et al* (2010) took 12 subjects through 6 sets of 10 high-intensity eccentric contractions and found that post-exercise inflammation was reduced on a low-carbohydrate diet,[45] although it is important to remember that a carbohydrate drink during exhaustive exercise has been linked with a decrease in IL-6 levels. [47]

There has been much research examining the use of antioxidants to decrease inflammation. For example, Phillips *et al* (2003) found that after eccentric exercise, subjects consuming antioxidant and fatty acid supplements had significantly lower levels of IL-6 and CRP.[48] However free radicals are involved in signalling roles during training adaptations and so antioxidant supplements may weaken these signals. There is currently much controversy in the antioxidant literature, so caution is generally recommended around high-dose supplementation long-term.[49]

2.4. Tumour Necrosis Factor Alpha (TNFA): -308 G>A

TNF codes for the pro-inflammatory cytokine, tumor necrosis factor (TNF). It is produced chiefly by activated macrophages and is a member of a group of cytokines that stimulate the acute phase inflammatory reaction. The primary role of TNF is in the regulation of immune cells: it is able to induce fever, apoptotic cell death, sepsis (through IL-1 & IL-6 production), inflammation and to inhibit tumour growth and viral replication. Like other inflammatory cytokines, TNF levels increase during and after intensive exercise.

A, the minor allele on the TNFA gene, has been shown to cause 2-fold greater levels of transcription compared to the G form. The A allele is therefore associated with increased circulating TNF levels. The AA genotype, although very rare (2%), has been associated with increases in CRP levels during a 20-week exercise programme: CRP levels are generally expected to decrease with regular exercise training. An A allele carrier, with greater TNF levels, may experience increased levels of fatigue and poorer recovery times.

Like other inflammatory cytokines, TNF has been shown to be increased during and after highly-intensity exercise. During 3 hours of cycling or inclined walking, plasma concentrations of IL-1β, IL-6 and TNF peaked at the end of exercise.[50] IL-1β and TNF were shown to be still elevated 24 hours later. Lakka *et al* (2006) noted that prior to a 20-week exercise programme, the AA genotype had greater baseline CRP levels. What's more, the exercise programme decreased CRP levels less in the AA individuals than other genotypes.[51] Nicklas *et al* (2005) took 213 older or overweight individuals with knee osteoarthritis through an 18 month walking and weight training exercise trial. It was found that walking distance and stair-climb time were better for individuals homozygous for the G allele.[52] Nieman *et al.* (2005) demonstrated that carbohydrate intake during 2.5 hours of cycling at 60% max power decreased plasma cortisol, adrenaline, IL-6 and IL-10 responses, but did not affect TNF alpha levels.[53]

2.5. Superoxide Dismutase 2 (SOD2): 208 T>C (Val16Ala)

The SOD2 gene codes for manganese superoxide dismutase (SOD2), which is a potent free radical scavenger within the cell, especially the mitochondria. The SOD2 enzyme converts superoxide free radicals to hydrogen peroxide. The mitochondria is commonly referred to as the workhorse of the cell and is therefore the site of many oxidative reactions during energy production, so free radicals are generated here. There is evidence that oxidation within the cell contributes to muscular fatigue and extreme exercise can cause increased lipid peroxidation and depleted levels of vitamin E. Long-term training can increase base levels of SOD2, whereas short-term bursts of intense activity will increase oxidative stress.

The SOD2 C allele has been associated with high oxidative stress biomarkers and people with this genotype who consume low levels of fruit and vegetables are at increased risk of developing long-

term disease. These levels of oxidative stress can be reduced by a diet that contains antioxidants from fruit and vegetables and supporting SOD2 activity.

In a study of 231 healthy students, it was found that SOD2 enzyme activity was 33% higher in CT or TT individuals compared to CC individuals and on average, SOD2 activity was 15% higher in females than males.[54] It has been shown that exercise training favourably increases baseline levels of antioxidant enzymes. Garcia-Lopez *et al.* (2007) demonstrated that mRNA levels of catalase, glutathione peroxidise (GP), MnSOD (mitochondrial) and CuZnSOD2 (cytosolic) were increased after 21 weeks of strength training, while endurance training only increased SOD2 and GP mRNA levels.[55] Mastaloudis et al (2004a) studied 22 runners during a 50 km ultramarathon. There were two groups: placebo or antioxidant (1g Vit C, 300mg Vit E).[56] F2-isoprostanes, a measure of lipid peroxidation, which were at similar levels between groups at baseline, increased during the run only in the placebo group. In the same group,[57] DNA damage increased mid-race, but returned to baseline 2-hours post-race.

2.6. Endothelial Nitric Oxide Synthase (eNOS): Asp298Glu (G>T)

The endothelium-derived nitric oxide (NO) plays a key role in the regulation of vascular tone and peripheral vascular resistance. It also has vasoprotective effects by suppressing platelet aggregation, leukocyte adhesion and smooth muscle cell proliferation.[58] "Impaired endothelial function, either as a consequence of reduced production/release or increased inactivation of endothelium-derived vasodilators, as well as interactions of NO with angiotensin, reactive oxygen species and oxidized lipoproteins, has detrimental functional consequences and is one of the most important cardiovascular risk factors".[58]

The T allele has been associated with decreased activity of the eNOS enzyme which is linked to an increase in the presence of free radicals, damaging to tissues.

The T allele affects proteolytic cleavage of the enzyme thereby reducing nitric oxide bio-availability in the blood vessel wall and promoting atherosclerosis, as a result it is associated with atherosclerosis, essential hypertension, end-stage renal disease and pre-eclampsia.[59–61] The T allele has also been associated with a number of cancers.[62,63]

GT and TT genotype individuals might show greater beneficial effects of n-3 PUFA consumption.[60]

3. Genetic variants associated with athletic performance

It is now two decades since the first gene was identified as an indicator of sporting performance.[64] This gene, ACE, has now been extensively studied and been shown to be predictive of endurance and power-oriented performance. Numerous other genes have also been studied and fourteen genetic polymorphisms have been included in the DNA Sport test. They have been identified as those with high scientific credibility and those which can make a difference to the training and lifestyle intervention of an athlete, based on knowledge of his/her genes.

3.1. Angiotensinogen (AGT): Met238Thr (T>C)

The angiotensinogen (AGT) protein, produced mainly by the liver, is an important component of the renin-angiotensin system. AGT is cleaved by renin to form angiotensin I, the precursor of angiotensin II that regulates vascular resistance and sodium homeostasis through vasoconstriction, determining blood pressure. High levels of AGT can lead to an increase in the production of angiotensin II resulting in hypertension.[65]

The Met238Thr (T>C) polymorphism within AGT results in a threonine amino acid, instead of a methionine, at position 238 of the protein. This variant has been associated with higher plasma levels of AGT, blood pressure regulation and the risk of hypertension-associated disorders.[27] AGT Met238Thr (T>C) has also been associated with elite power and strength performance.

When comparing the frequency of the AGT Met238Thr (T>C) variation between top endurance and power Spanish athletes as well as controls, Gomez-Gallego *et al.* (2009) showed that the CC genotype was significantly over-represented in power athletes. This indicated that the CC genotype may favour power sports performance, possibly due to the higher activity of angiotensin II that acts as a skeletal muscle growth gactor.[66] Zarębska *et al.* (2013) repeated this study in Polish athletes and found that the frequency of the CC genotype was significantly higher in power athletes in comparison to control and endurance groups.[67] This AGT polymorphism has recently been investigated with regards to response to a 12 week aerobics program.[68] Participants completed physical power tests before and after the intervention, such as sprint tests and jump tests. All power-related variables improved significantly in response to the aerobic training, however significantly greater improvements were seen in the jump-based power variables in C allele carriers in comparison to TT homozygotes.[68] This indicates that the AGT gene may influence power and strength improvements in response to training.

The C allele has been associated with significantly higher levels of plasma AGT and an increased resting blood pressure.[69] The Heritage Family Study investigated the AGT Met238Thr polymorphism and blood pressure response to an endurance exercise training program. T allele carries showed significantly greater decreases in diastolic blood pressure in comparison to CC homozygotes.[70] Additionally, a longitudinal study demonstrated that regular moderate-intensity exercise attenuates age-related increases in systolic blood pressure in TT homozygotes.[71]

AGT CC homozygotes are more susceptible to left ventricular cardiac hypertrophy with long term exercise training,[72] and even in response to a 17 week training program.[73] The AGT CC and ACE DD genotypes have also been shown to interact in the effect of exercise training on cardiac mass.[74]

3.2. Angiotensin I-Converting Enzyme (ACE): Insertion (I)/Deletion (D)

The angiotensin I converting enzyme (ACE) functions in regulating circulatory homeostasis, through the synthesis of the vasoconstrictor angiotensin II. ACE hydrolyses angiotensin I to angiotensin II, which also drives aldosterone synthesis, and the degradation of bradykinin, a potent vasodilator.

A polymorphism exists in the ACE gene that results in the insertion (I allele) or the absence (D allele) of a 287 base pair fragment. Inter-individual variation in ACE plasma levels can vary as much as a 5-fold, approximately half of this variation is thought to be due to the ACE I/D polymorphism.[75]

The D allele is associated with increased activity of the ACE enzyme, likely causing increased production of the angiotensin II vasoconstrictor and degradation of the vasodilator bradykinin. Higher circulating ACE activity has been significantly associated with baseline quadriceps muscle strength.[76] The I allele on the other hand has been associated with lower ACE activity.

The ACE DD genotype has been associated with greater lean body mass, muscle volume, and percentage of Type II fast twitch muscle fibres.[77,78] Various studies have shown that the D allele is over-represented in power and strength athletes.

The D allele or DD genotype has shown to be over-represented in short and middle distance swimmers.[79–81] Nazarov *et al.* (2001) showed a significantly higher frequency of the ACE D allele in short distance athletes (<1 minute),[82] similarly shown by Papadimitriou *et al.* (2009) in track and field sprinters.[83] Recently, the ACE D allele has been significantly associated with greater standing log jump results.[84] Giaccaglia *et al.* (2008) found following an 18-month walking and light weight training exercise programme involving elderly, obese individuals, those with the DD genotype had greater gains in knee extensor muscle strength in comparison to subjects with the II genotypes.[85] Following a 12-week high speed training program in older woman, Pereira *et al.* (2013) showed that D allele carriers had greater improvements in maximum strength and power.[86]

Individuals with the ACE DD genotype should be aware of an increased risk left ventricular hypertrophy as a result of exercise training.[64]

The I allele on the other hand has been linked to aerobic endurance athletic ability and a greater percentage of type I slow twitch muscle fibres,[78] higher VO2max, greater aerobic work efficiency, improved fatigue resistance, higher peripheral tissue oxygenation during exercise, greater aerobic power response to training, greater cardiac output and maximal power output in athletes.[87]

Myerson *et al.* (1999) showed that the frequency of the I allele increased with increasing running distance in Olympic runners, revealing that the I allele may be associated with improved endurance performance.[79] The ACE I allele has also been linked to elite endurance rowing,[88,89] and shown to be

over-represented in elite cyclists, long distance runners and handball players.[90] Collins *et al.* (2004) noted an association between the I allele and endurance performance of fastest triathlon finishers.[91] Similarly, Shenoy *et al.* (2010) showed a greater frequency of I alleles in triathletes compared to controls.[92] The II genotype is also over-represented in endurance runners,[93,94] with frequency of the allele increasing with running distance.[95] Znazen *et al.* (2016) recently noted that the ACE I/D polymorphism frequency was significantly different between power and endurance athletes, suggesting that this may play an important role in sporting adaption to physical training.[96] A recent meta-analysis by Ma *et al.* (2013) further solidified the role of the II genotype in endurance athlete status.[97]

A 2016 multi-cohort study examined the ACE I/D polymorphism and sprint time in elite athletes. ACE DD genotype carriers had faster 200m and 400m sprint times compared to those with the II genotype. The study found that the ACE D allele, in a dominant model, accounted 1.48 % of sprint time variance. Although multiple genetic variants and environmental factors influence sprint time, a combination of the ACE and ACTN3 gene variants may be the difference between a world record and only making the Olympic final.[98]

3.3. Bradykinin Receptor B2 (BDKRB2): Exon 1 +9/-9 (C>T)

Bradykinin is an endothelium-dependent vasodilator and acts via the Bradykinin B2 Receptor (BDKRB2). ACE is able to degrade the vasodilator bradykinin by forming a complex with BDKRB2, this is thought to play a role in the cross-talk between the renin-angiotensin and the kallikrein-kinin systems.

The absence (-9) of a 9 base pair repeat in exon 1 of BDKRB2 is associated with higher gene transcriptional activity and mRNA expression of BDKRB2 (T allele).[99] Levels of bradykinin may effect skeletal muscle glucose uptake and blood flow during exercise. The BDKRB2 -9 (T) allele has been positively associated with endurance exercise performance, likely due to enhanced skeletal muscle mechanical and metabolic efficiency (the amount of energy used per unit of power output) with this allele.[99]

In 2004, Williams *et al.* showed that the BDKRB2 -9 (T) allele is associated with greater muscle contraction efficiency in healthy individuals as well as Olympic track athletes.[99] This study also showed that a combination of the ACE I allele and BDKRB2 -9 (T) allele yields an additive affect, significantly associated with aerobic endurance capacity.

Saunders *et al.* 2006, showed that the BDKRB2 -9-9 (TT) genotype occurred at a significantly higher frequency in an ironman triathlete group compared to controls. The -9 (T) allele also appears to be associated with a faster finishing time.[100] A higher frequency of the BDKRB2 TT genotype has also been noted in marathon runners.[101]

The BDKRB2 +9+9 (CC) genotype has been associated with greater thirst and fluid weight loss during an Ironman triathlon event; this would not be advantageous for endurance activities.[102]

A subjects' BDKRB2 +9/-9 (C>T) genotype should be interpreted with caution as some studies have failed to replicate the association of this polymorphism with endurance athlete status.[103,104]

3.4. Vascular Endothelial Growth Factor (VEGF): -634 C>G

Vascular Endothelial Growth Factor (VEGF) is an endothelial cell proliferator involved in blood vessel growth. Angiogenesis is a key factor in the physiological adaptation to aerobic exercise, required to match the needs of the active tissue. Levels of VEGF affect peripheral circulation and capillary blood flow, muscle oxygenation and VO_2max (maximal oxygen consumption).[105] VEGF promotes angiogenesis in response to aerobic exercise, an important contributor to training-induced changes in VO_2max and increases in capillary-muscle fibre ratio and capillary density.[106] Coffey et al. (2006) noted and approximate 4-fold increase in muscle VEGF levels in response to both resistance and endurance exercise training sessions,[107] while Gustafsson *et al.* (2005) showed a 178% increase in muscle VEGF mRNA expression following dynamic resistance exercise.[108]

The -634 C>G polymorphism in the promotor region of the VEGF gene has been linked to altered expression of the VEGF protein.[109] This gene variant has been associated with aerobic capacity and endurance athlete status.[105]

The -634 C>G C allele is associated with greater expression of VEGF and a higher VO_2max level at baseline and in response to aerobic training.[105] Ahmetov *et al*. (2008) noted that the frequency of the VEGF C allele is significantly higher in endurance athletes in comparison to control subjects.[110] This was replicated in another large cohort of 1423 athletes and 1132 controls.[111] A correlation is also seen between the VEGF C allele and higher levels of aerobic performance (based on maximum power and VO_2max).[110] The combination of studies suggest that increased VEGF expression, as seen with the C allele, is associated with greater muscle efficiency, advantageous for aerobic performance.

3.5. Nuclear Respiratory Factor 2 (NRF-2); GABPB1: A>G

The GA binding protein transcription factor β subunit 1 (GABPB1), also known as nuclear respiratory factor 2 (NRF2) is one of the two nuclear respiratory factors, which induce mitochondrial biogenesis. The NRF2 transcription factor regulates genes involved in mitochondrial function, influencing respiratory capacity and the rate of ATP production during exercise. The stimulation of mitochondrial biogenesis by exercise is dependent on increased NRF2 gene expression. NRF2 also regulates mitochondrial transcription factor A (TFAM), cytochrome c, and heme biosynthesis proteins.[112]

An A>G variant within NRF2 (GABPB1) has been linked to VO_2max and endurance capacity. In 2007, He *et al.* showed that a greater training response to an 18-wk endurance training program was seen in NRF2 rs7181866 G allele carriers. When three SNPs on the NRF-2 gene (rs12594956, rs8031031 and rs7181866) those with the ATG haplotype had a 57% higher training response, determined by running

economy and VO_2max, compared non-carriers of the particular alleles.[112] Eynon *et al.* (2009) found a significantly higher proportion of the AG genotype in endurance athletes, compared to sprinters, and control subjects. This association was even more pronounced when looking at elite international athletes, suggesting an association between the G allele and endurance athlete status.[113] Maciejewska-Karłowska *et al.* (2012) observed that the frequency of the AG genotype was significantly higher in a group of rowers compared to controls.[114]

The collective studies support the notion that genetic variation within the NRF2 gene belongs to group of polymorphisms that are associated with endurance performance.

3.6. Peroxisome Proliferator-Activated Receptor-Gamma Coactivator-1 (PPARGC1A): Gly482Ser (G>A)

Peroxisome Proliferator-Activated Receptor-Gamma Coactivator-1 (PPARGC1A), otherwise known as PGC-1α, is a co-activator of the PPAR family of proteins and plays an essential role in mitochondrial function and biogenesis, fatty acid oxidation, energy homoeostasis and glucose utilisation, thermogenesis, adipocyte differentiation, angiogenesis, and Type I slow twitch muscle fibre specialization.[115] PPARGC1A is expressed in tissues with high energy demands, where a high mitochondrial density is required. Exercise training results in increased expression of PPARGC1A (preferentially in Type 1 muscle fibres), a key factor in exercise-induced mitochondrial biogenesis.[116]

A Gly482Ser (G>A) polymorphism exists in PPARGC1A that influences expression of this gene; the A allele resulting in decreased levels of PPARGC1A mRNA,[115] consequently associated with reduced aerobic improvements from exercise training. The GG genotype on the other hand has been associated with a greater VO_2max, reflective of aerobic capcity.[115] Interestingly, the frequency of the A allele has been observed to be higher in individuals with Type 2 diabetes.

Lucia *et al.* (2005) showed that the frequency of the unfavourable A allele was significantly lower in world class Spanish endurance athletes in comparison to controls.[117] Individuals with the G allele also had greater improvements in aerobic fitness following a 9 month intervention study in comparison to A allele carriers.[118] Akhmetov *et al.* (2007) observed a higher frequency of the PPARGC1A G allele in athletes in comparison to controls; the G allele also being associated high values of aerobic performance.[119] The high frequency of the G allele in endurance athletes was seen again in a large study combining multiple genetic variants linked to endurance athlete status.[111] This has similarly been repeated in Israeli,[120] Polish and Russian [121] endurance athletes.

Conversely, Gineviciene *et al.* (2016) recently found an association between the AA genotype and strength/power athlete status, with the AA genotype being particularly favourable for powerlifters.[122]

3.7. Peroxisome Proliferator-Activated Receptor-Alpha (PPARA): Intron 7 G>C

Peroxisome Proliferator-Activated Receptor-Alpha (PPARA) transcription factor is a regulator of lipid and glucose metabolism and energy homeostasis in the liver, skeletal muscle and heart. Activation of PPARA promotes the uptake, utilisation, and oxidation of fatty acids and plays an important role in the adaptive response to endurance training. PPARA expression levels are higher in type I (slow-twitch) muscle fibres than in type II (fast-twitch) muscle fibres.[123] Endurance training increases the use of non-plasma fatty acids in energy homeostasis and may enhance skeletal muscle oxidative capacity by influencing PPARA gene expression.

A G>C variation exists in intron 7 of the PPARA gene. The G allele leads to increased expression of PPARA and has been associated with endurance performance. Conversely the C allele is linked to anaerobic (power) performance predominantly reliant on muscle glucose metabolism.[123]

Ahmetov *et al.* (2006) noted a higher frequency of the PPARA GG genotype in endurance athletes, compared to an increasingly higher frequency of the C allele with increasing anaerobic component of physical performance. Individuals with the GG genotype also had a significantly higher percentage of slow twitch muscle fibres in comparison to CC homoxygotes.[123] The same authors found that the frequency of the PPARA G allele was significantly greater in elite rowers, and associated with higher values of aerobic performance, than in control subjects.[119] Maciejewska *et al*. (2011) similarly noted a higher frequency of the G in elite rowers compared to sedentary controls.

The PPARA C allele has been associated with exercise-induced left ventricular growth. Jamshidi *et al*. (2002) noted that individuals with the C allele had a significantly greater increase in left ventricular mass following a 10-week training program in comparison to GG homozygotes.[124]

3.8. Beta 2 Adrenergic Receptor (ADRB2): Arg16Gly (A>G); Gln27Glu (C>G)

The adrenergic receptors are a family regulatory proteins that mediate the physiological effects of the epinephrine and the norepinephrine. ADRB2 encodes the β2 adrenergic receptor which plays a part in the regulation of the cardiac, pulmonary, vascular, endocrine and the central nervous system.[125] Adrenaline acts predominantly via ABRB2 in order to maintain blood glucose levels during prolonged exercise, by promoting glycogenolysis.

A variant that causes an arginine to glycine change at position 16 of ADRB2 (A>G) has been associated with altered vasodilator responses to catecholamines during stress, and so modulates the pressor response (increasing cardiac output) to exercise, as driven by the sympathetic nervous system. A glutamine to glutamate polymorphism at position 27 of the gene (C>G) has been associated with endurance performance and the ability to lose weight with exercise training.

Kochanska-Dziurowicz *et al.* (2013) measured plasma concentrations of adrenaline, noradrenaline and ADRB2 gene expression before and after exercise in trained ice hockey players. Performed work by the athletes was found to be dependent on initial levels of noradrenaline in plasma and ADRB2 mRNA in peripheral blood mononuclear cells.[125] It has been suggested that the endogenous catecholamine stimulation of ADRB2 that occurs with heavy exercise may result in enhanced cardiovascular function in individuals with one particular genotype over another.[126]

Arg16Gly (A>G)

Snyder *et al.* (2006) found that individuals with Arg (A) allele had lower receptor density and resting cardiac output,[126] and suggest a correlation between these factors. Eisenach *et al.* (2004) noted a greater heart rate response to exercise in individuals with the Arg/Arg (AA) genotype, which may aid in maintaining an increased cardiac output during peripheral vasodilation (decreased total peripheral resistance).[127] In an investigation of the Arg16Gly variant in athletes, Wolfarth *et al.* showed that the 16Arg (A) allele was significantly overrepresented in elite endurance athletes compared to sedentary controls, suggesting a positive association between the Arg16Gly polymorphism and endurance performance status.[128] Tsianos *et al.* (2010) reported that marathon runners with the ADRB2 Arg (A) allele had significantly faster running times compared to (Gly) G allele carries, implicating this variant in endurance running. Performance.[101] The Arg (A) has also been associated with greater peak VO_2max in heart failure patients.[129]

Gln27Glu (C>G)

Moore *et al.* (2001) investigated the ADRB2 Gln27Glu variant in female elite endurance athletes post menopause. Individuals with the Gln (C) allele had a greater VO_2max than Glu/Glu (GG) homozygotes and were subsequently associated with elite endurance performance.[130] When looking at the variants in the ABRB2 gene in athletes of varying sporting demands (both power/strength and endurance), Sawczuk *et al.* (2013) found that the Arg 16Gly G allele and the Gln7Glu G allele, were overrepresented in strength/power athletes compared to controls.[131]

The Glu/Glu (GG) genotype has also been associated with greater fat deposition and obesity. Macho-Azcarate *et al.* 2003 noted that women with the Glu/Glu (GG) genotype had reduced fat oxidation in response to exercise in comparison to those with the Gln (C) allele.[132]

3.9. Thyrotropin Releasing Hormone Receptor (TRHR): rs7832552 C>T

The TRHR gene encodes the Thyrotropin-releasing hormone (TRH) receptor. TRH stimulates the release of thyroid-stimulating hormone (TSH) and prolactin from the anterior pituitary. TSH in turn stimulates the release of Thyroxin (T4) and Triiodothyronine (T3) from the thyroid gland. Thyroxin is necessary for the anabolic action of the growth factor GH-IGF1, essential in muscle protein balance and adaptive changes to load.[133] T3 and T4 are also involved in increasing metabolic rate and aiding catecholamines in the mobilization of fuels during exercise.

Two gene variants within TRHR (rs7832552 C>T and rs16892496 T>G), have been significantly associated with lean body mass (skeletal muscle mass).[133] These variants are in strong linkage disequilibrium and the minor T and G alleles have respectively been found to be favourable for increased lean body mass and therefore strength and power activities.

Liu *et al.* (2009) conducted a genome wide association study (GWAS) on lean body mass variation. TRHR was identified as an important genetic determinant of lean body mass. Subjects with the TT rs7832552 genotype had 2.55 kg greater lean body mass compared to those with the C allele.[133] CC This same association was observed in three replication studies, totalling more than 6000 participants, and meta analyses of the initial GWAS study and replication studies achieved significant association.

3.10. Alpha-actinin 3 (ACTN3): 577 R>X

Alpha-actinins are a family of actin-binding proteins that maintain the cytoskeleton. The ACTN3 gene encodes alpha-actinin 3, which is only present in type II (fast) muscle fibres and also has a low expression in brain tissue. Alpha-actinins are found at the Z-line of the muscle where they anchor actin filaments and help to maintain the structure of the sarcomere. Alpha-actinins also interact with a signalling factor that plays a role in the specialisation of muscle fibre type, diameter and metabolism.

The ACTN3 gene contains a polymorphism which results in two versions of ACTN3: a functional R allele and a null X allele. The genotype that is homozygous for the X allele (XX) is completely deficient in alpha-actinin 3. The percentage of people with two X alleles range from about 1% in African populations to about 18% in Europeans and 25% in East Asians.

It has been demonstrated that male and female elite Sprint athletes have significantly higher frequencies of the R alleles than endurance athletes and sedentary controls.[134,135] Additionally, when Roth *et al* (2008) studied elite and local black and white bodybuilders, they showed a significantly lower XX frequency in the bodybuilders compared to controls.[136] None of the black bodybuilders were homozygous for the X allele. In a cycling study, it was found that subjects who were not ACTN3 deficient had greater peak power outputs and ventilatory thresholds than cyclists with the XX genotype.[137]

When studying the ACTN3 gene for endurance performance, mixed results have been found. Shang *et al* (2010) showed that the XX genotype was significantly over-represented in the female endurance athletes compared to controls.[138] However, no relationship between genotype and endurance-status was found in the male athletes. Doring *et al* (2010) found that the XX genotype was similar in prevalence between 316 endurance athletes and 304 controls.[139] Additionally, Saunders *et al* (2007) found when studying 457 male Ironman triathletes compared with 243 controls, that the XX polymorphism was not associated with ultra-endurance performance.[140] These observations have also been replicated in professional cyclists and Olympic endurance runners.[141] Collectively these studies suggest that the XX genotype has not yet been shown to be a good marker for endurance performance.

3.11. Vitamin D Receptor (VDR): Taq1 T>C

The Vitamin D Receptor (VDR) gene encodes the nuclear hormone receptor for vitamin D3 and is expressed by cells in most organs. VDR activation is essential for the maintenance of calcium and phosphorus levels in the blood and bone. VDR regulates the expression of many genes involved in essential bodily functions and has been identified as a regulator of skeletal muscle;[142] VDR binds vitamin D metabolites in muscle cells.

Approximately 200 variants exist in the VDR gene. The VDR Taq1 polymorphism is a T>C (T>t) change; this variant has been shown to be in linkage disequilibrium with the VDR Bsm1 G>A (b>B) variant. VDR Taq1 is reported in DNA Sport, however VDR Bsm1 is also commonly referred to in the literature. The VDR Taq1 CC (commonly called tt) and VDR Bsm1 AA (commonly called BB) homozygotes have been associated with greater muscle strength.

Geusens *et al.* (1997) investigated VDR polymorphisms and muscle strength. Quadriceps strength was found to be significantly higher in non-obese elderly women with the VDR Bsm1 bb genotype (Taq1 TT).[143] In contrast Grundberg *et al.* (2004) found that healthy women with the VDR Bsm1 BB (Taq1 CC) genotype, had greater hamstring isokinetic strength than the bb (TT) genotype group.[144] Additionally, Wang *et al.* (2006) showed that VDR Bsm1 bb (Taq1 TT) genotypes demonstrated lower knee flexion concentric torque than the B allele (Taq1 C allele) carriers.[145] Windelinckx *et al.* (2007) demonstrated that men with the Bsm1 AA genotype (BB) and Taq1 CC genotype (tt) had higher isometric and concentric quadriceps strength in comparison to their counterparts.[146] Bahat *et al.* (2010) similarly replicated the association of the VDR Bsm1 BB (Taq1 CC) genotype with greater extensor strength of the knee.[147]

Vitamin D supplementation may improve muscle strength through an increase in the size and amount of type II (fast twitch) muscle fibres in individuals with low vitamin D status.[148] Sufficient Vitamin D status is important in athletes and has also been linked to the prevention of stress fractures.[142] The VDR Bsm1 BB genotype (Taq1 CC) has additionally been associated with lower bone mineral density.[149]

3.12. Cytochrome P450 1A2 (CYP1A2): -163 C>A

Caffeine is a central nervous system and metabolic stimulant that is used to reduce physical fatigue. In athletics, moderate doses of caffeine have been known to improve both sprint and endurance performance.[150] Cytochrome P450 1A2 (CYP1A2) is one of the main enzymes that catalyse the oxidation of caffeine in humans.

A C>A variant exists in intron 1 of CYP1A2, affecting caffeine metabolism. Individuals with the C allele are associated with a reduced ability to metabolise caffeine.[151]

Womack *et al.* (2012) investigated the ergogenic effects of caffeine and the CYP1A2 polymorphism in trained cyclists. Individuals with the AA genotype experienced a greater ergogenic effect and

enhanced performance benefit with caffeine ingestion, in comparison to C allele carriers. This study was performed using 6mg/kg of caffeine 1 hour prior to performance.[150] Pataky *et al.* (2016) further demonstrated that cyclists with the CYP1A2 A allele benefited from caffeine ingestion with or without a caffeine mouth rinse 1 hour prior to exercise.[152]

Conversely, Salinero *et al.* (2017) found no significant effect of the CYP1A2 genotype on performance with 3mg/kg caffeine intake 1 hour prior to exercise.[153]

Individuals with the CYP1A2 AA genotype are likely to metabolise and clear caffeine at a faster rate than those with the C allele. C allele carriers should experiment with taking caffeine in more than 1 hour before the start of a race or event in order to gain from the ergogenic effects. Individuals with the CYP1A2 AA genotype are likely to benefit from caffeine intake ≤ 1 hour before a race or event and depending on the length of the event, consider an additional dose.

3.13. Circadian Locomotor Output Cycles Kaput (CLOCK): 3111 T>C

Multiple variables influence sports or exercise performance; more recently, circadian rhythms and chronotype have been noted as considerable factors. Chronotype refers to morning preference, evening preference or neither, and affects several physiological variables. An individual's chronotype may affect the time of day they are most likely to be at peak performance.[154,155]

A systematic review by Vitale *et al.* (2017) on chronotype and exercise concluded that morning and evening preference effects athletic performance.[155] Individuals that have a morning preference chronotype perceived less effort when performing a submaximal physical task in the morning in comparison to individuals with evening preference or neither. Additionally, individuals with morning preference achieved better race times in morning events in comparison to those with evening preference or neither.

Circadian Locomotor Output Cycles Kaput (CLOCK) is an essential element of the persistence and period of circadian rhythms, and effects chronotype and sleep. The C allele of the CLOCK 311 T>C variant has been associated with evening preference and delayed sleep timing.[156–158] This gene has also been linked to weight loss and eating behaviour.[157]

Genetic variants that underlie chronotype (morning or evening preference) and the effect on performance, need to be further studied in order to confirm previous findings. Coaches and trainers should however consider the chronotype effect, habitual time of day of training sessions, and time of day of competition in the lead up to an event.[159]

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