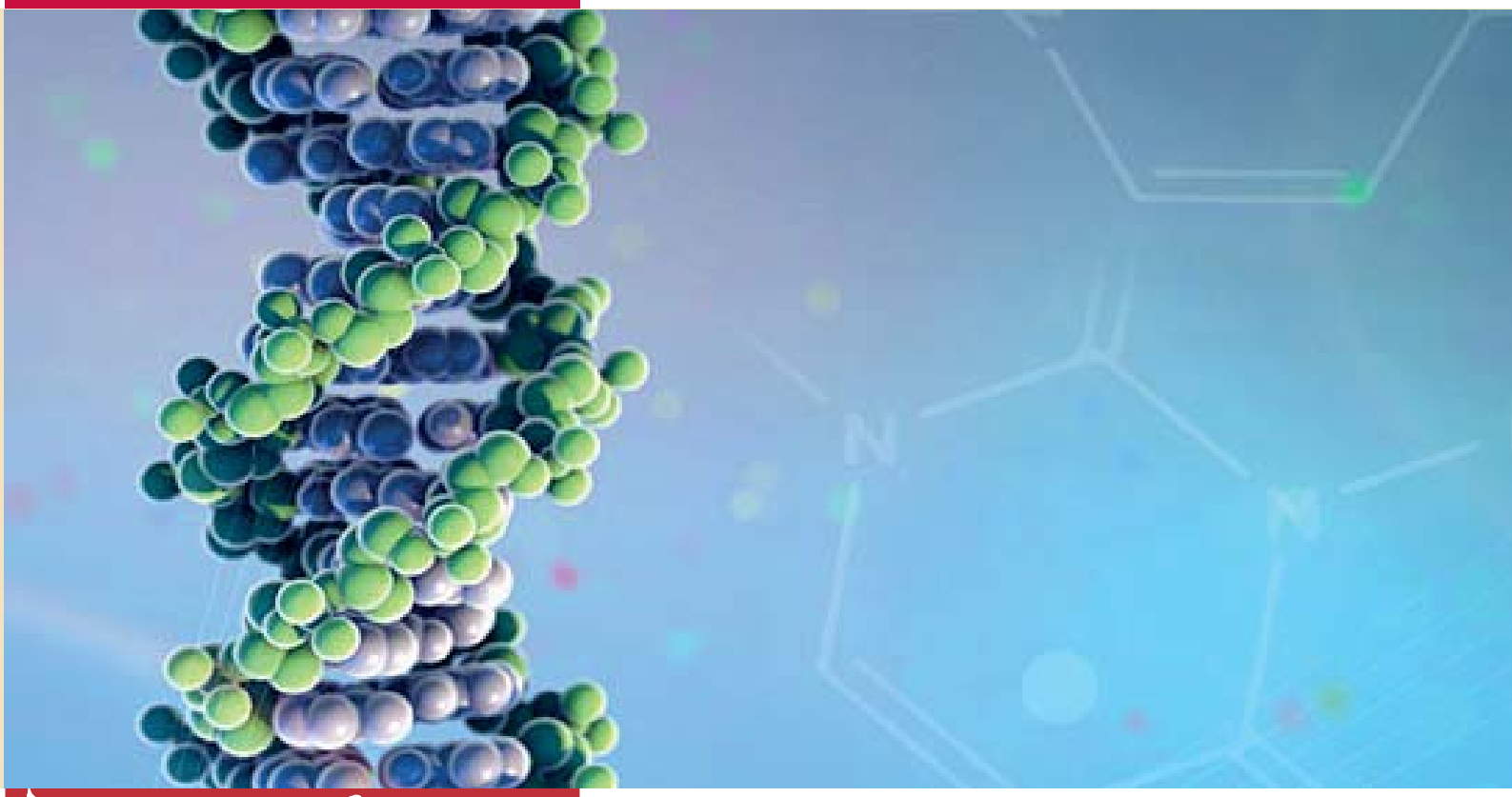
Master Thesis

Sannija Goļeva-Fjellet

Genotype frequency distribution of *ACE* I/D and *ACTN3* R577X polymorphisms in the Norwegian population

Do *ACE* I/D and *ACTN3* R577X polymorphisms influence self-reported physical activity levels?



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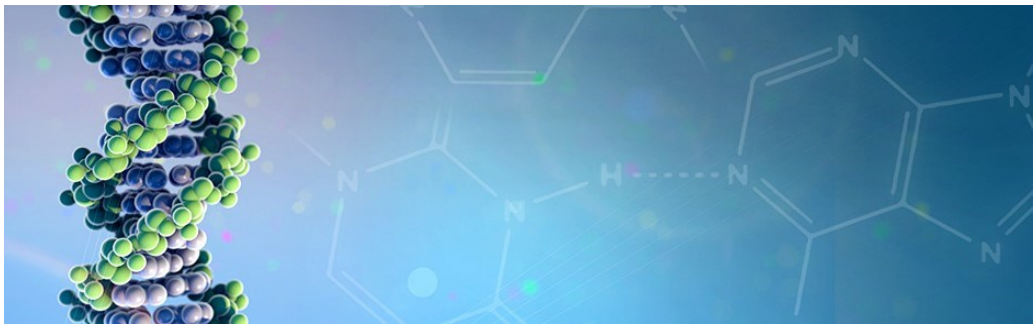

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Abstract

Physical inactivity is a global challenge as physical activity (PA) levels are lower than ever before in human history, and burden of non-communicable diseases continue to increase worldwide. PA is a complex behaviour, and unlike earlier beliefs that environmental factors are main contributor to PA behaviour, genetic factors are receiving increasingly more attention. It is likely that there are many genes, each with only small effect size, influencing the PA levels. These, in turn, interact with other biological and environmental factors to determine activity level of an individual. Genes that favour higher physical fitness have likely a more indirect contribution to PA behaviour.

Many potential candidate genes, influencing PA directly and indirectly, have been discovered. Despite many studies on the subject, results are still inconsistent, at least partly due to poor study design. *ACE* and *ACTN3* genes are the most widely investigated in field of exercise genomics. Main focus of many studies is the effect these genes have on athlete sprint and endurance performance. However, increasing number of studies are directed towards the potential effects in the general public. *ACE* insertion/deletion (I/D) and *ACTN3* R577X polymorphisms are of special interest. I-allele of the *ACE* gene, and X-allele for the *ACTN3* gene has been generally associated with improved endurance, while D-allele and R-allele for the *ACE* and *ACTN3* genes, respectively, has been associated with sprint and power related phenotypes. Both polymorphisms are also potential candidate genes for influencing PA levels.

Frequency of the different alleles for the *ACE* I/D and *ACTN3* R577X polymorphisms demonstrates a large variation in different population. The present study found that approximately 24% of the Norwegian subjects were homozygous for either D or I-allele for the *ACE* I/D polymorphism. Around 53% were heterozygous. For the *ACTN3* R577X polymorphism, the frequencies were approximately 31%, 49%, and 21% for RR, RX and XX genotype, respectively. Genotype frequencies for both genes did not deviate from Hardy-Weinberg Equilibrium, and were similar to what had previously been found in other Caucasian populations.

Female subjects showed significantly higher self-reported PA levels compared to male subjects. This corresponds well to other studies on the Norwegian population. There was no association between the two genes and self-reported PA levels in the study population of the present study. It is likely that other than *ACE* and *ACTN3* genes influence levels of self-reported PA in the Norwegian population.

Contents

Preface

This Master thesis is written as a part of the Master program at the Department of Environmental and Health Studies at the Telemark University College. The present study is one of two studies of a project investigating effects of genes on physical activity levels. Main focus of this particular study was to carry out genotyping analysis and determine genotype frequencies of the *ACE* and *ACTN3* genes. Second study, carried out by Anne Mari Bjurholt, focused on estimating self-reported PA levels in the Norwegian population, based on questionnaire data. The association study between the two genes and the PA levels was conducted on the combined data from both studies.

I would like to thank my family, which has always been there for me; Mona Sæbø for being an inspirational supervisor for my Master thesis, and to the teacher of my biology-classes at secondary school, Sandra Štrause, who introduced me to the exciting world of biology, anatomy and genetics. I would also like to acknowledge my colleagues at the library for providing me with literature; Karin Brekke Li and Frode Bergan for giving a helping hand at the lab when I needed it the most; and Øyvind Støren for helping me and Anne Mari with statistics. Thanks to Sarah for helping out with my English writing. The person I would like to thank the most and to whom I dedicate my Master thesis is my husband, Øyvind. Without his support and patience I would not be able start studying something I really love, and to go the whole way. Thank you so much!

Skien, May 2015

Sannija Goļeva-Fjellet

Terms and abbreviations

Adenosine triphosphate (ATP) – a high energy molecule that stores energy in all living cells

Aerobic exercise-exercise during which the aerobic energy pathway is the main contributor to energy; usually performed over prolonged period of time and are of low to moderate intensity (e.g. walking, running, etc.)

Anaerobic exercise- exercise during which the anaerobic energy pathways are main contributors to energy (e.g. strength training, sprinting etc.)

Ang I- Angiotensin I

Ang II- Angyotensin II

bp- base pair

BP- blood pressure

CAP-accreditation- The College of American Pathologists' laboratory accreditation

CLIA-certification- Clinical Laboratory Improvement Amendments- certification

Direct calorimetry- measures the heat produced and released by an individual; this method has a limited practical application due to expenses and time consumption

Eccentric exercise- exercise during which force is applied (e.g. weights are held) while the muscle is lengthening, usually used in relation to resistance training

Fatigability- susceptibility to get exhausted/fatigue

Haplotype – a set of linked genetic variants or alleles on a single chromosome or a part of a chromosome

Homeostasis- process within a biological system of maintaining stability by regulating its internal conditions

HUNT- The Nord-Trøndelag Health Study

HWE- Hardy Weinberg Equilibrium

Hypertension- high blood pressure

Indirect calorimetry-method for estimating energy expenditure by measuring consumption of O_2 and production of CO_2

KAM study- Kolorektal cancer, Arv og Miljø molecular epidemiological study

KO mice- Knockout mice that is bred with a gene/-s that is/are inactivated (in this case Actn3 gene) in order to observe/ study phenotypic changes

Linkage disequilibrium (LD)- non-random association between certain alleles. Alleles in LD are more likely to occur together

LV- left ventricle of the heart/ left ventricular

METs- metabolic equivalents, multiples of the resting metabolic rate. One MET equals the amount of oxygen consumed at rest (approx. 3.5 ml/min/kg VO_2)

Muscle hypertrophy- muscle growth; a biological adaptation to increased workload

NCDs- Non-communicable diseases

NORCCAP study- Norwegian Colorectal Cancer Prevention study

Overload- greater than normal intensity/load is needed in order to induce training related adaptations

PA- **physical activity**; any bodily movement produced by skeletal muscle requiring energy expenditure

PCR- polymerase chain reaction

Power output- rate at which work is done; Power output = (Force generated*Distance)/Time

QTL- Quantitative trait loci

RAS- Renin-angiotensin system

RAAS- Renin-angiotensin-aldosterone system

SBBP- Short Physical Performance Battery is an assessment tool for measuring lower extremity functioning and physical performance in elderly. It consists of repeated chair stands, balance stand and 4m walk.

SNP-single nucleotide polymorphism

Static (isometric) training- training in which the length of the muscles do not change

Vascular tone- the level of how constricted blood vessels are compared to the maximally dilated state

Vasoconstrictor- nervous signal or substance that makes the blood vessels to constrict/narrow

Vasodilator- nervous signal or substance that makes the blood vessels to dilate/expand

VO_{2max}- maximal oxygen uptake/consumption also called aerobic capacity; it is a measure of individual's capacity for aerobic energy production

Aim of the study

The aim of this study is to:

- genotype and determine the prevalence of the *ACE* and *ACTN3* alleles in the Norwegian population
- establish a baseline for future studies on genes and exercise on various groups of individuals such as those affected by diabetes, obesity and aging
- determine if there is an association between the *ACE* and *ACTN3* genotype and self-reported physical activity level

1 Introduction

Humans are designed for physical activity (PA), and early *Homo sapiens* could not have emerged and spread so successfully without the ability to perform physically demanding work. Many of the traits characteristic to humans, such as upright posture and bipedal locomotion, gave necessary evolutionary advantages in the harsh evolutionary environment. In modern societies, however, the technological progress (Bouchard et al., 2012) that started around 200 years ago with the Industrial Revolution (Levine, 2014), has made humans less active than ever before (Bouchard et al., 2012). According to some authors, *Homo sedentarius* would be a more appropriate description of human beings as a species nowadays (McArdle et al. (2010); Levine (2014)).

PA is a complex behaviour (Bray et al., 2011), and is defined as any bodily movement produced by skeletal muscle requiring energy expenditure (Bouchard et al., 2012). Any behaviour, including PA, is influenced by three factors: genetic (i.e. biological), environmental and an interaction of both, as demonstrated in Figure 1. Until recently, environmental factors have been receiving most attention in literature. However, many studies are now pointing towards the heritability of the PA levels in humans and other organisms (Lightfoot, 2011a). It has now become clear that despite of the significant impact of environmental factors on PA levels, genetics strongly influence whether a person is sedentary or physically active (De Moor et al., 2009). PA is likely influenced by several or many genes acting together (Bray et al., 2011), each with only a small effect size, needing a very large sample to detect such effects (De Moor et al., 2009). Biological factors are, in turn, often affected by both environmental and other biological factors and are therefore called complex traits (Brutsaert and Parra, 2009).

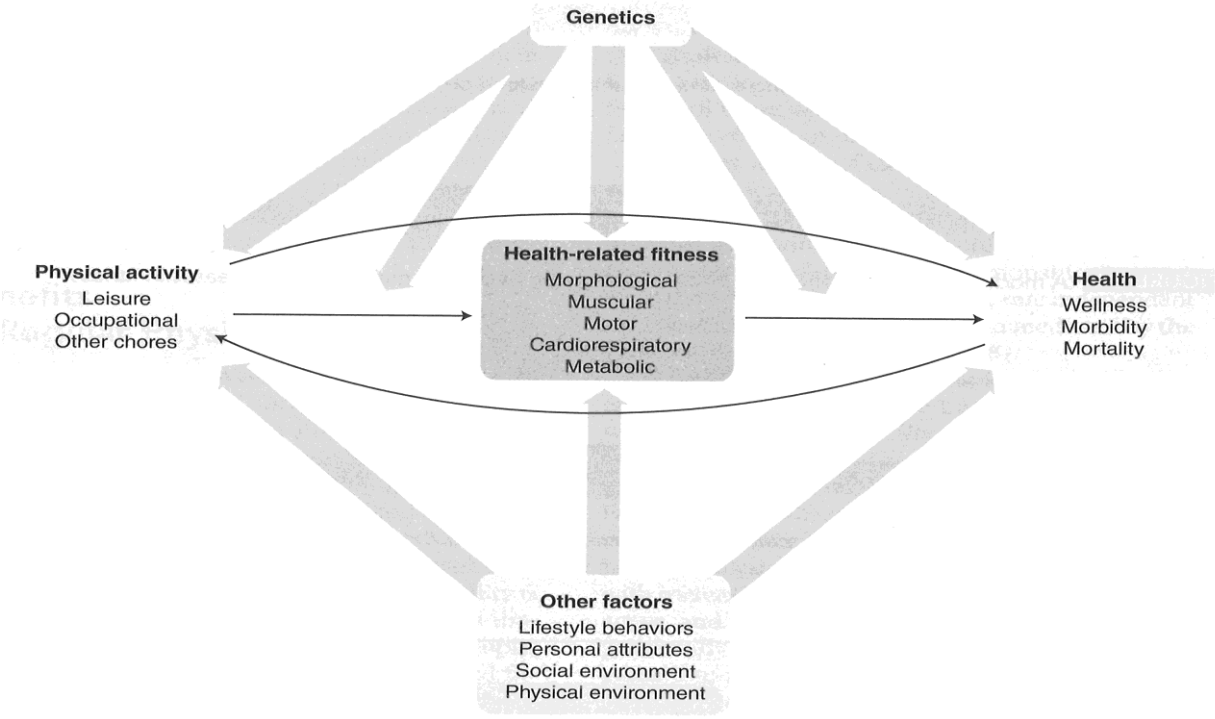

Figure 1: *The complex relationship between physical activity, health-related fitness and health. These are in turn influenced by environmental factors (e.g. social and physical environment) as well as genetics (Bouchard et al., 2012).*

Another factor possibly influencing PA participation is the level of one's fitness and how well an individual responds to exercise (Nicklas, 2010). Thus, the genes that favour fitness and trainability would probably increase the levels of PA (Stubbe et al., 2006). Also for trainability, large heterogeneity has been observed (Nicklas, 2010). Those experiencing no or little change in their physical fitness as a result of regular exercise (low responders) on one phenotype (e.g. endurance) will experience improvements in another phenotype (e.g. power), as response to exercise is a trait-specific phenomenon (Sarzynski et al., 2011).

An increasing number of companies offer their services in tailoring a gene-based training program for the general public, as well as athletes. Tests are normally based on data obtained on candidate gene studies and genome-wide association studies. A set of single nucleotide polymorphisms (SNPs) have been revealed having both favourable and less favourable alleles (Bouchard, 2012), covering traits associated with various endurance, power and injury phenotypes (DNAFit, 2014) or trainability (XRGenomics, 2014). By determining the genotype for these SNPs, individuals that are high and low responders to exercise could be

identified (Bouchard, 2012). Some of the tests are available online (DNAFit (2014); XRGenomics (2014)) or over the counter (Boots, 2014), while others are prescribed by physician (PathwayGenomics, 2014). With growing knowledge of the genetic basis of performance, individualizing training according to the genetic profile may become a way of maximizing the beneficial effects on health (Sarzynski et al., 2011) thereby improving performance in future athletes (Roth, 2007), promoting compliance to regular exercise in the general public (Nicklas, 2010), and decreasing negative health effects of inactivity (Lightfoot, 2011b).

An increasing amount of evidence implicates that lack of PA have many negative impacts on our health, such as an increasing burden of chronic diseases, loss of functional capacity (Bouchard et al., 2012) and psychological disorders (Levine, 2014). Physical inactivity is one of several contributing causes leading to non-communicable diseases (NCDs) which are chronic, non-transmittable diseases that kill around 36 million people globally (World Health Organization, 2010). Four main types of NCDs are cancers, chronic respiratory diseases, diabetes and cardiovascular diseases. Although most of the deaths caused by NCDs occur in low- and middle-income countries, also high-income countries, including Norway, are affected. These diseases affect mostly elderly people; however, all age groups are affected. Increased PA levels in everyday life is one of the ways to reduce NCDs (World Health Organization, 2013), which also combats other social and health issues caused by overweight and inactivity (World Health Organization, 2014).

Changes in physical and socioeconomic environments, both at work and at peoples' homes, have contributed to various types of low-intensity PA. Sitting has become the most common human behaviour for many individuals, especially in industrialized countries (Hamilton and Owen, 2012). Large interindividual differences in PA levels have been observed between different subgroups within a population (e.g. ethnicity, gender and age). Observed PA differences among different ethnic groups can be partly explained by the cultural, religious and socioeconomic differences (Katzmarzyk, 2012). A study from the Finnmark County, Norway found significantly different self-reported PA levels in Norwegian and Sami ethnic groups. The Sami population demonstrated a higher total PA score compared to the Norwegian population, with the main contributor being PA levels during work. More than 50% of the study participants of the Sami origin lived in the typical Sami municipalities, with reindeer industry being dominant (Hermansen et al., 2002). The Norwegian population, however, was found to be more active when compared to subjects from five large immigrant

groups in Oslo, Norway. Approximately 50% of immigrants reported that they were inactive compared to 20% Norwegians. Typically, immigrant men were more physically active than immigrant women, while the opposite was true for the Norwegian population. Subjects with lower levels of education tended to be more sedentary (Kumar, 2008), indicating social factors contributing to activity levels.

In general, women tend to be less physically active than men, due to both social factors (Katzmarzyk, 2012) as well as biological/genetic gender differences (Lightfoot, 2011a). However, it could vary between different populations, as some studies report the opposite (Aspenes et al. (2011); Kumar (2008)). Age-related differences are most likely influenced by social, psychological and biological mechanisms. Children tend to show the highest levels of PA, with levels decreasing in adolescents and adults (Katzmarzyk, 2012). PA has be found to be influenced by environmental factors to a higher degree during childhood, and has a decreasing influence with an increasing age (Stubbe et al., 2005). Biological factors that are proposed as possible causes to the observed differences are neurobiological changes and general physiological decline, to name a few (Katzmarzyk, 2012).

Despite of the large amounts of information available on the importance of PA, only one in five adults in Norway meets the recommended PA levels (Norwegian Institute of Public Health, 2009). It is recommended to adhere to a minimum of 150 minutes moderate intensity exercise/activities or 75 minutes of high intensity activities weekly as well as reduce sitting. The positive effect of exercise is dose-dependent, i.e. exercise above the recommended time gives additional health benefits. The Norwegian Directorate of Health also emphasizes the importance of resistance training of larger muscle groups at least two times a week. Regular resistance training improves muscle strength by adaptation of the neural system and/or inducing protein synthesis leading to increased muscle mass (McArdle et al., 2010). This, in turn, can decrease occurrence of injuries as well as improve recovery from them, but, most importantly – makes daily life activities easier to perform (Hubal et al., 2011). Endurance training, on the other hand, induces pulmonary, cardiovascular and metabolic adaptations (McArdle et al., 2010), and can provide various benefits. One of the positive effects of endurance training is a decrease in prevalence and mortality from coronary artery disease (Mersy, 1991).

It has been observed that some individuals experience little or no improvements in their maximal oxygen consumption (aerobic capacity, VO_{2max}) in response to standardized aerobic training programs, while others improve it by 100% compared to the initial levels (Bouchard

and Rankinen, 2001). There are similar variations in response to resistance training stimuli. Around 20-30% of both muscular fitness and VO_{2max} is thought to be genetically determined (McArdle et al., 2010). However, the exact mechanisms behind variations in trainability are not clear. Factors like age, gender or ethnic origin does not seem to have major influence (Bouchard and Rankinen, 2001), and yet undetermined genetic factors are likely the main cause for such variations. Individuals with the same genotypes are more likely to have similar responses to regular exercise compared to those with different genotypes (Sarzynski et al., 2011). Variations in trainability have been observed in both the general population (Bouchard, 2012) and athletes performing at top level (Ahmetov and Rogozkin, 2009). As lack of improvement in physical fitness in response to training may weaken individuals' compliance to training, identifying genetic markers that could predict individuals' response to aerobic and/or resistance training could maintain the motivation to start and pursue exercising (Nicklas, 2010). Advances in biotechnology have allowed to investigate various genes and genetic markers that could possibly be associated with various fitness phenotypes (Gibson, 2009), such as PA levels (Lightfoot, 2011a), muscle strength/size (Hubal et al., 2011) or aerobic capacity (Pitsiladis et al., 2011). Today's technology, such as high-throughput sequencing and genome-wide association studies (GWAS), allows faster data generation and analysis, improving our knowledge about genetics behind complex traits (Wolfarth et al., 2014), as well as making genotyping a lot more affordable (Roth and Thomis, 2011).

Table 1 demonstrates some of the most frequently studied genes associated with endurance, power and voluntary PA. Some of these genes are also thought to interact with exercise to influence body composition. Genes that commonly are associated with endurance phenotype are *ACE*, *ACTN3*, *ADRB2*, *BDKRB2* and *PPARA*; muscle power/strength –*ACE*, *ACTN3*, *MSTN*, *PPARA;* training response phenotypes – *ACE, ACTN3*, *BDKRB2*, *VDR*. Voluntary PA levels are thought to be influenced by *ACE*, *ACTN3*, *DRD1*, *LEPR*, *MC4R*, *MSTN*, *NHLH2*, *PAPSS2*, *VDR* (Lightfoot, 2011a). Genes that are thought to influence body composition in interaction with exercise/physical activity are *ACE*, *ADRB2* and *PPARA;* obesity- *MC4R, LEPR* (Sailors and Bray, 2011). Several of these genes are also a part of the genetic tests used for tailoring personalized training programs by several companies (DNAFit (2014); Sports Gene (2014); PathwayGenomics (2014)). Tests targeted towards weight management include *MC4R*, *ADRB2* (Sports Gene, 2014), *LEPR* and *PPARA* genes (PathwayGenomics, 2014). Athletic ability/physical fitness tests focus on *ACE*, *ACTN3* (Sports Gene (2014); DNAFit (2014)); *ADRB2*, *BDKRB2*, *PPARA*, *VDR* (DNAFit, 2014).

Table 1: *Genes frequently associated with fitness related traits (endurance and power), physical activity (PA).*

Candidate gene	Gene function	Associated with
ACE Angiotensin I converting enzyme	Blood pressure control; water and sodium regulation [a]	E[a], P[a,f], PA***, BC[e]
ACTN3 Alpha-actinin-3	Structural protein found in fast twitch fibers [a]	E[b], P[a,f], PA***
ADRB2 Adrenergic receptor 2	Part of adrenaline release regulation; mobilization of fuel during exercise [a]	E[a], BC[e]
BDKRB2 Bradykinin Receptor B2	Vasodilation, blood pressure control [a]	E[a]
DRD1 Dopamine receptor 1	Regulation of neuron growth, mediation of behavioural responses [c]	PA*
LEPR Leptin receptor	Stimulation of gene transcription, regulation of fat metabolism [c, e]	BC[c,e], PA***
MC4R Melanocortin-4 receptor	Regulation of energy homeostasis [e]	BC[c,e], PA***
MSTN Myostatin	Regulation of cell growth and differentiation; gene product of *MSTN* gene likely negatively regulates skeletal muscle growth [c]	P[f], PA**
NHLH2 Nescient helix-loop-helix 2	Transcription factor [c]	PA*
PAPSS2 3'-phosphoadenosine 5'-phosphosulfate synthase 2	Involved in initial muscle development [d]	PA**
PPARA Peroxisome proliferator-activated receptor alpha	Fat and carbohydrate metabolism regulation [a,e]	E/P[a], BC[e]
VDR Vitamin D receptor	Regulation of Vitamin D3 and Ca^{2+} levels in blood [a]	P[a], PA***

E- Endurance; P- Power; BC-body composition
*PA- Candidate genes for physical activity [d]; * three or more lines of evidence supporting candidacy [d];** potential candidate gene (at least two lines of evidence [d]; *** genes with limited support for candidacy [d]*
a)DNAFit (2014) b)Pitsiladis et al. (2011) c) NCBI (2015) d) Lightfoot (2011a) e)Sailors and Bray (2011) f)Hubal et al. (2011)

Among the genes described in Table 1, *ACE* and *ACTN3* are some of the most widely investigated genes in field of exercise genomics. These are potential candidate genes for PA levels as well as various traits related to elite athletic performance and exercise. Associations with the latter phenotype have received most of attention (Wolfarth (2011); Thomis (2011)), however, a number of studies have focused on the effects of the *ACE* and *ACTN3* genes, either together or seperately, in the general public as well. Associations for both genes have been found with trainability of cardiorespiratory traits (Montgomery et al. (1997); Myerson et al. (2001)), physical function (Buford et al. (2014); Pereira et al. (2013b); Alfred et al. (2011)) and muscle strength (Rodriguez-Romo et al. (2010); Pereira et al. (2013a); Lima et al. (2011); Moran et al. (2006)) having been investigated. Evidence for their status as candidate genes for PA levels as well as fitness related traits for these two genes, however, is limited, as replication studies are produced contradicting results (Loos et al., 2015).

Despite the large number of studies on many candidate genes, results are contradictory, causing entropy in the body of knowledge (Appell Coriolano and Duarte, 2012). It has been suggested the declaration a gene as a candidate gene for a particular trait should only occur if at least three or four independent lines of evidence supporting its candidacy are found in human and/or mouse models. Lines of evidence can be the functional relevance of a gene; location within a known quantitative trait loci (QTL); existing haplotype differences; expression difference, etc. (DiPetrillo et al. (2005); Lightfoot (2011a)). Both *ACE* and *ACTN3* genes are functionally relevant and are located within currently identified QTL associated with PA levels in mouse models, but not in human models. *ACTN3* also shows a haplotype difference (Lightfoot, 2011a). Both genes have been associated with improved endurance performance (Bouchard and Hoffman, 2011). Novak et al. (2009) suggested that endurance capacity might determine PA levels, and this was true in both mouse and human models. However, it has not yet been supported by other studies (Lightfoot, 2011a).

1.1 The *ACE* gene

The *ACE* gene is located on chromosome 17 (Wang et al., 2008), contains 26 exons and codes for angiotensin-converting enzyme (ACE), which acts on many different substrates. Two different species of ACE enzyme (somatic and germinal) are transcribed from the same gene, but each has different sites of action. The germinal form is expressed in testes only (Corvol, 1995), and it is the somatic ACE that is relevant for this study. ACE can be found as a

membrane bound enzyme in many types of tissues (Paul, Mehr et al. 2006), and as a freely circulating enzyme (ACE_{plasma}) in various body fluids (Corvol 1995).

The main physiological function of the enzyme is within renin-angiotensin system (RAS, Figure 2), which is a system that maintains normal blood pressure (BP, Coates (2003)). RAS gets activated by sympathetic stimulation or by blood volume and/or electrolyte loss in the body. Angiotensinogen, produced by liver, is cleaved by an enzymatic hormone renin (released by kidneys) into angiotensin I (Ang I). ACE, in turn, cleaves Ang I into angiotensin II (Ang II), which is an effective vasoconstrictor i.e. it causes blood vessels to constrict, increasing the BP. In addition, ACE catalyses degradation of a potent vasodilator bradykinin (Woods et al., 2000), causing a hypertensive reaction, i.e. increased BP as a result of decreased diameter of blood vessels (Puthucheary et al., 2011).

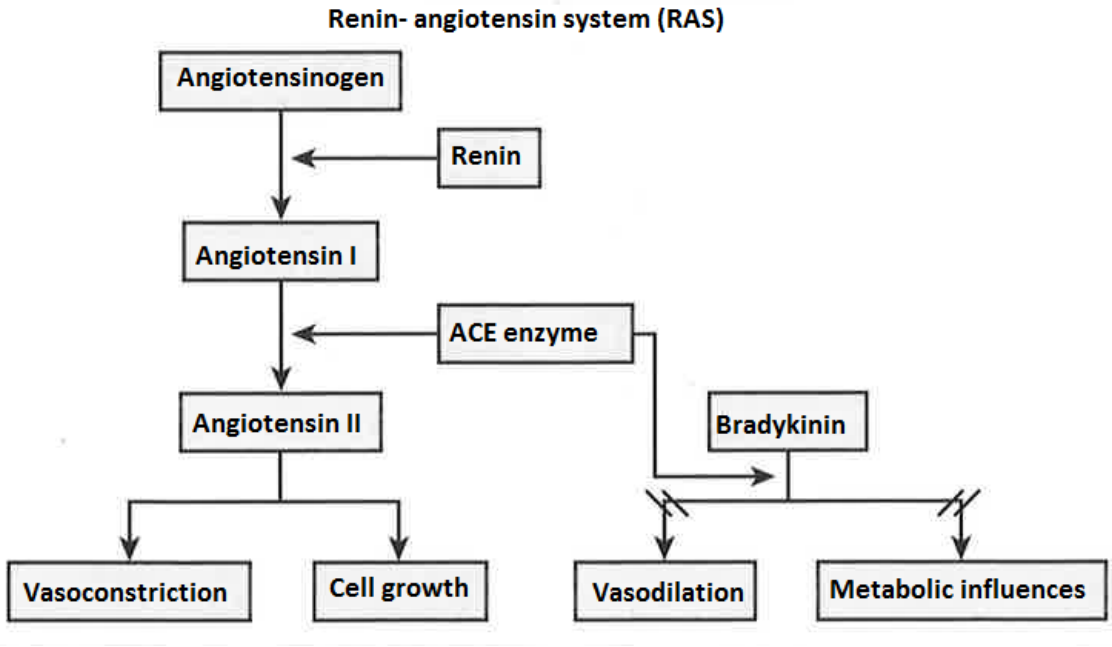

Figure 2: *Schematic diagram of the physiological pathways of the Renin-angiotensin-system (RAS) showing the complex physiological system ACE enzyme is a part of. In addition, ACE enzyme is catalyzing degradation of the potent vadodilator, bradykinin, from the kinin-kallikrein system (Roth, 2007)*

RAS is also a part of Renin-angiotensin-aldosterone system (RAAS) which is a system involved in sodium (Na^+) and potassium (K^+) regulation. In RAAS, Ang II stimulates excretion of the hormone aldosterone from the adrenal gland. Aldosterone excretion leads to reabsorption of Na^+, retention of water and excretion of K^+. As a consequence of increased blood plasma volume, the arterial BP rises (Sherwood, 2013). RAS and RAAS are endocrine

systems (Sherwood, 2013), however, local and cellular RAS exist as well. These are found in various types of tissues such as heart, lungs, brain (Paul et al., 2006) and skeletal muscle. Local and systemic RAS systems have been shown to interact (Paul et al., 2006).

Local muscle RAS may influence human performance (Jones and Woods, 2003). In contrast to the systemic action of endocrine RAS, local RAS has a metabolic role (Montgomery and Brull, 2000). It is likely that any differences in the expression of ACE may cause a variation in breakdown of BK and production of Ang II. Muscle RAS may affect performance as it plays a role in regulation of metabolism and vascular tone (Jones and Woods, 2003). Gordon et al. (2001) demonstrated that Ang II is necessary for skeletal muscle growth following overload during resistance training. However, RAS is likely not as important for normal maintenance of muscle mass. ACE may also have a similar impact on cardiac muscle growth (Gordon et al., 2001).

1.1.1 *ACE* I/D polymorphism

ACE insertion/deletion (I/D) polymorphism is a length polymorphism, where 287-bp *Alu* repeat is either present in intron 16 of the insertion allele (I-allele) or absent in the deletion allele (D-allele; Woods (2009)). *Alu* sequences are particularly rich in guanine and cytosine, and are common in human genome (Speicher et al., 2010). Frequency of the three existing genotypes (II, ID and DD) varies among populations, as shown in Figure 3 (for more detailed overview of genotype and allele prevalence see Appendix A).

In Caucasian populations, frequency distributions are found to be around 25% for each of the homozygous genotypes and 50% for the heterozygous genotype (i.e. a 1:2:1 ratio; Jones and Woods (2003)). It varies slightly, however, among the different populations of Caucasian ancestry (Rankinen et al., 2000b). Earlier reported *ACE* I/D polymorphism frequencies in healthy Norwegian subjects for DD and II genotype, respectively, are 32% and 21% in Tronvik et al. (2008); and around 27% and 23% in Bohn et al. (1993). Populations of African ancestry have, in general, a higher prevalence of the D-allele (Mathew et al. (2001); Barley et al. (1994)). Indian (Mathew et al., 2001), Samoan and Yanomami Indian (Barley et al., 1994), and Pima Indian (Foy et al., 1996) populations, on the other hand, have higher frequency of I-allele compared to Caucasian and African populations.

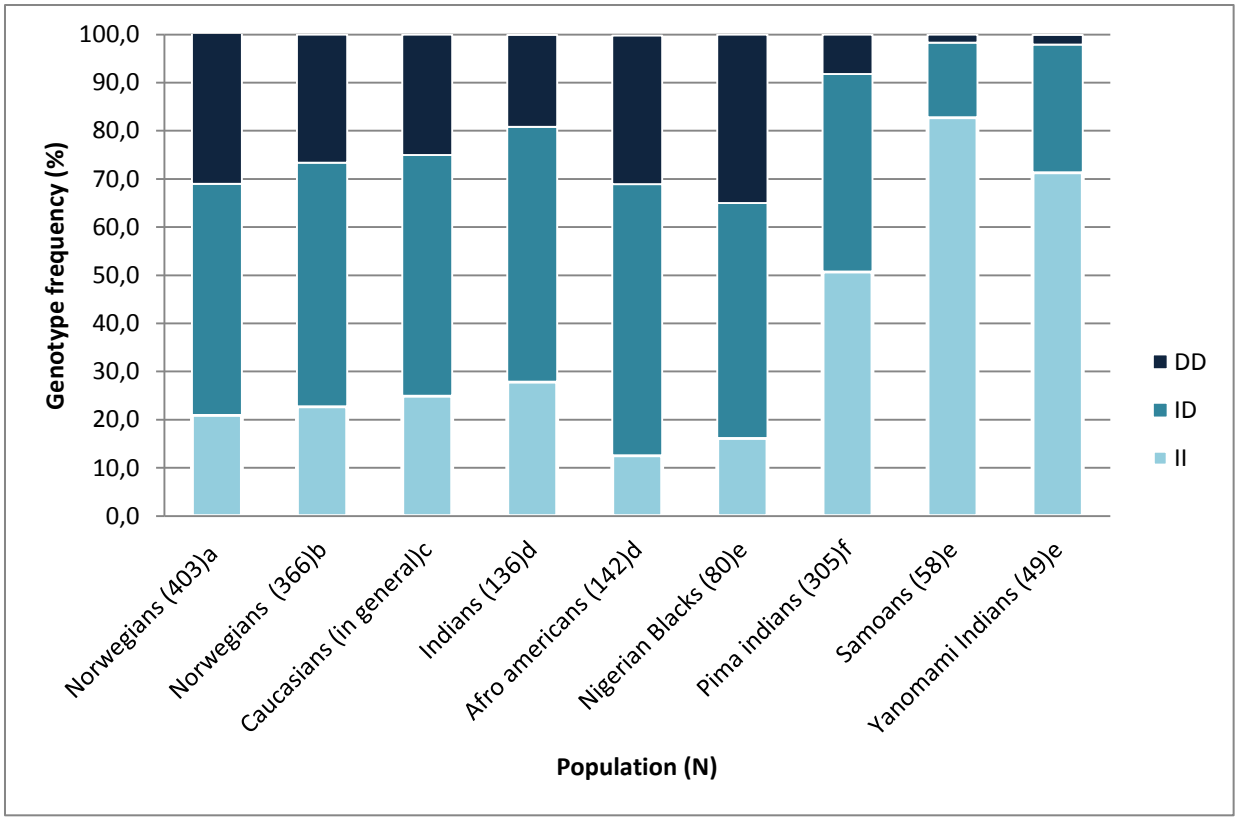

Figure 3: *Genotype frequencies (%) of the ACE I/D polymorphism in various populations. a) Tronvik et al. (2008); b) Bohn et al. (1993); c) Jones and Woods (2003); d) Mathew et al. (2001); e) Barley et al. (1994); f) Foy et al. (1996)*

One possible effect of the I/D polymorphism is the large interindividual differences in ACE_{plasma} concentrations found in healthy Caucasian subjects (Rigat et al., 1990). Subjects carrying the D-allele had been found to have higher ACE_{plasma} levels than the carriers of the I-allele. DD and II individuals have the highest and lowest ACE_{plasma} levels, respectively. Intermediate concentrations have been measured in heterozygotes. DD subjects have also been shown to have the highest degradation rate of BK (Brown et al., 1998), thus counteracting vasodilation. Studies on populations of different ethnic origins have showed racial differences in ACE_{plasma} activity associated with the *ACE* I/D polymorphism. The largest association between the polymorphism and ACE_{plasma} levels have been found in Caucasian subjects (up to 47% (Brown et al., 1998)). In Pima Indians (USA) only 6.5% of ACE_{plasma} levels could be explained by the *ACE* I/D polymorphism (Foy et al., 1996), while no significant association was found in black South African males (Payne et al., 2007).

From all genes associated with human performance, *ACE* I/D polymorphism (Wang et al., 2008) is the most widely studied for its association with endurance performance (Pitsiladis et

al., 2011). Despite many studies, results are neither conclusive nor consistent (Wang et al., 2008), creating a lot of debate (Woods, 2009). In general, the D-allele is associated with sprinting (Myerson et al. (1999) and short-distance swimming (Myerson et al. (1999); Woods et al. (2001)), among others. The I-allele, on the other hand, is associated with elite endurance rowing (Gayagay et al., 1998), long distance running (Myerson et al. (1999); Hruskovicova et al. (2006)), inline skating (Hruskovicova et al., 2006) and other endurance type of activities. Other studies, on the contrary, have found an opposite association for the D-allele in Israeli Caucasian athletes, i.e. frequency was highest among marathoners (77%) and lowest among sprinters (57%). The control group had intermediate frequencies (66%) (Amir et al., 2007). Similar findings were found in Iranian athletes (Shahmoradi et al., 2014). These results suggest that carrying D-allele may be beneficial for endurance performance in some ethnic groups (Amir et al., 2007). Despite the positive findings, several studies have not been able to find any association between *ACE* I/D polymorphism and athlete status, e.g. Rankinen et al. (2000b); Taylor et al. (1999); Orysiak et al. (2013); Ash et al. (2011).

Studies conducted on non-athletes have revealed that *ACE* I/D polymorphism may, among other things, influence degree of increase of strength (Folland et al., 2000) and aerobic power after a training program (Defoor et al., 2006); influence functional responses to exercising in older adults (Buford et al., 2014); determine physiological and skill parameters in adolescents (Moran et al., 2006); influence PA level in pubertal boys (Maestu et al., 2013) and adults (Winnicki et al., 2004).

Folland et al. (2000) found that increase in static strength after a nine-week strength training program was strongly genotype-dependent. Carriers of the D-allele (i.e. DD and ID genotype) showed larger increase in static strength compared to the II genotype, with heterozygotes having the largest increase (97% greater improvement compared to II subjects). The study population was healthy males without prior strength training history. There were no genotype-dependent differences in pre-training strength. This study indicates that the *ACE* gene plays a role in skeletal muscle hypertrophy as a result of overload, probably through the systemic and/or local skeletal muscle RAS. Presence of the D-allele seems to promote the hypertrophy, which is one of the most important factors in gained strength after a period of overload.

One of few studies investigating *ACE* I/D polymorphism's influence on physical, physiological and skills parameters in adolescents found that female homozygotes for the I-allele performed better at vertical jump tests and had greater handgrip strength. The authors did not, however, observe any effect in male teenagers. It was concluded that I-allele is

associated with a strength phenotype. Another conclusion was that in the general population the *ACE* I/D polymorphism has a modest influence on physical phenotype (Moran et al., 2006).

A study done by Vaughan et al. (2013) on men of Swiss descent showed that carriers of the I-allele (i.e. II and ID genotypes) experienced greater gains in endurance related parameters after a period of aerobic training. DD subjects, on the other hand, showed smaller or no improvement, depending on the parameter. The effect of the I-allele is likely exercise-type dependant, as changes occurred among men who were assigned to cycle, but not those who run. This study also discovered that during training and recovery from exercise, there is an I-allele dependant upregulation of a number of genes that are related to glucose and lipid metabolism.

Genetic variation within the *ACE* gene could also predict the adherence to training. This is, however, still in early stage of research (Nicklas, 2010), and the evidence for its candidacy as a gene determining levels of PA is still limited (Lightfoot, 2011a). Associations have been found between *ACE* I/D polymorphism and PA in pubertal boys. Subjects with DD genotype were found to be significantly more active (light PA and total PA measured by accelerometer) compared to II subjects who were more sedentary (Maestu et al., 2013). This contradicts the findings from a study by Winnicki et al. (2004) on adults. They found that DD subjects of both genders were more often sedentary compared to II subjects in mild hypertensive individuals. Heterozygotes had intermediate PA levels. PA levels were assessed by a questionnaire. According to the authors, family status, age and gender, in addition to the *ACE* I/D genotype, were significantly related to PA levels. No significant associations with PA were found for BMI, smoking, and other factors. Another study failed to find any association between *ACE* I/D polymorphism and leisure time PA in adults (Fuentes et al., 2002). Contradicting results between these studies could be a consequence of methodological differences.

A study of effects of two treatments - structured PA or health education - on physical function of older Caucasian adults revealed that treatment*genotype interaction was significant. Carriers of D-allele improved their gate speed as well as performance on the Short Physical Performance Battery (SPPB, see Terms and Abbreviations for more information) as a result of structured PA, compared to a group with the same genotype, but who received health education. II subjects in the exercise group, however, showed less improvement compared to II counterparts in the education group. Exercisers with II genotype experienced as much as

21.1% lower improvements in SBBP than the education group with the same genotype, and clearly demonstrated reduced responsiveness to exercise compared to carriers of the D-allele. This study was the first of its' kind to implicate that *ACE* I/D polymorphism is a source of variability in response to exercise in the elderly. The authors speculate that the chosen types of tests and exercise may favour carriers of D-allele, and results may differ if other test and training modes are used (Buford et al., 2014).

There are many suggested mechanisms by which *ACE* I/D polymorphism may exert its effects on fitness phenotypes. Firstly, lower degradation of BK, as observed among I-allele carriers, leads to increased delivery of substrates to the skeletal muscles increasing their efficiency (Jones and Woods 2003). Secondly, II subjects are shown to possess higher degrees of slow twitch muscle fibers which are engaged during aerobic exercise, compared to DD genotype, with a higher percentage of fast twitch fibers engaged during anaerobic exercise (Zhang et al., 2003). An increased number of the more efficient slow twitch fibers could be one of the explanations for the increased mechanical efficiency associated with the I-allele (Williams et al., 2000). Thirdly, increase in left ventricle (LV) mass after a strength and endurance training period is greatest in carriers of the D-allele and smallest in II genotype (Montgomery et al., 1997). Larger LV leads to increased contractile force of the heart, according to McArdle et al. (2010), and is a reversible adaptation.

1.2 The *ACTN3* gene

Although *ACTN3* R577X is not as well studied as *ACE* I/D, positive associations with fitness phenotypes have been found (Bray et al., 2009), and the body of evidence for the associations is continuously growing (Macarthur and North, 2011). Skeletal muscle genomics is still in its early stages, and *ACTN3* is the only structural protein that has been widely investigated for its association with muscular phenotypes. It is due to the role of structural proteins in maintaining the size and integrity of a muscle fiber that genetic variations may cause variations in muscle performance and size (Hubal et al., 2011). *ACTN3* gene is a protein coding gene located on chromosome 11. It encodes a protein with the official name alpha actinin 3 (α-actinin-3 further in text). *ACTN3* is a highly conserved gene (North et al., 1999) found in many species (Mills et al., 2001), but has the largest variation in mammals (MacArthur and North, 2004). α-actinin-3 is a member in the alpha-actin binding protein gene family (NCBI, 2015) consisting of four different α-actinin coding genes (Sjoblom et al., 2008), with the different isoform genes being scattered on different chromosomes (Beggs et

al., 1992) and producing at least six different proteins (Sjoblom et al., 2008). One of the isoforms, α-actinin-2, is mostly expressed in cardiac and oxidative muscle cells, while α-actinin-3 is almost exclusively expressed in fast muscle fibers (Mills et al., 2001) where it plays a role in the structure of a Z-line (Z-disk) found in sarcomeres (Sjoblom et al., 2008), as demonstrated in figure 4 and 5.

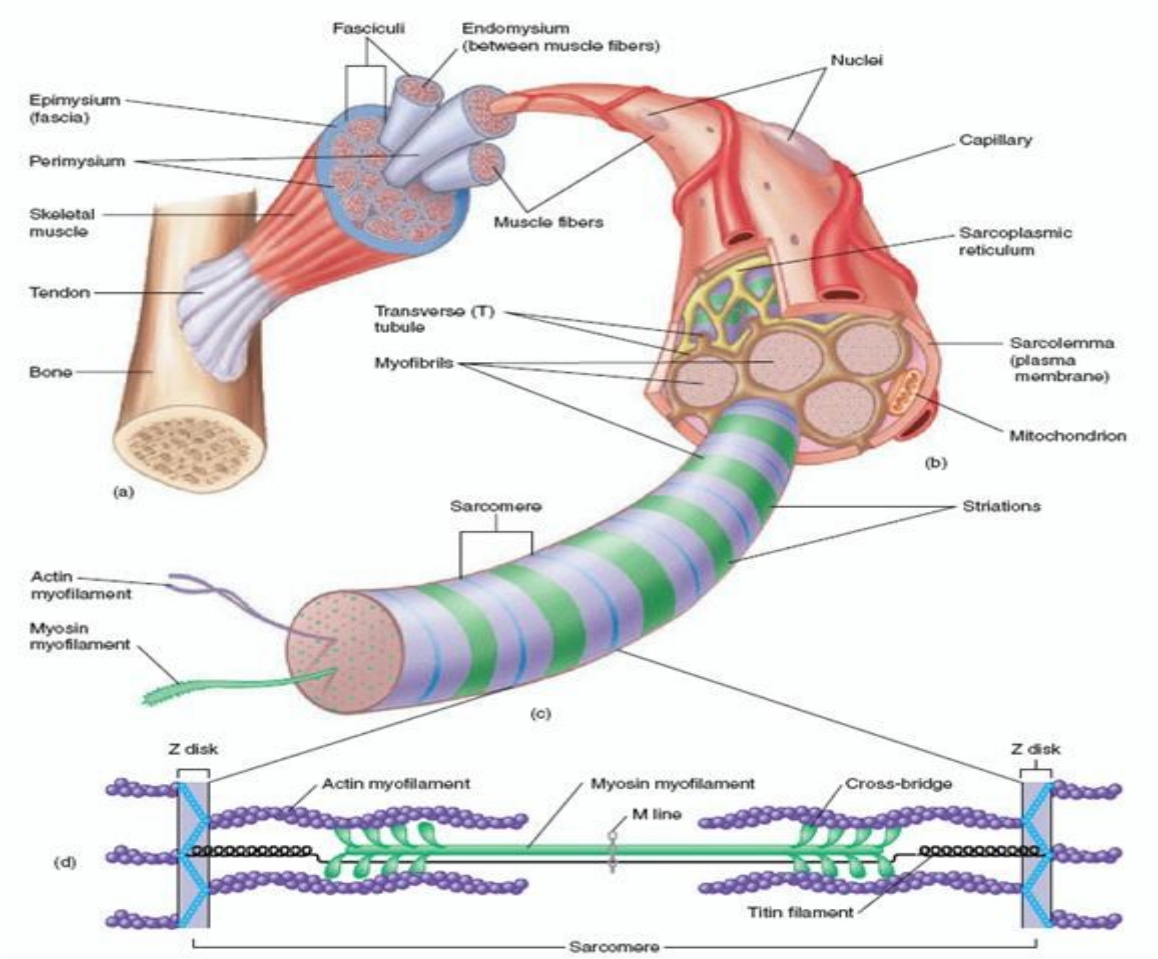

Figure 4: *Structure of skeletal muscle. a) Skeletal muscle consisting of muscle bundles (fasciculi) b) Individual muscle fibers within a fascicule c) An individual muscle fiber consisting of myofibrils which have a striated appearance due to the repeating units – sarcomeres d) Sarcomere consists of actin filaments, myosin filaments and titin filaments with Z-disks being boundaries between neighbouring sarcomeres (Picture is from APSU (2009); figure text adapted from McArdle et al. (2010)).*

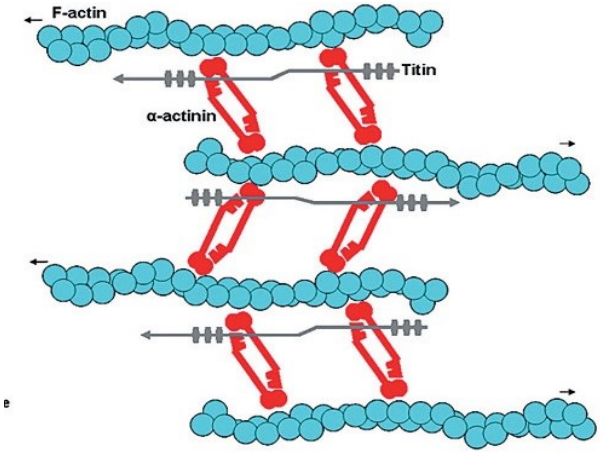

Figure 5: *Structure of Z-disk. α-actinin, showed in red, cross-links actin filaments (blue) of two subsequent sarcomeres. α-actinin interacts with the protein titin, which is shown in gray (Sjoblom et al., 2008).*

1.2.1 *ACTN3* R577X

R577X polymorphism, in particular, in *ACTN3* gene has been investigated for its influence on athletic performance (MacArthur and North, 2004). The R577X polymorphism is a transition mutation where cytosine has been transitioned to thymine in exon 16 of the *ACTN3*, converting arginine to a stop codon. This nonsense mutation (North et al., 1999) leads to a premature termination of the translation of mRNA to protein (Pierce, 2012). Due to the stop codon, less than 2/3 of the protein is synthesized and it is likely degraded rapidly. As the mutation is located at amino acid residue 577, the polymorphism is denoted by R577X (rs1815739; MacArthur and North (2004)). Initially, R577X polymorphism was thought to be an excellent candidate gene for neuromuscular disease as α-actinin-3 protein was lacking in biopsies taken from homozygotes for X-allele (XX). Biopsies from homozygotes for wild type R-allele (RR) and heterozygotes (RX), on the other hand, showed presence of the protein (North et al., 1999). However, as XX genotype was discovered also among healthy individuals, it became apparent that R577X polymorphism is common among the general public (MacArthur and North, 2004) and likely is not associated with an abnormal neuromuscular phenotype.

This suggests that α-actinin-3 may be redundant in humans as other factors, such as the α-actinin-2, likely compensate for the lack of protein in the Z-disks of the fast twitch fibers (North et al., 1999). Both proteins have similar structure, function, localisation and overlapping expression pattern (Mills et al., 2001). However, α-actinin-3 may still provide some evolutionary advantages that cannot be compensated for by α-actinin-2. After all, the *ACTN3* gene has been conserved through the evolutionary history of many mammals (North et al., 1999). As the *ACTN3* gene product is exclusively expressed in fast muscle fibers, it is likely essential for optimal high-speed contraction (Yang et al., 2007), and lack of it may have

negative consequences for sprinting (Yang et al., 2009). Associations between the R-allele and sprinting are confirmed by many studies, but the link between the null allele and endurance performance is not as clear (Yang et al., 2007).

X-allele has been found in all populations investigated (Yang et al., 2007), however, the frequency of the minor allele varies significantly in different populations (North et al., 1999). The X-allele may have been maintained in larger frequencies in some human populations due to recent positive selection (MacArthur and North, 2004), with highest frequency in Eurasia and lowest in Africa (Mills et al., 2001), as shown in Figure 6. See Appendix B for a more detailed overview of genotype and allele frequencies in different populations.

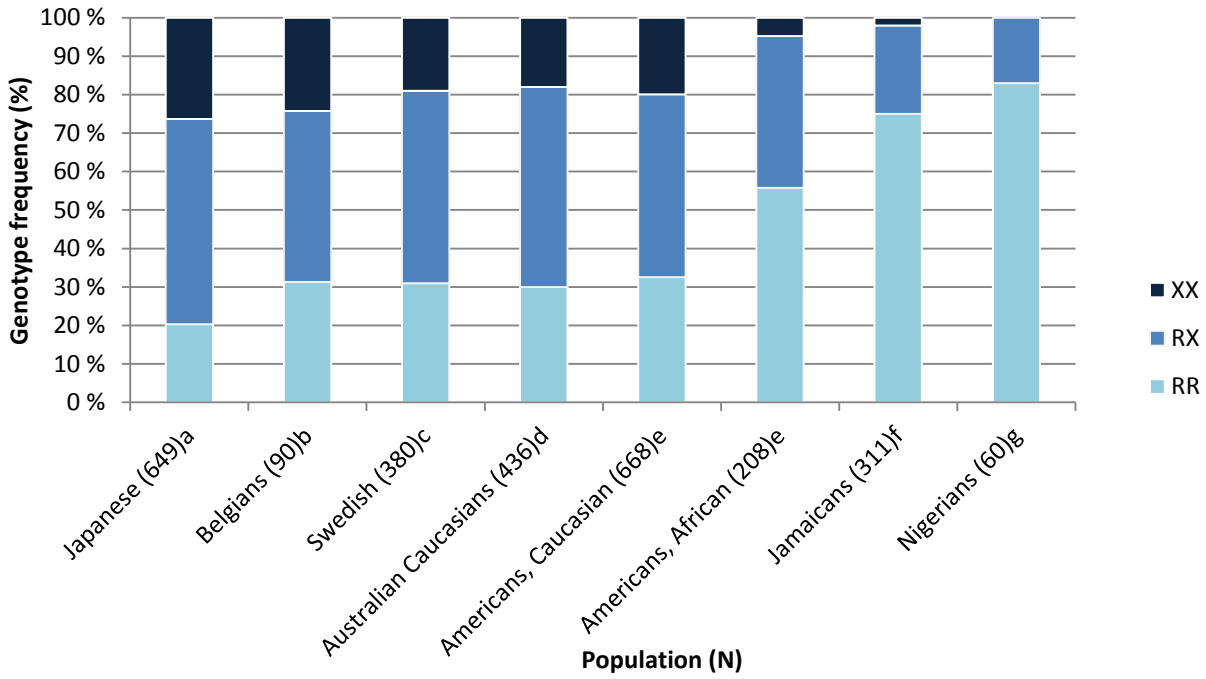

Figure 6: *Genotype frequencies (%) of the ACTN3 R577X polymorphism in various populations. a) Mikami et al. (2014); b) Vincent et al. (2008); c)Norman et al. (2009); d) Yang et al. (2003); e) Roth et al. (2008); f) Scott et al. (2010) g) Yang et al. (2007)*

Frequency of XX genotype in populations of European (Caucasian) ancestry is approximately 18% (Vincent et al., 2008). In the Swedish population, frequency of the 577X homozygotes is around 19% with an X-allele frequency of 44% (Norman et al., 2009), and is similar to those reported in other Caucasian populations (Garatachea et al. (2014), Eynon et al. (2014), Vincent et al. (2008), Yang et al. (2003), Roth et al. (2008)). Populations with lowest X-allele frequencies are Jamaican (0.14) with only 2% of the population being homozygotes for that allele (Scott et al., 2010); African Bantu (allele frequency: 0.10; XX genotype frequency: 1%)

((Mills et al. (2001); Yang et al. (2007)) and Kenyan/Nigerian populations (allele frequency: 0.09; XX genotype frequency: up to 1%; Yang et al. (2007)). Such large differences in the X-allele frequency among populations suggest that *ACTN3* may influence individuals' fitness under different environmental conditions (Yang et al., 2003). One of the possible explanations for such positive selection could be the shift towards more efficient muscle metabolism, i.e. slow-twitch traits in fast-twitch fibers (Yang et al., 2009).

Many studies have found higher frequency of the α-actinin-3 deficient XX genotype compared to RR genotype in endurance sporting disciplines (Pimenta et al. (2013); Yang et al. (2003); Eynon, Banting et al. 2014). RR genotype, on the contrary, is more common among athletes in sprint/power disciplines (Pimenta et al. (2013); Yang et al. (2003)) in Caucasian populations (Yang et al., 2009). In other populations, such as East African populations, lack of α-actinin-3 does not seem to influence muscle performance in athletes (Yang et al., 2007). Although some studies have managed to find associations between athlete performance and genotype, others have failed. In general, there are fewer studies finding associations between muscle strength and the *ACTN3* R577X genotype in men compared to women (Hubal et al., 2011). Women may have a larger effect from α-actinin-3 on muscle power than men (Yang et al., 2003), at least partly, due to hormonal difference in males and females (McArdle et al., 2010).

Lack of the α-actinin-3 may have an impact on the health of the general population (MacArthur et al., 2008), and a number of studies exist that have been investigating different aspects of muscle performance in the general public. *ACTN3* may have some subtle effects on skeletal muscle and, thus, might be one of the genetic factors contributing to normal variations in muscle function (North et al., 1999). Vincent et al. (2008) found that XX subjects demonstrated less rapid force generation in their fast twitch fibers compared to the RR genotype. As heterozygotes showed intermediate values in muscle strength measurements, the R-allele likely has additive effect on power enhancement. Similarly, soccer players with RX genotype demonstrated intermediate values compared to the homozygotes in all phenotypes tested, i.e. sprint (10m, 20m and 30m run), jump and VO_{2max}. Carriers of the R-allele demonstrated better results at strength tests, while XX homozygotes had higher VO_{2max} values, indicating better ability for endurance (Pimenta et al., 2013). Increased numbers of fast twitch (type IIx) fibers in RR subjects could be the explanatory factor for improved ability in performing dynamic movements (Vincent et al., 2008).

XX subjects seem to show higher sensation of muscle soreness after a acute eccentric exercise (see Terms and Abbreviations for explanation) compared to R-allele homozygotes, as discovered by Vincent et al. (2010). This is in agreement with the higher levels of indicator molecules of muscle damage in XX subjects. Such differences between the RR and XX genotypes were possibly caused by the structural differences in the muscles. RR subjects showed larger (80%) repair responses after the exercise, indicating that the protein product of *ACTN3* may have a protective function against muscle damage.

There are studies, however, where the findings make the effects of the R577X genotypes on muscle strength characteristics more unclear. XX subjects have been shown to have a larger response to resistance training after the intervention compared to RR counterparts despite the lower strength at the baseline. They speculate that greater increase in strength among XX individuals could be due to greater damage to the muscle after resistance training, caused by lack of α-actinin-3 in their fast-twitch muscles (Clarkson et al., 2005). Muscle damage is the stimulus necessary for the adaptive responses to occur (McArdle et al., 2010), and could make the fast-twitch muscles of X-allele homozygotes better at adapting to training. However, they are not able to develop as much power in their muscles as RR and RX subjects. *ACTN3* R577X could explain around 2% of baseline strength and strength after the intervention in this study, but there are likely many genes contributing to the genetic variation in muscle response to resistance training (Clarkson et al., 2005).

Due to their structural role in muscle fibers, the mechanisms by which the *ACTN3* nonsense polymorphism exerts its effects on performance may be easier to determine than for the *ACE* I/D polymorphism, where the polymorphism is located in an intron. Findings of the *ACTN3* knockout (KO) mice model have been useful for studying possible mechanisms. Studies on KO mice has showed that the α-actinin-2 is likely upregulated within these fast twitch muscle fibers, altering their metabolism towards more aerobic. Thus, metabolism in fast-twitch muscles becomes more similar to the metabolism of the slow twitch fibers that gets activated under endurance type of activities. Increased mitochondrial enzyme activity is likely to occur within fast twitch muscles lacking the α-actinin-3 protein, also indicating more efficient aerobic adenosine triphosphate production. A running test confirmed that the KO mice managed to run further before exhaustion than the wild type mice (MacArthur et al., 2007). Study by Norman et al. (2009) did not find differences in power output, fatigability or muscle fiber composition across the different R577X genotypes in moderately to well-trained individuals. However, similar to the KO mice study, expression of the α-actinin-2 was found

to be upregulated in XX individuals, compared with the wild-type homozygotes. The authors also suggest that *ACTN3* genotype may regulate training response as a result of repeated exercise.

Other changes observed in KO mice compared to their wild type counterparts were reduced fiber diameter and changed contractile properties of the fast twitch muscle. In addition, better ability to recover from fatigue and reduced grip strength were observed in KO mice, possibly due to lower muscle mass. There were no morphological or behavioural differences between the two types of mice. Similar change in metabolism is likely to occur in humans as well (MacArthur et al., 2008). In humans *ACTN3* homozygotes for the nonsense allele have been found to have around 5% fewer type IIx (fast twitch) fibers compared to the wild type homozygote. The same was true for relative muscle surface area covered by this type of fibers (Vincent, De Bock et al. 2008). These findings, however, could not be replicated by Norman et al. (2014). Another mechanism could be connected to use of muscle glycogen, which is utilized as the main carbohydrate energy source for working muscles (McArdle et al., 2010). Muscle glycogen use seems to be *ACTN3* R577X genotype dependent in fast-twitch fibers as glycogen levels remained higher in XX subjects compared to the RR and RX subjects despite being at similar levels prior to sprinting activity. This could indicate poorer utilization of the glycogen as a energy source in XX subjects (Norman et al., 2014).

1.3 Study design in exercise genomics

Most complex traits in sports science are equivalent to quantitative traits, i.e. traits that are measurable (VO_{2max}, muscle mass etc.), and these measurements are continuous variables (Gibson, 2009). In order to track down genes influencing complex traits two common approaches are used: GWAS and candidate gene studies (Gordish-Dressman and Devaney, 2011). However, pinpointing most important genetic factors is challenging due to the multifactorial nature of complex traits (Roth, 2007).

GWAS is a hypothesis-free approach meaning that no previous knowledge about candidate genes is necessary. In GWAS, a large number SNPs, evenly spread across the human genome, are analyzed using high-throughput microarray-based genotyping methods (Rankinen and Bouchard, 2012) in order to determine regions, QTL, associated with a particular quantitative trait (e.g. muscle strength). QTLs are co-transmitted together with a gene/genes associated with the specific phenotype of interest (Roth and Thomis, 2011), and are helpful for decreasing the number of genes needed to be investigated in order to find a candidate gene for

a trait. Instead of investigating all potential genes within a genome, one can focus on genes located within QTLs (Lightfoot, 2011a). GWAS is especially useful for studying complex traits (Roth and Thomis, 2011), such as physical performance. When QTL is found, other methods can be used to find allele responsible for the trait (Roth, 2007) as identified SNPs are not necessarily functional themselves, but they might be in linkage disequilibrium with the causal variant. One of the disadvantages of GWAS are the large number of subjects necessary in order to achieve high statistic power due to the multiple statistical tests computed during GWAS. With adequately large sample size, even genetic variants with small effect sizes can be detected (Roth and Thomis, 2011).

Most of the studies referred to in this study are genetic association studies, however. These types of studies investigate the effect of a polymorphic allele (SNPs, insertions/deletions etc.) on a particular trait. Knowledge about its location, function and possible mechanisms of action is necessary prior to the study (Roth and Thomis, 2011). In addition, the phenotype should be defined appropriately as it determines the statistical method used for the analysis (Gordish-Dressman and Devaney, 2011). Despite of the fact those, in most cases, effects of individual genes are small, up to 90% of studies on physical performance focus on candidate genes (Brutsaert and Parra, 2009). Candidate gene association studies, either based on randomly selected subjects or case-control study design (Roth and Thomis, 2011), often investigate genes known to be involved in metabolic pathways or physiological systems influencing physical fitness phenotypes (Bray, 2000). Many studies often focus on a single gene/polymorphism, although focus on multiple gene studies is increasing (Roth and Thomis, 2011). In approximately 15% of all physical performance candidate gene studies, *ACE* I/D polymorphism has been investigated. However, the results are still contradictory, as large sample size is required in order to pinpoint loci with small effects (Brutsaert and Parra, 2009).

Many polymorphisms associated with PA levels and other exercise related traits are relatively common. Recent studies support the rare variants-common disease hypothesis. Rare genetic variants contribute significantly to complex traits despite their low frequency. These are often located within coding or regulatory regions, and can have major effects on common traits/diseases. Such rare variants are normally not included in GWAS and other types of association studies (Rankinen and Bouchard, 2012). One should also consider that epigenetics is another potential factor determining the levels of activity (Lightfoot, 2011b).

The genetic data available indicates that it will, rather soon, be possible to determine subjects responding well to an exercise program, as well as those responding poorly or being intolerant

of exercise (Rankinen and Bouchard, 2012). However, wide application of genetic tests for improved performance or picking out talents is not yet possible, as research is still in its infancy (Roth, 2007). Predictability of such tests is low (Hubal et al., 2011), especially of those based on a single genetic marker, such as the over-the-counter test available in Norway. Predictability could only be improved by gaining more knowledge (Hubal et al., 2011) through large and properly designed studies (Rankinen and Bouchard, 2012).

2 Materials and methods

2.1 Study population

Genotype frequency of the *ACE* I/D and *ACTN3* R577X polymorphisms is the main focus of the present study. Frequency calculations are based on a sample of 1079 individuals whose EDTA blood samples were available (genotype study population further in text). Self-reported physical activity (PA) data were available for 1268 individuals (PA study population further in text; personal communication, Bjurholt, 2015). All subjects are from the Kolorektal cancer, Arv og Miljø molecular epidemiological study (the KAM study). Subjects filled out a detailed questionnaire on physical activity level and nutrition data, among others, as a part of the KAM study (Skjelbred et al., 2006). KAM study is approved by the Regional Ethics Committee and the Data Inspectorate. Only subjects from Telemark with no risk for adenomas or low risk adenomas were genotyped in this thesis.

KAM-study cohort, in turn, is based on the screening group from of Norwegian Colorectal Cancer Prevention Trial study (The NORCCAP study) as described elsewhere (Skjelbred et al., 2006). Briefly, approximately 21,000 subjects 50-64 years old of both genders were randomly drawn from population registers of the city of Oslo and the county of Telemark (clinicaltrials.gov, 2015) by their home address, and invited to participate. Detailed description of study design and exclusion criteria for the NORCCAP study has been published earlier (Colhoun et al. (2003); Bretthauer et al. (2002)). Attendance rate was high for Telemark County, i.e. approximately 70% (Bretthauer et al., 2002). Answers from the questionnaire from the NORRCAP study were later used to validate the responses on self-reported PA levels from KAM study, which is the basis of PA estimates in this study. Around 450 participants filled out the questionnaire at three separate occasions, two times during the NORRCAP study and once during KAM.

In order to analyse association between *ACE* I/D and *ACTN3* R577X polymorphisms and PA levels, data from genotype and PA population was combined. The new data set consisted of individuals with both genotype and PA data (N=926; the combined study population further in text), as shown in Figure 7.

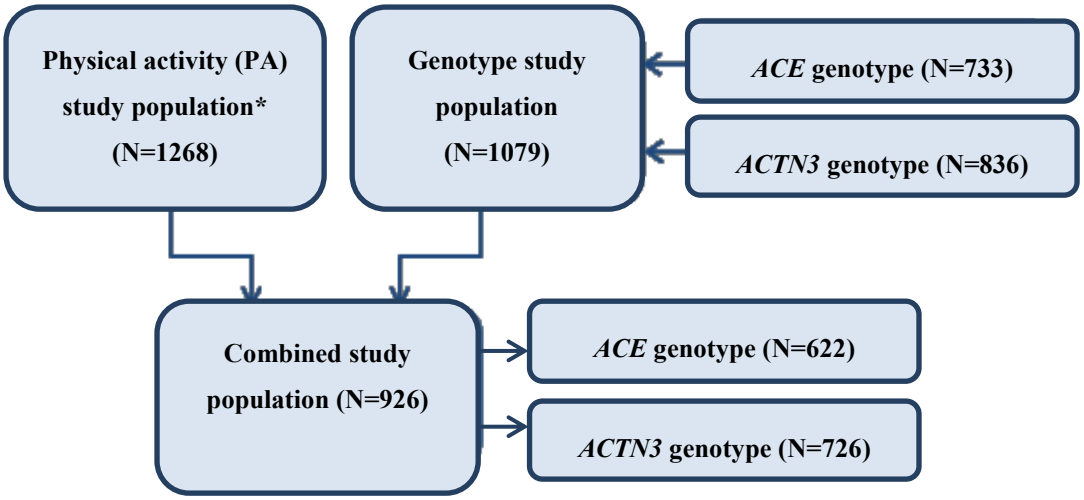

Figure 7: *Overview of the three different study populations (physical activity (PA), genotype and combined study populations) obtained from Kolorektal cancer, Arv og Miljø (the KAM study). Data from the genotype and the combined study population have been used for calculations in the present study.*
**unpublished data from the study of Bjurholt, A.M.*

Study is based on analysis of two separate study populations, i.e. the genotype and the combined study population. Genotype frequency data are based on genotype study population baseline characteristics of which is presented as means ± SD and the coefficient of variation, where appropriate, in Table 2. See Appendix C a) and b) for baseline characteristics for the PA study population and the combined study population.

Table 2: *Baseline characteristics of the genotype study population*

Variable	Total (N=1079)		Male (N=554)		Female (N=515)	
	mean ± SD	CV	mean ± SD	CV	mean ± SD	CV
Age (year)	55.8 ± 3.8	6.9	56.0 ± 3.8	6.8	55.5 ± 3.9	7.0
Anthropometric data						
Weight (kg)	78.7 ± 13.8	17.5	84.7 ± 11.9	14.0	72.0 ± 12.5	17.4
Height (cm)	173.1 ± 8.6	4.9	179.2 ± 6.1	3.4	166.7 ± 5.5	3.3
BMI (kg/m^2)	26.2 ± 3.7	14.3	26.4 ± 3.2	12.2	25.9 ± 4.6	17.6

SD- standard deviance; CV=Coefficient of Variance

2.2 Extraction of DNA

Salting out procedure, described in Miller et al. (1988) with some modifications, was used in order to extract genomic DNA from venous blood stored at -20^{o}C prior to extraction (Hansen et al., 2005). Three times the sample volume lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 1

mM EDTA, pH 7.4) was added to thawed, anticoagulated blood samples. Samples were then incubated at 3°C for 30-60 min and centrifuged. The pellet containing nucleated cells were then resuspended in 10 ml SE buffer (75 mM NaCl, 24 mM EDTA, pH 8.0). 500 µl 20% SDS and 50 µl protease K were added and samples were incubated over night at 40°C. After incubation 2.0 ml 6M NaCl was added to each sample and shaken for 15 seconds. Lysate was then centrifuged at 5000rpm for 15 min at 3°C in order to create a protein pellet. Two volumes of cold absolute ethanol were then added to supernatant. The precipitated DNA was then washed in 3 ml 70% ethanol, air dried and then moved to a 2.0 ml tube containing 200-1000 µl TE-buffer (10 mM Tris, 0.1 mM EDTA, pH 7.5). DNA was then dissolved by shaking it at 800 rpm for 24 hours at room temperature. Stock solution was stored at 4°C until use.

2.3 Quantification of DNA

DNA was quantified with Picodrop Microliter UV/Vis Spectrophotometer (Picodrop Limited, United Kingdom) by 260 nm absorbance. Purity of DNA was measured by 260/280 nm ratio. Each sample was measured twice, and average concentration value was used for making working solution with approximate DNA concentration of 100 ng/µl. Average DNA concentration of stock solutions was 585.6 ng/µl (range from 11.5 ng/µl to 1099.6 ng/µl), with average purity at 1.69 for 1079 samples.

2.4 Genotyping for *ACE* I/D polymorphism with PCR

Genotyping for both the *ACE* and *ACTN3* gene polymorphisms were done by being blind to subject identity.

The insertion/deletion (I/D) genotype for the *ACE* gene was identified by polymerase chain reaction (PCR) amplification with Eppendorf Mastercycler gradient (Eppendorf AG, Germany) and MJ Research PTC 200 Thermal Cycler (Bio-Rad Laboratories, Inc., USA). Each reaction mixture of 24.5 µl contained 2% dimethyl sulfoxide (DMSO), 1x PCR buffer, 0.2mM dNTP, 2mM MgCl, 0.2 pmol/µl of each primer, 0.5U/µl Taq polymerase. Approx. 100 ng of genomic DNA was added to each PCR reaction with total volume of 25.5 µL. Forward primer was 5'-CTGGAGACCACTCCCATCCTTTCT-3' and reverse primer 5'-GATGTGGCCATCACATTCGTCAGAT-3' (Rigat et al., 1992). Initial denaturation at 95°C for 3 min was followed by 30 cycles of denaturation (95°C, 15 sec), hybridization (53°C; 45

sec) and extension (72°C; 30 sec). After final elongation (72°C; 5 min) the PCR products were stored at 4°C until separating DNA fragments with gel electrophoresis. PCR resulted in three possible outcomes: DD (190 bp), ID (490 and 190 bp) and II (490 bp; Abigail (2012)).

There was one negative control per 39 samples. No positive controls were used initially. Samples that gave no results and samples that gave unclear results were re-analyzed, with samples that have been genotyped successfully as positive controls.

The I allele was generally weakly amplified in heterozygotes despite the addition of 2% DMSO in PCR reaction. DMSO is added into PCR reaction to facilitate denaturation of the DNA strand (Altshuler, 2006). In order to avoid mistyping of ID individuals as DD, another PCR reaction was carried out with insertion specific forward primer (5'-TTTGAGACGGAGTCTCGCTC-3') and standard reverse primer, as described by Shanmugam et al. (1993). Only samples with DD genotype were analyzed during this PCR reaction. Each reaction of 25.0 µl contained 12.5 µl AmpliTaq Gold® PCR Master Mix, 5% DMSO, 0.2 pmol/µl of each primer, and approx. 100 ng template DNA. PCR reaction conditions were as follows: initial denaturation at 95°C for 3 min was followed by 30 cycles of denaturation (92°C), hybridization (61°C) and extension (72°C) for 1 minute each. After final elongation (72°C; 7 min) the PCR products were stored at 4°C until analysis. Insertion specific PCR reaction yielded a 408bp long DNA fragment in carriers of the I-allele (II and ID genotypes) and no PCR product in DD subjects.

2.5 Separation and visualization of PCR products

PCR products were separated by 6**%** polyacrylamide gel electrophoresis (PAGE) for 30 min at 150V. Size standard Phi-X174 RF DNA/Hae III (Thermo Fisher Scientific Inc, USA) was used. Gel staining was conducted by adding GelRed® (Biotium, Inc., USA) directly in slot: 1 µl 6X Standard Loading Buffer (Omega Bio-tek Inc, USA) with 2XGelRed® + 4 µl sample, as suggested by Genaxxon (2011). Gels were visualized by UV light with Gel Doc system from Syngene (Division of Synoptics Ltd).

2.6 Genotyping for *ACTN3* R577X polymorphism

Genotyping of *ACTN3* R577X polymorphism was carried out with TaqMan® SNP Genotyping Assay, assay ID C____590093_1 (Applied Biosystems®, USA) on StepOne™

Real-Time PCR System (Applied Biosystems®, USA). Genotype calling was done by StepOne Software v2.0.

Each reaction of a total of 15 µl genotyping mixture contained 8.44 µl Genotyping Master Mix, 0.42 µl Assay mix (40x), 6.33 µl distilled H_20 and 1.5 µl of DNA template (approx. 100 ng/µl). Reaction conditions were as follows: 30 sec at 60°C followed by initial denaturation stage for 10 min at 95°C ; denaturation stage for 15 sec at 95°C followed by annealing at 60°C for 1 min in cycling stage with 40 cycles altogether; at final post read temperature was kept at 60°C for 30 sec (ThermoFisherScientific, 2013).

In addition to the duplicate samples, two no-template controls (water instead of DNA template) and two positive controls (one of each homozygous genotype) were used on each 48-well plate, as suggested in McGuigan and Ralston (2002) in order to ensure accuracy of the AD-assay. Genotypes of positive controls were previously determined by 23andMe, Inc., privately-held company offering personal genetic analysis service. Laboratory testing is done in CLIA-certified and CAP-accredited laboratory (see Terms and abbreviations). They use Illumina HumanOmniExpress-24 format chip (Illumnia, San Diego, CA, USA) for genotyping purposes (23andme.com). According to producer of this chip, it covers common genetic variations with strategically selected tagSNP and, thus, is well suited for GWAS (Illumina, 2014).

For some samples automated genotype calls were not created, likely due to some technical issues. Although there were clusters along the horizontal and vertical axes as well as at the diagonal, these yielded "undetermined" call on allele discrimination plot (see Appendix D). For many of these samples raw data were available, and thus, these results were read manually.

2.7 Determination of physical activity levels

Energy expenditure during PA is often measured in metabolic equivalents (METs) which compare oxygen consumption during an activity compared to the resting level. One MET is resting metabolic rate, i.e. amount of oxygen consumed during rest, which is set at 3.5 ml O_2/min/kg (for a subject weighing 70 kg). Two METs would then be oxygen consumption twice the resting level, and so on. METs provide a useful method of estimating functional capacity of a person (Jetté et al., 1990) and intensity of an activity (Ainsworth et al., 2000). Intensity of different activities is commonly classified as light when breathing is close

to/slightly above normal level and perspiration is minimal (up to 4 METs); moderate when breathing is above normal and there is a definite perspiration (5-8 METs); heavy when breathing and perspiration is heavy (8 and higher METs; Jetté et al. (1990)). In order to improve cardiorespiratory fitness, activities should be at 6 METs and higher (McArdle et al., 2010).

PA levels and physical capacity score in METs are calculated based on KAM study questionnaire data. Questions and response options are presented in Table 3. A total of 1268 individuals had responded on questions about their PA habits by choosing one of the provided alternatives as an answer (PA study population). Focus of the questions on PA in KAM study was on aerobic (endurance) type of exercise. As it did not map the habits of resistance and other type of anaerobic training, PA calculations in this study are based exclusively on aerobic PA.

Table 3: *Questions and response options on participants' physical activity habits in KAM study*

Question	Response options
Have you cycled or walked to work during the past five years?	No
	Once a week
	Several times a week
	Daily
Do you hike?	No
	Once a week
	Several times a week
Were you participating in sports or outdoor activities at a younger age?	No
	Yes
How often do you exercise for at least 20 minutes?	Never
	Less than once a week
	Once a week
	Several times a week
	Daily
If you exercise, do you perspire?	No
	Yes

MET levels were assigned to each activity (cycling, walking or exercise) depending on how often the particular activity was conducted (=PA level). By combining PA level with perspiration data, sum of physical capacity was calculated. This estimate was, in turn, used to determine participants' physical capacity score and MET category (unpublished data). PA levels and sum of physical capacity were later used in the analysis of association between the *ACE* I/D and *ACTN3* R577X genotypes and PA levels. In order to investigate associations between genotypes of *ACE* I/D and *ACTN3* R577X and PA, participants from the combined study population (n=926) were grouped in two categories based on their response on whether they reported participation in any kind of PA or not.

Several of subjects (n=455) filled out similar questionnaires at several occasions i.e. twice during the NORCCAP study and once during KAM-study. In order to validate answers on PA questions from KAM-study, responses were compared (data not shown). Questions about PA in both studies were rather similar, with only minor differences (see Appendix E for questions regarding PA from NORCCAP study).

2.8 Data analysis

Chi-square test (χ^2) was used in order to test whether or not the results were in Hardy-Weinberg equilibrium (HWE). Differences in genotype distribution between male and female subjects; between the genotype and the combined PA and genotype study populations; and between genotype frequencies in present study and populations from other studies were investigated contingency Chi-square test. Both tests were done by the R Commander package, R version 3.1.1. Associations between *ACE* I/D and *ACTN3* R577X genotype and physical activity was investigated with two-tailed t-test and univariate and multivariate general linear models with post hoc (Bonferroni) test in IBM SPSS Statistics, version 22 (Chicago, IL, USA). Level of significance for all calculations were set at p=0.05 for all tests.

3 Results

3.1 Frequency of *ACE* I/D genotype

According to Shanmugam et al. (1993) amplification of I-allele in heterozygotes is suppressed in PCR reaction of unknown reasons. After completing all runs with first protocol, heterozygotes were clearly underrepresented (38%), while homozygotes for D-allele were overrepresented (36%) compared to what was expected from the HWE (i.e. genotype frequency of around 25% for each homozygote and 50% for heterozygotes). After performing insertion-specific protocol, 733 out of 1079 samples were genotyped successfully; 173 were homozygous for the deletion (D) allele, 174 were homozygous for insertion (I) allele and 386 were heterozygous, i.e. 23.6%, 23.7% and 52.7% of the total genotype study population, respectively (see Table 4). Genotype was impossible to determine for 360 samples. Out of these, 345 yielded no PCR product even after repeated PCR run; 6 were genotyped as possible DD subjects; 6 were genotyped as possible ID subjects; 3 were genotyped as possible II subjects, but without certainty, and thus were not included in the statistical analysis.

Table 4: *ACE I/D genotype frequency distribution in the Norwegian population (the genotype study population)*

Genotype, n (%)	Total (N=1079)	Male (N=554)	Female (N=515)	Unknown gender (N=10)
	(n=733)	**(n=364)**	**(n=366)**	**(n=3)**
II	174 (23.7)	94 (25.8)	80 (21.9)	-
ID	386 (52.7)	193 (53.0)	191 (52.2)	2
DD	173 (23.6)	77 (21.2)	95 (26.0)	1

N-Total number of subjects in the genotype study population; n-number of subjects with successfully determined genotype.

The observed genotype frequencies did not deviate from HWE (χ^2=2.08, df=2,p=0.35). Allele frequencies were 0.55 for the D allele and 0.45 for the I-allele. Genotype frequencies did not differ significantly between male and female subjects (χ^2= 3.02, df = 2, p= 0.22).

3.2 Frequency of *ACTN3* R577X genotype

Out of 1079 samples, genotype was impossible to determine for 243 samples, thus the sample size for *ACTN3* R577X polymorphism was 836 subjects whose genotype was successfully determined. 30.9% (n=258) were homozygous for the R-allele, 48.6% (n=406) were

heterozygous, and 20.6% (n=172) were homozygous for the X-allele, as demonstrated in Table 5. Allele frequencies were 0.55 for the R-allele and 0.45 for the X allele.

***Table 5**: ACTN3 R577X genotype frequency distribution in the Norwegian population (genotype study population)*

Genotype, n (%)	Total (N=1079)	Male (N=554)	Female (N=515)	Unknown gender (N=10)
	(n=836)	**(n=407)**	**(n=425)**	**(n=4)**
RR	258 (30.9)	111 (27.3)	143 (33.6)	4
RX	406 (48.6)	204 (50.1)	202 (47.5)	-
XX	172 (20.6)	92 (22.6)	80 (18.8)	-

N-Total number of subjects in the genotype study population; n-number of subjects with successfully determined genotype

The observed *ACTN3* R577X frequencies in the Norwegian population were in HWE (χ^2= 0.15, df= 2, P= 0.93), and did not differ significantly between genders (χ^2= 4.49, df = 2, p-value = 0.11).

3.3 Associations between physical activity and *ACE* I/D and *ACTN3* R577X genotype

The combined study population included 926 subjects for whom the PA responses were available, in combination with one or both genotypes. Genotype frequencies from the combined study population did not differ significantly from frequencies found in the genotype study population (p= 0.90 and p=0.93 for the *ACE* and *ACTN3* genes, respectively). PA and genotype data for *ACE* I/D and *ACTN3* R577X for the combined study population are demonstrated in Table 6 (see Appendix F for questionnaire response summary). 74.1% (n=686) of the subjects had PA levels of over 10 METs, and 7.5% (n=69) had levels under 7 METs. Similarly, 7.0% (n=67) of the subjects had physical capacity score under 7 METs, while 74.1% (n=686) demonstrated capacity score above 10 MET. 34 (3%) subjects from the combined study population responded that they do not participate in any kind of PA (data not shown).

Table 6: *Physical activity (PA) level, physical capacity score, and genotype frequency distribution for ACE I/D and ACTN3R577X polymorphisms in the combined study population*

Variable	Total (N=926) *n* (%)	Male (N=476) *n* (%)	Female (N=450) *n* (%)
Total PA level			
< 7 MET	69 (7.5)	42 (8.9)	27 (6.0)
7 - 10 MET	171 (18.5)	109 (22.9)	62 (13.8)
> 10 MET	686 (74.1)	325 (68.3)	361 (80.2)
Total physical capacity score			
< 7 MET	65 (7.0)	39 (8.2)	26 (5.8)
7 - 10 MET	115 (12.4)	73 (15.3)	42 (9.3)
> 10 MET	746 (80.6)	364 (76.5)	382 (84.9)
	Genotype		
ACE	(n=622)	(n=307)	(n=315)
II	141 (22.7)	73 (23.8)	68 (21.6)
ID	333 (53.5)	167 (54.4)	166 (52.7)
DD	148 (23.8)	67 (21.8)	81 (25.7)
ACTN3	(n=726)	(n=354)	(n=372)
RR	222 (30.6)	101 (28.5)	121 (32.5)
RX	359 (49.5)	180 (50.9)	179 (48.1)
XX	145 (19.9)	73 (20.6)	72 (19.4)

Results are presented as means ± SD; CV=Coefficient of Variance; PA- physical activity; N-Total number of subjects in the combined PA and genotype study population; MET = metabolic equivalent.

Women were found to be significantly more physically active than men ($p<0.05$); however, difference was modest. No associations were found when tested between PA levels or physical capacity score across *ACE* and *ACTN3* genotypes; or when adjusted for gender and BMI. Similarly, no associations were found when combined effects of both polymorphisms were investigated. See Appendix G for self-reported PA data and genotype frequencies for *ACE* I/D and *ACTN3* R577X polymorphisms in the PA study population.

4 Discussion

Genes influence many aspects of physical fitness and exercise response. *ACE* and *ACTN3* genes are among the most studied genes in the field of exercise genomics. They are thought to have influence on traits such as muscle strength, endurance and trainability.

Genotype frequencies for *ACE* I/D polymorphism vary widely across populations. In Caucasian populations, in general, genotype frequency is 25% for II and DD genotypes and 50% for heterozygotes (Jones and Woods, 2003). In the present study approximately 24% were found to be homozygous for either I or D allele, and 53% were heterozygous for *ACE* I/D polymorphism. These results are consistent with two earlier studies on Norwegian subjects (Bohn et al. (1993); Tronvik et al. (2008)). Genotype frequencies did not deviate from HWE, indicating correct genotyping, sufficient sample size (Roth, 2007) and/or no stratification of the sample (Marchini et al., 2004). The study population of the present study is homogenous and large enough to be a good representative of a Norwegian population (Skjelbred et al., 2006). The results from the present study can be used as a reference group in further case-control or intervention studies.

In order to determine genotype for *ACE* I/D polymorphisms, two different PCR protocols were used (i.e. three-primer method). The first PCR reaction is designed for amplifying both alleles (I and D), while the other protocol is designed for amplifying the insertion allele only. Amplification of the I-allele can sometimes be suppressed in heterozygotes under PCR-reaction (Shanmugam et al., 1993). This was also observed in the present study as the DD genotype was overrepresented after the initial two-primer PCR protocol, and there was no concordance with HWE. However, genotyping results after running PCR protocol with insertion-specific forward primer showed many heterozygotes incorrectly genotyped as homozygotes for D-allele. Many studies use the three-primer genotyping method, which demonstrates genotyping consistency of 92-100% (Glenn et al. (2009); Shanmugam et al. (1993)). However, some studies (Defoor et al. (2006); Shahmoradi et al. (2014); Maestu et al. (2013)), including the earliest study of Norwegian population (Bohn et al., 1993), only used a two-primer-protocol, which have genotyping accuracy of 55% (Glenn et al., 2009). Genotyping errors could eventually lead to false associations, meaning that results from these studies should be treated with caution. Eisenmann et al. (2009) performed genotyping using PCR-restriction fragment length polymorphism protocols on two polymorphisms that are known to be in complete linkage disequilibrium with *ACE* I/D polymorphism. These polymorphisms were previously described by Glenn et al. (2009). This was done as other

standard methods (two-primer method and deletion-specific primer method) returned inconsistent results. Wang et al. (2013) reported using a TaqMan® assay for determining genotype for *ACE* I/D polymorphism by genotyping a SNP in perfect linkage disequilibrium with *ACE* I/D. Using AD-assay could be more advantageous in studies with large sample size as assays are known for producing reliable and reproducible results (Life Technologies, 2011). Standard PCR protocols are labour intensive as two separate PCR protocols are necessary for achieving 100% genotyping accuracy.

Many (n=345) of the total of 1079 samples in this study did not yield any results even after repeated PCR runs. DNA was extracted by modified salting out procedure, and the quality/quantity is generally sufficient for genotyping. It may, however, be necessary to purify the DNA for better results. One possible cause for lacking results is too low concentrations or no DNA in the working solution. However, this is unlikely, as only few samples had low concentrations in the stock solution. In addition, several of these yielded results for the *ACTN3* gene.

It is generally accepted that around 18% of the Caucasian population are α-actinin-3 deficient (Vincent et al., 2008). Frequencies of the RR genotype in Caucasian populations ranges from 27 to 39%, while for the XX genotype – 11 to 27%. Genotype frequencies for R577X polymorphism in the present study were 30.9, 48.6 and 20.6% for RR, RX, and XX genotype, respectively. Allele frequencies were 0.55 and 0.45 for R and X allele, respectively. Observed frequencies did not differ significantly from HWE, and were similar between genders. To the best of my knowledge, there are no previous studies on *ACTN3* R577X polymorphism conducted on Norwegian subjects. Findings of the present study are consistent with earlier findings in other Caucasian populations, such as the Swedish population (Norman et al., 2009). Minor allele frequency in the Swedish population have been reported to be 0.44, and 19% are homozygotes for XX genotype. The present population is representative for general Caucasian population, and can be used as a control group in further studies on people affected by diabetes, obesity or other conditions.

For the *ACTN3* R577X polymorphism, it was not possible to genotype 243 out of the 1079 samples. For most of the samples, a software problem was the main reason for missing data. However, for some samples too large variation in template concentrations within samples could potentially explain lack of results. It is generally suggested to normalize input of template DNA in reactions. The assay based methods, such as the one used in the present study, is a method of choice in many of the association studies on *ACTN3* gene. It is a robust

(McGuigan and Ralston, 2002) genotyping method yielding highly accurate results. The method is less labour intensive (Life Technologies, 2011) compared to conventional PCR methods.

Well designed studies are crucial for finding associations between genes and complex traits, such as PA. Results from previous studies suggest that up to 70% of PA participation can be explained by genetic factors (Stubbe et al., 2006), and genes influencing levels of PA are not deterministic genes, but susceptibility genes (Hagberg, 2010). This means the person is born with predisposition towards being inactive/active (Lightfoot, 2011b). Many studies have found associations between *ACE* I/D and *ACTN3* R577X polymorphism and elite physical performance and various traits in the general public. However, many studies have failed to find this association. Potential weaknesses for many of these studies are mixed cohorts (e.g. mixed nationalities (Cam et al., 2007); differences in study design; small population size; using results from studies that are not specifically designed to discover associations between genetic marker and complex traits (Rankinen et al., 2000b). Small sample sizes (Loos et al., 2015) have insufficient power to detect effects of a gene on a complex trait (Hattersley and McCarthy, 2005) and are at higher risk of finding false positive association (Rankinen et al., 2000a).

The NORRCAP study (samples of which later were basis for the KAM-study biobank) had a strong study design, partly due to large sample size of a homogenous study population and high attendance rate (around 70% for participants from Telemark county), as described in earlier studies (Gondal et al. (2003); Hansen et al. (2005)). The study design of the present study is strengthened further as the high minor allele frequencies reported for both polymorphisms in Caucasian populations, i.e. up to 50% for the I-allele in both *ACE* (Jones and Woods, 2003) and *ACTN3* gene (Clarkson et al., 2005) makes it possible to draw a balanced sample from the population by common random sampling strategies (Roth, 2007).

Findings of the present study found no association between the *ACE* I/D and *ACTN3* R577X polymorphism and levels of self-reported PA. Association between the polymorphisms in the present study was based on whether subjects reported any engagement in PA or not, i.e. active vs. sedentary. To get a proper evaluation of this association it is crucial to have a correct measure of the PA level. In the KAM cohort data from questionnaires were used. Significantly higher PA levels among women compared to men were found, which is consistent with findings from other studies on Norwegian subjects (Kumar (2008); Bertheussen et al. (2011)). These results differ, however, from what is generally accepted, i.e.

men are more active than women (Lightfoot, 2011a). The study population of this particular study demonstrated rather high PA levels. Around 70% of the combined study population demonstrated PA levels above 10MET, meaning that these persons are generally physically active. Compared to other populations, these are high levels of activity (De Moor et al., 2009). In addition, around 74% of the subjects had physical capacity score above 10 METs, at least partly confirming high PA levels, as physical capacity score was estimated by combining the PA level data with the intensity of the reported activities (i.e. perspiration data). Only 34 individuals (3%) from the combined study population did not report any participation in PA (i.e. were defined as sedentary), thus, giving a small sample size when tested against the active group for association between *ACE* and *ACTN3* genes and PA levels. However, results did not change considerably when low PA level group (under 7 METs, n=69 (7.5%)) was tested against the group with high self-reported PA levels (above 10 METs, n= 686 (74.1%)).

Although questionnaires are widely used and cheap tools for mapping PA behaviour, they present a major weakness - self-reported measures of PA are subjective (Ainsworth et al., 2015) as participants do not always recall their past activities accurately (Bray et al., 2011). As a result, the power to detect PA-gene relationships is potentially weakened. Nevertheless, questionnaires are often used in genetic studies (Bray et al., 2011). It has been shown that the self-reported PA levels are often over or underestimated compared to the real PA levels. In addition, levels of vigorous PA tend to be overestimated (Prince et al., 2008) which could potentially explain why so many individuals in the present study are highly active (MET above 10). All these factors could represent a potential weakness of the present study, especially as the questionnaire used in the KAM-study was not developed specifically for the purpose of determining PA levels. It had rather been designed to map participants' lifestyle in order to gain as much information on potential risk factors for development of colorectal cancer (Skjelbred et al., 2006).

According to Prince et al. (2008), the most accurate estimates of PA levels can be obtained by direct measures of PA (e.g. direct or indirect calorimetry, different wearable sensors etc.). However, as these methods are too expensive to use in large studies, instead questionnaires and other self-reporting tools are widely used (Bray et al., 2011). Direct methods are commonly being used to validate questionnaire data (Prince et al., 2008). The questionnaire from the KAM-study is, although slightly less detailed, fairly similar to the questionnaire used in The Nord-Trøndelag Health study (HUNT; Kurtze et al. (2008)). Results from the short questionnaire used in HUNT 1 were reproducible, and comparable with the other validated

assessment methods, such as the validated International Physical Activity Questionnaire, direct measurements of energy expenditure and VO_{2max} measurements. In addition, responses on PA questions from several occasions were available for many subjects in present study. In that way, it was possible to evaluate the reliability of the responses from KAM-study. There was a good resemblance in responses between the NORCCAP and KAM studies, indicating that participant PA habits had not changed notably between the different surveys and/or that participant's managed to recall their past physical activity well. Thus, results from current study should be valid, and can be used to compare PA levels in the present study with other studies.

Most of the potential candidate genes for their influence on PA levels have been picked out based on their functional relevance, and not due to their location within known QTL. A serious disadvantage of such an approach is the increased probability of finding false positive associations. As a countermeasure, it has been suggested to require at least three independent lines of evidence before declaring a candidate gene. Lines of evidence are, for instance, functional relevance, being located in known QTL and showing gene expression differences (Lightfoot (2011a); DiPetrillo et al. (2005)). Relatively few studies have been investigating relationship between *ACE* I/D polymorphism and self-reported PA level. To the best of my knowledge, there are no candidate gene studies that have been investigating association between PA and *ACTN3* R577X polymorphism directly. However, some indications for their candidacy have been found, but evidence for their candidacy is limited as these genes are not being supported by at least three lines of evidence. *ACE* and *ACTN3* are both functionally relevant genes, and they have been found to be located within an identified QTL in mice. *ACTN3*, in addition, have haplotype differences. However, none of them are located within QTL associated with PA in humans (Lightfoot, 2011a). DD genotype of the *ACE* I/D polymorphism have been associated with both higher (Maestu et al., 2013) and lower (Winnicki et al., 2004) PA levels compared to II genotype. Fuentes et al. (2002), on the other hand, did not find any association between *ACE* I/D and leisure time PA. *ACE* and *ACTN3* genes have also been thought to influence PA indirectly, i.e. through their effects on response to regular exercise or physical fitness. Gene variants that improve physical fitness faster are thought to promote PA (Stubbe et al., 2006).

Other genes than *ACE* and *ACTN3* likely influence the PA levels in the Norwegian population. In general, heritability of PA levels has been reported to be small to medium high, and higher in males than females. In the Norwegian population, on the contrary,

heritability estimates of exercise participation have been reported to be 27% and 56% in male and female subjects, respectively (Stubbe et al., 2006). However, any single gene will likely only have a modest influence on a particular complex trait (Brutsaert and Parra, 2009), such as PA levels. Due to the large sample size, homogenous study population and high frequency of the minor alleles of *ACE* I/D and *ACTN3* R577X polymorphisms, any such associations would have been found by present study.

Even though genes play an important role in determining the levels of PA, genetic control of PA is not fully understood. Studies have revealed only a small number of possible QTLs associated to regulation of PA, meaning either involvement of only few genes or, a more likely explanation, a large number of genes involved in determination of the PA each of which playing only a minor role. In addition, regulatory mechanisms in non-coding areas of a DNA may also play a role. At the time of writing, there were only two genes that met the three-line-of-evidence standard, i.e. *Drd1* and *Nhlh2*. There are at least four independent lines of evidence for each of the two genes, such as being located in QTL in mice, exhibiting at least partial haplotype differences, being functionally relevant (Lightfoot, 2011a). *Drd1* codes for dopamine receptor, and mediates behavioural responses as well as influence neuron growth (NCBI, 2015). *Nhlh2* codes for a transcription factor Nescient helix-loop-helix 2 (NCBI, 2015), and exact mechanisms for regulating PA are not known. It could be interesting to see whether these two genes could be linked to levels of PA in the Norwegian population.

5 Conclusion

To the best of my knowledge, this is largest study on *ACE* I/D polymorphism, and first study on *ACTN3* R577X polymorphism conducted on healthy Norwegian subjects. Results are based on large sample size acquired from a homogenous population. Genotype frequency of *ACE* I/D and *ACTN3* R577X polymorphisms found in the Norwegian population do not differ significantly from other Caucasian populations. The results of the present study are representative for a broader Norwegian population. Genotype frequencies from present study can serve as a baseline in further case-control or intervention studies investigating association between *ACE* I/D and *ACTN3* R577X polymorphism and fitness related traits.

Present study could not find any association between the *ACE* I/D and *ACTN3* R577X polymorphisms and self-reported physical activity levels. Thus, it is unlikely that *ACE* and *ACTN3* genes have an impact on self-reported PA levels in Norwegian population. However, findings are based entirely on participation in aerobic type of activities, as results for strength training were not available. It was found that female subjects are significantly more active than male subjects. Results correspond well to findings from other studies on Norwegian subjects.

6 References

23andme.com. *How it works: Our technology and standards* [Online]. 23andMe, Inc. Available: https://www.23andme.com/howitworks/ [Accessed 06.05.2015 2015].

Abigail, R. 2012. *Angiotensin converting enzyme (ACE), Actinin (ACTN3), Tumour necrosis factor (TNF) gene polymorphism associated with biomarkers and physical characteristics in young African cricket players of Zulu origin.* MSc. University of Zululand.

Ahmetov, I. I. & Rogozkin, V. A. 2009. Genes, athlete status and training -- An overview. *Med Sport Sci,* 54**,** 43-71.

Ainsworth, B. E., Cahalin, L., Buman, M. & Ross, R. 2015. The current state of physical activity assessment tools. *Prog Cardiovasc Dis,* 57**,** 387-95.

Ainsworth, B. E., Haskell, W. L., Whitt, M. C., Irwin, M. L., Swartz, A. M., Strath, S. J., O'brien, W. L., Bassett, D. R., Jr., Schmitz, K. H., Emplaincourt, P. O., Jacobs, D. R., Jr. & Leon, A. S. 2000. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc,* 32**,** S498-504.

Alfred, T., Ben-Shlomo, Y., Cooper, R., Hardy, R., Cooper, C., Deary, I. J., Gunnell, D., Harris, S. E., Kumari, M., Martin, R. M., Moran, C. N., Pitsiladis, Y. P., Ring, S. M., Sayer, A. A., Smith, G. D., Starr, J. M., Kuh, D. & Day, I. N. 2011. ACTN3 genotype, athletic status, and life course physical capability: meta-analysis of the published literature and findings from nine studies. *Hum Mutat,* 32**,** 1008-18.

Altshuler, M. L. 2006. *PCR troubleshooting: The essential guide*, Horizon Scientific Press.

Amir, O., Amir, R., Yamin, C., Attias, E., Eynon, N., Sagiv, M., Sagiv, M. & Meckel, Y. 2007. The ACE deletion allele is associated with Israeli elite endurance athletes. *Exp Physiol,* 92**,** 881-6.

Appell Coriolano, H. J. & Duarte, J. A. 2012. Studies on gene polymorphisms in sports fancy fashion or important field of research? *Int J Sports Med,* 33**,** 419-20.

APSU 2009. Gross Anatomy of Skeletal Muscle. *In:* MUSCLE, E. R. C. G. A. O. S. (ed.). www.apsu.edu: Austin Peay State University.

Ash, G. I., Scott, R. A., Deason, M., Dawson, T. A., Wolde, B., Bekele, Z., Teka, S. & Pitsiladis, Y. P. 2011. No association between ACE gene variation and endurance athlete status in Ethiopians. *Med Sci Sports Exerc,* 43**,** 590-7.

Aspenes, S. T., Nilsen, T. I., Skaug, E. A., Bertheussen, G. F., Ellingsen, O., Vatten, L. & Wisloff, U. 2011. Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Med Sci Sports Exerc,* 43**,** 1465-73.

Barley, J., Blackwood, A., Carter, N. D., Crews, D. E., Cruickshank, J. K., Jeffery, S., Ogunlesi, A. O. & Sagnella, G. A. 1994. Angiotensin converting enzyme insertion/deletion polymorphism: association with ethnic origin. *J Hypertens,* 12**,** 955-7.

Beggs, A. H., Byers, T. J., Knoll, J. H., Boyce, F. M., Bruns, G. A. & Kunkel, L. M. 1992. Cloning and characterization of two human skeletal muscle alpha-actinin genes located on chromosomes 1 and 11. *J Biol Chem,* 267**,** 9281-8.

Bertheussen, G. F., Romundstad, P. R., Landmark, T., Kaasa, S., Dale, O. & Helbostad, J. L. 2011. Associations between physical activity and physical and mental health--a HUNT 3 study. *Medicine and science in sports and exercise,* 43**,** 1220.

Bohn, M., Berge, K. E., Bakken, A., Erikssen, J. & Berg, K. 1993. Insertion/deletion (I/D) polymorphism at the locus for angiotensin I-converting enzyme and myocardial infarction. *Clin Genet,* 44**,** 292-7.

Boots. 2014. *YesNo Klassifisering av muskelegenskaper* [Online]. Boots Norge AS. Available: https://www.boots.no/butikk/s/products/view.html?ref=search&p=11324167 [Accessed 02.11.2014.

Bouchard, C. 2012. Genomic predictors of trainability. *Exp Physiol,* 97**,** 347-52.

Bouchard, C., Blair, S. & Haskell, W. 2012. Why study physical activity and health? *Physical activity and health.* Champaign, Ill: Human Kinetics.

Bouchard, C. & Hoffman, E. P. (eds.) 2011. *Genetic and Molecular Aspects of Sport Performance*: Wiley-Blackwell.

Bouchard, C. & Rankinen, T. 2001. Individual differences in response to regular physical activity. *Med Sci Sports Exerc,* 33**,** S446-S451.

Bray, M. S. 2000. Genomics, genes, and environmental interaction: the role of exercise. *J Appl Physiol,* 88**,** 788-92.

Bray, M. S., Fulton, J. E., Kalupahana, N. S. & Lightfoot, J. T. 2011. Genetic Epidemiology, Physical Activity, and Inactivity. *Genetic and Molecular Aspects of Sport Performance.*

Bray, M. S., Hagberg, J. M., Perusse, L., Rankinen, T., Roth, S. M., Wolfarth, B. & Bouchard, C. 2009. The human gene map for performance and health-related fitness phenotypes: the 2006-2007 update. *Med Sci Sports Exerc,* 41**,** 35-73.

Bretthauer, M., Gondal, G., Larsen, K., Carlsen, E., Eide, T. J., Grotmol, T., Skovlund, E., Tveit, K. M., Vatn, M. H. & Hoff, G. 2002. Design, organization and management of a controlled population screening study for detection of colorectal neoplasia: attendance rates in the NORCCAP study (Norwegian Colorectal Cancer Prevention). *Scand J Gastroenterol,* 37**,** 568-73.

Brown, N. J., Blais, C., Jr., Gandhi, S. K. & Adam, A. 1998. ACE insertion/deletion genotype affects bradykinin metabolism. *J Cardiovasc Pharmacol,* 32**,** 373-7.

Brutsaert, T. D. & Parra, E. J. 2009. Nature versus nurture in determining athletic ability. *In:* COLLINS, M. (ed.) *Med Sport Sci.* 2009/08/22 ed. Basel: Karger.

Buford, T. W., Hsu, F. C., Brinkley, T. E., Carter, C. S., Church, T. S., Dodson, J. A., Goodpaster, B. H., Mcdermott, M. M., Nicklas, B. J., Yank, V., Johnson, J. A. & PAHOR, M. 2014. Genetic influence on exercise-induced changes in physical function among mobility-limited older adults. *Physiol Genomics,* 46**,** 149-158.

Cam, S., Colakoglu, M., Colakoglu, S., Sekuri, C. & Berdeli, A. 2007. ACE I/D gene polymorphism and aerobic endurance development in response to training in a non-elite female cohort. *J Sports Med Phys Fitness,* 47**,** 234-8.

Clarkson, P. M., Devaney, J. M., Gordish-Dressman, H., Thompson, P. D., Hubal, M. J., Urso, M. L., Price, T. B., Angelopoulos, T. J., Gordon, P. M., Moyna, N. M., Pescatello, L. S., Visich, P. S., Zoeller, R. F., Seip, R. L. & Hoffman, E. P. 2005. ACTN3 genotype is associated with increases in muscle strength in response to resistance training in women. *J Appl Physiol,* 99**,** 154-163.

clinicaltrials.gov. 2015. *NORCCAP: Norwegian Colorectal Cancer Prevention Trial* [Online]. ClinicalTrials.gov. Available: https://clinicaltrials.gov/show/NCT00119912 [Accessed 14.05.2015 2015].

Coates, D. 2003. The angiotensin converting enzyme (ACE). *Int J Biochem Cell Biol,* 35**,** 769-73.

Colhoun, H. M., Mckeigue, P. M. & Davey Smith, G. 2003. Problems of reporting genetic associations with complex outcomes. *Lancet,* 361**,** 865-72.

Corvol, P., Williams, T.A.,Soubrier, F. 1995. Peptidyl Dipeptidase A: Angiotensin I-Converting Enzyme. *In:* BARRETT, A. J. (ed.) *Methods in Enzymology: Proteolytic Enzymes: Aspartic and Metallo Peptidases.* Academic Press.

De Moor, M. H., Liu, Y. J., Boomsma, D. I., Li, J., Hamilton, J. J., Hottenga, J. J., Levy, S., Liu, X. G., Pei, Y. F., Posthuma, D., Recker, R. R., Sullivan, P. F., Wang, L., Willemsen, G., Yan, H., De Geus, E. J. & Deng, H. W. 2009. Genome-wide association study of exercise behavior in Dutch and American adults. *Med Sci Sports Exerc,* 41**,** 1887-95.

Defoor, J., Vanhees, L., Martens, K., Matthijs, G., Van Vlerken, A., Zielinska, D., Schepers, D., Vlietinck, R. & Fagard, R. 2006. The CAREGENE study: ACE gene I/D polymorphism and effect of physical training on aerobic power in coronary artery disease. *Heart,* 92**,** 527-8.

Dipetrillo, K., Wang, X., Stylianou, I. M. & Paigen, B. 2005. Bioinformatics toolbox for narrowing rodent quantitative trait loci. *Trends Genet,* 21**,** 683-92.

DNAFit. 2014. DNAFit®. Available: http://www.dnafit.com/ [Accessed 02.11.2014.

Eisenmann, J. C., Sarzynski, M. A., Glenn, K., Rothschild, M. & Heelan, K. A. 2009. ACE I/D genotype, adiposity, and blood pressure in children. *Cardiovasc Diabetol,* 8**,** 14.

Eynon, N., Banting, L. K., Ruiz, J. R., Cieszczyk, P., Dyatlov, D. A., Maciejewska-Karlowska, A., Sawczuk, M., Pushkarev, V. P., Kulikov, L. M., Pushkarev, E. D., Femia, P., Stepto, N. K., Bishop, D. J. & Lucia, A. 2014. ACTN3 R577X polymorphism and team-sport performance: A study involving three European cohorts. *J Sci Med Sport,* 17**,** 102-106.

Folland, J., Leach, B., Little, T., Hawker, K., Myerson, S., Montgomery, H. & Jones, D. 2000. Angiotensin-converting enzyme genotype affects the response of human skeletal muscle to functional overload. *Exp Physiol,* 85**,** 575-9.

Foy, C. A., Mccormack, L. J., Knowler, W. C., Barrett, J. H., Catto, A. & Grant, P. J. 1996. The angiotensin-I converting enzyme (ACE) gene I/D polymorphism and ACE levels in Pima Indians. *J Med Genet,* 33**,** 336-7.

Fuentes, R. M., Perola, M., Nissinen, A. & Tuomilehto, J. 2002. *ACE gene and physical activity, blood pressure, and hypertension: a population study in Finland*.

Garatachea, N., Verde, Z., Santos-Lozano, A., Yvert, T., Rodriguez-Romo, G., Sarasa, F. J., Hernandez-Sanchez, S., Santiago, C. & Lucia, A. 2014. ACTN3 R577X

polymorphism and explosive leg-muscle power in elite basketball players. *Int J Sports Physiol Perform,* 9**,** 226-32.

Gayagay, G., Yu, B., Hambly, B., Boston, T., Hahn, A., Celermajer, D. S. & Trent, R. J. 1998. Elite endurance athletes and the ACE I allele – the role of genes in athletic performance. *Hum Genet,* 103**,** 48-50.

Genaxxon. 2011. *Genaxxon newsletter: GelRed® latest findings of our customers* [Online]. Genaxxon BioScience GmbH. Available: http://www.genaxxon.com/newsletter/12.04.2011.EASTER%20SURPRISE%20with%20Genaxxon%20bioscience.html [Accessed 12.02.2014.

Gibson, W. T. 2009. Key concepts in human genetics: understanding the complex phenotype. *Med Sport Sci,* 54**,** 1-10.

Glenn, K. L., Du, Z. Q., Eisenmann, J. C. & Rothschild, M. F. 2009. An alternative method for genotyping of the ACE I/D polymorphism. *Mol Biol Rep,* 36**,** 1305-10.

Gondal, G., Grotmol, T., Hofstad, B., Bretthauer, M., Eide, T. J. & Hoff, G. 2003. The Norwegian Colorectal Cancer Prevention (NORCCAP) screening study: baseline findings and implementations for clinical work-up in age groups 50-64 years. *Scand J Gastroenterol,* 38**,** 635-42.

Gordish-Dressman, H. & Devaney, J. M. 2011. Statistical and Methodological Considerations in Exercise Genomics. *Exercise Genomics.* Springer.

Gordon, S. E., Davis, B. S., Carlson, C. J. & Booth, F. W. 2001. ANG II is required for optimal overload-induced skeletal muscle hypertrophy. *Am J Physiol Endocrinol Metab,* 280**,** E150-9.

Hagberg, J. M. 2010. Exercise genes? And no, not Levi's 501s! *J Appl Physiol* [Online], 109. Available: http://jap.physiology.org/jap/109/3/619.full.pdf [Accessed 2010-09-01 00:00:00].

Hamilton, M. & Owen, N. 2012. Sedentary Behaviour and Inactivity Physiology. *In:* Haskell, W. L., Bouchard, C. & Blair, S. N. (eds.) *Physical activity and health.* 2nd ed. ed. Champaign, Ill: Human Kinetics.

Hansen, R., Saebo, M., Skjelbred, C. F., Nexo, B. A., Hagen, P. C., Bock, G., Bowitz Lothe, I. M., Johnson, E., Aase, S., Hansteen, I. L., Vogel, U. & Kure, E. H. 2005. GPX Pro198Leu and OGG1 Ser326Cys polymorphisms and risk of development of colorectal adenomas and colorectal cancer. *Cancer Lett,* 229**,** 85-91.

Hattersley, A. T. & Mccarthy, M. I. 2005. What makes a good genetic association study? *The Lancet,* 366**,** 1315-1323.

Hermansen, R., Njolstad, I. & Fonnebo, V. 2002. Physical activity according to ethnic origin in Finnmark county, Norway. The Finnmark Study. *Int J Circumpolar Health,* 61**,** 189-200.

Hruskovicova, H., Dzurenkova, D., Selingerova, M., Bohus, B., Timkanicova, B. & Kovacs, L. 2006. The angiotensin converting enzyme I/D polymorphism in long distance runners. *J Sports Med Phys Fitness,* 46**,** 509-513.

Hubal, M. J., Urso, M. L. & Clarkson, P. M. 2011. Genetic Aspects of Muscular Strength and Size. *In:* Pescatello, L. S. & Roth, S. M. (eds.) *Exercise Genomics.* Humana Press.

Illumina. 2014. *The HumanOmniExpress (OmniExpress) BeadChip, about* [Online]. Illumina, Inc. Available:

http://products.illumina.com/products/human_omni_express_beadchip_kits.ilmn [Accessed 29.09.2014.

Jetté, M., Sidney, K. & Blümchen, G. 1990. Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clinical Cardiology,* 13**,** 555-565.

Jones, A. & Woods, D. R. 2003. Skeletal muscle RAS and exercise performance. *Int J Biochem Cell Biol,* 35**,** 855-866.

Katzmarzyk, P. T. 2012. Physical Activity and Fitness With Age, Sex, and Ethnic Differences. *In:* Haskell, W. L., Bouchard, C. & Blair, S. N. (eds.) *Physical activity and health.* 2nd ed. ed. Champaign, Ill: Human Kinetics.

Kumar, B. N. 2008. The Oslo immigrant health profile. *Diabetes,* 3**,** 8.

Kurtze, N., Rangul, V., Hustvedt, B. E. & Flanders, W. D. 2008. Reliability and validity of self-reported physical activity in the Nord-Trondelag Health Study: HUNT 1. *Scand J Public Health,* 36**,** 52-61.

Larsen, I. K., Grotmol, T., Almendingen, K. & Hoff, G. 2006. Lifestyle as a predictor for colonic neoplasia in asymptomatic individuals. *BMC gastroenterology,* 6**,** 5.

Levine, J. A. 2014. Lethal sitting: homo sedentarius seeks answers. *Physiology (Bethesda),* 29**,** 300-1.

Life Technologies. 2011. TaqMan® SNP Genotyping Assays. *Product Bulletin* [Online]. [Accessed 26.09.2014].

Lightfoot, J. T. 2011a. Can you be born a couch potato? The genomic regulation of physical activity. *Exercise Genomics.* Springer.

Lightfoot, J. T. 2011b. Current understanding of the genetic basis for physical activity. *J Nutr,* 141**,** 526-30.

Lima, R. M., Leite, T. K., Pereira, R. W., Rabelo, H. T., Roth, S. M. & Oliveira, R. J. 2011. ACE and ACTN3 genotypes in older women: muscular phenotypes. *Int J Sports Med,* 32**,** 66-72.

Loos, R. J., Hagberg, J. M., Perusse, L., Roth, S. M., Sarzynski, M. A., Wolfarth, B., Rankinen, T. & Bouchard, C. 2015. Advances in Exercise, Fitness, and Performance Genomics in 2014. *Med Sci Sports Exerc*.

Macarthur, D. G. & North, K. N. 2004. A gene for speed? The evolution and function of α-actinin-3. *BioEssays,* 26**,** 786-795.

Macarthur, D. G. & North, K. N. 2011. The ACTN3 Gene and Human Performance. *Genetic and Molecular Aspects of Sport Performance.* Wiley-Blackwell.

Macarthur, D. G., Seto, J. T., Chan, S., Quinlan, K. G., Raftery, J. M., Turner, N., Nicholson, M. D., Kee, A. J., Hardeman, E. C., Gunning, P. W., Cooney, G. J., Head, S. I., Yang, N. & North, K. N. 2008. An Actn3 knockout mouse provides mechanistic insights into the association between alpha-actinin-3 deficiency and human athletic performance. *Hum Mol Genet,* 17**,** 1076-86.

Macarthur, D. G., Seto, J. T., Raftery, J. M., Quinlan, K. G., Huttley, G. A., Hook, J. W., Lemckert, F. A., Kee, A. J., Edwards, M. R., Berman, Y., Hardeman, E. C., Gunning, P. W., Easteal, S., Yang, N. & North, K. N. 2007. Loss of ACTN3 gene function alters

mouse muscle metabolism and shows evidence of positive selection in humans. *Nat Genet,* 39**,** 1261-5.

Maestu, J., Latt, E., Raask, T., Sak, K., Laas, K., Jurimae, J. & Jurimae, T. 2013. ACE I/D polymorphism is associated with habitual physical activity in pubertal boys. *J Physiol Sci,* 63**,** 427-34.

Marchini, J., Cardon, L. R., Phillips, M. S. & Donnelly, P. 2004. The effects of human population structure on large genetic association studies. *Nat Genet,* 36**,** 512-7.

Mathew, J., Basheeruddin, K. & Prabhakar, S. 2001. Differences in frequency of the deletion polymorphism of the angiotensin-converting enzyme gene in different ethnic groups. *Angiology,* 52**,** 375-9.

Mcardle, W. D., Katch, F. I. & Katch, V. L. 2010. *Exercise Physiology: Nutrition, energy, and human performance*, New York, NY: Lippincott Williams & Wilkins.

Mcguigan, F. E. & Ralston, S. H. 2002. Single nucleotide polymorphism detection: allelic discrimination using TaqMan. *Psychiatr Genet,* 12**,** 133-6.

Mersy, D. J. 1991. Health benefits of aerobic exercise. *Postgrad Med,* 90**,** 103-7, 110-2.

Mikami, E., Fuku, N., Murakami, H., Tsuchie, H., Takahashi, H., Ohiwa, N., Tanaka, H., Pitsiladis, Y. P., Higuchi, M., Miyachi, M., Kawahara, T. & Tanaka, M. 2014. ACTN3 R577X genotype is associated with sprinting in elite Japanese athletes. *Int J Sports Med,* 35**,** 172-7.

Miller, S. A., Dykes, D. D. & Polesky, H. F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res,* 16**,** 1215.

Mills, M., Yang, N., Weinberger, R., Vander Woude, D. L., Beggs, A. H., Easteal, S. & North, K. 2001. Differential expression of the actin-binding proteins, alpha-actinin-2 and -3, in different species: implications for the evolution of functional redundancy. *Hum Mol Genet,* 10**,** 1335-46.

Montgomery, H. E. & Brull, D. 2000. Gene–environment interactions and the response to exercise. *Int J Exp Pathol,* 81**,** 283-287.

Montgomery, H. E., Clarkson, P., Dollery, C. M., Prasad, K., Losi, M. A., Hemingway, H., Statters, D., Jubb, M., Girvain, M., Varnava, A., World, M., Deanfield, J., Talmud, P., Mcewan, J. R., Mckenna, W. J. & Humphries, S. 1997. Association of angiotensin-converting enzyme gene I/D polymorphism with change in left ventricular mass in response to physical training. *Circulation,* 96**,** 741-7.

Moran, C. N., Vassilopoulos, C., Tsiokanos, A., Jamurtas, A. Z., Bailey, M. E., Montgomery, H. E., Wilson, R. H. & Pitsiladis, Y. P. 2006. The associations of ACE polymorphisms with physical, physiological and skill parameters in adolescents. *Eur J Hum Genet,* 14**,** 332-9.

Myerson, S. G., Hemingway, H., Budget, R., Martin, J., Humphries, S. & Montgomery, H. 1999. Human angiotensin I-converting enzyme gene and endurance performance. *J Appl Physiol,* 87**,** 1313-1316.

Myerson, S. G., Montgomery, H. E., Whittingham, M., Jubb, M., World, M. J., Humphries, S. E. & Pennell, D. J. 2001. Left ventricular hypertrophy with exercise and ACE gene insertion/deletion polymorphism: a randomized controlled trial with losartan. *Circulation,* 103**,** 226-30.

NCBI. 2015. *NCBI Gene* [Online]. National Center for Biotechnology Information (NCBI). Available: http://www.ncbi.nlm.nih.gov/gene [Accessed 28.04.2015.

Nicklas, B. J. 2010. Heterogeneity of Physical Function Responses to Exercise in Older Adults: Possible Contribution of Variation in the Angiotensin-1 Converting Enzyme (ACE) Gene? *Perspect Psychol Sci,* 5**,** 575-584.

Norman, B., Esbjornsson, M., Rundqvist, H., Osterlund, T., Glenmark, B. & Jansson, E. 2014. ACTN3 genotype and modulation of skeletal muscle response to exercise in human subjects. *J Appl Physiol,* 116**,** 1197-203.

Norman, B., Esbjornsson, M., Rundqvist, H., Osterlund, T., Von Walden, F. & Tesch, P. A. 2009. Strength, power, fiber types, and mRNA expression in trained men and women with different ACTN3 R577X genotypes. *J Appl Physiol,* 106**,** 959-65.

North, K. N., Yang, N., Wattanasirichaigoon, D., Mills, M., Easteal, S. & Beggs, A. H. 1999. A common nonsense mutation results in alpha-actinin-3 deficiency in the general population. *Nat Genet,* 21**,** 353-4.

Norwegian Institute Of Public Health. 2009. *Physical activity in Norway - fact sheet* [Online]. Norwegian Institute of Public Health (NIPH). Available: http://www.fhi.no/artikler/?id=77146 [Accessed 01.03.2015.

Novak, C. M., Escande, C., Gerber, S. M., Chini, E. N., Zhang, M., Britton, S. L., Koch, L. G. & Levine, J. A. 2009. Endurance capacity, not body size, determines physical activity levels: role of skeletal muscle PEPCK. *PLoS One,* 4**,** e5869.

Orysiak, J., Zmijewski, P., Klusiewicz, A., Kaliszewski, P., Malczewska-Lenczowska, J., Gajewski, J. & Pokrywka, A. 2013. The association between ace gene variation and aerobic capacity in winter endurance disciplines. *Biol Sport,* 30**,** 249-53.

Pathway Genomics. 2014. *Pathway Fit®* [Online]. Pathway Genomics Corporation. Available: https://www.pathway.com/pathway-fit/ [Accessed 06.11.2014.

Paul, M., Mehr, A. P. & Kreutz, R. 2006. Physiology of local renin-angiotensin systems. *Physiol Rev,* 86**,** 747-803.

Payne, J. R., Dhamrait, S. S., Gohlke, P., Cooper, J., Scott, R. A., Pitsiladis, Y. P., Humphries, S. E., Rayner, B. & Montgomery, H. E. 2007. The impact of ACE genotype on serum ACE activity in a black South African male population. *Ann Hum Genet,* 71**,** 1-7.

Pereira, A., Costa, A. M., Izquierdo, M., Silva, A. J., Bastos, E. & Marques, M. C. 2013a. ACE I/D and ACTN3 R/X polymorphisms as potential factors in modulating exercise-related phenotypes in older women in response to a muscle power training stimuli. *Age (Dordr),* 35**,** 1949-59.

Pereira, A., Costa, A. M., Leitao, J. C., Monteiro, A. M., Izquierdo, M., Silva, A. J., Bastos, E. & Marques, M. C. 2013b. The influence of ACE ID and ACTN3 R577X polymorphisms on lower-extremity function in older women in response to high-speed power training. *BMC Geriatr,* 13**,** 131.

Pierce, B. A. 2012. *Genetics: a conceptual approach,* New York, W.H. Freeman.

Pimenta, E. M., Coelho, D. B., Veneroso, C. E., Barros Coelho, E. J., Cruz, I. R., Morandi, R. F., De, A. P. G., Carvalho, M. R., Garcia, E. S. & De Paz Fernandez, J. A. 2013. Effect of ACTN3 gene on strength and endurance in soccer players. *J Strength Cond Res,* 27**,** 3286-92.

Pitsiladis, Y., Wang, G. & Wolfarth, B. 2011. Genomics of aerobic capacity and endurance performance: Clinical implications. *Exercise genomics.* Springer.

Prince, S. A., Adamo, K. B., Hamel, M. E., Hardt, J., Connor Gorber, S. & Tremblay, M. 2008. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. *Int J Behav Nutr Phys Act,* 5**,** 56.

Puthucheary, Z., Skipworth, J. R. A., Rawal, J., Loosemore, M., Van Someren, K. & Montgomery, H. E. 2011. The ACE Gene and Human Performance 12 Years On. *Sports Med,* 41**,** 433-448.

Rankinen, T. & Bouchard, C. 2012. Genetic Differences in the Relationships Among Physical Activity, Fitness, and Health. *In:* Haskell, W. L., Bouchard, C. & Blair, S. N. (eds.) *Physical activity and health.* 2nd ed. ed. Champaign, Ill: Human Kinetics.

Rankinen, T., Pérusse, L., Gagnon, J., Chagnon, Y. C., Leon, A. S., Skinner, J. S., Wilmore, J. H., Rao, D. C. & Bouchard, C. 2000a. Angiotensin-converting enzyme ID polymorphism and fitness phenotype in the HERITAGE Family Study. *J Appl Physiol,* 88**,** 1029-1035.

Rankinen, T., Wolfarth, B., Simoneau, J. A., Maier-Lenz, D., Rauramaa, R., Rivera, M. A., Boulay, M. R., Chagnon, Y. C., Pérusse, L., Keul, J. & Bouchard, C. 2000b. No association between the angiotensin-converting enzyme ID polymorphism and elite endurance athlete status. *J Appl Physiol,* 88**,** 1571-1575.

Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P. & Soubrier, F. 1990. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest,* 86**,** 1343-6.

Rigat, B., Hubert, C., Corvol, P. & Soubrier, F. 1992. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). *Nucleic Acids Res,* 20**,** 1433.

Rodriguez-Romo, G., Ruiz, J. R., Santiago, C., Fiuza-Luces, C., Gonzalez-Freire, M., Gomez-Gallego, F., Moran, M. & Lucia, A. 2010. Does the ACE I/D polymorphism, alone or in combination with the ACTN3 R577X polymorphism, influence muscle power phenotypes in young, non-athletic adults? *Eur J Appl Physiol,* 110**,** 1099-106.

Roth, S. M. 2007. *Genetics primer for exercise science and health,* Champaign, Ill, Human Kinetics.

Roth, S. M. & Thomis, M. A. 2011. Fundamental Concepts in Exercise Genomics. *Exercise Genomics.* Springer.

Roth, S. M., Walsh, S., Liu, D., Metter, E. J., Ferrucci, L. & Hurley, B. F. 2008. The ACTN3 R577X nonsense allele is under-represented in elite-level strength athletes. *Eur J Hum Genet,* 16**,** 391-4.

Rupert, J. L., Devine, D. V., Monsalve, M. V. & Hochachka, P. W. 1999. Angiotensin-converting enzyme (ACE) alleles in the Quechua, a high altitude South American native population. *Ann Hum Biol,* 26**,** 375-80.

Sailors, M. H. & Bray, M. S. 2011. The Interaction Between Genetic Variation and Exercise and Physical Activity in the Determination of Body Composition and Obesity Status. *Exercise Genomics.* Springer.

Sarzynski, M. A., Rankinen, T. & Bouchard, C. 2011. Twin and Family Studies of Training Responses. *In:* Claude Bouchard & Hoffman, E. P. (eds.) *Genetic and Molecular Aspects of Sport Performance.* Wiley-Blackwell.

Scott, R. A., Irving, R., Irwin, L., Morrison, E., Charlton, V., Austin, K., Tladi, D., Deason, M., Headley, S. A., Kolkhorst, F. W., Yang, N., North, K. & Pitsiladis, Y. P. 2010. ACTN3 and ACE genotypes in elite Jamaican and US sprinters. *Med Sci Sports Exerc,* 42**,** 107-12.

Shahmoradi, S., Ahmadalipour, A. & Salehi, M. 2014. Evaluation Of ACE Gene I/D polymorphism in Iranian elite athletes. *Adv Biomed Res,* 3.

Shanmugam, V., Sell, K. W. & Saha, B. K. 1993. Mistyping ACE heterozygotes. *PCR Methods Appl,* 3**,** 120-1.

Sherwood, L. 2013. Introduction To Human Physiology. International ed of 8th revised ed edition ed.: Brooks/Cole.

Sjoblom, B., Salmazo, A. & Djinovic-Carugo, K. 2008. Alpha-actinin structure and regulation. *Cell Mol Life Sci,* 65**,** 2688-701.

Skjelbred, C. F., Saebo, M., Wallin, H., Nexo, B. A., Hagen, P. C., Lothe, I. M., Aase, S., Johnson, E., Hansteen, I. L., Vogel, U. & Kure, E. H. 2006. Polymorphisms of the XRCC1, XRCC3 and XPD genes and risk of colorectal adenoma and carcinoma, in a Norwegian cohort: a case control study. *BMC Cancer,* 6**,** 67.

Speicher, M. R., Antonarakis, S. E. & Motulsky, A. G. 2010. *Vogel and Motulsky's Human Genetics,* Berlin, Heidelberg, Springer-Verlag Berlin Heidelberg.

Sports Gene. 2014. *Sports Gene* [Online]. Sports Gene Llc. Available: http://www.sportsgene.ee/references [Accessed 30.04.2015. 2015].

Stubbe, J. H., Boomsma, D. I. & De Geus, E. J. 2005. Sports participation during adolescence: a shift from environmental to genetic factors. *Med Sci Sports Exerc,* 37**,** 563-70.

Stubbe, J. H., Boomsma, D. I., Vink, J. M., Cornes, B. K., Martin, N. G., Skytthe, A., Kyvik, K. O., Rose, R. J., Kujala, U. M., Kaprio, J., Harris, J. R., Pedersen, N. L., Hunkin, J., Spector, T. D. & De Geus, E. J. C. 2006. Genetic Influences on Exercise Participation in 37.051 Twin Pairs from Seven Countries. *PLoS ONE,* 1**,** e22.

Taylor, R. R., Mamotte, C. D., Fallon, K. & Van Bockxmeer, F. M. 1999. Elite athletes and the gene for angiotensin-converting enzyme. *J Appl Physiol,* 87**,** 1035-7.

Thermofisher Scientific. 2013. *Thermocycler Protocol* [Online]. Thermo Fisher Scientific Inc, . Available: http://www.thermoscientificbio.com/applications/pcr-and-pcr/introduction-to-qpcr/ [Accessed 23.10.2014.

Thomis, A. I. M. 2011. Genes And Strength And Power Phenotypes. *In:* Claude Bouchard & Hoffman, E. P. (eds.) *Genetic and Molecular Aspects of Sport Performance.* Wiley-Blackwell.

Tronvik, E., Stovner, L. J., Bovim, G., White, L. R., Gladwin, A. J., Owen, K. & Schrader, H. 2008. Angiotensin-converting enzyme gene insertion/deletion polymorphism in migraine patients. *BMC Neurol,* 8**,** 4.

Vaughan, D., Huber-Abel, F. A., Graber, F., Hoppeler, H. & Fluck, M. 2013. The angiotensin converting enzyme insertion/deletion polymorphism alters the response of muscle energy supply lines to exercise. *Eur J Appl Physiol,* 113**,** 1719-29.

Vincent, B., De Bock, K., Ramaekers, M., Van Den Eede, E., Van Leemputte, M., Hespel, P. & Thomis, M. A. 2008. ACTN3 (R577X) genotype is associated with fiber type distribution. *Physiol Genomics,* 32**,** 58.

Vincent, B., Windelinckx, A., Nielens, H., Ramaekers, M., Van Leemputte, M., Hespel, P. & Thomis, M. A. 2010. Protective role of alpha-actinin-3 in the response to an acute eccentric exercise bout. *J Appl Physiol,* 109**,** 564-73.

Wang, G., Mikami, E., Chiu, L. L., A, D. E. P., Deason, M., Fuku, N., Miyachi, M., Kaneoka, K., Murakami, H., Tanaka, M., Hsieh, L. L., Hsieh, S. S., Caporossi, D., Pigozzi, F., Hilley, A., Lee, R., Galloway, S. D., Gulbin, J., Rogozkin, V. A., Ahmetov, Ii, Yang, N., North, K. N., Ploutarhos, S., Montgomery, H. E., Bailey, M. E. & Pitsiladis, Y. P. 2013. Association analysis of ACE and ACTN3 in elite Caucasian and East Asian swimmers. *Med Sci Sports Exerc,* 45**,** 892-900.

Wang, P., Fedoruk, M. N. & Rupert, J. L. 2008. Keeping Pace with ACE Are ACE Inhibitors and Angiotensin II Type 1 Receptor Antagonists Potential Doping Agents? *Sports Med,* 38**,** 1065-1079.

Williams, A. G., Rayson, M. P., Jubb, M., World, M., Woods, D. R., Hayward, M., Martin, J., Humphries, S. E. & Montgomery, H. E. 2000. The ACE gene and muscle performance. *Nature,* 403**,** 614.

Winnicki, M., Accurso, V., Hoffmann, M., Pawlowski, R., Dorigatti, F., Santonastaso, M., Longo, D., Krupa-Wojciechowska, B., Jeunemaitre, X., Pessina, A. C., Somers, V. K., Palatini, P. & Group, H. S. 2004. Physical activity and angiotensin-converting enzyme gene polymorphism in mild hypertensives. *Am J Med Genet A,* 125A**,** 38-44.

Wolfarth, B. 2011. Genes And Endurance Performance. *In:* Claude Bouchard & Hoffman, E. P. (eds.) *Genetic and Molecular Aspects of Sport Performance.* Wiley-Blackwell.

Wolfarth, B., Rankinen, T., Hagberg, J. M., Loos, R. J., Perusse, L., Roth, S. M., Sarzynski, M. A. & Bouchard, C. 2014. Advances in exercise, fitness, and performance genomics in 2013. *Med Sci Sports Exerc,* 46**,** 851-9.

Woods, D. 2009. Angiotensin-converting enzyme, renin-angiotensin system and human performance. *Med Sport Sci,* 54**,** 72-87.

Woods, D., Hickman, M., Jamshidi, Y., Brull, D., Vassiliou, V., Jones, A., Humphries, S. & Montgomery, H. 2001. Elite swimmers and the D allele of the ACE I/D polymorphism. *Hum Genet,* 108**,** 230-2.

Woods, D., Humphries, S. E. & Montgomery, H. E. 2000. The ACE I/D polymorphism and human physical performance. *Trends Endocrinol Metab,* 11**,** 416-20.

World Health Organization. 2010. Global Recommendations on Physical Activity for Health. Geneva: World Health Organization.

World Health Organization. 2013. *Fact sheet: Noncommunicable diseases* [Online]. World Health Organization. Available: http://www.who.int/mediacentre/factsheets/fs355/en/ [Accessed 02.11.2014.

World Health Organization. 2014. *Physical Inactivity: A Global Public Health Problem* [Online]. Word Health Organization. Available: http://www.who.int/dietphysicalactivity/factsheet_inactivity/en/ [Accessed 02.11.2014.

XRGenomics. 2014. XRGenomics LTD Available: http://www.xrgenomics.com/ [Accessed 02.11.2014.

Yang, N., Garton, F. & North, K. 2009. Alpha-actinin-3 and performance. *Med Sport Sci,* 54**,** 88-101.

Yang, N., Macarthur, D. G., Gulbin, J. P., Hahn, A. G., Beggs, A. H., Easteal, S. & North, K. 2003. ACTN3 genotype is associated with human elite athletic performance. *Am J Hum Genet,* 73**,** 627-31.

Yang, N., Macarthur, D. G., Wolde, B., Onywera, V. O., Boit, M. K., Lau, S. Y., Wilson, R. H., Scott, R. A., Pitsiladis, Y. P. & North, K. 2007. The ACTN3 R577X polymorphism in East and West African athletes. *Med Sci Sports Exerc,* 39**,** 1985-8.

Zhang, B., Tanaka, H., Shono, N., Miura, S., Kiyonaga, A., Shindo, M. & Saku, K. 2003. The I allele of the angiotensin-converting enzyme gene is associated with an increased percentage of slow-twitch type I fibers in human skeletal muscle. *Clin Genet,* 63**,** 139-44.

7 Appendix

Appendix A: Genotype and allele frequencies of the ACE I/D polymorphism

Table 7: *Genotype (%) and allele frequencies of the ACE I/D polymorphism in various populations.*

Population	N	Genotype frequency (%)			Allele frequency		Reference
		II	ID	DD	I	D	
Caucasians (in general)	-	25	50	25	0.50	0.50	Jones and Woods (2003)
Caucasian (Germany)	120	26	47	27	0.50	0.50	Rankinen, Wolfarth et al. (2000)
Caucasians (USA)	80	24	49	28	0.48	0.52	Foy et al. (1996)
Caucasians (Finland)	80	18	51	30	0.44	0.56	Rankinen et al. (2000b)
Caucasians (Canada)	100	22	42	36	0.43	0.57	Rankinen et al. (2000b)
African Americans	142	13	56	31	0.41	0.59	Mathew et al. (2001)
Nigerian Blacks	80	16	49	35	0.41	0.59	Barley et al. (1994)
Indians (incl. Pakistani)	136	28	53	19	0.55	0.45	Mathew, Basheeruddin et al. (2001)
Pima Indians (USA)	305	51	41	8	0.71	0.29	Foy et al. (1996)
Na-Dene (South America, lowlands)	50	58	32	10	0.74	0.26	Rupert et al. (1999)
Quechua (South America, above 3000 m.a.s.l.)	63	59	38	3	0.78	0.22	Rupert et al. (1999)
Yanomami Indians (South America)	49	71	27	2	0.85	0.15	Barley, Blackwood et al. (1994)
Samoans (Polynesia)	58	83	16	2	0.91	0.09	Barley, Blackwood et al. (1994)

m.a.s.l.-meters above sea level

Appendix B: Genotype and allele frequencies of the ACE I/D polymorphism

Table 8: *Genotype (%) and allele frequencies of the ACTN3 polymorphism in various populations*

		Genotype frequency (%)			Allele frequency		
Population	**N**	**RR**	**RX**	**XX**	**R**	**X**	**Reference**
Caucasians (Poland)	354	39	50	11	0.64	0.36	Eynon et al. (2014)
Caucasians (Russia)	335	35	41	23	0.56	0.44	Eynon et al. (2014)
Caucasians (Sweden)	380	31	50	19	0.56	0.44	Norman et al. (2009)
Caucasians (Australia)	436	30	52	18	0.56	0.44	Yang et al. (2003)
Caucasians (Spain)	283	32	50	18	0.57	0.43	Garatachea et al. (2014)
Caucasians (USA)	668	33	48	20	0.56	0.44	Roth et al. (2008)
Caucasians (USA)	469	27	46	27	0.50	0.50	Clarkson et al. (2005)
Caucasians (Dutch)	90	31	44	24	0.53	0.46	Vincent et al. (2008)
Asians	55	36	33	31	0.53	0.47	Clarkson et al. (2005)
Hispanics	25	20	44	36	0.42	0.58	Clarkson et al. (2005)
PNG Highlander	39	44	41	15	0.64	0.36	Mills et al. (2001), Yang et al. (2007)
Ethiopians	198	42	47	11	0.66	0.35	Yang et al. (2007)
Aboriginal Australians	87	52	38	10	0.71	0.29	Mills et al. (2001), Yang et al. (2007)
African Americans	208	56	39	5	0.76	0.25	Roth, Walsh et al. (2008)
Jamaicans	311	75	23	2	0.87	0.14	Scott et al. (2010)
African Bantu	88	78	21	1	0.90	0.10	Mills et al. (2001), Yang et al. (2007)
Kenyans	158	84	15	1	0.92	0.09	Yang et al. (2007)
Nigerians	60	83	17	0	0.92	0.09	Yang et al. (2007)

PNG Highlander- Papua New Guinea Highlanders

Appendix C: Descriptive data for the physical activity (PA) and the combined study population

a) Table 9: *Descriptive data for the PA study population*

Variable	Total (N=1268)		Male (N=692)		Female (N=577)	
	mean ± SD	CV	mean ± SD	CV	mean ± SD	CV
Age (year)	56.4 ± 3.9	6.86	56.7 ± 3.8	6.69	56.1 ± 3.9	7.02
Anthropometric data						
Weight (kg)	79.1 ± 14.0	17.72	85.0 ± 12.4	14.65	72.0 ± 12.5	17.29
Height (cm)	173.6 ± 8.7	4.99	179.4 ± 6.2	3.45	166.7 ± 5.5	3.31
BMI (kg/m^2)	26.2 ± 3.8	14.36	26.4 ± 3.4	12.77	25.9 ± 4.2	16.07

SD- standard deviation; CV=Coefficient of Variance

b) Table 10: *Descriptive data for the combined study population*

Variable	Total (N=926)		Male (N=476)		Female (N=450)	
	mean ± SD	CV	mean ± SD	CV	mean ± SD	CV
Age (year)	55.8 ± 3.8	6.86	56.0 ± 3.8	6.73	55.6 ± 3.9	6.99
Anthropometric data						
Weight (kg)	78.7 ± 13.7	17.39	84.8 ± 11.9	14.01	72.2 ± 12.4	17.22
Height (cm)	173.1 ± 8.5	4.92	179.2 ± 6.1	3.39	166.7 ± 5.5	3.29
BMI (kg/m^2)	26.2 ± 3.8	14.32	26.4 ± 3.2	12.22	25.9 ± 4.2	16.27

SD- standard deviation; CV=Coefficient of Variance

Appendix D: Genotype calls for *ACTN3* R577X from TaqMan® SNP Genotyping Assay

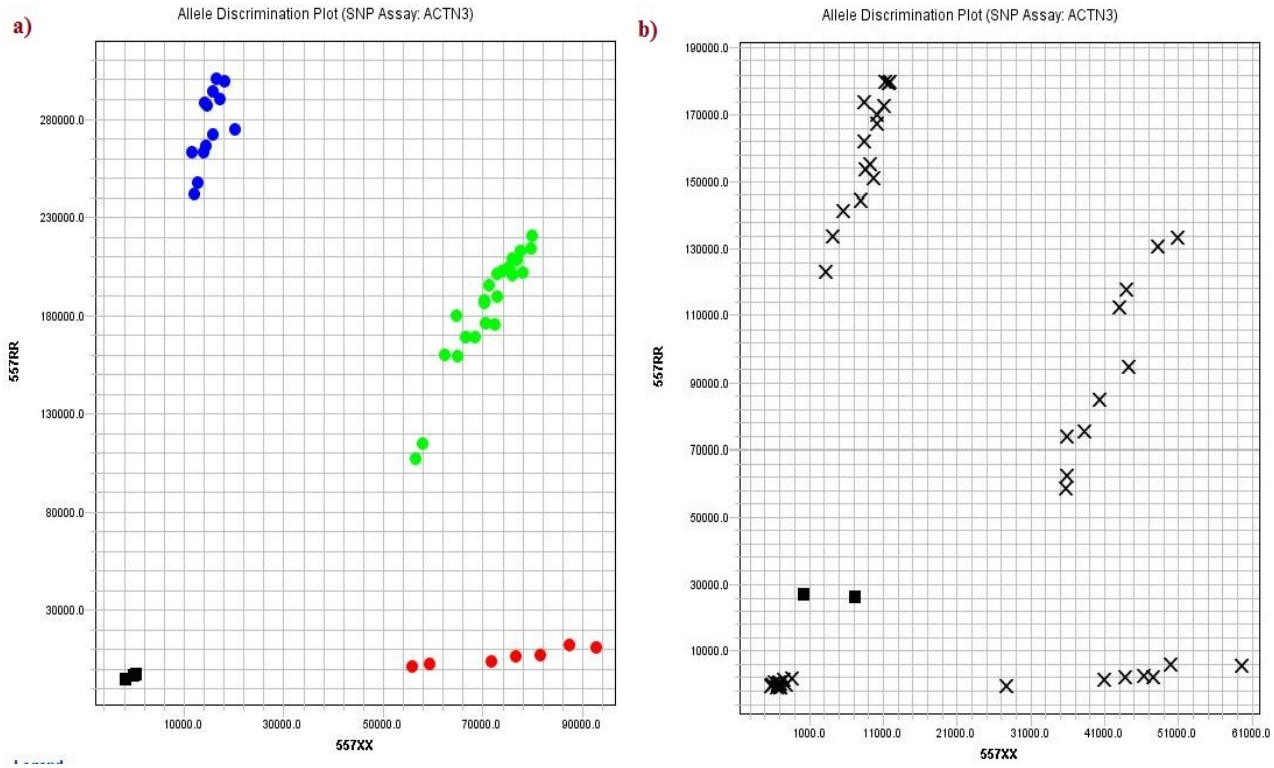

Figure 8: *a) normal genotype calls with clustering along the vertical axis (homozygocity for R-allele;●), horizontal axes (homozygocity for X-alele; ●) and diagonal (heterozygocity; ●).*

b) Undetermined (×) calls with clustering along the horizontal, vertical axes and diagonal.

■ *- positive controls*

Appendix E: The NORCCAP physical activity questionnaire

***Table 11**: Questions, reply options and encoding used in the NORCCAP study covering anthropometric data and PA levels.*

Questions	Reply options	Encoding	
Height in cm		kg/m^2	
Weight in kg			
During the last three years have you acquired any chronic diseases that have limited your level of activity? E.g. hip disorder or cardiovascular disease.	[Yes] [No]	[1] [2]	
How often do you have physical activity for more than 20 minutes (e.g. walking, cycling, jogging or swimming) ? Put only one mark.[1]	[Never] [<1 time/week] [1-2 times/week] [3-4 times/week] [5-7 times/week] [>7 times/week]	[1] [2] [3] [4] [5] [6]	[A]
How often do you have physical activity for more than 20 minutes (e.g. walking, cycling, jogging or swimming)? Put only one mark.[1]	[Never] [<1 time/week] [1-2 times/week] [3-4 times/week] [5-7 times/week] [>7 times/week]	[1] [2] [3] [4] [5] [6]	[B]
Total score for exercise		= [A]+[B]	

[1] *These answers were added up to a new variable to give each participant a total score for physical exercise. E.g.: If person NN answered [Never] to the question on: physical exercise without sweating and [1-2 times/week] to the question on: physical exercise with sweating, the total score for exercise would be: 1+3=4 (min-max 2-12).*

Adapted from Larsen et al. (2006)

Appendix F: Response summary to KAM-study questionnaire

***Table 12**: Response summary of the combined study population to KAM-study questionnaire*

Questions and reply options	Response summary					
	Total (N=926)		Male (N=476)		Female (N=450)	
	n (%)	CV	*n* (%)	CV	*n* (%)	CV
Cycling/walking to/from work		96.1		99.6		92.4
Never	588 (66.1)		338 (71.3)		250 (60.1)	
Once a week	66 (7.4)		28 (5.9)		38 (9.1)	
Several times a week	136 (15.3)		59 (12.4)		77 (18.5)	
Daily	100 (11.2)		49 (10.3)		51 (12.3)	
Hiking		98.6		98.9		98.2
Never	221 (24.2)		123 (26.1)		98 (22.2)	
Once a week	467 (51.2)		245 (52.0)		222 (50.2)	
Several times a week	225 (24.6)		103 (21.9)		122 (28.9)	
Exercise (> 20 min)		100		100		100
Never	86 (9.3)		52 (10.9)		34 (7.6)	
Less than once a week	206 (22.2)		125 (26.3)		81 (18.0)	
Once a week	231 (24.9)		112 (23.5)		119 (26.4)	
Several times a week	340 (36.7)		158 (33.2)		182 (40.4)	
Daily	63 (6.8)		29 (6.1)		34 (7.6)	
Perspiration during exercise		93.7		93.1		94.4
Yes	576 (66.4)		310 (70.0)		266 (59.1)	
No	292 (33.6)		133 (30.0)		159 (35.3)	

CV-coefficient of variance

Appendix G: Physical activity and genotype data for the PA study population

***Table 13**: Response summary to KAM-study questionnaire, physical activity (PA) data, and genotype frequencies for the PA study population*

Variable		Total (N=1268)		Male (N=689)		Female (N=579)	
		N (%)	CV	*N* (%)	CV	*N* (%)	CV
PA data							
Cycling/walking to/from work			96.3		99.0		93.3
	Never	814 (66.6)		485 (71.1)		329 (60.9)	
	Once a week	91 (7.4)		41 (6.0)		50 (9.3)	
	Several times a week	191 (15.6)		90 (13.2)		101 (18.7)	
	Daily	126 (10.3)		66 (9.7)		60 (11.1)	
Hiking			98.8		99.0		98.6
	Never	304 (24.3)		171 (25.1)		133 (23.3)	
	Once a week	627 (50.0)		346 (50.7)		281 (49.2)	
	Several times a week	322 (25.7)		165 (24.2)		157 (27.5)	
Exercise (> 20 min)			100		100		100
	Never	118 (9.3)		75 (10.9)		43 (7.4)	
	Less than once a week	284 (22.4)		171 (24.8)		113 (19.5)	
	Once a week	302 (23.8)		156 (22.6)		146 (25.2)	
	Several times a week	468 (36.9)		235 (34.1)		233 (40.2)	
	Daily	96 (7.6)		52 (7.5)		44 (7.6)	
Perspiration during exercise			94.5		94.0		95.0
	Yes	796 (62.8)		453 (69.9)		343 (62.4)	
	No	402 (31.7)		195 (30.1)		207 (37.3)	
Total PA level, *n* (%)							
< 7 MET		98 (7.7)		63 (9.1)		35 (6.0)	
7 - 10 MET		239 (18.8)		150 (21.8)		89 (15.4)	
> 10 MET		931 (73.4)		476 (69.1)		455 (78.6)	
Total capacity score, *n* (%)							
< 7 MET		91 (7.2)		59 (8.6)		32 (5.5)	
7 - 10 MET		159 (12.5)		100 (14.5)		59 (10.2)	
> 10 MET		1018 (80.3)		530 (76.9)		488 (84.3)	
Genotype, *n* (%)							
	ACE	(*n*=622)		(*n*=307)		(*n*=315)	
	II	141 (22.7)		73 (23.8)		68 (21.6)	
	ID	333 (49.4)		167 (54.4)		166 (52.7)	
	DD	148 (23.8)		67 (21.8)		81 (25.7)	
	ACTN3	(*n*=726)		(*n*=354)		(*n*=372)	
	RR	222 (30.6)		101 (28.5)		121 (32.5)	
	RX	359 (49.4)		180 (50.8)		179 (48.1)	
	XX	145 (20.0)		73 (20.6)		72 (19.4)	