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Kristina Tiainen

Genetics of Skeletal Muscle Characteristics and Maximal Walking Speed among Older Female Twins

JYVÄSKYLÄN YLIOPISTO

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ABSTRACT

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The purpose of this study was to examine the contribution of genetic and environmental on individual differences in skeletal muscle characteristics and maximal walking speed. The aim was also to identify chromosomal areas that account for skeletal muscle characteristics and maximal walking speed and to study whether the regions are same for these traits.

Maximal isometric muscle strength of different muscle groups (hand grip, knee extensor, and ankle plantar flexion), leg extensor power, lower leg muscle cross-sectional area (CSA) and maximal walking speed were measured in the laboratory from 103 monozygotic and 114 dizygotic female twin pairs 63-76 years of age and without severe disabilities. Zygosity was confirmed by a battery of 10 highly polymorphic gene markers. The statistical analyses were carried out using quantitative genetic modeling and maximum likelihood methods in genome-wide linkage analysis.

Genetic effects accounted on average for 20-30% of the variance in isometric muscle strength, leg extensor power, and maximal walking speed and 75% of the variance in lower leg muscle CSA, while the remaining variance was due to environmental effects. Maximal isometric muscle strength of different muscle groups and leg extensor power shared a genetic effect in common, indicating that they are at least partly regulated by the same genes. The common genetic effect for isometric knee extensor strength and leg extensor power indicated that different muscle contractions are partly regulated by the same genes. Strength, power and walking speed had a genetic effect in common which accounted for 52% of the variance in strength, 36% in power and 34% in walking speed. The genome-wide linkage analysis showed the highest multipoint logarithm of the odds (LOD) score for isometric muscle strength on chromosome 15, for leg extensor power on chromosome 8, for lower leg muscle CSA on chromosome 20 and for walking speed on chromosome 13.

The results suggest that some people may be more prone to low muscle strength and functional limitation in old age due to genetic predisposition, but this does not rule out the possibility that changes in the lifestyle of predisposed individuals may also have a major effect. Approximately half of the variance in each trait was explained by environmental effects, which suggests that physical activity is important to improve performance and preventing functional limitation. Further studies and fine-mapping are needed for more in-depth analyses of the linkage analysis findings to identify the genes responsible for the genetic effects on these traits.

Key words: Strength, power, muscle mass, gait, genetic effect, environmental effect, genes, genome-wide scan, twin

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following papers, which will be referred to by their Roman numerals.

I Tiainen, K., Sipilä, S., Alén, M., Heikkinen, E., Kaprio, J., Koskenvuo, M., Tolvanen, A., Pajala, S., Rantanen, T. 2004. Heritability of maximal isometric muscle strength in older female twins. Journal of Applied Physiology 96, 173–180.

II Tiainen, K., Sipilä, S., Alén, M., Heikkinen, E., Kaprio, J., Koskenvuo, M., Tolvanen, A., Pajala, S., Rantanen, T. 2005. Shared genetic and environmental effect on strength and power in older female twins. Medicine & Science in Sports & Exercise 1, 72–78.

III Tiainen, K., Pajala, S., Sipilä, S., Kaprio, J., Koskenvuo, M., Alén, M., Heikkinen, E., Tolvanen, A., Rantanen, T. 2006. Genetic effects in common on maximal walking speed and muscle performance in older women. Scandinavian Journal of Medicine & Science in Sports (DOI: 10.1111/j.1600-0838.2006.00553.x.).

IV Tiainen, K., Perola, M., Kovanen, V., Sipilä, S., Rikalainen, K., Widen, E., Kaprio, J., Rantanen, T., Kujala, U. M. 2006. Genome-wide linkage analysis for maximal walking speed and skeletal muscle characteristics in older women. Submitted for publication.

ABBREVIATIONS

A	Additive genetic effect
ACE	Quantitative genetic model including additive genetic effect, shared environmental effect and non-shared environmental effect
Acom	Additive genetic effect in common
ADE	Quantitative genetic model including additive genetic effect, non-additive genetic effect and non-shared environmental effect
Aspe	Specific additive genetic effect
BMI	Body mass index
C	Shared environmental effect
CE	Quantitative genetic model including, shared and non-shared environmental effects
Ccom	Shared environmental effect in common
cM	Centimorgan
CNTF	Ciliary neurotrophic factor
CNTFR	Ciliary neurotrophic factor receptor
COL1A1	Type 1 collagen alpha 1
CSA	Cross-sectional area
Cspe	Specific shared environmental effect
D	Non-additive (dominance) genetic effect
DE	Quantitative genetic model including non-additive genetic effect and non-shared environmental effects
df	Degree of freedom
Δdf	Differences in degrees of freedom
DNA	Deoxyribonucleic acid
DZ	Dizygotic
E	Non-shared environmental effect
Ecom	Non-shared environmental effect in common
Espe	Specific non-shared environmental effect
FITSA	Finnish Twin Study on Aging
GDF8	Growth differentiation factor-8, myostatin
HRT	Hormonal replacement therapy
IGF1	Insulin-like growth factor 1
IGF2	Insulin-like growth factor 2
LOD	Logarithm of the odds
-2LL	-2 times log-likelihood
Δ-2LL	Differences in -2 times log-likelihood
MZ	Monozygotic
N	Newton
SD	Standard deviation
SNP	Single nucleotide polymorphism
VDR	Vitamin D receptor
W	Watt
XZ	Uncertain zygosity

CONTENTS

1 INTRODUCTION

Several quantitative and qualitative changes occur in the human body with aging. Skeletal muscle mass and muscle strength decline, functional performance, such as walking ability is impaired and the risk of disability is increased (Rantanen et al. 1999a, Foldvari et al. 2000, Bean et al. 2003, Lauretani et al. 2003). Skeletal muscle as the largest organ in the human body plays an important role in body movement and metabolism. The functions of skeletal muscles are contractibility, locomotion activity, breathing, maintenance of posture and body position, support for soft tissues, metabolism, storing proteins and the maintenance of body temperature. Muscle mass and strength reach their peak at the age of 30 years and begin to decline thereafter (Rantanen et al. 1998b, Frontera et al. 2000). Around the age of 60 years the deterioration accelerates due to structural and functional changes in the neuromuscular system and the diminished use of muscles (Rantanen et al. 1998b, Frontera et al. 2000). Walking speed decreases with aging which is partly explained by the age-related decline in muscle strength and power (Himann et al. 1988, Bohannon 1997).

Sarcopenia, the loss of skeletal muscle mass (Rosenberg 1989), is a multifactorial age-related phenomenon that impairs the metabolism and physical function (Roubenoff 2003). As a consequence of the loss of muscle mass, muscle strength also decreases. The prevalence of sarcopenia increases in the older age and is a significant risk factor for functional limitation and disability. Both men and women lose muscle mass and strength with aging, but among women sarcopenia may be a more prevalent public health problem because women with an equal level of disability will live longer than men (Leveille et al. 2000).

Large individual differences in muscle mass, strength and power have been observed in all age groups. These differences may be due to environmental or genetic factors, or both. Twin studies provide an opportunity to estimate and to differentiate sources of familial resemblance that may arise from shared genes, shared environments, or both (Plomin et al. 2001). Thus far, information about the heritability of muscle characteristics is limited, particularly in the old age. Heritability describes the extent to which interindividual differences in a phenotype are explained by genetic differences in a certain popula-

tion at a certain time (Plomin et al. 2001). Studies of the heritability of muscle characteristics have mainly addressed hand grip strength in different age groups, and the results suggest that the heritability of muscle strength varies from 20% to 70% (Arden & Spector 1997, Carmelli & Reed 2000, Katzmarzyk et al. 2001, Frederiksen et al. 2002). However, heritability may change during the lifespan. Earlier studies (e.g. Izquierdo et al. 1999, Bamman et al. 1999, Goodpaster et al. 2001, Newman et al. 2003a) have observed that muscle characteristics, such as muscle mass and strength, show a relatively high phenotypic correlation. The correlation may be partly due to a common genetic background. Previous studies have investigated the heritability of muscle characteristics separately. There is no scientific evidence to show whether there are genes that have a general effect on muscle function in old age or if the contribution of genes is trait-specific.

Maintaining walking ability is an important factor for functional independence and quality of life in old age. Several cross-sectional studies (Rantanen et al. 1998a, Rantanen et al. 2001, Lauretani et al. 2003, Visser et al. 2005) and longitudinal studies (Rantanen et al. 1999b, Onder et al. 2005) have shown a strong association between a decreased muscle strength, a reduced walking speed and increased walking disabilities. Severe walking disabilities increase with aging. Among American women aged 65-74 years the prevalence of severe walking disabilities was 2%, whereas among those aged 85 years or over the prevalence was 9% (Rantanen et al. 1999a). Environmental factors, such as physical activity and exercise training, are known to have an effect on walking speed, whereas the effect of genetic factors is limited. Genetic effects accounted for 17% of the variation in maximal walking speed among older female twins (Pajala et al. 2005). Despite the association between strength and walking speed, no previous information was found about whether the observed association is explained by shared genetic effects.

The associations of genes and gene variants with muscle characteristics and functional performance is currently under intensive investigation. Walking speed, muscle mass, strength and power are complex multifactorial phenotypes that are affected by many different genes. Earlier studies have investigated the associations of candidate gene polymorphisms with these phenotypes, but data from genome-wide linkage scans are limited. Genes that have an effect on the trait are not similarly active in all individuals all the time, and thus their level of activity and importance in relation to the trait may change depending on age and gender. It is important, therefore, to identify the chromosomal areas and candidate genes relating to skeletal muscle characteristics and functional performance also among older people.

The present study examined the contribution of genetic and environmental effects on skeletal muscle characteristics and maximal walking speed in older female twins. In addition, genome-wide linkage analysis was used to identify chromosomal areas that account for skeletal muscle characteristics and maximal walking speed and whether these regions are the same for these traits.

2 REVIEW OF THE LITERATURE

2.1 Skeletal muscle characteristics in old age

2.1.1 Muscle mass

Cross-sectional studies have shown significant differences in muscle mass between young and older subjects (Lexell et al. 1988, Janssen et al. 2000, Trappe et al. 2003). Muscle mass reaches its peak at the age of 25-30 years (Lexell et al. 1988, Janssen et al. 2000) and thereafter begins to decline. The decline accelerates at the end of the fifth decade when approximately 10% of the muscle mass has been lost. Thirty years later, at the age of 80 years, on average 40% of the peak muscle mass has disappeared (Lexell et al. 1988). Comparing age groups, Janssen et al. (2000) observed that among individuals 18-88 years of age lower body muscle mass, as measured by magnetic resonance imaging, decreases 1.9 kg per decade among men and 1.1 kg per decade among women. The data on changes in muscle mass with aging obtained from longitudinal studies are limited. Hughes et al. (2001) and Davies et al. (2002) estimated the muscle mass of the whole body using measurement of creatinine from 24-hour urine collections, and observed 1% annual decline in muscle mass among women and men 60-70 years of age. Frontera et al. (2000) also reported an average 1% loss of muscle mass per year among sedentary healthy 65-year-old men using a computer tomography scan of the quadriceps and knee flexor muscles.

Hughes et al. (2001) reported a significantly higher loss of muscle mass in older men (12.9%) compared to older women (5.3%) during a 10-year follow-up. Zamboni et al. (2003) evaluated whole body muscle mass, using dual energy X-ray absorptiometry and observed that men 68 to 78 years of age lost more leg muscle mass (3.6%) than women of same age (2.4%) in a two-year follow-up. However, men have more muscle mass than women throughout the lifespan. In a cross-sectional study by Janssen et al. (2000), the proportion of muscle mass among men 18-29 years of age was 42% and among men aged 60-69 years it was 34%. The corresponding proportions in women were significantly lower among

young women (34%) and older women (27%). Thus, among women, even minor changes in muscle mass may result in a limited on functional performance. In addition, the decline in muscle mass among women accelerates significantly during the menopause due to changes in hormonal status, whereas in men the decline is more gradual throughout the adult lifespan (Phillips et al. 1993).

2.1.2 Sarcopenia

Definition and prevalence of sarcopenia

Rosenberg (1989) was the first researcher who named the age-related loss of muscle mass as sarcopenia. The term sarcopenia comes from Greek, sarx for flesh and penia for loss. Sarcopenia is a multifactorial, universal phenomenon that is always related to advancing age. Thus, it is different from an acute disuse atrophy, where muscle mass is reduced but fiber number and specific force are maintained (Barton & Morris 2003). It is also distinct from cachexia, which is the cytokine-associated wasting of protein and energy stores caused by the effects of inflammatory diseases such as cancer, chronic pulmonary disease or acquired immune deficiency syndrome (Thomas 2005). Nowadays, sarcopenia is also often attached to declined in muscle strength, because muscle mass and strength are closely related to each other.

Although sarcopenia has been under intensive investigation during recent years, it has no generally accepted clinical definition. Baumgartner et al. (1998) defined sarcopenia as an appendicular skeletal muscle mass per height squared (kg/m^2) of less than two standard deviations (SD) below the mean of a healthy young reference group or less than 7.26 kg/m^2 in men and less than 5.45 kg/m^2 in women. This definition is almost analogous with the definition of osteopenia. However, the present definition of sarcopenia is not based on any medical observations or medical thresholds, but it is a deviation-based definition.

Using the relative skeletal muscle index as a definition, the prevalence of sarcopenia increased from 13-24% among individuals less than 70 years of age to over 50% among those more than 80 years of age (Baumgartner et al. 1998). In the study by Iannuzzi-Sucich et al. (2002) using the same definition, the overall prevalence of sarcopenia was 22.6% among women 64-93 years of age and 26.8% among men of same age. In the subgroup analysis of women and men over 80 years of age, the prevalence increased to 31.0% and 52.9%, respectively.

Etiology and consequences of sarcopenia

The etiology of sarcopenia is not completely clear. Many potentially important mechanisms in the etiology of sarcopenia have been suggested (Figure 1), such as lack of regular physical activity, diseases, hormonal changes, loss of neuromuscular function, changes in protein metabolism, changes in body composition and nutritional status, apoptosis, and an altered gene expression (Morley et al. 2001, Doherty 2003, Marcell 2003, Roubenoff 2003).

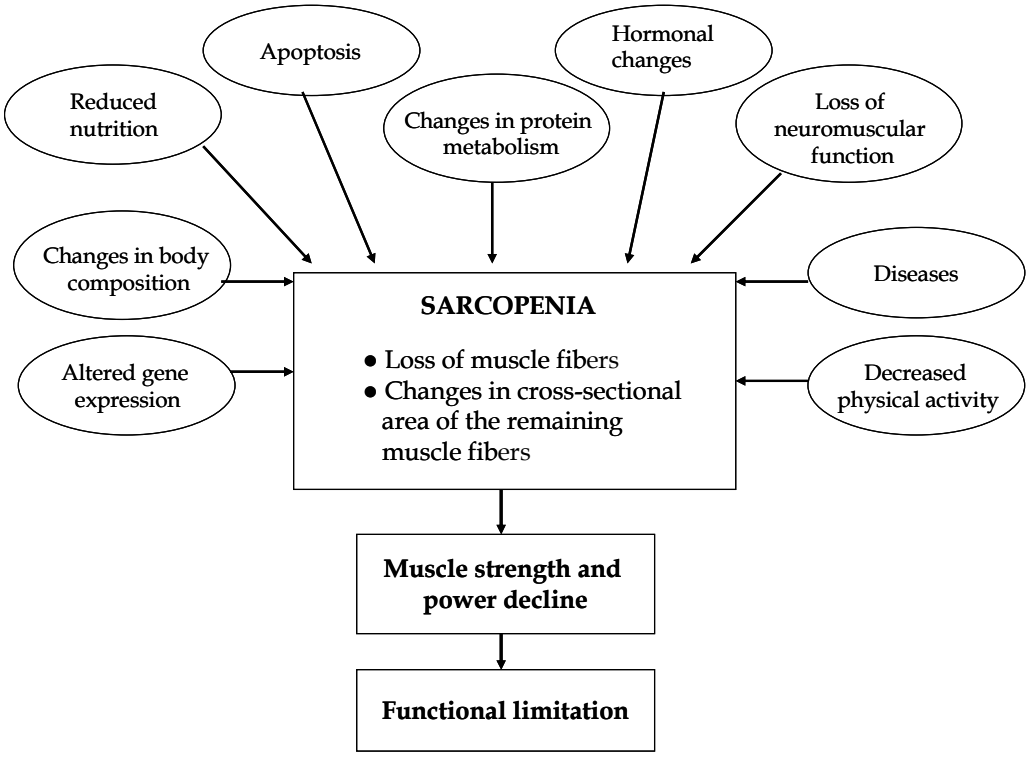


FIGURE 1 Main factors underlying sarcopenia (modified from Roubenoff 2003).

Endocrine activity changes during aging (Lamberts et al. 1997). The production of anabolic hormones, such as sex hormones (estrogen and testosterone), insulin, insulin-like growth factor-1 (IGF1) and growth hormone, decreases. These changes are related to normal aging and they do not necessarily need any medical treatment. In women, the most dramatic hormonal changes occur during menopause, on average at the age of 51 years. Menopause is an age-related condition and its most evident hormonal change is the significant decline in the secretion of estrogen due to ovarian failure (Rannevik et al. 1986). Among women, the level of testosterone also decreases, but the decline is minor compared to that of estrogen (Rannevik et al. 1986). Earlier studies have demonstrated the importance of estrogen in the maintenance of bone tissue and thus in the prevention of osteoporosis (Prestwood et al. 2000, Paschalis et al. 2003). The decline in ovarian estrogen secretion may be related to the loss of muscle mass and strength, which are known to accelerate after menopause. The estrogen receptor alpha has also been found in skeletal muscles (Lemoine et al. 2003) suggesting that estrogen could also have an effect on muscle tissue. The literature reports conflicting results on the association between hormonal replacement therapy (HRT) and skeletal muscle mass. In some cross-sectional studies, HRT was not significantly associated with muscle mass or muscle strength (Ribom et al. 2002, Aubertin-Leheudre et al. 2005). However, it has been observed that HRT may maintain or even improve muscle strength among post-menopausal women and thus prevent functional limitation and disability (Phillips et al.

1993, Greeves et al. 1999, Skelton et al. 1999, Uusi-Rasi et al. 2003). Placebo-controlled studies have also produced conflicting results. In the study by Sipilä et al. (2001) HRT had positive effects on muscle mass. Among 50- to 57-year-old women who were in early postmenopause, the lean tissue cross-sectional area (CSA) of the quadriceps muscles increased by 6.3% in response to the use of HRT for 12 months, while CSA was not changed during the same period in nonuser. In contrast, Walker et al. (2001) and Kenny et al. (2005) observed no change in muscle mass or strength in response to HRT therapy.

In apoptosis, a highly regulated cell death, a single cell dies without death or disruption of the surrounding tissue (Dirks & Leeuwenburgh 2004). Recent studies have suggested that apoptosis also occurs in muscle cells with advancing age (Dirks & Leeuwenburgh 2002, Dirks & Leeuwenburgh 2004). Apoptosis has an important role in tissue homeostasis and it is also essential for normal development. However, to understand the role of apoptosis in the pathogenesis of sarcopenia, more research is required.

Reduced appetite and food intake are associated with aging and are important factors in the development and progression of sarcopenia and functional disabilities (Morley 2001, Morley et al. 2001). Morley (2001) termed this physiological decline in food intake as the anorexia of aging. The reasons and consequences of a reduced nutrition status may include decreased physical activity, diseases, changes in metabolism in general and changes in basal metabolic rate, and a reduced energy requirement and expenditure. Malnutrition is always harmful for older people. In some cases, also weight loss can cause adverse changes in functional performance. When older persons lose weight they lose both fat and muscle mass, that may worsen muscle strength and functional performance (Newman et al. 2005). On the other hand, sarcopenia may also occur with obesity (sarcopenic obesity). The prevalence of obesity, defined as the body mass index (BMI) of greater than or equal to 30 kg/m^2, decreases with aging. However, this does not necessarily mean that older people lose fat mass with aging. Lean body mass decreases with aging, while the proportion of body fat and abdominal fat increases (Zamboni et al. 2003). Obese people may maintain or even increase their fat mass but lose skeletal muscle mass, which may increase the risk of functional limitation (Baumgartner 2000). A person who has simultaneously sarcopenia and obesity has almost three times greater risk for disability compared to a person without sarcopenic obesity (Baumgartner et al. 2004). Increased fat infiltration into the muscle with aging is associated with poorer muscle strength and an increased risk for disability (Sipilä & Suominen 1993, Visser et al. 2002, Visser et al. 2005).

Many diseases such as diabetes decrease muscle mass and strength. Insulin has an important anabolic effect primarily by reducing protein degradation and stimulating protein synthesis (Perriott et al. 2001, Clark et al. 2003). The results from a large population-based study by Abbatecola et al. (2005) showed an association between an increased insulin resistance and poor muscle strength. As a consequence of the diseases that limit exercise or cause disability, physical activity decreases that reduce muscle mass, strength and functional perform-

ance. Decreased physical activity is a risk factor for the accumulation of body fat and insulin resistance, both of which are major risk factors for diabetes.

Early life development may also influence sarcopenia. It has been suggested that a lower birth weight is associated with lower muscle strength in later life and thus expose to sarcopenia. Sayer et al. (2004) found that a higher birth weight was associated with a better hand grip strength in later life among over 1400 men and women aged 65 years or more suggesting that sarcopenia may have prenatal origins. Also in the study by Kuh et al. (2002), birth weight was strongly associated with hand grip strength among men and women aged 53 years. The results supported the importance of prenatal influences on muscle development with potential consequences even in late life.

Major changes occur in skeletal muscle gene expression profiles with aging (Welle et al. 2003, Park & Prolla 2005). Older women's muscles show an increased amount of oxidative deoxyribonucleic acid (DNA) damage compared with younger women's muscles (Welle et al. 2003, Welle et al. 2004, Short et al. 2005). Age-related reduction in muscle mitochondrial DNA and increased DNA oxidation are related to a declining mitochondrial adenosine tri-phosphate production rate in skeletal muscle (Short et al. 2005). These changes are also associated with aerobic capacity and glucose tolerance (Short et al. 2005). The research about the genetics of sarcopenia is undergoing. In the study by Roth et al. (2004) a polymorphism in vitamin D receptor translation start site was associated with fat-free-mass and sarcopenia among men aged 58-93 years.

Because skeletal muscle mass and muscle strength have an effect on functional performance, sarcopenia also impairs physical function. Sarcopenic women and men had approximately four times higher rates of disability compared with persons with a normal muscle mass (Baumgartner et al. 1998). Although muscle mass and strength are strongly associated, Lauretani et al. (2003) observed that muscle strength but not muscle mass, was associated with a reduced lower extremity performance.

Sarcopenia is also associated with increased mortality. Metter et al. (2002) observed in their 25-year longitudinal study among healthy men that decreased muscle strength was a more important contributor to mortality than muscle mass. Newman et al. (2006) also found that muscle strength was more closely associated with mortality than muscle mass among men and women 70-79 years of age.

The prevention of muscle loss would be one of the most important interventions to avoid sarcopenia. Age-related sarcopenia may be prevented or treated with lifestyle interventions and pharmacological treatment. Maintaining physical activity may be the most important strategy to prevent age-related sarcopenia. Several studies have shown significant improvements in muscle mass and strength as a consequence of resistance training, even among very old men and women (e.g. Ferri et al. 2003, Kraemer et al. 2004, Binder et al. 2005). Pharmacological treatment is also possible, however, medications may have adverse effects.

The main observations associated with sarcopenia are a loss in the number of skeletal muscle fibers (Lexell et al. 1988) and change in the CSA of the remaining fibers (Aniansson et al. 1986). A reduction in the size of fibers, mostly of type II (fast-twich) fibers is also an important factor, but to a lesser extent (Lexell et al. 1988). Muscle fiber number reaches its peak on average at the age of 25 years. The reduction in fiber numbers is linear at the age of 50 years and accelerates thereafter. On average, 40% of fibers are lost by the age of 80 years (Lexell et al. 1988). Although age-related muscle atrophy is mainly caused by loss in the number of muscle fibers, the reduction in fiber CSA is also significant. Reduction in fiber CSA from the age of 20 to 80 years was, on average, 26% (Lexell et al. 1988). The steeper decline in power than strength may, at least partly, be due to the greater effects of aging on type II (fast-twitch) than type I (slow-twitch) fibers, both in the reduction in fiber numbers and size (Lexell et al. 1988).

A primary factor in the age-associated reductions observed in contractile muscle strength might be the loss of alpha-motor neuron input to muscle. Although the reduction in muscle CSA explains most of the decline in muscle strength, additional factors, such as changes in the number and size of motor units, also explain the age-related changes in muscle strength (Klein et al. 2001). The reduced number of motor units, the significant enlargement of surviving motor units, and motor unit remodeling decrease the conduction velocity of motoneuron innervation (Campbell et al. 1973, Wang et al. 1999).

In the study by Doherty et al. (1993) the number of motor units was significantly reduced among men and women 60-81 years of age compared with those aged 20-38 years. Even healthy people have lost half of their motor units at the age of 60 years compared with people in their 20's. The size of remaining motor units was significantly larger among older compared to younger people (Doherty et al. 1993). Aging particularly affects the largest and fastest conducting motor units, potentially decreasing velocity and consequently muscle power more than muscle strength (Wang et al. 1999).

2.1.3 Muscle force production

When the skeletal muscle is active the filaments slide past one another and the muscle shortens. Maximal isometric muscle strength has been defined as the maximum voluntary contraction performed at a specific joint angle against an unyielding resistance (Enoka 1994). In this condition whole-muscle length will not change. When force production is greater than external load, muscle shortens during the contraction, and this phenomenon is called a concentric contraction. If the load is greater than the produced muscle force, the muscle lengthens, and this phenomenon is called an eccentric contraction (Enoka 1994). Muscle power is the product of force generation and the speed of muscle contraction i.e. the ability of the neuromuscular system to produce the greatest possible force as fast as possible (Enoka 1994). According to the force-velocity curve, the

highest power is attained when muscle contraction happens at approximately one third of maximum force. At higher force levels, speed decreases and at higher speed levels force decreases thus resulting in a lower power output (Enoka 1994).

Due to the quantitative and qualitative changes in the neuromuscular system with aging skeletal muscle strength and power among older people are at a lower level than among younger people. Tracy & Enoka (2002) and Bazzucchi et al. (2004) reported that older healthy 70-year-old women have, on average, 30% lower maximal isometric knee extensor strength compared to younger women. Maximal isometric muscle strength decreases after the age of 30 years, and the decrease accelerates around the age of 60 years. On the basis of the results of the longitudinal studies, the decrease in isometric muscle strength is 1-2% per year after the age of 65 years (Bassey 1998, Rantanen et al. 1998b, Frontera et al. 2000, Hughes et al. 2001). In a large longitudinal study by Frederiksen et al. (2006) the annual decline in hand grip strength was 0.59 kg for men and 0.31 kg for women between the age of 50 to 85 years. The decrease in muscle strength among women at the age of 60 years is more significant than among men, who show a more gradual decrease over adult age (Phillips et al. 1993, Samson et al. 2000). Samson et al. (2000) showed an accelerated decline in muscle strength in women above the age of 55 years. Among healthy women, isometric knee extensor strength decreased by 10.3% between the age of 20 and 55 years. Between 55 and 80 years the decrease in strength was greater, 40.2%. In men the decline in strength was linear and more gradual. The decline in muscle power may be steeper than the decline in isometric muscle strength. Cross-sectional data suggest deterioration in maximal power of 2-4% per year among healthy men and women 65-89 years of age (Skelton et al. 1994). Macaluso & De Vito (2003) observed 62% lower maximal power in the lower leg in healthy women 65-74 years of age compared to women 18-30 years of age.

Decline in strength and power with aging may be greater in the lower than upper extremities (Hughes et al. 2001, Candow & Chilibeck 2005). In the longitudinal study by Hughes et al. (2001) the isokinetic muscle strength of the knee extensors and flexors among older women decreased by 1.4% and 1.6% per year, respectively, compared to the strength of the elbow muscle where the decrease was 0.2%. Also, Frontera et al. (2000) showed in a 12-year longitudinal study a greater decline in the muscle strength of the knee extensors and flexors compared to the elbow extensors and flexors among sedentary healthy 65-year-old men. A cross-sectional analysis, including 37 mobility-limited men and women 65-93 years of age, showed a strong relationship between the power of the upper and lower body muscles (Herman et al. 2005). The correlations of strength and power between the upper and lower limbs varied between 0.69-0.89, suggesting that upper limb muscle power could be used as a surrogate measure of total body power.

2.2 Walking ability in old age

Maintaining walking ability is important for functional independence and the quality of life in old age. Walking speed is also good predictor of future functional limitation and disability (Guralnik et al. 1995, Rantanen et al. 2001, Studenski et al. 2003, Visser et al. 2005). Data from longitudinal studies on walking ability and changes with aging are limited. Walking speed decreases with aging, maximal walking speed more than normal-paced walking speed (Bohannon 1997). Mean maximal walking speed among healthy 30-year-old women and men was 2.3 m/s and 2.5 m/s, respectively, while maximal walking speed among healthy women and men 60- 70 years of age had decreased to 1.7 m/s and 2.0 m/s, respectively (Bohannon et al. 1996, Bohannon 1997). In the study by Tiedemann et al. (2005) the mean six-meters normal walking speed in women aged 75-98 years was 1.04 m/s and among same-age men 1.12 m/s. The gender differences also remain in old age.

Walking ability is a task that can be evaluated with respect to many different aspects. The movements of body segments during walking can be evaluated with kinematic parameters, such as step length, stride length, step width and duration of stance, swing and double-support phases. Also the cadence of the gait, initiation and stopping as well as ability to step over and cross obstacles are important aspects of gait. Walking ability may also be assessed by measuring walking time, velocity or distance. Short walking tests, such as 4-30 meters tests, assess peak velocity, whereas a longer walking test, such as the 400 meters or six-minute test, assess endurance and aerobic capacity rather than walking speed. Walking speed tests have been widely used also among older people (Rantanen et al. 2001, Steffen et al. 2002, Visser et al. 2002, Ble et al. 2005, Onder et al. 2005, Tiedemann et al. 2005).

A walking test could be performed at a normal or maximal walking speed. In a normal-paced walking speed test, the subjects are instructed to walk at their normal comfortable speed. During maximal walking speed trials, the persons are asked to walk as fast as possible without compromising safety and without running. Also, questionnaires and interviews are used to evaluate walking ability and walking speed. Self-reported functional status has been used, especially in large population based studies. Although the self-reported functional status does not fully agree with the results of the functional tests, the information from questionnaires and interviews also enables subjects whose functional status would be too low to perform the functional test, such as a walking test, to take part in the study (Sakari-Rantala et al. 2002). Compared with other self report items, self-report walking ability may be the best predictor of functional limitation among older people (Alexander et al. 2000, Reuben et al. 2004, Cesari et al. 2005, Sayers et al. 2005).

2.2.1 Factors underlying walking limitations

Age

Severe walking disabilities increase with aging. Rantanen et al. (1999a) observed that the prevalence of severe walking disabilities was only 2% in women aged 65-74 years, while among women 85 years or older the prevalence had climbed to 9%. In a Finnish nationally representative study, The Health 2000 study (Aromaa & Koskinen 2002), only 2% of individuals 55-64 years of age had difficulty in walking at a speed of 0.8 m/s, but every fourth of those aged 75-84 years had difficulty in reaching the velocity of 0.8 m/s. In the city of Helsinki, 0.8 m/s is the minimum walking speed required to cross the street during the green light of signaled intersections. Although walking ability largely depends on physiological characteristics, it is also a complex cognitive task that is partly determined by higher-level cognitive functions (Hausdorff et al. 2005).

Diseases

Musculoskeletal and neurological age-associated changes and many chronic conditions impair walking ability. Age-related changes in the senses may also have an effect on walking. Diseases, such as diabetes and asthma, cause many structural and functional changes in the neuromuscular system and thereby increase the risk of functional limitation and disability. Pulmonary diseases and heart diseases also reduce muscle strength and thus decrease aerobic capacity and increase the risk for functional limitations.

In the study by Resnick et al. (2002), men and women aged 70-79 years with diabetic neuropathy had significantly worse maximal walking speed (0.99 m/s) compared with non-diabetic controls (1.34 m/s). Andersen et al. (2004) observed muscle weakness at the ankle and knee extensor muscles among type 2 diabetic patients aged 70 and over. The authors suggested that a distal neuropathic process may underlie the impaired motor performance. A large cross-sectional study among women aged 65 years or over showed that women with diabetes had a 2.3 times greater prevalence of severe walking limitation, assessed with a self report, than healthy individuals (Volpato et al. 2002). Also, Menz et al. (2004) reported lower walking speed among older men and women with diabetic peripheral neuropathy. The normal walking speed in the 20-meter test was 0.98 m/s among patients with diabetes and 1.21 m/s among individuals without diabetes. The difference was even more evident on an irregular surface, where the walking speed was 1.12 m/s in individuals without diabetes and 0.84 m/s in patients with diabetes.

Lower-extremity peripheral arterial disease is a common chronic condition that severely compromises walking ability by intermittent claudication. Peripheral arterial disease is commonly associated with diabetes and causes increased risk for functional disabilities among older persons. Newman et al. (2003b) compared a 400-meter walking time among older men and women with and without peripheral arterial disease. The walking time was 5 minutes 50 seconds

among individuals with peripheral arterial disease and 5 minutes and 31 seconds in those without the disease. Atypical leg symptoms due to undefined illnesses may be the reason for a significant impairment in walking ability among older patients with peripheral arterial disease (Collins et al. 2005).

Aging has an effect on the range of motion by limiting the mobility of the joint. The reduction in the range of hip extension and ankle plantar flexion may cause difficulty in walking and, in consequently reduce walking speed (Kerrigan et al. 1998). Osteoarthritis may not only affect the strength of the muscles around the unhealthy joint, but it also has a generic effect on functional performance, such as walking. Pain, especially in the lower extremities, causes walking difficulties and decreases walking speed. Foot and leg pain is independent predictor of functional status among older people (Barr et al. 2005). In the Women's Health and Aging Study, older women with moderate knee pain walked four meters at a maximal velocity of 0.92 m/s, whereas those with severe knee pain walked at the velocity of 0.83 m/s (Lamb et al. 2000).

Balance and muscle strength

To walk, sufficient postural balance and muscle strength are needed. Rantanen et al. (1999a) showed in their cross-sectional study that the risk for severe walking disabilities was 10 times higher among older women with both strength and balance impairment compared with women with no impairments. An individual with poor balance needs more strength to maintain a stabile upright position during walking than a person whose balance is adequate. In walking, older people do not increase their speed and stride length to the same extent as younger people. Shkuratova et al. (2004) suggested that this could be a strategy to maintain walking stability.

Age-related changes in muscle characteristics, such as a decreased size and number of muscle fibers and a reduced conduction of motor unit, slow walking speed. Several cross-sectional studies have shown that muscle weakness in the lower extremities is associated with a reduced walking speed and an increased risk of disability, particularly among older people (Rantanen et al. 1998a, Foldvari et al. 2000, Bean et al. 2003, Lauretani et al. 2003). Knee extensors and flexors are particularly important for walking (Buchner et al. 1996, Rantanen & Avela 1997, Rantanen et al. 1998a, Tiedemann et al. 2005). A decreased strength of ankle plantar flexions also slows walking speed (Kerrigan et al. 1998). The muscle strength of lower extremities explained 22% of the variation in normal walking speed among men and women aged 60-96 years (Buchner et al. 1996). It has been suggested that the association between muscle strength and power and walking speed is curvilinear and includes thresholds. Above the threshold of reserve capacity, an increase in strength or power has no effect on walking speed, but acts as a safety margin. Below the threshold, changes in strength or power also cause changes in walking speed (Buchner & de Lateur 1991, Rantanen & Avela 1997, Rantanen et al. 1998a).

The ability to walk and maintain a well-balanced posture under changing circumstances requires sufficient strength as well as sufficient velocity to pro-

duce the required force. It has been suggested that a poor muscle power rather than strength is more closely correlated with the risk of mobility limitation, such as a decreased walking speed, among older persons (Foldvari et al. 2000, Bean et al. 2003, Cuoco et al. 2004, Petrella et al. 2005, Sayers et al. 2005). Although muscle power is closely related to muscle strength, it has been suggested that muscle power is a separate factor that may have a greater effect on functional performance (Bean et al. 2002). In the study by Bean et al. (2003) low muscle power and muscle strength both increased the risk of functional disabilities. However, the decreased muscle power was associated with a 2-3-fold greater risk of functional disabilities than the decreased muscle strength. However, Lauretani et al. (2003) reported that power was no better an indicator for poor mobility than muscle strength.

2.3 Research in genetic and environmental effects

2.3.1 Quantitative genetic modeling to estimate variance components

Quantitative genetic modeling estimates to which extent the observed differences among individuals are due to genetic or environmental variation without specifying the genetic or environmental effects (Plomin et al. 2001). Genotype is the genetic constitution of an individual. It is also the combination of alleles at particular loci. A trait observed in an individual which is the result of the interaction of genotype and environmental effects is called a phenotype (Plomin et al. 2001). Heritability is a statistical estimate for the size of a genetic effect and describes the extent to which individual differences in a phenotype are explained by genetic variation in a certain population at a certain time (Plomin et al. 2001). Therefore, the heritability of a phenotype may change during the lifespan. Heritability refers to the genetic contribution to individual differences at the population level, not to the phenotype of an individual (Plomin et al. 2001).

In genetic modeling, total variance in a trait is decomposed into components representing the genetic and environmental variances. Typically, genetic effects are classified into additive genetic effects (A) and non-additive genetic effects (D). Additive genetic effects refer to the individual alleles summed over the contributing loci and non-additive effects refer to interactions between alleles at the same loci or different loci. Additive genetic effects and shared environmental effects could be considered together as familial effects. Environmental effects are classified into shared environmental effects (C) and non-shared environmental effects (E). Shared environmental effects are common to both twins or family members in general, for example, things that are related to the rearing environment, where certain factors have affected both individuals in the same way in their childhood and then tracked over to adulthood behavior. Non-shared environmental effects are exposures that are not shared by family members and thereby contribute only to within-pair differences in a trait. In

addition, non-shared environmental effects contain measurement error and are thus always included in genetic modeling (Neale & Cardon 1992).

Differences between individuals may be due to environmental or genetic factors or both. Different family study designs such as the classic twin study design, adoption design and nuclear family design provide an opportunity to test the relative influence of nature and nurture. The classic twin study, in which twins have been reared together since birth, provides an opportunity to differentiate sources of familial resemblance that may arise from shared genes, shared environments, or both. The mathematical modeling of the data is based on the fact that two types of twinning occur. Monozygotic (MZ) twins share all of their genes (100%), while dizygotic (DZ) twins share on average 50% of their segregating genes. Consequently, in DZ twin pairs genetic effects contribute to both similarity and differences, whereas among MZ twin pairs they only contribute to similarity. Greater similarity between MZ twin pairs compared to DZ twin pairs is evidence for the genetic influence on the trait (Neale & Cardon 1992, Posthuma et al. 2003).

Family studies extend the MZ-DZ design to include parents, siblings and offspring in the analysis. Family studies assess the resemblance between genetically related parents and offspring and between siblings living together and can assess intergenerational similarity and transmission of genetic and social effects from one generation to the next. A family resemblance that can be due to either heredity or to a shared family environment can be estimated with correlations and covariances among family members (Plomin et al. 2001).

Although many phenotypes"run in families", family resemblance can be due to either nature or nurture. Adoption of a sibling into a different family creates pairs of genetically related individuals with individual family environments. Their resemblance estimates the contribution of genetics to family resemblance. Adoption also produces family members who share the family environment but are not related genetically. Consequently, it is possible to estimate the contribution of family environment to family resemblance (Plomin et al. 2001). Similarities between the adoptee and biological parents suggest the importance of genetic influence, because the similarity is due to shared genes and not shared environmental effects. Similarities between the adoptee and adoptive parents represent the effects of the shared family environment effects because they are not genetically related (Thomas et al. 2004). The weaknesses of adoption designs are the poor generalizability of the results and limited availability of adoptees, but on the other hand the design is relatively powerful.

Twin similarity for a categorical trait, such as a disease, can be examined using twin concordance-discordance design. Concordance describes the presence of the same trait in both members of the pair. It is also an index of the risk of a member of a pair to have a disease or an observed trait given that the other member of the pair has that trait (Strachan 2000, Plomin et al. 2001).

Twin studies are based on the assumptions of twinning mechanisms, random mating, an equal environment and representativeness (Kyvik 2000, Rijsdijk & Sham 2002). If the twins arose from one fertilized egg, the pair is MZ and if they arose from two eggs fertilised by two different sperms, the pair is DZ (e.g.

Hall 2003). The basis for all quantitative genetic modeling is that the twinning is correctly assessed. Nowadays DNA samples provide a reliable method of assessing zygosity.

The equal environments assumption, which is the most basic assumption of the twin method, requires that MZ and DZ twins share environmental effects relevant to the trait under study to the same extent. Consequently environmentally caused similarity is roughly the same for both types of twins reared in the same family. If the equal environmental assumption does not hold, the greater similarity of MZ pairs compared with DZ pairs could not be inferred to result solely from genetic influences. Thus similarity could partly be explained by environmental effects rather than by genetic effects (Plomin et al. 2001). Criticism has been leveled at the equal environments assumption. If MZ twins are treated more similarly than DZ twins, the MZ correlation will increase compared to the DZ correlation. As a result, the twin method will overestimate the genetic effect. The genetic effects could also be underestimated if MZ twins experience greater environmental differences, as this will increase the variability between them (Plomin et al. 2001). The equal environments assumption has rarely been found to be violated, but needs to be assessed in each twin study.

Another important assumption is representativeness. The selection criteria that are used to define the study population will affect the generalizability of the results to the entire population. Both MZ and DZ twins are assumed to represent the target population. If the sample is representative, the prevalence and incidence of diseases and means and variances of the measured traits should be the same as in the general population, and there should be no differences by zygosity.

Random mating with respect to the trait under study occurs when the mating is not influenced by any environmental or genetic effects. However, for example a tall child often has both a tall mother and a tall father, therefore positive assortative mating occurs. Spouses may resemble each other because they share environments (social homogamy). However, if people choose spouses who are alike (i.e. phenotypic assortment), it would affect the estimates of the relative roles of genes end environment. The assortative mating is worth notice because it increases the genetic variance in the population and has effect on the estimates of heritability. Although the correlation between spouses is rather low, assortative mating increases the genetic variability because its effects will accumulate in the later generations. Assortative mating raises the DZ correlation and increases their similarity. This will decrease the difference of the correlation between MZ and DZ twins. If the assortative mating is not taken into account it may cause underestimation of the heritability and overestimation of the shared environmental effects (Plomin 2001, Rijsdijk & Sham 2002).

Heritability estimates are population-specific and can differ between age groups and gender. Heritability can also be affected by the measurement method. As the result, variation between the results of heritability may differ quite widely among studies. If violation of the assumptions occurs, it will lead to incorrect estimates of the genetic effect and the shared environmental effect.

When the assumptions of twin study are taken into account, it is a powerful method to examine the genetic and environmental effects on the trait.

2.3.2 Search for genomic regions that contribute to the traits of interest with genome-wide linkage analysis

Genome-wide linkage analysis is a statistical technique used to search for genomic regions that contribute to the disease or other traits of interest and to help in localizing and identifying genes related to traits. In linkage analysis the distance between the DNA marker locus and a disease or observed trait locus is modeled in small numbers of large multigenerational families or large numbers of small families such as sibpairs consisting of both affected and unaffected family members (Sham 1998, Vink & Boomsma 2002, Thomas 2004).

The basic principle in linkage analysis is to find the DNA marker locus whose alleles are inherited with the observed trait locus. A genetic marker is a segment of DNA with a known physical location on a chromosome. The most common types of DNA markers are common mutations in a single-base pair (Single Nucleotide Polymorphism, SNP) or a variable number of repeats of two or more base pairs (microsatellites) (Vink & Boomsma 2002). A SNP is the most prevalent type of polymorphism in the human genome. In a SNP, a single base pair at a particular nucleotide site differs among individuals with a minor allele frequency of at least 5% (Hartl & Jones 2002). Microsatellites are landmarks of a known location in the genome. They are short non-coding repetitive DNA sequences that are repeated many times within the genome. The evidence for a linkage is derived using statistical procedures that estimate the co-segregation of the trait and DNA marker. In linkage analyses, a single marker (two-point linkage analysis) or several markers simultaneously (multipoint linkage analysis) can be used. The multipoint analysis improves the statistical power of the analysis.

The statistical methods used in linkage analysis can be divided in regression analyses and maximum likelihood methods. Several software packages, such as Merlin, Solar and Genehunter can be used to perform linkage analysis. Linkage is usually reported as a logarithm of the odds (LOD) score. A LOD score is a statistical estimate of whether the two genetic loci (the disease gene and the marker locus) are physically close enough on a particular chromosome that they are likely to be inherited together. Positive scores are evidence for linkage and negative scores are evidence against linkage. A significant LOD score will be found if the marker locus is linked to the trait. A LOD score of 3 is considered statistically significant evidence of linkage and it is equivalent to a p value of 0.0001 (Vink & Boomsma 2002, Teare & Barret 2005).

A limited statistical power can be a problem in linkage analyses of complex traits with multiple contributing loci (Vink & Boomsma 2002). The major advantage of linkage analysis is that it is systematic in the sense that several hundred, typically 400 to 1000, microsatellite markers or several thousands SNPs can be used to scan the entire genome (Teare & Barret 2005). When linkage analysis has identified an interesting chromosomal region surrounding a

marker with a significantly high LOD score, the investigation can continue by fine-mapping, i.e. investigating more markers at a higher density to find the specific genes related to the trait (Teare & Barret 2005).

2.4 Genetics of skeletal muscle characteristics and maximal walking speed

Most of the previous studies carried out among older twins have used grip strength as the indicator of the strength phenotype. In these studies the genetic effects have accounted for 22-52% of the variance in hand grip strength (Reed et al. 1991, Arden & Spector 1997, Carmelli & Reed 2000, Frederiksen et al. 2002). A family study by Katzmarzyk et al. (2001) investigated hand grip strength among 635 males and 629 females aged 7-69 years from 502 families. The heritability of hand grip strength was 48% in a population with a wide age range suggesting a significant familial resemblance. Data on the heritability of lower extremities are limited. The results of twin studies suggest that one third to one half of the interindividual variation in muscle strength and power is accounted for by genetic effects (Pérusse et al. 1987, Arden & Spector 1997). Among younger individuals, 45-year-old sibpairs, the heritability of hip flexors and knee extensors was 42% (Zhai et al. 2004).

It has been observed that the relative contributions of genetic and environmental effects to the variability of strength can change over the years. During a 10-year follow-up, the heritability of isometric hand grip strength decreased from 35% to 22%, while the shared environmental effects increased from 39% to 45% among male twins who were on average 63 years of age at baseline (Carmelli & Reed 2000). Also, diseases have an effect on the heritability of muscle strength. Frederiksen et al. (2002) found that excluding individuals with chronic diseases increased the heritability estimate from 52% to 62%. This may be explained by a reduced environmental variability.

Muscle CSA and lean body mass, which are close approximates of a total body muscle mass, seem to be under a relatively strong genetic regulation. A total of 66-92% of the variation in muscle CSA was explained by genetic effects depending on the muscle measured, assessment method, and age of the subjects (Loos et al. 1997, Thomis et al. 1997). Among boys and girls 10-14 years of age, genetic effects accounted for 87-95% of the variation in circumference of the muscles of the upper and lower extremities (Loos et al. 1997). A heritability of more than 85% was observed for arm CSA among male twins 17-30 years of age by Thomis et al. (1997 and 1998). Genetic effects accounted for 52-84% of variation in lean body mass among older female twins (Seeman et al. 1996, Arden & Spector 1997, Nguyen et al. 1998). In a study by Schousboe et al. (2004), the heritability of lean body mass was 61% among female twins aged 18-67 years. Forbes et al. (1995) reported a heritability of lean body mass of 70% in twin pairs of same sex over the age range of 7-85 years.

Only a few studies have investigated genetic effects on walking speed, and even fewer on maximal walking speed. In the Finnish Twin Study on Aging (FITSA) among older female twins, genetic effects accounted for 17% of the total variance in maximal walking speed (Pajala et al. 2005). Normal walking speed, which was a part of the lower-extremity sum score, was found to have a moderate genetic effect among older male twins (Carmelli et al. 2000).

2.5 Candidate genes for skeletal muscle characteristics and maximal walking speed

The next step after obtaining evidence for heritability by genetic modeling is to identify the chromosomal areas involved in traits by genome-wide linkage analysis. A high heritability estimate justifies genome-wide linkage analysis. Likewise, if the modeling has shown common genetic effects for the observed traits, then at least in part, the same chromosomal regions and genes in these areas can be said to be related to these traits. Rather little data are available from genome-wide linkage scans on the chromosomal areas contributing to muscle strength, power, CSA and walking speed.

The associations of genes with muscle characteristics and functional performance are currently under an intensive investigation. In their reviews Wolfarth et al. (2005) and Beunen & Thomis (2004) listed candidate genes associated with muscle strength, power and muscle mass. The candidate genes included myostatin, also designated growth differentiation factor-8 (GDF8), the ciliary neurotrophic factor (CNTF), the ciliary neurotrophic factor receptor (CNTFR), insulin-like growth factor 2 (IGF2), vitamin D receptor (VDR), type 1 collagen alpha 1 (COL1A1), and angiotensin converting enzyme.

Myostatin, a member of the transforming growth factor-beta family of proteins, is a muscle-specific negative regulator of muscle mass and muscle strength (Tobin & Celeste 2005). A linkage analysis by Huygens et al. (2004) also suggested that the myostatin pathway may be an important factor for muscle strength. Seibert et al. (2001) found an association between the R153 allele of the myostatin gene with lower muscle strength among women aged 70-79 years. However, they did not observe any relationship between the polymorphism and muscle mass. Other studies have suggested that inhibiting the function of the myostatin may restore skeletal muscle mass and strength (Tobin & Celeste 2005, Walsh & Celeste 2005).

CNTF, a member of the cytokine family, is a neurotrophic factor which is important for neuronal and muscle development and growth, as pointed out in the reviews of Vergara and Ramirez (2004) and Gordon et al. (2005). Roth et al. (2001) indicated the importance of the CNTF heterozygote G/A genotype on muscle strength, power and quality of muscle throughout the adult age lifespan. It has been suggested that the CNTF G/A genotype influences characteristics of motor unit during a submaximal contraction of knee extensor muscles

(Conwit et al. 2005). CNTFR has an essential role in muscle regeneration and motoneuron survival after muscle damage (Kami et al. 2000). Roth et al. (2003) observed that C174T polymorphism in the CNTFR was significantly associated with fat-free mass among men and women with a mean age of 53 years.

Skeletal muscle is a target organ for vitamin D, and a deficiency of vitamin D is associated with muscle weakness. VDR has been found to affect muscle cell maturation and functioning by binding to vitamin D metabolites in human skeletal muscle cell (Janssen et al. 2002, Pfeifer et al. 2002). Grundberg et al. (2004) examined two polymorphisms of VDR, poly A repeat and BsmI. Among women aged 20-39 years, homozygotes for a shorter poly A repeat and/or the absence of the linked BsmI restriction site had a 11.5% higher muscle strength than women homozygotes for a longer poly A repeat and/or the presence of the linked BsmI restriction site. In the study by Roth et al. (2004) the VRD translation initiation start site (FokI) polymorphism was associated with sarcopenia in men aged 50 years and older.

The COL1A1 gene codes for a protein type 1 collagen, the major collagen type in bone, tendons and in the intramuscular connective tissue. In bone COL1A1 Sp1 polymorphism is associated with osteoporosis and bone mineral density (Grant et al. 1996, Suuriniemi et al. 2006). Van Pottelbergh et al. (2001) have shown an association between COL1A1 Sp1 polymorphism and muscle strength. Presence of the s allele was associated with significantly lower grip strength among community-dwelling men over age of 70 years.

Insulin-like growth factors, such as IGF2, are peptides that have an important role in regulating growth, and influencing cell differentiation and regeneration, not only during the developmental period but also throughout the lifespan (O'Dell & Day 1998). These peptides are also potent regulators of muscle by participating in the formation and maintenance of skeletal muscle characteristics (Frost & Lang 2003). Lower plasma IGF1 levels and higher plasma interleukin-6 levels were associated with lower muscle strength and power in the study by Barbieri et al. (2003) among 526 persons aged 20-102 years. Also, Cappola et al. (2001) observed that low serum IGF1 levels were related to poor knee extensor strength among older women. A study that compared men aged 21-31 years and men aged 62-77 years suggested that the IGF1 gene expression may decline with aging (Welle et al. 2002). The variation in ApaI polymorphic site within the IGF2 gene may be associated with muscle development during growth and may also have an effect on muscle mass and muscle function in later life (Schrager et al. 2004). Individuals homozygous for the G allele had significantly greater muscle mass, strength and power at the age of 35 years and 65 years than individuals homozygous for the A allele (Schrager et al. 2004). Sayer et al. (2002) observed that the ApaI polymorphism of the IGF2 was significantly associated with hand grip strength and birth weight in men 64-74 years of age. Results also showed that birth weight has independent effects on adult hand grip strength among men. A similar association was not seen in women suggesting that adulthood environment may play a more important role in older women than men.

Angiotensin converting enzyme is a key component of the renin-angiotensin system and an essential hormonal regulator of blood pressure. Most of the genetic studies have focused on an insertion/deletion polymorphism of angiotensin converting enzyme. However, the results on the association between the polymorphisms in the angiotensin converting enzyme gene and muscle characteristics are conflicting. Frederiksen et al. (2003b) found no association between the insertion/deletion polymorphism of the angiotensin converting enzyme and the level of hand grip strength in a follow-up study among men and women aged 73 years and over. No association between the polymorphism and the level or change of physical activity was found either in a study among elderly Danish people by Frederiksen et al. (2003a), either. Similarly, Thomis et al. (2004) found no evidence for an association between the insertion/deletion polymorphism of the angiotensin converting enzyme gene and change in muscle strength in response to 10 weeks of strength training among young men. However, Folland et al. (2000) observed a significant association between the insertion/deletion polymorphism and changes in muscle strength in response to strength training of nine weeks among young men. Improved muscle strength was associated with the presence of deletion allele. Williams et al. (2000) showed that the insertion allele was associated with improved muscle performance among young men during the 11 weeks of aerobic training. There is some evidence that pharmacological intervention using angiotensin converting enzyme inhibitors may have an effect on muscle characteristics. Onder et al. (2002) observed in a three-year follow-up study that treatment with angiotensin converting enzyme inhibitors slowed down the decline in muscle strength in 80-year-old women. The decline was significantly less among angiotensin converting enzyme inhibitor users compared to continuous or intermittent users of other antihypertensive drugs.

For walking ability, which is a more multifactorial phenotype than muscle strength, no candidate genes have been suggested. However, Cappola et al. (2001) found a weak association between low serum IGF1 levels and a slow walking speed among community-dwelling women aged 70-79 years. It is possible that some of the genes which are related to muscle strength and power are also related to walking speed. In addition, genes related to neural function may have an effect on walking speed. However, evidence for these associations is lacking.

Obviously, muscle CSA, muscle strength, muscle power and walking speed are complex multifactorial phenotypes affected by many different genes. Genes that have an effect on the trait are not similarly active in all individuals and their activity and importance in relation to the trait may change depending on age and gender. Therefore, it is important to identify the chromosomal areas and candidate genes relating to skeletal muscle characteristics and walking speed also among older age groups.

3 AIMS OF THE STUDY

The main purpose of this study was to examine to what extent genetic and environmental effects accounted for skeletal muscle characteristics and maximal walking speed in a genetically controlled population. In addition, I wished to identify the chromosomal regions contributing to skeletal muscle characteristics and maximal walking speed and whether these areas are same for these traits.

The specific aims of the study were:

1. To examine genetic and environmental effects on maximal isometric muscle strength (hand grip, knee extensor, and ankle plantar flexion), leg extensor power, lower leg muscle CSA and maximal walking speed. (Studies I, II, and IV)

2. To examine whether maximal isometric knee extensor strength, leg extensor power and maximal walking speed share genetic or environmental effects in common. (Studies II and III)

3. To identify the chromosomal regions contributing to skeletal muscle characteristics (isometric knee extensor strength, leg extensor power, lower leg muscle CSA) and maximal walking speed and to examine whether skeletal muscle characteristics and maximal walking speed are regulated by the same chromosomal areas. (Study IV)

4 PARTICIPANTS AND METHODS

4.1 Participants

4.1.1 Recruitment

This study is part of the FITSA study, a study of genetic and environmental effects on the disablement process in older female twins. The participants were recruited from the Finnish Twin Cohort, which was launched in 1974 and is located at the Department of Public Health at the University of Helsinki. The Finnish Twin Cohort study, consisting of some 14 000 pairs of known zygosity born before 1958, is a longitudinal study of the genetic and environmental factors of chronic diseases and risk factors. The Finnish Twin Cohort (Kaprio et al. 1978, Kaprio & Koskenvuo 2002) contained 1260 respondent female twin pairs born in 1924-1937 and first studied in 1975. Of this group, an invitation to take part in the present study was sent in the year 2000 to every female MZ twin pair (n= 178), every third female DZ twin pair (n= 212) and to 24 female twin pairs with uncertain zygosity (XZ). Altogether 414 female twin pairs were selected solely on the basis of age and zygosity. To be recruited for the study, both individuals in the pair had to agree to participate (Figure 2).

Twin pairs arrived from all over Finland the evening preceding the laboratory measurements and stayed overnight in a hotel. Both of the individuals in the pair came to the laboratory at the same time and received their individual test schedules on arrival (Figure 3).

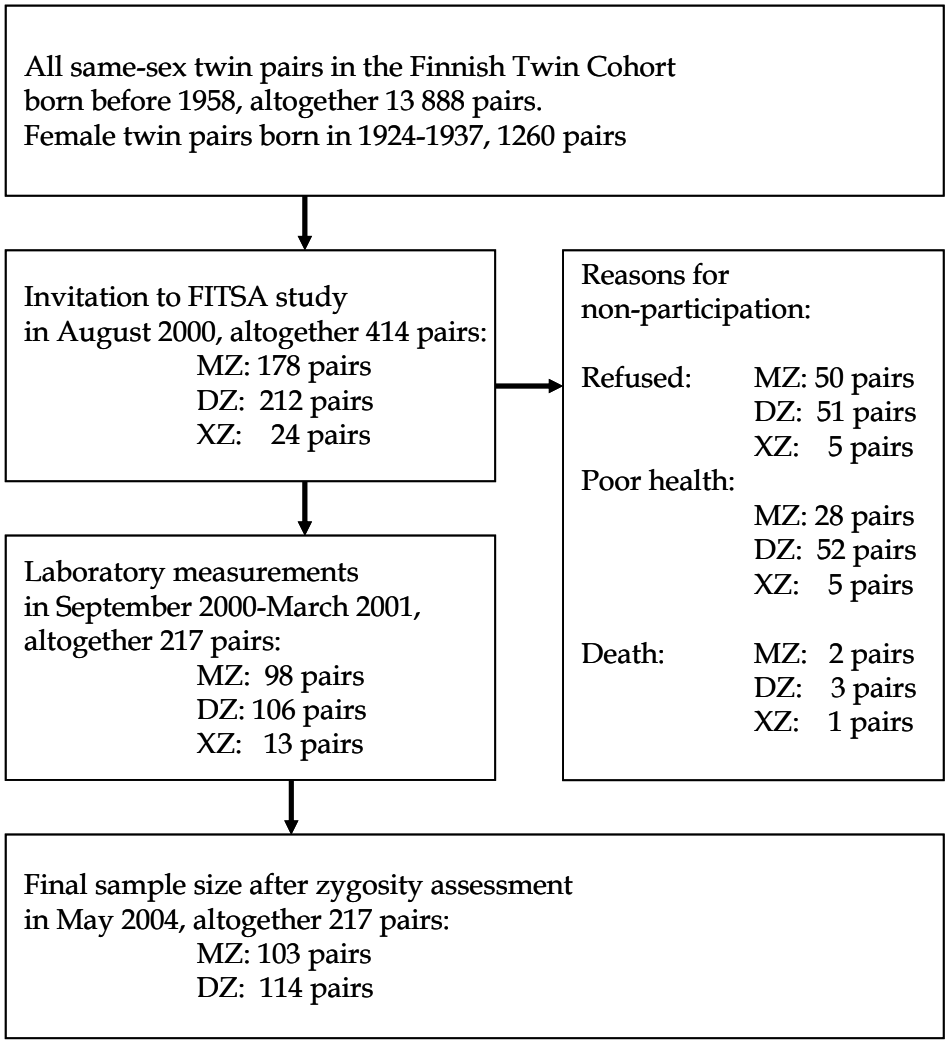


FIGURE 2 Recruitment of the participants.

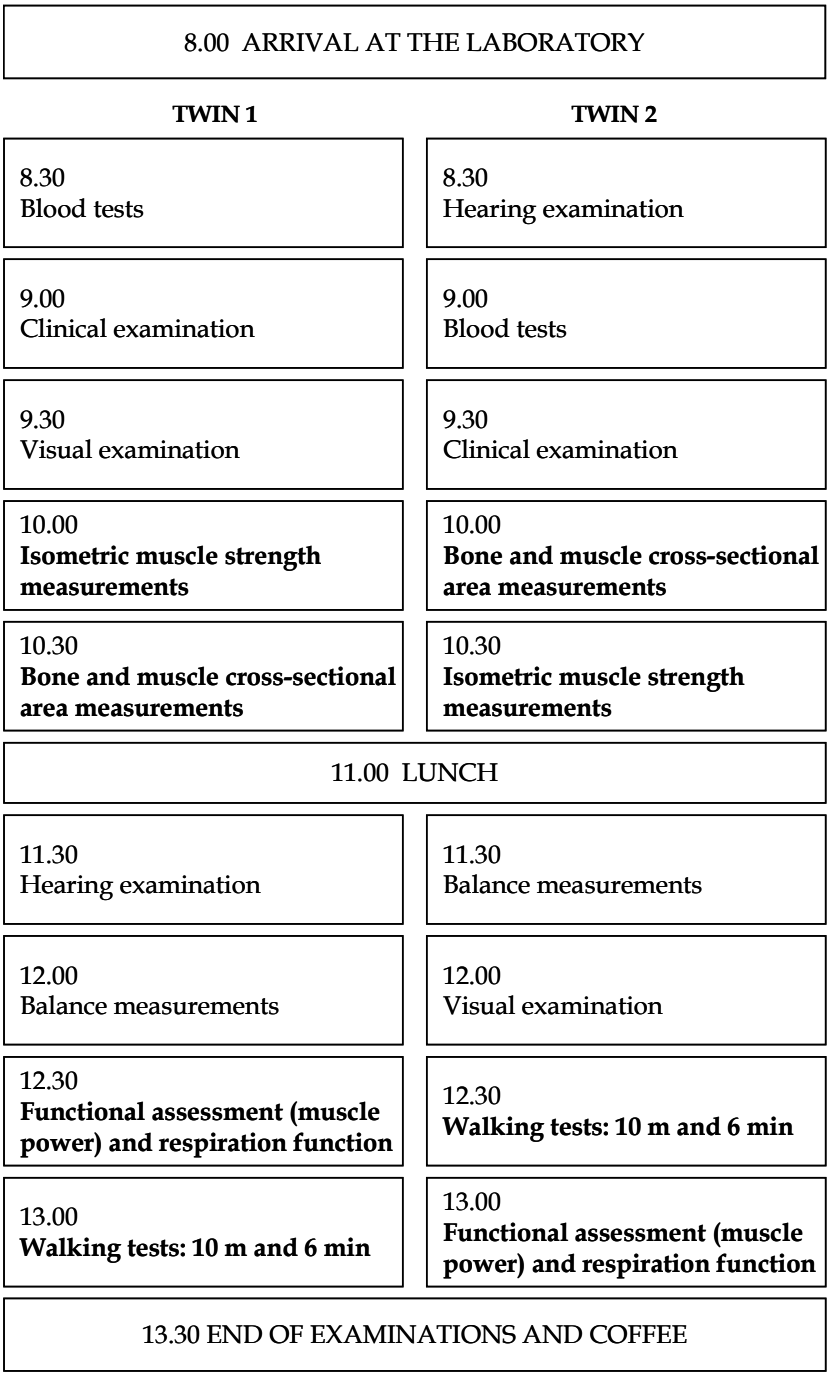


FIGURE 3 Test schedule for twin 1 and twin 2.

4.1.2 Zygosity

The zygosity was determined at the baseline study in 1975 by a validated questionnaire (Sarna et al. 1978) and confirmed in the FITSA study using DNA extracted from a venous blood sample by a battery of 10 highly polymorphic gene markers. These analyses were carried out by the Paternity Testing Laboratory of the National Public Health Institute in Helsinki, Finland. As a result of the DNA

determination four XZ twin pairs were classified as MZ and nine as DZ. After the zygosity analyses of DZ and MZ twins, one DZ twin pair was classified as MZ. After all the zygosity determinations, the final study sample of this study comprised 103 MZ and 114 DZ female twin pairs.

4.1.3 Ethical aspects

The study was approved by the ethics committee of the Central Hospital District of Central Finland. Before the laboratory examinations, the subjects were informed about the measurements and they provided a written informed consent. The informed consent contained also information about the DNA sample. This study was carried out according to good clinical and scientific practice.

4.1.4 Total numbers of pairs in different studies

The zygosity assessment from DNA was completed in May 2004. Because the zygosity assessment continued during the study period, the total number of MZ and DZ twin pairs varied between studies (Table 1). Studies I and II were written up before all the DNA zygosity assessments had been completed.

TABLE 1 Total numbers of monozygotic (MZ) and dizygotic (DZ) twin pairs used in studies I-IV.

Studies	MZ pairs	DZ pairs
I	97	102
II	101	116
III	103	114
IV	-	94

4.2 Measurements

4.2.1 Isometric muscle strength

The maximal voluntary isometric muscle strength measurements were performed on the dominant side in a sitting position using an adjustable dynamometer chair (Good Strength, Metitur Ltd, Jyväskylä, Finland). The dominant side was determined as the side of the dominant hand for both the maximal isometric muscle strength and muscle power measurements. *Hand grip strength* was measured with a dynamometer fixed to the arm of the chair with the elbow flexed at 90°. *Knee extensor strength* was measured at a knee angle of 60° from full extension with the ankle fastened by a belt to a strain-gauge system. In *the ankle plantar flexion* strength measurement, the ankle was set at an angle of 90° and was fastened by a belt to a strain gauge-system. The leg was elevated to the horizontal position and the knee was set at an angle of 20° from full extension. After familiarization with the measurement procedure, three to five maximal

efforts, each separated by a one-minute rest, were conducted. During the measurements the subjects were verbally encouraged to produce their maximum. The data were digitized into Newtons (N), recorded and stored on a computer using the Good Strength software package (Metitur Ltd, Jyväskylä, Finland). For each subject the best performance with the highest value was accepted as the result. In our laboratory using these equipments, the coefficient of variation between two consecutive measurements performed two weeks apart has earlier been 6.1% for hand grip strength and 6.3% for knee extensor strength (Rantanen et al. 1997). The coefficient of variation in the ankle plantar flexion measurements in the present study was 15.9%.

4.2.2 Leg extensor power

Leg extensor power was measured using the Nottingham Leg Extensor Power Rig, according to the published guidelines (Bassey et al. 1990), in an upright sitting position with the active leg towards the push-pedal in front of the seat. First, the dominant leg was measured, followed by the non-dominant leg. The subject was instructed to push the pedal as hard and fast as possible. Two to three practice trials were allowed for the participants to familiarize themselves with the method. Five to nine maximal efforts per leg each separated by a 30-second rests interval were conducted. The average power of each push was calculated according to published guidelines (Bassey et al. 1990) using The Leg Rig software package (University of Nottingham, Medical Faculty Workshops, Queen's Medical Centre, Nottingham, UK) and expressed in watts (W). For each subject, the best performance with the highest value was accepted as the result. In the present study, the intraclass correlation coefficient in the leg extensor power was 0.92 and the coefficient of variation was 8% between two measurements one week apart.

4.2.3 Lower leg muscle cross-sectional area

The lower leg muscle CSA on the dominant side was measured using peripheral quantitative computed tomography (XCT-2000, Stratec Medizintechnik, Pforzheim, Germany). Two millimeter thick tomography slices were taken at 55% upwards from the joint surface of the distal tibia obtained from a scout view. The whole CSA of lower leg without subcutaneous fat tissue and bones was included in the lower leg muscle CSA. The image processing and calculation of parameters were done using Bonalyse 1.3 (Bonalyse Ltd, Jyväskylä, Finland). In our laboratory the coefficient of variation in the lower leg muscle CSA measurements was 1%

4.2.4 Maximal walking speed

Maximal walking speed over 10 meters was measured in the laboratory corridor using photocells for timing. Three meters were allowed for acceleration. Participants were instructed to "walk as fast as possible, without compromising

your safety". The use of a walking aid was allowed if needed. The test was done twice and the faster performance was documented as the result. For the analyses, walking speed (m/s) was calculated by dividing the 10 meter distance by the time taken to walk it. The coefficient of variation in the maximal walking speed measurement in the present study was 5% (Pajala et al. 2005).

4.2.5 Health and medication

To ensure the safety of measurements, subjects' present health status, chronic conditions and exercise eligibility were carefully evaluated by a physician during a 30-minute clinical examination. Cardiovascular, musculoskeletal and neuromuscular status, in particular, was evaluated and attention was paid to risk factors and contraindications for the exercise and the functional performance measurements. Study physician was always available during the measurements. Self-reports of acute and chronic diseases, HRT, corticosteroid treatment and the current level of physical activity had been obtained earlier by questionnaires and was confirmed by the physician during the clinical examination.

4.2.6 Physical activity level

The participants were classified as sedentary, moderately active or active on the basis of their physical activity self-report. Physical activity was measured using the scale developed by Grimby (1986), with slight modifications. Those reporting no other activity but light walking two or fewer times a week, were rated as *sedentary*. Those reporting walking or other light exercise at least three times a week, but no exercise more intensive than that, were rated as *moderately active*. If a participant reported moderate or vigorous exercise at least three times per week she was rated as *active*.

4.2.7 Genotyping

DNA was extracted from EDTA- anticoagulated whole blood according to standard procedures. In linkage analysis 397 microsatellite markers, spaced on average 10.0 centimorgans (cM) apart, were genotyped on all 22 autosomes and X chromosome. The marker set used was the ABI PRISM Linkage mapping Set MD10 (Applied Biosystems). Standard PCR-protocols were used for the amplification of fragments using 10 ng of genomic DNA as a template. The program PEDCHECK (O'Connell & Weeks 1998) was used to detect genotypes violating the Mendelian segregation of alleles.

4.3 Statistical methods

4.3.1 General statistical methods

Descriptive statistics were obtained using the Mx program, version 1.52a (Neale & Cardon 1992, Neale et al. 2003), Stata (Stata Corporation, 2001) and SPSS (2001) softwares before the quantitative genetic modelling and linkage analyses. The normality of data was checked, equality of the means and variances between the MZ and DZ twins was tested and age and weight-adjusted intraclass correlation coefficients were calculated. The level of statistical significance was set at $p < 0.05$.

4.3.2 Genetic modeling (Studies I, II, and III)

Univariate genetic analysis (Studies I, II, and III). To quantify the genetic and environmental contributions to each of the skeletal muscle characteristics and maximal walking speed univariate genetic analysis was used. The possible combinations of the variance components A, D, C and E in the genetic models are ACE, ADE, AE, CE, DE and E. Non-additive genetic effects and shared environmental effects cannot be estimated simultaneously when only data on twin pairs raised together are available (Neale & Cardon 1992).

The expected correlation between additive genetic effects in MZ and DZ twins is 1.0 and 0.5, respectively. The expected correlation between non-additive genetic effects is 1.0 and 0.25. For shared environmental effects the correlation is 1.0 and for non-shared environmental effects zero for both MZ and DZ twins (Figure 4, Neale & Cardon 1992). In genetic modeling, the aim is to build up a model which fits the data well with the least possible explanatory components.

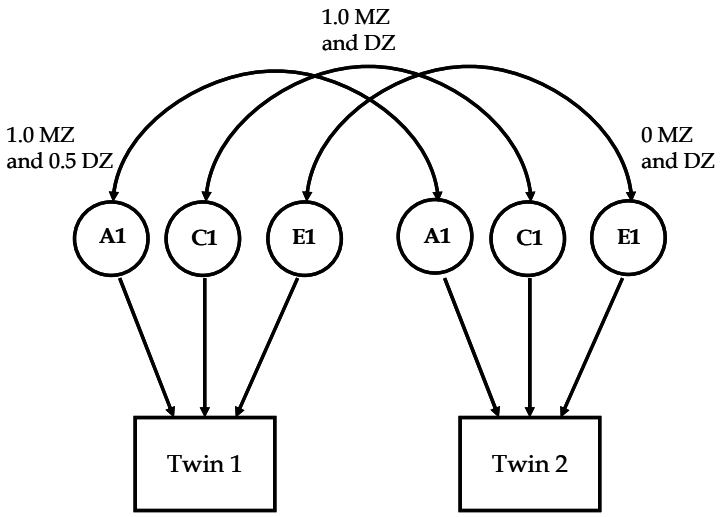


FIGURE 4 Path diagram of univariate ACE model with additive genetic effect (A), shared environmental effect (C) and non-shared environmental effect (E). Correlations between A, C and E for monozygotic (MZ) and dizygotic (DZ) twins are also shown in the figure.

Cholesky decomposition model (Studies II and III). To evaluate whether two different muscle contractions share a genetic component or whether the genetic effects are specific for each measurement, a bivariate Cholesky decomposition model was used. Cholesky modeling was also used to evaluate whether isometric knee extensor strength and maximal walking speed as well as leg extensor power and maximal walking speed share a genetic component in common. In such an ACE model (Figure 5), genetic effect A_1 is shared by both observed traits, while genetic effect A_2 loads only onto one of the traits. The shared environmental (C_1, C_2) and non-shared (E_1, E_2) environmental effects have similar patterns of loadings. In the present study, the analysis was started with the hypothetic full ACE model including all the plausible parameters. To obtain a more parsimonious model, the full model was modified by dropping the weakest (i.e. parameter estimate zero or very small) non-significant parameters one at a time, until the model with the best fit was reached. Biological plausibility was also used as a guideline in selecting the parameters to be dropped (Neale & Cardon 1992, Neale et al. 2003).

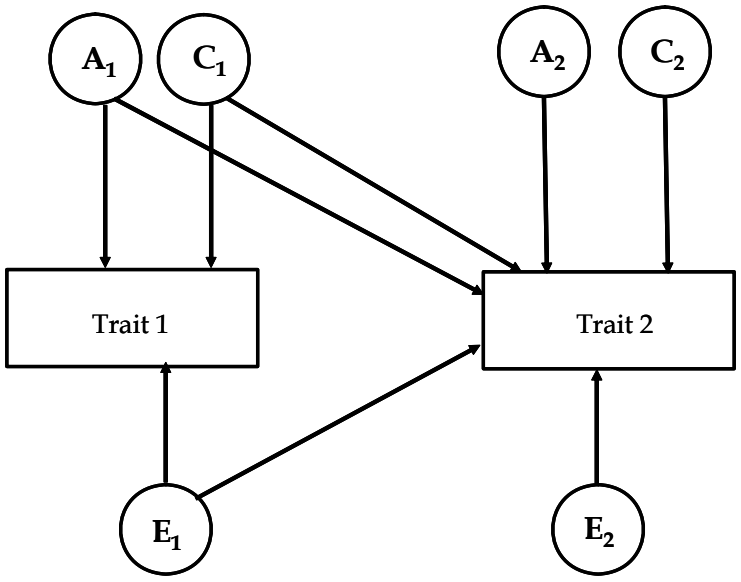


FIGURE 5 The full Cholesky decomposition ACE model with additive genetic effects (A_1, A_2), shared environmental effects (C_1, C_2) and non-shared environmental effects (E_1, E_2).

Independent pathway model (Studies I and III). An independent pathway model (Figure 6) was used to evaluate whether 1) isometric hand grip, knee extension and ankle plantar flexion strength or 2) isometric knee extensor strength, leg extensor power and maximal walking speed share a genetic component or whether the genetic effect is specific for each trait, and to what extent the effects in each case are common or trait-specific. The full independent pathway model consists of genetic and environmental effects which are common to all the observed traits (Acom, Ccom, Ecom) as well as genetic ($Aspe_1$, $Aspe_2$, $Aspe_3$), shared environmental ($Cspe_1$, $Cspe_2$, $Cspe_3$), and non-shared environmental

($Espe_1$, $Espe_2$, $Espe_3$) effects, which are specific for each measure. The analysis was started with the hypothetic full independent pathway model. To obtain a more parsimonious model the full model was modified by dropping the weakest (i.e. parameter estimate zero or very small) non-significant parameters one at the time, until the model with the best fit was reached. Quantitative genetic modeling was carried out using the Mx program, version 1.52a (Neale & Cardon 1992, Neale et al. 2003).

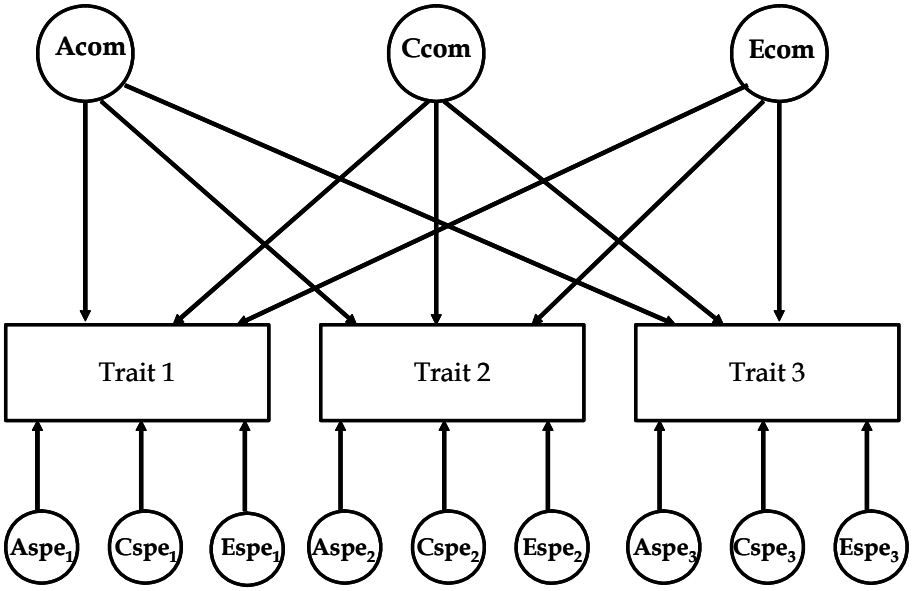


FIGURE 6 The full independent pathway model for three traits with additive genetic effect (Acom), shared environmental effect (Ccom) and non-shared environmental effect (Ecom) in common. In addition, traits have specific additive genetic effects ($Aspe_1$, $Aspe_2$, $Aspe_3$), shared environmental effects ($Cspe_1$, $Cspe_2$, $Cspe_3$) and non-shred environmental effects ($Espe_1$, $Espe_2$, $Espe_3$).

4.3.3 Genome-wide linkage analysis (Study IV)

Multipoint linkage analysis for skeletal muscle characteristics and maximal walking speed were done using the variance-components models implemented in MERLIN (Abecasis et al. 2002), using age as a covariate. In MERLIN the phenotypic variance that is explained by the estimated identity-by-descent is shared at a chromosomal position. The analysis assumed joint multivariate normality of phenotypic values, additive genetic effects and no interaction between genes and the residuals.

5 RESULTS

5.1 Characteristics of participants

Table 2 summarizes the physical characteristics of 206 MZ and 228 DZ twin individuals. No statistically significant differences between the MZ and DZ twin individuals were observed in means or variances of age, body height or weight, or body mass index (BMI).

In all twins the prevalence of knee (29%), hip (14%) or foot and ankle osteoarthritis (12%), coronary heart disease (12%), asthma (8%), cerebrovascular disease (7%) or diabetes (6%) did not differ systematically between MZ and DZ twins. Altogether, 22% of the subjects were HRT users and 4% corticosteroid users. Twenty-eight per cent were classified as sedentary, 50% as moderately active and 22% as active on the basis of a self-reported physical activity questionnaire. Neither the use of medication nor the level of current physical activity differed on average between the MZ and DZ twins.

TABLE 2 Means and standard deviations (SD) for physical characteristic in monozygotic (MZ) and dizygotic (DZ) twin individuals.

	MZ individuals n= 206		DZ individuals n= 228		Equality of means
Variables	mean	SD	mean	SD	p-value
Age (years)	68.3	3.7	68.9	3.1	0.25
Weight (kg)	69.6	11.8	70.6	12.2	0.48
Height (cm)	158.0	6.4	159.1	5.8	0.20
BMI (kg/m^2)	28.0	4.8	28.0	4.7	0.94

BMI, body mass index

Means and standard deviations for isometric muscle strength, leg extensor power and maximal walking speed did not differ significantly between MZ and DZ twin individuals (Table 3). Age explained on average 2% of the total vari-

ance in isometric muscle strength, 4% in lower leg muscle CSA and 5% in leg extensor power and maximal walking speed.

TABLE 3 Means and standard deviations (SD) for skeletal muscle characteristics and maximal walking speed in monozygotic (MZ) and dizygotic (DZ) twin individuals.

	MZ individuals			DZ individuals			Equality of means
Measurements	n	mean	SD	n	mean	SD	p-value
Isometric muscle strength (N)							
Hand grip	206	189.7	61.4	228	191.6	52.5	0.78
Knee extensor	199	296.3	81.0	217	287.0	85.6	0.34
Ankle plantar flexion	199	221.7	86.5	210	217.2	81.6	0.66
Leg extensor power (W)	199	102.9	36.0	220	97.7	32.6	0.21
Lower leg muscle CSA (cm^2)	199	60.1.	8.9.	216	60.0.	9.5	0.97
Maximal walking speed (m/s)	199	1.7	0.4	219	1.7	0.3	0.40

CSA, cross-sectional area

5.2 Genetic and environmental effects on skeletal muscle characteristics and maximal walking speed (Studies I, II, and IV)

Table 4 indicates intraclass correlation coefficients for muscle characteristics and maximal walking speed. The age-adjusted intraclass correlation coefficient for lower leg muscle CSA was at least twice as high in the MZ twins than in the DZ twins, which suggested the contribution of additive genetic effects. The age and weight-adjusted intraclass correlation coefficient for isometric knee extensor strength was almost twice as high than the correlation for the DZ twins, which suggested also the contribution of additive genetic effects. Greater age and weight-adjusted intraclass correlation coefficients were also observed in the MZ compared to DZ twin pairs for the other measured muscle characteristics and maximal walking speed.

TABLE 4 Age and weight-adjusted intraclass correlations coefficients (95% confidence intervals) for skeletal muscle characteristics and maximal walking speed in monozygotic (MZ) and dizygotic (DZ) twin pairs.

Variable	MZ	DZ
Isometric hand grip strength	0.428 (0.270-0.586)	0.364 (0.204-0.524)
Isometric knee extensor strength	0.490 (0.324-0.627)	0.275 (0.089-0.442)
Isometric ankle plantar flexion strength	0.528 (0.384-0.671)	0.455 (0.300-0.609)
Lower leg muscle CSA	0.722 (0.627-0.817)*	0.323 (0.151-0.495)*
Leg extensor power	0.622 (0.483-0.730)	0.395 (0.224-0.542)
Maximal walking speed	0.622 (0.483-0.730)	0.395 (0.224-0.542)

*Age-adjusted intraclass correlation
CSA, cross-sectional area

Table 5 shows the standardized estimates of the univariate genetic models for the most acceptable model of the measured phenotypes. In the univariate genetic modelling for isometric hand grip and ankle plantar flexion strength, the CE model showed the best fit for the data. For isometric knee extensor strength and lean tissue CSA, the most parsimonious model was the AE model. For leg extensor power and maximal walking speed, the most acceptable model was the ACE model.

TABLE 5 Results of the most acceptable univariate genetic models for skeletal muscle characteristics and maximal walking speed.

	Standardized estimates (95% CI)		
Variables	a^2	c^2	e^2
Isometric hand grip strength		40 (27–51)	60 (49–73)
Isometric knee extensor strength	49 (34–61)	-	51 (39–67)
Isometric ankle plantar flexion strength	-	47 (36-58)	53 (42-64)
Leg extensor power	30 (0-66)	29 (0-57)	41 (31-54)
Lower leg muscle CSA	75 (58-96)	-	25 (19-35)
Maximal walking speed	15 (0-51)	44 (1-63)	41 (31-54)

a, additive genetic effects
c, shared environmental effects
e, non-shared environmental effects

In the final independent pathway model for hand grip, knee extensor and ankle plantar flexion strength, an additive genetic effect in common (Acom) accounted for 14% of the total variance in hand grip strength and 31% of the total variance in knee extensor strength (Figure 7). A shared environmental effect in common (Ccom) explained 48% of the variance in ankle plantar flexion strength, 10% in hand grip strength and 12% in knee extensor strength. The non-shared environmental effect in common (Ecom) accounted for 6-23% of the total variance in ankle plantar flexion, hand grip and knee extensor strength. In addition, each strength measure had its own specific non-shared environmental effect ($Espe_1$, $Espe_2$, $Espe_3$), that explained 29-52% of the total variance.

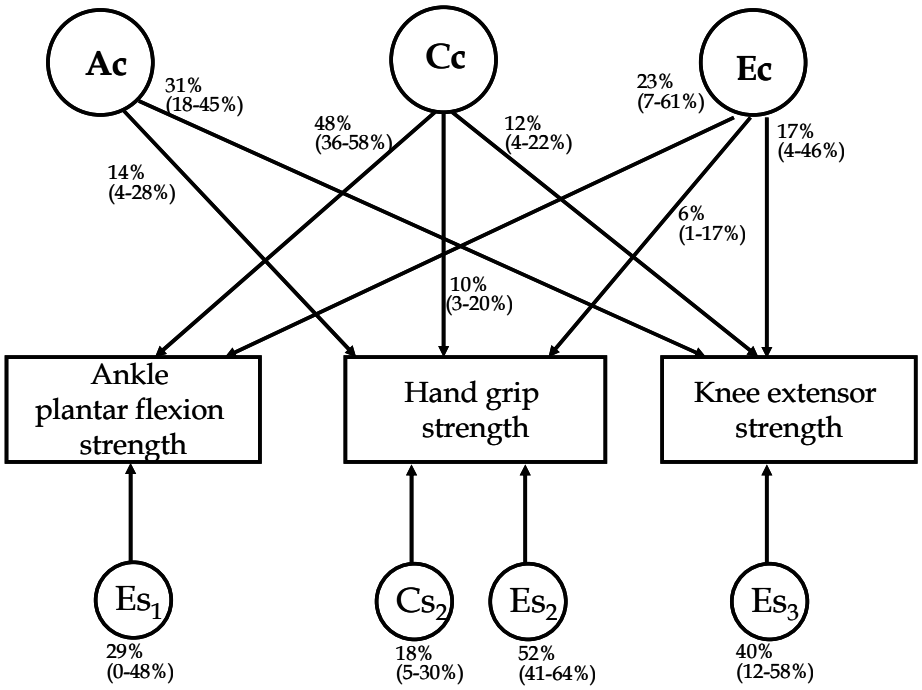


FIGURE 7 Reduced ACE model for maximal isometric ankle plantar flexion, hand grip and knee extensor strength among older female twins. The percentages (95% confidence interval) are proportions of common additive genetic (Acom), shared environmental (Ccom) and non-shared environmental effects (Ecom). In addition, the model includes a specific shared environmental effect for hand grip strength ($Cspe_2$) and specific non-shared environmental effects for ankle plantar flexion, hand grip and knee extensor strength ($Espe_1$, $Espe_2$, $Espe_3$).

5.3 Shared genetic and environmental effects on isometric knee extensor strength and leg extensor power (Study II) and isometric knee extensor strength, leg extensor power and maximal walking speed (Study III)

In the final reduced ACE model for isometric knee extensor strength and leg extensor power (Figure 8), strength and power had an additive genetic effect in common (A_1) explaining 48% of the total variance in strength and 32% in power. These two muscle contractions also had a non-shared environmental effect in common (E_1), which accounted for 52% of the total variance in strength and 4% in power. Leg extensor power had also a specific shared environmental effect (C_2) explaining 28% and a non-shared environmental effect explaining 36% of the total variance in power.

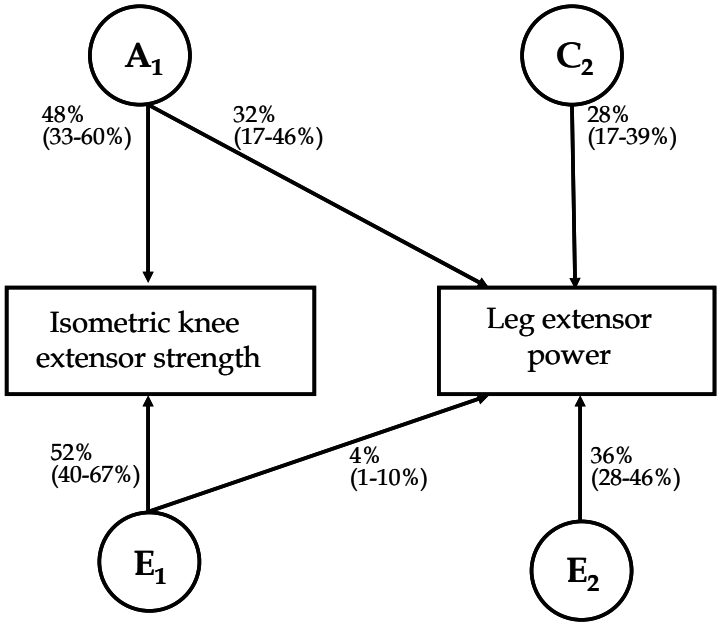


FIGURE 8 The most parsimonious Cholesky decomposition model for isometric knee extensor strength and leg extensor power consists of an additive genetic effect (A_1) and a non-shared environmental effect (E_1) in common. In addition, leg extensor power has its own shared environmental effect (C_2) and non-shared environmental effect (E_2). The percentages (95% confidence intervals) are the proportions of genetic and environmental effects.

To examine whether maximal walking speed and isometric knee extensor strength and maximal walking speed and leg extensor power share genetic or environmental effects in common we used bivariate genetic modeling. In the bivariate model the reduced ACE model was selected as the most parsimonious and theoretically acceptable model for strength and walking speed as well as for power and walking speed (Table 6, Figure 9).

TABLE 6 Results of the bivariate genetic analyses for isometric knee extensor strength, leg extensor power and maximal walking speed.

	Model fit		Model comparison		
Model	-2LL	df	Δ-2LL	Δdf	p-value
Isometric knee extensor strength and maximal walking speed					
ACE	4933.90	817			
AE	4863.33	822	70.57	5	<0.01
CE	4940.57	820	77.23	3	<0.01
Reduced ACE	4935.67	821	3.67	4	0.45
Leg extensor power and maximal walking speed					
ACE	4207.46	820			
AE	4217.96	823	10.50	3	0.02
CE	4210.73	823	3.26	3	0.35
Reduced ACE	4209.93	823	2.47	3	0.29

A, additive genetic effects; C, shared environmental effects; E, non-shared environmental effects
-2LL, -2 times log-likelihood
Δ-2LL, differences in -2LL compared with ACE-model
df, degree of freedom
Δdf, differences in df compared with ACE-model

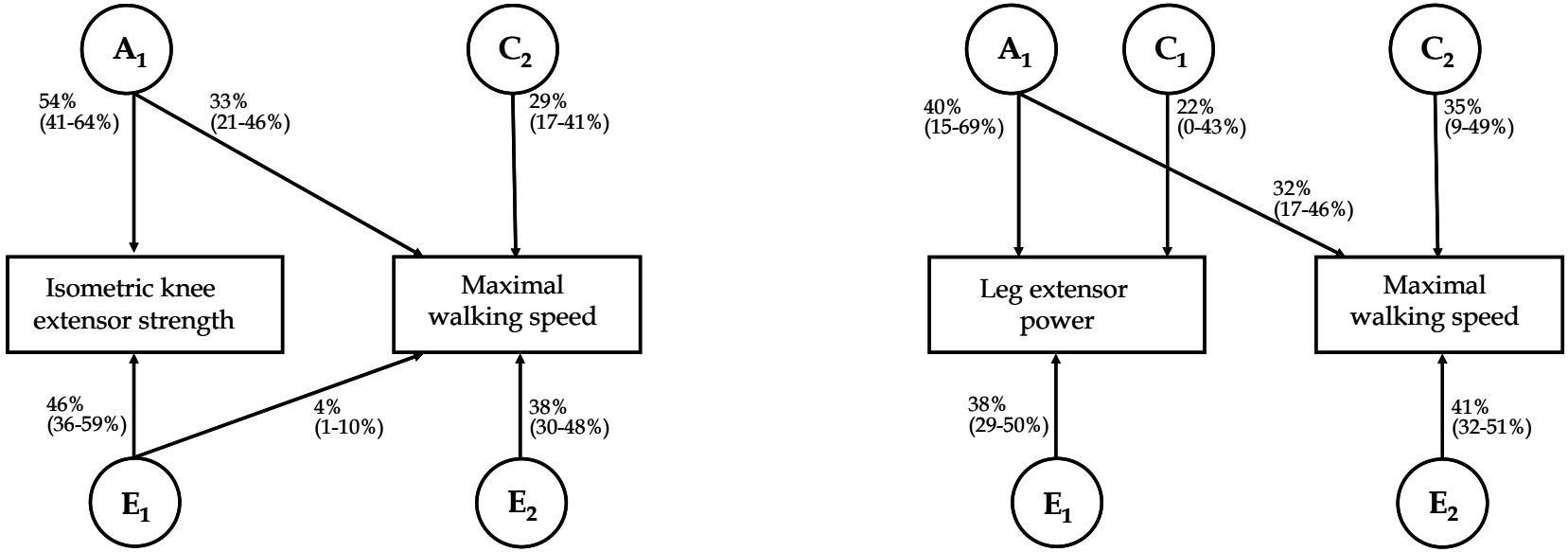


FIGURE 9 The most parsimonious Cholesky decomposition models for isometric knee extensor strength and maximal walking speed and for leg extensor power and maximal walking speed among older female twins. Both models consist of a genetic effect in common (A_1) for muscle characteristics and walking speed. The remaining variance in both models was accounted for by trait-specific environmental (C_1, C_2, E_1, E_2) effects. The percentages (95 % confidence intervals) are the proportions of genetic and environmental effects.

Next, all three traits were modeled together. In the trivariate genetic modeling, isometric knee extensor strength, leg extensor power and maximal walking speed had an additive genetic effect in common (Acom) which accounted for 52% of the total variance in strength, 36% in power and 34% in walking speed (Figure 10). Strength and power had also a non-shared environmental effect in common (Ecom) explaining 13% of the total variance in strength and 14% in power. The remaining variance was accounted for by trait-specific genetic and environmental effects. The estimates for a specific shared environmental effect ($Cspe_2$) or a specific genetic effect ($Aspe_2$) of leg extensor power was no longer statistically significant; however, either one, but not both, could be dropped from the model, indicating the presence of trait-specific familial effects on leg extensor power.

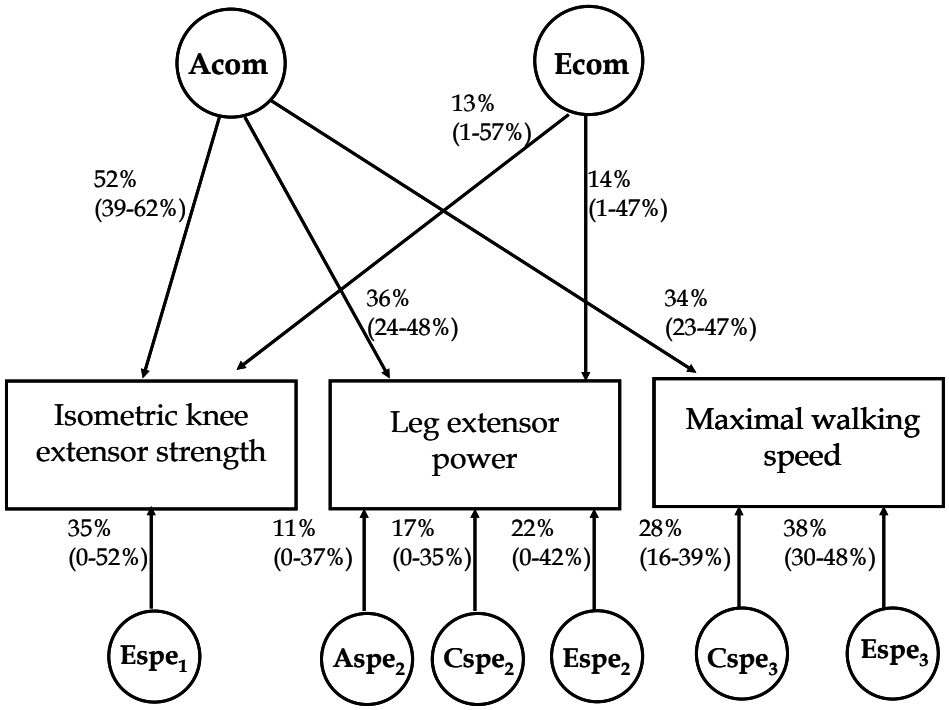


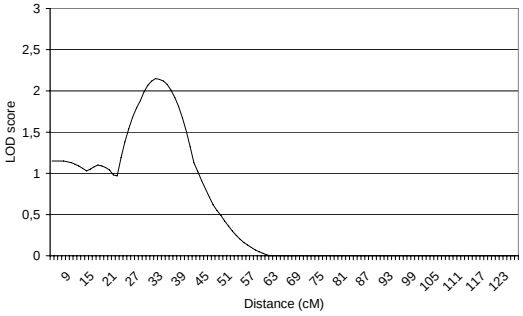
FIGURE 10 Reduced ACE model for maximal isometric knee extensor strength, leg extensor power and maximal walking speed among older female twins. The percentages (95 % confidence intervals) are the proportions of common additive genetic (Acom) and non-shared environmental effects (Ecom). In addition, the model includes trait-specific genetic ($Aspe_2$) and environmental ($Cspe_2$, $Cspe_3$, $Espe_1$, $Espe_2$, $Espe_3$) effects.

5.4 Genome-wide linkage analysis for skeletal muscle characteristics and maximal walking speed (Study IV)

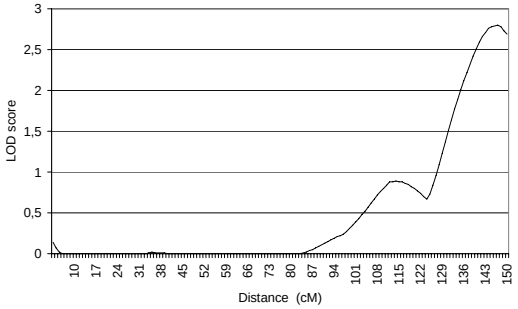
The suggestive evidence for linkage to isometric knee extensor strength was observed on chromosome 15q14 (LOD score= 2.15, Figure 11a), for leg extensor power on chromosome 8q24.22 (LOD score= 2.80, Figure 11b), for lower leg

muscle CSA on chromosome 20q13.13 (LOD score= 2.88, Figure 11c) and chromosome 9q34.11 (LOD score= 2.68, Figure 11d) and for maximal walking speed on chromosome 13q21.31 (LOD score= 2.30, Figure 11e). The phenotypes are known to be multifactorial and, expectedly, the LOD scores remained fairly low.

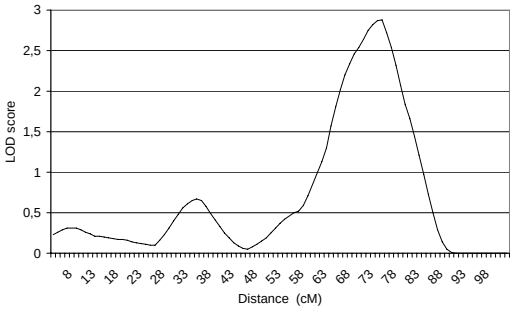
a) Isometric knee extensor strength:
Chromosome 15



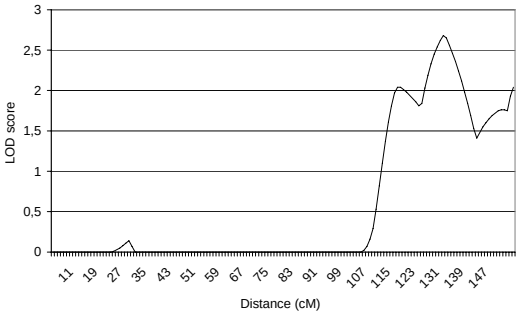
b) Leg extensor power:
Chromosome 8



c) Lower leg muscle CSA:
Chromosome 20



d) Lower leg muscle CSA:
Chromosome 9



e) Maximal walking speed:
Chromosome 13

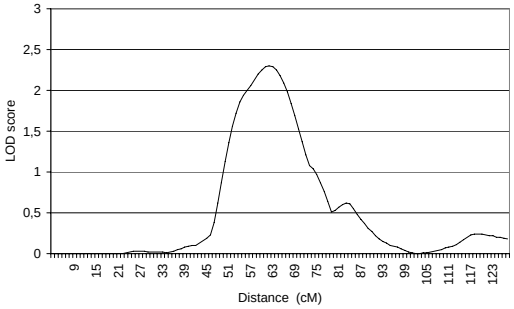


FIGURE 11 LOD scores for genome-wide linkage analysis for a) maximal walking speed on chromosome 13, b) isometric knee extensor strength on chromosome 15, c) leg extensor power on chromosome 8, and d) lower leg muscle CSA on chromosome 20 and e) on chromosome 9. X-axis indicates distance in cM from pter and y-axis indicates LOD score values.

The chromosomal areas of suggestive linkage to isometric muscle strength and leg extensor power were close each other on chromosome 15. LOD scores higher than 1.0 were seen both for isometric muscle strength and leg extensor power on chromosome 15 on the area containing 7.82-26.82 cM from pter.

6 DISCUSSION

This study examined genetic and environmental effects on skeletal muscle characteristics and maximal walking speed in older female twins using quantitative genetic modeling. In addition, genome-wide linkage analysis was used to identify chromosomal areas which have an effect on skeletal muscle characteristics and maximal walking speed and whether these regions are the same for these traits.

Genetic effects accounted for 20-30% of the variance in isometric muscle strength, leg extensor power and maximal walking speed and, 75% of the variance in CSA among healthy women 63 to 76 years of age, while the remaining variance was due to environmental effects. The maximal isometric muscle strength of different muscle groups shared a genetic effect in common, indicating that they are at least partly regulated by the same genes. The common genetic effect for leg extensor power and isometric knee extensor strength suggested that these different muscle contractions are partly regulated by the same genes. Isometric knee extensor strength, leg extensor power and maximal walking speed also had a genetic effect in common which accounted for 52% of the variance in strength, 36% in power and 34% in walking speed. The genome-wide linkage analysis showed suggestive evidence for linkage to isometric muscle strength on chromosome 15, for leg extensor power on chromosome 8, for lower leg muscle CSA on chromosomes 20 and 9 and for maximal walking speed on chromosome 13. As far as we know, this is the first study to examine genetic and environmental effects in common for physiological traits, such as muscle strength and power and a functional trait, such as walking speed by quantitative genetic modeling. This study was also the first to identify the chromosomal areas related to the three skeletal muscle characteristics and walking speed from the whole genome.

6.1 Genetic and environmental effects on muscle characteristics and maximal walking speed

The topic of the present study, muscle strength and walking speed in older women, is important. Skeletal muscle plays an essential role in many biological functions, including movement and metabolism. Adequate muscle strength and the ability to walk are important to maintain an independent life with aging. In particular, women at the age of 50-60 have a significant menopause related decline in muscle mass and strength. The subjects of the present study were women 63-76 years of age. Older women form a risk group for sarcopenia and further disability. Walking speed shows the level of present functional status, but changes in walking speed also predict future functional limitation and disability (Guralnik et al. 2000, Onder et al. 2005).

Recent studies (Reed et al. 1991, Arden & Spector 1997, Carmelli & Reed 2000, Frederiksen et al. 2002) have shown that the heritability of isometric muscle strength, mainly measured in hand grip, among older twins has varied between 22-52%. In the present study among women aged 63-76 years, the heritability of hand grip strength (14%) was more moderate compared with that found in earlier studies. Because hand grip strength has been widely used as an indicator of the strength phenotype, data on the heritability of the lower extremities are rather limited. In earlier studies (Pérusse et al. 1987, Arden & Spector 1997) one third to one half of the interindividual variation in muscle strength and muscle power has been accounted for by genetic effects. The heritability of isometric knee extensor strength and leg extensor power observed in the present study was on a similar level. Differences between results of earlier studies and the present study may be explained in part by differences in environmental variability, methods of measuring, study samples and modeling approaches. The subjects of the present study were also relatively healthy, independent older women. Diseases may influence heritability estimates. In a study among 1 757 Danish twin pairs aged 45-96 years, the age and sex adjusted heritability of hand grip strength was 0.52 (Frederiksen et al. 2002). When individuals with diseases were excluded, heritability increased to 0.62. In the present study participants were relatively healthy, thus diseases did not have a major effect on heritability estimates.

Quantitative genetic modeling can not specify the factors behind the observed genetic or environmental effects. However, on the basis of earlier studies, suggestions may be made about factors that might be included in genetic and environmental effects. For example, there is an association between muscle force production and muscle mass (e.g. Bamman et al. 1999, Izquierdo et al. 1999, Goodpaster et al. 2001, Newman et al. 2003a). For muscle CSA, 66% to 92% of the variance was explained by genetic effects (Loos et al. 1997, Thomis et al. 1997). In lean body mass, which predominantly consists of skeletal muscle, genetic effects have been reported to account for 52% to 84% of the variance among older female twins (Arden & Spector, 1997, Nguyen et al. 1998). For an-

thropometric factors, genetic effects accounted for 70-80% of the variance in height (Silventoinen et al. 2000, Silventoinen et al. 2003) and 58-78% of the variance in BMI (Stunkard et al. 1990, Schousboe et al. 2004). It is thus plausible that anthropometric factors could form part of the common genetic effect for strength, power and walking speed. However, in the present study, anthropometric factors did not play a significant role because their effect was largely controlled for by using standardized measurement positions and by taking body weight into account as a covariate in some of the statistical analyses.

The finding of a common genetic background for isometric knee extensor strength and leg extensor power as well as two muscle contractions and walking speed could also be explained by the influence of neural factors on both muscle contractions. There is some evidence to show that neural factors regulating physical movements are at least moderately regulated by genes (Simonen et al. 1998). Also, diseases with genetic predisposition, such as osteoarthritis and diabetes, may partly explain the common genetic effect. Genetic effects accounted for 39-65% of the variance in the prevalence of osteoarthritis in older women (Kaprio et al. 1996, Spector et al. 1996). Type 2 diabetes (Kaprio et al. 1992) and asthma (Nieminen et al. 1991, Räsänen et al. 1997) are also diseases with a significant genetic predisposition. Genetic factors also have an effect on blood pressure levels (Hernelahti et al. 2004, Hottenga et al. 2006). These diseases cause structural and functional changes in body functions, including skeletal muscle, thereby increasing the risk of functional limitation and disability. In the present study, the most common self-reported diseases were osteoarthritis of the knee (29%), the hip (14%) and foot and ankle (12%), heart diseases (12%) and asthma (8%). It is also possible that some people had diseases, not yet diagnosed but already affecting muscle characteristics and functional performance, which may contribute to the genetic component.

A physically active lifestyle is often adopted in childhood. Family members often resemble each other so that either active or sedentary life styles accumulate in the same families (Pérusse et al. 1989, Simonen et al. 2004). The adoption of a physically active lifestyle in childhood influences the level of physical activity throughout the lifespan even into older age (Hirvensalo et al. 2000). In childhood, individual differences in physical activity level and sports participation were largely accounted for by shared environmental effects whereas genetic effects had no influence on them (Franks et al. 2005, Stubbe et al. 2005). The impact of genetic effects increases in adulthood. In the study by Stubbe et al. (2005) among male and female twins 13-16 years of age, shared environmental effects accounted for the main part of the interindividual differences in sports participation. At the age of 17-18, the contribution of environmental effects declined and genetic effects accounted for 36% of the variance, while after the age of 18 years the proportion of genetic effects was 85%. In the study by Simonen et al (2004) genetic effects accounted for half of the variation in the level of adulthood exercise among male twins aged 35-70 years. Characteristics which make it either easy or inconvenient to exercise are partly genetically determined (Wolfarth et al. 2005). Some people may be more interested in

physical activity and training because their inherited physical characteristics make it easier for them to exercise than for the others.

6.2 Genome-wide linkage analysis for muscle characteristics and maximal walking speed

In whole genomic searches, assumptions about the candidacy of particular genes or regions are not made. Instead, these analyses are a true search for genetic effects along chromosomes. In the present study, chromosomal areas for muscle characteristics with LOD scores of 2.0 and higher were found on chromosomes 8, 9, 15 and 20. Earlier studies have shown linkage for maximal oxygen uptake (Bouchard et al. 2000) and plasma cholesterol levels (Feitosa et al. 2005) on chromosome 8q24. Chagnon et al. (2001) found a suggestive linkage for BMI on chromosome 9q34. In the present study, partly in the same areas on chromosome 8 and 9, the LOD scores 2.0 or higher for leg extensor power and lean tissue CSA were found. These findings suggest that these chromosomal areas might harbor genes related to muscle phenotypes. Within the specified areas on chromosomes 8, 9, and 20 are located some genes related to the ubiquitination/deubiquitination of proteins, which is an essential regulatory mechanism in the intracellular degradation of proteins. The balance between protein synthesis and protein degradation pathways controls the maintenance of muscle mass. The levels of ubiquitinated proteins are known to increase in atrophying muscle (Wing 2005). Outside the protein synthesis machinery the ubiquitination/deubiquitination of proteins have an important contribution to many other biological processes, including cell cycle progression, DNA repair, organelle biogenesis, vesicular trafficking, transcriptional activation and signal transduction (D'Andrea & Pellman 1998, Quesada et al. 2004). Additionally, several extracellular matrix genes and transcription factor genes harbor chromosomes 8, 9 and 20. On chromosome 15, the area with LOD scores of 2.0 and higher for isometric muscle strength contains e.g. genes related to signal transduction and the function of synapses and neurons, and are expressed both in the cells of the central nervous system and in muscle tissue itself (LeBeau et al. 2003, Nonami et al. 2005). On that chromosomal area are also localized nicotinic receptor genes and other genes which may have important role in the spinal cord and skeletal muscle during early developmental periods (Keiger et al. 2003). These receptors may also have a role in the central regulation of autonomic functions (Tribollet et al. 2001).

The highest LOD score for walking speed was found on chromosome 13. This chromosomal area harbors genes involved in the regulation of synapse formation as well as synapse dynamics and signaling via synapses. Some of these genes are highly expressed in the cells of the central nervous system (Angst et al. 2001, Junghans et al. 2005). On that chromosomal area are localized, for example, several cadherin superfamily genes which are important fac-

tors also in many other biological processes. Cadherins are transmembrane proteins, which have an important role in cell-cell adhesion and synaptic functions. Several cadherins have been implicated in regulation of myogenesis, the differentiation of skeletal muscle (Yagi & Takeichi 2000, Angst et al. 2001, Kang et al. 2003, Krauss et al. 2005)

Even though quantitative genetic modeling showed an additive genetic effect in common for muscle strength, power and walking speed, the results of the genome-wide linkage analysis did not show chromosomal areas in common for these traits. For strength and power we observed an overlapping area on chromosome 15. However, the LOD scores in this area were not especially high. This could be due to the examined traits which may be affected by several, and not necessarily the same genes.

In their reviews Wolfarth et al. (2005) and Beunen & Thomis (2004) summarized the candidate genes for muscle characteristics found in association studies.These genes are located on chromosomes 2 (GDF8), 11 (IGF2, CNTF), 12 (VDR) and 17 (COL1A1, angiotensin converting enzyme). Earlier studies have not suggested candidate genes for walking ability or walking speed. In the present study, the highest LOD scores did not match chromosomal areas where candidate genes for muscle characteristics are located. However, in the earlier candidate gene studies the associations were relatively weak so it is not surprising that the present study did not provide evidence of linkage in these loci. The reasons for differences between the results may be due to the nature of muscle characteristics and walking speed, and limited statistical power. The examined traits are multifactorial and consequently affected by multiple genes. It is also possible that different genes play a role in muscle strength determination in men than women (Peeters et al. 2005), which may influence the results of the present analysis

6.3 Methodological considerations

In the present study, the results of the univariate and multivariate analyses differed somewhat. The univariate analyses were considered preliminary analyses, and served as the basis for the multivariate analysis. The difference between the results of the univariate and multivariate genetic analyses may be due to low statistical power in the univariate analyses to discriminate genetic effects from the shared environmental effects. Multivariate analysis takes into account all the information available, and thereby increases the statistical power of the analysis and improves the reliability of the results. However, if the requirements of twin analysis are strictly applied, the size of the present sample may be insufficient to discriminate genetic effects from shared environmental effects, which might be seen in our results. The proportions of genetic and environmental effects varied to some extent between the different univariate and multivariate analyses. For isometric knee extensor strength, the proportion of genetic effects varied between 31% and 54%. For leg extensor power, the propor-

tion of genetic effects varied between 30% and 47% and for maximal walking speed between 15% and 34%. Isometric hand grip strength, ankle plantar flexion strength and lower leg muscle CSA were included in only one model. However, if the genetic effects and shared environmental effects are discussed together, as familial effects, the variation from model to model is minor, ranging between 43-52% for isometric knee extensor strength, 59-64% for leg extensor power and 59-62% for maximal walking speed.

The number of the MZ and DZ pairs differed slightly between analyses. Even minor differences in the number of the MZ and DZ pairs included in the model may to some extent influence the results. The first article reported the results for 97 MZ and 102 DZ twin pairs. After the DNA zygosity assessment of XZ pairs, four XZ twin pairs were classified as MZ and nine as DZ. When zygosity assessments of MZ and DZ pairs were completed one DZ twin pair was ascertained to be a MZ pair. After all the zygosity assessments had been done, the final sample of this study consisted of 103 MZ and 114 DZ female twin pairs. Before the zygosity assessment was completed in May 2004, two articles had already been written. However, the changes in zygosity determination were rather small and are not likely to have had a significant effect on the results.

The requirement that both individuals of the pair had to participate may have resulted in the exclusion of twin pairs in which one or both sisters had poor health or mobility who were unable to travel to the study centre. This may have resulted in overestimation of twin similarity, which probably increased the proportion of the shared environmental effect and decreased the relative contribution of genetic effects. Future studies should include larger numbers of subjects or other types of relatives to ensure the clear identification of the relative proportions of genetic and shared environmental effects.

Although the main limitation of the present study was low sample size and hence limited statistical power, the advantage was that the sample was population-based and drawn from the relatively isolated Finnish population. An isolated population has reduced environmental and genetic heterogeneity offering a population with restricted confounders and exhibiting less genetic variability (Jorde et al. 2000, Peltonen et al. 2000). The present study connects the results of the functional performance tests with genotypic information, which could also be counted among its strengths.

A further advantage of the present study was that the tests commonly used in studies on the functional capacity of older people were done in the laboratory, in standardized circumstances and with valid and reliable methods. The measurements used in the present study to evaluate muscle strength and power and walking speed have proved to be suitable and safe methods among older persons (Arden & Spector 1997, Rantanen et al. 1997, Bassey 1998). The measurements give a reliable view of the level of the subject's present muscle strength and functional performance but also predict conceivable functional limitations and disabilities (Rantanen et al. 1998b, Bean et al. 2002, Studenski et al. 2003). The most problematic measurement, from the viewpoint of reliability, was the isometric ankle plantar flexion strength test. The coefficient of variation

measured in the present study was 15.9%. Ankle plantar flexion, as a performance, may have been a more unfamiliar movement for the participants than those involved in the other measurements, causing the greater variability in performance between repeated measurements. However, it is unlikely that this had a major effect on the results of the genetic modeling. The measurement error, which is included in the non-shared environmental effects, increases the relative contribution of the non-shared environmental effects in the model. In the present study, non-shared environmental effects did not differ among the isometric muscle strength measurements.

This study included only female twin pairs, which limits the generalizability of the results. Because heritability estimates can differ between age groups and genders, the results can only be generalized to older women. However, our results give new and important information about genetic and environmental effects on muscle strength in older women, an increasing population group. The lower level of muscle strength among women compared to men throughout the lifespan, in part explains the greater prevalence of functional disability among women (Leveille et al. 2000).

6.4 Future directions

In the future, it would be interesting to investigate longitudinally from childhood to old age what happens to the relative proportion of genetic and environmental effects with aging. This question has been discussed in only one earlier study, which found that a transition occurs in the relative proportions of genetic and environmental effects with aging among men (Carmelli & Reed 2000). The present study suggested that some people may be more prone to functional limitation in old age due to their genetic predisposition. They may be genetically predisposed to frailty or their genetic risk may have increased with advancing age or the gene-environment interaction may have exposed them to the functional limitation. To acquire knowledge of possibilities for rehabilitation and interventions to prevent functional limitations and disabilities it would be useful to investigate the contribution of the genetic component to variation in training response. The results may throw light on the role of genetic effects in explaining the variability in responses to training and help to plan more effective training interventions. Further research is also needed to confirm whether the chromosomal regions found in the present study in older Finnish women, resemble those in other populations, including younger individuals and men. Finally, fine-mapping studies to confirm the contribution of the most interesting chromosomal regions would be needed.

7 MAIN FINDINGS AND CONCLUSIONS

The main findings of the present study can be summarized as follows:

1. Genetic effects accounted on average for 20-30% of the interindividual variance in isometric muscle strength, muscle power, and maximal walking speed and 75% of the variance in lower leg muscle CSA, while the remaining variance was due to environmental effects.

2. The maximal isometric muscle strength of different muscle groups shared genetic effects in common, indicating that these phenotypes are partly regulated by the same genes.

3. The observed genetic effect in common for isometric knee extensor strength and leg extensor power indicated that these two muscle contractions phenotypes are partly regulated by the same genes.

4. Maximal isometric knee extensor strength, leg extensor power and maximal walking speed had a genetic effect in common which accounted for about one-half of the variance in strength and one-third of the variance both in power and walking speed.

5. Suggestive evidence of linkage was observed for isometric muscle strength on chromosome 15, for leg extensor power on chromosome 8, for lower leg muscle CSA on chromosomes 20 and 9 and for maximal walking speed on chromosome 13. The LOD scores higher than 1.0 were seen for isometric muscle strength and leg extensor power on chromosome 15 on the area containing 7.82-26.82 cM from pter.

In conclusion, the results of the present study suggest that some people may be more prone to functional limitation in old age due to their genetic predisposition, but this does not rule out the possibility that changes in the lifestyle of predisposed subjects may also have a major effect. Approximately half of the variance in each trait was explained by environmental effects, which indicates the importance of physical activity and other lifestyle factors in improving performance and preventing functional limitation. The study also found interesting chromosomal areas related to muscle characteristics and walking speed. Further research is needed to confirm whether the chromosomal regions found in the present study in older Finnish women resemble those in other populations, including younger individuals and men. The next step would be confirmatory fine-mapping studies of the most interesting chromosomal regions.

YHTEENVETO

Lihasvoiman ja kävelynopeuden periytyvyys iäkkäillä naiskaksosilla

Lihasvoima on yksi kävelykyvyn tärkeimmistä osatekijöistä. Parhaimmillaan lihasvoima on 30 ikävuoden kohdalla ja alkaa sen jälkeen heikentyä. Huomattavaksi lihasvoiman heikentyminen muuttuu 50–60 vuoden iässä. Naisilla on koko elämän ajan vähemmän lihasmassaa ja lihasvoimaa kuin miehillä. Sukupuolten välinen lihasmassan ja lihasvoiman ero selittää naisten suurentunutta sarkopenian riskiä, joka altistaa heidät myös lisääntyneille toimintakyvyn ongelmille, kuten kävelyvaikeuksille. Hidastunut kävelynopeus lisää toiminnanvajausten riskiä ja ennustaa toiminnanvajausten ilmaantuvuutta.

Yksilöiden väliset erot lihasvoimassa ja toimintakyvyssä selittyvät perimällä ja eroilla ympäristötekijöissä. Ympäristötekijät eivät vaikuta kaikkiin ihmisiin samalla tavalla, vaan ihmisten ominaisuuksiin vaikuttavat myös geneettiset tekijät. Kaksostutkimuksessa verrataan toisiinsa perimältään 100-prosenttisesti samanlaisia identtisiä kaksosia ja perimältään 50-prosenttisesti samanlaisia ei-identtisiä kaksosia. Identtisten kaksosten suurempi samankaltaisuus verrattuna ei-identtisiin kaksosiin on osoitus siitä, että perimä selittää yksilöiden välisiä eroja tutkittavissa ominaisuuksissa. Lihasvoiman periytyvyyttä on aikaisemmin tutkittu lähinnä mittaamalla käden puristusvoimaa, jonka yksilöiden välisistä eroista geneettiset tekijät selittävät 22–52%. Geneettisten tekijöiden selitysosuus yksilöiden välisten lihasvoimaerojen selittäjänä saattaa kuitenkin muuttua ikääntyessä niin, että ympäristötekijöiden merkitys kasvaa geneettisten tekijöiden merkityksen vähentyessä. Tutkittaessa myös ikääntyneitä pystytään selvittämään, pysyykö geneettisten tekijöiden osuus vaihtelun selittäjänä vakaana läpi elämän vai tapahtuuko siinä muutoksia.

Koko perimän kattavia kytkentäanalyysejä ei ole aikaisemmin tehty lihasmassalle, lihasvoimalle ja kävelynopeudelle. Aikaisemmat assosiaatiotutkimukset ovat viitanneet siihen, että kromosomeissa 2, 11, 12 ja 17 olisi geenejä, joilla saattaa olla yhteyttä lihasmassaan ja lihasvoimaan. Kävelynopeuteen mahdollisesti vaikuttavia perimän alueita on tutkittu huomattavasti vähemmän.

Tämän väitöskirjatutkimuksen tarkoituksena oli selvittää miten geneettiset tekijät ja ympäristötekijät selittävät 63–76-vuotiaiden naiskaksosten lihasvoimassa, voimantuottotehossa, pohkeen alueen lihasten poikkipinta-alassa ja maksimaalisessa kävelynopeudessa havaittuja yksilöiden välisiä eroja geneettisesti kontrolloidussa väestössä. Lisäksi selvitettiin mitkä kromosomaaliset alueet ovat yhteydessä lihasvoimaan, voimantuottotehoon, pohkeen alueen lihasten poikkipinta-alaan sekä kävelynopeuteen ja ovatko nämä alueet yhteisiä tutkittaville ominaisuuksille.

Tutkittavat valittiin Suomen kaksoskohorttitutkimukseen (The Finnish Twin Cohort Study) osallistuneista 63–76-vuotiaista naiskaksosista. Tutkimukseen osallistui 103 identtistä ja 114 ei-identtistä 63–76-vuotiasta naiskaksosparia.

The Finnish Twin Cohort Study -tutkimuksen alkutilanteessa vuonna 1975 tsygoottisuus määritettiin kyselylomakkeen avulla. Tsygoottisuus varmistettiin laboratoriomittausten yhteydessä DNA-näytteellä. Laboratoriossa tehtäviin mittauksiin kuuluivat maksimaalinen isometrinen lihasvoima (käden puristusvoima, polven ojennusvoima ja nilkan ojennusvoima), alaraajojen voimantuottoteho, pohkeen alueen lihasten poikkipinta-ala sekä maksimaalinen 10 metrin kävelynopeus. Geneettisten ja ympäristötekijöiden selitysosuutta selvitettiin geneettisen mallinnuksen avulla. Kytkentäanalyysiä käytettiin selvitettäessä, mitkä perimän alueet ovat yhteydessä tutkittaviin ominaisuuksiin.

Tämän tutkimuksen tulokset osoittavat, että 20–30% yksilöiden välisistä isometrisen lihasvoiman, alaraajojen voimantuottotehon ja maksimaalisen kävelynopeuden eroista selittyy geneettisillä tekijöillä. Pohkeen alueen lihasten poikkipinta-alassa havaituista yksilöiden välisistä eroista geneettiset tekijät selittävät 75%. Eri lihasryhmien tuottamalla isometrisellä lihasvoimalla ja kahdella erilaisella lihassupistustavalla, isometrisellä lihasvoimalla ja alaraajojen voimantuottoteholla, näyttäisi olevan yhteistä geneettistä taustaa. Tutkimustulokset viittaavat siihen, että osittain samat geenit vaikuttavat näihin luustolihasten ominaisuuksiin. Isometrisellä lihasvoimalla, alaraajojen voimantuottoteholla ja maksimaalisella kävelynopeudella havaittiin olevan yhteistä geneettistä taustaa, joka selitti yksilöiden välisistä eroista isometrisessä lihasvoimassa 52%, alaraajojen voimantuottotehossa 36% ja kävelynopeudessa 34%. Kytkentäanalyysi osoitti, että kromosomissa 15 on isometriseen lihasvoimaan, kromosomissa 8 alaraajojen voimantuottotehoon, kromosomeissa 20 ja 9 pohkeen alueen lihasten poikkipinta-alaan sekä kromosomissa 13 kävelynopeuteen yhteydessä olevia geenejä. Kromosomissa 15 havaittiin alue, joka oli yhteydessä sekä isometriseen lihasvoimaan että alaraajojen voimantuottotehoon, vaikkakin LOD score -arvot jäivät melko alhaisiksi.

Geneettisen mallinnuksen tulokset viittaavat siihen, että osittain samat geenit ovat yhteydessä isometriseen lihasvoimaan, voimantuottotehoon sekä maksimaaliseen kävelynopeuteen iäkkäillä naiskaksosilla. Osa väestöstä saattaa olla geneettisestä perimästään johtuen muita alttiimpia toimintakyvyn heikkenemiselle. Ympäristötekijöiden suuri selitysosuus korostaa kuitenkin edelleen fyysisen aktiivisuuden, kuntoutuksen, harjoittelun sekä muiden ympäristötekijöiden tärkeyttä fyysisen toimintakyvyn ylläpidossa ja parantamisessa iäkkäillä naisilla. Vaikka kytkentäanalyyseissä ei löytynyt viitteitä yhteisistä perimän alueista lihasvoimille ja kävelynopeudelle, tulos ei ole ristiriidassa geneettisen mallinnuksen tulosten kanssa. Tulokset vahvistavat nykykäsitystä, että lihasvoima ja kävelynopeus ovat monitekijäisiä ominaisuuksia, joihin vaikuttavat useat eri geenit. Käytetty aineisto on mitä ilmeisimmin liian pieni osoittamaan osaa kytkennöistä. Lisätutkimuksia tarvitaan tarkentamaan nyt löydettyjä mielenkiintoisia perimän alueita suuremmalla tutkimusjoukolla ja tarkemmilla analysointimenetelmillä.

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