The role of genetic variation in selected human musculoskeletal ageing traits.

Submitted by Garan Jones to the University of Exeter
as a thesis for the degree of
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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature:

Abstract

Loss of muscle mass and function, termed sarcopenia, occurs commonly with advancing age. This loss of strength can have a profound impacts on an individual's life expectancy and quality of life. Population genetic studies can provide information on underlying biological mechanisms, but little was known about the genetic contributions to sarcopenia.

By using data from multi-national community based studies of 256,523 individuals of European ancestry aged 60 years or older I have identified 15 genomic risk loci for muscle weakness with age. I have shown that the genetic contributions to muscle weakness in later life have novel characteristics not seen in studies of muscle strength at younger ages. I have also shown that for a section of the older population meeting the criteria for sarcopenia, there is a substantial auto-immune component separate from diagnosed autoimmune conditions, such as Rheumatoid arthritis. Analysis of sex-specific cohorts has highlighted that the underlying genetics contributing to muscle weakness with age differ between the sexes. Additional research on the shared pathways between age-related traits and muscle weakness with age has shown that diabetes, rheumatoid arthritis and life courses traits, for example birth weight, share at least some of the same biological pathways. Biological pathways implicated included transcription regulation, processing of misfolded proteins, cell growth and development.

In conclusion I have identified several common genetic variants associated with sarcopenia in humans, which has highlighted an autoimmune component and several shared casual pathways with traits ranging from life-course and growth traits through to later life conditions such as Rheumatoid arthritis and diabetes.

These findings should inform efforts to prevent and treat muscle loss with advancing age, and may more personalised approaches to intervention.

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List of abbreviations

AChR	Acetylcholine receptor
ALM	Appendicular Lean Mass
ASM	Appendicular Skeletal Muscle mass
AWGS	Asian Working Group for Sarcopenia
BIA	BioImpedance Analysis
BMI	Body Mass Index
CART	Classification and Regression Trees
CHD	Coronary Heart Disease
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
CSA	Cross-Sectional Area
CT	Computed Tomography
DEXA	Dual-Energy X-ray Absorptiometry
eQTL	expressed Quantitative Trait Loci
ER	Endoplasmic Reticulum
FLoSS	Family Longevity Selection Score
FUMA	Functional Mapping and Annotation of Genome-Wide Association Studies
GTEx	Genotype-Tissue Expression

GWAS	Genome-Wide Association Study
HES	Hospital Episode Statistics
HLA	Human Leukocyte Antigen
IBD	Identical By Descent
LD	Linkage Disequilibrium
MAC	Minor Allele Count
MAF	Minor Allele Frequency
MAGMA	Multi-marker Analysis of Genomic Annotation
MM	Mismatch probe
MRI	Magnetic Resonance Imaging
MSK	Musclo-Skeletal
MuSK	MUscle-Specific tyrosine Kinase
NMJ	Neuromuscular Junctions
OR	Odds Ratio
PCA	Principal Component Analysis
PM	Prefect Match probe
pQTL	protein Quantitative Trait Loci
ROS	Reactive Oxidative Stress
SASP	Senescence-Associated Secretory Phenotype
SD	Standard Deviation

SMI	Skeletal Mass Index
SMM	Skeletal muscle mass
SNP	Single Nucleotide Polymorphism
SNV	Single Nucleotide Variant
UPR	Unfolded Protein Response
WHR	Waist-Hip Ratio
YLD	Years Lived with Disability

List of study abbreviations

ARIC	Atherosclerosis Risk in Communities
AWGS	Asian Working Group on Sarcopenia
BASE-II	Berlin Aging Study II
BPROOF	B-Vitamins for the prevention of Osteoporotic fractures
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
CHS	Cardiovascular Health Study
CSHA	Canadian Study of Health and Aging
EPIC	European Prospective Investigation into Cancer and Nutrition
EWGSOP	European Working Group on Sarcopenia in Older People
FHS	Framingham Heart Study
FNIH	Foundation for the National Institutes of Health
HRS	Health Retirement Study
IWGS	International Working Group on Sarcopenia
LASA	Longitudinal Aging Study Amsterdam
LLFS	Long-Life Family Study

MrOS	Osteoporotic Fractures in Men
NHGRI	National Human Genome Research Institute
ROSMAP	Religious Orders Study and Memory and Aging Project
SAGE	Sarcopenia in Geriatric Elderly
SHIP	Study of Health in Pomerania
TSHA	Toledo Study for Healthy Aging
UKB	United Kingdom Biobank
WLS	Wisconsin Longitudinal Study

Acknowledgements

This work is dedicated to my children, Morgan and Molly, and in memory of my father.

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1 Introduction

1.1 The ageing process

Ageing has been described as the accumulation of molecular and cellular damage over time (D. Harman 1981; Denham Harman 1992). In the multicellular organisms, such as humans, this process is characterised by a gradual decrease in physical and mental capacity, an increased risk of multi-morbidity and then finally death (López-Otín et al. 2013). Benjamin Gompertz described aging as a process leading to an exponential increase in mortality over time (Gompertz 1825). The Gompertz model as the “law of mortality” has been superseded by an appreciation that other models exist across a range of different species (O. R. Jones et al. 2014), however it’s simple intrinsic message that for much of the adult human lifespan, age-specific mortality rates increase exponentially is still relevant (Kirkwood 2015).

One of the hallmarks of human ageing is the progressive loss of muscle, and the increased risk of associated musculoskeletal disorders of ageing, such as frailty. Musculoskeletal (MSK) ageing itself has been defined by Dawson et al, as comprising of four components; osteoporosis, osteoarthritis, sarcopenia and frailty (Dawson and Dennison 2016). These conditions have been shown to have a substantial impact on individual mobility, pain and disability (Leveille 2004; Blyth and Noguchi 2017). Osteoarthritis and Osteoporosis have been intensively analysed in the UK Biobank dataset (Zengini et al. 2018; Kemp et al. 2017), however lesser studied components such as sarcopenia and frailty are just as relevant to the health burden of MSK ageing in older adults and are the focus of the thesis.

Sarcopenia has been characterised as the age-related loss of skeletal muscle mass and function (Cruz-Jentoft et al. 2010, 2019). Sarcopenia, as defined by the Foundation for the National Institutes of Health criteria (grip strength less than 26 Kg in men and under 16 Kg in women) (S. A. Studenski et al. 2014) has been estimated to increase UK healthcare costs by £2.5 billion (Pinedo-Villanueva et al. 2019). In addition, sarcopenia has been associated with a higher risk of all-cause mortality (Beaudart et al. 2017) and functional decline (Beaudart et al. 2017). As increasing life expectancy results in a larger aging population, with increased risks of multi-morbidity, hospitalisation, and premature death, later life conditions such as frailty and sarcopenia have become more common and of greater impact to the health system.

In this chapter I will discuss the background to the musculoskeletal system ageing process, alongside the underlying mechanisms. I will outline the relationship between loss muscle mass and reduced function, and how these components of sarcopenia relate to frailty and health in older adults, and the methods for defining these conditions. The impact of these age related conditions will also be discussed. Finally I will outline the specific aims of my thesis, which included investigating the mechanisms of sarcopenia in older adults of European ancestry using genetics.

1.2 Sarcopenia and dynapenia

The name sarcopenia (Greek ‘sarcx’ or flesh and ‘penia’ or loss) was proposed in 1989 by Rosenberg (I. H. Rosenberg 1997). However it is only since 2016 that sarcopenia has been recognised as an independent condition by the International

Classification of Diseases, with an ICD-10 code of M62.84 (“ICD-10 Version:2019” n.d.).

The health burden of musculoskeletal disorders such as sarcopenia, rheumatoid arthritis, osteoarthritis and frailty are substantial with the conditions associated with incident disability and an increased risk of co-morbidities (Choong and Brooks 2012; Duffield et al. 2017).

Low muscle strength in mid-life has been shown to be strongly predictive of later life disability (T. Rantanen 2003) with musculoskeletal (MSK) disorders being a major cause of Years Lived with Disability (YLDs) (Vos et al. 2012), and a recognised global health burden, whose full impact is often underestimated (Hoy et al. 2015).The Milan EXPO survey highlighted that it is physical performance rather than muscle mass that decreases more rapidly with age. Individuals older than 75 years have approximately 60% of their muscle strength and 30% of their function (Francesco Landi et al. 2017).

Sarcopenic individuals over the age of 80, have a substantially higher mortality risk (HR: 2.32, 95% CI:1.01-5.43) when compared to controls without sarcopenia (F. Landi et al. 2013). In addition the economic impact of sarcopenia is substantial, with a recent estimate of the cost of hospitalisations in the US for patients with sarcopenia as $40.4 Billion annually (Goates et al. 2019).

1.2.1 European Working Group on Sarcopenia in Older People

The European Working Group on Sarcopenia in Older People (EWGSOP) published their first definition of Sarcopenia in 2010 (Cruz-Jentoft et al. 2010), followed by a revised version in 2019 (Cruz-Jentoft et al. 2019). This allowed the characterisation of sarcopenia as a syndrome with progressive generalised loss of skeletal muscle and function, which increased risk of physical disability, a lowered quality of life, and multi-morbidities resulting in death.

In the original definition, sarcopenia was diagnosed by low muscle mass (Skeletal Mass Index – SMI 7.26 Kg/m^2 for males and 5.5 kg/m^2 for females), plus the presence of either/or low muscle strength (handgrip strength - < 30 Kg for males and < 20 Kg for females) and low physical performance (Figure 1-1).

The low muscle mass measured by dual-energy X-ray absorptiometry (DEXA) had a cut-off derived from 2 standard deviations below the mean Skeletal Mass Index (SMI) based on measurements from 229 non-Hispanic white men and women aged 18-40 years of age who were participants in the Rosetta study (1986-1992) (Cruz-Jentoft et al. 2010; Baumgartner et al. 1998). While low grip strength, a clinical predictor of loss of muscle function, was defined based on 2 standard deviations below the mean based measured in 1,030 (469 mean and 561 women) participants from the InCHIANTI study by isometric dynamometry (Lauretani et al. 2003).

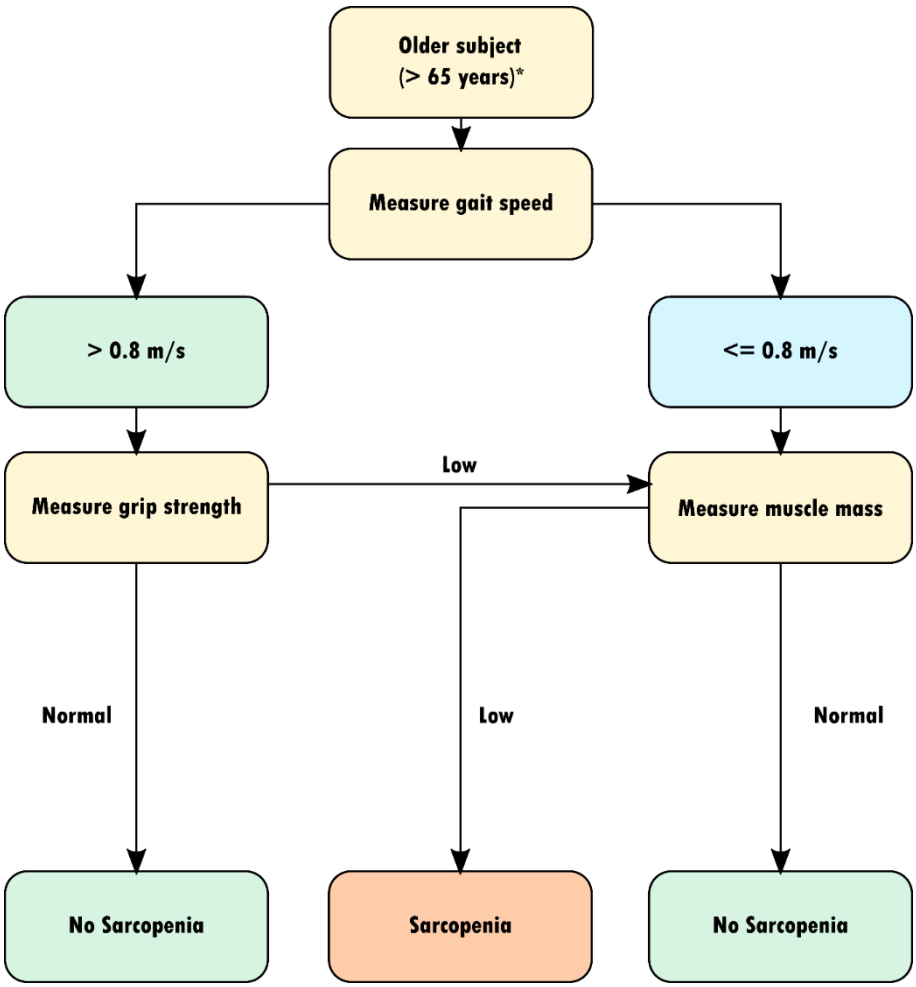

Figure 1-1 : EWGSOP (2010) flowchart for defining sarcopenia

* Comorbidity and individual circumstances must also be considered, Adapted from (Cruz-Jentoft et al. 2010)

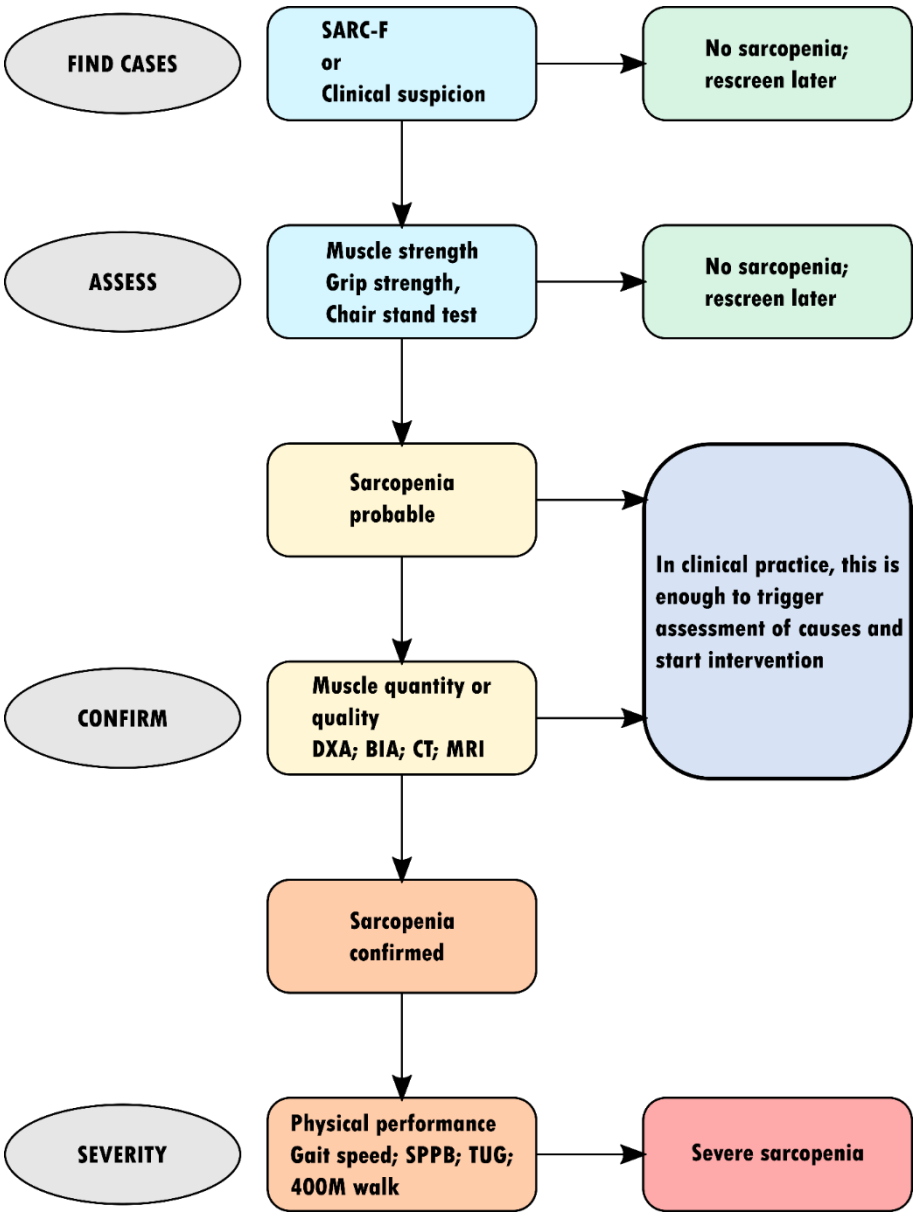

Figure 1-2 : EWGSOP2 (2019) flowchart for defining sarcopenia

Adapted from (Cruz-Jentoft et al. 2019)

The European Working Group on Sarcopenia in Older People was revised in 2019 (Cruz-Jentoft et al. 2019), with the introduction of the SARC-F questionnaire (Malmstrom et al. 2016; Gülistan Bahat et al. 2018) (discussed in more detail in section 1.4.2 1.2.4 Additional definitions of sarcopenia) and expanded on the concepts of probable sarcopenia and severe sarcopenia (Figure 1-2). The original 2010 definition included the categorisation of sarcopenia into “Presarcopenia”, “Sarcopenia” and “Severe sarcopenia” (Cruz-Jentoft et al. 2010) as shown in Table 1-1:

Table 1-1 : EWGSOP1 Stages of sarcopenia

Stage	Muscle Mass	Muscle Strength	Performance
	CT / MRI / DEXA / BIA / Total potassium	Handgrip strength / Knee flexion / Peak expiratory flow	SPPB / Usual gait speed / Timed get up and go test / Stair climb test
Presarcopenia	Low		
Sarcopenia	Low	Low OR	Low
Severe sarcopenia	Low	Low	Low

EWGSOP1 (2010) definition, sarcopenia can be characterised by the loss of muscle mass and either muscle strength or performance (Cruz-Jentoft et al. 2010). CT=Computed tomography; MRI=Magnetic resonance imaging; DEXA=Dual energy X-ray absorptiometry; BIA= Bioimpedance analysis

Under the EWGSOP1 definition, pre-sarcopenia is characterised by low muscle mass without any particular loss of function or performance. This is identified by accurate measurement of muscle mass loss, such as by Dual-energy X-ray absorptiometry (DEXA) or Bioimpedance analysis (BIA). Sarcopenia itself requires the additional observation (Cruz-Jentoft et al. 2010) of either low muscle

strength (for example of handgrip strength via a Dynameter, or Knee flexion) or a low score on tests measuring physical performance (for example the Short Physical Performance Battery – SPPB (JM et al. 1994)), see Table 1-2.

Table 1-2 : Measurements of muscle mass and function in research and practice

Variable	Research	Clinical practice
Muscle mass	Computed tomography (CT)	BIA
	Magnetic resonance imaging (MRI)	DXA
	Dual energy X-ray absorptiometry (DEXA)	Anthropometry
	Bioimpedance analysis (BIA)	
	Total or partial body potassium per fat-free soft tissue	
Muscle strength	Handgrip strength	Handgrip strength
	Knee flexion/extension	
	Peak expiratory flow	
Physical performance	Short Physical Performance Battery (SPPB)	SPPB
		Usual gait speed
	Usual gait speed	Get-up-and-go test
	Timed get-up-and-go test	
	Stair climb power test	

(Cruz-Jentoft et al. 2010)

The EWGSOP updated the original definition of sarcopenia at a meeting in early 2018, releasing their new definition in 2019. This was to reflect the substantial additional clinical evidence and scientific feedback from the initial EWGSOP definition of 2010 (Cruz-Jentoft et al. 2019).

Four areas were mentioned specifically:

1) Recognition that although sarcopenia is still a condition associated with ageing and older people, that it can begin earlier in life and that it has multifactorial causes (Sayer et al. 2004, 2008).
2) Low muscle strength and functional has been shown to be more important component of sarcopenia than the loss of muscle mass.
3) Muscle mass and muscle quality can be difficult to measure accurately.
4) Clearer diagnostic criteria and cut-offs were required for use in the mainstream medical practice.

The updated 2019 definition, EWGSOP2, instead of “pre-sarcopenia” identifies “probable sarcopenia” as reduced handgrip strength and/or increased time on the five times chair stand test (> 15 seconds). The additional observation of a low Skeletal Mass Index is required to confirm sarcopenia. Severe sarcopenia is once again indicated by a combination of the three metrics of low muscle strength, quantity and physical performance (Cruz-Jentoft et al. 2019).

1.2.2 Foundation for the National Institutes of Health

In 2012 the Foundation for the National Institutes of Health (FNIH) sarcopenia project published a series of guidelines for their definition of sarcopenia (S. A. Studenski et al. 2014). The primary outcome selected for sarcopenia was mobility impairment, as measured by gait speed. A gait speed of less than or equal to 0.8 m/s was used as the primary cut-point due to its impact on overall survival (S. Studenski et al. 2011) and disability (Abellan Van Kan et al. 2009).

The FNIH aggregated data from nine cohorts (26,625 participants; mean age – male: 75.2 years, mean age – female: 78.6 years) in order to derive their cut-points for sarcopenia.

For both the low grip strength and appendicular lean mass (ALM), Classification and Regression Trees (CART), or decision tree analysis was used to recursively partition participants into mutually exclusive groups. These groups are defined by a predictor cut-point range within which participants have similar outcome probabilities (Breiman et al. 1984). The outcomes used for the FNIH definition being prevalence of slowness and then maximum grip strength.

Cross validation was used to select the cut-points for both the grip and lean mass CART analyses. The pooled data was randomly partitioned into 10 equally sized mutually exclusive subsets. Each of these subsets, containing 90% of the data, were analysed by the decision tree. The prediction error from each of the subsets was calculated and the tree pruned in order to select the nodes with the smallest prediction error (Alley et al. 2014; Cawthon et al. 2014) - Figure 1-3.

The grip strength cut-offs used pooled data from 9,897 men and 10,950 women (Alley et al. 2014), while for the low appendicular lean mass data from 7,582 men and 3,688 women was analysed (Cawthon et al. 2014).

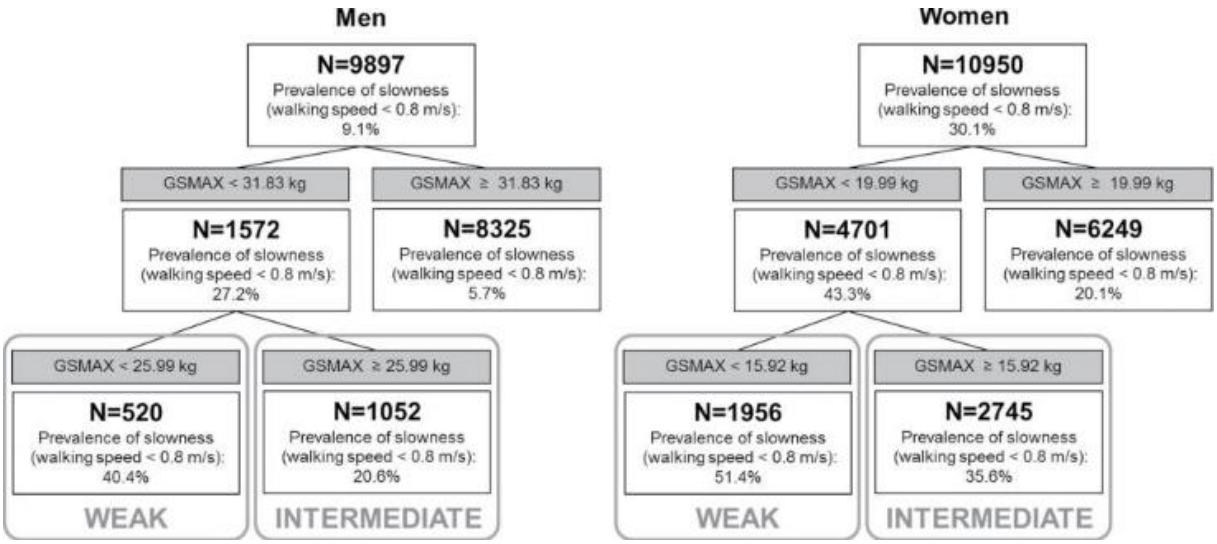

Figure 1-3 : Classification tree for FNIH gait speed and grip strength

(Alley et al. 2014)

The odds ratio of mobility impairment, as measured by low gait speed, for males with a grip strength of less than 26 Kg was 7.6 (CI 95% 6.1-9.5) when compared to men with normal grip strength (≥ 32 Kg). For women the odds ratio for low gait speed with a low grip strength of less than 16 Kg was 4.4 (CI 95% 3.9-5.0).

For ALM the odds ratio of weakness, as measured by low grip strength, was 6.9 (CI 95% 5.4-8.9) for men and 3.6 (CI 95% 2.9-4.3) for women.

The definition proposes a gait speed of less than 0.8 m/s with less than 26Kg grip strength for men and less than 16 Kg for women, in addition to a low lean appendicular muscle mass (adjusted for BMI) of less than 0.789 for men and less than 0.512 for women, Table 1-3.

Table 1-3 : FNIH cut-points for Weakness and Low Lean Mass in Men and Women

Cutpoint	Men	Women
Weakness		
Recommended: grip strength (GSMAX)	<26 kg	<16 kg
Alternate: grip strength adjusted for BMI ($GSMAX_{BMI}$)	<1.0	<0.56
Appendicular lean body mass		
Recommended: ALM adjusted for BMI (ALM_{BMI})	<0.789	<0.512
Alternate: ALM	<19.75 kg	<15.02 kg

Notes: ALM = appendicular lean mass; BMI = body mass index. (S. A. Studenski et al. 2014)

1.2.3 International definitions of sarcopenia

In 2014 the Asian Working Group for Sarcopenia (AWGS) published a report on specific diagnostic cut-offs for the definition of sarcopenia in Asian populations (L.-Y. K. L.-K. Y. Chen et al. 2014).

Recommended cut-offs for lean muscle mass derived from DEXA were 7.0 Kg/m2 for men and 5.4 Kg/m2 for women. Bio-impedance analysis (BIA) suggested 7.0 Kg/m2 for men and 5.7 Kg/m2 for women. In addition a gait speed of less than 0.8 m/s and low handgrip strength (less than 26 Kg for men and less than 18 Kg for women) were also found to indicate sarcopenia in Asian participants. As the published work highlights there is a wide range of ethnicities in Asia, when

compared to the single northern European lineage which is often the focus of diagnostic criteria (L.-Y. K. L.-K. Y. Chen et al. 2014), and the AWGS acknowledge these limitations in proposing their definitions for sarcopenia (Figure 1-4).

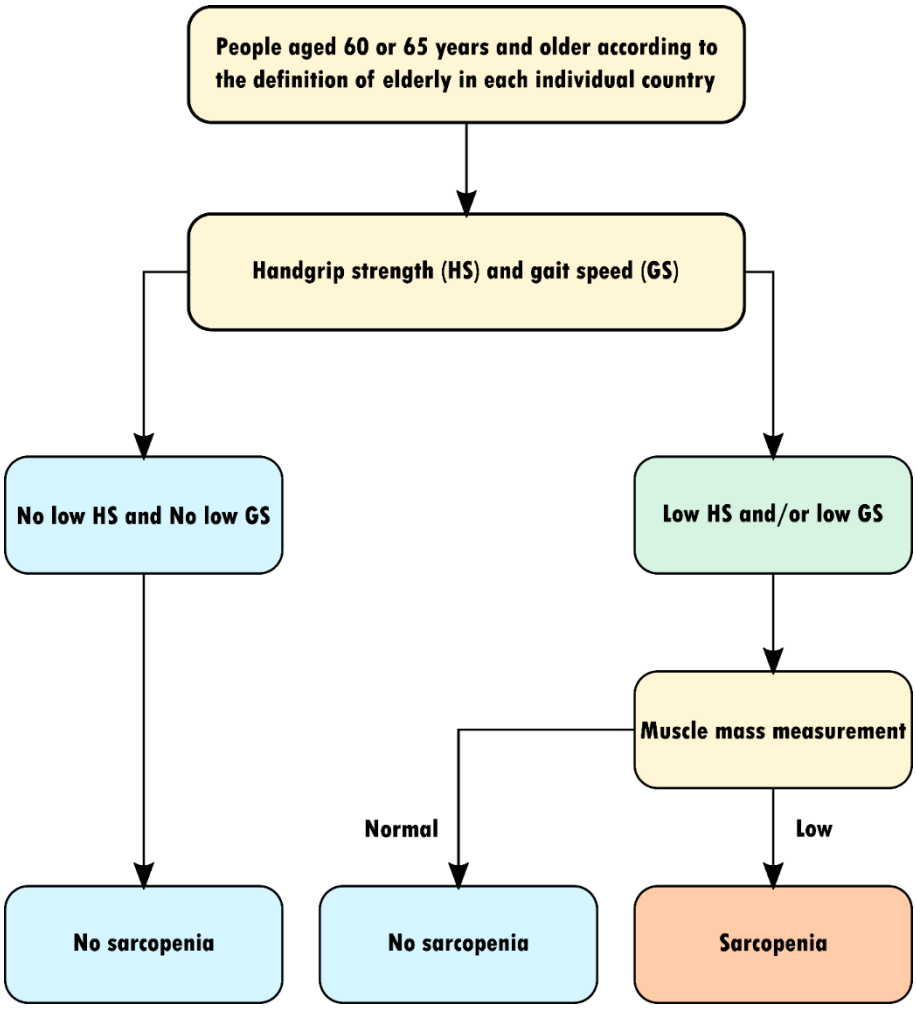

Figure 1-4 : AWGS (2014) flowchart for defining sarcopenia

Adapted from (L.-Y. K. L.-K. Y. Chen et al. 2014)

There is currently no single recognised set of definitive criteria for sarcopenia, with definitions being provided by a number of consortia. As noted by the AWGS in their 2014 definition, the proposed cut-offs for defining sarcopenia for the Asian population follows similar methodology for measuring physical performance but provides different cut-offs to the definitions for the European population

(EWGSOP/FNIH). This reflects the differences in ethnicity, body size, lifestyles and cultural background (L.-Y. K. L.-K. Y. Chen et al. 2014). Woo *et al*, 2014, noted that overall observations of anthropometric measures (BMI, ASM/height^2, and grip strength) in a diverse set of studies on community dwelling adults over 65 years of age with Asian participants (Beijing Chinese, Singapore Chinese, Hong Kong Chinese, Japanese, Malays and Indians) were significantly lower when compared to similar studies with participants of European ancestry (J. Woo et al. 2014), however gait speed was equivalent. Global variation in grip strength has been investigated regarding a consensus definition of sarcopenia by Dodds et al. They found that although developed regions closely followed the normative grip data from a cohort of 12 British studies (Dodds et al. 2014), this was not the case for developing regions (Figure 1-5) (Dodds et al. 2016).

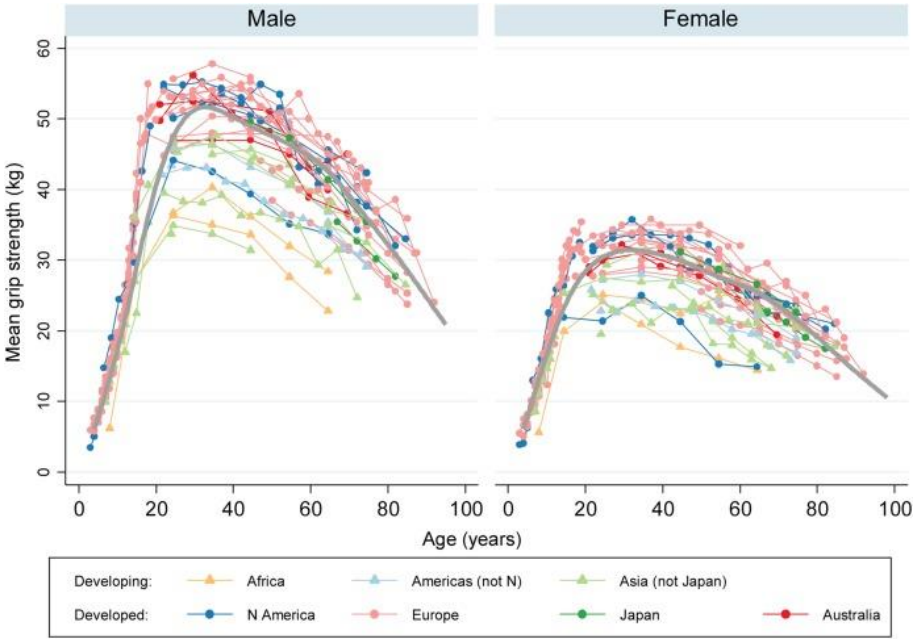

Figure 1-5 : Grip strength mean values by UN region

(Dodds et al. 2016)

Grey curve shows the mean values from data for 12 British studies (Dodds et al. 2014).

1.2.4 Additional definitions of sarcopenia

The SARC-F (Malmstrom et al. 2016) incorporates five observations in order to score the likelihood of sarcopenia: ability to climb stairs, rising from a chair, lifting and carrying strength, assistance in walking and finally the number of falls in the past year. A combined score of equal to or greater than 4 is predictive of sarcopenia and adverse outcomes.

Evaluation of the screening ability of the SARC-F for sarcopenia, found that although sensitivity is poor, its specificity is high (Ida, Kaneko, and Murata 2018; Gülistan Bahat et al. 2018). This allows for the selection of patients who would benefit from a more rigorous assessment and possible confirmation of a sarcopenia diagnosis, however the low sensitivity means that SARC-F is not reliable enough on its own to act as a screening tool.

SARC-F is concerned primarily with muscle function and doesn't take muscle mass into consideration. Extensions to the SARC-F questionnaire have increased its performance as a screening tool by including calf circumference measurements (Barbosa-Silva et al. 2016), or as part of a more comprehensive algorithm as with the EWGSOP2 definition (Cruz-Jentoft et al. 2019). SARC-F combined with calf circumference (SARC-CalF) has been proposed as a suitable enhancement to the stand alone SARC-F questionnaire in order to improve screening performance (Barbosa-Silva et al. 2016).

An additional sarcopenia definition proposed by the International Working Group on Sarcopenia (IWGS) was also considered for inclusion in this study. In 2009 the IWGS met in Rome, Italy in order to provide a consensus definition of

sarcopenia (Fielding et al. 2011), which was published in 2011. The IWGS specified that sarcopenia should be considered in older patients who are long-term bedridden, cannot independently rise from a chair or who have a gait speed of less than 1 m/s. They defined sarcopenia as a gait speed of less than 1 m/s and low appendicular muscle mass of ≤ 5.67 Kg/m^2 in women and ≤ 7.23 kg/m^2 in men (Fielding et al. 2011).

The IWGS definition was developed for use in clinical settings, such as hospital or care homes. By contrast the EWGSOP definition in 2010 focused on any community dwelling individual over the age of 65. A study by Lee *et al* comparing the two definitions for diagnosing sarcopenia in 408 individuals over the age of 65 recruited as part of the I-Lan Longitudinal Ageing Study (ILAS), found that agreement between the two definitions was only fair (kappa = 0.448 by relative appendicular skeletal muscle index and kappa = 0.471 by Skeletal Mass Index) (W.-J. Lee et al. 2013). Although the comparison focused almost exclusively on the loss of muscle mass, the discrepancy between the IWGS and EWGSOP definitions, as well as EWGSOP focus on community diagnosis, made the EWGSOP and FNIH better candidates for my research with the UK Biobank.

1.2.5 Prevalence and impact

A systematic review by Mayhew et al, 2019 found that the prevalence of sarcopenia in community-dwelling older adults varied across a wide range (9.9% to 40.4%) depending on the sarcopenia definition (Mayhew et al. 2019) - Figure 1-6.

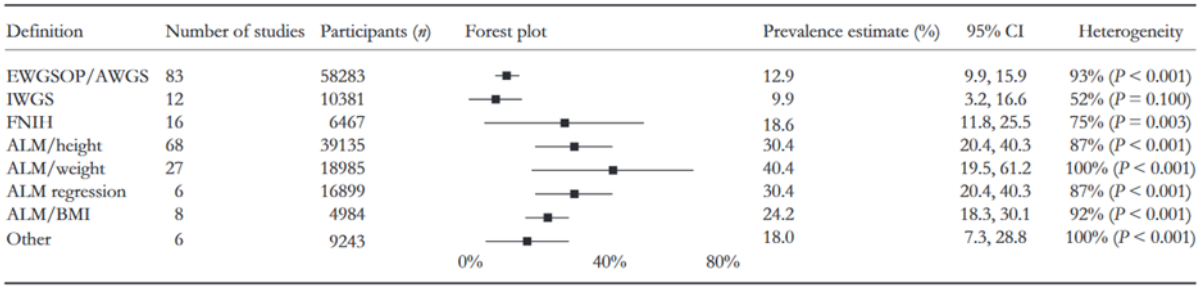

Figure 1-6 : Sarcopenia prevalence estimates

(Mayhew et al. 2019).

Prevalence of sarcopenia under the EWGSOP combined low grip strength and low Skeletal Muscle Index (SMI), of European ancestry and between the ages of 60 and 70, in the UK Biobank is 12.7% for females and 4.3% for males. A recent review of sarcopenia prevalence in community dwelling adults over 60, based on a number of different definitions returned an estimate of 10% (95% CI: 8-13%) for females and 10% (95% Ci: 8-12%) for males (Shafiee et al. 2017). It should be noted that out of the 35 studies included in the analysis (n=58404 individuals; 32642 male, 25762 female) 21 studies were from Asian populations while 14 studies were categorised as Non-Asian. Sarcopenia was defined using one of EWGSOP, AWGS or IWGS criteria (depending on the study population) and so accounted for regional differences. The Health and Retirement study (HRS) however has a sarcopenia prevalence of 39% in females and 27% in males in

the over 60 age group. It should be noted that HRS has substantially older cohort compared to UK Biobank.

Sarcopenia of older adults in nursing homes has also been investigated and found to be extremely prevalent. In a meta-analysis of 12 studies (2685 participants) using the EWGSOP1 definition of sarcopenia, an estimated pooled prevalence was 41% (I^2=96%, 95% CI 32%-51%, Figure 1-7).

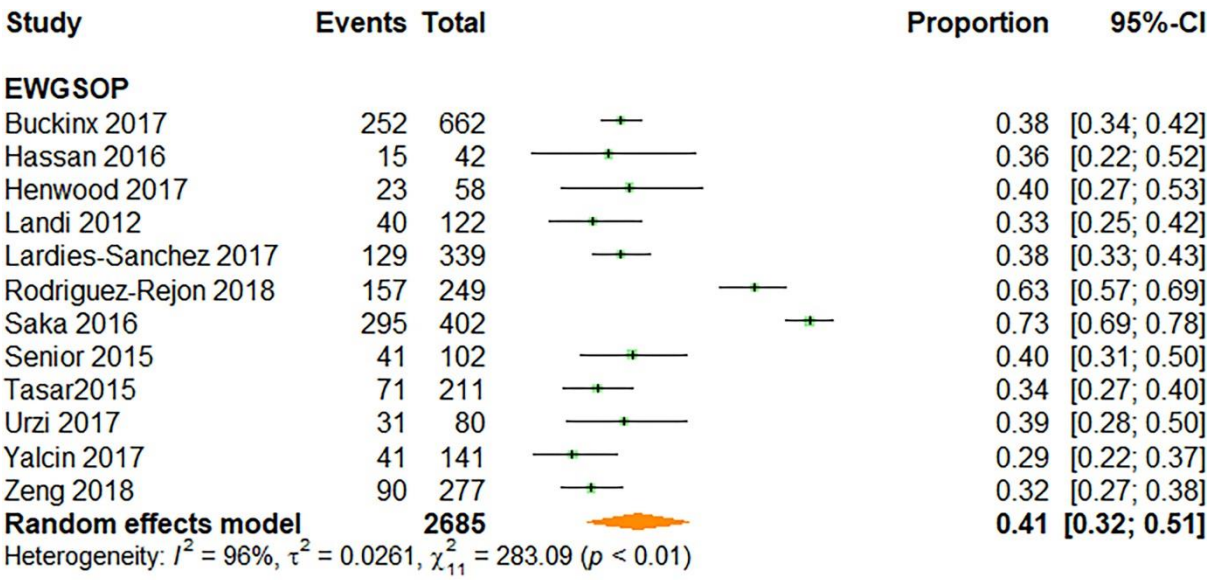

Figure 1-7 : Prevalence of EWGSOP1 sarcopenia in nursing homes.

(Shen et al. 2019)

Data from a study of geriatric inpatients (Sarcopenia in Geriatric Elderly - SAGE) between April 2013 and May 2015 (Jens Reiss et al. 2016) has been used to compare the prevalence of sarcopenia as defined by both EWGSOP versions 1 and 2 (J. Reiss et al. 2019). The study found that the overall prevalence of sarcopenia diagnosed using EWGSOP2 was significantly lower than EWGSOP1, 18.1% versus 27.7% of participants. This discrepancy was mainly due to sex-specific differences, with sarcopenia prevalence in women found to be 22.1% (EWGSOP1) against 17.4% (EWGSOP2), however in men the percentage

meeting the definition of sarcopenia was 37.9% for EWGSOP1 and only 19.4% for EWGSOP2.

Of greater concern was the lack of overlap between the individuals defined as sarcopenic under EWGSOP1 compared to EWGSOP2. From the 19 women defined as having sarcopenia under EWGSOP1, 10 individuals did not reach the EWGSOP2 criteria and of the 15 women meeting the EWGSOP2 definition, 6 did not reach the EWGSOP1 cut-offs. With the males a similar pattern was observed with 11 out of the 22 men meeting the EWGSOP1 definition, but being judged as non-sarcopenic by the EWGSOP2 algorithm. However there were no new males diagnosed with sarcopenia under the EWGSOP2 definition (J. Reiss et al. 2019). The small, non-representative nature of the study should be taken into account, however it does highlight the variability in prevalence depending on the algorithm and definitions used.

A study of sarcopenia prevalence in community dwelling men (Uppsala Longitudinal Study of Adult Men, ULSAM; N=287 males, aged 85-89 years) found that the definition used, EWGSOP1 or EWGSOP2, resulted in different individuals being identified as sarcopenic, despite the overall prevalence of 20% and 21% respectively being similar (Sobestiansky, Michaelsson, and Cederholm 2019).

1.2.6 Overlap between frailty and sarcopenia

The Rapid Geriatric Assessment, comprising of the SARC-F (Jean Woo, Leung, and Morley 2014), FRAIL (J. E. Morley, Malmstrom, and Miller 2012), Simplified Nutritional Appetite Questionnaire (SNAQ) (Rolland et al. 2012) and Rapid Cognitive Screen (RCS) (Malmstrom et al. 2015) tools, was used to screen 11,344 individuals over the age of 65 in Missouri, USA between 2015 and 2019. The study found that most individuals who screened positive for sarcopenia or frailty, also returned positive for the other condition, highlighting the large overlap between the two conditions (Sanford et al. 2020), see Figure 1-8.

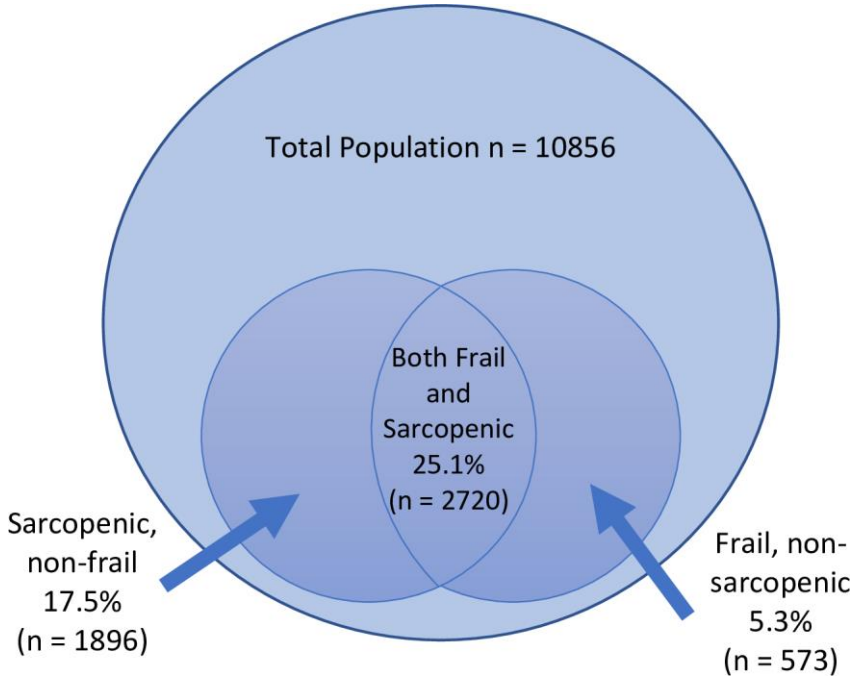

Figure 1-8 : Overlap between sarcopenia and frailty

(Sanford et al. 2020); sarcopenia as defined by SARC-F, frailty defined by FRAIL

1.3 Frailty

Frailty is a heterogenous phenotype (Junius-Walker et al. 2018) with symptoms including loss of muscle mass and function, unintentional weight loss, exhaustion, cognitive impairment and decline in motivation. A combination of these traits can be measured using indices such as the Fried index of frailty (L. P. Fried et al. 2001) to provide a definitive phenotype. Frailty is often characterized by a state of increased vulnerability following a stressor event, with implications for multiple adverse outcomes, such as falls or additional co-morbidities (Clegg et al. 2013).

The Rockwood scale is a 7 point Clinical Frailty Scale originally developed to categorise frailty for 2305 participants of the Canadian Study of Health and Aging (CSHA) over a 5 year time frame (Rockwood et al. 2005). A number of tools were used in order to assign participants to a category including the CSHA Frailty Index (derived from a count of 70 deficits), CSHA Function Scale (based on daily living activities and the ability to maintain independence) and clinical history of falls, co-morbidity, cognitive impairment or dementia.

The methodology used to create the CSHA Frailty Index has been developed into a standardised process (Searle et al. 2008). Williams *et al* used these methods to produce a list of frailty indices (FIs) derived from 49 self-reported questionnaire items in the UK Biobank data, concluding that FIs provided a valid measure of frailty with the UK Biobank participant. Survival analysis showed that a 0.1 FI increment was associated with a 65% increase in mortality risk (hazard ratio = 1.65, 95% confidence interval: 1.62-1.68) (D. M. Williams et al. 2019).

Frailty measured by five criteria (weight loss, exhaustion, grip strength, low physical activity and slow walking pace) was also measured in the entire UK

Biobank (493,737 healthy community volunteers, age range 37-73 years) by Hanlon *et al*. The study found that 16,538 (3%) met the criteria for frail, 185,360 (38%) for pre-frail and 291,839 (59%) as not frail (Hanlon et al. 2018). It should be noted that the UK Biobank is a community based study of predominately young healthy volunteers. Analysis has shown that UK Biobank participants were more likely to be female and older than the general population, and to live in less socioeconomically deprived areas. At age 70-74 rates of all-cause mortality were 55.5% lower in women and 46.2% lower in men compare to the general population of the same age range. There is also a “healthy volunteer” selection bias within the UK Biobank compared to the general population (Fry et al. 2017). However despite this bias previous studies have found that results from analyses in UK Biobank are generalizable to the population, especially in the context of prospective analysis of incident disease (Fry et al. 2017).

The Fried frailty phenotype and Rockwood frailty index should be regarded as complementary measures of frailty, rather than overlapping metrics, as they record the presence of frailty by very different methods (Cesari et al. 2014) - Table 1-4.

Table 1-4 : Characteristics of Fried frailty phenotype and Rockwood frailty index

Frailty phenotype (Fried)	**Frailty Index (Rockwood)**
Signs, symptoms	Diseases, activities of daily living, results of a clinical evaluation
Requires clinical assessment for measurements such as grip strength.	Possible to obtain using existing records, for example electronic frailty index
Categorical variable	Continuous variable
Pre-defined set of criteria	Unspecified set of criteria
Frailty as a pre-disability syndrome	Frailty as an accumulation of deficits
Meaningful results potentially restricted to non-disabled older persons	Meaningful results in every individual, independently of functional status or age

(Adapted from Cesari et al, 2014)

1.3.1 Prevalence

A systematic review and meta-analysis of frailty in community-dwelling older adults worldwide, using mainly the Fried criteria for frailty, found that frailty rates were significantly higher in women (44.8 cases per 1000 person years, 95% CI 36.7-61.3; heterogeneity between studies I^2=97.7%) than men (24.3 cases per 1000 person years, 95% CI 19.6-30.1; I2=8.94%). The meta-analysis involved 101,259 participants in which 73.3% were women (Ofori-Asenso et al. 2019).

A systematic review of frailty in community dwelling older adults has placed the prevalence at 17.4% in middle (US$1006-US$3955) and low (≤ US$1005) income countries, Figure 1-9.

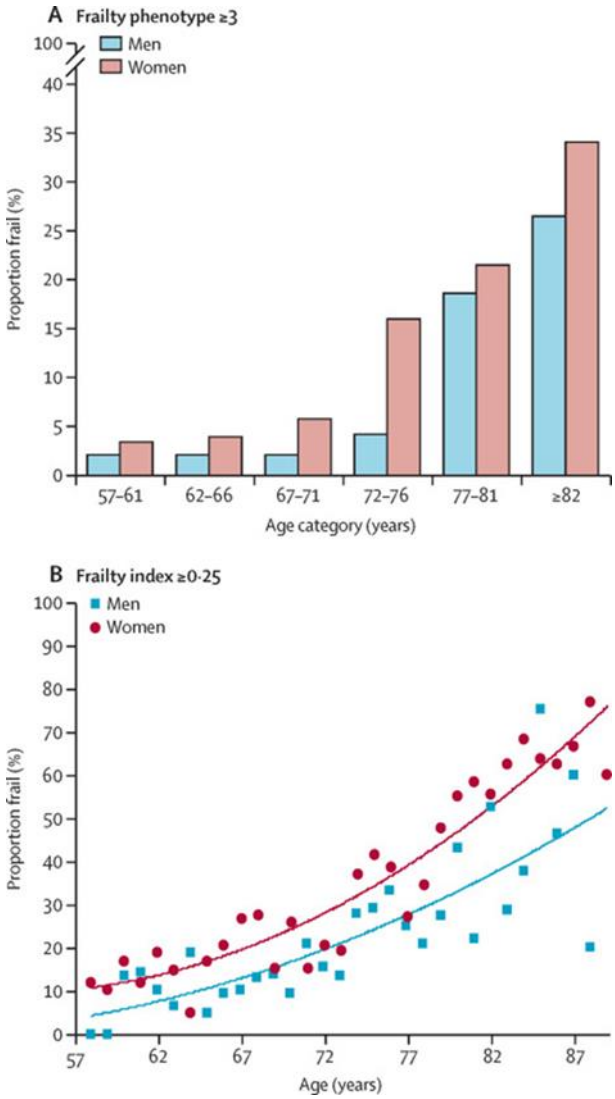

Figure 1-9 : Frailty prevalence for men and women by age

(Hoogendijk et al. 2019)
Prevalence in the Longitudinal Aging Study Amsterdam wave F (2005-6) based on either frailty phenotype (A) or frailty index (B).

1.4 Measuring the Musculoskeletal ageing process

1.4.1 Grip strength

Because the strength of both upper and lower limbs can be used as a proxy for overall muscle function (Aadahl et al. 2011; Bohannon et al. 2012), the most convenient and currently recommended clinical measure is provided by maximum grip strength using a handheld dynamometer (Beaudart et al. 2019) - Figure 1-10.

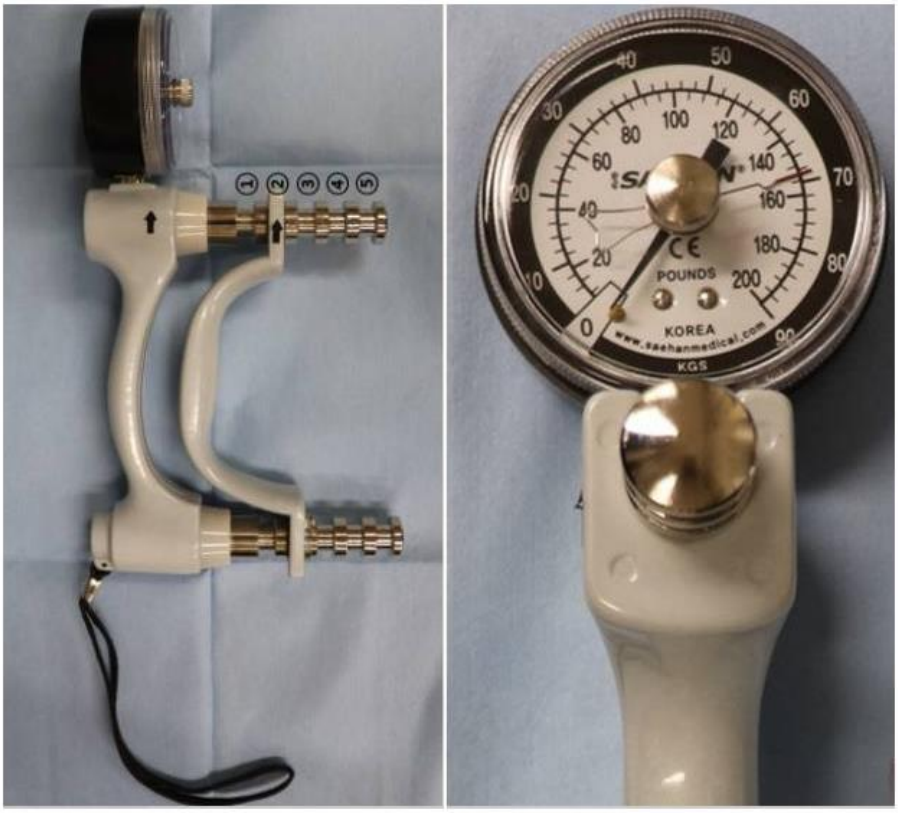

Figure 1-10 : Hydraulic type dynamometer (Jamar)

(S. H. Lee and Gong 2020)

Handgrip strength has been found to be a strong indicator of mortality, across a range of studies of community dwelling adults (mixed ancestry), with a hazard

ratio of 1.67 (95% confidence interval 1.45-1.93) when comparing the weakest to strongest quartiles from 14 studies (N=53,476) (Cooper, Kuh, and Hardy 2010).

The 2010 European Working Group on Sarcopenia in Older People (EWGSOP) used grip strength as measured by a hand dynameter as its preferred measure of muscle strength (Cruz-Jentoft et al. 2010).

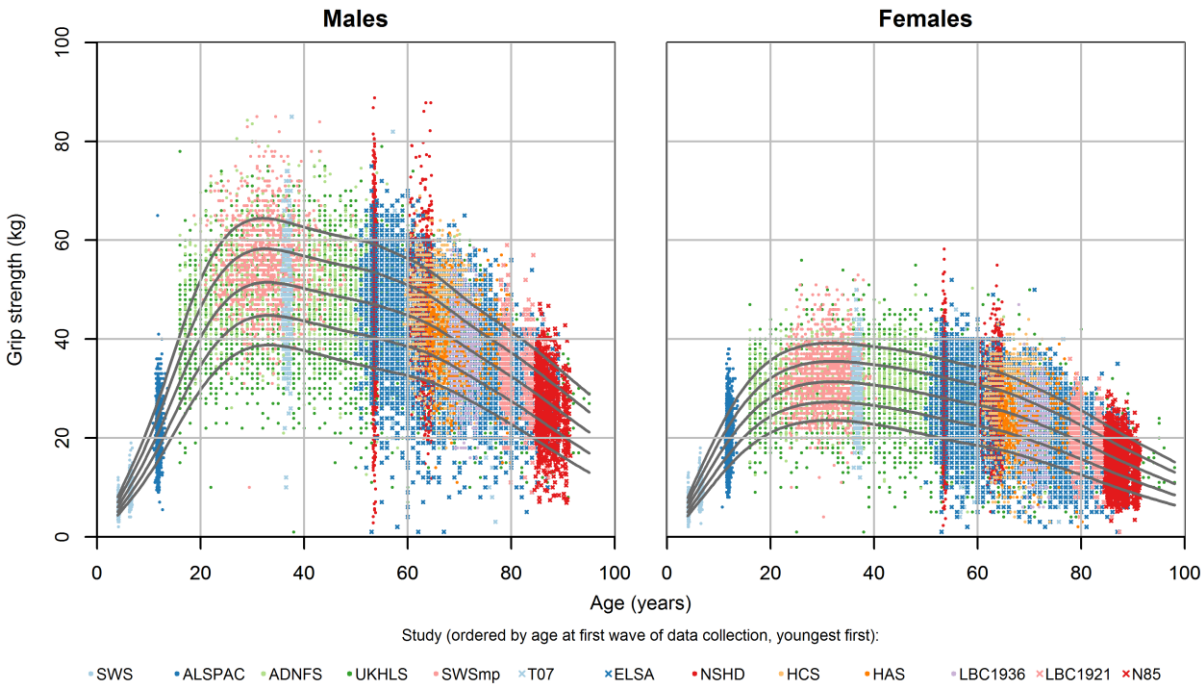

Figure 1-11 : Grip strength across life course from 12 British studies

(Dodds et al. 2014) : 49,964 participants, age range 4-90 years

A study in 2014 by Dodds *et al*, on handgrip strength within the British population used 12 general population studies (N=49,964) with a wide age range (4-90 years). Peak mean grip was observed for both sexes at the age of 32 (51.9 Kg for males and 31.4 Kg for females) and this was used to calculate the prevalence of weak grip strength at different age range - Figure 1-11. Weak grip strength was defined as either a T-score of -2 (32 Kg or less for males and 19 Kg or less for

females) or -2.5 (27 Kg or less for males and 16 Kg or less for females) - Figure 1-12.

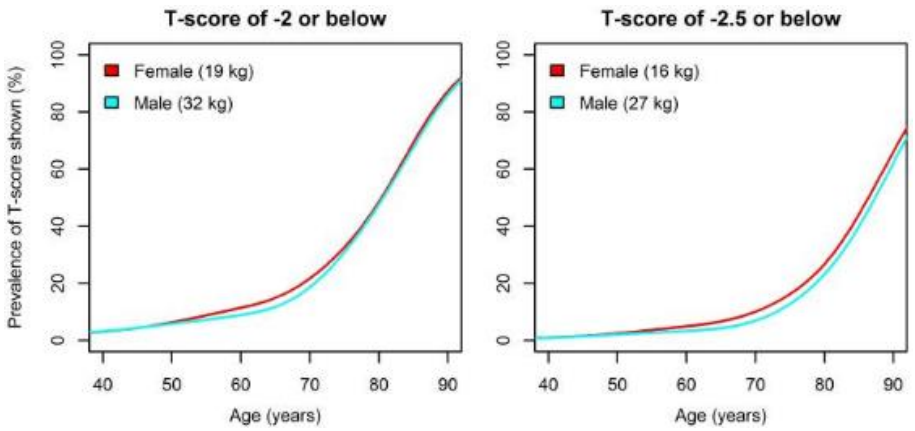

Figure 1-12 : Prevalence of low grip strength in 12 British studies

(Dodds et al. 2014)

23% of males and 26.6% of females had a grip strength lower than the weakest grip strength definition of a T-score of -2.5 by the age of 80, with the cut-offs of 27Kg for males and 16Kg females being roughly equivalent to the more stringent FNIH definition of low grip strength in the context of sarcopenia (26Kg for males, 16 Kg for females).

1.4.2 Appendicular Lean Muscle mass

Appendicular lean muscle mass is a commonly used metric for healthy aging and physical performance decline with age (Ian Janssen, Heymsfield, and Ross 2002; Goodpaster et al. 2006).

Dual-energy x-ray absorptiometry (DEXA) is used clinically to measure body composition, with estimates of lean muscle mass being derived from the raw data

(J. Kim et al. 2002). Computed tomography (CT)(Georgiou et al. 2020), bioimpedance analysis (BIA) (Scafoglieri et al. 2017) and magnetic resonance imaging (MRI) (Tavoian et al. 2019) are also used in studies of lean muscle mass.

1.4.3 Short Physical Performance Battery

The Short Physical Performance Battery (SPPB) is a series of physical tests used to gauge balance and muscle strength. SPPB has been shown to be suitable for diagnosing older adults with severe sarcopenia when used in isolation (Phu et al. 2020), and has been proposed for diagnostic use due to it being easy to administer. It consists of a series of balance tests in the following positions; side by side (feet touching), semi tandem (one foot slightly in front of the other but still overlapping) and tandem (heel of one foot directly in front of the toes of the other foot). The positions have to be maintained for 10 seconds. Gait speed along an 8 foot course, and the ability to rise from a chair are also tested. Tests are scored on a scale of 0-4 based on quartiles of results from the original SPPB paper (Guralnik et al. 1994).

A meta-analysis of studies using SPPB to predict all-cause mortality (17 studies, n= 16,534, mean age 76 ± 3 years) found that a score of less than 10 is predictive, with the lowest class of score between 0-3 having a very high increased risk of mortality (OR 3.25, 95% CI 2.86-3.79) (Pavasini et al. 2016).

1.4.4 Gait speed

A recent cohort study of 904 participants from New Zealand, over almost five decades, found that a slower gait speed in mid-life (45 years of age) is associated with accelerated biological aging, including neurocognitive functions (Rasmussen et al. 2019). This included key sarcopenia indicators such as low grip strength (Beta=0.36, 95% CI, 0.25-0.46) and poor performance on the chair-stand test (Beta=0.34, 95% CI, 0.27-0.40).

Gait speed is a good predictor of loss of physical function with age (Abellan Van Kan et al. 2009) and long term survival (S. Studenski et al. 2011).

1.5 Musculoskeletal system and ageing

Over the age of 70 years, individuals have an average rate of muscle mass loss of around 0.5-1% per year, with the majority of over 70 year olds retaining only 80% of their muscle mass compared to individuals in the 20-30 years age bracket (Mitchell et al. 2012). There is a reduction in muscle fibre numbers and fibre size with age, with the Type 2a (fast-twitch) myofibres being the most affected (W. J. Evans and Lexell 1995). Studies on the variability of the morphology of the Type 2 and Type 1 fibres have shown that in older individuals Type 2 fibres exhibit a significantly wider range of cross-sectional area, when compared to younger individuals – something not seen to such an extent with the Type 1 fibres (J Lexell and Taylor 1991; W. J. Evans and Lexell 1995). However there is enormous variability between individuals, and the factors that affect individual susceptibility to loss of muscle function are not fully understood.

Sarcopenia, the age related loss of muscle mass and function and dynapenia, the age-associated loss of muscle function independent of atrophy are thought to be caused by a number of factors. Identification of suitable biomarkers has implicated a multifactorial pathogenesis for sarcopenia, with the involvement of a diverse set of pathways including the neuromuscular junction, endocrine system, growth factors, protein synthesis and lysis, behaviour mediated pathways, inflammation and redox-related factors (Curcio et al. 2016).

1.5.1 Muscle fibres

Myofibres (skeletal muscle fibres - Figure 1-13 : Structure of muscle fibre, (Betts et al., n.d.)) are post-mitotic multinucleated cells formed during embryonic and foetal development from the fusion of myoblasts (Mintz and Baker 1967), and continue to regenerate damaged muscle by a similar fusion mechanism throughout life by myoblasts produced from the muscle satellite cells (Zammit 2008). With advancing age muscle re-growth is reduced by the lower potential for satellite cells to proliferate, differentiate into myoblasts and fuse with the diminished myofibre (Suetta et al. 2013).

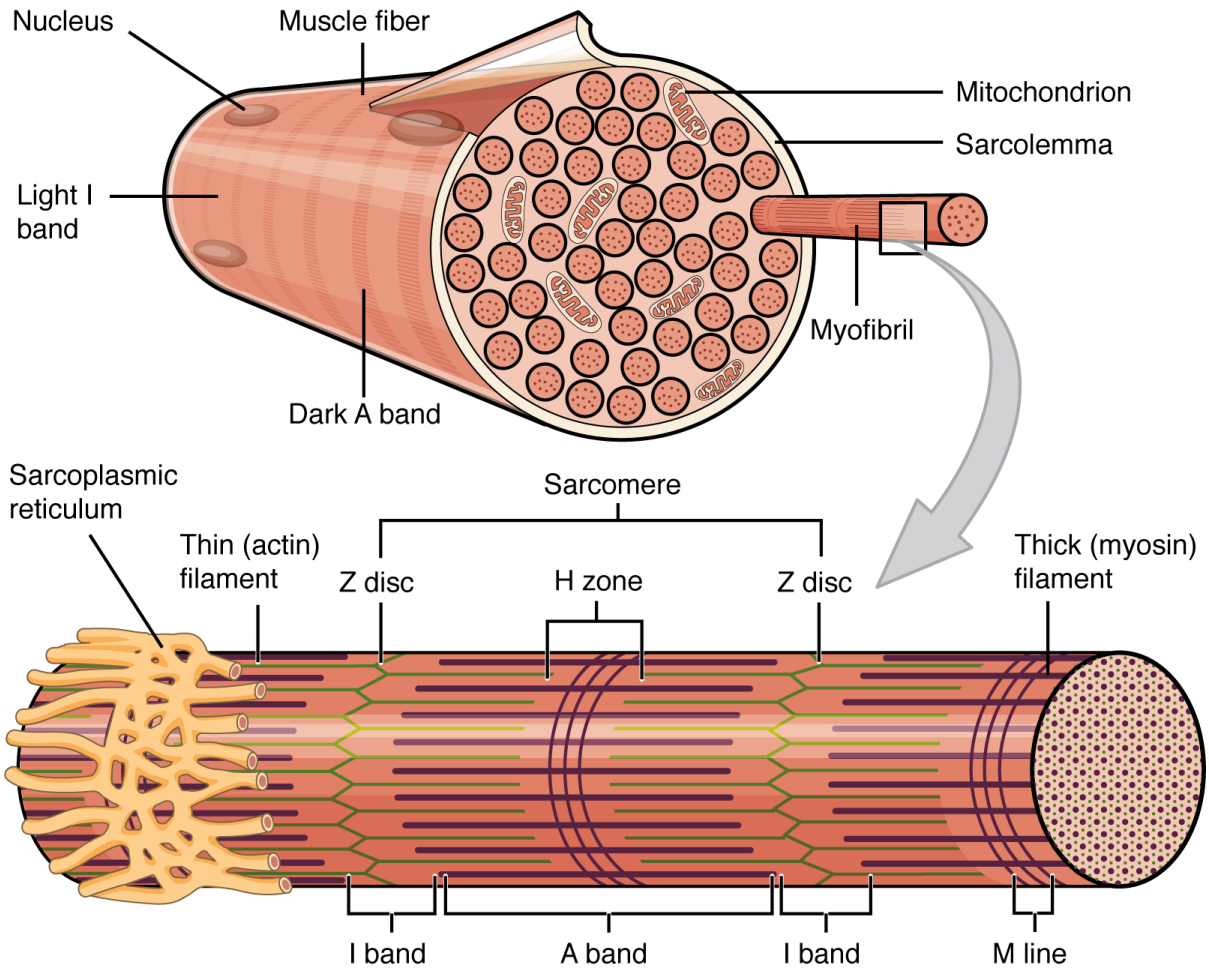

Figure 1-13 : Structure of muscle fibre

(Betts et al., n.d.)

Myofibres are categorised into various types based on their characteristic response to stimuli, metabolism, contractile cycle and expression of specific proteins, in particular the heavy myosin protein (Figure 1-13) (Schiaffino and Reggiani 2011).

Table 1-5 : Basic types of muscle fibres in Humans

	Slow-twitch	Fast-twitch	
Fiber type	Type 1	Type 2A	Type 2X
Contraction speed	Slowest	Fast	Fast
Fatigue endurance	Highest	Low	Low
Metabolism	Oxidative	Oxidative	Glycolytic
Indicative Mysosin	MYH7	MYH2	MYH1

Adapted from (Talbot and Maves 2016); Intermediate types with characteristics falling between the main categories exist, for example Type 1C co-express MYH7 and MYH2 heavy chain Myosin.

Aging muscle is characterised by the atrophy of muscle fibres, cell death of the muscle fibre and alterations to the grouping of muscle fibre types (Doherty 2003).

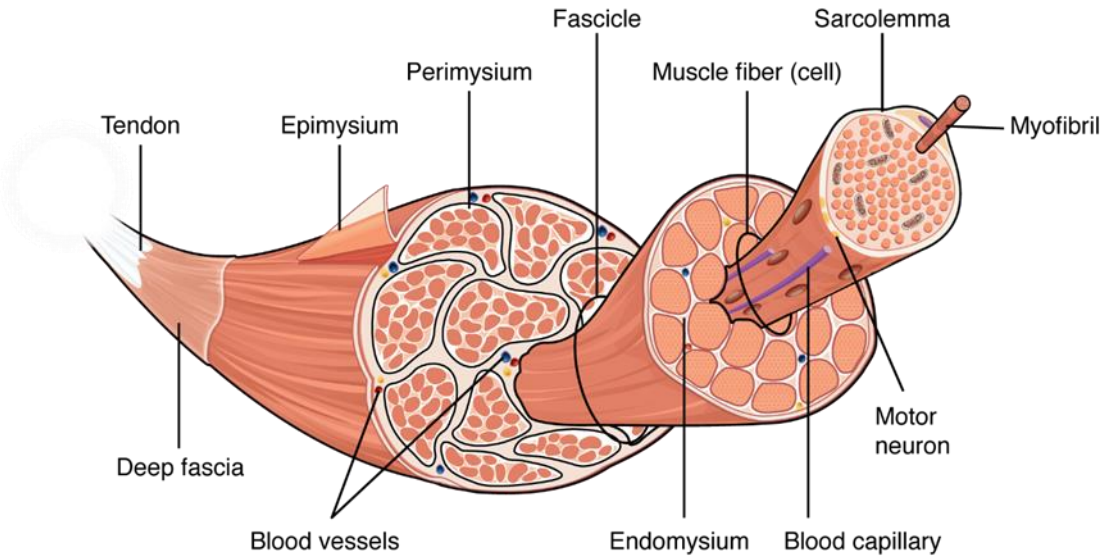

Figure 1-14 : Structure of skeletal muscle

(Betts et al., n.d.)

Muscle fibres are grouped together with a motor neuron and form the basic unit of a mammalian skeletal muscle, the motor unit (Sherrington 1925). Each muscle

fibre within the motor unit is innervated by an axon from the alpha motor neuron and all muscle fibres are of the same phenotype, for example Type 1 or 2A. Motor units are organised into fascicle (Figure 1-14), with motor units of the same type rarely being adjacent to one another (Edström and Larsson 1987).

In older individuals the cross-sectional area (CSA) of Type 2 fibres are significantly smaller when compared to younger individuals (approximately 35% smaller; $P < 0.001$, measurements from five cross sectional of 375 Type 2 fibres from 20 individuals, age 19 to 84 years), while the CSA of the Type 1 fibres between the young and older individuals showed only a 6% decrease (J Lexell and Taylor 1991). The study authors also observed that aged fibres had a highly significant variation in the mean CSA for both Type 1 and 2 fibres, with an increased number of both atrophied and enlarged (hypotrophied) dysfunctional fibres.

1.5.2 Satellite cells

Skeletal muscle satellite cells are essential for muscle growth and development. They are stem cells providing the reserve of myoblasts for myofibre regeneration in the adult, and reside in niches on the surface of the muscle fibre underneath the basal lamina (Relaix and Zammit 2012) - Figure 1-15.

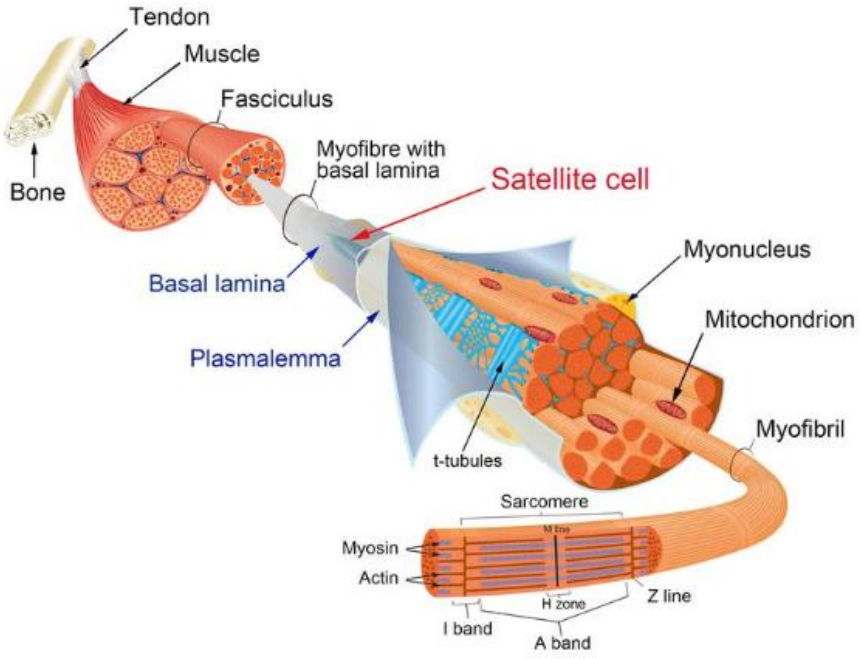

Figure 1-15 : Skeletal muscle satellite cells

(Relaix and Zammit 2012)

The reduced capacity of skeletal muscle in older adults to repair itself appears to be correlated with a loss of satellite cell function, such as their ability to migrate to the site of injury (Collins-Hooper et al. 2012) or respond to signalling molecules correctly (JV 2012). In a small study (N=105, number of cases=6) sarcopenic men have been shown to have a lower Satellite cell (SC) density and lower SC to muscle fibre ratio than controls (Patel et al. 2015).

1.5.3 Denervation and re-innervation

Denervation and re-innervation of muscle fibres has been shown to increase with age (Rowan et al. 2012), alongside the loss of functioning motor units (McNeil et al. 2005). Neuron cell death leads to denervation of the motor unit, with re-innervation achieved by axonal sprouting from nearby motor neurons. This results in absorption of the orphaned muscle fibres into an enlarged motor unit (Gordon, Hegedus, and Tam 2004). This process of adaptive axonal sprouting

breaks down with extreme age leading to the loss of the motor unit, atrophy of the muscle fibres (Rowan et al. 2012) and a subsequent reduction in overall muscle function.

The underlying mechanisms for this loss of motor neurons are not fully understood with a number of possible causes including dysregulation of mitochondria and oxidative stress, inflammation and neurodegeneration of motor neurons and the attending Schwann cells (Gonzalez-Freire et al. 2014).

1.5.4 Neuromuscular Junction

Innervation of the muscle fibres occurs at the Neuromuscular Junctions (NMJ; Figure 1-16) where the motor neuron forms a pretzel-like structure against the muscle fibre cell. This structure is covered by the terminal Schwann cell and these cells have a role in regenerating the neuronal connection when denervation occurs (Son and Thompson 1995; Reynolds and Woolf 1992), as do the Schwann cells forming the remaining endoneurial tube from an axon undergoing a dying-back (Wallerian degeneration (Conforti, Gilley, and Coleman 2014)) event (Nguyen, Sanes, and Lichtman 2002; A. F. Rosenberg et al. 2014).

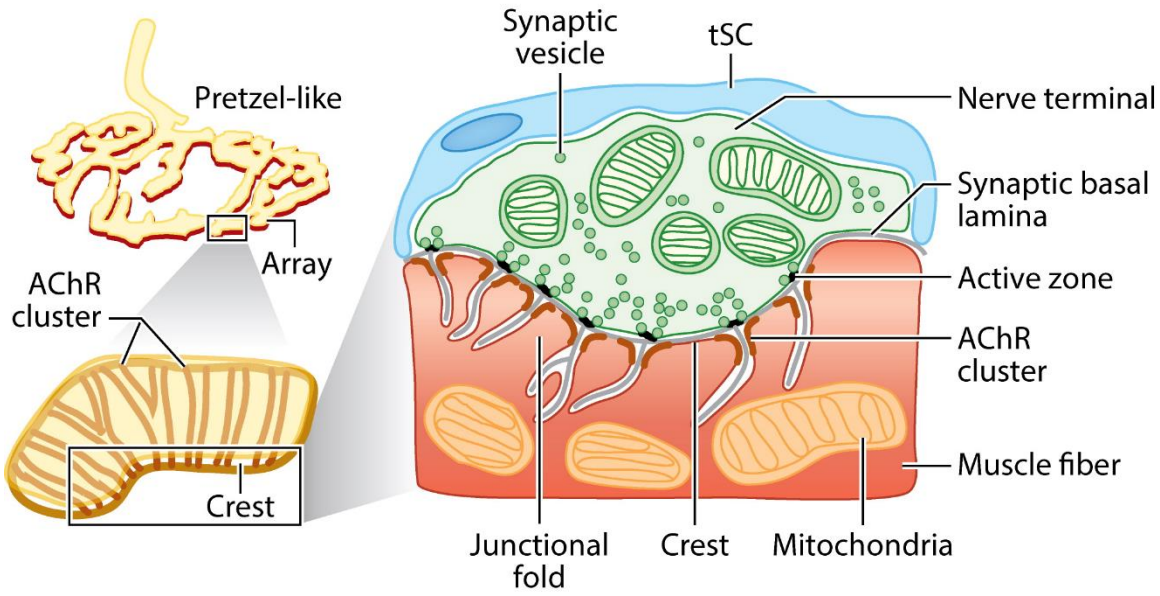

Figure 1-16 : Structure of the Neuromuscular Junction

(L. Li, Xiong, and Mei 2018): tSC -Terminal Schwann Cells, AChR – Acetylcholine receptors

In ageing mammals the postsynaptic site has been observed to fragment into numerous, unconnected sections resulting in decreased Acetylcholine receptor (AChR) density. Additionally the AChR clusters increasingly are not contacted by an axon, or the terminal axons were misshapen. Finally innervation of the same NMJ by multiple axons is also observed and associated with lower motor unit function from fibre atrophy (Valdez et al. 2010; Hepple and Rice 2016).

Neuromuscular junction dysfunction could be the result of an increased breakdown of Agrin, a protein responsible for activation of MuSK (muscle-specific tyrosine kinase), itself involved in the stabilization of the acetylcholine receptor (Bütikofer et al. 2011).

1.5.5 Muscle morphology

The structure of skeletal muscle undergoes changes with age. The intermingling of fibres from different motor units seen in young adulthood, gives way to increasing grouping of fibres of the same type which alters the functionality of the muscle (Figure 1-17) (Hepple and Rice 2016).

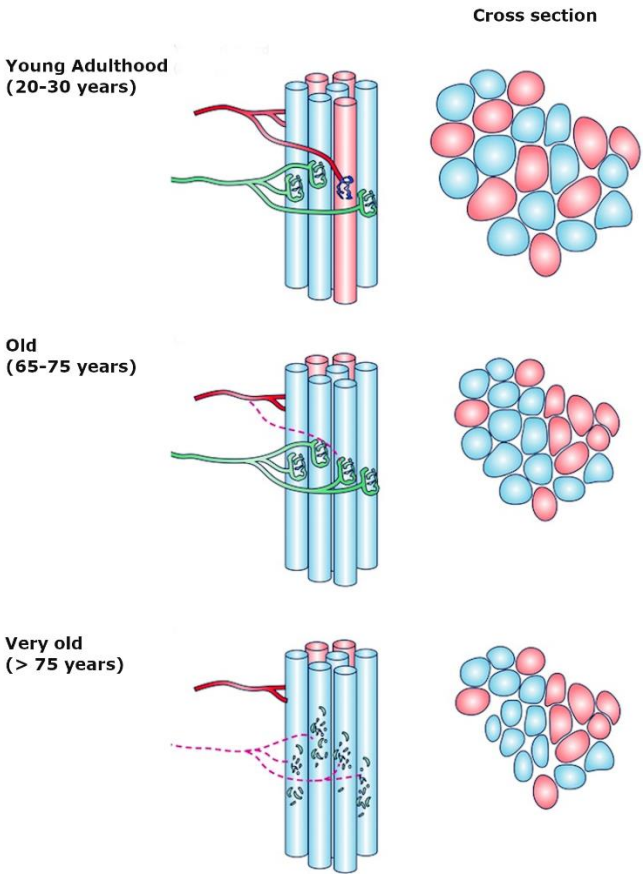

Figure 1-17 : Morphology of ageing muscle

(Hepple and Rice 2016)

The loss of muscle function due to age has been shown to be related to the changes in the population balance of the fibres, with reduced numbers of Type 2 fibers (Porter, Vandervoort, and Lexell 1995).

1.5.6 Muscle-lipid system

Lipids are stored in skeletal muscle as either adipocytes or intramyocellular lipid droplets (Engelke et al. 2018). Adipocytes can be located in between the muscle groups as perimuscular adipose tissue or within the muscle groups as intramuscular adipose tissue (Figure 1-18)

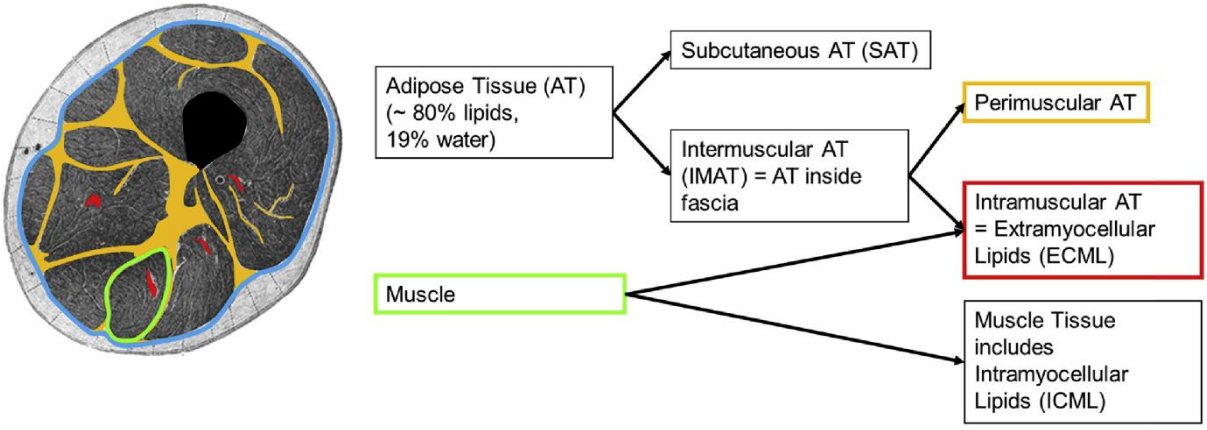

Figure 1-18 : Muscle-lipid system

(Engelke et al. 2018); Cross section of the thigh muscle. Blue outlines the muscle fascia. Green outlines a single muscle Fascicle. Adipose tissue includes intramuscular (red) and perimuscular (yellow)

Accumulation of intramuscular fat increases with age resulting in progressive loss of strength (Delmonico et al. 2009). The infiltration of lipids into the skeletal muscle (myosteatosis) may be due to a number of mechanisms, including a shared origin of osteoblasts and adipocytes from the mesenchymal progenitors with adipogenesis favoured by hormone disruption.

1.5.7 Immune response

Aging is associated with a chronic state of low-grade inflammation characterised by increased levels of pro-inflammatory mediators such as C-reactive protein (CRP), tumour necrosis factor α (TNF-α) and interleukin 6 (IL-6) (Paik et al. 2013; Puzianowska-Kuźnicka et al. 2016). Sarcopenic individuals have shown increased levels of TNF-α and IL-6 when compared to matched controls of similar age (age > 60) (Bian et al. 2017).

Aged tissues accumulate senescent cells, cells that have ceased to divide due to damage or stress. These cells release a range of cytokines, growth factors and proteases cumulatively referred to as the Senescence-associated secretory phenotype (SASP) (Neves et al. 2015).

IL-6 is a component of the SASP (Coppé et al. 2008) and is involved in the transition of muscle satellite cells to an active state. In healthy skeletal muscle this promotes myogenesis (Serrano et al. 2008) upon acute tissue stress, for example in response to exercise. However chronic IL-6 signalling due to the pro-inflammatory state and accumulation of senescent cells observed in older individuals can have a detrimental effect (J. Wang et al. 2017).

C-reactive protein (CRP) is a biomarker for systemic inflammation, and has also been seen to have increased levels in individuals with sarcopenia (Fujikawa et al. 2017) and in particular sarcopenic obesity – a combination of decreased muscle mass and function alongside infiltration of fat cells into muscle tissue and overall high fat mass (Stenholm et al. 2008).

1.5.8 Protein turnover

Protein synthesis and breakdown in skeletal muscle is thought to be due to the interaction of three distinct systems – ubiquitin-proteasome pathway, calpain system and the autophagy mechanism (Tipton, Hamilton, and Gallagher 2018).

One of the best characterised protein synthesis pathways in muscle is the PI3-Kinase/AKT/mTOR cascade (Bodine et al. 2001) (Figure 1-19).

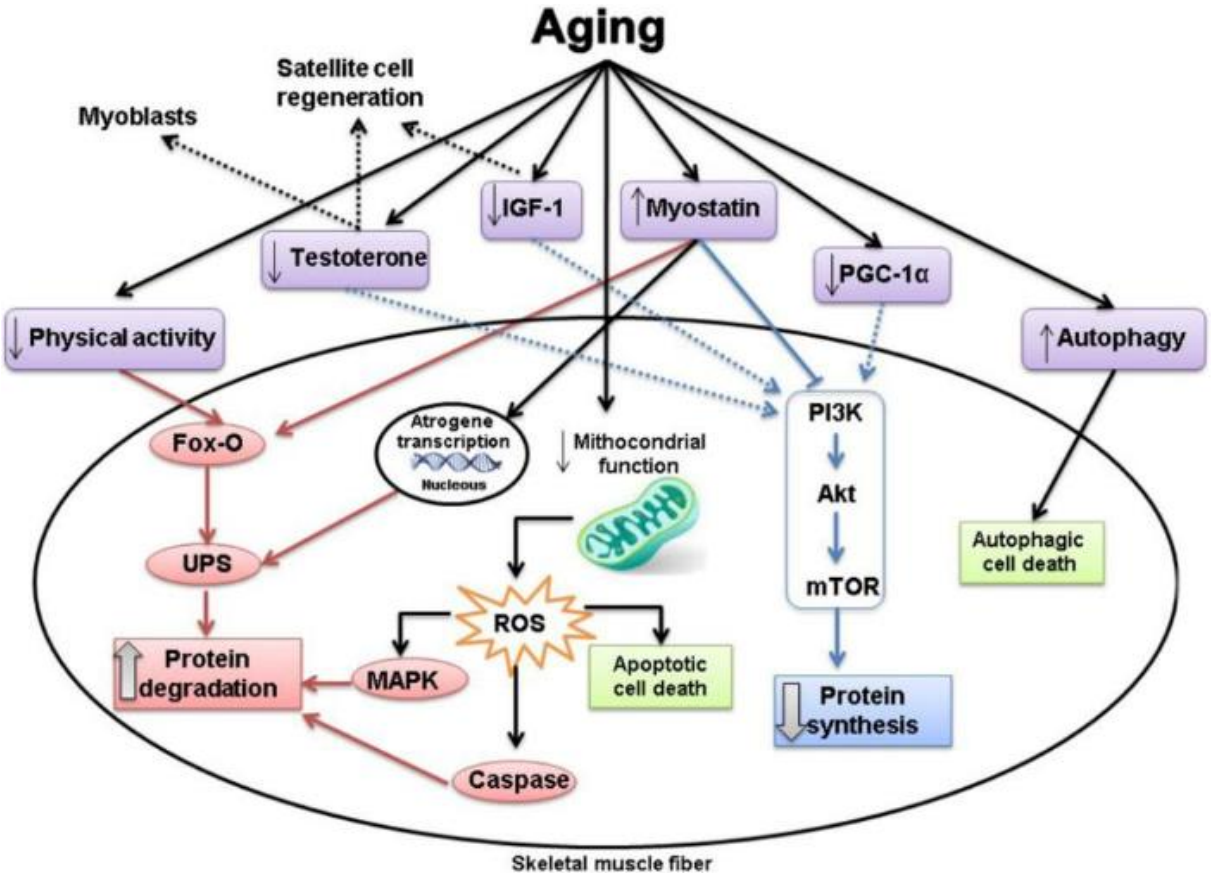

Figure 1-19 : Aging and skeletal muscle protein synthesis and degradation

(Gomes et al. 2017)

The mTOR cascade has been shown to regulate skeletal muscle mass (Yoon 2017), and there are two distinct mTOR complexes, *mTORC1* and *mTORC2*

(Laplante and Sabatini 2012). *mTORC1* in particular has been linked to sarcopenia (H. Tang et al. 2019) and neuromuscular dysfunction in ageing skeletal muscle (H. Tang et al. 2019). This makes a dysfunctional mTOR pathway as a possible candidate for the development of sarcopenia in later life.

A study on the transcriptional markers of Unfolded Protein Response (UPR) pathway in older adults (75 ± 5 years) compared to young adults (27 ± 5 years) showed that after exercise the activation of the pathway was significantly lower in the older participants (Hart et al. 2019).

1.5.9 Oxidative stress

Endogenous reactive oxygen species (ROS) have a central role in the free radical theory of aging (Denham Harman 1992; Kregel and Zhang 2007). Accumulation of ROS and their secondary products increases in skeletal muscle with age, with associated oxidative damage (Jackson and McArdle 2011).

1.5.10 Mitochondria

Mitochondria dysfunction has been postulated as a possible underlying causative mechanism in sarcopenia and weakness associated with skeletal muscle and age (Alway, Mohamed, and Myers 2017).

Sarcopenic skeletal muscle has fewer mitochondria and reduced mitochondrial respiratory complex activity. In addition intracellular levels of NAD+, a major

regulator of oxidative mitochondrial metabolism (Cantó, Menzies, and Auwerx 2015), are lower in sarcopenic muscle (Migliavacca et al. 2019).

1.5.11 Central Nervous System

Age related changes to the nervous system can lead to reduced muscle strength by atrophy of the grey and white matter of the brain, domaminergic degeneration or loss of volume of subcortical structure such as the nasal ganglia and cingulate cortex which have an important role in mediating movement (Manini, Hong, and Clark 2013; B. C. Clark 2019).

1.5.12 Vasculature

The microvasculature of the skeletal muscles is reduced with age (Ryan et al. 2006) and reduced levels of physical activity (Hedman et al. 2002). Studies of sarcopenic older adults has shown that they have a lower capillary-to-muscle fibre ratio (Patel et al. 2015). Skeletal muscle satellite cells, which are essential for repair and regeneration of myofibres, have been shown to be spatially located at a further distance from the muscle fibre capillaries in older men (average age 67 years) when compare to younger controls (average age 24 years) (Nederveen et al. 2016).

1.5.13 Bone

Bone is a highly dynamic tissue with an estimated turnover of the entire human skeleton every ten years (W. C. Lee et al. 2017), through a cycle of resorption and bone formation.

The tissue comprises of two main components, a range of specialised and precursor cells (Table 1-6), and the extracellular matrix (ECM).

Table 1-6 : Bone cell types

Cell type	Function
Mesenchymal stem cells	Multipotent stem cells able to differentiate into mesodermal lineage, for example osteoblasts (Ullah, Subbarao, and Rho 2015).
Pre-osteoblasts	Osteoblast-committed progenitor cells actively proliferate before differentiation.
Osteoblasts	Specialised cuboidal bone-matrix secreting cells, with a large Golgi apparatus and increased rough endoplasmic reticulum. Crucial for bone formation and remodelling (Neve, Corrado, and Cantatore 2011).
Osteocytes	Embedded in the bone matrix and metabolically quiescent – respond to strain and stress on the matrix.
Osteoclasts	Osteoclast precursors derived from circulating mononuclear monocyte macrophages. Multinucleated Osteoclasts formed from the fusion of precursors are essential for bone resorption and repair (Schell et al. 2006).
Bone lining cells (BLC)	Post-mitotic flat osteoblast lineage cells, can be reactivated as functional osteoblasts and pre-osteoblasts (Matic et al. 2016).

Osteoporosis can be defined as “systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture” (“Consensus Development

Conference: Diagnosis, Prophylaxis, and Treatment of Osteoporosis" 1993). There is evidence that sarcopenia and osteoporosis share common underlying mechanisms such as increased inflammation, and sensitivity to anabolic hormone secretion (Cruz-Jentoft et al. 2010; Reginster et al. 2016).

Osteoblasts are responsible for secretion of certain hormone and signalling molecules related to skeletal muscle mass, such as osteocalcin (A. J. Lee, Hodges, and Eastell 2000).

1.5.14 Hormones and signalling molecules

Muscle cells secrete a wide range of signalling molecules termed myokines (B. K. Pedersen et al. 2003). They can be characterised as cytokines and other peptides that are expressed and released by the muscle fibres, often in response to muscle contraction, and exert either paracrine or endocrine effects (Bente Klarlund Pedersen et al. 2007).

These include the interleukins interleukin-6 (IL-6), interleukin-7 (IL-7) and interleukin-15 (IL-15); insulin-like growth factor 1 (IGF-1); Myostatin, follistatin and decorin; as well as additional myokines such as osteoglycin (OGN)(Guo et al. 2017).

Testosterone has been shown to decrease in males with age (androgen deficiency), with a corresponding increase in luteinising hormone (LH), follicle-stimulating hormone (FSH) and sex hormone-binding globulin (SHBG) (John E. Morley et al. 1997). Testosterone can induce an increase in muscle mass by

hypertrophy of myofibres (Sinha-Hikim et al. 2002) and upregulating the proliferation of satellite cells (Bhasin et al. 2003).

Specific muscle force (maximum voluntary force per cross-sectional area) declines significantly in post-menopausal women, with the decline in men delayed until a later age (over 60 years of age) and only reaching the level seen in post-menopausal women, after the age of 75. Hormone replacement therapy (either sequential oestrogen / progestin or oestrogen alone) has been shown to reduce this muscle weakness (Phillips et al. 1993). A cross-sectional study of 144 women aged 30-70 found that prevalence of sarcopenia (as defined by appendicular lean mass adjusted by the square of the participant's height or ALM index ≤ 5.67 Kg/m^2, International Working Group on Sarcopenia (Fielding et al. 2011)) in early (n=31, 50 ± 3 years) and late (n=30, 50 ± 4 years) perimenopausal, and early (n=26, 55 ± 3 years) and late (n=27, 62 ± 4 years) postmenopausal as 3%, 30%, 27% and 32%. This is compared to the control group of premenopausal women (n=30, 38 ± 6 years) with a prevalence of 7%. Measured Follicle Stimulating Hormone (FSH) was found to be negatively correlated with ALM index ($r = -0.28$, $p = 0.003$), while oestradiol was not significantly correlated ($r = 0.088$, $p= 0.34$) (Park et al. 2020). Sex hormone-binding globulin (SHBG) regulates the amounts of biologically active testosterone and oestradiol by binding to them with different affinities (Laurent et al. 2016).

Insulin-like Growth Factor-1 (IGF-1) is secreted by many tissues including the liver and skeletal muscle and has no specific target tissue after entering the bloodstream, despite this tissues capable of producing their own IGF-1 seem to be affected to a lesser extent by lower circulating IGF-1 levels (Sjögren et al. 1999).

IGF-1 and growth hormone (GH or somatotropin, *GH1*) form part of the GH/IGF-1 axis which amongst a wide range of pleiotropic effects, promotes growth. Levels of GH and IGF-1 decline after reaching adulthood to low levels in the over 60s in humans (Zadik et al. 1985; Junnila et al. 2013), a period referred to as the "somatopause"(Lombardi et al. 2005; Bartke 2008). Decreased GH/IGF-1 has been linked to extended longevity in a range of organisms including humans (Fontana, Partridge, and Longo 2010). IGF-1 is a regulator of the PI3K/AKT/mTOR pathway, via its receptor.

Higher systemic levels of GDF11 (Growth Differentiation Factor 11, a member of the Transforming Growth Factor β – TGFβ family) have been shown to impair satellite cell proliferation and so inhibit muscle fibre regeneration (Y. S. Lee and Lee 2013). Myostatin (GDF8) and GDF11 both bind to activing type II receptors and activate the SMAD 2/3 pathway (Trendelenburg et al. 2009; Paul Oh et al. 2002), which in turn is an important regulator of muscle mass in adult humans (Sartori et al. 2009). GDF11 levels have been shown to increase with age in both humans and mice and inhibits the regeneration of skeletal muscle (Egerman et al. 2015).

Loss of the bone derived hormone Osteocalcin has been shown in the female mouse model (homozygous negative *Ocn* -/-) to result in decreased skeletal muscle mass (in particular the hindlimb muscle). The decrease in muscle mass appears to be the result of loss of cross-sectional area from the myofibers, via signalling of the *Gprc6a* receptor (Oury et al. 2011) and dysregulation of protein synthesis, however it should be noted that muscle function appeared to be maintained (Mera et al. 2016). In older mice exogenous osteocalcin treatment

was shown to increase muscle mass, although muscle strength (Mera et al. 2016).

1.6 Genetics of associated traits

Previous studies have investigated the genetics of measurements used to define sarcopenia including maximal grip strength and appendicular lean muscle mass. Additional work is underway within the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium for metrics such as the Short Physical Performance Battery.

A Genome-Wide Association Study (GWAS) is a methodology for testing variants across the genomes from many participants in order to discover genotype-phenotype associations with a given trait. The variant or genotype data being commonly provided by SNP (Single Nucleotide Polymorphisms) arrays containing millions of oligonucleotide probes specific for certain variants or alleles – further details are available in Chapter 2 - Methods.

Significant associations between the genotype and phenotype identify genomic risk loci, often covering a region with multiple variants due to the variants being inherited together (Linkage Disequilibrium). GWAS have successfully identified risk loci for a wide range of traits and diseases (Tam et al. 2019).

1.6.1 Maximal grip strength

Twin/Sibling studies with young adult, middle aged (59-69) or extreme age (over 90) participants have shown grip strength to be a highly heritable trait with h^2 between 30-65% (Matteini et al. 2010; Reed et al. 1991; Silventoinen et al. 2008).

Linear grip strength has been investigated in the UK Biobank (UKB) dataset by both Tikkanen (2018) and Willems (2017). Willems utilised data from the May 2015 imputed interim genotyping release, restricting the analysis to biallelic Single Nucleotide Variants (SNVs) with a Minor Allele Frequency (MAF) ≥ 1% from 142,035 UKB participants self-identifying as having white ancestry. Replication was undertaken with 53,145 European individuals from the CHARGE consortium (Willems et al. 2017).

By contrast Tikkanen used the full UKB July 2017 imputed genotyping release with only unrelated individuals who clustered by principal component analysis with European ancestry and self-reported as white British descent. Genetic markers with a minor allele count of equal to or less than 30 and/or a low imputation quality (<0.8) were excluded. The initial discovery Genome Wide Association Study (GWAS) was carried out on a random sub-sample of 223,315 UKB participants, replication was undertaken using the remaining 111,610 individuals and then a meta-analysis was undertaken with both datasets for the Mendelian Randomization and pathway analysis (Tikkanen et al. 2018).

Willems *et al*, identified 16 genomic loci (a region containing a number of genetic variants or polymorphisms, often in linkage disequilibrium, which shows significant association with the trait of interest) associated with maximal hand grip strength. These included variants proximal to *TGFA* (rs958685 - Transforming Growth Factor Alpha, involved in cell proliferation, differentiation and development), *POLD3* (rs72979233 - DNA Polymerase Delta 3, Accessory Subunit, involved in DNA replication and repair), *ERP27* (rs11614333 - Endoplasmic Reticulum Protein 27, part of the Unfolded Protein Response of the ER) and *HOXB3* (rs2288278 - Homeobox B3 part of the cell development

pathways). The study also highlighted the HLA (rs78325334) region. Fifteen of the 16 loci had MAF of over 5%, with only one significant locus close to *DEC1* (MAF=3%) having a MAF of less, and so a high prevalence in the general population. The authors highlighted the possible roles for the proximal genes in the structure and function of skeletal muscle (*ACTG1* encodes a component of the costmere, part of the Z-disc of the sarcomere) and cell growth (*TGFA* – Transforming Growth Factor Alpha).

Tikkanen identified 101 loci associated with maximal grip strength in their discovery dataset (N = 223,315, $P < 5 * 10^{-8}$), and achieved replication for 64 of these (N = 111,610, P < 0.01). The most significant associations in the discovery analysis where seen for *FTO* (rs1421085), *ATXN2L* (rs12928404), *SLC39A8* (rs13107325) and *ADCY3* (rs10203386). Although 7 of the loci are located on chromosome 6, only two are within the HLA region, defined as between the start of the *MOG* gene GRCh37 Chr6:29624758 and the end of the *COL11A2* gene GRCh37 Chr6:33160276. These were *HLA-DRB1* (rs2760975) and *PRRC2A* (rs2260051), although only *PRRC2A* was significant in both the discovery and replication datasets.

The study implicated the *FTO* locus, rs1421085 (long associated with obesity(Loos and Yeo 2014)) and a possible regulatory variant, rs12928404, close to *ATXNL2L* and *ATP2A1*. ATP2A1 is a SERCA Ca(2+) ATPase, an intracellular pump embedded in the sarcoplasmic reticula of muscle cells (Hovnanian 2007; Winther et al. 2013) and is known to be involved in Brody disease, a rare inherited disorder of skeletal muscle function where exercise induces uncontrollable muscle relaxation, stiffness or cramps (Odermatt et al. 1996). *ATP2A1* was identified by the gene-based analysis as the most significant

gene for grip strength. It should be noted that rs12928404 is an eQTL for *TUFM* in a number of tissues. *TUFM* encodes a protein product with a mitochondrial import signal (Christian and Spremulli 2012) and is involved in protein synthesis.

Tikkanen concluded that grip strength shared biological pathways with frailty and that in addition to genes related directly to muscle function, neuro-development and maintenance was also linked to maximal grip strength.

1.6.2 Lean muscle mass

A 2017 meta-analysis of lean muscle mass analysed 38,292 participants of European ancestry from 20 cohorts in the discovery set, with whole body lean mass measured by either dual energy X-ray absorptiometry (DEXA, 21,074 participants) or bioelectrical impedance (BIA, 17,218 participants) (Zillikens et al. 2017). In a similar manner appendicular lean muscle mass was estimated from 15 cohorts with 28,330 participants (6 cohorts using BIA, the majority measured with DEXA).

Replication was analysed using 33 studies with 63,475 participants (47,227 of European ancestry and 16,248 African American, South Asian or Korean ancestry) for whole body lean muscle mass and for appendicular lean muscle mass

Five variants were associated with whole body lean mass and were replicated, rs2943656 (*IRS1* - Insulin Receptor Substrate 1), rs9991501 (*HSD17B11*-Hydroxysteroid 17-Beta Dehydrogenase 11), rs2287926 (*VCAN* – Versican),

rs4842924 (*ADAMTSL3* - ADAMTS Like 3) and rs9936385 (*FTO* - FTO Alpha-Ketoglutarate Dependent Dioxygenase). The variants for *IRS1*, *VCAN* and *ADAMTSL3* were replicated in the appendicular lean muscle mass cohort (Zillikens et al. 2017). *ADAMTSL3* has previously been associated with height (H. L. Allen et al. 2010) and Zillikens *et al* proposed that the variants at this genomic risk locus could be directly involved in muscle mass via growth and development pathways rather than height, since their analysis adjusted for height. *VCAN* is involved in chondrocyte differentiation and joint development (Choocheep et al. 2010), *IRS1* is part of the insulin signalling pathway and is highly expressed in skeletal muscle (Long et al. 2011), while *FTO* is required for myogenesis (X. Wang et al. 2017).

1.7 Biomarkers associated with sarcopenia

Age related biomarkers have been analysed in the UK Biobank cohort (396,707), using the EWGSOP2 definition of sarcopenia (Cruz-Jentoft et al. 2019). Under the revised definition low grip strength (<26 kg in men and <16 kg in women) and low muscle mass (appendicular lean muscle mass (kg) divided by height (m) as measured by bioimpedance; < 7.0 kg/m^2 for men and < 5.5 kg/m2 for women) is categorised as sarcopenia. The addition of self-reported slow gait speed was used as a proxy for a gait speed of less than or equal to 0.8 m/s, as EWGSOP2 defines participants with all three criteria as having severe sarcopenia.

For the biomarker analysis both those with severe sarcopenia (3 criteria) and confirmed sarcopenia (2 criteria) were analysed together to give a prevalence of

0.9% in female participants (n=209,722, cases=1,990, mean age 55.8 ± 8 years) and 0.1% in male participants (n=186,935, cases=112, mean age 60.6 ± 7.4 years) (Petermann-Rocha, Gray, et al. 2020).

Out of the initial 33 biomarkers, 20 were associated with sarcopenia in women and 18 in men (Figure 1-20). Overall the results highlight the complex multifactorial nature of muscle loss and weakness with age. IGF-1 (GH/IGF-1 axis) was associated with sarcopenia in this study, and have previously been linked to muscle wastage (Giovannini et al. 2008). Higher levels of sex hormone-binding globulin (SHBG) were also associated with sarcopenia in the analysis and has previously been observed alongside reduced appendicular skeletal muscle mass in older men (Baumgartner et al. 1999). Lower levels of testosterone were associated with sarcopenia in the female participants, although the coefficient is inconclusive in the male participants. This could be due to the low average age of the participants and the earlier and more profound onset of the menopause in women compared to the andropause in men (Horstman et al. 2012).

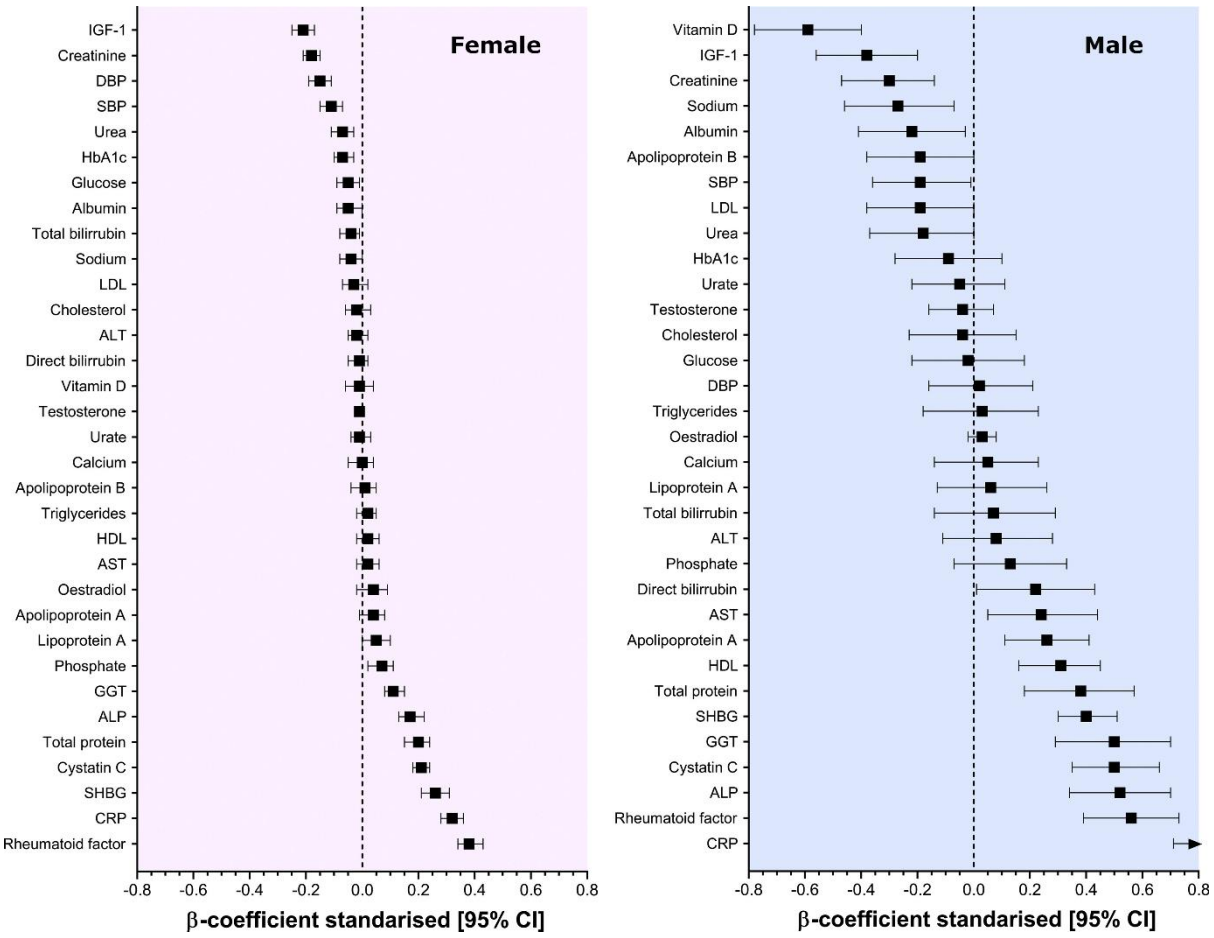

Figure 1-20 : Association between age-related biomarkers and sarcopenia

(Petermann-Rocha, Gray, et al. 2020)

Biomarkers of renal function, derived from the turnover of muscle mass such as lower creatinine and higher cystatin C were also observed to be associated with sarcopenia. Inflammatory markers such as rheumatoid factor and C-reactive protein (CRP) were also associated, which highlights a possible role of the immune system in the development of sarcopenia and age-related loss of muscle mass and function.

1.8 Summary

Current understanding of sarcopenia and muscle weakness directly related to age is that there is a multifactorial pathogenesis (Figure 1-21) with a diverse set of interacting pathways resulting in a pathological end state.

Various studies have shown the extreme range of muscle fibre cross-sectional area in aged muscle, in particular in Type 2 fibres (J Lexell and Taylor 1991) and the accumulating presence of both atrophied and hypotrophic fibres (Jan Lexell, Taylor, and Sjöström 1988). Age related denervation and re-innervation of the neuromuscular junction (NMJ) is only one possible cause of this dysfunction and associated muscle wasting (Tintignac, Brenner, and Rüegg 2015) and reasons include oxidative stress and inflammation (J. Wang et al. 2017), and incomplete or misconfiguration of the NMJ during a re-innervation (Hepple and Rice 2016).

My work aims to understand some of the inheritable causes to the underlying mechanisms responsible for loss of muscle mass and function, and in turn sarcopenia. In addition this thesis will provide evidence for the genetic contribution to sarcopenia and loss of muscle function with age, which will provide plausible pathways to explain the variability in muscle function between older individuals, and the reasons for certain individuals' susceptibility to physical frailty.

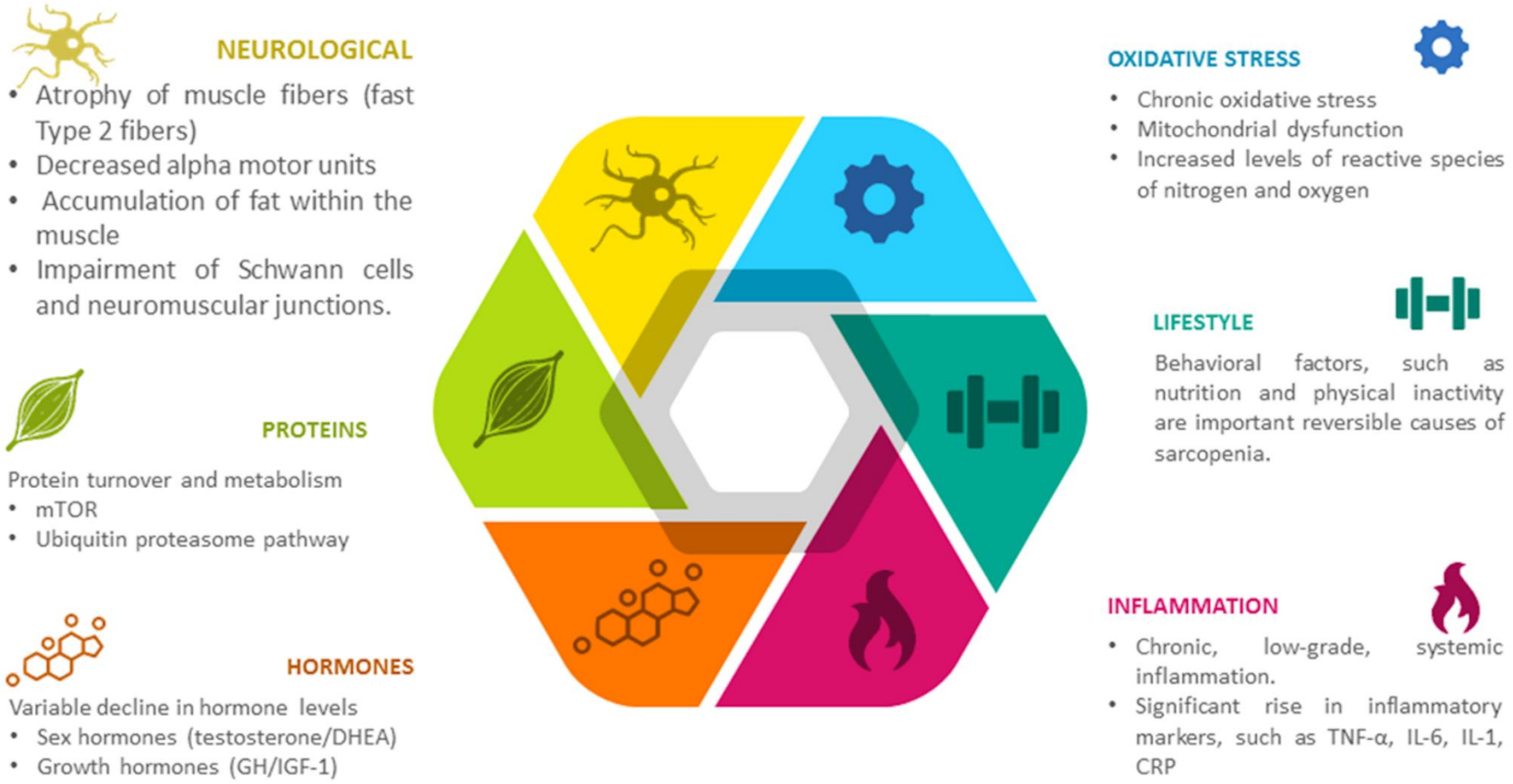

Figure 1-21 : Multifactorial pathogenesis of dynapenia and sarcopenia

1.9 Aims and objectives

There have been previous large scale studies of grip strength (Willems et al. 2017; Tikkanen et al. 2018), however these have been across all age ranges and of the continuous trait of grip strength. The focus of my thesis is older individuals and using the defined cut-offs for low grip strength derived from sarcopenia definitions, as proxies for whole body loss of muscle function.

In the course of my research I have investigated low strength in older adults in order to identify mechanisms that result in some older individuals having a higher susceptibility to physical weakness / frailty than others. I hypothesize that these specific pathways that result in weakness with age are distinct from overall strength across the life course.

Previous studies using the diagnostic criteria from the definitions of the sarcopenia have been unable to robustly identify significant risk loci, although sample size was undoubtedly a factor.

By using the definitions of low grip strength and lean muscle mass provided by international sarcopenia consortia I have found evidence that sarcopenia does have a multifactorial pathogenesis with multiple routes to the pathogenic state and that it does indeed have a distinct genetic component set apart from those seen for maximal grip strength.

1.9.1 Chapter Aims

Chapter 3: HLA and sarcopenia; to identify alleles of the HLA locus that are associated with Sarcopenia and Dynapenia in older adults.

In the first results chapter I have investigated the HLA region and HLA haplotypes, and their relationship to sarcopenia. I have shown how different measures of sarcopenia, either low grip strength or appendicular lean muscle mass, or both metrics taken together, are associated with different sets of HLA haplotypes. I also show how combinations of HLA types can increase risk of meeting the definition of sarcopenia.

Chapter 4: GWAS meta-analysis of 22 CHARGE cohorts for dynapenia, as characterised by low grip strength, in order to identify genomic risk loci associated with dynapenia

In this results chapter I use data from 256,523 individuals of European ancestry aged 60 years or older from 22 international cohorts in order identify 15 genomic risk loci associated with muscle weakness. These loci are involved in growth and development of the musculo-skeletal system, control of the immune system, regulation of transcription and protein metabolism.

Chapter 5: Sex-stratified analysis of GWAS meta-analysis of 22 CHARGE cohorts for dynapenia. By investigating sex stratified subsets of the meta-analysis I show that the genomic risk loci for muscle weakness with age has distinct loci for each sex.

Chapter 6: Mendelian Randomisation analysis of meta-analysis against a range of traits associated with aging and frailty to investigate possible relationships and causal mechanisms.

In my final results chapter I show that there are shared casual pathways between muscle weakness with age and traits such as age of menarche, birth weight, and Rheumatoid arthritis.

2 Methods

2.1 UK Biobank study

The UK Biobank is a large population based prospective cohort with extensive baseline data captured during recruitment including physical measurements, biochemical assays and genomic information (Sudlow et al. 2015). The number of participants, general composition reflecting the UK population - although with a healthy volunteer bias, and age range (40-70 years) made the UK Biobank the most appropriate available source of data given the overall aim of the thesis, which is studying the effect of inheritable factors in low muscle function with age.

Integration of the study with national health records and subsequent additional measures such as Magnetic Resonance Imaging (MRI) of participants and monitoring of physical activity, has increased the utility of the study and allowed researchers unprecedented access to a large population based cohort with comprehensive phenotyping (Bycroft et al. 2018).

Recruitment to the UK Biobank study throughout the period 2006 – 2009, involved mailed invitations to 9.2 million potential participants, aged between the ages of 40 to 69 years and living within approximately 25 miles of one the 22 study assessment centres. With a response rate of 5.47%, 503,325 individuals were recruited to take part in the study (N. Allen et al. 2012).

Figure 2-1: UK Biobank assessment centres

www.ukbiobank.ac.uk

Genetic data derived from the two microarray panels used by UK Biobank was available on 488,377 UK Biobank participants after genotype calling and quality control performed centrally by the UK Biobank team (Bycroft et al. 2018).

For the main focus of my thesis I analysed participants over the age of 60 at baseline, and whose genotypes clustered by principal component analysis of the 1000 genomes data, into a group identified as European ancestry (Thompson et al. 2019a) (clustering of populations is explained in further in detail in section 2.1.3). Those participants of European ancestry form the largest ancestral group within the UK Biobank and the constituent studies available through the CHARGE consortium, and so give the greatest statistical power. This is especially important given the stratification of the

cohorts into older individuals in order to separate age-related sarcopenia and muscle weakness from cachexia, or loss of muscle function and mass due to a chronic non-age related condition, which in turn reduces the overall study sample size.

2.1.1 Description of cohort

9,238,453 individuals registered with the National Health Service (NHS), were selected for inclusion in the UK Biobank on the criteria of aged between 40 and 69 years, and had a postal address within 25 miles of one of the 22 assessment centres (see Figure 2-1: UK Biobank assessment centres). From these initial invitations 503,317 participants joined the study and attended one of the assessment centres (participation rate 5.45%) (Fry et al. 2017). The low response rate reduces the ability of the study to accurately reflect the overall general population.

2.1.2 Healthy volunteer bias

A comparison of the prevalence of self-reported health conditions between the participants of the UK Biobank (2006 – 2010) and individuals from the Health Survey for England (2006, 2009 and 2010) highlighted that UK Biobank participants are healthier than the general population (see Table 2-1) (Fry et al. 2017). Health conditions such as cardiovascular disease are more prevalent in the 55-64 age range for both males and females in the general population, compared to the UK Biobank participants (18.5% and 15.2% for males and females in the Health Survey for England study compared to 11.5% and 5.0% for males and females in the UK Biobank cohort). This trend is consistent across a range of long term health conditions, with the exception of Chronic Obstructive Pulmonary Disease (COPD) in older females in the UK Biobank (0.1% in HSE compared to 0% in UK Biobank), although the difference is marginal. The authors noted that despite this, the assessment of exposure-disease relationships may be generalizable and does not require the participants to represent the population at large (Fry et al. 2017).

Table 2-1: UK Biobank healthy participant bias

	Men				**Women**			
Self-Reported Disease	Age 45–54 Yrs		Age 55–64 Yrs		Age 45–54 Yrs		Age 55–64 Yrs	
	UKB	HSE	UKB	HSE	UKB	HSE	UKB	HSE
Cardiovascular disease	4.6	10.9	11.5	18.5	2.4	10.3	5.0	15.2
Ischemic heart disease	2.8	3.6	7.9	10.6	0.9	1.3	2.6	3.5
Stroke	0.8	1.2	1.9	3.0	0.6	0.9	1.0	2.3
Angina	1.8	2.4	5.3	8.0	0.7	1.2	2.1	3.2
Myocardial infarction	1.7	2.1	4.5	6.3	0.3	0.7	0.9	1.6
Abnormal heart rhythm	1.5	5.7	3.1	6.3	1.4	5.7	2.2	7.3
Hypertension	21.2	27	34.4	39	15.4	16	27.4	29
Diabetes	4.5	8.1	7.8	10.5	2.4	3.5	6.3	8.0
Chronic kidney disease	0.2	1.1	0.3	1.5	0.2	1.2	0.2	1.9
Asthma	11.7	12	9.9	13	13.0	16	11.8	15
COPD	0.1	1	0.4	3	0.1	0	0.4	2

HSE=Health Survey for England 2006/2009/2010 data; UKB=UK Biobank Self-reported health conditions; fields in green show lower incidence for each condition compared to the alternative dataset (in orange); adapted from (Fry et al. 2017)

2.1.3 Population structure

The UK Biobank mapped self-reported ethnicity of 141,670 samples against the results of Principal Component Analysis (PCA) of 101,284 SNPs in order to identify underlying population structure (Sze and Schloss 2016; Bycroft et al. 2018).

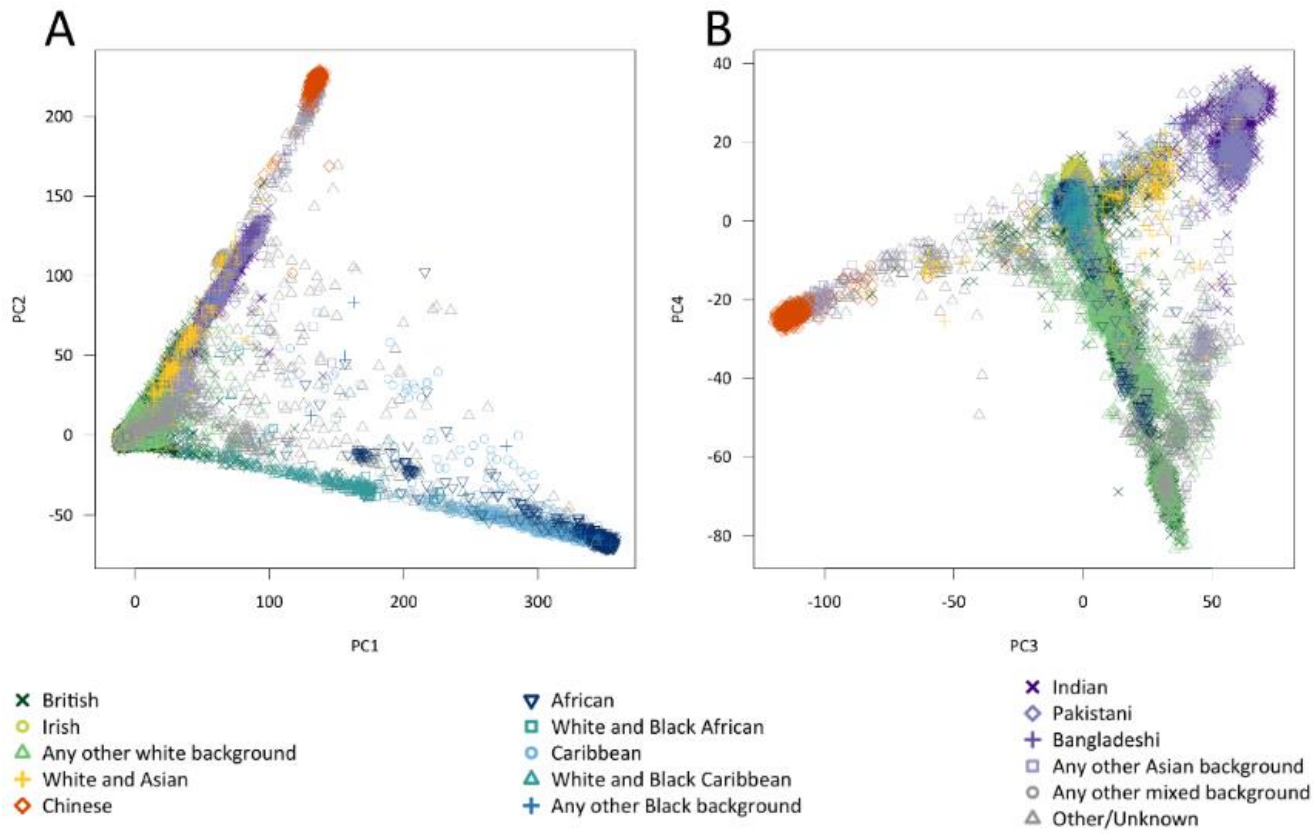

Figure 2-2: Genetic principal components in UK Biobank

Chart A shows the first principal component (PC1) against the second (PC2); Chart B shows the third principal component (PC3) against the fourth (PC4). Charts are overlaid with the UK Biobank self-reported ethnicity (Sze and Schloss 2016).

Analysis throughout this thesis focused on individuals identified as European ancestry due to this population being the largest group in the UK Biobank cohort. This was due to the required statistical power to detect the large number of variants with small effect

sizes associated with a complex trait, such as low grip strength. Some initial investigation was conducted into using studies that are part of the CHARGE consortium with predominantly Korean participants, however the lack of a similar population in the UK Biobank and the small size of the Korean study made this unfeasible.

In order to obtain these individuals principal components were generated using the 1000 Genomes Cohort high confidence SNPs by Dr Andrew Wood (University of Exeter Medical School). The individual loadings from these variants were then used to project the UK Biobank data into the same principal component space, with K-means clustering from principal components 1 to 4 (Pilling et al. 2017). Each participant could then be assigned to one of the five super-populations from the 100-genome project (European, African, East-Asian, South-Asian and Admixed) (Auton et al. 2015).

2.1.4 UK Biobank older people cohort

The focus of the thesis was on older adults in the UK Biobank and so only participants between the age ranges of 60 to 70 were included in the subsequent analysis. The lower bound for the age range was selected as one of the common chronological ages used to define the onset of old age, and 23% of the global burden of disease linked to people over this age (Prince et al. 2015). Individuals in this age category (60-70) are often referred to as “young-old” and exhibit the earliest signs of functional decline specific to the ageing process (K.-L. Chou and Chi 2002). By stratifying the analysis by this age group I aim to reduce the influence of incidence of loss of muscle function

and mass due to chronic illnesses that are not age-related (cachexia) and to still investigate the earliest detectable signs of age-related changes.

In addition participants without values for the required fields to define either low grip or sarcopenia (maximum linear grip strength in either hand and skeletal muscle mass, calculated according to the equation by Janssen et al (Ian Janssen et al. 2000)) were excluded. Analysis was confined to participants falling within the 1000 Genome European reference population cluster as described above.

Definitions of sarcopenia and low grip are discussed in more detail in the Introduction (see Section 1.2) and the specific methods sections for each of the analysis chapters. Briefly, the European Working Group on Sarcopenia in Older People 2010 (Cruz-Jentoft et al. 2010) and the Foundation for the National Institutes of Health sarcopenia (Dam et al. 2014) definitions were used for cut-offs and analysis throughout the thesis. Participants who had withdrawn permission for use of their data were removed at the start of the project.

2.2 CHARGE consortium

The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) was formed by five founding members in 2008 to coordinate GWAS meta-analyses of a range of phenotypes associated with aging (Psaty et al. 2009). By meta-analysing GWAS from multiple separate cohorts the statistical power to detect significant associations can be increased (Zeggini and Ioannidis 2009), and the results are more generalizable across multiple populations.

We submitted a study design for consideration by the constituent member cohorts, in 2018. Twenty two studies, including our own analysis using UK Biobank, were

recruited from CHARGE cohorts (see Table 2-2). The CHARGE consortium cohorts involved in the meta-analysis are described in the following sections 2.2.1 to 2.2.16.

Table 2-2: CHARGE consortium cohorts - participant numbers

Study	Females EWGSOP (<20kg)	Males EWGSOP (<30kg)	Females FNIH (<16kg)	Males FNIH (<26kg)	Females Total	Males Total	Total
ARIC	650	289	217	134	2,025	1,630	3,655
BASE-II	55	19	5	4	779	752	1,531
BPROOF	328	145	137	64	1,244	1,275	2,519
CHS	601	201	231	88	1,855	1,206	3,061
EPIC-Norfolk	926	503	326	219	3,962	3,546	7,508
FHS	400	142	153	60	1,461	1,245	2,706
HRS	2,380	1,244	1,089	665	6,164	4,650	10,814
InCHIANTI	162	75	90	48	458	361	819
LASA I	121	77	43	37	249	255	504
LASA II	192	85	68	36	632	589	1,221
Long Life Family Study	824	586	446	397	1,788	1,571	3,359
MrOS Gothenburg	0	35	0	10	0	941	941
MrOS Malmo	0	29	0	12	0	891	891
ROSMAP 1	661	198	346	129	1,096	473	1,569
ROSMAP 2	197	48	108	32	266	95	361
Rotterdam Study I	511	234	253	121	853	587	1,440
Rotterdam Study II	273	137	121	58	684	565	1,249
Rotterdam Study III	146	86	41	36	844	640	1,484
SHIP	96	35	43	8	523	513	1,036
TSHA	844	447	525	316	1,218	882	2,100
UK Biobank	24,229	8,807	8,966	3,941	105,597	94,968	200,565
WLS	993	585	393	319	3,770	3,420	7,190
Total	**34,589**	**14,007**	**13,601**	**6,734**	**135,468**	**121,055**	**256,523**
	EWGSOP total=	**48,596**	***FNIH total=***	**20,335**			

2.2.1 Atherosclerosis Risk in Communities

The Atherosclerosis Risk in Communities (ARIC) Study is a population-based prospective cohort study of cardiovascular disease and includes a total of 15,792 participants aged 45-64 years at baseline (1987-89), chosen by probability sampling from four US communities (The ARIC investigators 1989). Cohort members completed five clinic examinations, conducted approximately three years apart between 1987 and 1998, with a fifth visit conducted from 2011 – 2013. Clinic examinations included assessment of cardiovascular risk factors, self-reported medical family history, employment and educational status, diet, physical activity, comorbidity, clinical and laboratory measurements.

2.2.2 Berlin Aging Study II

Berlin Aging Study II (BASE-II) is a multidisciplinary study initiated in 2009 investigating factors related to human aging (Bertram et al. 2014). All subjects are recruited from the Berlin metropolitan area and underwent an extensive phenotypic assessment, including a 2-day internal medicine examination (follow-up will be completed in 2020).

2.2.3 B-Vitamins for the prevention of Osteoporotic fractures

The B-Vitamins for the prevention of Osteoporotic fractures (B-PROOF) study is a randomized, placebo-controlled, double-blind trial that studied the effect of vitamin B12 and folic acid supplementation on osteoporotic fractures in 2,919 people of 65 years

or over, and having homocysteine levels of 12-50 µmol/L. Participants were recruited between 2008 and 2011 and followed during a 2-3 year period (J.P. et al. 2011).

2.2.4 Cardiovascular Health Study

The Cardiovascular Health Study (CHS) is a population-based, prospective cohort study of risk factors for development and progression of CHD and stroke in older adults ages 65 years or older (Linda P. Fried et al. 1991). A cohort of 5,201 non-institutionalized men and women were selected and enrolled from randomly generated Medicare eligibility lists in 4 U.S. communities in 1989-90; an additional 687 predominantly African American participants were recruited and enrolled in 1992-93. Clinic examinations were performed at study baseline, at annual visits through 1999, and again in 2005-2006. Participants were contact by telephone annually between exams, and every 6 months after the exams ended. Multiple physical and biological tests have been performed, including assessment of physical function.

2.2.5 European Prospective Investigation of Cancer Norfolk

The European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study (“The EPIC (European Prospective Investigation into Cancer) Norfolk Cohort,” n.d.) is a prospective population-based cohort study which recruited 25,639 men and women aged 40-79 years at baseline between 1993 and 1998 from 35 participating general practices in Norfolk, UK. Individuals attended for a baseline health check including the provision of blood samples for concurrent and future analysis. Further health check visits have been conducted since the baseline visit. Participants have

contributed information about their diet, lifestyle and health through questionnaires and health checks over two decades.

2.2.6 Framingham Heart Study

The Framingham Heart Study (FHS) is a single-site, community-based cohort study that was initiated in 1948 to investigate risk factors for cardiovascular disease and other major illnesses. The FHS includes three generations: the original cohort followed since 1948 (Gen1) (Dawber and Kannel 1966); their offspring and spouses of the offspring, followed since 1971 (Offspring or Gen2) (Feinleib et al. 1975); and children of the offspring enrolled in 2002 (Third Generation or Gen3) (Splansky et al. 2007). The Original cohort enrolled 5,209 men and women who comprised two-thirds of the adult population then residing in Framingham, MA, USA. The Offspring cohort comprises 5,124 persons who have been examined about every 4 to 8 years. Examination 1 of the Gen3 occurred between 2002 and 2005 and involved 4,095 participants. Offspring spouses not previously enrolled who were a biological parent of a Gen 3 participant were enrolled into the New Offspring Spouse cohort to complete family pedigrees. All cohorts continue under active surveillance. The FHS follows two multi-ethnic cohorts, Omni group 1 and Omni group 2 to reflect the current diversity of the town of Framingham, MA.

2.2.7 Health and Retirement Study

The Health Retirement Study (HRS) is representative American cohort with participants over age 50 to monitor factors related to aging and retirement (Fisher and Ryan 2018; Juster and Suzman 1995). A random subset of ~26,000 participants were

selected in three phases (phase 1 in 2006, phase 2 in 2008, and phase 3 in 2010) to collect biological specimen between 2006 and 2010.

2.2.8 InCHIANTI

The InCHIANTI study is a population-based epidemiological study aimed at evaluating the factors that influence mobility in the older population living in the Chianti region in Tuscany, Italy (Ferrucci et al. 2000). 1616 residents were selected from the population registry of Greve in Chianti (a rural area: 11,709 residents with 19.3% of the population greater than 65 years of age), and Bagno a Ripoli (Antella village near Florence; 4,704 inhabitants, with 20.3% greater than 65 years of age). The participation rate was 90% (n=1453), and the subjects ranged between 21-102 years of age.

2.2.9 Longitudinal Aging Study Amsterdam

Longitudinal Aging Study Amsterdam (LASA) is an ongoing, population-based cohort of individuals 55 years and older living in the Netherlands. The design and rationale is described elsewhere (Hoogendijk et al. 2016; Huisman et al. 2011). In short, 3017 participants (55-84 years old) were included at baseline (1992-1993) and two additional cohorts were added in 2002-2003 and 2012-2013 with respectively 1002 and 1023 participants. Follow-up visits were conducted every 3 years. Trained interviewers collected data on cognitive, emotional, physical and social functioning during a home interview. Subsequently, all participants were invited for a medical interview during which further diagnostic examinations were done and blood samples were drawn.

2.2.10 Long-Life Family Study

The Long-Life Family Study is a family-based cohort study of exceptional longevity that recruited families at four study centers (Boston, New York, Pittsburgh and Denmark) from 2006 to 2009. In brief, the LLFS recruited selected families with multiple exceptionally old living individuals, totaling 4559 individuals, which included long-lived probands and their siblings (n = 1445), their offspring (n = 2329) and spouse controls (n = 785). The probands were ≥79 years old in the USA, and ≥90 years old in Denmark. Families were selected to participate in the study based on The Family Longevity Selection Score (FLoSS) (Sebastiani et al. 2009) which calculated the rank sibships by current age or age at death of siblings, the size of the sibship and the number of alive individuals available for study. A proband's family was eligible if the FLoSS reached a score of 7 or higher, which met the following criteria: (1) the proband, at least one living sibling, and one of their living offspring (minimum family size of 3) were all able to give informed consent, and (2) were willing to participate in the interview and examination including the blood sample for serum and DNA extraction. The age of the 3359 participants in the current analyses was: 77.6 +/- 12.9 (range: 60-110).

2.2.11 MrOS

The Osteoporotic Fractures in Men (MrOS) study is a multicenter, prospective study including older men in Sweden, Hong Kong and the United States. The MrOS Sweden study (n=3014) consists of three sub-cohorts from three different Swedish cities (n=1005 in Malmö, n=1010 in Gothenburg, and n=999 in Uppsala) (Mellström et al.

2006). Study subjects (men aged 69 to 81 years) were randomly identified using national population registers. A total of 45% of the subjects who were contacted participated in the study. To be eligible for the study, the subjects had to be able to walk without assistance, provide self-reported data, and sign an informed consent. In this study 941 unrelated participants from Gothenburg and 891 unrelated participants from Malmö were included.

2.2.12 ROSMAP

The Religious Orders Study (ROS) and Memory and Aging Project (MAP)

The ROS, started in 1994, enrolled Catholic priests, nuns, and brothers, from about 40 groups in 12 states (A. Bennett et al. 2012). The follow-up rate of survivors exceeds 90%. Participants were free of known dementia at enrollment, agreed to annual clinical evaluations, and signed both an informed consent and an Anatomic Gift Act form donating their brains at time of death. Participants take a neuropsychological test battery. DNA was extracted from whole blood, lymphocytes, or frozen post-mortem brain tissue. Genotyping was performed at the Broad Institute's Center for Genotyping and the Translational Genomics Research Institute and the Children's Hospital of Philadelphia.

The Rush Memory and AP, started in 1997, enrolled older men and women from assisted living facilities in the Chicago area with no evidence on dementia at baseline (A. Bennett et al. 2012). The follow-up rate of survivors exceeds 90%. Participants agreed to annual clinical evaluations, and signed both an informed consent and an Anatomic Gift Act form donating their brains at time of death. Participants were invited to take a neuropsychological test battery. DNA was extracted from whole blood,

lymphocytes, or frozen postmortem brain tissue. Genotyping was performed at the Broad Institute's Center for Genotyping and the Translational Genomics Research Institute and the Children's Hospital of Philadelphia.

2.2.13 Rotterdam Study

The Rotterdam Study is an ongoing prospective population-based cohort that investigates occurrence, determinants, and consequences of diseases in an ageing population (Ikram et al. 2017). The first baseline measurement of Rotterdam Study started in 1990. After two expansions in 2000 and 2006, it comprised 14,926 participants aged 45 years and over by the end of 2008. Follow-up visits were held every 3-5 years.

2.2.14 Study of Health in Pomerania

The Study of Health in Pomerania (SHIP) is a population-based project in West Pomerania, the north-east area of Germany (Volzke et al. 2011). A sample from the population aged 20 to 79 years was drawn from population registries. First, the three cities of the region (with 17,076 to 65,977 inhabitants) and the 12 towns (with 1,516 to 3,044 inhabitants) were selected, and then 17 out of 97 smaller towns (with less than 1,500 inhabitants), were drawn at random. Second, from each of the selected communities, subjects were drawn at random, proportional to the population size of each community and stratified by age and gender. Only individuals with German citizenship and main residency in the study area were included. Finally, 7,008 subjects were sampled, with 292 persons of each gender in each of the twelve five-year age strata. In order to minimize drop-outs by migration or death, subjects were selected in

two waves. The net sample (without migrated or deceased persons) comprised 6,267 eligible subjects. Selected persons received a maximum of three written invitations. In case of non-response, letters were followed by a phone call or by home visits if contact by phone was not possible. The SHIP population finally comprised 4,308 participants (corresponding to a final response of 68.8%).

2.2.15 Toledo Study for Healthy Aging

Data were taken from the Toledo Study for Healthy Aging (TSHA), a population-based study conducted on 2,488 individuals aged 65 years and older. Study participants were selected by a two-stage random sampling from the municipal census of Toledo, covering both institutionalized and community dwelling persons from rural and urban settings. Data were collected from 2006 to 2009 and then in a second wave between 2013-2015, and included information on social support, activities of daily living, comorbidity, physical activity, quality of life, depressive symptoms, and cognitive function. In addition, a nurse collected anthropometric data, conducted tests of physical performance (walk speed, upper and lower extremities strength, and the stand-and-sit from a chair test) and obtained a blood sample.

2.2.16 Wisconsin Longitudinal Study

The Wisconsin Longitudinal Study (WLS) is a one-third sample of all 1957 Wisconsin high school graduates and a randomly selected sibling (Herd, Carr, and Roan 2014). These respondents were originally empaneled with an in-person questionnaire at age 18 (1957), which was followed with a mail survey of parents in 1964, telephone survey in 1975, mail and telephone surveys in 1993 and 2004 and in-person interviews in

2011, where data on grip strength was collected from participants. The WLS has a high response rate, exceeding 80 percent in most rounds of data collection. Between In 2006-11, the WLS collected saliva samples from respondents using Oragene kits (Rylander-Rudqvist 2006). After quality control, a total of 9,012 graduate and sibling respondents were genotyped at ~710,000 markers (before imputation) utilizing the Omni-Express beadchip.

Cohort details and definitions used for the analyses can be found in the methods sections for each analysis chapter (also available in Supplementary Table 2-1: Summary of cohorts and muscle strength phenotypes used).

2.3 Genotype data in the UK Biobank

2.3.1 Description of microarray technology

The concept of detecting a specific sequence of DNA by hybridization with a known DNA sequence, can be traced back to early colony hybridization methods of the 1970s (Grunstein and Hogness 1975). The original design of DNA genotyping “printed” arrays, where the oligonucleotide probes are printed directly onto a supporting glass surface have been mainly superseded by two technologies. These modern microarrays are more suitable for large scale genotyping and can be categorized into the photolithographic In Situ-Synthesized spot chips as developed by Affymetrix (Axiom technology, now part of Thermo-Fisher Scientific) alongside Agilent / Roche and the bead array technology offered by Illumina (Miller and Tang 2009). The genotyping for UK Biobank was undertaken using two bespoke designs based on the Axiom technology, as described later.

2.3.2 In situ-synthesized oligonucleotide microarrays

Microarrays produced by this technology are synthesized in-situ on the microarray wafer surface by the repeated exposure of the surface to ultra-violet (UV) light, using a lithographic mask in order to target specific areas of the wafer for the extension of the underlying probes.

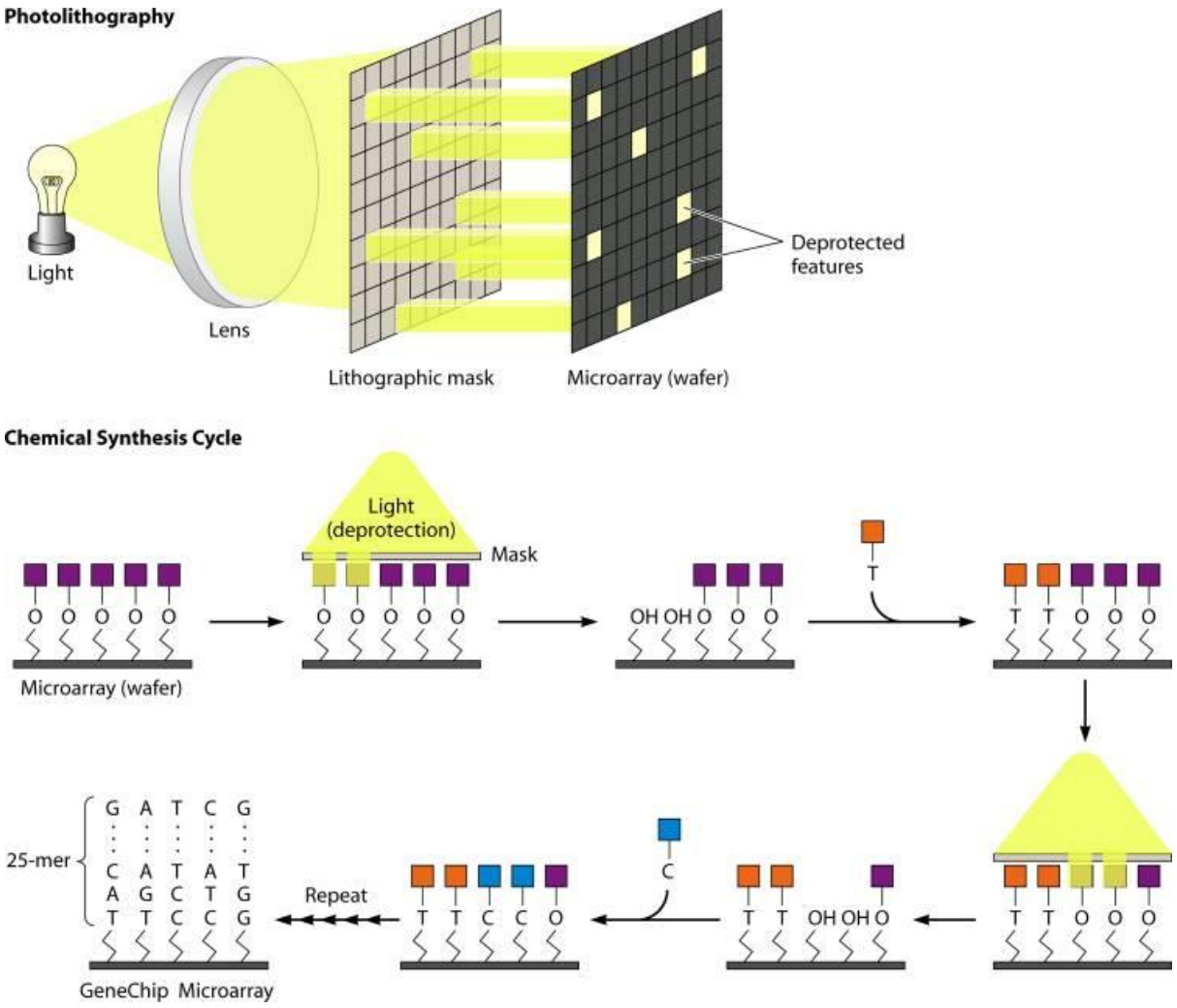

Figure 2-3: Affymetrix Axiom oligonucleotide microarray synthesis

(Miller and Tang 2009)

The linker molecule on the base layer has a reactive hydroxyl group that is protected by a UV labile cap. Addition of the required nucleotide occurs when the UV light is focused by the lithographic mask onto an area of the wafer with the site of the probe to be extended. This activates the hydroxyl group which then binds covalently to the supplied nucleotide. After washing the process is repeated in order to extend the oligonucleotide probes. Each probe is typically around 25 nucleotides in length (Miller and Tang 2009).

Due to the limited length of the probe sequence multiple probes are manufactured as a probe set containing 8 to 16 pairs of probes. Each pair consists of a perfect match to the sequence to be captured (PM) and a mismatch (MM) probe with the 13th position (of the 25mer probe sequence) changed to the complement nucleotide. This configuration helps increase the signal intensity, reduce the background noise and so increase sensitivity and specificity of the array (H. Liu, Bebu, and Li 2010).

2.3.3 Hybridization of probe with genomic DNA

For genotyping, genomic DNA from the participant is extracted, amplified, fragmented and denatured to produce single stranded DNA before being loaded onto the microarray chip. This process will include various quality control steps including quantification of the amount of DNA available (Biosystems 2018). The following is an overview of the process specific to the Affymetrix Axiom genotyping microarray plates and the GeneTitan MC instrument used for the hybridization and imaging, as used by UK Biobank.

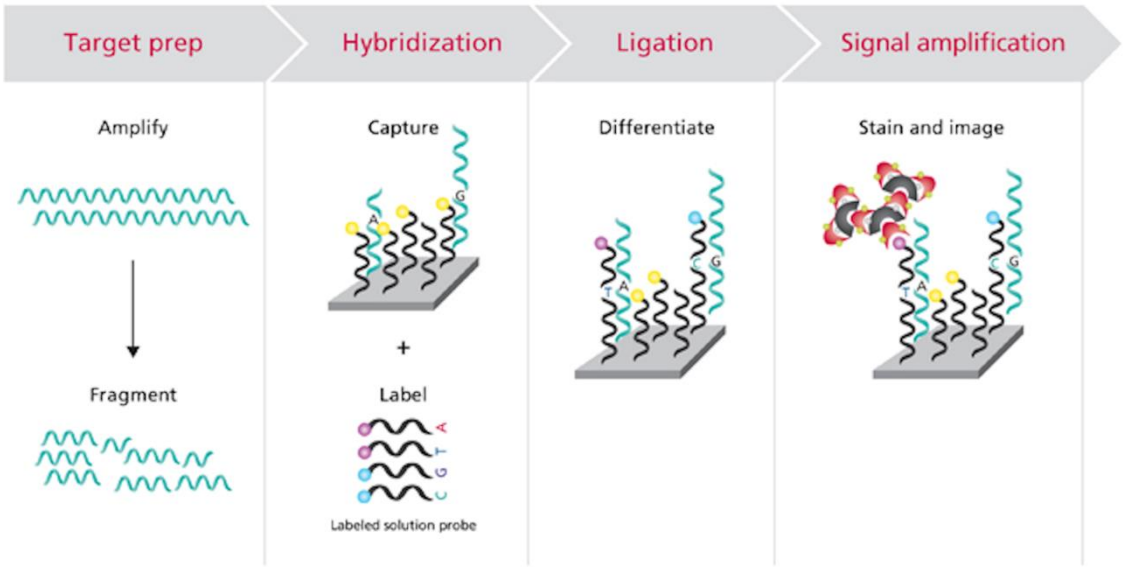

Figure 2-4: Axiom genotyping microarray reaction

(Shapero et al. 2011)

After hybridization with the probes, the plate is washed to reduce background noise from non-hybridized fragments. Then each polymorphic nucleotide is queried through a multi-colour (two differently labelled sets of ligation probes, Hapten-1 and Hapten-2) ligation event. This is followed by staining and imaging (Shapero et al. 2011). Figure 2-5 shows an example of an image of an Axiom genotyping microarray. In this case the custom design used the UK Biobank Affymetrix Axiom array as a basis (area highlighted in teal) (Emami et al. 2020).

Figure 2-5: Custom germline cancer Axiom microarray

(Emami et al. 2020)

2.3.4 UK Biobank microarrays

The UK Biobank includes genetic data for 488,377 participants, which were genotyped by two different microarrays, with overlapping coverage. The initial set of 49,950 participants were part of the UK Biobank Lung Exome Variant Evaluation (UK BiLEVE) cohort (Wain et al. 2015) and were analysed using the Applied Biosciences UK BiLEVE Axiom array. The larger set of 438,427 participants were then genotyped using the Applied Biosystems UK Biobank Axiom array (Bycroft et al. 2017). This array has been designed with specific markers to aid in HLA typing (7,348), coverage for eQTLs (expression Quantitative Trait Loci; 17,115 markers), autoimmune and inflammatory pathway associated variants (258), as well as a range of variants with known trait associations as found in the NHGRI GWAS catalogue (8,136) for a total number of 820,967 markers on the array (Affymetrix 2014). Despite the genotyping for UK Biobank participants being analysed on two different arrays, there is a significant

overlap between the two panels with approximately 95% of the probes in common (Bycroft et al. 2018).

UK Biobank has recently released sequencing data, which has been used to review the accuracy of the microarray genotyping. The study found that for common SNPs (Minor Allele Frequency > 1%) the Axiom chip had an average sensitivity of 99.8%, specificity of 99.7% and precision of 99.0%. The BiLEVE chip had an average sensitivity of 99.7%, specificity of 99.7% and precision of 98.7%. However for rare variants (MAF < 0.001%) sensitivity falls to 29.5% for the Axiom chip and 4.4% for the BiLEVE chip (Weedon et al. 2019).

2.3.5 Imputation (HRC v1.1)

Although data from microarrays such as the UK Biobank Axiom array may contain information on over 800,000 markers, this is still only a small fraction of the variation present in the genome. The concept of linkage disequilibrium (the non-random inheritance of combinations of alleles) can be used alongside external panels of population matched haplotypes (which have been extensively genotyped or sequenced) to predict or impute the missing sites based on the available subset of SNPs in the raw data (Marchini and Howie 2010).

The Haplotype Reference Consortium (HRC) produced the first version of its 64,976 haplotypes, based in whole genome sequencing (WGS) data from twenty studies (32,611 samples), in 2016 (McCarthy, Das, Kretzschmar, Delaneau, Wood, Marchini, et al. 2016). The studies consisted mainly of samples from European ancestry, although the more diverse 1000 Genomes Project Phase 3 cohort has also been incorporated into the reference.

The reference allows imputation against 44,187,567 sites at a minimum Minor Allele Frequency (MAF) of 0.0077% or a Minor Allele Count (MAC) of equal to or greater than five from the combined data (McCarthy, Das, Kretzschmar, Delaneau, Wood, Marchini, et al. 2016). The imputation of variants by HRC reference panel resulted in 39,235,157 SNPs for the UK Biobank participants (Bycroft et al. 2018). Imputation accuracy is discussed in detail in section 3.7.

For our GWAS meta-analysis we requested that participating cohorts imputed their variants against the HRC v1.1 reference panel prior to submission.

2.4 Quality control and filtering of variants

2.4.1 Minor Allele Frequency

Due to technical limitations it was prudent to filter variants obtained from genotyping and imputation. Although the UK Biobank microarray panels include genotype probes for rare alleles (MAF < 1%), the number of false positive calls can reach approximately 60% for loci with a MAF of between 0.001% - 0.005% (equivalent to approximately 10 heterozygous individuals out of the 500,000 UKB participants). At the extremely rare alleles (MAF < 0.0005%, approximately 5 heterozygous individuals in the UKB data), recent analysis has found that all variants calls were false positives (Wright et al. 2019), see Table 2-3 and Figure 2-6.

Table 2-3: Evaluation of rare alleles in UK Biobank

MAF Bin (%)	FP	TP	Unclear	Total
0–0.0005	511	0	11	522
0.0005–0.001	607	8	59	674
0.001–0.005	1,598	218	210	2,026
0.005–0.01	138	204	73	415
0.01–0.05	66	456	48	570
0.05–0.1	2	129	5	136
0.1–0.5	6	189	7	202
0.5–1	0	40	0	40
Total	2,928	1,244	413	4,585

(Wright et al. 2019)

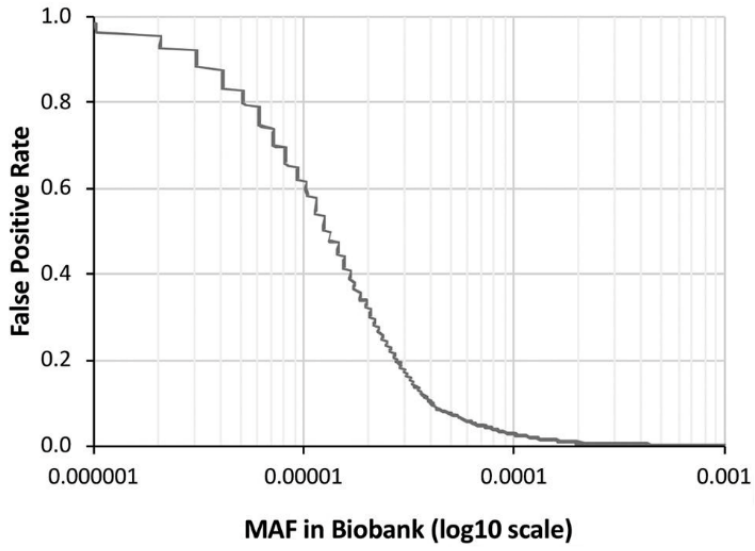

Figure 2-6: False positive rate for rare alleles in UK Biobank

(Wright et al. 2019)

Rare alleles have long been thought to be a potential source of the “missing heritability” observed in complex traits, alongside larger structural variations and variants with

reduced penetrance (Maher 2008). Larger studies like the UK Biobank and techniques such as GWAS meta-analysis, which increase the number of participants, should provide a solution for rarer alleles; however as Wright et al have shown this is still an issue for very rare alleles (presumably with larger effect sizes) (Wright et al. 2019).

For most cases I filtered the GWAS analyses, keeping only variants that exceeded a Minor Allele Frequency of 1% or 0.01. I requested that individual cohort data for the GWAS meta-analysis was uploaded without any MAF filter, in order to preform quality control checks in-house.

2.4.2 Hardy-Weinberg equilibrium (HWE)

In a randomly mating population, the frequency of a biallelic site with alleles ***p*** and ***q*** will reach Hardy-Weinberg equilibrium (Hardy 1908) or ***p2***, ***2pq***, and ***q2***, as long as a number of assumptions are true. The assumptions are that the population size is sufficiently large to allow random mating (and genetic drift is negligible), an absence of natural selection, no gene flow or migration, no mutation of the site, the locus is on an autosomal chromosome and finally that mating is random.

HWE can be used to discard variants deviating significantly from the principles of random selection and is generally used in GWAS to filter genotyping errors (Turner et al. 2011). Deviation from HWE may also highlight underlying population stratification and selection bias (Wigginton, Cutler, and Abecasis 2005). During the UK Biobank SNP quality control step variants with a significant deviation from HWE were flagged and set to missing for the initial interim release. For the full release these SNPs were investigated further and a substantial number were then passed for release (Sze and Schloss 2016).

For the various cohorts of the CHARGE GWAS meta-analysis full details of their specific filtering steps can be found in the summary appendix. However in general variants that showed departure from HWE were discarded in line with existing methodology best practice (Turner et al. 2011).

2.4.3 Population stratification

Population stratification can arise by non-random mating, possibly due to geographic isolation of subpopulations or bottle-neck effects. Distinct populations can be then observed from the genotype allele frequencies. Such distortions of genotype allele frequencies can confound the relationship between a variant and an observed trait (Hellwege et al. 2017).

Population stratification is accounted for by the Linear Mixed Model used by BOLT-LMM, by using principal component analysis to adjust both the phenotype and genotype to weighted values, which then undergo standard association testing (Sze and Schloss 2016). This mixed-model association method has been shown to reliably handle geographic population structure, family relatedness and cryptic relatedness (P.-R. Loh et al. 2015).

2.4.4 Sex discrepancy

Initial quality control for any GWAS includes a check for sample handling errors (Marees et al. 2018). One of the easiest to perform is to check for the reported sex against that predicted from the genotype data. Software tools for analysing genotype data often provide a suitable function, such as GWASTools (Turner et al. 2011) and

PLINK (S. Purcell et al. 2007). These checks can also reveal sex chromosome anomalies, such as Turner or Kleinfelter syndrome.

2.4.5 Sample relatedness

Pairwise kinship can be inferred from the genotyping data using as few as 100,000 SNPs. By reviewing whether or not two individuals share alleles at a specific locus that are identical by descent (IBD), and the overall ratio of IBD alleles, the family relationships can be revealed. For example, parent-child pairs should share one IBD allele at every locus (within a margin of error for technical artefacts and random mutations). In a similar method the sharing of alleles between siblings should overall show a ratio of shared alleles of 25% for zero shared alleles, 50% for one shared allele and 25% for two shared alleles. This information can then be used to inform the analysis plan (Turner et al. 2011).

The UK Biobank used the KING algorithm, as it is resistant to falsely identifying relatedness due to confounding by population structure (Manichaikul et al. 2010).

2.4.6 Low call rate (Missingness) and heterozygosity

Storage of DNA and technical issues can result in a low genotyping efficiency or call rate. SNPs exhibiting an overall call rate of less than 98-99% are generally considered poor quality and should be removed from the analysis (Turner et al. 2011).

Sample contamination can be checked by examining the heterozygosity over common (MAF ≥ 0.05) autosomal markers, with a high heterozygosity (>3 Standard Deviations above the mean) being indicative of contamination. Plots of the B allele frequency

(BAF) fluorescence intensity and the log(R) ratio (LRR – a normalised measure of overall signal strength) against the chromosomal position, can also show abnormal clustering of values in the case of contamination (Igo et al. 2016). Runs of Homozygosity (ROH) or long tracts of homozygous genotypes, caused by the inheritance of identical haplotypes from a common ancestor, as well as a mixed ethnic ancestry can also confound the measures of heterozygosity and so these plots should be examine with the study population in mind (Ceballos et al. 2018).

2.4.7 Imputation quality

Although modern genotyping arrays consist of millions of probes capable of providing the direct genotype for these SNPs, this information can be expanded by imputation to provide greater genome wide coverage and statistical power for the following GWAS (Marchini and Howie 2010).

Imputation accuracy is dependent on the size of the imputation panel (combination of population specific panels can boost the performance especially with regards to rare alleles), the algorithm used to impute the missing variants (for example BEAGLE (B. L. Browning and Browning 2009) or IMPUTE (B. N. Howie, Donnelly, and Marchini 2009)), minor allele frequency (with rarer alleles being more difficult to accurately model) and the degree of genetic diversity between the study population and the reference panel used (Marchini and Howie 2010).

2.4.8 Imputation of HLA alleles

HLA*IMP:02 software (Dilthey et al. 2013) was used to impute 11 loci within the MHC region with a multi-population reference panel. Reference panels were constructed specifically for each of the loci from a number of datasets (Table 2-4) (Bycroft et al. 2018).

Table 2-4 : UK Biobank HLA loci imputation references

HLA locus	Reference datasets merged	Number of SNPs used	Number of reference individuals
HLA-A	CEU+58,GSK,YRI,1000G,T1DGC	661	8,085
HLA-B	CEU+58,GSK,YRI,1000G,T1DGC	927	9,120
HLA-C	CEU+58,GSK,YRI,1000G,T1DGC	908	7,732
HLA-DRB1	CEU+58,GSK,YRI,1000G,T1DGC	626	8,869
HLA-DRB3	GSK,KC,SW	849	880
HLA-DRB4	GSK,KC,SW	849	865
HLA-DRB5	GSK,KC,SW	801	808
HLA-DQA1	CEU+58,GSK,YRI,T1DGC,PA	747	6,242
HLA-DQB1	CEU+58,GSK,YRI,1000G,T1DGC	623	8,491
HLA-DPA1	T1DGC,PA,SW	794	6,067
HLA-DPB1	GSK,T1DGC,PA,SW	691	6,176

CEU+58 = British 1958 cohort, HapMap CEU and CEPH CEU+; GSK = GlaxoSmithKline dataset; YRI = HapMap YRI; 1000G = 1000 Genomes project; T1DGC = Type 1 Diabetes Genetics Consortium; KC = King's College African-American individuals; SW = Karolinska Institutet Swedish individuals; PA = Pillai Pan-Asian dataset (Bycroft et al. 2018).

2.5 Genome wide association software

2.5.1 BOLT-LMM

The BOLT-LMM algorithm using a modified form of the linear mixed models that have become the most common method for association testing in Genome-Wide Association Studies (GWAS) (P.-R. Loh et al. 2015).

Due to the standard linear mixed model assuming that, for a complex trait, all variants are causal with small effect sizes (Goldstein 2009), the algorithmic complexity approaches $O(MN^2)$ or $O(M^2N)$ – where M is the number of samples and N is the number of SNPs - this becomes prohibitively expensive in computational requirements when applied to the large biobanks, such as the UK Biobank (P.-R. Loh et al. 2018).

Even although recent meta-analysis GWAS have identified thousands of significant loci for complex traits such as height and BMI (Yengo et al. 2018), this is still a limited set of variants and so BOLT-LMM adapts the standard linear model to account for non-Gaussian prior distributions of effect size to better model the contributing genetic architecture to a phenotype (P.-R. Loh et al. 2015).

The BOLT-LMM algorithm has four main steps (P.-R. Loh et al. 2015), each reducing the complexity to O(MN) or to linear complexity from the exponential complexity of the standard approach, $O(M^2N)$.

1a. Estimation of variance parameters

1b. Computation of infinitesimal mixed-model association statistics

2a. Estimation of Gaussian mixture-model parameters

2b. Computation of Gaussian mixture-model association statistics

BOLT-LMM was used to provide the GWAS results for Chapter 4 and 5.

2.5.2 LMOR adjustment

The Linear Mixed Model utilized by BOLT-LMM uses principal component correction along with logistic regression in order to account for population stratification (J. Yang et al. 2014). When used with binary traits this results in estimates of genetic effect on a different scale to the odds ratio derived from logistic regression. This makes direct comparison between studies, for example in meta-analysis of GWAS, problematic (Cook, Mahajan, and Morris 2017).

Prior to meta-analysis by METAL studies that had used BOLT-LMM for association testing (UK Biobank, HRS and WLS were transformed by using the LMOR function (Lloyd-Jones et al. 2018b) to odds ratios.

2.5.3 Case-Control balance

Due to the reliance of BOLT-LMM on basing the calculation of p-values on a chi-squared distribution, inflated Type 1 errors (false positives) can occur when the case-control ratios are severely imbalanced. The impact of the Type 1 error inflation for binary traits depends on the sample size, case fraction and minor allele frequency (MAF). Simulation studies have shown that for case fractions > 10% and MAF > 0.1%, in the UK Biobank data (N=459K), BOLT-LMM p-values are correct and not significantly affected by the Type 1 error inflation (P.-R. Loh et al. 2018). The prevalence of the low grip phenotypes within the UK Biobank, stratified into the 60-70 age cohort, has a suitable number of cases.

For a smaller sub-sample (N=150K) Type 1 error inflation is an issue at higher p-value thresholds than 5 x 10^{-8}, for example the occasionally used “suggestive” p-value threshold of 1 x 10^{-6}. However the inflation is not significant at case fractions > 10%, MAF > 1% and sample size of 150K (p-value threshold = 5 x 10^{-8}) (P.-R. Loh et al. 2018).

2.5.4 PLINK

The PLINK toolset (S. Purcell et al. 2007) was used for additional analysis and confirmation of results. Analysis of allosomes (chromosomes X and Y) and mitochondria SNPs to the development of low grip strength was preformed using PLINK on directly genotyped UK Biobank data.

2.6 GWAS Meta-analysis

2.6.1 METAL

Meta-analysis was preformed using the METAL software from the Center for Statistical Genetics, at the University of Michigan (Willer, Li, and Abecasis 2010a).

Meta-analysis was performed by one of two methods, the sample size method (the default method in METAL and for our analyses) which uses the direction of effect and the P-value to produce a signed Z-score (extremely negative Z-scores therefore indicate a small P-value). The allele specific Z-scores are then combined using a weighting scheme based on the effective sample size – either provided in the configuration or data files.

The Standard Error method was also used for exploratory analysis, and in this approach the β-coefficients were weighted by their estimated standard errors (Willer, Li, and Abecasis 2010a).

2.7 Linkage disequilibrium

2.7.1 LDlink

Calculation of linkage disequilibrium for interpretation of the relationship between genomic risk loci was occasionally performed as a secondary analysis. LDlink was used for this purpose (Machiela and Chanock 2015).

LDpair was the most commonly used module, with the reference population set to GBR (1000 Genomes population reference – European ancestry – British in England and Scotland) (Auton et al. 2015).

2.7.2 Linkage disequilibrium metrics

In order to assess correlation between variants LDlink calculates a number of statistics including R^2, D' and goodness-of-fit (Chi-square and p-value). If R^2 is greater than 0.1 then linkage disequilibrium is present, with 0.6 being an indicator of moderate LD and 0.8 a measure of high LD.

D' is an indicator of allelic segregation for two genetic variants, essentially whether or not two alleles at different sites are inherited together. With a range between 0 and 1, a value of 0 indicates alleles are not linked while a value of 1 means that at least one of the expected haplotype combination has not been found.

R^2 measures the correlation of alleles for two variants, giving a value between 0 and 1. R^2 of 0 indicates that the alleles are independent, while a value of 1 shows that the allele of one variant predicts an allele in a second variant, they are therefore in linkage disequilibrium. R^2 accounts for the Minor Allele Frequency while D' does not.

2.7.3 Heritability estimates

The LD Score Regression software, LDSC, was used to provide heritability estimates for the GWAS meta-analysis traits, for example EWGSOP low grip strength. LDSC overcomes confounding bias due to population stratification or relatedness, and accounts for the issue of polygenicity common in complex trait analysis (heritable component is due to many variants with individual small effect sizes) by utilizing linkage disequilibrium to control for these. European LD scores from the 1000 Genomes project were used as the LD reference (B. K. Bulik-Sullivan, Loh, Finucane, Ripke, Yang, Patterson, et al. 2015). Further details are available in the specific methods section of Chapter 4.

2.8 Annotation of results

2.8.1 FUMA

Annotation and functional mapping of variants was undertaken using FUMA (Watanabe, Taskesen, Van Bochoven, et al. 2017). Summary statistics from both the GWAS and Meta-analysis GWAS for low grip strength and sarcopenia was annotated using a range of modules available through FUMA.

FUMA's default settings were used for lead SNP and candidate SNP identification, see Table 2-5.

Table 2-5 : FUMA settings

Meta-analysis GWAS	
Sample size - combined	254894
Sample size - female only	133222
Sample size - male only	119123
Maximum P-value of lead SNP (<)	5.00E-08
Maximum P-value cut-off (<)	0.05
r^2 threshold to define independent significant SNPS (≥)	0.6
2nd r^2 threshold to define lead SNPs (≥)	0.1
Reference panel population	UKB release2b 10K European
Include variants in reference panel (non-GWAS tagged SNPs in LD)	Yes
Minimum Minor Allele Frequency (≥)	0
Maximum distance between LD blocks to merge into a locus (< kb)	250
MHC region	Included
MAGMA analysis	GTEX v7/v8

2.8.2 MAGMA

MAGMA (v1.08) (de Leeuw et al. 2015) was used by FUMA for gene and gene-set analysis. MAGMA uses a multiple regression approach (multiple linear principal components regression) in order to incorporate linkage disequilibrium between genetic markers for a gene. MAGMA uses a linear regression model even when the phenotype to be analysed is binary; although the F-test used to compute the gene p-value assumes a continuous trait, testing has shown that the results remain accurate (de Leeuw et al. 2015).

2.8.3 GTEx

FUMA uses data from the Genotype-Tissue Expression (GTEx) project in order to annotate lead SNPs with quantitative trait loci (QTL) that are significantly associated with a measurable phenotype. For eQTLs the associated phenotype is the expression of a gene and GTEx provides tissue specific eQTLs for a large number of variants (Lonsdale et al. 2013; “The GTEx Consortium Atlas of Genetic Regulatory Effects across Human Tissues” 2020). Initial exploratory annotation of GWAS meta-analysis was done in GTEx v.7, subsequent final analysis in GTEx v.8.

2.9 Multiple testing

The large number of simultaneous tests in Genome-Wide Association Studies, results in a multiple testing problem which has to be taken into account when drawing inferences from the results.

The following methods were used for correction of multiple testing:

2.9.1 Bonferroni

The aim of the Bonferroni correction is to control the probability of obtaining at least one false positive result. The *p* value is adjusted by dividing the statistical threshold of 0.05 by the number of tests conducted – in the case of GWAS this is the number of SNPs.

A genome-wide significance threshold was proposed by the International HapMap Consortium based on an estimation of the "effective number of independent tests" of 150 per 500 kbp (kilo base pairs) in the CEU (Utah residents with European ancestry), CHB (Han Chinese) and JPT (Japanese) populations at a MAF ≥ 0.05 (5%). The additional diversity of the African population, YRI (Nigerian), increased the test numbers to 350 per 500 kbp (Belmont et al. 2005). For the Non-African populations this results in a significance threshold of 5.5×10^{-8} for a 3 Gb human genome, which has become the de facto standard for GWAS significance. For African populations a more stringent threshold is required, with 1.0×10^{-8} being proposed (Hoggart et al. 2008).

The significance threshold for GWAS was estimated by Dudbridge et al using a permutation procedure on GeneChip 500K Affymetrix array data from the Wellcome Trust Case-Control Consortium. They found that a genome-wide significance threshold to be approximately 7.2×10^{-8} in the UK population of European ancestry,

based on 359,491 available SNPs (Dudbridge and Gusnanto 2008), which confirms the estimation by the HapMap consortium.

However due to Linkage Disequilibrium (LD) many SNPs are correlated and so cannot be considered as independent tests (Vergara-Lope et al. 2019). Bonferroni correction can therefore be considered conservative and may discard real associations.

2.9.2 Benjamini-Hochberg

The Bejamini-Hochberg false discovery rate (FDR) (Benjamini and Hochberg 1995) controls for the proportion of Type 1 errors (incorrectly rejecting the null hypothesis), or false positives in an analysis. I used this method to provide adjusted p-values for the Two-Sample Mendelian Randomisation analysis, over the more stringent Bonferroni correction. Applying Benjamini-Hochberg FDR adjustment to GWAS is problematic since the method assumes that the tests (in this case the SNPs) are independent of each over (Marees et al. 2018).

2.10 Genetic correlation

Cross-trait Linkage Disequilibrium score regression can be used to test whether two traits share any genomic variants representing underlying pathways or mechanisms.

The LDSC software (B. K. Bulik-Sullivan, Loh, Finucane, Ripke, Yang, Patterson, et al. 2015) was used to investigate genetic correlation between muscle strength or function traits, and a range of phenotypes of interest. LDSC uses reference linkage disequilibrium data in order to help reduce bias due to cryptic relatedness and population stratification (B. K. Bulik-Sullivan, Loh, Finucane, Ripke, Yang, Patterson,

et al. 2015). GWAS summary statistics can then be used to compare the underlying genetic correlation between two traits of interest.

LD score regression intercept provides a measure of the bias due to population stratification and cryptic relatedness. The heritability of a trait is expressed on a liability scale so that it is comparable across studies (h2).

2.11 Mendelian randomisation

Mendelian randomization allows the inference of causal associations between heritable complex traits, such as low grip strength and diabetes, by using genetic instruments (D. M. Evans and Davey Smith 2015; Burgess, Foley, and Zuber 2018; Pingault et al. 2018). Genetic Instruments are Instrumental Variables or variables associated with the risk factor of interest, which are not related to any confounders and affect the outcome only through the risk factor. In the case of Genetic Instruments, genomic variants associated with the risk factor are used since these are set at birth.

By using genetic instruments from our previous multi-cohort, community based Genome-Wide Association Study (GWAS) meta-analysis of low grip strength in older people (256,523 Europeans aged 60 years and over) (G. Jones et al. 2020) Mendelian randomization can be used to infer causal relationships with a potentially modifiable biomarker, such as increased levels of circulating vitamin D being associated with lower risk of multiple scelorosis (OR=0.963; 95%CI=0.945-0.981)(Jiang, Ge, and Chen 2019), without the confounding of outcome and exposure that can be problematic in observational studies. The methodology used is discussed in greater detail in Chapter 6.

2.11.1 Two Sample MR

Two sample Mendelian randomization (MR) can be used to perform MR using GWAS summary results, where the SNP-exposure effects and the SNP-outcome effects are obtained from separate studies(Pierce and Burgess 2013). This allows the use of large pre-existing GWAS analyses to infer casual inferences between two traits, which are not measured in the same set of samples(Hemani et al. 2018). For Chapter 6 the R package TwoSampleMR (https://mrcieu.github.io/TwoSampleMR/) was used, further specific details are available in Chapter 6 methods.

2.12 GWAS Catalogue

The NHGRI-EBI GWAS Catalog of published genome-wide association studies was used to manually annotate and investigate related traits for lead SNPs from the GWAS meta-analysis presented in Chapters 4 and 5 (Buniello et al. 2019a).

2.13 Statistical and general programming languages

Throughout this thesis I have used STATA 15.1 and R 3.4.9 to 3.5.1 for statistical analysis. STATA was used for the majority of the regression analysis and interpretation of data. R was used for more specialised packages such as TwoSampleMR, as well as visualisation and reformatting of data.

Linux shell scripts were used to run various software and packages on the ISCA Linux cluster. In addition shell scripts were used to format data for input into these packages and to filter results on given parameters. When greater error control or specialised libraries were required I also occasionally used Python scripts. Examples of scripts and code used in the main parts of the GWAS Meta-analysis and associated studies can be found at:

https://github.com/pasted/gw_meta_analysis_low_muscle_strength

3 Analysis 1: Sarcopenia and the HLA complex

Sarcopenia and variation in the Human Leukocyte Antigen complex

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3.1 Overview

The underlying mechanisms which result in the state of sarcopenia in older adults is thought to include an autoimmune component. In this analysis we show that sarcopenia is associated with alleles of six genes from the Human Leukocyte Antigen region, this is independent of a wide range of known autoimmune conditions, including Rheumatoid Arthritis.

My contribution to this article included analysis and interpretation of the data – alongside the co-authors, review of literature and preparation of the subsequent paper for publication.

3.2 Abstract

Background

Aging is characterized by chronic inflammation and loss of muscle mass and strength, termed sarcopenia. Human Leukocyte Antigen (HLA) types are major drivers of autoimmune disease, although with limited penetrance. We tested whether HLA types and related genetic variants are associated with sarcopenia in autoimmune disease free older people.

Methods

Data from 181,301 UK Biobank European descent volunteers aged 60 - 70 with measured hand-grip strength and impedance. Logistic regression analysis estimated HLA types sarcopenia associations, adjusted for confounders and multiple testing.

Results

Of 100 HLA types (allele frequency >1%), six increased sarcopenia likelihood, after multiple statistical testing correction. Participants with two HLA-DQA1*03:01 alleles had 19.3% increased sarcopenia likelihood (Odds Ratio 1.19, 95% Confidence Intervals 1.098-1.295, p=2.84*10-5), compared to no alleles. HLA-DRB4*01:03 homozygotes had a 15.4% increased likelihood of sarcopenia (OR 1.1539, CI 1.079-1.234, p=2.66*10-5). Participants with at least 6 of the possible 12 HLA alleles associated with sarcopenia had 23% increased likelihood of sarcopenia (OR 1.23 CI 1.123-1.346, p=7.28*10-6).

Of 831 non-coding genetic variants in the HLA region previously implicated in disease, 4 were associated with sarcopenia, including rs41268896 and rs29268645 (ORs = 1.08, CI 1.051-1.107, p=1.06*10-8 and 1.07, CI 1.037-1.09 p=1.5*10-6, respectively).

Conclusion

Variation in specific HLA types and non-coding SNPs are associated with sarcopenia, in 60 to 70 year old carriers free of diagnosed autoimmune diseases, with cumulative risks having substantial effects. More work is also needed on whether some patients with sarcopenia have evidence of autoimmune processes and might benefit from targeted treatment.

Keywords

Sarcopenia, Human Leukocyte Antigen, inflammation, Muscle

3.3 Introduction

Immuno-senescence and 'Inflammaging' are characterized by a low grade chronic pro-inflammatory state associated with ageing, which results from an imbalance between the inflammatory and anti-inflammatory networks (Franceschi et al. 2007)(Bektas et al. 2017). The Human Leukocyte Antigens (HLA) have a major role in mediating the chronic inflammatory pathway in autoimmune disease via antigen presentation to the CD4+/8+ T cells (Dendrou et al. 2018). The HLA complex has long been associated with autoimmune disorders and infectious disease (Shiina et al. 2009). Examples of associations between certain HLA types and autoimmune disease include HLA-B*27 and spondyloarthritis (SpA), HLA-B*51 and Behcet's disease (Bodis, Toth, and Schwarting 2018), and HLA-DRB1*15:01 and multiple sclerosis (Goodin et al. 2018)(Moutsianas et al. 2015). However, autoimmune diseases are generally not monogenic or fully penetrant, but rather are complex polygenic diseases influenced by environmental factors (despite having over 110 linked genes, excluding HLA types, the heritability of multiple sclerosis is approximately 28% (Goodin 2016)).

Sarcopenia, loss of muscle mass and strength during ageing, is associated with reduced physical functioning and is associated with increased risks of morbidity (including falls and fractures) and mortality (Cruz-Jentoft et al. 2010) (Brown, Harhay, and Harhay 2016). The European Working Group on Sarcopenia in Older People (EWGSOP) defines age-related Sarcopenia to include both low muscle mass and low muscle function (Cruz-Jentoft et al. 2010). The Baltimore Longitudinal Study of Aging (BLSA) found evidence that this can occur as early as age 40 in both men and women (Metter et al. 1997), with progressive decline over time. Previous studies have presented evidence for the role of inflammation in sarcopenia (Dalle, Rossmeislova, and Koppo 2017)(Westbury et al. 2018), and it is becoming apparent that contributors

to disease vary in penetrance from Mendelian disorders to complex diseases (Bastarache et al. 2018).

While muscle loss is a classical feature of multiple sclerosis and some other autoimmune conditions, little is known of whether the carrier status genetic predisposition to autoimmunity accelerates muscle loss in later life. We aimed to investigate the impact of HLA types on susceptibility to sarcopenia in individuals without a diagnosed autoimmune condition in the exceptionally large UK Biobank study.

3.4 Methods

We used data from 451,447 UK Biobank participants of European descent, confirmed by principal components analyses of genome-wide genetic information from a selection of participants who self-identified as 'white European' (Pilling et al. 2017). The UK Biobank is a volunteer study where participants visited one of 22 assessment centres across the UK. A range of physiological and questionnaire data was collected, including genetic data from blood draws (Sudlow et al. 2015). Genotyping data was generated on the initial ~50,000 participants using the Affymetrix UK BiLEVE Axiom array and the ~450,000 participants of the remaining cohort were genotyped using the Affymetrix UK Biobank Axiom array - the two arrays sharing over 95% similarity (http://www.ukbiobank.ac.uk).

3.4.1 Phenotype definitions

Sarcopenia was classified using the EWGSOP definition (Cruz-Jentoft et al. 2010), based on having both low hand grip strength and skeletal muscle mass index (SMI). For grip strength the highest value from hand grip strength, left and hand grip strength, right was taken as the selected metric. Low grip strength was defined as under 30kg in Males and under 20kg in Females, as measured by Jamar J00105 hydraulic hand dynamometer.

Skeletal muscle mass (SMM) was calculated using the following equation from Janssen *et al* (I Janssen et al. 2000);

$$\text{SMM (kg)} = [(\text{Ht}^2 \mid R \times 0.401) + (\text{gender} \times 3.825 + (\text{age} \times -0.071)] + 5.102$$

Where Ht is standing height in centimetres measured at the initial assessment; *R* is BIA resistance in ohms for the whole body taken by Tanita BC418MA body composition analyser at the initial assessment visit; for sex, men = 1 and women = 0; and age is in years. A Skeletal muscle mass index (SMI) was then calculated from the SMM.

$$SMI = SMM/Ht\text{-}in\text{-}meters^2$$

Low SMI was defined by Janssen *et al.* as under 8.87 in Males and under 6.42 in Females. Analysis was restricted to the age range of 60-70. We excluded a small number participants with max grip strength or lean mass (from BIA) greater than 4 standard deviations from the mean (SMI > 46.61; max grip > 73.91).

3.4.2 Auto-immune disease exclusions

The HLA region has been associated with a wide range of autoimmune diseases (Matzaraki et al. 2017; Dendrou et al. 2018), and a number of these diseases impact the measurements used by this study in order to define sarcopenia, such as muscle weakness (through the proxy of grip strength) and lean muscle mass. In order to resolve any association between the HLA region and sarcopenia in later life, participants with diagnosed autoimmune disease were excluded. One additional factor to consider is that treatment of many autoimmune conditions may involve anti-inflammatory steroids, such as glucocorticoids (Fullerton and Gilroy 2016). Glucocorticoids being one of the most common causes of drug-induced myopathy (Gupta and Gupta 2013).

A list of 57 (including subtypes) autoimmune diseases were generated from previous review articles (Cárdenas-Roldán, Rojas-Villarraga, and Anaya 2013) and the ICD-10 codes used to exclude participants from the initial analysis (Supplementary Table s3-1). This list was not exhaustive and certain autoimmune conditions not included are mentioned in the discussion. In addition, UK Biobank self-reported data was also used based on the autoimmune conditions in the ICD-10 list. Following the initial analysis any additional autoimmune diseases associated with the HLA types shortlisted, were added to the exclusion criteria and formed the basis of the secondary analysis.

3.4.3 HLA imputation

Imputation of HLA types was performed centrally by the UK Biobank team. In brief, HLA*IMP:02 (Dilthey et al. 2013) was used to impute the four-digit HLA types from genotype information, with a number of modifications: localization feature turned off; graph sampling error (mS) and graph building error (mB) probabilities were both set to 0.001; and the number of sampled haplotype pairs was set to 5 (Motyer and Leslie 2016). Individuals are therefore coded as 0, 1 or 2 depending on the number of HLA alleles carried for each gene. The methods allow for imprecise coding (e.g. 1.92) to indicate the confidence in HLA type imputation. Some HLA type codes indicate unknown or other HLA types (e.g. *99:01), and these were not included in analyses. HLA-types below 1% frequency were excluded from analyses.

3.4.4 Statistical Analysis

Logistic regression analyses were performed for each HLA type against sarcopenia, adjusted for age, sex, genotype array, and the first five principal components for

ancestry. Cohorts were restricted to the age range 60 to 70 and of European descent. Participants with known autoimmune disease were excluded as previously described, based on Hospital Episode Statistics data (ICD-10 codes) and self-reported fields.

HLA types were modelled first assuming an additive effect, and secondly comparing participants with 2 alleles to those with 0. This was in order to model the effect of recessive modes of inheritance of the HLA types within the cohort.

Participants with imprecise HLA imputations were recoded for the second, categorical analysis (i.e. estimated allele dose between 0 and 0.25 set to 0, values between 0.75 and 1.25 set to 1, and finally between 1.75 and 2 to 2; other doses were set to missing due to imprecise imputation). Correction for multiple testing was applied using the Benjamini-Hochberg method. Statistical analyses were performed in STATA (v14.1) and R (v3.3.2). Charts and figures were generated with package metafor (v2.0).

After analysing each HLA type for its association with sarcopenia we performed a literature search to identify any autoimmune diseases implicated by the significant HLA types not already included in the autoimmune exclusion criteria. A second analysis was performed after participants with diagnoses of the autoimmune diseases identified had been added excluded; these are the final results presented.

3.4.5 Non-coding SNPs previously linked to a phenotype in previous Genome-Wide Association Studies.

The HLA types assessed so far were based on the protein-coding sequence of the HLA protein expressed. We also investigated non-coding SNPs within the HLA region that have previously been associated with traits in the GWAS catalogue; SNPs in the HLA region (Shiina et al. 2009) between GRCh.v38: Chr6:29545629 (start of GABBR1

transcript ENST00000355973.7, minus 10Kb) and GRCh.v38: Chr6:33419924 (end of KIFC1 transcript ENST00000428849.6, plus 10 Kb) were downloaded from the NHGRI-EBI GWAS Catalogue(Burdett, T; Hall, PN; Hastings, E; Hindorf, LA; Junkins, HA; Klemm, AK; MacArthur, J; Manolio, TA; Morales, J; Parkinson H; Welter 2018). SNPs labelled with one or more of the following contexts were excluded stop_gained, synonymous_variant, missense_variant, non_coding_transcript_exon_variant, frameshift_variant or inframe_deletion, in order to prioritise variants with a possible regulatory role over variants involved in changes to protein coding. Only SNPs with genome wide significance with a reported trait (p-value < $5*10^{-8}$) were included in the analysis. These were tested for their association with sarcopenia using the methods described above for HLA type analysis. P-values < $1*10^{-5}$ were deemed to be significant (a commonly used cut-off for suggestive associations in GWA studies). Associations between these SNPS and sarcopenia-associated HLA types were also calculated. The GTEx database ("GTEx Analysis V7 (DbGaP Accession Phs000424.v7.P2) Single-Tissue Cis-EQTL Data" 2018) was used to identify genes with expression affected by the genetic variants.

3.5 Results

We selected 196,099 UK Biobank participants of European descent aged 60-70 with complete phenotype, diagnosis, and genotype data for investigation. Of these, 14,798 participants had at least one diagnosed autoimmune disease (including type-1 diabetes, multiple sclerosis, and rheumatoid arthritis, see methods for details) and were excluded from analyses. The remaining 181,301 participants were included in the analysis. The mean age was 64.1 years and 95,340 were female (Table 1). Of 85,961 men included in the analysis 3,510 were defined as having sarcopenia (4.08%), and of 95,340 women there were 11,540 defined as sarcopenic (12.10%).

Table 3-1: UK Biobank participant characteristics

	n	min-max	mean (SD)
Age of study participant (years)	181,301	60-70	64.11 (2.85)
BMI (Kg/m2)	181,301	12.81-68.41	27.52 (4.46)
Grip strength (Kg)	181,301	0-73	31.09 (10.71)
Skeletal muscle mass (Kg)	181,301	8.57-46.13	21.96 (6.00)
	n	**%**	
Gender			
Female	95,340	52.59	
Male	85,961	47.41	
Highest education level attained			
None	48,224	26.94	
Secondary	28,870	16.13	
College-level	26,810	14.98	
Professional/University	75,075	41.94	
Smoking status			
Never	90,242	50.01	
Previous	75,618	41.9	
Current	14,602	8.09	
EWGSOP Sarcopenia			
No	166,251	91.70	
Yes	15,050	8.30	

Note: UK Biobank participants aged 60-70 of European descent with complete grip strength, skeletal mass, genotype (HLA), and autoimmune diagnosis data. Participants with a diagnosis of autoimmune diseases were excluded from analyses.

Of 100 HLA types with allele frequency >1%, six were associated with sarcopenia (EWGSOP definition) in logistic regression models adjusted for age, sex, genotyping array type, and population structure (genetic principal components 1-5), after accounting for multiple testing (FDR<5%) (Figure 3-1)

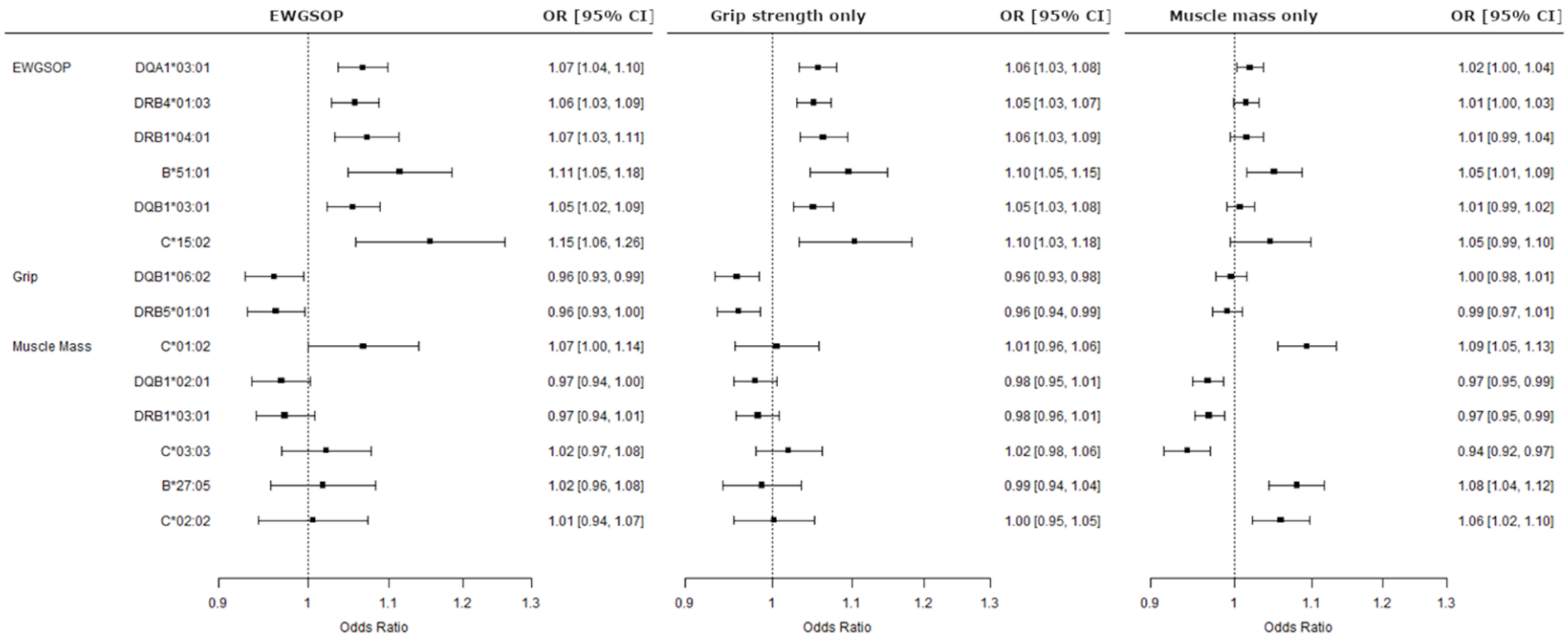

Figure 3-1: Forest plot of HLA types associated with Sarcopenia phenotypes.

Note: Additive logistic regression analysis of 97 HLA-types associated with sarcopenia (Benjamini-Hochberg correction for multiple testing applied). OR=Odds Ratio per allele of HLA-type, CI=Confidence Interval, EWGSOP=Combined sarcopenia definition: low grip and muscle mass. HLA types passing the FDR cut-off of 0.05 for each phenotype are marked with asterisks (*)

We compared participants with two alleles of each HLA-type to those with zero alleles, with the assumption of larger effects with a recessive mode of inheritance; participants homozygous for HLA-DQA1*03:01 have 19.3% (Odds Ratio 1.19, 95% Confidence Intervals 1.098-1.295, $p=2.84*10^{-5}$) increased likelihood of sarcopenia, compared to those without HLA-DA1*03:01 (Supplementary Table s3-1) and HLA-DRB4*01:03 homozygotes had a 15.4% increased likelihood of sarcopenia (Odds ratio 1.15, CI 1.079-1.234, $p=2.66*10^{-5}$). When the six HLA-types were combined, participants with at least six alleles (of a possible 12 - each person can have up to two alleles of each HLA type) had 23% increased likelihood of sarcopenia (n= 5,685 participants of 181,301; OR 1.23, 95% CI 1.123-1.346, $p=7.28*10^{-6}$).

Seven HLA-types were associated with the sarcopenia definition based on grip strength alone, and six were associated with the sarcopenia muscle mass definition, with no overlap between the alleles associated with the two phenotypes (Figure 3-1) (Supplementary Table s3-2). All of the top HLA type associations for the EWGSOP definition of sarcopenia were also present in the low grip phenotype, with the exception of HLA-C*15:02 which did not reach significance after correction for multiple testing in the low grip phenotype analysis. Conversely, the HLA types associated with low muscle mass (HLA-B*27:05 OR 1.079, 95% CI 1.042-1.117, $p=1.64*10^{-5}$; HLA-C*01:02 OR 1.093, 95% CI 1.054-1.134, $p=1.65*10^{-6}$ and HLA-C*02:02 OR 1.059, 95% CI 1.022-1.096, $p=1.46*10^{-3}$) were distinct from those associated with the EWGSOP definition of sarcopenia.

In sensitivity analyses we investigated the effect of adjusting for height and weight; all the reported risk increasing associations between HLA-types and sarcopenia

remained significant, with nominal changes to effect sizes (Supplementary Table s3-3). However, the three protective HLA-types associated with the sarcopenia grip definition were non-significant after adjusting for height and weight ($p > 0.05$).

3.5.1 Non-coding Single Nucleotide Polymorphisms in HLA region

We analysed 831 non-coding SNPs within the HLA region previously implicated in human traits at genome wide significance ($p<5*10^{-8}$) available in the UK Biobank genotype data (frequency ≥1%). We found 216 SNPs nominally ($p<0.05$) associated with the EWGSOP definition of sarcopenia (Supplementary Table s3-4). 10 were associated with p-value $<1*10^{-5}$ (commonly used cut-off for suggestive associations in GWA studies). Of these, we identified 4 independent signals (Table 3-2), after removing those in linkage disequilibrium (R^2 >0.4, see Supplementary Table s3-5). This included rs41268896 and rs2293751 with p-values $< 5*10^{-8}$ (ORs = 1.079 and 1.069, respectively).

Table 3-2: Non-coding SNPs within the HLA region associated with EWGSOP definition of sarcopenia (p ≤ 1.0 x 10-5) with gene expression information.

RS id	A1/A0	CHR:POS	Trait	OR	95% CI	p-value	Expressed gene(s)	Tissue type*
rs41268896 ¥	A/G	6:32070069	Atopic dermatitis	1.08	1.05-1.11	1.06E-08	ATF6B, CYP21A1P, BAG6, **HLA-DQA2**, PRRT1	MS, AS, AT, WB, SN
rs2844479	C/A	6:31572956	Height	1.07	1.04-1.09	6.11E-07	BAG6, ATF6B, CSNK2B, LY6G5C, CYP21A1P	AT, MS, HLV, TH, EM
rs9268645 †	G/C	6:32408527	T1 diabetes	1.06	1.04-1.09	1.50E-06	**HLA-DQA2**, **HLA-DQB2**, **HLA-DQB1**, **HLA-DRB6**, **HLA-DRB9**	MS, WB, WB, MS, TH
rs2072633	G/A	6:31919578	Coronary artery disease	1.06	1.03-1.08	3.58E-06	CYP21A1P, ATF6B, **HLA-DQA2**, PSORS1C1, C2	AS, MS, WB, TH, TE

Note: Additive model; A1=Effect allele, A0=Reference allele, POS=build 37 base pair, OR=Odds Ratio, CI=95% Confidence Intervals. Trait = the top identified trait from the GWAS catalogue. * Tissue type: Adipose Subcutaneous=AS; Artery Tibial=AT; Colon Sigmoid=CS; Esophagus Muscularis=EM; Muscle_Skeletal=MS; Nerve Tibial=NT; Skin Not Sun Exposed Suprapubic=SN; Thyroid=TH; Whole Blood=WB; Heart Left Ventricle=HLV; Testis=TE. † correlated with DQA1*03:01 (R^2=0.42). ¥ in LD with protein QTL for AFT6A (R^2=0.8 with rs8111 in UKB) (Sun et al. 2018)

We interrogated the GTEx eQTL (expression quantitative trait loci) database of SNP-expression associations to determine the likely genes affected by these 4 genetic variants (Table 3-2, see Supplementary Table s3-6 for full details). This included specific HLA genes (HLA-DQA2, HLA-DQB2, HLA-DQB1, HLA-DRB6 and HLA-DRB9), in addition to other genes with plausible mechanisms of action in sarcopenia (*ATF6B*, *CYP21A1P*, *CYP21A2*, *BAG6*, *PRRT1*, *CSNK2B*, *LY6G5C*, *PRRC2A*, *PSORS1C1*, C2). We also searched for protein QTLs in a recent paper by Sun *et al.* (Sun et al. 2018) and found that rs41268896 is highly correlated (R^2=0.8 in UKB) with rs8111, a pQTL for ATF6A.

We also investigated the combined effect of the 4 non-coding SNPs; participants with at least 4 effect alleles (of a possible 8), showed an increased likelihood of sarcopenia of 11% (n= 74,820 participants of 181,301; OR 1.11, 95% CI 1.075-1.151, $p=1.06*10^{-9}$).

3.6 Discussion

To the best of our knowledge, this is the first large human population study of HLA effects on sarcopenia in older people without autoimmune disease. We identified 6 HLA types associated with sarcopenia in 181,301 UK Biobank participants of European descent aged 60-70. Although these have modest effect sizes (per allele odds ratios from 1.054 to 1.15) they are common in the study population, ranging in frequency from 3.7% to 24.8%. In combination, participants with more than six of the HLA-types had markedly increased likelihood of sarcopenia (27%). These results suggest that lifetime exposure to mild chronic pro-inflammatory state may contribute to risk of sarcopenia.

We also identified 4 non-coding genetic variants in the HLA region of chromosome 6 associated with sarcopenia. These are known to affect the expression of multiple genes, including *HLA* genes, in multiple tissues including skeletal muscle. Although more evidence is required for a clear causal link, this suggests that regulation in expression of HLA genes may also be an important driver of sarcopenia, not just the protein sequence (HLA-types). When taken in combination participants with more than four of the effect alleles, had an 11% increased likelihood of sarcopenia.

The EWGSOP definition of sarcopenia, which combines low grip strength and low muscle mass, shares many of the associated HLA alleles with those seen in low grip strength definition alone. In contrast, there is little overlap with the low muscle mass definition. Often, grip strength alone is used to identify “frail” participants in population studies (L. P. Fried et al. 2001) as it is the maintenance of function that appears most important.

HLA-types associated with sarcopenia are also associated with conditions linked to joint pain and loss of function, such as rheumatoid arthritis (DRB1*04:01), ankylosing spondylitis (C*15:02), Behcet's disease (B*51:01, joint swelling and pain prevalence in 45-60% of cases (Zeidan et al. 2016)), and neuropathic pain (DQB1*03:02, although a meta-analysis (Veluchamy et al. 2018) only showed association to the two-digit allele DQB1*03). Other HLA alleles associated with sarcopenia include DQA1*03:01, which has been linked to pemphigus vulgaris, a painful autoimmune condition which can affect the skin, mouth and groin with blisters. DQB1*03:01 has only been shown to have an association previously as a protective allele against primary biliary cholangitis, although this study provides evidence of a novel association with sarcopenia. In this analysis we excluded participants with a self-reported or hospital diagnosis of autoimmune conditions, including those mentioned here, suggesting that the associations observed here are not due to diagnosed conditions. More work is needed on whether these associations are due to a general pro-inflammatory effect or represent sub-clinical manifestations of specific autoimmune processes. More work is also needed on whether some patients with sarcopenia have evidence of autoimmune processes and might benefit from targeted treatment.

Some HLA types were protective for sarcopenia: increasing copies of DQB1*06:02, DRB5*01:01 and DRB1*15:01 were all associated with reduced likelihood of sarcopenia, and have previously been implicated in a number of studies in the development of multiple sclerosis (Goodin et al. 2018), although mainly as part of an extended haplotype including all three types.

When we compared participants carrying two alleles of each HLA to those with zero we found far greater effect sizes; for example the likelihood of being sarcopenic for DQA1*03:01 rises from 6% to 19.3%, suggesting recessive effects. DRB4*01:03 has

been reported as appearing with increased frequency in a limited study of Brazilian patients with polyarteritis nodosa (de Lira Freire et al. 2009). Association of DRB4*01:03 with rheumatoid arthritis has mixed evidence in the literature with recent studies showing no association (K. Kim et al. 2016), while slightly older and smaller studies in a different population showing a link (Louthrenoo et al. 2015). HLA-DQA1*03:01 has been previously shown to increase the risk of type 1 diabetes (Noble and Valdes 2011), when present as part of the DR4 haplotype or in conjunction with DQB*02:01 (Tollefsen et al. 2012).

The analysis of low grip strength alone reinforced the associations seen in the EWGSOP sarcopenia definition analysis, with only a single additional association for DQA1*01:02. This type was protective against low grip strength and reported as associated with a multiple sclerosis-like condition in transgenic mice (Kaushansky et al. 2015). HLA alleles were only associated with low muscle mass using the additive model, C*01:02 and B*27:05, both of which have previously been linked to forms of arthritis – psoriatic arthritis (Chandran et al. 2013) and spondyloarthritis (Ma et al. 2017). Neither type appeared in the associations for the combined EWGSOP phenotype of sarcopenia, suggesting potential independent effects.

In our analysis of non-coding genetic variation in the HLA-region we identified 4 genetic variants associated with sarcopenia. These SNPs also affect expression of genes other than HLAs, including *ATF6B*, which encodes a transcription factor in the unfolded protein response (UPR) pathway during endoplasmic reticulum (ER) stress and there has been speculation that ER stress may impair autophagy and myogenesis activity resulting in sarcopenia (Potes et al. 2017) (Deldicque 2013). rs41268896 is strongly correlated with the rs8111 (R^2=0.8), known to affect protein levels of ATF6A (Sun et al. 2018), which binds as a heterodimer with ATF6B. The rs41268896 A allele

increases *ATF6B* expression, whilst the rs8111 T allele (co-inherited) decreases ATF6A protein levels, suggesting a complex relationship; more work is required to understand this. The *BAG6* protein product is also involved in elimination of misfolded proteins, including class-I HLA products (Yamamoto et al. 2017) (Y. Xu et al. 2013). *CYP21A2* has a role in producing cortisol and aldosterone as part of the hypothalamic-pituitary-adrenal (HPA) axis, disruption to the HPA has been linked to decline in physical function and aging (Gaffey et al. 2016)(Doleschall et al. 2014).

The exclusion of autoimmune conditions, based on a previous literature, was not exhaustive. Further exclusions, for example polymyalgia rheumatica (ICD-10 M35.3), would help reinforce the evidence that sarcopenia has an auto-immune component separate from any other underlying autoimmune condition. Polymyalgia rheumatica (and the closely related condition, giant cell arteritis, GCA) in particular has been shown to involve muscle weakness, stiffness and functional impairment (Buttgereit et al. 2016), which would impact the low grip measurements used in the study.

In addition GCA susceptibility has previously been associated with HLA-DRB1*04 (odds ratio = 2.69, P = 1.5×10−11), while HLA-DRB1*15 and HLA-DRB1*16 have been noted to have a protective effect (OR = 0.65, P = 8.2×10−6)(Mackie et al. 2015).

The common treatment for GCA are steroids, such as the glucocorticoids (Simon, Ninan, and Hissaria 2021), however glucocorticoid-induced myopathy is one of the most common causes of drug induced muscle weakness (Gupta and Gupta 2013) and so a sensitivity analysis of self-reported drug use could help to resolve any bias in the results due to treatments that impact muscle function and therefore the definitions of sarcopenia used in this analysis.

The strengths of this study include the large number of older participants with consistent sarcopenia measurements and medical records data available for analysis. However, it is a volunteer study and will therefore be healthier than the general population: effects of HLA types on sarcopenia may therefore be underestimated in this study. Additionally, autoimmune diagnoses may be under-reported as diagnoses are either self-reported or from hospital in-patient records only; further studies will be required. Future work should clarify the associations between HLA-types, circulating inflammatory cytokines, and frailty, ideally in a longitudinal study.

3.7 Conclusions

In this large study of older participants we identified 16 HLA-types associated with sarcopenia, especially with low hand grip strength, in the absence of autoimmune diagnoses. Chronic, low-grade autoimmune phenotypes may be present in these individuals, predisposing them to sarcopenia. Additional analysis of non-coding variants showed that SNPs involved the regulation of HLA, ER stress response and immune function genes are also associated with sarcopenia. Further studies into the long-term effects of HLA variation are required. More work is also needed on whether some patients with sarcopenia have evidence of autoimmune processes and might benefit from targeted treatment.

4 Analysis 2: Genome-wide meta-analysis of muscle weakness in older adults

Genome-wide meta-analysis of muscle weakness identifies 15 susceptibility loci in older men and women

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Nature communications, 12(1), pp.1-11

4.1 Overview

Low grip strength is a good predictor of overall muscle function and is a commonly measured metric, making a multi-cohort Genome-Wide Association Study meta-analysis possible. By using the low grip cut-offs as defined by the European Working Group on Sarcopenia and genotyping data from members of the CHARGE consortium I have shown that there are fifteen genomic risk loci with low grip strength, 12 of which have not previously been associated with a continuous measure of grip strength, in older adults (age > 65) of European ancestry.

This analysis highlights the multiple pathways to the physical components of frailty and sarcopenia.

My contribution included leading the writing of the paper and primary analyst for the multi-cohort study. In addition I coordinated the meta-analysis and helped draft the analysis plan. The resulting paper has been accepted for publication at Nature Communications: Jones, G., Trajanoska, K., Santanasto, A.J., Stringa, N., Kuo, C.L., Atkins, J.L., Lewis, J.R., Duong, T., Hong, S., Biggs, M.L. and Luan, J.A., 2021. Genome-wide meta-analysis of muscle weakness identifies 15 susceptibility loci in older men and women. Nature communications, 12(1), pp.1-11.

4.2 Abstract

Low muscle strength is an important heritable indicator of poor health linked to morbidity and mortality in older people. In a genome-wide association study meta-analysis of 256,523 Europeans aged 60 years and over from 22 cohorts we identified 15 loci associated with muscle weakness (European Working Group on Sarcopenia in Older People definition: n=48,596 cases, 18.9% of total), including 12 loci not implicated in previous analyses of continuous measures of grip strength. Loci include genes reportedly involved in autoimmune disease (*HLA-DQA1* $p=4*10^{-17}$), arthritis (*GDF5* $p=4*10^{-13}$), cell cycle control and cancer protection, regulation of transcription, and others involved in the development and maintenance of the musculoskeletal system.

4.3 Introduction

Age-associated loss of muscle strength (termed dynapenia) (Brian C Clark and Manini 2012) is one of the characteristic changes occurring with advancing age, and muscle weakness is considered a fundamental component of frailty and sarcopenia (Manini and Clark 2012). Individuals over 70 years old typically demonstrate up to 20% lost muscle mass compared with individual in their twenties (Mitchell et al. 2012) Although definitions of reduced muscle function in older people have focused on loss of muscle mass ("sarcopenia") evidence now shows that muscle weakness itself is often more predictive of negative health outcomes (Cawthon et al. 2019). Muscle weakness causes difficulties in daily functioning (i.e. disability) and low muscle strength (measured as hand grip strength, considered a biomarker of general dynapenia) is predictive of future morbidity and mortality (Mitchell et al. 2012) over the long term (Taina Rantanen et al. 1999). Despite intensive research, causes of and contributors to muscle weakness in later life remain to be fully elucidated (Cruz-Jentoft et al. 2019). Importantly, muscle strength is heritable, and can thus be studied using genetic analysis.

Previously a genome-wide association study (GWAS) by the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) consortium identified two loci associated with maximum hand grip strength (as a quantitative trait) in 27,581 Europeans aged 65 and over (Matteini et al. 2016). Another study on maximum hand grip strength (divided by weight) in mostly middle-aged UK Biobank participants (334,925 people aged 40 to 70, mean aged 56) identified and replicated 64 loci, many of which are known to have a role in determining anthropometric measures of body size (Tikkanen et al. 2018; Willems et al. 2017). These previous studies that considered grip strength as a continuous phenotype across young and old individuals

may not provide insights into the age related loss of muscle strength that leads to a magnitude of weakness sufficient to call it a disease.

Given the limited data on genetic contributions to a clinically meaningful level of muscle weakness in older adults, we aimed to determine the genetic variants and investigate causal pathways associated with low measured grip strength in 256,523 older adults (aged 60+ years) of European ancestries from the CHARGE consortium. The primary analysis was based on the established 2010 European Working Group on Sarcopenia in Older People (EWGSOP) definition of low grip strength (Chapter 4), and results were compared to an analysis of the alternative Foundations of the National Institutes of Health (FNIH) definition based on its association with functional outcomes, with additional analyses stratified by sex (Chapter 5).

4.4 Methods

4.4.1 GWAS of low grip strength in older people

I conducted a GWAS meta-analysis of low grip strength in participants aged 60 years or older of European ancestry from 22 studies yielding a combined sample of 254,894 individuals. Individual studies used different genotyping platforms and imputation was predominantly performed using the Haplotype reference consortium (HRC) v1.1 panel.

Over 70 year olds were not specifically excluded from the meta-analysis, with the analysis plan criteria being individuals over the age of 60. However due to the nature of the recruitment for the largest dataset, the UK Biobank, the upper age in this particular sample subset was 70.

We excluded individuals below the age of 60 with low grip strength due to potential confounding with conditions causing Cachexia (such as cancer), or similar. In doing so we hoped to highlight age-specific associations to low grip strength, and their impact on healthy ageing.

Two definitions of low muscle hand grip strength were utilized at the time of analysis. The primary analysis was of the 2010 EWGSOP criteria for sarcopenic grip strength (Grip strength < 30 Kg Male; < 20 Kg Female).

GWAS was performed by each cohort individually using regression models, adjusted for age, sex (except in sex-specific models), and population substructure, accounting for relatedness and technical covariates as required by the individual study. No adjustment for anthropometric measures was made in the primary analysis, but the effects were explored in sensitivity analyses. Fixed-effects inverse variance weighted meta-analysis was performed using METAL (Willer, Li, and Abecasis 2010b) using the GWAS summary statistics generated by each cohort, with genomic control for

population structure (see Supplementary Methods for details, available online at https://www.nature.com/articles/s41467-021-20918-w and in Appendix). The following quality control filters were applied: minor allele frequency (MAF) > 0.01, imputation info score of > 0.4, and the variant present in at least two studies (UK Biobank – the largest included cohort - plus at least one other). The final analysis therefore included 9,678,524 genetic variants. Associations that achieved a $p<5*10^{-8}$ were considered statistically significant, with those reaching the more stringent threshold of $p<5*10^{-9}$ highlighted.

Distinct loci were initially defined as two significant variants separated by >500kb. To identify independent signals at each locus we used FUMA (Functional Mapping and Annotation of Genome-Wide Association Studies) (Watanabe, Taskesen, van Bochoven, et al. 2017), which uses Linkage Disequilibrium (LD) information to determine independence (r^2 threshold = 0.1 for independent significant single nucleotide polymorphisms (SNP)). We used Linkage Disequilibrium Score Regression (LDSC, v1.0.0) to estimate the level of bias (i.e. from population stratification and cryptic relatedness) in the GWAS, and the SNP-based heritability of low grip strength (B. K. Bulik-Sullivan, Loh, Finucane, Ripke, Yang, Consortium, et al. 2015).

4.4.2 Locus overlap with diseases and anthropometric traits

The GWAS catalog of published locus-trait associations (Buniello et al. 2019b) was searched to identify whether low grip strength-associated loci determined from our meta-analysis are known to influence other traits or diseases. In addition, we performed sensitivity analyses in the UK Biobank sample to determine whether associations between variants and low grip strength identified in the meta-analysis

were robust to adjustment for the following traits or diseases: height, weight, body mass index, osteoarthritis, rheumatoid arthritis, and osteoporosis (prevalent diseases were from the self-reported data at baseline or in the Hospital Episode Statistics). Analyses were performed in STATA (v 15) using logistic regression models adjusted for age, sex, principal components 1 to 10, assessment center, and genotyping array (the UK Biobank using two different Affymetrix microarrays that shared >95% of sites - Supplementary Methods).

4.4.3 Gene Ontology Pathways, Tissue Enrichment, and eQTL analyses

I utilized FUMA to perform functional interpretation of the GWAS results (Watanabe, Taskesen, van Bochoven, et al. 2017). In particular, FUMA performs gene-set analysis (using Multi-marker Analysis of GenoMic Annotation (MAGMA)(de Leeuw et al. 2015a)) to identify pathways enriched amongst the significant genes (weighted by the SNP-associations in proximity to them), in addition to searching eQTL databases to identify SNPs that significantly alter the expression of genes in various tissues.

We used MetaXcan to determine whether gene-level transcriptomic associations in GTEx v7 skeletal muscle data were enriched in the GWAS summary statistics for low grip strength (Barbeira et al. 2018). The analysis included 7,512 genes with measured expression in the dataset; we applied Benjamini-Hochberg multiple-testing correction, with adjusted p-values < 0.05 deemed to be significant.

LD Score Regression applied to specifically expressed genes (LDSC-SEG) allows the identification of enriched tissue activity associated with GWAS results (Finucane et al. 2018). We applied LDSC-SEG (v1.0.0) to the GWAS summary statistics using the datasets `Multi_tissue_gene_expr` and `Multi_tissue_chromatin` provided by the authors. We applied Benjamini-Hochberg multiple-testing correction for the 703 tests

(n gene expression = 205, n chromatin = 498), with adjusted p-values < 0.05 deemed to be significant.

4.4.4 Data Availability

The GWAS summary statistics and supporting information on low grip strength in older people are available on the Musculoskeletal Knowledge Portal (http://musculoskeletalgenomics.org/). All relevant additional data is available on request from the authors.

Supplementary result tables and methods available online at https://www.nature.com/articles/s41467-021-20918-w, and in attached Appendix / Supplementary Tables.

4.5 Results

4.5.1 Study description

The meta-analysis comprised 256,523 individuals of European descent aged 60 years or older at assessment from 22 independent cohorts with maximum hand grip strength recorded - including the UK Biobank, the US Health and Retirement Study (HRS), the Framingham Heart Study (FHS), and others. 46,596 (18.9%) of all participants had muscle weakness (dynapenia) based on hand grip strength (EWGSOP definition: grip strength < 30 Kg Male; < 20 Kg Female). Individual study characteristics are described in the Supplementary Information and in Supplementary Table s4-1.

This chapter describes the primary analysis of EWGSOP definition low grip strength. Subsequent additional analyses described in later chapters: these include analysis of males and females separately, and investigation of casual pathways using mendelian randomisation.

4.5.2 GWAS of low muscle strength identifies 15 loci

We found 15 genomic risk loci to be associated ($p<5*10^{-8}$; 8 loci $p<5*10^{-9}$) with EWGSOP definition low hand grip strength in our GWAS meta-analysis of 22 cohorts (N=256,523, n=48,596 cases), adjusted for age, sex, and technical covariates (Figure 4-1, Table 4-1).

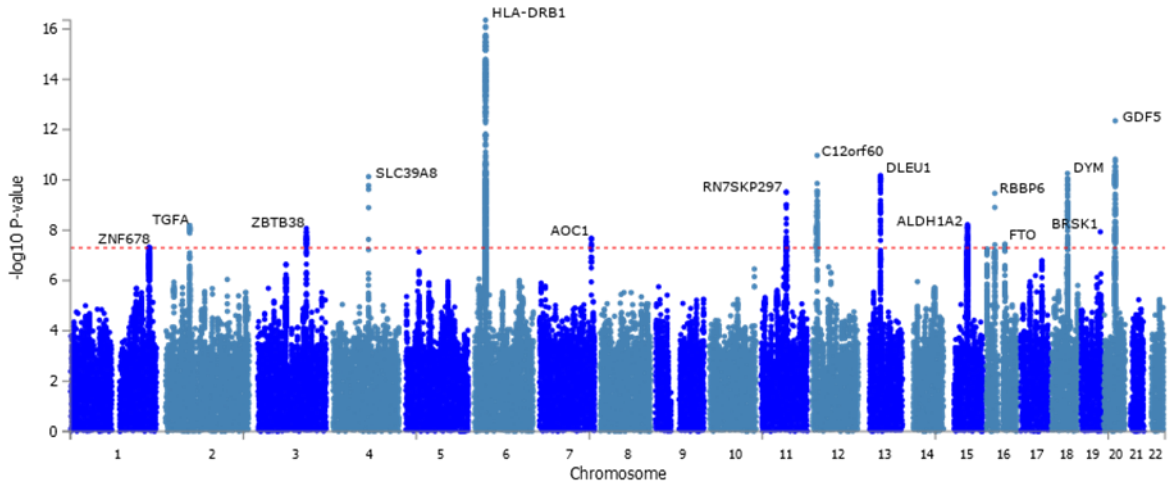

Figure 4-1: Manhattan plot of low grip strength genome-wide association study -log10 p-values

The p-values are from a fixed-effects meta-analysis of 256,523 Europeans aged 60 or older from 22 cohorts. The outcome was low hand grip strength grip strength cutoff (males <30 kg, females <20 kg). The x-axis is the chromosomal location, and the y-axis is the –log10 p-value for each genetic variant. The horizontal red line is the threshold for genome-wide significance (p<5*10-8). Fifteen genomic loci cross the threshold, and the lead variant (most significantly associated with low strength) is described in table 1. The nearest gene is displayed for each locus.

Table 4-1: Genomic risk loci associated with low grip strength in 256,523 older men and women

RSID	Chr	BP (b37)	EA	OA	EAF	OR	*p*-value	Nearest gene	GTEx increased	GTEx decreased
rs34415150	6	32560477	G	A	0.18	1.087	**4.4*10-17**	*HLA-DRB1*	***HLA-DQA2****(Snse)* †*; HLA-DRB6; HLA-DQB2; HLA-DOB*	***HLA-DQA1****(Snse)* †*; HLA-DRB1; HLA-DQB1; HLA-DQB1-AS1*
rs143384	20	34025756	A	G	0.59	1.056	**4.5*10-13**	*GDF5*	***UQCC1****(Musk/Cfib)* †*; FAM83C; CPNE1*	*GDF5; RPL36P4*
rs62102286	18	46592408	T	G	0.56	1.050	**5.5*10-11**	*DYM*		***DYM****(Wb)* †
rs3118903	13	51099577	A	G	0.22	1.059	**6.7*10-11**	*DLEU1*		*RNASEH2B-AS1*
rs13107325	4	103188709	T	C	0.07	1.094	**7.4*10-11**	*SLC39A8*		*UBE2D3*
rs11236213	11	74394369	G	A	0.69	1.052	**3.0*10-10**	*RN7SKP297*	*KCNE3; POLD3*	*RP11-864N7.4; CHRDL2*
rs34464763	12	15032860	A	T	0.39	1.056	**3.2*10-10**	*C12orf60*	*RP11-233G1.4*	*ERP27; SMCO3; C12orf60; MGP*
rs143459567	16	24600412	T	C	0.04	1.126	**3.4*10-10**	*RBBP6*		
rs2899611	15	58327347	G	T	0.50	1.044	6.0*10-9	*ALDH1A2*		*ALDH1A2*
rs958685	2	70703847	C	A	0.49	1.044	6.5*10-9	*TGFA*	*TGFA* (Ts)†	*TGFA* (Bcor, Bcau, Bhyp, Bacc)†
rs7624084	3	141093285	T	C	0.56	1.044	8.5*10-9	*ZBTB38*	*ZBTB38* (Skse, Esom, Snse)†	*ZBTB38* (Wb, Thy, Adips, Ts)†
rs79723785	19	55818225	C	T	0.02	1.182	1.2*10-8	*BRSK1*	***HSPBP1****(Art)**	
rs10952289	7	150524681	T	C	0.66	1.044	2.1*10-8	*AOC1*	*AOC1* (Haa, Hlv, Liv); *TMEM176B* (Haa)†	*AOC1* (Esom, Adipv, Thy, Esog)†
rs8061064	16	53912364	A	T	0.46	1.042	3.6*10-8	*FTO*		
rs12140813	1	227776827	T	C	0.19	1.052	4.8*10-8	*ZNF678*	*JMJD4*	*SNAP47*

Chr= chromosome; BP= base pair position, genome build 37; EA= effect allele; OA= other allele; EAF= effect allele frequency; OR= Odds Ratio of having low grip strength (EWGSOP criteria) per allele; *p*-value= fixed-effects meta-analysis p-value, values < $5*10^{-9}$ highlighted in bold; Nearest gene= on GRCh37; GTEx

increased/decreased= Top four genes with known expression associations with the lead SNP in GTEx v8, ordered by p-value. *rs79723785 is a splicing QTL for HSPBP1. † GTEx v8 differential expression by tissue; Gene names in bold indicate that the SNP is the lead eQTL for that gene in the stated tissue (note: in many cases although the lead SNP is not the lead eQTL, it is often correlated with it) – Ts = Testis; Bput = Brain - Putamen (basal ganglia); Bcor = Brain – Cortex; Bcau = Brain - Caudate (basal ganglia); Bhyp = Brain – Hypothalamus; Bacc = Brain – Anterior cingulate cortex; Asub = Adipose – subcutaneous; Wb = Whole blood; Esom = Esophagus – mucosa; Skse = Skin sun exposed lower leg; Snse = Skin non-sun exposed lower leg; Thy = Thyroid; Adips = Adipose – Subcutaneous; Adipv = Adipose - Visceral (Omentum); Esog = Esophagus - Gastroesophageal Junction; Haa = Heart - Atrial Appendage; Hlv = Heart - Left Ventricle; Liv = Liver; Musk = Muscle - Skeletal; Cfib = Cells - Cultured fibroblasts; Art = Artery - Tibial.

The strongest associations were with variants close to *HLA-DQA1* (rs34415150, beta/log-OR per G allele=0.0833, $p=4.4*10^{-17}$), *GDF5* (rs143384, beta per A allele=0.0545, $p=4.5*10^{-13}$) and *DYM* (rs62102286, beta per T allele=0.0487, $p=5.5*10^{-11}$). Twelve of the fifteen lead SNPs from the GWAS have not previously been identified in studies of continuous grip strength in all ages (Buniello et al. 2019b) (see Table 4-2 and Supplementary Table s4-2).

This included the three most strongly associated variants near *HLA-DQA1* (previously implicated in rheumatoid arthritis: see Table 2), *GDF5* ('Growth differentiation factor 5': previously implicated in height, waist hip ratio, muscle mass, and osteoarthritis) and *DYM* ('Dymeclin': implicated in in height). Six other variants were previously linked to height and four to osteoarthritis. None were significantly ($p<5*10^{-8}$) associated with lean muscle mass, although rs10952289 near AOC1 is nominally associated with appendicular lean muscle mass ($p=6*10^{-4}$) (Zillikens et al. 2017). The test of cohort heterogeneity in METAL for all 15 lead SNPs was not statistically significant (nominal het $p>0.05$). Full summary statistics for the meta-analysis are available for download (http://musculoskeletalgenomics.org/).

Table 4-2: Low grip loci that appear in the GWAS catalog of published variant-trait associations

Genetic variant	*p*-value	Nearest Gene	Height	BMI	WHR	Bone	Grip strength	OA	RA	Neuro
rs34415150	4.42*10-17	*HLA-DRB1*							rs6931277 (R^2=0.82)	rs6931277 (R^2=0.82)
rs143384	4.47*10-13	*GDF5*	rs143384		rs143384	rs6060373 (R^2=0.75)		rs143383 (R^2=0.82)		
rs62102286	5.49*10-11	*DYM*	rs9967417 (R^2=0.83)							
rs3118903	6.71*10-11	*DLEU1*	rs3116602 (R^2=0.94)		rs3118910 (R^2=0.94)	rs157165 (R^2=0.91)				rs1262778 (R^2=0.81)
rs13107325	7.42*10-11	*SLC39A8*		rs13107325	rs13107325		rs13107325	rs13107325		
rs11236213	3.01*10-10	*RN7SKP297*			rs11236213					
rs34464763	3.15*10-10	*C12orf60*				rs2430690 (R^2=0.98)	rs10846071 (R^2=0.98)	rs4764133* (R^2=0.93)		
rs143459567	3.41*10-10	*RBBP6*				rs34365165 (R^2=0.93)				
rs2899611	6.01*10-9	*ALDH1A2*								
rs958685	6.52*10-9	*TGFA*					rs958685	rs3771501 (R^2=0.90)		
rs7624084	8.51*10-9	*ZBTB38*	rs6440003 (R^2=0.98)		rs7632381 (R^2=0.98)	rs1991431 (R^2=0.91)				rs9846396 (R^2=0.91)
rs79723785	1.16*10-8	*BRSK1*						rs4252548 (R^2=1.0)		
rs10952289	2.10*10-8	*AOC1*	rs2110001 (R^2=0.85)							

rs8061064	3.55*10-8	*FTO*								
rs12140813	4.76*10-8	*ZNF678*	rs1390401 (R^2=1.0)		rs6672530 (R^2=1.0)					

Data from the GWAS catalog (www.ebi.ac.uk/gwas) extracted in March 2020. BMI = Body Mass Index; WHR = Waist-Hip ratio or Hip circumference adjusted for BMI; Bone=includes bone mineral density and bone size (e.g. neck of femur) traits; Grip=Increasing Handgrip Strength (linear); OA = Osteoarthritis; RA = Rheumatoid Arthritis; Neuro = Neurological disease; R2=correlation with lead low grip Genetic Variant – information from LDlink-pair GBR population. * SNP has been independently associated with osteoarthritis of the hand (den Hollander et al. 2017).

Overall, three of the fifteen identified lead variants (or proxies) have not previously been implicated in anthropometric or musculoskeletal phenotypes in the GWAS catalogue (see Table 2). This included: *RBBP6* ('RB binding protein 6, ubiquitin ligase': a tumor suppressor gene), *ALDH1A2* ('Aldehyde Dehydrogenase 1 Family Member A2': involved in the synthesis of retinoic acid), and a variant near *FTO* ('FTO Alpha-Ketoglutarate Dependent Dioxygenase': involved in the oxidative demethylation of different forms of RNA). Although the lead *ALDH1A2* SNP itself has not been identified in previous GWAS, other independent variants (R^2<0.6) at the same locus (e.g. rs3204689) have been found to be associated with osteoarthritis (Table 4-2).

The LDSC regression intercept was not significantly greater than 1, suggesting no bias due to population stratification or cryptic relatedness (B. K. Bulik-Sullivan, Loh, Finucane, Ripke, Yang, Consortium, et al. 2015). The single nucleotide polymorphism (SNP) based heritability (h^2) of low grip strength was 0.044 (SE 0.0027), i.e. 4.4%, by LD Score Regression.

4.5.3 Subset of overall grip strength variants are associated with low grip strength

After reviewing the 64 published variants associated with continuous measures of grip strength in the UK Biobank cohort (Tikkanen et al. 2018), for all ages (40 to 70 years), we found that only three of these SNPs were significant genome-wide ($p<5*10^{-8}$) in the EWGSOP low grip strength GWAS (Supplementary Table s4-3). The top association was rs13107325 (*SLC39A8* linear grip $p=4.4*10^{-23}$, T effect allele = 0.006 unit decrease in linear grip strength; low grip strength meta-analysis $p=7.4*10^{-11}$, T effect allele = 0.0897 unit increase in risk of low grip strength with age), then rs2430740 (*C12orf60* linear grip $p=6*10^{-12}$, G effect allele = 0.002 unit decrease in

linear grip strength; low grip strength meta-analysis p=2.6*10^{-9}, G effect allele = 0.045 unit increase in risk of low grip strength with age) and finally rs11236203 (*POLD3*, linear grip p=8.4*10^{-10}, G effect allele = 0.002 unit decrease in linear grip strength; low grip strength meta-analysis p=2.8*10^{-8}, G effect allele = 0.043 unit increase in risk of low grip strength with age). Two less-significant associations (< 1*10^{-6}) were seen with rs3821269 (*TGFA* linear grip p=3.5E*10^{-15}, A effect allele = 0.002 unit decrease in linear grip strength; low grip strength meta-analysis p=8.0*10^{-8}, A effect allele = 0.04 unit increase in risk of low grip strength with age) and rs1556659 (*ENSG00000232985* linear grip p=1.1*10^{-11}, C effect allele = 0.002 unit decrease in linear grip strength; low grip strength meta-analysis p=3.5*10^{-7}, C effect allele = 0.04 unit increase in risk of low grip strength with age).

A previous GWAS by the CHARGE consortium identified two loci associated with maximum hand grip strength recorded in 27,581 Europeans aged 65 or older (Matteini et al. 2016). We found that neither locus was associated with low grip strength in our analysis (rs752045 EWGSOP p=0.67, rs3121278 EWGSOP p=0.15).

4.5.4 GWAS of low grip strength based on FNIH criteria

In secondary analysis we performed GWAS using the low grip strength definition published by the FNIH (S. A. Studenski et al. 2014). This criterion uses lower grip strength cut-offs (<26Kg for males and <16Kg for females) than the EWGSOP definition (Cruz-Jentoft et al. 2010), resulting in fewer cases (n=19,345, 7.6% of total). Five loci were significant in the analysis (p<5*10^{-8}), only one of which was not identified in the EWGSOP low grip strength analysis described previously (see Table 4-3, either the same SNP or in high LD with the EWGSOP lead SNP at that locus, for example

rs3771501 and rs958685 R^2=0.90). This single base-pair deletion (rs1403785912 - chr9:4284961:T:-) mapped to *GLIS3* (“GLIS family zinc finger 3” - a repressor and activator of transcription), and may be specifically associated with more “extreme” weakness (EWGSOP p-value = $1.2*10^{-3}$).

Table 4-3: Genomic risk loci associated with low grip strength FNIH definition

RSID	Chr	Position	EA	OA	EAF	Effect	P-value	Nearest gene*	GTEx gene expression increased ‡	GTEx gene expression decreased
rs34415150	6	32560477	G	A	0.18	0.1158	5.61E-16	HLA-DRB1	***HLA-DQA2****(Snse); HLA-DRB6; HLA-DQB2; HLA-DOB*	***HLA-DQA1****(Snse); HLA-DRB1; HLA-DQB1; HLA-DQB1-AS1*
rs3771501	2	70717653	A	G	0.47	0.0697	5.04E-11	TGFA	*TGFA (Ts)†*	*TGFA (Bcor, Bcau, Bhyp, Bacc)†*
rs1403785912*	9	4284961	AT	A	0.52	0.0765	4.32E-09	GLIS3	*RP11-358M14.2**	
rs12456780	18	46947541	T	A	0.33	0.0633	1.29E-08	DYM		*DYM*
rs191252760	4	61454905	G	A	0.02	0.1863	3.17E-08	AC095061.1		*ADGRL3*

Gene names in bold indicate that the SNP is the lead eQTL for that gene in the stated tissue (note: in many cases although the lead SNP is not the lead eQTL, it is often correlated with it). † GTEx v8 differential expression by tissue – Ts = Testis; Bcor = Brain – Cortex; Bcau = Brain - Caudate (basal ganglia); Bhyp = Brain – Hypothalamus; Bacc = Brain – Anterior cingulate cortex; Asub = Adipose – subcutaneous; Wb = Whole blood; Esom = Esophagus – mucosa; Skse = Skin sun exposed lower leg; Snse = Skin non-sun exposed lower leg; Thy = Thyroid; Adips = Adipose – Subcutaneous; Adipv = Adipose - Visceral (Omentum); Esog = Esophagus - Gastroesophageal Junction; Haa = Heart - Atrial Appendage; Hlv = Heart - Left Ventricle; Liv = Liver.

* SNP not found in GTEx v8 - rs3934283 used as proxy (r2=0.995, FNIH low grip p-value=3.02*10-6)

4.5.5 Mitochondrial variants and low grip strength

Analysis of 116 mitochondrial genetic variants (MAF>0.01) available in the UK Biobank directly genotyped microarray data identified two associated with EWGSOP-defined low hand grip strength ($p<0.00043$, i.e. Bonferroni-adjustment for mitochondrial variants). rs41518645 is a missense variant (p.Asp171Asn) in *MT-CYB*, identified in Plink logistic regression analysis ($p=0.0003$). rs201950015 is intronic, located between genes *CO1* and *ATP6/8* ($p=0.00042$). No variants achieved "genome-wide" significant ($p<5*10^{-8}$). See Table 4-4.

Table 4-4: Mitochondrial variants associated with EWGSOP low grip strength in UK Biobank participants

AffxID	BP	A1	A2	MAF	OR	SE	P	RSID	Annotation	Nearest Gene(s)
Affx-79381710	15257	A	G	0.02138	1.16	0.04101	0.00029	rs41518645	Exonic, missense (p.Asp171Asn)	CYB
Affx-79443447	7476	T	C	0.01825	1.168	0.04413	0.000418	rs201950015	Intergenic	CO1 [..] ATP6/8

4.5.6 Gene Expression and Pathways

I used data from the Genotype-Tissue Expression project (GTEx) v8 to identify whether the variants associated with low grip strength affect expression of genes (Table 4-1; Supplementary Table s4-4). Of the top 15 EWGSOP-associated variants 12 are eQTLs for at least one gene. For 8 of these, the nearest gene to the variant by chromosomal location is known to have altered expression, but other genes in the locus may also be affected. This is consistent with a recent study showing that the "nearest gene" is often a good candidate for being a causal pathway (Stacey et al. 2019). For the top two loci (*HLA-DQA1* and *GDF5*) the variants are eQTLs for these nearest genes, however for the *SLC39A8* locus the lead SNP (rs13107325) is not an eQTL for *SLC39A8*, but is an eQTL for *UBE2D3* ('Ubiquitin Conjugating Enzyme E2 D3') in the aorta.

In MAGMA analysis I found 80 GO processes enriched in low grip strength-associated genes (see Supplementary Table s4-5 for details), mainly involved in the immune system and antigen presentation.

MetaXcan (Barbeira et al. 2018) identified 24 genes with expression in skeletal muscle significantly enriched in the low grip strength GWAS, after Benjamini-Hochberg adjustment for multiple testing (Supplementary Table s4-6). The International Mouse Phenotyping Consortium database (www.mousephenotype.org) was used to investigate the possible phenotypes associated with the genes highlighted by MetaXcan, with many having clear effects on relevant phenotypes such as "growth", "lean mass", "body weight", "angiogenesis", "thymus involution", and "lipid metabolism" (Supplementary Table s4-7).

We used LDSC-SEG to determine tissue-specific gene expression and chromatin modification enrichment in the low grip strength GWAS results (Finucane et al. 2018). We found no significant enrichment for the genetic determinants of low grip strength in expression profiles and epigenetic changes after adjustment for multiple testing, although genes expressed in the tibial nerve were borderline enriched (Benjamini-Hochberg-adjusted p-value 0.1). See Supplementary Table s4-8 for details.

4.5.7 Low grip strength loci independence from musculoskeletal traits and diseases

To determine whether the genetic variants associated with low grip strength identified in the GWAS were independent of anthropometric traits or musculoskeletal co-morbidities I performed regression analyses in the UK Biobank cohort with adjustment for the following covariates: height, weight, skeletal muscle mass (determined using bioimpedance analysis), osteoarthritis, Rheumatoid arthritis, osteoporosis, Dupytrens contracture (one or more fingers permanently bent), and rhizarthrosis (arthritis of the thumb). The association between eight of the fifteen EWGSOP loci and low grip strength was attenuated after adjusting for height, including rs143384 (initial UKB $p=3.7*10^{-11}$; adjusted UKB $p=3.8*10^{-2}$) and rs7624084 (initial UKB $p=9.3*10^{-7}$; adjusted UKB $p=4.9*10^{-1}$). Adjustment for weight or BMI did not substantially attenuate any of the associations. Overall, the associations were not attenuated by adjustment for osteoarthritis, Rheumatoid arthritis, osteoporosis, Dupytrens, or rhizarthrosis. See Supplementary Information for diagnostic codes, and Supplementary Table s4-9 for detailed results.

4.6 Discussion

In this study of 256,523 Europeans aged 60 years and over we identified 15 genetic loci - 3 novel - associated with the EWGSOP definition of low grip strength, plus two additional loci for the FNIH definition that used a more extreme definition for muscle weakness. Only three of these were previously implicated in previous analyses of continuous strength measures, suggesting that the genetic causes of clinically meaningful weakness at older ages are partly distinct. We found prominent overlaps with osteoarthritis and Rheumatoid arthritis, but also with cardiovascular disease and type-2 diabetes. Additional links to asthma and allergy were also found. The novel pathways implicated appear to include hallmark mechanisms of ageing (López-Otín et al. 2013), for example cell cycle control related to the cancer control retinoblastoma pathway. However other ageing pathways such as telomere length (Kuo et al. 2019), and many lifespan-associated loci including *APOE* (Timmers et al. 2019), were not associated. Three of the low grip strength-associated genetic signals identified have not appeared in GWAS prior to the time of analysis, further demonstrating that low strength in older people may have distinct genetic underpinnings.

The strongest association found was rs34415150, near the *HLA-DQA1* gene (Figure 4-2). Genetic variants at this locus have been implicated in a wide range of conditions, including autoimmune diseases such as Rheumatoid arthritis (Cortes et al. 2019), and continuous grip strength (Tikkanen et al. 2018). HLA haplotypes *HLA-DQA1**03:01 and *HLA-DRB1**04:01, have been previously linked to sarcopenia in older UK Biobank participants (G. Jones et al. 2019).

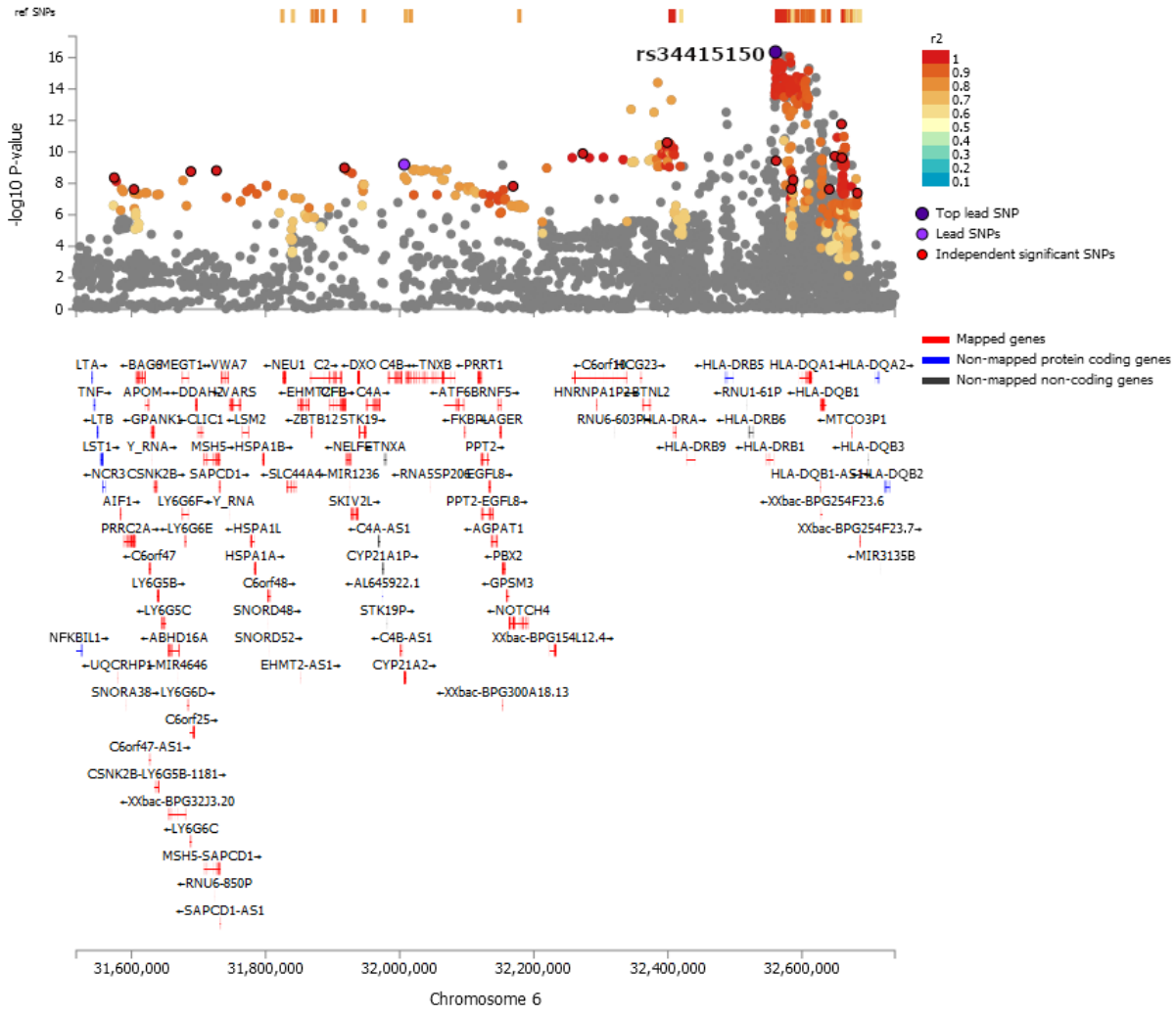

Figure 4-2: HLA-DQA1 risk loci for low grip strength (rs34415150)

HLA-DQA1 is associated with chronic inflammation in muscle of untreated children with juvenile dermatomyositis (inflammatory myopathies in children, which one of the characteristics is muscle weakness) (Y.-W. Chen et al. 2008). Additionally, in a multi-trait analysis of age-related diseases *HLA-DQA1* was confirmed and it may therefore contribute to underlying ageing mechanisms as a “geroscience locus” (Melzer, Pilling, and Ferrucci 2019).

Overall five of the fifteen genomic risk loci for EWGSOP low grip strength have been previously associated, or in linkage disequilibrium with variants associated with osteoarthritis (rs143384 – *GDF5*, rs13107325 – *SLC39A8*, rs34464763 – *C12orf60*, rs958685 – *TGFA* and rs79723785 - *BRSK1*), of which two are also linked to adiposity, a known risk factor for arthritis (Murphy et al. 2008). We found that rs143384 in the 5' untranslated region of growth/differentiation factor 5 (*GDF5*) was the second most strongly associated variant with low grip strength, see Figure 4-3. *GDF5* is a protein in the transforming growth factor beta (TGF-β) family, with key roles in bone and joint development (Francis-West et al. 1999; Capellini et al. 2017).

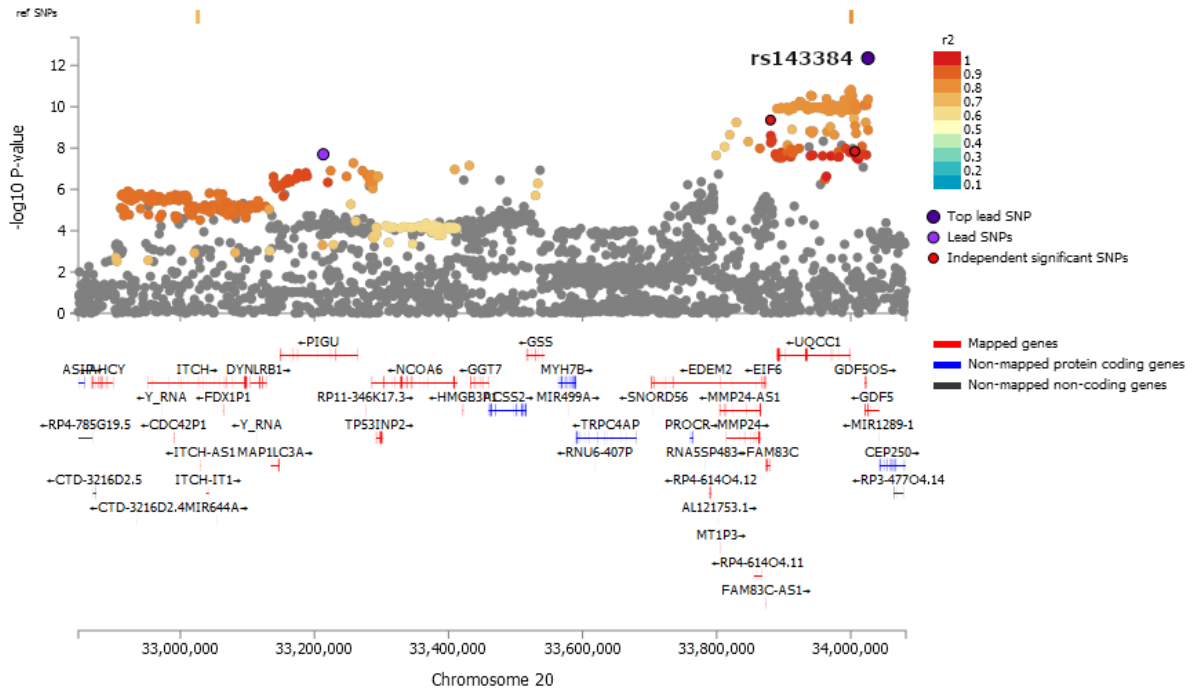

Figure 4-3: GDF5 risk locus for low grip strength (rs143384)

It was the first locus identified for osteoarthritis (Miyamoto et al. 2007), with a reported odds ratio of 1.79, as well as one of the first identified for height (Sanna et al. 2008). GDF5 is known to reduce expression of cartilage extracellular matrix-degrading

enzymes in human primary chondrocytes (Uhalte et al. 2017), thereby may be an potential intervention target for avoiding weakness at older ages, although work is needed to determine when intervention would be most effective. In follow-up analyses we found that the association between rs143384 and low grip strength was independent of prevalent osteoarthritis in UK Biobank participants, although we cannot rule out an effect of sub-clinical osteoarthritis. However, the association was completely attenuated after adjustment for standing height, suggesting the effect of the variant is mediated by developmental traits such as bone length. Mice with loss of *Gdf5* function exhibited severely impaired knee development, and the regulatory region pinpointed to mediate this effect in humans includes osteoarthritis-associated genetic variants (Pregizer et al. 2018). Although the observed association between variants mapping to *GDF5* and low strength might be mediated by hand osteoarthritis pain compromising grip strength, there is evidence of a direct effect of GDF5 on muscle (Traoré et al. 2019).

The locus on Chr 18 is proximal to the DYM gene (see Figure 4-4). Loss of function mutations in this gene are associated with Dyggve-Melchior-Clausen syndrome and Smith-McCort dysplasia respectively. Mice lacking this gene present with chondrodysplasia resulting from impaired endochondrial bone formation and abnormalities of the growth plate that begin to manifest shortly birth (Osipovich et al. 2008). In homozygous mutant mice, and in patients with loss of function mutations in this gene, both the axial and the appendicular skeleton are affected. As is noted in Table 4-2, this gene is also associated with height in the GWAS catalogue. Interestingly, this gene is highly expressed in skeletal muscle in humans (Paupe et al., n.d.), however its function in muscle is not completely understood.

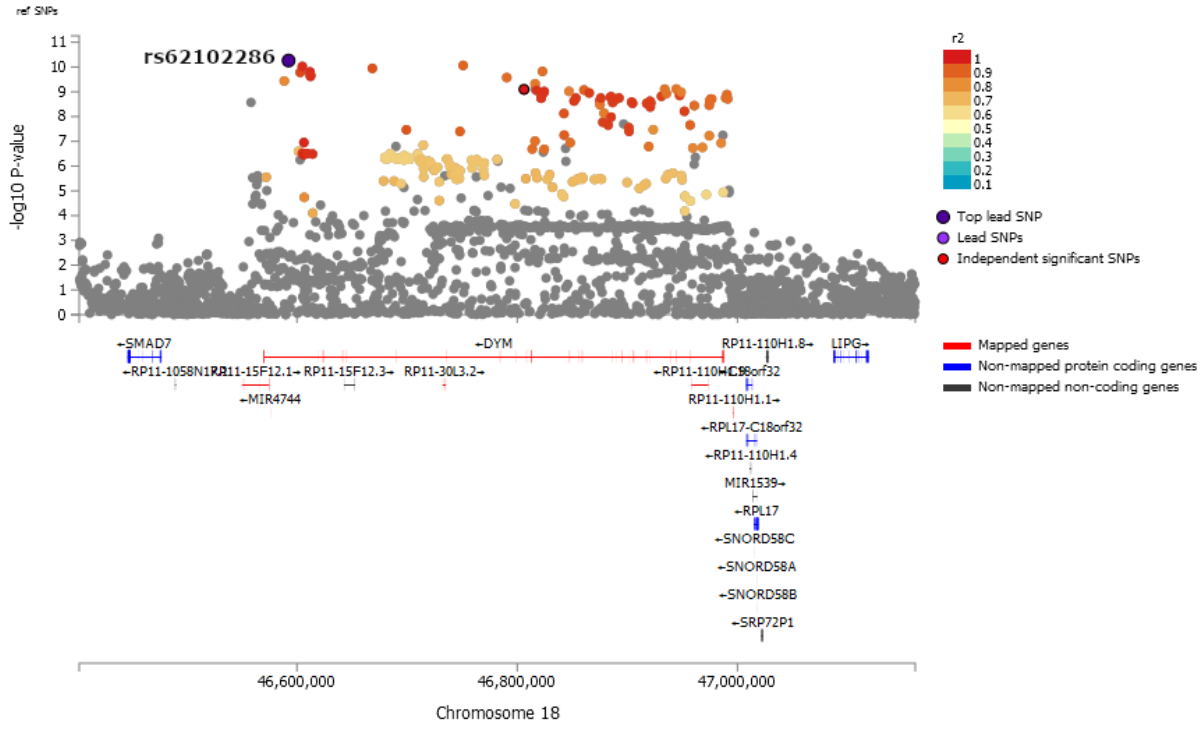

Figure 4-4: DYM risk locus for low grip strength (rs62102286)

SLC39A8 encodes for the metal ion transporter ZIP8 which has been shown to be upregulated in chondrocytes present in osteoarthritic cartilage (J.-H. Kim et al. 2014). The lead SNP rs13107325 is a missense variant within *SLC39A8* which has been previously associated with osteoarthritis (Tachmazidou et al. 2019).

On chromosome 12 genetic variants known to affect expression of *MGP* (Matrix Gla Protein) in the tibial nerve (among other tissues) are associated with osteoarthritis of the hand but not of the hip or knee (den Hollander et al. 2017). MGP is an inhibitor of arterial and soft tissue calcifications, with links to atherosclerosis (Herrmann et al. 2000). Consistent with this, older women with severe abdominal aortic calcification have greater decline in grip strength over 5 years (Rodríguez et al. 2018). More

recently a study suggested MGP may also regulate muscle development and atrophy (Ahmad et al. 2017). Variants that result in non-functional MGP have been shown to result in the autosomal recessive condition, Keutel syndrome (Munroe et al. 1999). Keutel syndrome is characterised by brachytelephalangism, hearing loss, peripheral pulmonary stenosis and abnormal cartilage calcification (Cormode, Dawson, and Lowry 1986). The variable shortening of the terminal phalanges may influence grip strength, whether or not this phenotype is found in carriers of variants associated with the rs34464763 genomic risk locus would require further research.

We also identified variants known to affect Transforming Growth Factor Alpha (*TGFA*) expression (increased in the testis, decreased in the brain), which is implicated in cell proliferation, differentiation and development. This locus has previously been identified for overall strength (Willems et al. 2017), and suggestive evidence from a study of 1,323 participants linked rs2862851, a variant in linkage disequilibrium with the lead SNP rs958685 (R^2=0.90, D'=1.0), with increased risk of osteoarthritis in the knee (OR=1.4, $p=3.1*10^{-4}$) (Cui et al. 2017).

The low grip strength locus on chromosome 15 is near *ALDH1A2*, which has a key role in the pathogenesis of osteoarthritis (Styrkarsdottir et al. 2014). Low grip strength-associated variants at this locus have previously been identified for severe osteoarthritis of the hand (Styrkarsdottir et al. 2014) The lack of this gene in mice is perinatally lethal, however at embryonic day 18.5 mice lacking this gene do present with numerous cartilage gene defects (Vermot et al. 2003). We also identified variants that affect expression of *CHRDL2* (Chordin Like 2) in the thyroid, known to interact with mouse Gdf5, which is upregulated in human osteoarthritic joint cartilage cell line (Nakayama et al. 2004). In addition, down-regulation of *CHRDL2* expression has been

linked to the progression of severe osteoarthritis in the knee joint (C.-H. Chou et al. 2015), suggesting a role for CHRDL2 in cartilage repair.

Two of the identified loci (*RBBP6* 'RB Binding Protein 6, Ubiquitin Ligase' and *ZBTB38* 'Zinc Finger And BTB Domain Containing 38') form an axis involved in DNA replication and chromosomal stability (Miotto et al. 2014). RBBP6 ubiquitinates the transcriptional repressor ZBTB38, destabilizing it and reducing its action on the replication factor MCM10. In mice, *Zbtb38* is highly expressed in skeletal muscle, loss of this methyl-CpG-binding protein (which is also known as *Cibz*), promotes myogenic differentiation. Conversely, expression of *Zbtb38* is decreased in satellite cells during muscle regeneration (Oikawa et al. 2011), suggesting that like other members of this gene family, this gene is involved in cellular differentiation. Variants associated with low grip strength in our analysis are known to decrease *ZBTB38* expression in whole blood and skeletal muscle (among others) and increase expression in the skin.

Mitochondrial dysfunction is a hallmark of aging, yet we found no variants associated with low strength at genome-wide significance levels. Variants in *MT-CYB* were nominally significant (p=0.0003): MT-CYB (mitochondrial cytochrome b) is part of the mitochondrial respiratory chain, and essential for Complex III formation. Monogenic diseases associated with *MT-CYB* include exercise intolerance and "Additional features include lactic acidosis, muscle weakness and/or myoglobinuria" (UniProt n.d.). Recent work by Cohen *et al.* have highlighted the importance of mitochondrial peptides such as humanin in many age-related diseases (Yen et al. 2013), however we found no variants in these genes associated with low grip strength passing multiple testing correction.

We observed minimal overlap between loci associated with low grip strength and general anthropometric traits such as height and continuous measures of strength. The SNP-based heritability estimate for EWGSOP low grip strength in older adults was 4.4% (SE 0.3%). This compares to 13% (SE 0.4%) SNP-based heritability for continuous grip strength in UK Biobank participants aged 40 to 70 (Tikkanen et al. 2018). This may be partly explained by our using a binary cut-off for low grip compared to the quantitative grip analysis. Additionally, we observed only limited negative genetic correlation between low grip strength and lean muscle mass (~30%): taken together, these results emphasize that the genetics of weakness and overall strength are distinct.

We chose to not adjust for body size in our primary analysis in case interesting or novel effects were masked, but in follow-up analysis determined that all except two of the loci identified were predominantly independent of participant height and weight.

There are a number of limitations to our analyses. The data included are predominantly from subjects at the younger end of the age 60 plus demographic, and is not enriched for frail individuals. Our analysis was limited to relatively common variants (prevalence > 1%) in subjects with European ancestries only. The analyses of the FNIH strength cut-points also have limited power, given the low prevalence of the phenotype, although studying the extremes of a continuous trait can provide increased power if stronger associations are uncovered. We did not include additional analysis of the revised EWGSOP2 low grip cut-points (Cruz-Jentoft et al. 2019) as these are almost identical to the FNIH criteria, which many cohorts had already analysed; we future analyses should include this. Analysis of rare and structural variants, and analyses in other ancestral groups will give a more complete picture of the genetic landscape for low grip strength.

To conclude, genetic variation in 15 loci are related to muscle weakness in people aged 60 plus, of European descent, with limited overlap with loci associated with the full range of muscle strength in 40 to 70 year olds. Novel loci implicated may be involved in hallmark pathways of ageing including cell cycle control and inflammation, along with loci implicated in arthritis and pathways involved in the development and maintenance of the musculoskeletal system.

Contributions

D.E.A., B.G.W., E.B., M.L.G., M.G., I.D., N.v.d.V, L.C.P.G.M.d.G, B.M.P., C.L., N.J.W., S.B., N.M.v.S, M.H, J.M.Z., D.M., M.K., D.A.B., A.S.B., P.L.D.J., A.G.U., A.T., L.R.M., F.G.G., J.C., P.H., L.B., C.O., J.M.M, L.F., D.K., F.R., and D.P.K. designed and managed individual studies. B.G.W., N.M.v.S, M.H, D.A.B., A.S.B., P.L.D.J., L.R.M., F.G.G., J.C., and P.H. collected data. G.J., K.T., A.J.S., N.S, J.R.L, J.M.M, G.A.K, L.F., D.M., D.K., F.R., D.P.K. and L.C.P. reviewed the analysis plan. G.J., K.T., A.J.S., N.S, C.K., T.V.D., S.H., M.L.B., J.L., C.S., K. L. L. , T.T, M.K.W., R.C., M.N., S.G., J.Y., M.C., S.W., K.S., A.T., and L.C.P. analyzed the data. G.J. and L.C.P. performed the meta-analysis. G.J., C.A-B and L.C.P. performed the pathway and other analyses. J.M.M, G.A.K, L.F., D.M., D.K., F.R., D.P.K., and L.C.P. supervised the overall study design. G.J., K.T., A.J.S., N.S, C.K., J.L.A., J.M.M, G.A.K, L.F., D.M., D.K., F.R., D.P.K. and L.C.P. wrote the manuscript. G.J., K.T., A.J.S., N.S, C.K., J.L.A., J.R.L, T.V.D., S.H., M.L.B., J.L., C.S., K. L. L., T.T, M.K.W., R.C., M.N., S.G., J.Y., M.C., S.W., K.S., E.K., C.A-B., D.E.A., B.G.W., E.B., M.L.G., M.G., D.S., I.D., N.v.d.V, L.C.P.G.M.d.G, B.M.P., M.C.O., A.E.F., C.L., N.J.W., S.B., N.M.v.S, M.H, Q.T., J.M.Z., D.M., M.K., D.A.B., A.S.B., P.L.D.J., A.G.U., U.V., T.K., A.T., L.R.M., F.G.G., J.C., P.H., L.B., C.O., J.M.M, G.A.K, L.F., D.M., D.K., F.R., D.P.K. and L.C.P. reviewed the manuscript.

5 Analysis 3: Stratified analysis of muscle weakness in older people

Genome-wide meta-analysis of muscle weakness identifies 15 susceptibility loci in older men and women

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5.1 Overview

Low grip strength is a commonly used proxy for measuring overall muscle function, particularly in regards to the age-related condition, sarcopenia (Aadahl et al. 2011; Bohannon et al. 2012; S. H. Lee and Gong 2020). The prevalence of sarcopenia, or loss of muscle mass and function primarily due to age varies considerably from population to population, age and sex (S. A. Purcell et al. 2020; Shafiee et al. 2017).

It is known that skeletal muscle protein turnover differs between men and women with age (G. I. Smith and Mittendorfer 2016; G. I. Smith et al. 2012), as is the influence of sex on different substrate utilization in skeletal muscle mitochondria (Montero et al. 2018; Mark A. Tarnopolsky 2008; Horton et al. 1998). We hypothese that the mechanisms leading to weakness due to ageing may differ between men and women and so we investigated the genomic risk loci associated with low grip strength in older adults in a large community based meta-analysis with cohorts separated by sex.

Using sex-stratified analysis of the GWAS meta-analysis from 22 cohorts (135,468 females and 121,055 males) including genotyping data from members of the CHARGE consortium and the low grip cut-offs as defined by the European Working Group on Sarcopenia I have shown that there are eight genomic risk loci associated with low grip strength in older women (age > 60 of European ancestry). Although there is substantial overlap with our previous analysis on both sexes, there is one unique risk locus intronic to *PKD1*.

This analysis highlights the multiple pathways to the physical components of frailty and sarcopenia.

My contribution included leading the writing of resulting paper and primary analyst for the multi-cohort study. In addition I coordinated the meta-analysis and helped draft the analysis plan. A substantial part of this chapter has been published as a section of our paper “Genome-wide meta-analysis of muscle weakness identifies 15 susceptibility loci in older men and women”.

5.2 Abstract

Prevalence of age-associated loss of muscle strength varies considerably between sexes, age ranges and population setting. We undertook sex-stratified analysis of low grip strength, as a proxy for overall muscle weakness, in participants from 22 studies (n=256,523) of European ancestry, in order to investigate the underlying differences in age related muscle weakness between the sexes.

Eight genomic risk loci were found to be associated with muscle weakness in the female only (n=135,468) cohort. Of these seven risk loci were shared by those associated with low grip strength in our previous analysis of 256,523 older adults of European ancestry over the age of 60. This includes genes involved in arthritis, autoimmune disease and development of the muscloskeletal system.

One locus appears to be unique for low grip strength in older women, when compared to either the analysis of both sexes together or men on their own, and is an expression quantitative trait locus for the gene *PKD1*, or Polycystin 1. This transmembrane protein has a role in multiple signaling pathways and is associated with the kidney disease, Autosomal Dominant Polycystic Kidney Disease (ADPKD). However the lead SNP, rs7185040, has not previously been associated with this disease.

The separate analysis of the male only cohort found three variants associated with muscle weakness in older adults. Two had at least nominally ($p < 0.01$) been associated with muscle weakness in the combined cohort, while the third rs774787160 which is intronic to *DSCAM* (Down syndrome cell adhesion molecule) had not previously been associated and was novel to the male only analysis.

Further analysis of the allosomes or sex chromosomes and mitochondrial variants found no additional variants associated with muscle weakness, beyond the two

mitochondrial candidate SNPs previously seen in the combined study (Chapter 4).

In conclusion, there were a small number of genetic variants associated with weakness at other ages in either males or females, highlighting specific pathways that may be sex-specific. These included hallmarks pathways of ageing including cell cycle control and inflammation, along with loci implicated in arthritis and pathways involved in the development and maintenance of the musculoskeletal system. However, the majority of loci and pathways appear to overlap between males and females.

5.3 Introduction

The prevalence of sarcopenia and age related muscle weakness is highly variable based on the criteria used to define sarcopenia (S. A. Purcell et al. 2020), and the population characteristics (Shafiee et al. 2017). Some studies have shown differences in prevalence between the sexes. For example, the risk of developing sarcopenia has been shown to be higher in women compared men of a similar age (age adjusted OR=1.20, 95% CI 0.98 to 1.47)(L. Yang, Smith, and Hamer 2019), in a large community based study (n=3404; 54.1% women; mean age 63.4 ± 7.7, low grip strength as defined by EWGSOP 2010 as the main criteria).

The underlying mechanisms for these observations does have evidence in the current literature with differences in skeletal muscle physiology and atrophy been noted between the sexes (Rosa-Caldwell and Greene 2019), including higher sensitivity of muscle cells in males to inflammation-mediated atrophy compared to female muscle cells (Wallengren et al. 2015; Cosper and Leinwand 2011), which in turn are more susceptible to atrophy from disuse(Callahan et al. 2014). This heterogeneity between the responses of skeletal muscle to challenges in a sex specific manner, may help explain some of the variation in prevalence rates amongst different study populations.

Pre-frail elderly (>60 years of age) have been showed to accrue impairment of mitochondrial function in skeletal muscle, when compared to active elderly people (Andreux et al. 2018). Mitochondrial dysfunction has long formed the basis for one of the main theories of aging, through the resulting Reactive Oxidative Stress (ROS) and cellular damage (Denham Harman 1992).

Montero et al, observed higher levels of fat oxidation over carbohydrate in female compared to male skeletal muscle mitochondria (Montero et al. 2018), and this

confirmed earlier studies on lipid versus carbohydrate / protein oxidation during exercise in males and females (M. A. Tarnopolsky et al. 1995; L. J. Tarnopolsky et al. 1990; Blatchford, Knowlton, and Schneider 1985).

Although previous studies have investigated the genetic basis for hand grip strength (Willems et al. 2017; Tikkanen et al. 2018; Matteini et al. 2016) as a quantitative trait, there has been less focus on low hand grip strength (as a proxy for loss of overall muscular function) in older adults and in particular any underlying differences between the sexes.

The evidence for underlying differences in major mechanisms for loss of muscle function with age, as well as the lack of previous investigations into the casual pathways associated with sex and definitions of low muscle function, are to be examined by this study. We aim to investigate the genomic risk loci associated with low grip strength in older people stratified by sex, using a large community based cohort with 135,468 females and 121,055 males of European descent aged over 60 years old. In addition we will analyse the allosomal and mitochondrial genotypes from UK Biobank participants for associations with low grip strength.

5.4 Methods

5.4.1 GWAS of low grip strength in older people

Initially I conducted a GWAS meta-analysis of low grip strength in participants aged 60 years or older of European ancestry from 22 studies yielding a combined sample of 254,894 individuals. Individual studies used different genotyping platforms and imputation was predominantly performed using the Haplotype reference consortium (HRC) v1.1 panel. Details on methods used for individual studies is available in the supplementary methods. Following the original GWAS meta-analysis on both sexes, sex-stratified analysis was undertaken on the 135,468 females and 121,055 males.

The primary analysis was of the 2010 EWGSOP criteria for sarcopenic grip strength (Grip strength < 30 Kg Male; < 20 Kg Female). In secondary analysis we considered a more data-driven definition with more extreme thresholds by the Foundation for the National Institutes of Health (FNIH) sarcopenia project 2014 (Grip strength < 26 Kg Male; < 16 Kg Female) for comparison.

GWAS was performed by each cohort individually using regression models, adjusted for age, and population substructure, accounting for relatedness and technical covariates as required by the individual study. For UK Biobank cohort GWAS was performed using BOLT-LMM, with co-variants for age, sex (if applicable), microarray used, and assessment center. For each CHARGE cohort methods used are included in the supplementary information, however the GWAS was minimally adjusted for age, sex and the first ten principal components to account for population structure.

No adjustment for anthropometric measures was made in the primary analysis, due to possible collider bias. Fixed-effects inverse variance weighted meta-analysis was performed using METAL (Willer, Li, and Abecasis 2010b) using the GWAS summary statistics generated by each cohort, with genomic control for population structure (see Methods for details). The following quality control filters were applied: minor allele frequency (MAF) > 0.01, imputation info score of > 0.4, and the variant present in at least two studies (UK Biobank – the largest included cohort - plus at least one other). The final analysis therefore included 9,678,524 genetic variants. Associations that achieved a $p<5*10^{-8}$ were considered statistically significant, with those reaching the more stringent threshold of $p<5*10^{-9}$ highlighted.

Distinct loci were initially defined as two significant variants separated by >500kb. To identify independent signals at each locus we used FUMA (Functional Mapping and Annotation of Genome-Wide Association Studies) (Watanabe, Taskesen, van Bochoven, et al. 2017), which uses Linkage Disequilibrium (LD) information to determine independence (r^2 threshold = 0.1 for independent significant single nucleotide polymorphisms (SNP)).

5.4.2 Gene Ontology Pathways, Tissue Enrichment, and eQTL analyses

I utilized FUMA to perform functional interpretation of the GWAS results (Watanabe, Taskesen, van Bochoven, et al. 2017). In particular, FUMA performs gene-set analysis (using Multi-marker Analysis of GenoMic Annotation (MAGMA) (de Leeuw et al. 2015)) to identify pathways enriched amongst the significant genes (weighted by the SNP-associations in proximity to them), in addition to searching eQTL databases to identify SNPs that significantly alter the expression of genes in various tissues.

5.4.3 Analysis of variants on sex-chromosomes and mitochondria

Additive association testing with the two low grip definitions (FNIH and EWGSOP) in the sex- chromosomes X and Y, as well as the mitochondrial chromosome was performed in PLINK 1.9.3 (S. Purcell et al. 2007) on the UK Biobank dataset. The analysis was stratified by sex and as a combined (all participant) cohort, of participants of European ancestry and 60 years of age, or older.

Mitochondrial genotypes were direct from microarray data and not imputed, whereas the sex chromosomes X and Y were imputed. Only variants with a Minor Allele Frequency (MAF) of greater than 1% were considered, due to loss of genotyping accuracy at lower allele frequencies and the low heterozygosity generally exhibited by rare variants (MAF < 1%) – this is especially important for the analysis as higher heterozygosity has been associated with healthy ageing (K. Xu et al. 2019). In addition imputation panels are still focused on variants with MAF > 1%, although incorporation of rarer haplotypes has been an aim of more recent panels (Bomba, Walter, and Soranzo 2017).

5.5 Results

5.5.1 Sex stratified analysis identifies different risk loci

In sex-stratified analysis there were eight significant genomic risk loci associated with EWGSOP low grip strength in females only (total N=132,443 with n=33,548 cases, 25.3%; see Figure 5-1, Table 5-1). Seven of the eight loci were either present in the main analysis or were correlated with corresponding variants, for example rs201754 (chr13:51078446, Female only) and rs3118903 (chr13: 51099577, both sexes) with D'=0.97 and r^2=0.91. rs7185040 (chr16: 2145787), mapped to gene *PKD1*, was only significant in the analysis of females only, although the association is borderline in the analysis of males and females together (females p = $3*10^{-8}$; combined analysis p = $5.5*10^{-8}$).

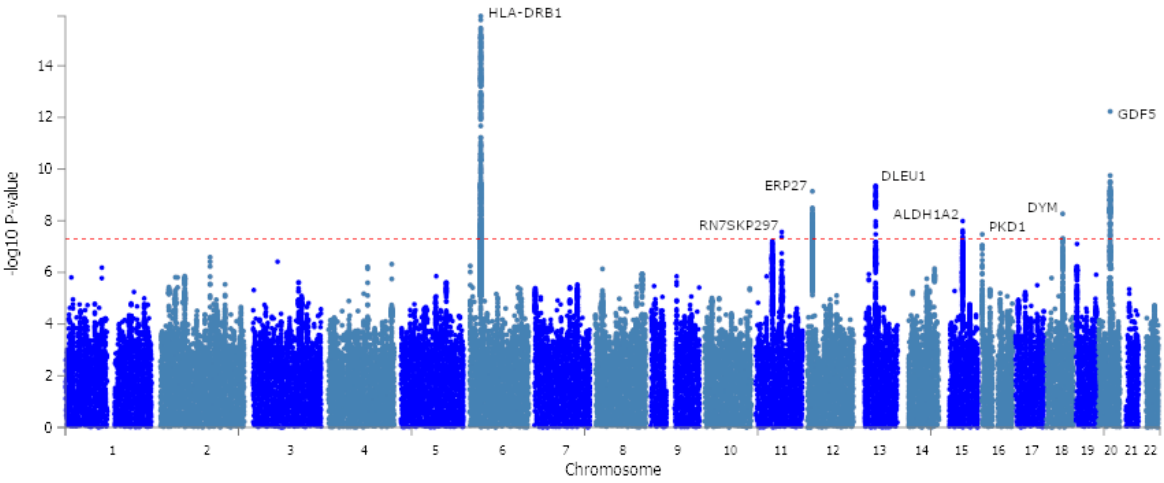

Figure 5-1: Manhattan plot of low grip strength female only genome-wide association study

The p-values are from a fixed-effects meta-analysis of 135,468 females of European ancestry, aged 60 or older from 22 cohorts. The outcome was low hand grip strength

grip strength cutoff (females <20 kg). The x-axis is the chromosomal location, and the y-axis is the –log10 p-value for each genetic variant. The horizontal red line is the threshold for genome-wide significance (p<5*10-8). Eight genomic loci cross the threshold, and the lead variant (most significantly associated with low strength) is described in Table 5-1. The nearest gene is displayed for each locus.

Table 5-1: Genomic risk loci associated with EWGSOP low grip strength in 135,468 women

			EWGSOP Female					**EWGSOP All**		**EWGSOP Male**		
RSID	**Chr**	**BP**	**P-value**	**EA**	**OA**	**EAF**	**Log OR**	**P-value**	**Log OR**	**P-value**	**Log OR**	**Nearest gene***
rs34415150	6	32560477	1.2E-16	G	A	0.18	0.1001	4.42E-17	0.0833	1.02E-03	0.0582	HLA-DRB1
rs143384	20	34025756	5.87E-13	A	G	0.59	0.0657	4.47E-13	0.0545	2.45E-03	0.0401	GDF5
rs201754	13	51078446	4.43E-10	C	T	0.22	0.0668	3.30E-10	0.0554	3.97E-02	0.0321	DLEU1
rs4764133	12	15064363	3.9E-09	T	C	0.38	0.054	1.92E-09	0.0454	2.42E-02	0.03	ERP27
rs2899611	15	58327347	1.03E-08	G	T	0.51	0.0515	6.01E-09	0.0431	5.33E-03	0.0363	ALDH1A2
rs11236213	11	74394369	2.72E-08	G	A	0.69	0.054	3.01E-10	0.0504	3.13E-04	0.0508	RN7SKP297
rs7185040	16	2145787	3.36E-08	C	A	0.18	0.0642	5.57E-08	0.052	3.91E-02	0.0348	PKD1
rs550258	18	46860643	4.81E-08	T	C	0.35	0.0511	8.51E-10	0.0473	2.23E-03	0.0414	DYM

Chr= chromosome; BP= base pair position, genome build 37; EA= effect allele; OA= other allele; EAF= effect allele frequency; Log OR= Log Odds Ratio of having low grip strength (EWGSOP criteria) per allele; *p*-value= fixed-effects meta-analysis p-value, values < $5*10^{-9}$ highlighted in bold; Nearest gene= on GRCh37;

The analysis of males only (total N=118,371 with 13,327 cases, 11.3%) identified three genomic loci associated with the EWGSOP low grip strength definition. One of these variants, rs35225200, was in linkage disequilibrium with a lead SNP from the combined analysis (rs13107325 R^2=0.89, D'=1.0), which means that this is not an independent signal. While the two remaining lead variants appeared to be distinct signals (see Table 5-2): rs774787160 is a common deletion variant (Effect allele frequency 0.73) mapped to gene *DSCAM* and was not associated with low grip strength in females (males p = $1*10^{-8}$; females p = 0.9) and rs145933237 mapped to *MIR466* (MicroRNA 466, short non-coding RNA involved in post-transcriptional regulation of gene expression), which was only nominally associated in females (males p = $2*10^{-8}$; females p=0.01). The reduced number of cases in males could contribute to the fewer associations observed, and the borderline significance (p-values close to $5*10^{-8}$).

The results from sex-stratified analyses need to be interpreted with caution. The largest cohort in our analysis, UK Biobank, has been shown to have a sex-differential participation bias for a number of traits, including BMI, which may impact the sex-stratified analysis of muscle weakness with age (Pirastu et al. 2021). Pirastu et al suggest adjusting the analysis either utilising the Heckman correction, or due to limitations the authors have also proposed a new genomic structural equation model that corrects for collider bias induced by sample selection.

The sex-differential participation bias appears to be specific to the participant selection criteria for each study, for example the iPSYCH showed the lowest heritability estimate and the least participation and survival bias out of the cohorts in the paper. This is a result of the random sampling of participants from routinely collected neonatal dried

blood spots born between 1981 and 2005 in Denmark (C. B. Pedersen et al. 2018). Replication in cohorts with different selection criteria would help to confirm the results.

Table 5-2: Genomic risk loci associated with EWGSOP low grip strength in 121,055 men

			EWGSOP Male					EWGSOP All		EWGSOP Female		
RSID	**Chr**	**Position**	**P-value**	**EA**	**OA**	**EAF**	**Effect**	**P-value**	**Log OR**	**P-value**	**Log OR**	**Nearest gene***
rs774787160	21	41996684	1.39E-08	A	ACACATCCAAAAG	0.73	0.0983	2.99E-03	0.0275	9.27E-01	-0.001	DSCAM
rs145933237	3	31188133	2.05E-08	C	G	0.02	0.2688	2.04E-06	0.1344	1.06E-02	0.0898	hsa-mir-466
rs35225200	4	103146888	2.91E-08	C	A	0.08	0.1289	1.67E-10	0.0859	8.46E-05	0.0647	SLC39A8

Chr= chromosome; Position= base pair position, genome build 37; EA= effect allele; OA= other allele; EAF= effect allele frequency; p-value= fixed-effects meta-analysis p-value, values < 5*10-9 highlighted in bold; Log OR=Log Odds Ratio per effect allele; Nearest gene= on GRCh37

5.5.2 Sex stratified analysis using FNIH definition

Using the FNIH low grip strength definition (<26Kg for males and <16Kg for females), on the sex-stratified cohorts found that while 4 genomic risk loci were associated with low grip strength in females (n=13,601), there were no significant associations observed for the males (n=6,734). Of the four risk loci identified in the female FNIH analysis, three had previously been associated with the less stringent EWGSOP low grip definition in the female cohort (rs34415150 - FNIH $p = 6.1*10^{-14}$, EWGSOP $p = 1.2 * 10^{-16}$; rs143384 - FNIH $p = 5.3 * 10^{-9}$, EWGSOP $p = 5.9 * 10^{-13}$ and rs552086 – FNIH $p = 4.9 * 10^{-8}$, EWGSOP $p = 4.8 * 10^{-8}$). The remaining risk locus rs3771512 is an eQTL for TGFA and although a genomic risk loci was not identified at this location in the sex-stratified EWGSOP cohort, previous analysis using the combined male and female participants (Chapter 4) had identified a risk locus, rs958685, correlated with rs3771512 ($R^2 = 0.39$, D' = 0.96). see Table 5-3.

Table 5-3: Genomic risk loci associated with FNIH low grip strength in 135,468 women

			FNIH Female					FNIH All		FNIH Male		
RSID	**Chr**	**BP**	**P-value**	**EA**	**OA**	**EAF**	**Log OR**	**P-value**	**Log OR**	**P-value**	**Log OR**	**Nearest gene***
rs34415150	6	32560477	**6.09E-14**	G	A	0.18	0.1343	**5.61E-16**	0.1168	**9.68E-06**	0.1095	HLA-DRB1
rs143384	20	34025756	5.27E-09	A	G	0.59	0.0796	**1.04E-06**	0.0526	1.15E-01	0.0294	GDF5
rs3771512	2	70697720	3.73E-08	C	A	0.70	0.0792	3.21E-08	0.0628	2.53E-02	0.0438	TGFA
rs552086	18	46806432	4.9E-08	G	C	0.33	0.0765	1.82E-08	0.0626	2.49E-02	0.0432	DYM

Chr= chromosome; BP= base pair position, genome build 37; EA= effect allele; OA= other allele; EAF= effect allele frequency; OR= Odds Ratio of having low grip strength (FNIH criteria) per allele; p-value= fixed-effects meta-analysis p-value, values < 5*10-9 highlighted in bold; Nearest gene= on GRCh37;

5.6 Analysis of sex chromosomes and mitochondrial variants

In our previous analysis of the association between mitochondrial variants and low grip strength (Chapter 4), we found two variants that reached nominal significance (Benjamini-Hochberg adjusted False Discovery Rate) - rs41518645 and rs201950015 – in the combined sex cohort.

We found no variants showing significant association with muscle weakness in the sex chromosomes, or allosomes for either the combined sex cohort or the sex stratified cohorts (MAF >= 1%).

No additional mitochondrial variants were associated (FDR adjusted p-value) with low grip strength in either of the sex-stratified cohorts.

5.6.1 Gene Expression and Pathways

MAGMA analysis using gene prioritization, identified 91 GO biological processes associated with the female only analysis (Table 5-4), with substantial overlap (51 of the pathways are common between the combined analysis – with 80 annotated GO pathways and the female only analysis – with 91 annotated GO pathways) in the processes seen for the combined meta-analysis (Chapter 4). This included pathways related to the immune system and antigen presentation. The combined analysis identified GO biological processes related to metal ion metabolism and homeostasis (Cadmium, Zinc, and Copper), which were not present in the female only analysis.

By contrast the MAGMA analysis of the male only cohort did not find any significant associations with GO biological processes.

Table 5-4: GO pathways associated with low grip strength in women (EWGSOP low grip strength)

GO pathway	Number of genes in pathway	Overlapping genes	P-value	Adjusted P-value
Adaptive immune response	600	69	7.82E-41	5.75E-37
Positive regulation of immune response process	1147	55	1.11E-14	4.09E-11
Regulation of immune response	1072	52	3.98E-14	9.75E-11
Positive regulation of immune response	871	46	6.49E-14	1.19E-10
Regulation of immune system process	1606	65	9.97E-14	1.46E-10
Antigen processing and presentation of peptide antigen	186	21	5.03E-13	6.16E-10
Antigen processing and presentation	221	22	1.83E-12	1.92E-09
Activation of immune response	692	37	1.43E-11	1.31E-08
Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	100	14	2.18E-10	1.78E-07
Antigen receptor mediated signaling pathway	294	21	2.77E-09	2.03E-06

5.7 Discussion

Our sex-stratified meta-analysis of 256,523 Europeans aged 60 years and over identified differences in the genomic risk loci. For these genomic risk loci this indicates that the underlying casual pathways are consistent between the two analyses including inflammation and immune response (rs34415150, HLA-DRB1), cell growth (rs143384, GDF5) and conditions such as osteoarthritis (rs2899611, ALDH1A2).

The one variant that was present in the female only results that was not correlated with a matching variant in the combined analysis was rs7185040. This variant is an eQTL for the downregulation of *PKD1*. Polycystin-1 (PC1, the protein encoded by *PKD1*) has been shown in the mouse model to have a role in osteoblastogenesis, with knock-out mutants of *pkd1* causing bone defects (Lu et al. 2001) and osteopenia(Xiao et al. 2010). Low grip strength has previously been associated with low bone mineral density in women (Dixon et al. 2005).

Mutations in *PKD1* and *PKD2* genes are the common cause of Autosomal Dominant Polycystic Kidney Disease (ADPKD) (Ong and Harris 2015), a disease with varying levels of severity dependent on the particular variations involved and is one of the most common inherited kidney disorders with an estimated prevalence of one in 2500 individuals of European ancestry (Willey et al. 2017). ADPKD is a progressive systemic disorder, affecting primarily the kidney where accumulation of cysts results in end-stage kidney disease around the sixth decade of life, but can also involve hypertension, liver cysts (Hogan et al. 2015), and intracranial aneurysms (Chapman et al. 2015). PKD1 belongs to the TRPP (Transient receptor potential protein) superfamily of cation channel proteins. One member of this family, PKD1L2 (Polycystic kidney disease gene 1-like 2) has been associated with chronic neuromuscular

impairments including neuromuscular junction degeneration, and polyneuronal innervation in the mouse model skeletal muscle (Mackenzie et al. 2009).

Whether the association with muscle weakness observed at the *PKD1* genomic risk locus is causal or due to a secondary effect from the burden of kidney disease is difficult to confirm and could be heavily confounded. Fine-mapping of the genomic risk locus could identify causal variants, as could eQTL analysis and network analysis of the gene pathways associated with the locus (Broekema, Bakker, and Jonkers 2020).

Horizontal pleiotropy between the traits of muscle weakness with age and PKD1 risk, could be tested for using Mendelian randomization Egger and Steiger filtering methods (Cho et al. 2020; Bowden, Davey Smith, and Burgess 2015).

However sarcopenia has previously been shown to be associated with renal dysfunction and chronic kidney disease (R. Yang et al. 2016; Ida et al. 2019; Ortiz and Sanchez-Niño 2019).

Although only seven out of the fifteen genomic risk loci identified in the combined analysis presented previously (Chapter 4) were also found in the female only analysis, most of the missing associations could be due to a power issue and small effect sizes observed. However one association with a comparatively strong signal in the combined analysis, rs13107325, an eQTL for *UBE2D3* was not observed in the female only results. *UBE2D3* or Ubiquitin Conjugating Enzyme E2 D3, is involved in the ubiquitin-proteasome pathways, ubiquitination of regulatory molecules such as cyclin D1 (Mittal et al. 2011) or *TP53* (Saville et al. 2004), and expression of *UBE2D3* downregulates human telomerase reverse transcriptase (hTERT) (H. Yang et al. 2016).

The FNIH low grip definition is more stringent than the EWGSOP definition (<26Kg for males and <16Kg for females compared to < 30 Kg for males and < 20 Kg for females) and shares three of the genomic risk loci with the EWGSOP female only analysis. However further investigation of the presence of one genomic risk locus in the FNIH female only analysis (rs3771512, intronic to TGFA) is required. Since this SNP is found to be correlated with rs958685, the lead variant of the risk locus in the combined sex EWGSOP meta-analysis (Chapter 4), but is not present in the EWGSOP female only analysis.

By contrast only one of the three genomic risk loci identified in the male only cohort was observed in our original meta-analysis on both sexes together. This was the genomic risk locus for *UBE2D3* (rs13107325 combined sex, rs35225200 male only R^2=0.89). Out of the remaining two loci rs145933237 is nominally (p=0.01) Two other risk loci rs774787160 and rs145933237 appear to be specific to the male cohort.

The lead variant rs774787160 (merged with rs59596901 on October 12, 2018 - Build 152 of dbSNP) is a deletion/insertion variant, intronic to *DSCAM* (Down syndrome cell adhesion molecule). The delins variant has not previously been reported to have a clinical significance (Clinvar March 2021, (Landrum et al. 2018)). *DSCAM* has previously been shown to be involved in axonal growth, via interactions with *DCC* and *UNC5* (Purohit et al. 2012; G. Liu et al. 2009). Mouse models have established that *DSCAM* knock-outs have impaired motor control (Lemieux et al. 2016).

The closest gene to the second genomic risk locus, rs145933237, is *hsa-mir-466* or *miRNA-466* (MicroRNA-466). MicroRNAs are key regulators of biological processes (Gebert and MacRae 2019) and are involved in skeletal muscle myogenesis (M. Xu et al. 2020; Callis, Chen, and Wang 2007). The mouse homologue *miR-466* has been

shown to be down-regulated (approximately 4-fold) in mice with high levels of physical activity (Dawes et al. 2015). *GADL1* is also associated with rs145933237 (although it is not the nearest gene), glutamic acid decarboxylase–like 1 is involved in plasma levels of carnosine, muscle strength and age-related degenerative changes in the mouse model (Mahootchi et al. 2020).

It is interesting to note that despite the issue of analysis power with regard to the male cohort, there appears to be little overlap with the top associations from both the female only cohort and the combined sex analysis. In particular the lack of signal associated with the immune response and the HLA region, may highlight that autoimmunity is a key mechanism for low grip strength in older women while other mechanisms such as neuronal health may have a greater role in men.

Previous research has found sexual dimorphism in substrate utilization in skeletal muscle mitochondria and our sex-stratified analysis did not associate mitochondrial variants with low grip strength in older adults.

Evidence for sexual dimorphism in protein turnover with aging muscle does find some support, with risk loci associated with low grip strength in women being proximal to genes involved in the Unfolded Protein Response pathways, for example *ERP27* (Galligan and Petersen 2012).

Limitations to our analysis include the dominance of healthy responders from the largest contributing study, the UK Biobank, which may not be representative of the population. The sample size for sex-specific analysis is limited, especially for men. Given the largest sample population is of European ancestry, study is restricted to this population at this time.

Due to the limitations with imputed genotyping data our analysis is limited to relatively common variants (minor allele frequency > 1%) in European populations. Analysis of rare and structural variants should be possible in future at least with the UK Biobank data, with the release of sequencing data. This will provide greater resolution to the underlying casual pathways and genetic risk factors.

In conclusion, there were a small number of genetic variants associated with weakness at other ages in either males or females, highlighting specific pathways that may be sex-specific. These included hallmarks pathways of ageing including cell cycle control and inflammation, along with loci implicated in arthritis and pathways involved in the development and maintenance of the musculoskeletal system. However, the majority of loci and pathways appear to overlap between males and females.

5.8 Data Availability

The GWAS summary statistics and supporting information on low grip strength in older people are available on the Musculoskeletal Knowledge Portal (http://musculoskeletalgenomics.org/). All relevant additional data is available on request from the authors.

6 Analysis 4: Analysis of casual risk factors for low grip strength using Mendelian Randomization

Genome-wide meta-analysis of muscle weakness identifies 15 susceptibility loci in older men and women

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6.1 Abstract

Low muscle strength is an important heritable indicator of poor health linked to morbidity and mortality in older people. Using Mendelian randomization on genome-wide association study meta-analysis of 256,523 Europeans aged 60 years and over for low grip strength (European working group on sacropenia definition), we report possible overlapping causal pathways, including diabetes susceptibility, haematological parameters, and the immune system. Our findings reinforce the multifactorial causes of weakness in older people, and the impact of growth and development earlier in the lifecourse on muscle weakness in later life.

6.2 Introduction

Musculoskeletal disorders associated with ageing, such as sarcopenia are often associated with disability and the occurrence of multiple co-morbidities (Duffield et al. 2017). Sarcopenia (Santilli et al. 2014) has been associated with a diverse range of conditions including diabetes (Scott et al. 2016), cardiovascular disease (G. Bahat and Ilhan 2016) and cognitive decline (M. Kim and Won 2019; I. Lee et al. 2018), amongst others.

Long-term conditions (LTCs) and their association with probable sarcopenia (as defined by the European Working Group on Sarcopenia in Older People low grip definition) has previously been investigated in an observational study using the entire UK Biobank cohort (age 40-70) (Dodds et al. 2020). The study found that the prevalence of conditions from a number of LTC categories (including musculoskeletal/trauma, endocrine/diabetes and neurological/psychiatric) in participants meant that those participants were more likely to also have low grip strength. Participants with multi-morbidity had almost twice the odds of being clinically weak (OR 1.96; 95% CI 1.91-2.02) (Dodds et al. 2020). However this study included the entire UK Biobank age range (40-70), and associations found by observational epidemiological studies could be confounded (Meuli and Dick 2018).

6.2.1 Mendelian randomization

Mendelian randomization or the random assortment of genes from parents to offspring, can be used to test the association between a trait and a genetic polymorphism that can control for some of the problems and limitations of traditional observational epidemiological studies (G. D. Smith and Ebrahim 2003). Confounding

- where the effects of the studied exposure on an outcome have become mixed with the effects from an additional exposure or factor (Skelly, Dettori, and Brodt 2012)) and reverse causation - where the outcome influences the exposure (Zapf, Dormann, and Frese 1996)), are two limitations that can be addressed using Mendelian randomization (G. D. Smith and Ebrahim 2003).

The genetic information or instrumental variable (IV) is inherited randomly from the parent's genes, and affects the outcome through the exposure - free from influence of any additional confounders that can directly affect the exposure or outcome (Didelez and Sheehan 2007). See Figure 6-1: Directed acyclic graph of Mendelian Randomization methodology

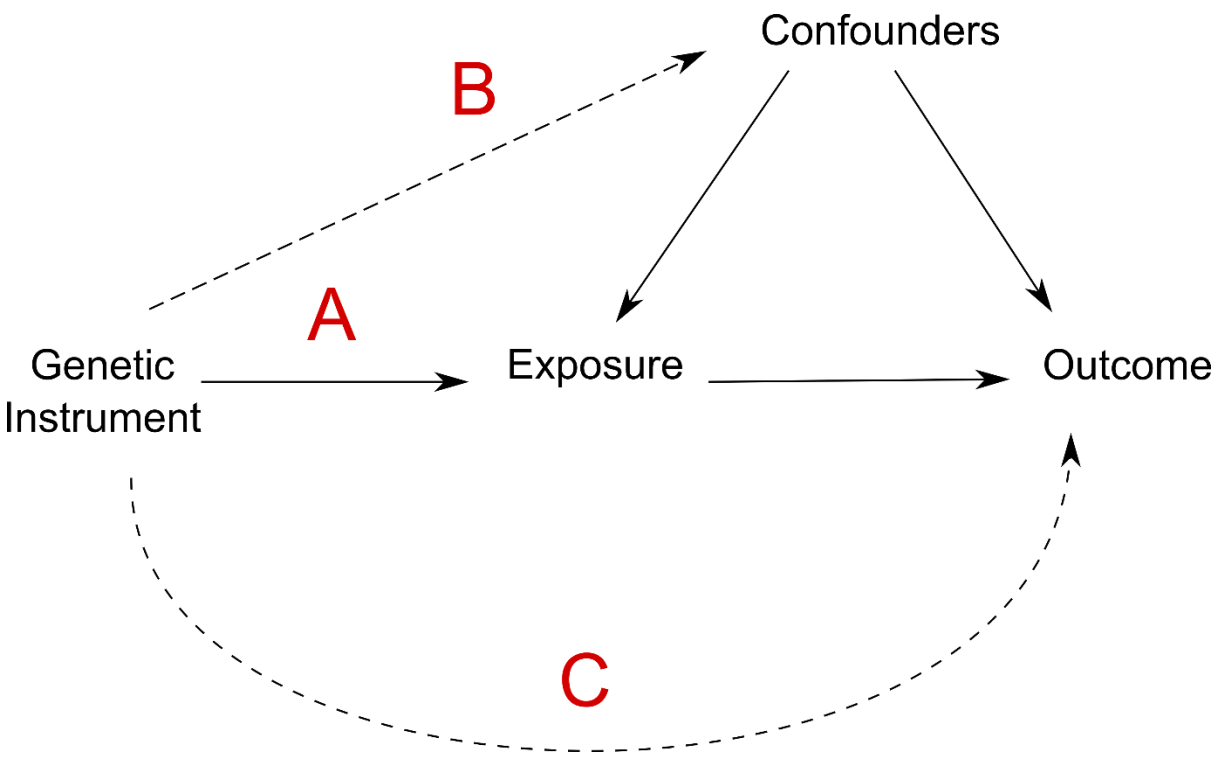


Figure 6-1: Directed acyclic graph of Mendelian Randomization methodology

Mendelian randomization does require that the instrumental variable (genetic variants) are:

a. Associated with the exposure / risk factor
b. Not associated with any confounders of the exposure or outcome (correlated pleiotropy if the IV is associated with a confounder)
c. Has no effect on the outcome directly, only through the exposure (uncorrelated pleiotropy if the IV is acting directly on the outcome)

6.2.2 Confounders

Confounding is a typical hazard of observational studies and it can occur when there is an apparent causal relationship between an exposure and an outcome, which is actually due to the confounder.

In such cases the confounder must be related to both exposure and outcome, it must not be an intermediary step in the causal pathway between exposure and outcome, and finally it must be distributed unequally between the study groups (Meuli and Dick 2018). Randomised clinical trials are resistant to confounding due to the equal distribution of the confounding factor between the study groups, equally Mendelian randomization relies on the random assignment of genotypes prior to birth before any influence by environmental or lifestyle factors that often act as confounders.

This therefore leads to an additional assumption for Mendelian randomization,

d. Variants (Instrumental variables) are randomly assigned amongst people

This is Mendel's second law or the "The Law of Independent assortment" (Morgan 1915), which states that the inheritance of one trait is independent of – randomized in respect to – the inheritance of other traits, as long as they are not in linkage disequilibrium.

6.2.3 Horizontal pleiotropy

Mendelian randomization requires that a number of assumptions have to be made about the exposure and outcome. One essential criteria is that the genetic instruments used act on the outcome exclusively through the exposure being analysed. If the instruments do act on the outcome directly as well as through the exposure or risk factor, this is termed "horizontal pleiotropy" (Solovieff et al. 2013; Ebrahim and Davey Smith 2008).

Egger regression can be used within a Mendelian randomization analysis in order to detect horizontal pleiotropy, and treats the bias resulting from such pleiotropy in a MR study as similar to small study bias in a meta-analysis (Bowden, Davey Smith, and Burgess 2015). The degree of this bias can be measured using the intercept from regression of standard normal deviates against precision (Egger et al. 1997).

6.2.4 Methods for MR analysis

The primary measure used for MR investigations was Inverse-Variance Weighted (IVW) regression, although for secondary analysis two other measures were also looked at MR-Egger and Weighted median. These methods allowed us to investigate the robustness of the IVW regression result. See Figure 6-2 for example.

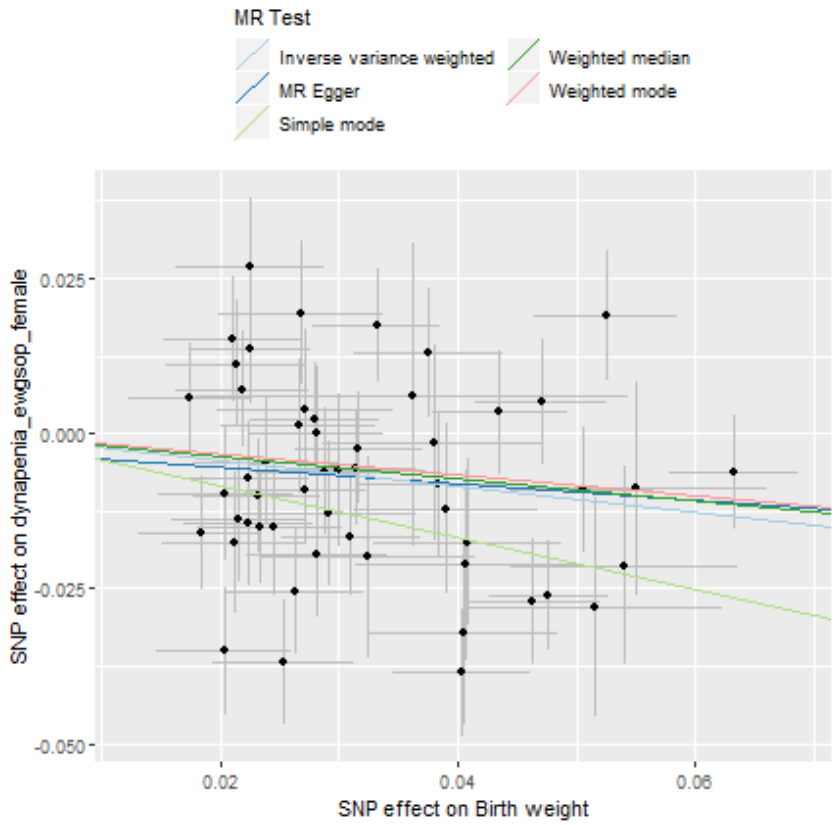


Figure 6-2 : Plot of Mendelian randomization analysis of Birth Weight SNPs on low grip strength (EWGSOP) in females only

The Inverse Variance Weighted (IVW) measure uses the ratio estimate of the causal effect of the exposure on the outcome for each genetic variant and their standard errors to calculate the estimate through a fixed effect meta-analysis model (Burgess, Butterworth, and Thompson 2013).

The IVW method does have problems correctly calculating the estimate when the Instrumental Variables are weak, or when there are variants with small effect sizes (Burgess and Thompson 2012)

6.2.5 Genetic correlation

The problem of confounding can be addressed by using the complementary method of cross-trait Linkage Disequilibrium (LD) score regression whichs takes into account all variants in LD with the lead genomic risk loci. This method is suitable when the heritably of the traits is distributed across thousands of variants with small effects (B. K. Bulik-Sullivan, Loh, Finucane, Ripke, Yang, Patterson, et al. 2015; B. Bulik-Sullivan et al. 2015). This provides a measure of genetic correlation between the two traits. While MR tests the casual effects of genetic instruments for the exposure trait with regard to the outcome trait, genetic correlation provides evidence of any shared underlying mechanisms or pathways, with no suggestion of the direction of any casual effect. Genetic correlation provides evidence of the pleiotropic action of any shared genes or pathways between casual loci in the two traits (van Rheenen et al. 2019).

6.3 Methods

6.3.1 GWAS of low grip strength in older people

I conducted a GWAS meta-analysis of low grip strength in participants aged 60 years or older of European ancestry from 22 studies yielding a combined sample of 254,894 individuals, as described in Chapter 4.

Two definitions of low muscle hand grip strength were utilized at the time of analysis. The primary analysis was of the 2010 EWGSOP criteria for sarcopenic grip strength (Grip strength < 30 Kg Male; < 20 Kg Female). Given the known differences in strength between males and females (on average) we also performed sex stratified analyses.

We used Linkage Disequilibrium Score Regression (LDSC, v1.0.0) to estimate the level of bias (i.e. from population stratification and cryptic relatedness) in the GWAS, and the SNP-based heritability of low grip strength (B. K. Bulik-Sullivan, Loh, Finucane, Ripke, Yang, Consortium, et al. 2015).

6.3.2 Genetic correlations and Mendelian randomization

We investigated the genetic correlation between the low grip strength trait and 10 diseases - chosen because they are common, chronic diseases of aging (Jaul and Barron 2017) - using LDSC (v1.0.0) (B. K. Bulik-Sullivan, Loh, Finucane, Ripke, Yang, Consortium, et al. 2015) and published GWAS summary statistics for the following: Alzheimer's disease (Jansen et al. 2019), breast cancer (Michailidou et al. 2017), chronic kidney disease (Pattaro et al. 2016), coronary artery disease (van der Harst and Verweij 2018), osteoporotic fracture risk (Trajanoska et al. 2018), osteoarthritis (Zengini et al. 2018), prostate cancer (Schumacher et al. 2018), rheumatoid arthritis

(Okada et al. 2014), stroke (Malik et al. 2018), and type-2 diabetes (Mahajan et al. 2018). We also calculated genetic correlations with the following anthropometric traits: height (Yengo et al. 2018), body mass index (BMI) (Yengo et al. 2018), waist:hip ratio (WHR) (Pulit et al. 2019), whole-body lean mass (Zillikens et al. 2017), and appendicular lean mass (Zillikens et al. 2017).

I also undertook a phenotype-wide Mendelian randomization (MR) association study to examine the causal effect of 83 traits on low hand grip strength. We used the `TwoSampleMR` (v0.4.23) package in R(Hemani et al. 2018) to perform the analysis of genetic instruments from the 83 traits, which including those traits with clear biological rationale (for example, adiposity) and others that are more exploratory for hypothesis generation (for example, puberty timing). GWAS studies were selected on the basis of number of available associated SNPs, and the study population was of European ancestry.

The process for running 'TwoSampleMR' included harmonisation of summary statistics from the provided GWAS to ensure that the effect of the SNP on the exposure and on the outcome corresponded to the same allele. Palindromic SNPs for which the effect allele strand could not be predicted from allele frequency were discarded from the trait Genetic Risk Score (GRS).

Selection of a trait for analysis as an outcome also required that the summary statistics had at least 5 SNPs associated with that trait, after harmonization.

Inverse Weighted Variance was used as the primary measure of association. Follow up sensitivity analysis of the identified traits was by the MR-Egger and using weighted median estimation methods provided in the package. Additional analysis by weighted median and weighted mode Mendelian randomization (MR) was plotted alongside MR

Egger and IVW in order to manually check for agreement between the methodologies, and so confirm findings of the main analysis. MR Egger was used to check for horizontal pleiotropy, evidence of which would violate one of the assumptions required for the Mendelian randomization analysis.

6.3.3 Data Availability

The GWAS summary statistics and supporting information on low grip strength in older people are available on the Musculoskeletal Knowledge Portal (http://musculoskeletalgenomics.org/) and the GWAS catalogue (www.ebi.ac.uk/gwas accession numbers GCST90007526, GCST90007527, GCST90007528, GCST90007529, GCST90007530 and GCST90007531)All relevant additional data is available on request from the authors.

6.4 Results

6.4.1 Study description

The meta-analysis of low grip strength in older people comprised 256,523 individuals of European descent aged 60 years or older at assessment from 22 independent cohorts with maximum hand grip strength recorded - including the UK Biobank, the US Health and Retirement Study (HRS), the Framingham Heart Study (FHS), and others. The methods and results for this study are described fully in Chapter 4. Individual study characteristics are described in the Supplementary Information and in Chapter 2 - Methods.

6.4.2 Genetic correlations and Mendelian randomization

We assessed ten common age-related diseases for their genetic correlation with low grip strength (Figure 6-3) using published genome-wide summary statistics and LDSC(B. K. Bulik-Sullivan, Loh, Finucane, Ripke, Yang, Patterson, et al. 2015). The largest genetic overlap (rG, or SNP genetic correlation estimate) was with osteoarthritis (29.7% genetic correlation, SE 6.3%), but also strong positive correlations with coronary artery disease (19.5%, SE 3.0%), type-2 diabetes (15.8%, SE 3.1%) and rheumatoid arthritis (12.7%, SE 7.9%).

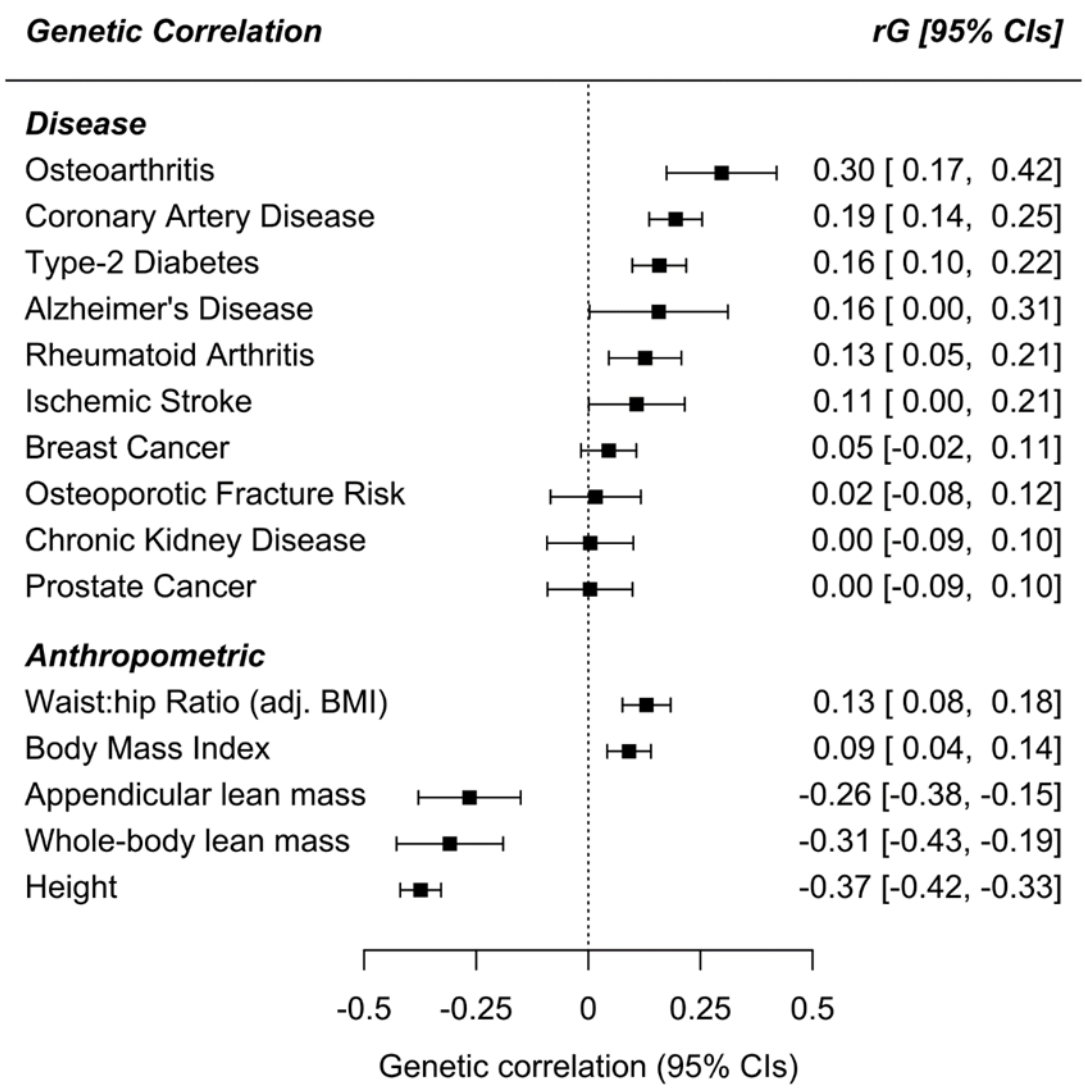


Figure 6-3: Low grip strength genetic correlations with ten common diseases and five anthropometric traits

Genome-wide genetic correlations between low muscle strength and published summary statistics for common age-related diseases and low muscle strength risk factors. Full results available in Supplementary Table s6-1.

We observed no significant genetic correlation after multiple-testing correction with the remaining diseases examined (osteoporotic fracture risk, Alzheimer's disease, stroke, chronic kidney disease, breast cancer and colorectal cancer). We also determined genetic correlations with five anthropometric traits (Figure 6-3) and found significant positive correlations with waist:hip ratio (13.0%, SE 2.7%) and BMI (9.1%, SE 2.5%), i.e. greater adiposity correlated with weakness in 60+ year olds. Significant negative correlations were observed with lean muscle mass (whole body: -30.9%, SE 6.1%; and appendicular: -26.5%, SE 5.8%) and with height (-37.4%, SE 2.3%). See Table 6-3.

Table 6-1: LDSC genetic correlations

Phenotype	rg	se	p	Reference
Osteoarthritis	0.2972	0.0626	2.0E-06	Zengini 2018 (PMID: 29559693)
CAD	0.1948	0.0301	9.1E-11	van der Haarst 2018 (PMID: 29212778)
Type-2 Diabetes	0.1582	0.0307	2.7E-07	Mahajan 2018 (PMID: 30297969)
AD	0.157	0.0787	4.6E-02	Jansen 2019 (PMID 30617256)
RA	0.1267	0.0413	2.1E-03	Okada 2014 (PMID: 24390342)
Ischemic Stroke	0.1078	0.0546	4.8E-02	Malik 2018 (PMID: 29531354)
Breast Cancer	0.0453	0.0315	1.5E-01	Michailidou 2017 (PMID: 29059683)
OFR	0.0164	0.0514	7.5E-01	Trajanoska 2018 (PMID: 30158200)
CKD	0.0042	0.0491	9.3E-01	Pattaro 2016 (PMID: 26831199)
Prostate Cancer	0.0037	0.0484	9.4E-01	Schumacher 2018 (PMID: 29892016)
WHR	0.1298	0.0274	2.3E-06	Pulit 2019 (PMID: 30239722)
BMI	0.0908	0.0249	2.7E-04	Yengo 2018 (PMID: 30124842)
ALMM	-0.265	0.0582	5.3E-06	Zillikens 2017 (PMID: 28724990)
WBLMM	-0.3089	0.0607	3.6E-07	Zillikens 2017 (PMID: 28724990)
Height	-0.3739	0.0232	2.1E-58	Yengo 2018 (PMID: 30124842)
Grip strength (Linear measure)	-0.952	0.0167	0.0E+00	Analysis in UK Biobank Europeans

Phenotype = GWAS for genetic correlation with low grip strength in older people: CAD=Coronary Artery Disease; AD=Alzheimer's Disease; RA= Rheumatoid Arthritis; OFR=Osteoporotic Fracture Risk; CKD=Chronic Kidney Disease; WHR= Waist:hip Ratio (adj. BMI); BMI= Body Mass Index; ALMM= Appendicular lean muscle mass; WBLMM= Whole-body lean muscle mass; Grip strength shown in this table is the linear measurement of grip at all ages rg = genetic correlation, se = standard error of rg, p = p-value for rg;

We examined 83 traits (Supplementary Table s6-5) in Mendelian randomization analysis to find evidence for shared causal pathways with weakness (low grip EWGSOP) at older ages: primary results presented are betas from inverse variance-weighted regression using the `TwoSampleMR` R package(Hemani et al. 2018) (Figure 6-4; Supplementary Table s6-2).

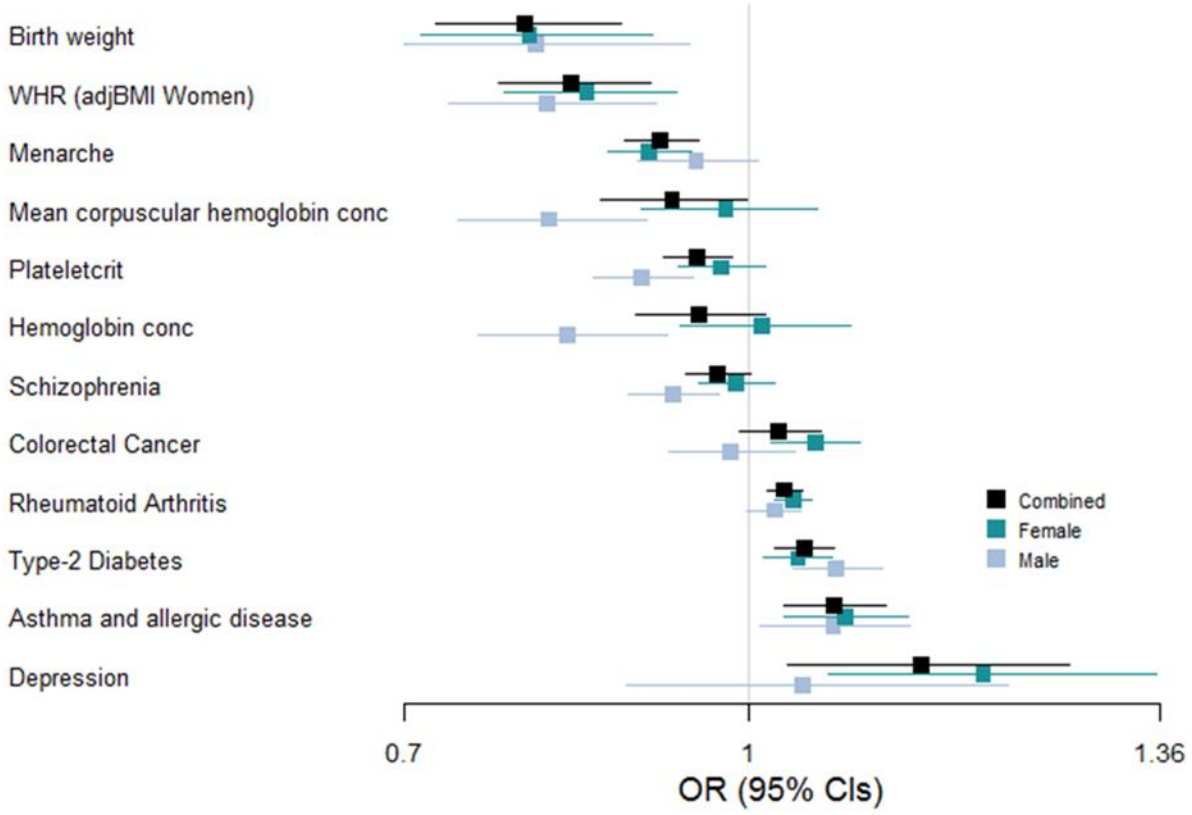


Figure 6-4: Traits sharing causal pathways with low grip strength at older ages identified in Mendelian Randomization analysis

In Figure 6-4, Mendelian randomization analysis estimates whether 83 exposures may share causal pathways with low grip strength in people aged 60 and older. Those identified as significant (multiple testing-adjusted $p < 0.05$) in at least one analysis (all participants, or males or females separately) are included in the figure. *WHR (adj. BMI in Women) = Waist-Hip Ratio SNPs identified in GWAS analysis of females only, adjusted for Body Mass Index. N = meta-analysis of 256,523 Europeans (biologically

independent samples) aged 60 or older from 22 cohorts. Data presented as Odds Ratios + /− 95% Confidence Intervals. Unadjusted p value (two-sided) from IVW regression analysis of exposure SNPs effect on low grip strength (EWGSOP definition). Full results of Mendelian randomization available in Supplementary Table s6-2, See Table 6-2 and Table 6-3.

Table 6-2: Mendelian randomization analysis of low grip strength in older people (EWGSOP)

		IVW MR - Combined				IVW MR - Female only				IVW MR - Male only			
Exposure	**nsnp**	**ivw or**	**ivw or lower ci**	**ivw or upper ci**	**ivw pval**	**ivw or**	**ivw or lower ci**	**ivw or upper ci**	**ivw pval**	**ivw or**	**ivw or lower ci**	**ivw or upper ci**	**ivw pval**
Menarche	298	0.9235	0.8915	0.9567	9.84E-06	0.9136	0.8780	0.9506	8.40E-06	1.0770	1.0393	1.1161	4.46E-05
Birth weight	53	0.8042	0.7273	0.8893	2.16E-05	0.8088	0.7145	0.9156	7.98E-04	0.8151	0.6999	0.9492	8.53E-03
Rheumatoid Arthritis	61	1.0316	1.0168	1.0467	2.44E-05	1.0386	1.0223	1.0551	2.66E-06	1.0224	0.9989	1.0465	6.21E-02
WHR (adjBMI Women)	45	0.8455	0.7820	0.9142	2.57E-05	0.8589	0.7876	0.9367	5.89E-04	0.8237	0.7380	0.9193	5.38E-04
Type-2 Diabetes	288	1.0488	1.0233	1.0750	1.50E-04	1.0433	1.0138	1.0737	3.79E-03	1.0770	1.0393	1.1161	4.46E-05
Asthma and allergic disease	34	1.0743	1.0308	1.1195	6.76E-04	1.0841	1.0308	1.1401	1.68E-03	1.0739	1.0109	1.1408	2.07E-02
PCT	193	0.9555	0.9262	0.9857	4.17E-03	0.9757	0.9383	1.0145	2.16E-01	0.9066	0.8638	0.9516	7.24E-05
Depression	89	1.1510	1.0349	1.2801	9.50E-03	1.2051	1.0705	1.3567	2.02E-03	1.0469	0.8938	1.2263	5.70E-01
Colorectal Cancer	53	1.0269	0.9917	1.0634	1.36E-01	1.0581	1.0203	1.0972	2.32E-03	0.9842	0.9309	1.0406	5.76E-01
MCHC	51	0.9333	0.8713	0.9996	4.88E-02	0.9802	0.9065	1.0598	6.15E-01	0.8252	0.7466	0.9120	1.66E-04
HGB	89	0.9569	0.9018	1.0153	1.45E-01	1.0118	0.9402	1.0888	7.55E-01	0.8426	0.7639	0.9295	6.23E-04
Schizophrenia	111	0.9733	0.9459	1.0016	6.43E-02	0.9889	0.9565	1.0224	5.12E-01	0.9338	0.8955	0.9738	1.37E-03

Significant associations for low grip strength for selected traits based on the EWGSOP definition for sarcopenia in older people. Inverse Variance Weighted (IVW) TwoSampleMR test. Menarche = Increasing age of Menarche; Birth weight = Increasing birth weight (KG); MCHC = Increasing Mature red cell Mean corpuscular haemoglobin concentration; HGB = Increasing Mature red cell Haemoglobin concentration; PCT = Increasing Platelet Plateletcrit; WHR (adjBMI Women) = Increasing Waist-Hip Ratio adjusted for Body Mass Index from GWAS for women only; All other traits = increasing incidence. nsnp = number of single nucleotide polymorphisms; ivw or = inverse variance weighted odds ratio; ivw pval = inverse variance weighted p-value

Table 6-3: IVW Mendelian randomization analysis of risk factors for ageing traits and diseases are associated with weakness in older people (EWGSOP low grip strength, both sexes)

Trait	Number of SNPs	Adjusted p-value*	MR Egger p-value	Unadjusted p-value	Odds ratio	OR lower CI	OR upper CI
Menarche	298	5.32E-04	0.0133	9.84E-06	0.923	0.891	0.957
Birth weight	53	5.32E-04	0.8606	2.16E-05	0.804	0.727	0.889
Rheumatoid Arthritis	61	5.32E-04	0.0002	2.44E-05	1.032	1.017	1.047
WHR (adjBMI Women)	45	5.32E-04	0.0113	2.57E-05	0.846	0.782	0.914
Type-2 Diabetes	288	2.48E-03	0.4736	1.50E-04	1.049	1.023	1.075
Asthma / allergic disease	34	9.35E-03	0.0247	6.76E-04	1.074	1.031	1.120
Platelet [PCT] Plateletcrit	193	4.94E-02	0.2659	4.17E-03	0.955	0.926	0.986

IVW=Inverse Variance Weighted TwoSampleMR test; *Benjamini-Hochberg FDR adjusted p-value; MR Egger p-value of intercept test (a low p-value indicates that the IVW estimate is biased and suggests either directional pleiotropy or failure of the InSIDE - Instrument Strength Independent of Direct Effect - assumption). Odds ratios = age of onset (Menarche), Kg (Birth weight), waist cm/ hip cm (WHR), % Volume occupied by platelets in the blood (Platelet crit), Binary traits are odds ratios of occurrence.

We found significantly increased likelihood of weakness (multiple testing-adjusted p-values<0.05) with genetically predicted rheumatoid arthritis (Odds Ratio=1.03, Benjamini-Hochberg adjusted p-value=$5.3*10^{-4}$), presence of type-2 diabetes (OR=1.05, BH p =$2.5*10^{-3}$), or incidence of asthma and allergic disease (OR=1.07, BH p=$9.4*10^{-3}$) (Table 6-2). Genetic predisposition to greater age of menarche (OR=0.92, BH p=$5.3*10^{-4}$; see Figure 6-5), increased birth weight (OR=0.80, BH p=$5.3*10^{-4}$) and increased waist-hip ratio (WHR) adjusted for BMI in women only (OR=0.85, BH p=$5.3*10^{-4}$) were protective of low grip strength as defined by the EWGSOP definition in both sexes (after adjustment for multiple testing).

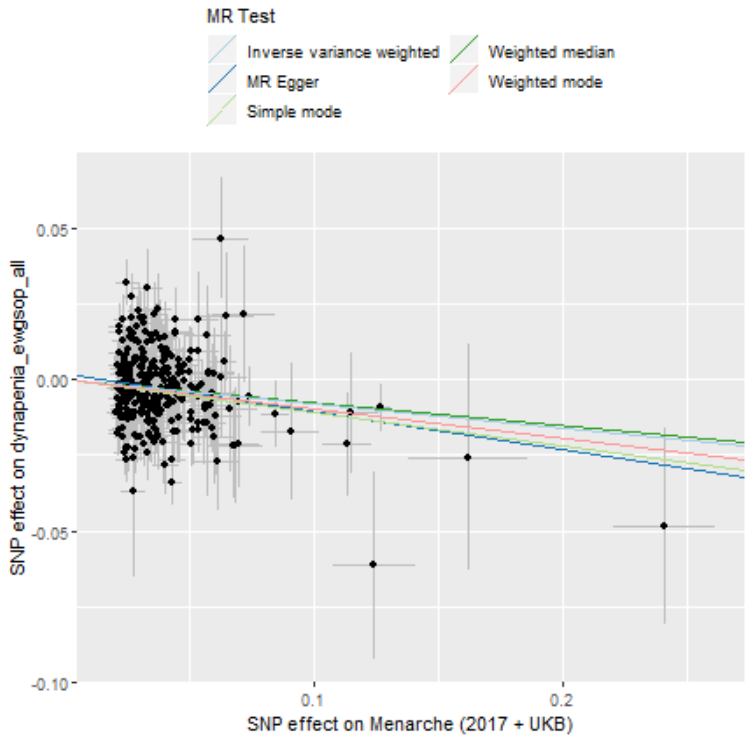


Figure 6-5: Mendelian randomization association between low grip strength and age of onset of menarche (EWGSOP, both sexes)

SNP effect of age of onset of Menarche against SNP effect on low grip strength (EWGSOP – combined) for each variant available from the 2017 UK Biobank GWAS for age at Menarche (Day et al. 2017).

For each significant analysis we also examined the results from the weighted-median and MR-Egger tests to check consistency and for horizontal pleiotropy. Horizontal pleiotropy arises when a single genetic variant or instrument influences multiple traits, and is one of the limitations of the Mendelian Randomization methodology (Hemani, Bowden, and Davey Smith 2018).

Only birth weight had an MR-Egger beta that was inconsistent with the main effect (-0.03 compared to -0.2), although the intercept did not significantly deviate from 0 (p=0.1) and the MR-Egger confidence intervals overlap the IVW effect (95% CIs -0.33

to 0.27). Additionally, the WHR association should be interpreted with caution, as the analysis of WHR variants associated with both sexes were not statistically significant (nominal IVW p=0.01, Table 6-4).

Table 6-4: MR Egger analysis of risk factors for ageing traits and diseases are associated with weakness in older people (EWGSOP low grip, both sexes)

Trait	MR Egger Beta	MR Egger ORs	MR Egger Beta SE	MR Egger p-value
Menarche	-0.124	0.883	0.050	0.0133
Birth weight	-0.027	0.973	0.154	0.8606
Rheumatoid Arthritis	0.043	1.044	0.011	0.0002
WHR (adjBMI Women)	-0.300	0.741	0.114	0.0113
Type-2 Diabetes	0.018	1.018	0.025	0.4736
Asthma / allergic disease	0.128	1.137	0.054	0.0247
Platelet [PCT] Plateletcrit	-0.036	0.965	0.032	0.2659

IVW=Inverse Variance Weighted TwoSampleMR test; *Benjamini-Hochberg FDR adjusted p-value; Odds ratios = age of onset (Menarche), Kg (Birth weight), waist cm/ hip cm (WHR), % Volume occupied by platelets in the blood (Platelet crit), Binary traits are odds ratios of occurrence.

The analysis of females only, identified depression (OR=1.21, BH $p=2.75*10^{-2}$), and colorectal cancer (OR=1.06, BH p=2.75*10-2) (Table 6-5, full results in Supplementary Table s6-3). Although the MR-Egger intercept for depression was not statistically different from 0, there is potential for horizontal pleiotropy confounding this result (seen on Figure 6-6).

Table 6-5: IVW Mendelian randomization of risk factors for ageing traits and diseases are associated with weakness in older people (EWGSOP low grip, Female only)

Trait	Number of SNPs	Adjusted p-value	Unadjusted p-value	Odds ratio	OR lower CI	OR upper CI
Rheumatoid Arthritis	61	2.20E-04	2.66E-06	1.039	1.022	1.055
Menarche	298	3.49E-04	8.40E-06	0.914	0.878	0.951
WHR (adjBMI Women)	45	1.63E-02	5.89E-04	0.859	0.788	0.937
Birth weight	53	1.65E-02	7.98E-04	0.809	0.715	0.916
Asthma / allergic disease	34	2.75E-02	1.68E-03	1.084	1.031	1.140
Depression	89	2.75E-02	2.02E-03	1.205	1.071	1.357
Colorectal Cancer	53	2.75E-02	2.32E-03	1.058	1.020	1.097
Type-2 Diabetes	288	3.93E-02	3.79E-03	1.043	1.014	1.074

IVW=Inverse Variance Weighted TwoSampleMR test; *Benjamini-Hochberg FDR adjusted p-value Odds ratios = age of onset (Menarche), Kg (Birth weight), waist cm/ hip cm (WHR). Binary traits are odds ratios of occurrence.

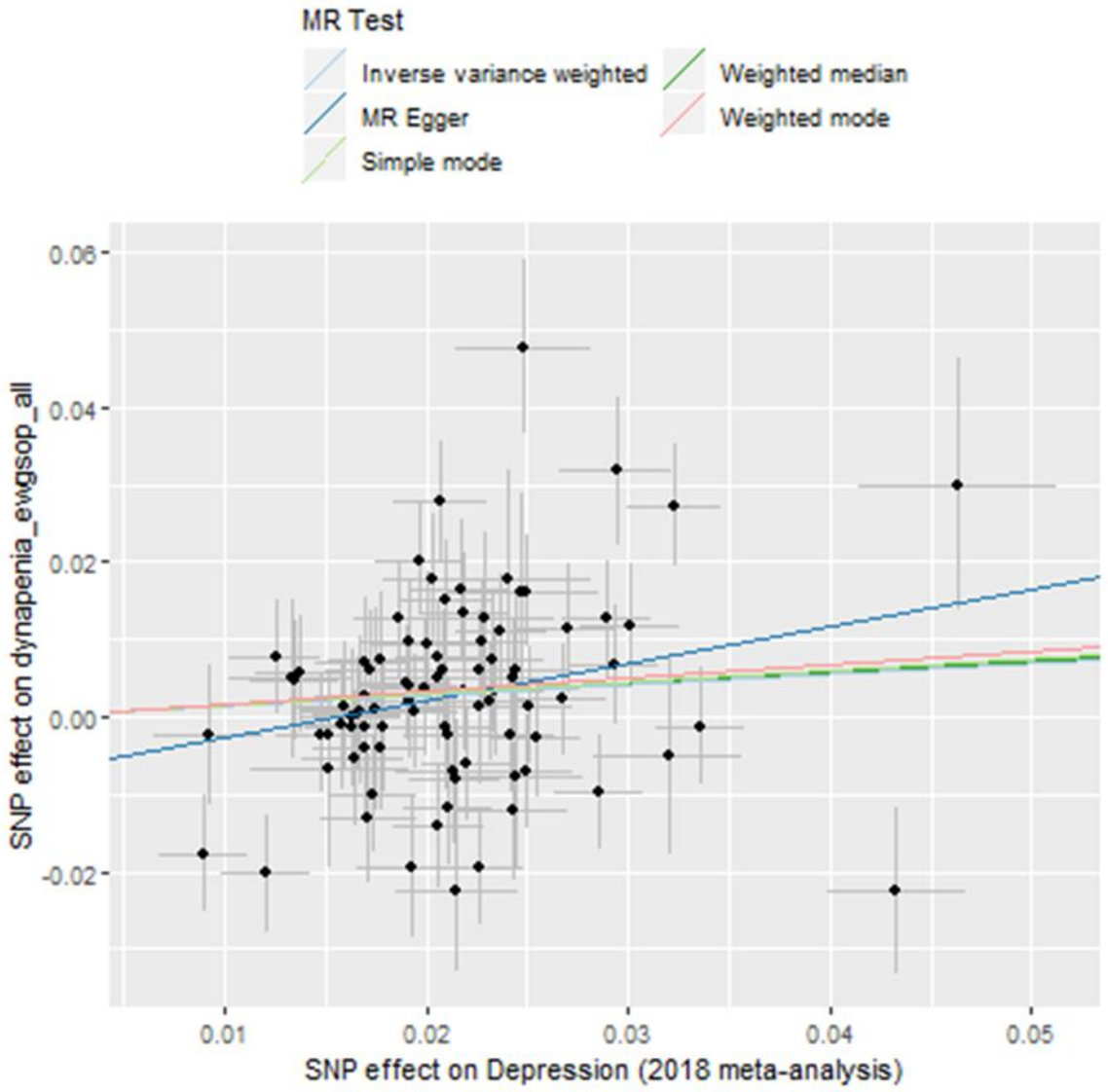


Figure 6-6: Plot of Mendelian randomisation analysis of depression SNPs on low grip strength (EWGSOP) in females only

Scatter plot of estimated SNP effects on low grip strength European Working Group on Sarcopenia definition against estimated SNP effects on Depression; GWAS meta-analysis N = 132,443 female participants (biologically independent samples) with n=33,548 cases of EWGSOP defined low grip strength; Data points indicate SNP effect on each trait +/- Standard Error

In the males, increased incidence of type-2 diabetes significantly increased odds of EWGSOP low grip (OR=1.08, BH $p=3.0*10^{-3}$) whereas greater plateletcrit (the volume occupied by platelets in the blood as a percentage) (OR=0.91, $p=3.0*10^{-3}$) and other hematological parameters appear to be protective (Table 6-6, full results in Supplementary Data s6-4).

Table 6-6: IVW Mendelian randomization of risk factors for ageing traits and diseases are associated with weakness in older people (EWGSOP low grip, Male only)

Trait	Number of SNPs	Adjusted p-value	Unadjusted p-value	Odds ratio	OR lower CI	OR upper CI
Type-2 Diabetes	288	3.00E-03	4.46E-05	1.077	1.039	1.116
Platelet [PCT] Plateletcrit	193	3.00E-03	7.24E-05	0.907	0.864	0.952
MCHC	51	4.59E-03	1.66E-04	0.825	0.747	0.912
WHR (adjBMI Women)	45	1.03E-02	5.38E-04	0.824	0.738	0.919
HGB	89	1.03E-02	6.23E-04	0.843	0.764	0.929
Schizophrenia	111	1.90E-02	1.37E-03	0.934	0.895	0.974

IVW=Inverse Variance Weighted TwoSampleMR test; *Benjamini-Hochberg FDR adjusted p-value MCHC = Increased Mature red cell mean corpuscular hemoglobin concentration; HGB = Increased Mature red cell hemoglobin concentration; WHR (adjBMI Women) = Increasing Waist-Hip ratio

To explore the effect of genetic predisposition to low grip strength at older ages we created an unweighted genetic risk score (GRS) in the UK Biobank European sample by summing the number of low grip strength-associated alleles (15 genetic variants so 30 alleles, mean number of alleles = 12.6, SD=2.3). We first confirmed the association with low grip strength in UK Biobank participants (OR per allele 1.036: 95% CIs 1.030 to 1.041, $p=6*10^{-40}$).

6.5 Discussion

Genetic correlation between two heritable traits provides a quantitative description of their relationship, and can help highlight shared underlying biological pathways and the causality of the relationship (van Rheenen et al. 2019). We found prominent genetic correlation (rG) between low grip strength and the common conditions, osteoarthritis and Rheumatoid arthritis, but also with cardiovascular disease and type-2 diabetes.

Many of these conditions were also observed in our Mendelian randomization analysis to share casual pathways with low grip strength with additional links to other immune related conditions, for example asthma and allergy, also been found.

Our previous GWAS meta-analysis of 256,523 Europeans aged 60 years and over, found little overlap at the individual locus level between low grip strength at older ages (see Chapter 4) and other diseases. We did observe significant genetic correlations, especially with osteoarthritis (30% overlap). However in Mendelian Randomization analysis we did not observe a causal relationship between osteoarthritis and low grip strength: taken together, this suggests that osteoarthritis shares causal risk factors and biological pathways with low grip strength at older ages, such as obesity, but may not cause it. Although our results were robust to adjustment for osteoarthritis (including rhizarthrosis – arthritis of the thumb) this may suggest that arthritis in the hand needs to be accounted for in measures of muscle weakness, as hand grip strength may not always reflect muscle strength elsewhere, e.g. lower-extremity strength.

Our Mendelian Randomization analyses highlighted specific traits and diseases which may share causal pathways with weakness at older ages. This included growth and

development traits such as increased birth weight, higher waist:hip ratio, and later pubertal timing (age at menarche) in women (highly genetically correlated – 75% – with age at voice breaking in men (Day et al. 2017)), where greater values were protective. Although puberty timing is highly polygenic, it is strongly genetically correlated with BMI in adults (-35%) (Day et al. 2017). These results are consistent with the observation that growth and development traits are associated with strength trajectories in later life (Kuh et al. 2019).

Raised red blood cell parameters - especially plateletcrit, the proportion of blood occupied by platelets - appear to be protective in males but associations were attenuated or non-significant in females. A number of studies have recently reported a link between raised platelet to lymphocyte ratio, inflammation, and sarcopenia cross-sectionally (Liaw et al. 2017). However our results suggest that plateletcrit (total platelet mass) across the lifecourse (rather than after sarcopenia onset) may be different, with the binding of platelets to lymphocytes shown to regulate the inflammatory response (Zamora et al. 2017).

Lastly, only four of the conditions we investigated (which included coronary artery disease, and some common cancers) appear to causally increase risk of weakness in older people: depression, asthma/allergic diseases, Rheumatoid arthritis, and type-2 diabetes. These are diverse conditions and further underlines the multifactorial causes of weakness in older people. The presence of depression and asthma in our results suggests that treatment of these conditions, alongside treatment for muscle weakness and wastage may be a viable alternative for a more positive outcome.

Some Mendelian Randomization analyses have limited power due to the lack of strong instruments, and therefore null results for these analyses should be interpreted with

caution. Ideally the GWAS summary statistics used for the exposures would not include studies, such as UK Biobank that are also used for in the low grip GWAS meta-analysis, due to the assumptions of Two sample MR and population stratification. Additional MR tests can be used to test for violations of the IV assumptions. Steiger filtering can be used to avoid the inclusion of SNPs that have a reverse casual effect (Hemani, Tilling, and Davey Smith 2017). This can be summarised as if a SNP causes the exposure and the exposure causes the outcome, then the effect of the SNP on the exposure should be larger than the effect of the SNP on the outcome. If this is not true then you can infer that the instrument is not influencing the exposure primarily, and could be evidence of reverse causality. It should be noted that measurement error could cause issues with this type of filtering. Co-localisation, where the same casual variant influences two or more traits / phenotypes, can also be investigated (Giambartolomei et al. 2014). Although it should be noted that testing co-localisation requires a similar LD structure between the exposure and outcome, also multiple casual variants within the same region can cause issues.

7 Discussion and conclusions

My thesis aims to improve on current knowledge of the role of inherited genetic variation in the development of musculoskeletal traits in human ageing.

By understanding the mechanisms involved in under-studied traits, such as low grip strength in older people, the development of interventions to delay or prevent the pain and disability resulting from the common musculoskeletal changes of ageing are facilitated. My analysis has added to the literature surrounding muscle function and ageing in older people and has been used by other researchers to investigate similar conditions, such as the effect of exercise on gene regulation in post-menopausal women ("Heavy-load exercise engages about 13% of the skeletal muscle transcriptome in postmenopausal women", Gautvik, K.M et al, submitted for review March 2021).

The recent release of a number of large biobanks, such as the UK Biobank, has allowed me the opportunity to find evidence of underlying mechanisms, for example the influence of the inflammation and growth / development pathways on low strength in older people. By meta-analysing Genome-Wide Association Studies from a wide-range of population based cohorts provided by the CHARGE consortium, I have been able to find associations for a greater number of variants with one of the physical components of both frailty and sarcopenia, low grip strength, than any previous study. Genetic Risk Scores (GRS) for this trait could have a clinical utility in predicting those most at risk of developing these debilitating conditions. However further study on the absolute effect in an independent population would be required. As would the suitability to other ancestral populations, other than European ancestry.

Sex stratified analysis of the GWAS meta-analysis has highlighted differences in the genomic risk loci for each sex. While Mendelian randomization has shown how the genetic instruments derived from the low grip strength in older people has a casual influence from certain age-related traits, with important sex specific differences.

Previous literature on life course epidemiology has highlighted how my analysis can be interpreted with relationship to environmental interactions throughout the subject's life course, with particular emphasis on critical periods of risk and the accumulation of risk throughout life (Ben-Shlomo 2002). Due to the focus of the study on muscle weakness in later life, the accumulation of lifetime risk can be significant and critical periods may be highlighted by the traits associated by Mendelian randomization, such as birth weight and age of menarche. By examining the results of the analysis within the life course epidemiology framework, critical periods for intervention and observation can be identified.

7.1 New contributions

I have taken the knowledge in this field forward by

- Discovery of 15 genomic risk loci associated with muscle weakness in older people and provided evidence for possible underlying mechanisms.

- Conducting a sex stratified analysis using data from the 22 cohorts on muscle weakness in older people, and showing that there are differences in the genomic risk loci between the sexes.

- Using Mendelian randomisation and genetic correlation between age related traits and conditions, indications of shared pathways with conditions such as

Rheumatoid arthritis, and diabetes. Life course traits such as age of onset of menarche were also implicated in later life muscle weakness.

- Found associations between HLA haplotypes and sarcopenia. Showed that certain HLA haplotypes are distinct to the separate components of low grip strength and lean muscle mass.

- That a combination of HLA alleles gives a 23% increased likelihood of sarcopenia.

7.2 Sarcopenia and variation in the Human Leukocyte Antigen complex

The underlying mechanisms which result in the state of sarcopenia in older adults is thought to include an autoimmune component, however the genetic contribution of immune system genes to sarcopenia is understudied (Tan et al. 2012). My analysis of 181,301 UK Biobank participants of European descent aged 60-70 (95,340 women, 12.10% defined as sarcopenic under EWGSOP criteria and 85,961 men, 4.08% EWGSOP sarcopenic) found an associated between the EWGSOP definition of sarcopenia (combined low grip strength and low lean muscle mass) and six HLA haplotypes.

When these six haplotypes were combined, the presence of at least 6 alleles, out of a possible 12 resulted in a 23% increased likelihood of sarcopenia.

7.2.1 Impact of findings

My analysis of UK Biobank participants using multiple definitions of sarcopenia highlighted that there is a substantial autoimmune component to sarcopenia in older

people, after accounting for common known autoimmune conditions. In addition I have shown that the two main clinical metrics used in the definition of sarcopenia, low grip strength and skeletal muscle mass, have different HLA haplotypes associated with them.

My study provides evidence of the involvement of the HLA genes in these traits in older people, independent of other known autoimmune conditions. Whether or not this interaction is due to Immuno-senescence and “Inflammaging” (Franceschi et al. 2018) is open to debate, although this does provide a mechanism by which the progression of sarcopenia and loss of muscle function with age could be attributed to.

Although I have tried to account for known auto-immune conditions, confounding with undiagnosed auto-immune conditions is possible. In my analysis HLA types associated with sarcopenia, such as DRB1*04:01, have also been previously associated with Rheumatoid arthritis (Viatte et al. 2015). In Chapter 6 I show that muscle weakness in older people shares underlying genetic correlation with Osteoarthritis (rG=0.30, 95% CI=0.17-0.42) and Rheumatoid arthritis (rG=0.13, 95% CI=0.05-0.21). I also show that rheumatoid arthritis shares a causal relationship with low grip strength in older people using Mendelian Randomization (p=2.44*10-5; Odds Ratio=1.032). Although in the MR analysis we did not exclude diagnosed rheumatoid arthritis or other auto-immune conditions from the low grip GWAS meta-analysis that provided the Instrumental Variables, these findings provide additional support for an auto-immune component to muscle weakness in older people.

Although six HLA types were found associated with low skeletal muscle mass on its own there was no overlap with the seven HLA types associated with low handgrip strength on its own. Despite this distinct separation between the two sets of results,

only HLA types seen in the low grip results were found in the combined EWGSOP results.This does suggest that not only is low grip strength the primary informative measure in the combined definition, but also that there may be distinct immune system pathways underlying the causes for the two separate metrics.

In addition by investigating the non-coding SNPs available in the genotyping data from the HLA region, 4 loci associated with the EWGSOP definition of sarcopenia were identified. Expression data identified that these SNPs were associated with various HLA types and proteins involved in the unfolded protein response. The unfolded protein response has previously been suggested as one of plausible mechanisms involved in sarcopenia (Deldicque 2013)(Gnimassou, Francaux, and Deldicque 2017), and the eQTLs (*BAG6*, *ATF6B*) and pQTLs (*ATF6A*) associated with sarcopenia by my analysis supports this. These preliminary results were also found in the meta-analysis presented in Chapter 4.

7.2.2 Future directions

Future plans could include a follow up GWAS excluding known auto-immune conditions and a subsequent MR analysis to try and quantify the influence of undiagnosed auto-immune conditions such as rheumatoid arthritis. Although the UK Biobank provides ICD-10 codes for auto-immune conditions from Hospital Episode Statistics (HES) data, a similar comprehensive resource was not always available for CHARGE cohorts that were part of the meta-analysis. Reanalysing the meta-analysis could also be informative if auto-immune conditions could be accounted for.

Myasthenia Gravis, an autoimmune disorder of the neuromuscular junction which

results in muscle weakness can be diagnosed by using an immunological test for the serum antibodies for skeletal muscle acetylcholine receptor. The sensitivity of such auto-antibody tests approaches 85% (Meriggioli and Sanders 2009). Late onset Myasthenia Gravis has an association with the HLA haplotype DRB1*15:01 (Odds Ratio=2.38; $p=7.4*10^{-5}$; n cases=369, n controls=651), amongst a number of additional HLA haplotypes (Maniaol et al. 2012). By understanding the auto-immune mechanism involved in Myasthenia Gravis various therapies have been developed including complement inhibitors, or depleting the antigen producing cells (Koneczny and Herbst 2019).

My analysis supports the hypothesis that there is an autoimmune component to sarcopenia, separate from any existing condition, in certain individuals. These therapies could provide the basis for treatment for a proportion of older people with muscle weakness and sarcopenia. With increased knowledge of the autoimmune targets and the antibodies involved, sensitive serum based tests could be developed.

7.3 Genome-wide meta-analysis of muscle weakness identifies 15 susceptibility loci in older men and women

Low grip strength is a good predictor of overall muscle function and is a commonly measured metric, making a multi-cohort Genome-Wide Association Study meta-analysis possible. By using the low grip cut-offs as defined by the European Working Group on Sarcopenia and genotyping data from members of the CHARGE consortium I have shown that there are fifteen genomic risk loci for low grip strength, 12 of which

have not previously been associated with a continuous measure of grip strength, in older adults (age > 60 of European ancestry).

Our analysis highlights the multiple pathways to the physical components of frailty and sarcopenia, including the immune response, growth and differentiation, and protein turnover.

7.3.1 Impact of findings

Our GWAS meta-analysis of low grip strength in older people is one of the largest population based studies on the primary component of sarcopenia. The genomic risk loci associated with low grip strength, have a range of potential casual genes which highlights the multifactorial nature of the underlying causes of sarcopenia.

Given the small effect sizes observed in our study, large population based studies are required in order to discover such associations. The previous analysis by the CHARGE consortium looking at maximum hand grip strength in a smaller meta-analysis of 27,581 Europeans aged 65 or older found two loci (rs752045 and rs3121278) (Matteini et al. 2016) neither of which were significant in our meta-analysis of low grip cut-offs according to the EWGSOP criteria. Although the Matteini et al CHARGE study analysed the contribution of maximal hand grip strength in older people, rather than low grip cut-offs, the lack of overlap with risk loci in my GWAS meta-analysis in Chapter 4 (as well as the maximal grip strength study using UK Biobank participants of all ages for 12 of the 15 risk loci (Tikkanen et al. 2018)) is an interesting observation. A possible reason for this is the use of maximal linear grip trait rather than a cut-off derived from sarcopenia definitions.

In addition to the secondary analysis using the FNIH sarcopenia cut-offs, a meta-analysis of maximal grip strength using our cohorts (although many of the CHARGE cohorts are in both studies) and participant criteria would have helped to confirm this difference in risk loci between maximal grip strength, seen in other studies, and low grip cut-offs in older people.

One of the risk loci, lead SNP rs13107325, was proximal to *SLC39A8*, a gene with range of pleiotropic effects on multiple conditions. For example the *SLC39A8* A391T missense mutation has been shown to increase risk of Crohn's disease, cardiovascular and metabolic diseases, as well as Parkinson's and neuropsychiatric disease. SLC39A8 encodes the ZIP8 metal influx transporter and it is the disruption of Manganese homeostasis which is thought to be an underlying mechanism in the development of many of these conditions (Nakata et al. 2020).

However our lead SNP rs13107325 is not an eQTL for *SLC39A8* but instead *UBE2D3*. Fine mapping of our genomic risk loci would help to gather further evidence of casual genes and either support our current interpretation of multiple casual pathways to low muscle strength in older people or possibly help to connect these seemly diverse loci into a single pathway.

7.3.2 Future directions

During the design of the analysis protocol low grip strength based on EWGSOP or FNIH cut-offs, was selected as the outcome due to its highly informative nature with regards to presence of sarcopenia (Cruz-Jentoft et al. 2010), general function of

skeletal muscle throughout the body (Bohannon et al. 2012), and general availability within the CHARGE cohorts.

Previous literature has noted that participants unable to complete grip measurement tests, have the highest mortality rates per 1000 person years (18.63; 11.03 to 31.46 95% CI) (Cooper et al. 2014). Inclusion of participants unable to complete the grip tests due to inflammation or weakness, as opposed to accidental damage to the hand, may help to resolve loci involved in the loss of muscle function.

A follow-up study on Skeletal Muscle Mass and possibly gait speed would be required to fulfil the full criteria for the EWGSOP 2010 version (Cruz-Jentoft et al. 2010) and FNIH (S. A. Studenski et al. 2014) definitions of sarcopenia. In addition to a cohort with cases defined by the combined criteria (low grip strength, low skeletal muscle mass and slow gait speed), analysing these separately in a large population based meta-analysis would also be informative. Although the CHARGE consortium are currently (march 2021) meta-analysing the Short Physical Performance Battery (SPPB), which incorporates gait speed as a measure of frailty, the UK Biobank only includes limited data on gait speed (self-reported, slow / normal / fast). This would mean that in order to use the UK Biobank cohort in any follow up study including gait speed as a trait, either the accelerometer data available for a proportion of the data would have to be used or the limited self-reported questionnaire data.

Follow-up studies on the same cohorts at later time points would allow a more robust analysis of the progress of muscle weakness with age, and provide valuable insights into the ageing process. Given the large number of cohorts involved this may be impractical to repeat the required measurements for all of the cohorts, however for the UK Biobank cohort repeat measurements (grip strength, lean muscle mass) are

available for the 100,000 participants involved in the UK Biobank imaging enhancement project (Littlejohns et al. 2020).

Since the development of the meta-analysis low grip strength protocol and subsequent meta-analysis, the European Working Group on Sarcopenia in Older People has released a revised version of their sarcopenia definition(Cruz-Jentoft et al. 2019) (EWGSOP2). Although the low grip strength cut-offs in EWGSOP2 are very similar to the FNIH definition we used as our secondary analysis (and hence why we didn't try and review the protocol at the last minute; FNIH Males <26 Kg, Females < 16Kg; EWGSOP2 Males < 27Kg, Females < 16 Kg), low grip strength is only part of the overall definition. When the entire algorithm for predicting sarcopenia is taken into account for both EWGSOP1 and EWGSOP2 the difference in prevalence within the UK Biobank for all ages is 8.14% and 0.36% respectively (Petermann-Rocha, Chen, et al. 2020). Given this significant difference in prevalence a future project using similar sized meta-analysis would provide further resolution of the underlying pathways in sarcopenia in older people.

In addition it should be noted that the cohorts involved in the meta-analysis are population and community based. Replication of the findings in cohorts based on hospital and care home residents would be essential in order to apply our findings to the forms of secondary sarcopenia associated with chronic illness found in these populations (Welch et al. 2018).

Replication of the results from the European population should be replicated for a range of different populations and ethnicities. There are different criteria for some ethnicities such as the Asian Working Group on Sacropenia (AWGS)(L.-Y. K. L.-K. Y. Chen et al. 2014), although most definition shared similar metrics, for example grip

strength and skeletal muscle mass. Despite this a number of extremely large population based biobanks with deep phenotyping and genotyping similar to that of the UK Biobank, with greater proportion of different ethnicities are becoming available("The 'All of Us' Research Program" 2019; Hunter-Zinck et al. 2020). Trans-ethnic meta-analysis can identify additional candidate risk loci as well as filter out noise due to population specific factors (Sakaue et al. 2020). For example a recent trans-ancestry meta-analysis of coronary heart disease (CAD) using both participants from the Biobank Japan and UK Biobank produced a polygenic risk score (PRS) that outperformed population specific PRS, identified additional risk loci and help to fine map the candidate genes associated with CAD across both populations (Koyama et al. 2020).

Our GWAS meta-analysis included annotation of the lead SNPs with FUMA, which included a number of approaches to identify casual variants (Watanabe, Taskesen, Van Bochoven, et al. 2017). However there also additional approaches that could be used to fine map the casual variants associated with low grip strength in older people. These include statistical methods such as a penalised regression (only considers SNPs whose effect size is not reduced to zero after being penalised), an heuristic LD method (where variants that pass a linkage disequilibrium threshold with the peak SNP are considered together, this approach was used in the FUMA annotation) or a Bayesian fine mapping approach (Schaid, Chen, and Larson 2018).

One common approach is to increase the SNP density and so the number of informative sites. The UK Biobank has recently started to release Exome sequencing and variant data for the majority of the participants (Szustakowski et al. 2020), and this

will in time be followed by the sequencing of the entire dataset. Such data allows high variant density analysis of the variants associated with sarcopenia and traits like low grip strength. Novel approaches such as aggregating loss of function and missense variants for a particular gene in order to resolve any association with a trait can be attempted(Fuchsberger et al. 2016). In addition analysis of rarer alleles with a Minor Allele Frequency of less than 1% is possible, as well as better identification of structural variants such as Copy Number Variants (CNV). Association of CNVs with anthropometric traits has previously been published on, using UK Biobank genotyping data (Macé et al. 2017). However sequencing data will enable smaller structural variants to be called.

7.4 Sex-stratified genomic analysis of low grip strength

Low grip strength is a commonly used proxy for measuring overall muscle function, particularly in regards to the age-related condition, sarcopenia (Aadahl et al. 2011; Bohannon et al. 2012; S. H. Lee and Gong 2020). The prevalence of sarcopenia, or loss of muscle mass and function primarily due to age varies considerably from population to population, age and sex (S. A. Purcell et al. 2020; Shafiee et al. 2017).

With the observation that sexual dimorphism in skeletal muscle protein turnover with age has been previously noted (G. I. Smith and Mittendorfer 2016; G. I. Smith et al. 2012), as has the influence of sex on different substrate utilization in skeletal muscle mitochondria (Montero et al. 2018; Mark A. Tarnopolsky 2008; Horton et al. 1998), we

investigated the genomic risk loci associated with low grip strength in older adults in a large community based meta-analysis with cohorts separated by sex.

Using sex-stratified analysis of the GWAS meta-analysis from 22 cohorts (135,468 females and 121,055 males) and the low grip cut-offs as defined by the European Working Group on Sarcopenia and genotyping data from members of the CHARGE consortium I have shown that there are eight loci associated with low grip strength in older women (age > 60) of European ancestry. Although there is substantial overlap with our previous analysis on both sexes, there is one unique risk locus intronic to *PKD1*.

7.4.1 Impact of findings

Although the overlap between the female only cohort risk loci and the results from the GWAS meta-analysis presented in chapter 4 were to be expected, the lack of associated risk loci overlap for the male only cohort with the original analysis is unexpected.

Case numbers were substantially lower for the male only cohort for both the EWGSOP and FNIH definition for low grip strength (N=121,055; n cases EWGSOP=14,007 or 1.6%; n cases FNIH=6,734 or 5.6%) when compared to the female only cohort (N=135,468; n cases EWGSOP=34,589 or 25.5%; n cases FNIH=13,601 or 10%). This was despite sex-specific cut-offs for low grip strength.

Therefore the lower number of overall risk loci identified may be expected in the male–only cohort due to sample size and number of cases. The non-significance of the two risk loci (rs774787160 and rs145933237 from the male-only results) in the female-only cohort could attenuate the association in the combined analysis, masking the

association. This highlights the importance of analysing the cohorts in a sex-stratified manner.

Both the novel male-only risk loci (rs774787160 and rs145933237) have plausible mechanisms for low grip strength through their nearest genes DSCAM (axonal growth and evidence of impaired motor control in model systems (Purohit et al. 2012; G. Liu et al. 2009; Lemieux et al. 2016)) and MIR466 (cellular apoptosis (Cao et al. 2018)). However neither lead SNP has an eQTL associated with it. In the case of rs145933237, for example, additional interesting genes are associated with the risk locus, including *GADL1* (glutamic acid decarboxylase–like 1 associated with plasma levels of carnosine, muscle strength and age-related degenerative changes) (Mahootchi et al. 2020). Additional fine mapping the sex-specific loci would help to identify the casual genes associated with the risk locus.

Such fine mapping could lead to specific treatments for age associated muscle weakness. For example, *GADL1* has a role in the biosynthesis of carnosine, and carnosine deficiency can be treated by β-Alanine supplementation(Saunders et al. 2017). It is interesting to note that *CARNS1*, another gene involved in carnosine metabolism, has an association with maximal handgrip strength (Mahootchi et al. 2020) and that carnosine itself has a substantial literature on its role in aging (Hipkiss, Baye, and de Courten 2016; Aydın et al. 2016; Chaleckis et al. 2016).

There are seven risk loci associated with the female-only cohort, which are also observed in the original combined analysis. The remaining risk locus rs7185040, an eQTL for *PKD1*, which is associated with Chronic Kidney Disease (CKD). One of the clinical implications of my findings is that potential treatments for clinical weakness may be sex-specific.

7.4.2 Future Directions

Increasing the size of the meta-analysis by including other large biobanks in a subsequent analysis will help find additional associations with low grip strength in older people. This would be particularly valuable for the male only cohort were sample size and number of cases may be an issue.

Trans-ethnic meta-analysis with large population based biobanks such as the Japanese Biobank (Nagai et al. 2017), has been used to increase the power and sensitivity of similar studies (Koyama et al. 2020).

Fine mapping of the risk loci for each sex stratified analysis would help to identify the casual genes, and suggest additional pathways involved in muscle weakness in older people.

7.5 Mendelian Randomization analysis of causal pathways associated with low grip in older people

I used Mendelian randomization on genome-wide association study meta-analysis of 256,523 Europeans aged 60 years and over for low grip strength (European working group on sacropenia definition), and found that there are a number of possible overlapping casual pathways, which include metabolic diseases such as diabetes, haematological parameters, and the immune system. These findings reinforce the multifactorial causes of weakness in older people, and the impact of growth and development earlier in the life-course on muscle weakness in later life.

7.5.1 Impact of findings

The analysis of aging related traits and chronic diseases of aging by Mendelian Randomization (MR) and genetic correlation confirmed the relationship with the aging immune system, and highlight certain measures of the human life course as important indicators of muscle weakness in later life.

Indicators like birth weight, age of menarche and waist-hip ratio, can provide public health intervention markers, if they are reliable and consistent predictors of later ill health (Belbasis et al. 2016; Hsieh et al. 1990). The life course traits identified by the MR analysis as associated with muscle weakness in older people, have been shown by previous literature to be associated with each other. Birth weight has an impact on age of menarche (Adair 2001; Ruth et al. 2016) and reproductive aging (Tom et al. 2010). Body Mass Index (BMI) predisposes to a range of later life diseases (Locke et al. 2015) and adiposity genetic loci have been associated with the timing of menarche onset (Fernández-Rhodes et al. 2013). In addition observational studies have linked waist-to-hip ratio and BMI to an earlier onset of menarche (Bratke et al. 2017; Żurawiecka and Wronka 2020).

A higher Waist-to-hip ratio or central adiposity has been shown to increase mortality risk in older individuals (60-69 years) who otherwise have a normal weight (Hazard ratio=1.41; 95% CI=1.25-1.61)(Bowman et al. 2017). MR analysis of waist-hip ratio has previously associated this trait with increased risk of both Type 2 diabetes and coronary heart disease (Emdin et al. 2017) in the UK Biobank cohort. The European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct case-cohort study found an association between earlier age of menarche and a higher risk of type 2 diabetes, this association was only partially explained by the known association

between BMI and type 2 diabetes (42% increase risk independent of BMI) (Elks et al. 2013).

This does suggest that muscle weakness in older people shares a causal pathway with these life-course traits, and metabolic factors may underlie at least part of the multifactorial nature of sarcopenia and muscle weakness with age.

Other associations found by the MR analysis include immune system traits and autoimmune diseases. Rheumatoid arthritis (Smolen, Aletaha, and McInnes 2016), as well as Asthma and allergic disease (Han, Krempski, and Nadeau 2020) have a basis in the dysregulation of the immune system (Ludwig et al. 2017). In addition traits such as raised platelet to lymphocyte ratio have been linked to inflammation and sarcopenia in community-dwelling older people (Liaw et al. 2017). This does provide evidence for a role of immuno-senescence and “inflammaging” in the underlying pathways resulting in muscle weakness in older people, and in turn sarcopenia.

7.5.2 Future Directions

Further testing of significant associations for robustness, the effect of heterogeneity outliers could be investigated using software such as Radial MR (Bowden et al. 2018), which can indicate if directional pleiotropy is present by calculating a suitable Q statistic. Analysis of the factors / biomarkers that have been identified in independent ageing cohorts with a longer follow up period, could provide an indication of the prognostic value in individuals who later become sarcopenic.

7.6 Summary

With an ageing world population, it becomes increasingly important to effectively diagnose and treat diseases associated with musculoskeletal ageing, such as sarcopenia.

My analysis of HLA types and their association with different definitions and components of sarcopenia has shown that there are specific risk haplotypes for muscle weakness in older people. By investigating low skeletal muscle mass and handgrip strength separately I was able to provide evidence that different HLA types are associated with each component. When these are looked at together, as a combined definition of sarcopenia, HLA types associated with low grip strength predominate. In addition, I was also able to show that specific HLA types in combination can result in 27% increased likelihood of sarcopenia. This suggests that a lifetime exposure to low level pro-inflammatory state contributes to the risk of developing sarcopenia in later life.

The results from my meta-analysis of Genome Wide Association Studies (GWAS) for low grip strength of 22 cohorts from the CHARGE consortium provided fifteen genomic risk loci. The majority of these have not previously been associated with continuous measures of grip strength in previous studies, which provides evidence that these loci are specific to low grip strength in older people.

The analysis of candidate genes associated with the genomic risk loci shows that there are a number of separate pathways involved, with previous literature on ageing mechanisms providing supporting evidence for potential pathways of pathogenesis.

The sex-specific analysis of low grip strength in older people highlighted the differences in genomic risk loci found to be significantly associated with the low grip definitions for each sex.

Although the female only analysis overlapped almost completely with the previous combined analysis, there were far fewer risk loci associated with low grip strength in the male only cohort. The presence of two unique loci for the male only analysis (*DSCAM* and *MIR466* / *GADL1*) and one for the female only analysis (*PKD1*) does allow a tentative conclusion that different pathways are casually attributable to low grip strength in older people, for each sex. This is further reinforced by the lack of lack of overlap in genomic risk loci between the combined results and the male only results.

Mendelian randomization has allowed me to investigate the casual pathways associated with low grip strength and provides further evidence of the multifactorial nature of the underlying mechanisms at work in the development of low grip strength in older people.

The genomic risk loci identified by the study do provide potential targets for treatment, in the same way that the *ADAMTS7* loci has been identified in Coronary Artery Disease (CAD) as a therapeutic target (Müller et al. 2016; Reilly et al. 2011). Pathways and traits associated through Mendelian randomization, such as Type 2 Diabetes, also provide possible routes for intervention (Massimino et al. 2021). In addition, events earlier in the life-course such as birth weight and age of menarche provide critical periods where observation and possible intervention may provide alleviation of later life diseases, such as sarcopenia (Mikkelsen et al. 2019).

7.7 Limitations

7.7.1 Limitations of UK Biobank

The UK Biobank constitutes the largest cohort in the meta-analysis, as well as the only data source for the analysis of HLA types and sarcopenia. UK biobank is known to have a healthy volunteer selection bias (Fry et al. 2017) (discussed in detail in Methods – chapter 2), which along with the young average age (approximately 64 years for the HLA analysis in Chapter 3) for the onset of sarcopenia and age-related reduction in muscle function, would reduce the number of cases and although this distorts the data with regards to the general UK population, it does mean a lower likelihood of false-positives.

In addition the underlying population structure within the UK Biobank genotyping data can also introduce bias into any analysis (Haworth et al. 2019). A recent study by Haworth et al, found that polygenic scores for traits such as BMI and height were influenced by geographic location of birth of UK Biobank participants (Haworth et al. 2019). However an analysis of birth location did not find maximum grip strength associated with birth location, unlike a number of traits such as walking pace, body mass index and various measures of limb/body fat (Cook, Mahajan, and Morris 2020). A follow up study on whether birth location is associated with the low grip cut-off of sarcopenia would provide evidence for any bias due to population structure.

7.7.2 Limitations of meta-analysis datasets

Despite the relatively large sample size for the GWAS meta-analysis, we have restricted the analysis to a single ethnicity – European ancestry. Although initially I had hoped to include a secondary analysis with contributors from CHARGE cohorts with

mainly Asian ancestry, the available number of cases was not enough to undertake the analysis. Future work may include the use of the multi-ethnic biobanks with a larger proportion of participants from other populations, although some possible candidates such as the NIH 'All of us' biobank does not include the measurements required (grip strength) (All of Us Research Program Investigators et al. 2019).

The age range of the studies included in the meta-analysis was fairly young for the onset of sarcopenia and so analysis of a cohort with older ages would provide a better representation of the more common onset age of sarcopenia.

7.7.3 Limitations of microarrays

As noted in the Methods – Chapter 2, microarray technology has limitations when used to analyse alleles whose minor version is present in the study population at lower frequencies (minor allele frequency less than 1%) (Weedon et al. 2019). The UK Biobank has recently started to release Exome sequencing data which could be used instead of microarray data in order to investigate rarer alleles.

In addition there are other types of genomic variation that can be detected by SNP array technology, such as structural and copy number variant, that were not investigated for their association with the traits in question. Although these could be detected using the UK Biobank data and suitable methods, this was not possible for the various CHARGE consortium studies without asking the analysts for each study to undertake this additional methodology. Given the time constraints on the meta-analysis this was not performed, however previous literature has shown how these larger variants have been associated with anthropometric traits (Macé et al. 2017) and so future analysis with a large meta-analysis may be informative.

7.7.4 Limitations of sarcopenia definitions

In the Introduction – Chapter 1, I discussed the various definitions for sarcopenia. The definitions are derived from measures that are specific to certain populations, for example the European Working Group on Sarcopenia (EWGSOP) used data from people of European ancestry. However often the baseline population used to calculate the cut-offs has a low sample size. For example the EWGSOP low grip definition (2010) was defined based on 2 standard deviations below the mean based measured in 1,030 (469 mean and 561 women) participants from the InCHIANTI study (Lauretani et al. 2003).

With both the population specific nature and small sample size of the initial dataset used in the definition, bias is possible. I have tried to account for this by restricting the analysis to similar populations as in the original definition, community based participants of European ancestry.

7.7.5 Limitations of sex-specific analysis

Sample sizes for each sex-specific GWAS meta-analysis (135,468 females and 121,055 males) is by definition smaller than the combined analysis (256,523 participants). The number of cases in for each of the sex-specific analysis was imbalanced with 25.5% (34,589) cases in the female cohort, compared to 11.6% (14,007) cases in the male cohort despite the EWGSOP sarcopenia definition taking into account sex. The most likely reason could be the lower ages of participants in the study compared to the age of participants for the sarcopenia definitions.

The sex-specific analysis, due to being a sub-set of the original meta-analysis, only used data from the autosomes (chromosomes 1-22) and the directly genotyped data for mitochondria. The allosomes, or sex-chromosomes were not analysed as genotyping data for the sex-chromosomes was not available for many of the CHARGE studies collaborating in the meta-analysis, and future work on these may provide additional genomic risk loci unique to each sex.

7.8 Conclusion

The progressive loss of skeletal muscle function and mass is a common result of the ageing process in humans, leading to deliberating states such as sarcopenia and frailty. There have been a number of proposed underlying mechanisms for this loss of musclo-skeletal function and my thesis has provided evidence for the contribution of genes involved in the immune system, unfolded protein response, cell cycle control, transcription regulation, as well as the growth and development pathways. In addition I have highlighted that there are different pathways contributing to muscle weakness with age, for each sex.

My analysis of the Human Leukocyte Alleles associated with sarcopenia provided evidence of that autoimmune pathways are contributing to clinical weakness in older people, with the difference between the two phenotypes of muscle mass and function providing two separate sets of risk alleles. Results from the investigation also showed that combinations of certain HLA alleles could increase an individual's risk for sarcopenia by up to 23%.

While investigating the casual pathways, I found that metabolic disease, such as diabetes and autoimmune conditions such as rheumatoid arthritis and allergic disease, had an association with muscle weakness with age.

In addition there were well characterised differences between the casual pathways between the sexes. Muscle weakness in older women being influenced by life course traits such as age of the onset of menarche and birth weight, whereas in older men there was a relationship with measures of blood cell volume and concentration. Further analysis of shared genetic correlation between muscle weakness with age and a

number of common diseases, also found the relationship to diabetes and rheumatoid arthritis but also coronary heart disease and osteoarthritis. There was also substantial genetic overlap with anthropometric traits like waist to hip ratio and height.

Taken together my thesis gives insight into the mechanisms involved in muscle weakness in later life. This provide plausible targets for intervention as well as genetic markers to help characterise individual risk of muscle weakness with age.

The association between certain HLA types and sarcopenia could provide treatment opportunities mediated through anti-inflammatory mechanisms, or through addressing the loss of immune tolerance and resulting autoimmune response.

8 Appendix

Supplementary methods for Chapters 4, 5 & 6. Originally published as part of (G. Jones et al. 2021). Abridged version, LocusZoom plots courtesy of Dr. Luke Pilling.

8.1 Supplementary Methods

8.1.1 Cohorts

22 studies with a total of 254,894 participants of European ancestries and age 60 or older were included in the analysis low grip strength. See Supplementary Table 8-1 for number of cases/controls in each cohort. Here follows individual study descriptions:

8.1.2 Atherosclerosis Risk in Communities (ARIC) study

The Atherosclerosis Risk in Communities (ARIC) Study is a population-based prospective cohort study of cardiovascular disease and includes a total of 15,792 participants aged 45-64 years at baseline (1987-89), chosen by probability sampling from four US communities(The ARIC investigators 1989). Cohort members completed five clinic examinations, conducted approximately three years apart between 1987 and 1998, with a fifth visit conducted from 2011 – 2013. Clinic examinations included assessment of cardiovascular risk factors, self-reported medical family history, employment and educational status, diet, physical activity, comorbidity, clinical and laboratory measurements. The present analyses were restricted to participants of European descent and utilized measurements from the Visit 5 grip strength assessment. Grip strength in kilograms of force was assessed using Jamar Hydraulic Hand Dynamometer in the participant's preferred hand (usually the dominant). The better of two trials was used for the dynapenia definitions and subsequent association analyses.

Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA). Exclusions at the individual level included removing first-degree relatives, ancestry outliers, samples with low call rate (<95%) or unexpected duplicates, and samples with a mismatch between called and phenotypic sex. SNPs were excluded due to having a low call rate for the combined sample (<95%), no chromosome location, being monomorphic in both the European and African descended genotyped individuals, and having an HWE p-value <10-6. Before imputation, additional quality control was completed using the "HRC/1KG Imputation Preparation and Checking Tool" developed by Will Rayner (version 4.2.5). SNPs with incorrect strand or reference/alternate allele designations were fixed accordingly and further SNPs were excluded due to mismatches with the 1000 Genomes reference panel, having allele frequency differences >0.2 from the European 1000 Genomes data, and being palindromic with frequencies >0.4. A total of 752,325 SNPs were submitted for imputation on the Michigan Imputation Server using the Haplotype Reference Consortium (HRC) r1.1 2016 reference panel with the following options chosen: phasing, Eagle v2.3; population, EUR (for quality control purposes); mode, Quality Control & Imputation.

After filtering for age, phenotype, genotype availability and removing all related individuals, 3655 individuals remained, with a minimum age of 66 and a maximum of 90. The number of cases and controls for each analysis/dynapenia (low grip strength due to ageing) definition is as follows: for FNIH, 351 cases and 3,304 controls; for EWGSOP, 939 cases and 2,716 controls. Logistic regression analyses were conducted using the '--logistic-snp' option on FAST (version 2.4)(Chanda et al. 2013) with imputed continuous dosage data. Covariates for adjustment included age, sex

(except in the sex-stratified analyses), study site and PCs 1-4. Only SNPs with a MAF ≥0.02 were included in association analyses.

8.1.3 Berlin Aging Study II (BASE-II)

BASE-II is a multidisciplinary study initiated in 2009 investigating factors related to human aging (Bertram et al. 2014). All subjects are recruited from the Berlin metropolitan area and underwent an extensive phenotypic assessment, including a 2-day internal medicine examination (follow-up will be completed in 2020). The BASE-II research project (Co-PIs are Lars Bertram, Ilja Demuth, Denis Gerstorf, Ulman Lindenberger, Graham Pawelec, Elisabeth Steinhagen-Thiessen, and Gert G. Wagner) is supported by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF) under grant numbers #16SV5536K, #16SV5537, #16SV5538, #16SV5837, #01UW0808, 01GL1716A and 01GL1716B. Another source of funding is the Max Planck Institute for Human Development, Berlin, Germany. Additional contributions (e.g., equipment, logistics, personnel) are made from each of the other participating sites.

Genome-wide SNP genotyping was performed using the "Genome-Wide Human SNP Array 6.0" [Affymetrix, Inc]) on the full BASE-II dataset (Bertram et al. 2014). For full QC and imputation procedures see (REF: PMID 26821332). Briefly, genome-wide imputation of unobserved genotypes was carried out on the QC'ed data using IMPUTE2 v2.2.2 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html) based on precompiled 'ALL 1000G Phase1 integrated haplotypes' reference panels (December 2013 release) [14]. A total of 27,213,648 SNPs were imputed, but only autosomal SNPs with an IMPUTE info value ≥0.35 and minor allele frequencies ≥1% were

retained for subsequent analyses. Overall, genotype and hand grip strength data were available in 1,531 unrelated (i.e. IBD/IBS sharing >12.5% using –genome in PLINK1.9) BASE-II participants aged 60+. To calculate PCs to be used as co-variates in the genome-wide analyses, LD pruning was applied to pre-imputation genotype data using PLINK (--indep-pairwise 1500 150 0.2) resulting in 117835 SNPs. Eigenvalues and eigenvectors were then calculated using PLINK (–pca). Based on the resulting scree plot, we selected the first 10 PCs as covariates. For the actual GWAS we used SNPTEST (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html) v2.5.4-beta3 (-frequentist 1 -method score -cov_names PC1 PC2 PC3 PC4 PC5 PC6 PC7 PC8 PC9 PC10 age -cov_names sex (for combined phenotypes only)).

8.1.4 B-Vitamins for the PRevention Of Osteoporotic Fractures (BPROOF) study

The B-PROOF study is a randomized, placebo-controlled, double-blind trial that studied the effect of vitamin B12 and folic acid supplementation on osteoporotic fractures in 2,919 people of 65 years or over, and having homocysteine levels of 12-50 µmol/L. Participants were recruited between 2008 and 2011 and followed during a 2-3 year period (J.P. et al. 2011). Genotyping was done by using the Illumina Omni-express array. Imputations to HRC1.1 were performed using the Michigan Imputation Server with standard settings. The GWAS analyses were performed using rvtest.

8.1.5 Cardiovascular Health Study (CHS)

The Cardiovascular Health Study is a population-based, prospective cohort study of risk factors for development and progression of CHD and stroke in older adults ages

65 years or older (Linda P. Fried et al. 1991). A cohort of 5,201 non-institutionalized men and women were selected and enrolled from randomly generated Medicare eligibility lists in 4 U.S. communities in 1989-90; an additional 687 predominantly African American participants were recruited and enrolled in 1992-93. Clinic examinations were performed at study baseline, at annual visits through 1999, and again in 2005-2006. Participants were contact by telephone annually between exams, and every 6 months after the exams ended. Multiple physical and biological tests have been performed, including assessment of physical function. The current analysis included 3,061 CHS participants of European ancestry for whom genotype and grip strength data were available.

Blood samples were drawn from all participants at their baseline examination and DNA was subsequently extracted from available samples. Genotyping was performed at the General Clinical Research Center's Phenotyping/Genotyping Laboratory at Cedars-Sinai among CHS participants who consented to genetic testing and had DNA available using the Illumina 370CNV BeadChip system. CHS was approved by institutional review committees at each field center and individuals in the present analysis had available DNA and gave informed consent including consent to use of genetic information.

Imputation to the HRC r1.1 2016 panel was performed on the Michigan imputation server. SNPs were excluded for variance on the allele dosage ≤0.01. The GWAS was conducted using a custom program written in R. Logistic regression models adjusted for age, sex (for the combined analyses), field center, and principal components were fit.

8.1.6 European Prospective Investigation of Cancer (EPIC) Norfolk

The European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study (https://dx.doi.org/10.22025/2019.10.105.00004) is a prospective population-based cohort study which recruited 25,639 men and women aged 40-79 years at baseline between 1993 and 1998 from 35 participating general practices in Norfolk, UK. Individuals attended for a baseline health check including the provision of blood samples for concurrent and future analysis. Further health check visits have been conducted since the baseline visit. Participants have contributed information about their diet, lifestyle and health through questionnaires and health checks over two decades. The Norwich Local Research Ethics Committee granted ethical approval for the study. All participants gave written informed consent. Grip strength measurement was taken at the third (2004-2006), the fourth (2012-2016) and the fifth (2016-2018) health checks. To maximum the sample size, we take grip and related measures from a late health check visit if it is unavailable at previous visit. Related samples, ethnic outliers and participants whose age was less than 60 years old were excluded from further analysis.

DNA has been extracted from all EPIC participants and stored blood has been analysed for an extensive range of classical and novel biomarkers. Samples were genotyped using Affymetrix Axiom array at Cambridge Genomic Services, Department of Pathology, University of Cambridge, UK, and samples were excluded if lower call rate (<95%), or heterozygosity outliers, or gender mismatch, or failed channel contrast (DishQC <0.82), or unusually high number of singleton genotypes, or impossible IBD values. SNPs were removed if lower call rate (<95%), or MAF=0, or failed HWE ($p<10^{-6}$), or clusters failed Affymetrix SNPolisher standard tests and thresholds, or MAF significantly affected by plate, or duplicates or unflippable. A total of 21044 samples

were forwarded to imputation using HRC r1 reference panel, and using UK10K+1000Gp3 reference panel for additional SNPs.

GWAS analysis was performed on 7508 participants using SNPTEST v2.5.4-beta3 (-method newml) under an additive model with covariates age, sex, and the first 10 principal components.

8.1.7 Framingham Heart Study

The Framingham Heart Study (FHS) is a single-site, community-based cohort study that was initiated in 1948 to investigate risk factors for cardiovascular disease and other major illnesses. The FHS includes three generations: the original cohort followed since 1948 (Gen1) (DAWBER and KANNEL 1966); their offspring and spouses of the offspring, followed since 1971 (Offspring or Gen2) (Feinleib et al. 1975); and children of the offspring enrolled in 2002 (Third Generation or Gen3) (Splansky et al. 2007). The Original cohort enrolled 5,209 men and women who comprised two-thirds of the adult population then residing in Framingham, MA, USA. The Offspring cohort comprises 5,124 persons who have been examined about every 4 to 8 years. Examination 1 of the Gen3 occurred between 2002 and 2005 and involved 4,095 participants. Offspring spouses not previously enrolled who were a biological parent of a Gen 3 participant were enrolled into the New Offspring Spouse cohort to complete family pedigrees. All cohorts continue under active surveillance. The FHS follows two multi-ethnic cohorts, Omni group 1 and Omni group 2 to reflect the current diversity of the town of Framingham, MA.

In the 1990s and early 2000s, DNA samples were collected in the Original, Offspring, Third Generation, and Offspring Spouse cohorts of the FHS. All individuals provided consent for genotyping. NHLBI funded genotyping using 550,000 SNPs (Affymetrix 500K mapping array plus Affymetrix 50K supplemental array). Imputations were performed with miniMACH3 using the HRC release 1.1 reference panel for SNPs passing the following criteria: call rate ≥ 97%, pHWE ≥ 10-6, < 100 Mendel errors, and MAF ≥ 1%.

Grip strength was recorded by trained technicians at the time of Offspring exam 7, exam 8 and exam 9 and Gen 3 exam 2, using a Jamar dynamometer. The maximum of six trials (three trial in each hand) was used. For participants with hand grip data at more than one examination, the first exam the individual was age 60 or older was used for analysis. The Boston University Medical Campus IRB approved the content of all examinations and participant provided informed consent at all attended exam.

8.1.8 Health and Retirement Study (HRS)

The Health Retirement Study is representative American cohort with participants over age 50 to monitor factors related to aging and retirement (Fisher and Ryan 2018; Juster and Suzman 1995). A random subset of ~26,000 participants were selected in three phases (phase 1 in 2006, phase 2 in 2008, and phase 3 in 2010) to collect biological specimen between 2006 and 2010. The DNA samples were genotyped at the Center for Inherited Disease Research (CIDR) on Illumina HumanOmni2.5 array, Phases 1-2 on HumanOmni2.5-4v1 and Phase 3 on HumanOmni2.5-8v1. Both arrays are designed to human genome build 37. Imputation analyses were performed using IMPUTE version 2.3 (B. Howie et al. 2012).

Genotyping data were available for 15,708 samples (6519 males and 9189 females) in the combined Phase 1-3 dataset. 15,454 remained after filtering related or duplicated samples, genotyping controls, and those with a missing call rate ≥ 2%. 12,940 (84%) were Whites. Of which, 10,919 reached 60 years old. The grip strength was measured using a medley spring-type hand dynamometer. Two measurements were taken from each hand and the maximum at the most recent visit was used to conduct association analyses. Grip strength ≥ 90 kg was considered unreal and set to missing based on actual data distribution. Dynapenia was determined based on the EWGSOP or FNIH definition. 10,814 white participant (4650 males and 6164 females) had both genotyping and grip strength data. 3624 (34%) and 1754 (16%) met the EWGSOP and FNIH criteria, respectively. Women were more likely to have dynapenia than men by the EWGSOP (39% vs. 27%) or FNIH (18% vs. 14%).

In genome-wide association analyses, the EWGSOP or FNIH dynapenia (low grip strength cut-off) was associated with SNPs, one at a time, in a linear mixed effects model, including additive allelic effect of the candidate SNP, sex (if not sex-specific), age at measurement, plus random polygenic and environment effects. The analyses were conducted using the BOLT-LMM version 2.2 (P.-R. Loh et al. 2015), with precomputed LD scores from Europeans in the 1000 Genomes.

8.1.9 InCHIANTI study

The InCHIANTI study is a population-based epidemiological study aimed at evaluating the factors that influence mobility in the older population living in the Chianti region in Tuscany, Italy. The details of the study have been previously reported (Ferrucci et al. 2000). Briefly, 1616 residents were selected from the population registry of Greve in

Chianti (a rural area: 11,709 residents with 19.3% of the population greater than 65 years of age), and Bagno a Ripoli (Antella village near Florence; 4,704 inhabitants, with 20.3% greater than 65 years of age). The participation rate was 90% (n=1453), and the subjects ranged between 21-102 years of age. Overnight fasted blood samples were for genomic DNA extraction. Maximal isometric hand grip strength was measured in kilograms using a hand-held dynamometer (Smith & Nephew, Agrate Brianza, Milan, Italy). The subject was seated in front of a bench with the tested arm supported on the bench and the elbow flexed to 45°. Participants were asked to perform the task twice with each hand, and the maximum strength attained during the four trials was used for the present analyses. Dynapenia was determined using the FNIH criteria and EWGSOP criteria. The study protocol was approved by the Italian National Institute of Research and Care of Aging Institutional Review and Internal Review Board of the National Institute for Environmental Health Sciences (NIEHS). All participants provided written informed consent.

Genome-wide genotyping was conducted using Illumina Infinium HumanHap 550K SNP arrays (Melzer et al. 2008). Genotyping was completed for 1210 subjects with a sample call rate >97%, heterozygosity rates > 0.3 and correct sex specification. 495,343 autosomal SNPs that passed quality control (MAF>1%, completeness >99%, HWE > 10-4) were used for imputation using as reference the Haplotype Reference Consortium (HRC) panel, version 1.1. The imputation was done using Minimac, and the process was facilitated by the Michigan Imputation server (Das et al. 2016). Associations between grip strength and SNP dosages was performed using logistic regression in PLINK 1.9, adjusting for age, sex (except sex-specific analyses), study site, and 10 principal components.

8.1.10 LASA: Longitudinal Aging Study Amsterdam

Longitudinal Aging Study Amsterdam (LASA) is an ongoing, population-based cohort of individuals 55 years and older living in the Netherlands. The design and rationale is described elsewhere (Hoogendijk et al. 2016; Huisman et al. 2011). In short, 3017 participants (55-84 years old) were included at baseline (1992-1993) and two additional cohorts were added in 2002-2003 and 2012-2013 with respectively 1002 and 1023 participants. Follow-up visits were conducted every 3 years. Trained interviewers collected data on cognitive, emotional, physical and social functioning during a home interview. Subsequently, all participants were invited for a medical interview during which further diagnostic examinations were done and blood samples were drawn. LASA has been approved by the Medical Ethics Committee of VU University Medical Center. All participants gave written informed consent.

DNA was isolated from buffy coats or full blood samples drawn at baseline. Genotyping was done using two arrays: Axiom-NL Array (Affymetrix Inc, Santa Clara, CA., USA) and Infinium Global Screening Array (GSA) (Illumina Inc, San Diego, CA., USA). Quality control (QC) was done separately for each array using Ricopili (Rapid Imputation for COnsortias PIpeLIne for GWAS), an established tool developed by the Psychiatric Genomics Consortium (Lam et al. 2019). After QC, the data was imputed using as reference the Haplotype Reference Consortium (HRC) panel, version 1.1 (McCarthy, Das, Kretzschmar, Delaneau, Wood, Haplotype Reference Consortium, et al. 2016). The imputation was done using Minimac 3, and the process was facilitated by the Michigan Imputation server (Das et al. 2016).

Grip strength was measured during the medical interview using a dynamometer (Takei TKK 5001, Takei Scientific Instruments Co. Ltd., Tokyo, Japan) and it was recorded in the nearest 1 kg. Dynapenia was determined using the FNIH criteria and EWGSOP criteria.

The analyses were performed separately per genotyping array and were adjusted for age, sex (except sex-specific analyses), 10 principal components and cohort (for the GSA array). Logistic regression in Plink v.1.9 was used.

8.1.11 Long-Life Family Study

The Long Life Family Study is a multi- centre international study on the genetics and familial components of exceptional survival. Between 2006 to 2009, LLFS successfully enrolled and rigorously phenotyped probands, their siblings, and children of families demonstrating exceptional survival (4,953 individuals from 539 families; N=1727 probands; 3226 offspring). LLFS includes: Field centres at Boston University, Columbia University, University of Pittsburgh, and University of Southern Denmark, at Laboratory Core (University of Minnesota) and a Coordinating Center (Washington University in St. Louis). In brief, the LLFS recruited selected families with multiple exceptionally old living individuals, the probands were ≥79 years old in the USA, and ≥90 years old in Denmark. Families were selected to participate in the study based on The Family Longevity Selection Score (FLoSS) (Sebastiani et al. 2009) which calculated the rank sibships by current age or age at death of siblings, the size of the sibship and the number of alive individuals available for study. A proband's family was eligible if the FLoSS reached a score of 7 or higher, which met the following criteria: (1) the proband, at least one living sibling, and one of their living offspring (minimum

family size of 3) were all able to give informed consent, and (2) were willing to participate in the interview and examination including the blood sample for serum and DNA extraction. The age of the 3359 participants who were aged 60 years and older with valid grip strength and genotyped data in the current analyses was: 77.6 ± 12.9 (range: 60-110).

Grip strength was measured using a JAMAR® hand-held dynamometer (Sammos Preston Rolyan, Bolingbrook, IL). Two trials for each were conducted, and the maximum value for the strongest hand was used in analyses.

Genotype data were produced on 4,716 of the consented LLFS subjects using the Illumina 2.5 million HumanOmni array, with the purpose of utilizing them in genome-wide association (GWA) analyses. Genotypes were called using Bead Studio. LLFS QC of the genotypes included using the package GRR (Graphical Representation of Relationships)(Abecasis et al. 2001) to check familial relationships and sample switches based on Identity-by-State genotypes. Corrections to familial relationships were made as warranted by the data. After GRR, additional QC included removing 18 samples with autosomal call rates less than 97.5%, leaving 4,693 (4,597 with EU ancestry) subjects for further analysis. To determine and exclude outliers with respect to ancestral population, we generated principle components (PCs) with smartpca2016 (Price et al. 2006) from 112,639 Tag SNPs in 4,597 (of 4,693) LLFS samples of European Ancestry and included 2,504 haplotypes from 1000 genomes Cosmopolitan panel to support ancestral clustering. 18 additional individuals of the 4,597 samples with EU ancestry were identified as PCs outliers and removed prior to imputation. Further, we calculated sample heterozygosity and removed 5 outliers (3 overlap in

PCA outliers) with excess heterozygosity, i.e. heterozygosity > median + 3*IQR, thereby leaving 4,577 samples for imputation.

SNP QC included using Loki (Heath 1997) to identify all Mendel errors in SNPs among families. 3,647 SNPs were identified as outliers with respect to total number of detected Mendelian errors and were excluded. All other genotype calls resulting in Mendel errors were set to missing, which occurred 153,363 times in our data. SNPs with a call rate lower than 98% were also excluded (n=83,774; 1,188 of these were also Mendelian outlier SNPs). Applying both the call rate and Mendelian error criteria, 86,233 autosomal SNPs were removed, leaving 2,225,478 SNPs in the cleaned genotyped data passing QC criteria.

From the SNPs that passed LLFS QC procedures, we applied the following criteria, per the MI server, to ensure the highest quality imputation. The imputation 'scaffold' for the MI server included SNPs from our cleaned genotyped SNPs (n=2,225,478) which met the following additional criteria: 1) Hardy-Weinberg equilibrium (p>=1E-06); 2) No allele mismatch when compared with 1000HG; 3) No position mismatch when compared with 1000HG (e.g. assembly GRCh37); 4) No association with known inversions between GRCh37 and GRCh38 (identified by order reversal between rs-named SNPs in b138 and b144 from NCBI annotation downloads); and 5) Monomorphic markers.

The cleaned binary files (one for each chromosome) were then uploaded to the imputation server for pre-phasing and imputation in VCF format (after bgzip and tabix in the UNIX environment). The final number of clean autosomal SNPs used for imputation was 1,559,272. Autosomal Imputation was performed on the Michigan Imputation Server (https://imputationserver.sph.umich.edu) by chromosome (e.g. 22

simultaneous jobs). The reference panel used was HRC r1.1 2016, phasing was performed with Eagle v2.3, population was set to EUR., and the mode option employed quality control and imputation.

GWAS was done using R package GENESIS via Bioconductor. The protocol in GENESIS is to first fit a null model without genotype data, which adjusts the outcome based on the covariates, principal components, and family correlations (GENESIS function 'fitNullModel'). Genetic relatedness matrix is established using sample of independent genotyped SNPs, then specified as the covariance matrix in the mixed model. Population stratification is adjusted for by a set of principal components specific to the outcome, which are derived from the same sample of independent SNPs. This data is then used in SNP association tests (GENESIS function 'assocTestSingle'), so that the mixed models only need to be run once. Tests were adjusted for Age, sex (if combined sex analysis), field centre, principal components (for population stratification). Betas, SEs, and Odds ratios were separately calculated from the Score and Score SE statistics output by GENESIS using the following equations: (https://support.bioconductor.org/p/119621/)

(1) $Beta = \frac{Score}{Score.SE^2}$

(2) $SE = \frac{1}{Score.SE}$

(3) $OR = \exp(Beta)$

8.1.12 MrOS (Osteoporotic Fractures in Men) study

The Osteoporotic Fractures in Men (MrOS) study is a multicenter, prospective study including older men in Sweden, Hong Kong and the United States. The MrOS Sweden study (n=3014) consists of three sub-cohorts from three different Swedish cities (n=1005 in Malmö, n=1010 in Gothenburg, and n=999 in Uppsala) (Mellström et al. 2006). Study subjects (men aged 69 to 81 years) were randomly identified using national population registers. A total of 45% of the subjects who were contacted participated in the study. To be eligible for the study, the subjects had to be able to walk without assistance, provide self-reported data, and sign an informed consent. The study was approved by the ethics committees at the Universities of Gothenburg, Lund, and Uppsala. Informed consent was obtained from all study participants. In this study 941 unrelated participants from Gothenburg and 891 unrelated participants from Malmö were included.

A Jamar® hydraulic hand dynamometer (5030J1, Jackson, MI, USA), with adjustable handgrip, was used in the grip strength test. Participants were made to sit in a standard chair with the arm resting on a moveable table with the dynamometer in an upright position. Two trials were performed on each hand. The better of the two results (presented as kilograms of force) was used in the analyses. Grip strength was not measured if the subject had current arthritis or pain in the wrist or hand or had undergone fusion, arthroplasty, tendon repair, synovectomy or related surgery of the upper extremity in the 3 months preceding the test. The coefficient of variation was 0.5%.

Genotyping, imputation and quality controls of MrOS Gothenburg were performed using the Illumina HumanOmni1_Quad_v1-0 B array. Genotypes were called using the Illumina's BeadStudio calling algorithm. The sample quality control exclusion criteria were sample call rate < 97%, excessive autosomal heterozygozity, first and second degree relatives, genotypic sex mismatch using X and Y chromosome probe intensities and gross chromosome abnormalities. Genotyped SNPs with GenTrain scores <0.6, cluster separation scores <0.4, call rates <97%, or MAF <0.01 were excluded. Also, autosomal SNPs with Hardy-Weinberg Equilibrium P-value <10-4 were excluded and genotype clusters for SNPs on chrX, chrY, chrXY and chrMT were reviewed manually. 714543 autosomal SNPs passed quality control.

Genotyping, imputation and quality controls of MrOS Malmö were performed using the HumanOmniExpress-12v1_B build 36. The sample quality control exclusion criteria were sample call rate < 97.5%, missing data, excessive autosomal heterozygozity, familiar relationship (one sample excluded), genotypic sex mismatch, non-caucasians and gross chromosome abnormalities. SNPs with call rates<95% were excluded. 725409 autosomal SNPs passed quality control.

The genotype data for both MrOS Gothenburg and MrOS Malmö were pre-phased first without a reference panel, using SHAPEIT2. The imputation was done using Sanger Imputation Service to the Haplotype Reference Consortium release 1.1.

Associations between grip strength and SNP dosages were obtained using logistic regression in PLINK 1.9, adjusting for age.

8.1.13 ROSMAP: the Religious Orders Study (ROS) and Memory and Aging Project (MAP)

The ROS, started in 1994, enrolled Catholic priests, nuns, and brothers, from about 40 groups in 12 states (A. Bennett et al. 2012). The follow-up rate of survivors exceeds 90%. Participants were free of known dementia at enrollment, agreed to annual clinical evaluations, and signed both an informed consent and an Anatomic Gift Act form donating their brains at time of death. Participants take a neuropsychological test battery. DNA was extracted from whole blood, lymphocytes, or frozen post-mortem brain tissue. Genotyping was performed at the Broad Institute's Center for Genotyping and the Translational Genomics Research Institute and the Children's Hospital of Philadelphia.

The Rush Memory and Aging Project, started in 1997, enrolled older men and women from assisted living facilities in the Chicago area with no evidence on dementia at baseline (A. Bennett et al. 2012). The follow-up rate of survivors exceeds 90%. Participants agreed to annual clinical evaluations, and signed both an informed consent and an Anatomic Gift Act form donating their brains at time of death. Participants were invited to take a neuropsychological test battery. DNA was extracted from whole blood, lymphocytes, or frozen postmortem brain tissue. Genotyping was performed at the Broad Institute's Center for Genotyping and the Translational Genomics Research Institute and the Children's Hospital of Philadelphia.

8.1.14 Rotterdam Study

The Rotterdam Study is an ongoing prospective population-based cohort that investigates occurrence, determinants, and consequences of diseases in an ageing population (Ikram et al. 2017). The first baseline measurement of Rotterdam Study started in 1990. After two expansions in 2000 and 2006, it comprised 14,926 participants aged 45 years and over by the end of 2008. Follow-up visits were held every 3-5 years. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians. Imputations to HRC1.1 were performed using the Michigan Imputation Server with standard settings (McCarthy, Das, Kretzschmar, Delaneau, Wood, Marchini, et al. 2016; Das et al. 2016).

8.1.15 Study of Health in Pomerania (SHIP)

The Study of Health in Pomerania (SHIP) is a population-based project in West Pomerania, the north-east area of Germany (Volzke et al. 2011). A sample from the population aged 20 to 79 years was drawn from population registries. First, the three cities of the region (with 17,076 to 65,977 inhabitants) and the 12 towns (with 1,516 to 3,044 inhabitants) were selected, and then 17 out of 97 smaller towns (with less than 1,500 inhabitants), were drawn at random. Second, from each of the selected communities, subjects were drawn at random, proportional to the population size of each community and stratified by age and gender. Only individuals with German citizenship and main residency in the study area were included. Finally, 7,008 subjects

were sampled, with 292 persons of each gender in each of the twelve five-year age strata. In order to minimize drop-outs by migration or death, subjects were selected in two waves. The net sample (without migrated or deceased persons) comprised 6,267 eligible subjects. Selected persons received a maximum of three written invitations. In case of non-response, letters were followed by a phone call or by home visits if contact by phone was not possible. The SHIP population finally comprised 4,308 participants (corresponding to a final response of 68.8%). The medical ethics committee of the University of Greifswald approved the study protocol, and oral and written informed consents were obtained from each of the study participants. Handgrip strength was assessed in the second five-year follow-up of the study (SHIP-2) and used for this project.

Non-fasting blood samples were drawn from the cubital vein in the supine position. The samples were taken between 07:00 AM and 04:00 PM, and serum aliquots were prepared for immediate analysis and for storage at -80 °C in the Integrated Research Biobank (Liconic, Liechtenstein). The SHIP samples were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0. Hybridisation of genomic DNA was done in accordance with the manufacturer's standard recommendations. Genetic data were stored using the database Caché (InterSystems). Genotypes were determined using the Birdseed2 clustering algorithm. For quality control purposes, several control samples where added. On the chip level, only subjects with a genotyping rate on QC probesets (QC callrate) of at least 86% were included. Finally, all arrays had a sample callrate > 92%. The overall genotyping efficiency of the GWA was 98.55 %. Imputation of genotypes was performed using the HRCv1.1 reference panel and the Eagle and minimac3 software implemented in the Michigan Imputation Server for pre-phasing and imputation, respectively. SNPs with a Hardy-Weinberg-Equilibrium p-value

<0.0001, a call rate <0.95, and monomorphic SNPs were removed before imputation. The GWAS were performed sex-stratified using EPACTS-3.2.6-patched adjusting for age, the (two) handgrip measurement devices, and the first 10 genetic principal components.

8.1.16 Toledo Study for Healthy Aging (TSHA)

Data were taken from the Toledo Study for Healthy Aging, a population-based study conducted on 2,488 individuals aged 65 years and older. Study participants were selected by a two-stage random sampling from the municipal census of Toledo, covering both institutionalized and community dwelling persons from rural and urban settings. Data were collected from 2006 to 2009, and included information on social support, activities of daily living, comorbidity, physical activity, quality of life, depressive symptoms, and cognitive function. In addition, a nurse collected anthropometric data, conducted tests of physical performance (walk speed, upper and lower extremities strength, and the stand-and-sit from a chair test) and obtained a blood sample. The diagnosis of the frailty syndrome was based on the Fried criteria (weakness, low speed, low physical activity, exhaustion, and weight loss). For this analysis, participants from the second wave of data collection 2013 – 2015 were included.

Genotyping was performing using Illumina Infinium Global Screening Array. The call rate was 0.98, 3.4% of the sample presented a call rate lower than 95% and were excluded from the analysis. Imputation Panel: HRC (Version r1.1 2016) using Michigan Imputation Server. GWAS was performed using SNPTEST logistic regression adjusted for age, sex, and 6 EVs.

8.1.17 UK Biobank (UKB)

Between 2006 and 2010, 503,325 volunteers (aged 40 to 70 years old) were recruited from across the United Kingdom to the UK Biobank study. Genetic data was available on 488,377 UK Biobank participants after genotype calling and quality control performed centrally by the UK Biobank team (Bycroft et al. 2018). We selected 451,447 participants identified as 'white European' through self-report and verified through principal components analysis based on genotypes. Briefly, principal components were generated in the 1000 Genomes Cohort using high-confidence SNPs to obtain their individual loadings. These loadings were then used to project all of the UK Biobank samples into the same principal component space and individuals were then clustered using principal components 1 to 4 (see (Thompson et al. 2019b) for details). Imputation of 39,235,157 genetic variants from the Haplotype Reference Consortium panel was performed using IMPUTE4 centrally by the UK Biobank team (Bycroft et al. 2018). After filtering for variants with MAF ≥0.1%, missingness <1.5%, imputation quality >0.1 and with Hardy-Weinberg equilibrium (HWE) $P>1x10^{-6}$ within the European-descent participants 11,516,125 imputed autosomal variants were eligible for the analyses. We used BOLT-LMM v2.3.2 to model the associations between imputed variants (dosages) and each phenotype (P.-R. Loh et al. 2015) using LD Score provided with the package for European populations. Although this means we have not used a logistic regression model, this approach has the advantage that the linear mixed effects model approach robustly accounts for relatedness and population structure.

8.1.18 Wisconsin Longitudinal Study (WLS)

The Wisconsin Longitudinal Study (WLS) is a one-third sample of all 1957 Wisconsin high school graduates and a randomly selected sibling (Herd, Carr, and Roan 2014). These respondents were originally empaneled with an in-person questionnaire at age 18 (1957), which was followed with a mail survey of parents in 1964, telephone survey in 1975, mail and telephone surveys in 1993 and 2004 and in-person interviews in 2011, where data on grip strength was collected from participants. The WLS has a high response rate, exceeding 80 percent in most rounds of data collection. Between In 2006-11, the WLS collected saliva samples from respondents using Oragene kits (Rylander-Rudqvist 2006). After quality control, a total of 9,012 graduate and sibling respondents were genotyped at ~710,000 markers (before imputation) utilizing the Omni-Express beadchip. Genotyping was complete at Johns Hopkins' Center for Inherited Disease Research (CIDR) and data cleaning was performed in collaboration with the Genetic Analysis Center at the University of Washington. The detailed procedures employed to generate the genetic data are available on the WLS website (https://www.ssc.wisc.edu/wlsresearch/documentation/GWAS). Genotype imputation using the Haplotype Reference Consortium (HRC) v1.1 panel was performed.

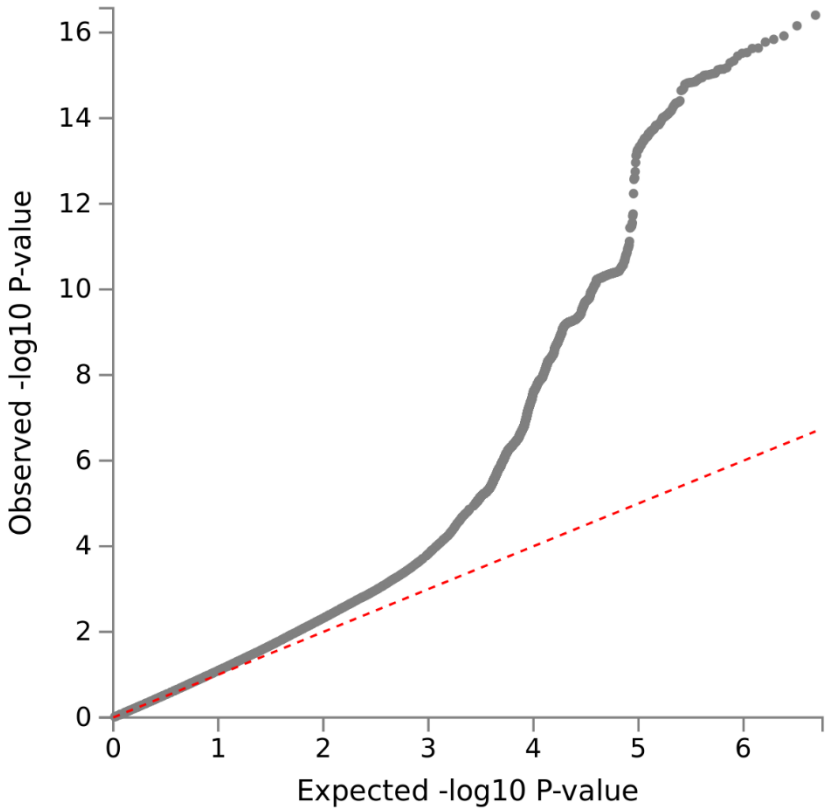
In the sample aged 60 or over at grip assessment (n=7,190) BOLT-LMM was used to model associations between the imputation variants and low grip strength phenotypes. Covariates used were age at grip strength measurement, age at DNA collection, 10 principal components, and sex.

WLS Data, documentation and other material are accessible at http://www.ssc.wisc.edu/wlsresearch/.

8.1.19 GWAS meta-analysis methods

Each cohort used the optimum GWAS method available at the time. Most used logistic regression models or equivalent to derive Odds Ratios. Some (HRS, UK Biobank and WLS) were able to use BOLT-LMM to maximise discovery power by including all related participants (appropriate adjustment made in the Linear Mixed Model methods but larger sample sizes are required for the method to be robust). While BOLT-LMM was developed for quantitative traits, it can be used for binary traits as long as they are sufficiently balanced (cases >10% of sample) (P. R. Loh et al. 2018). We therefore converted the regression coefficients from BOLT-LMM to Odds Ratios using a method published by Lloyd-Jones in 2018 (Lloyd-Jones et al. 2018a) prior to meta-analysis. This method uses the genetic effect (the beta), allele frequency, and sample prevalence to estimate the Odds Ratio from the Beta. We used the published R function to perform the transformation from the HRS, UK Biobank and WLS results (https://github.com/lukelloydjones/ORShiny/blob/master/shiny_lmor_func.R). Finally, inverse variance-weighted meta-analysis with genomic control was performed by METAL (Willer, Li, and Abecasis 2010b) on the log-Odds Ratios.

Figure 8-1: QQ plot of EWGSOP low grip GWAS results



8.1.20 Supplementary Figures 2A-O: LocusZoom plots for the 15 EWGSOP low grip loci

The LocusZoom online tool (http://locuszoom.org) was used to plot the regions around the 15 loci (panels A to O) significantly ($p<5*10^{-8}$) associated with low grip strength in this GWAS meta-analysis. The results can be explored using the below link. https://my.locuszoom.org/gwas/532795/?token=ad786aade6e44e90935921594de5185b

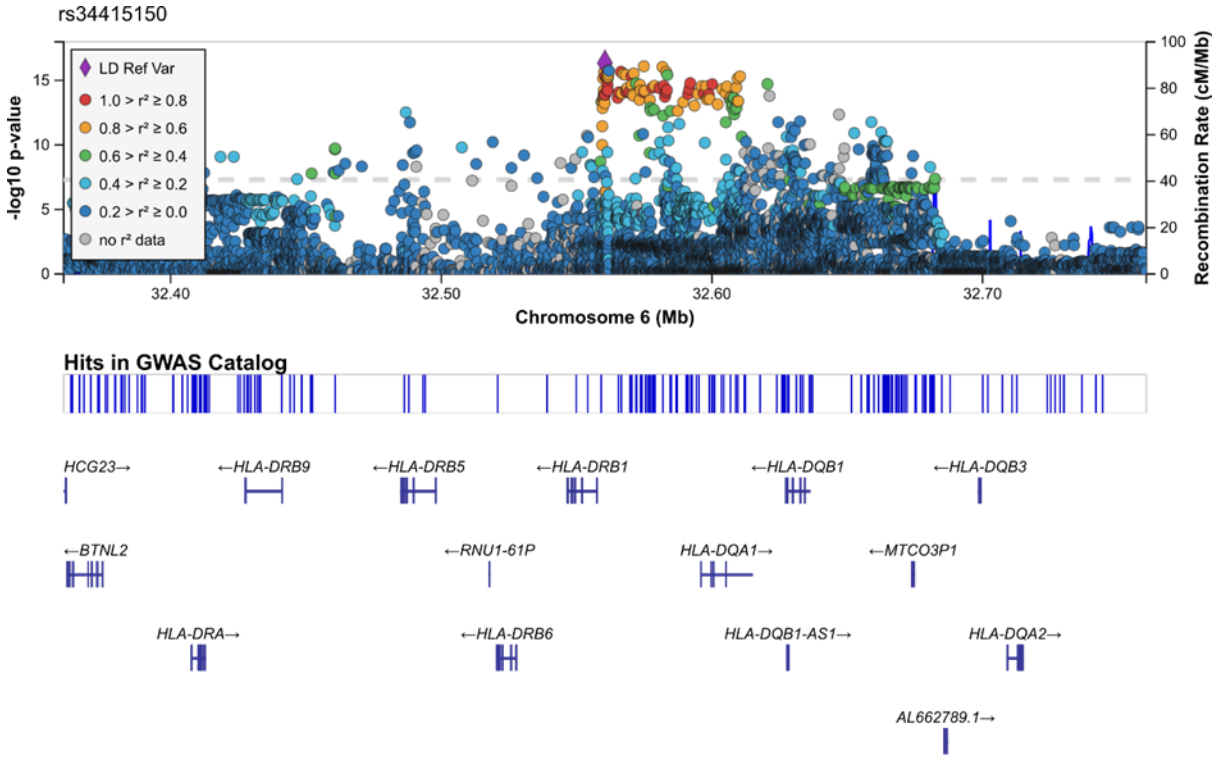


Figure 8-2: rs34415150 (chr6:32560477)

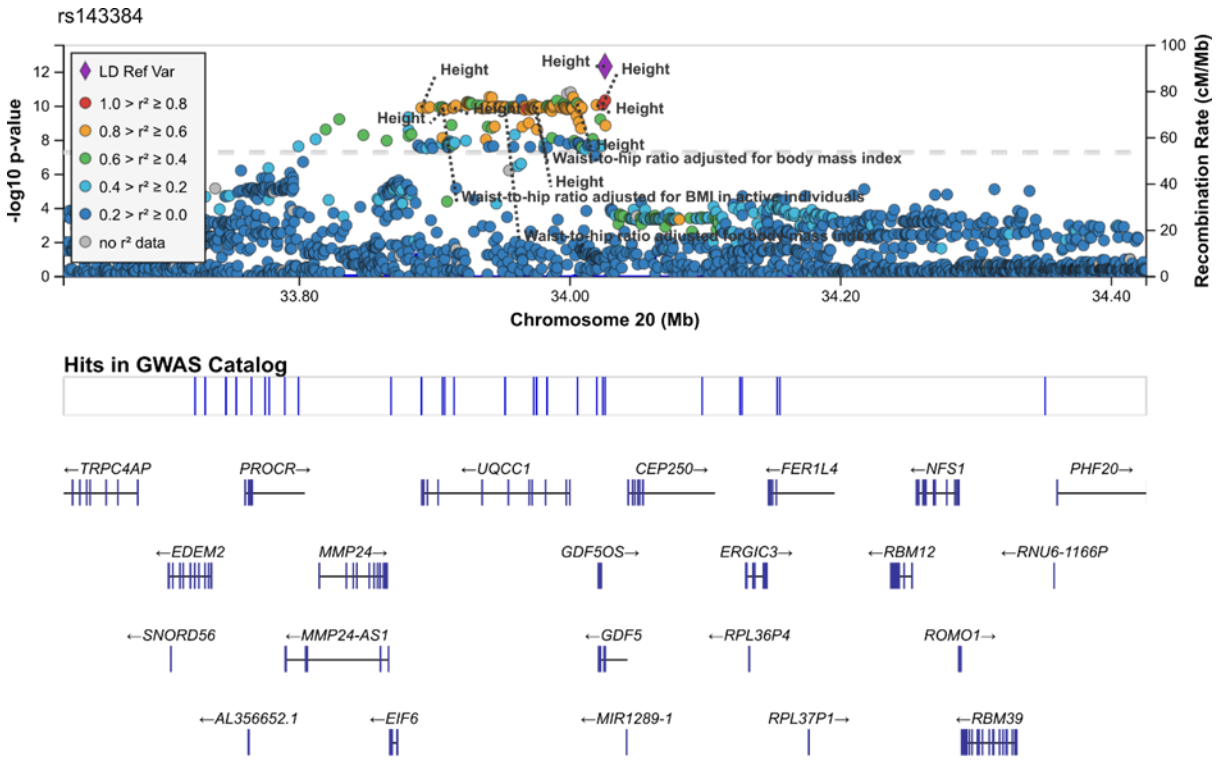


Figure 8-3: rs143384 (chr20:34025756)

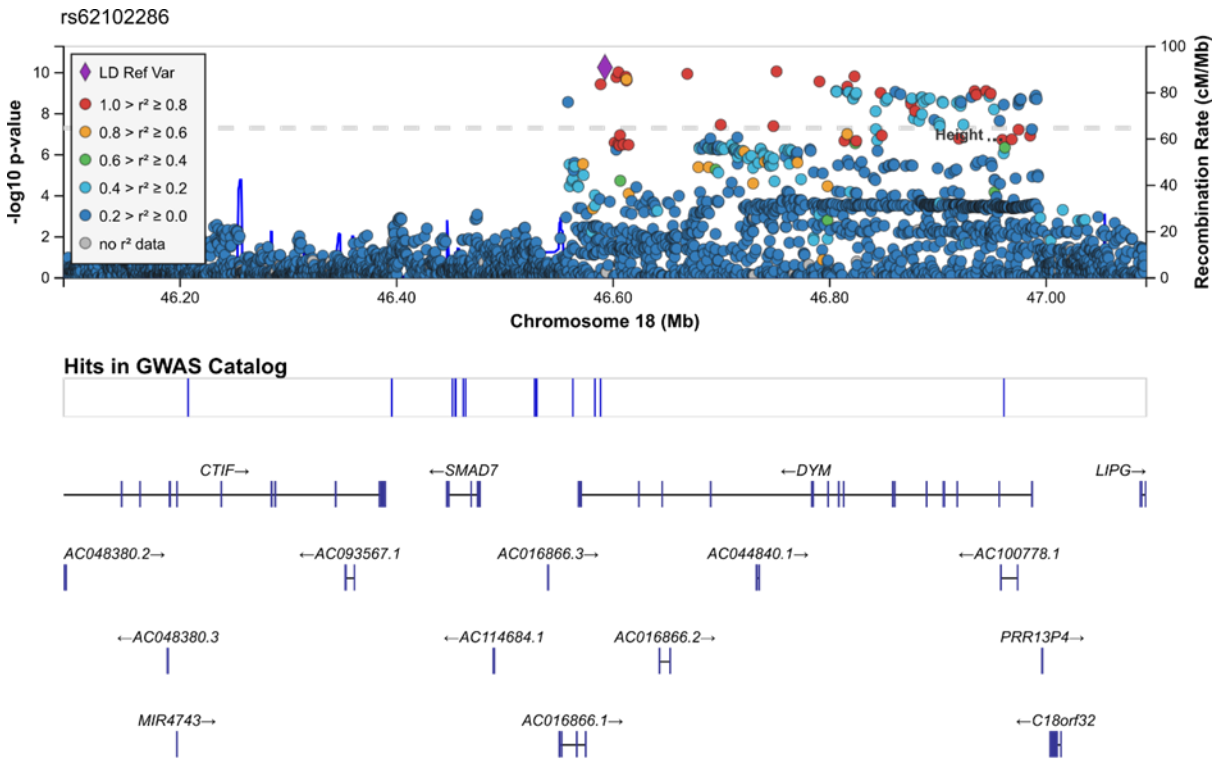


Figure 8-4: rs62102286 (chr18:46592408)

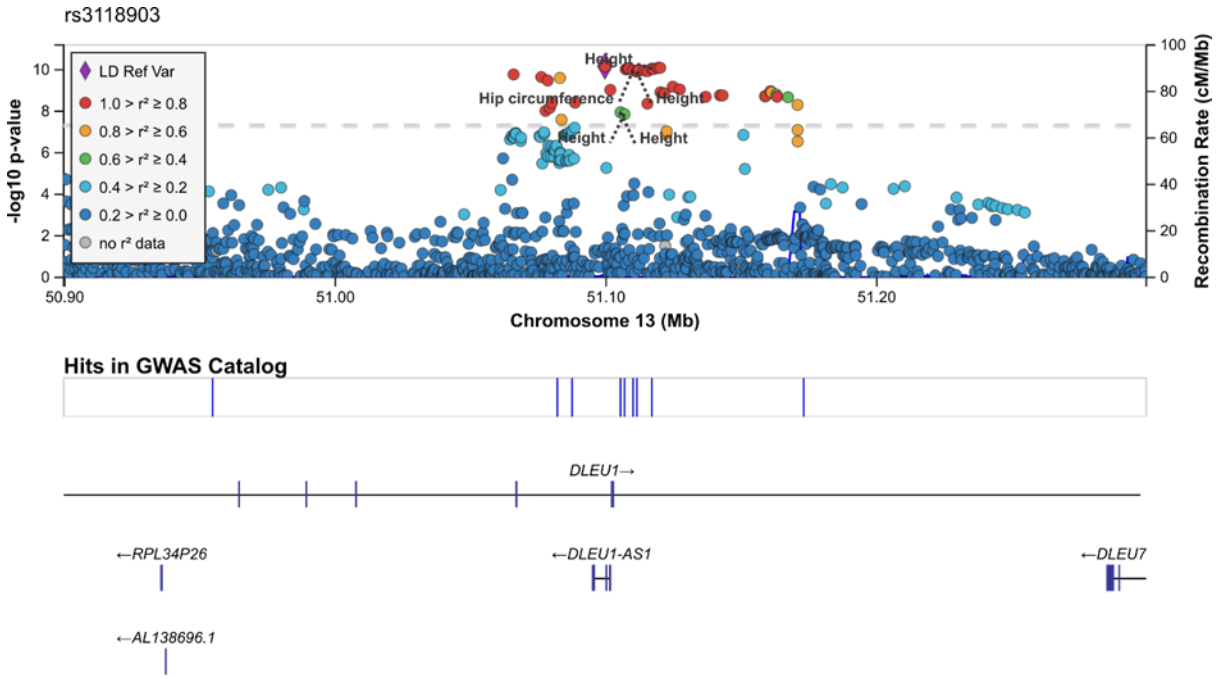


Figure 8-5: rs3118903 (chr13:51099577)

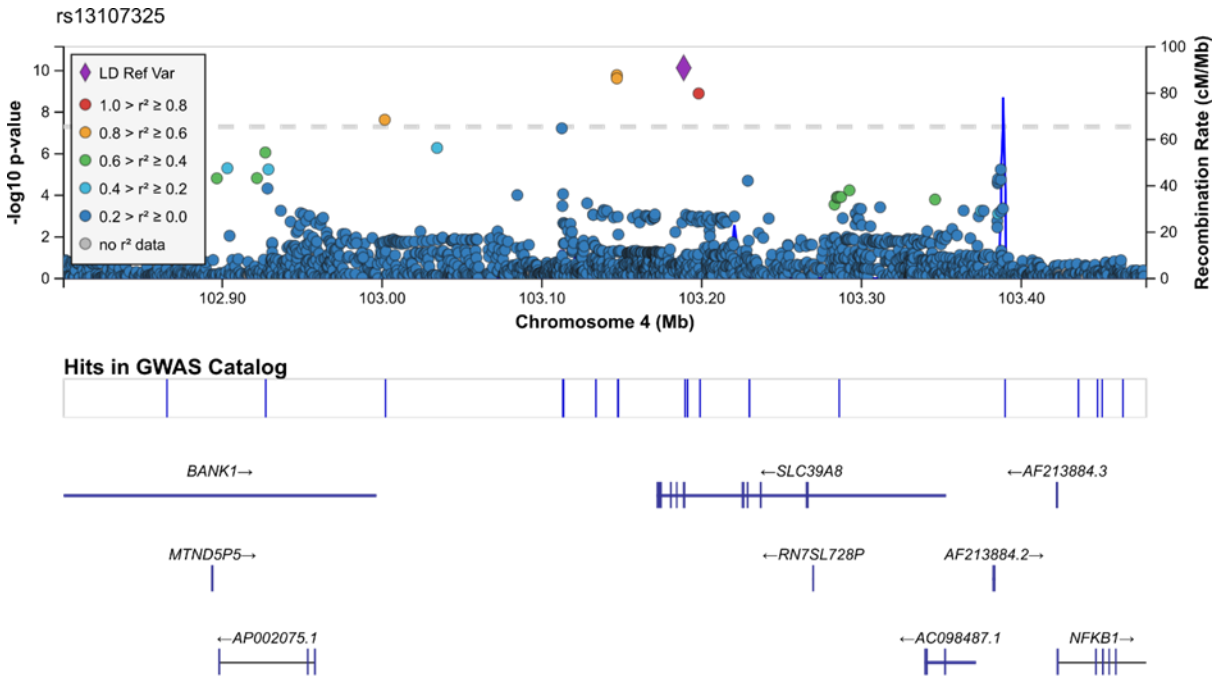


Figure 8-6: rs13107325 (chr4:103188709)

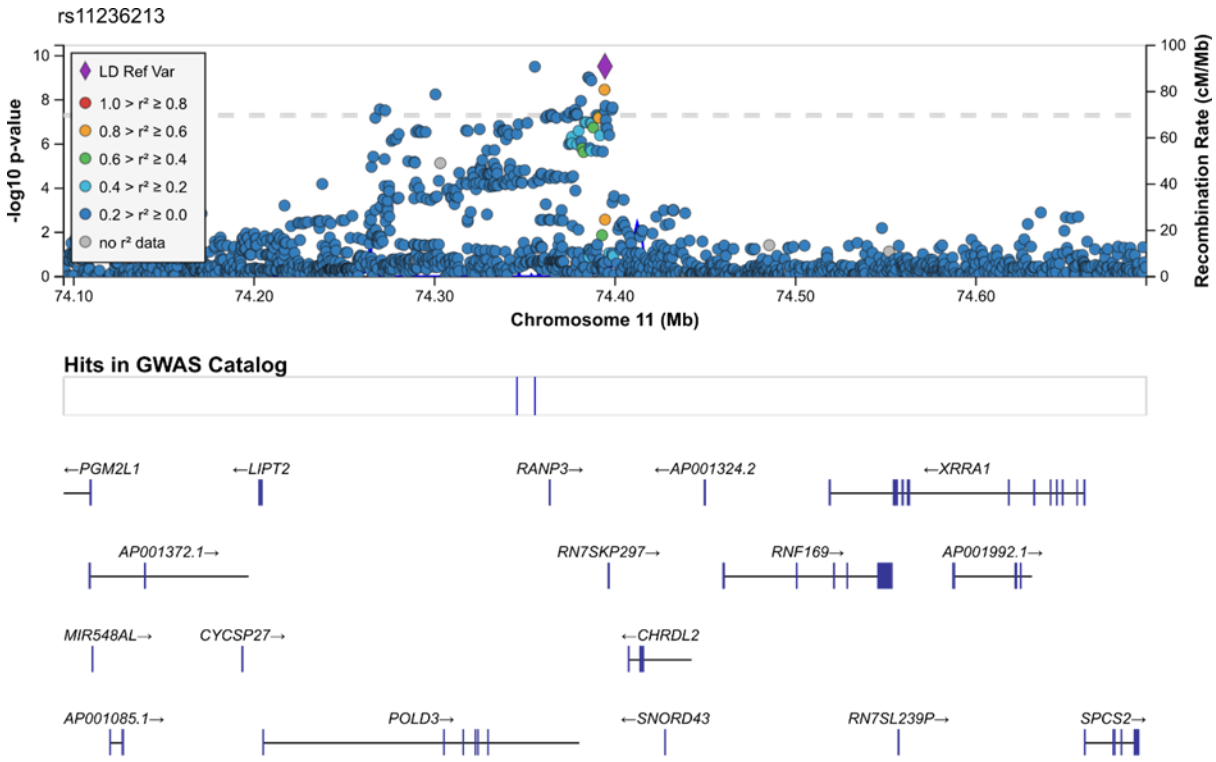


Figure 8-7: rs11236213 (chr11:74394369)

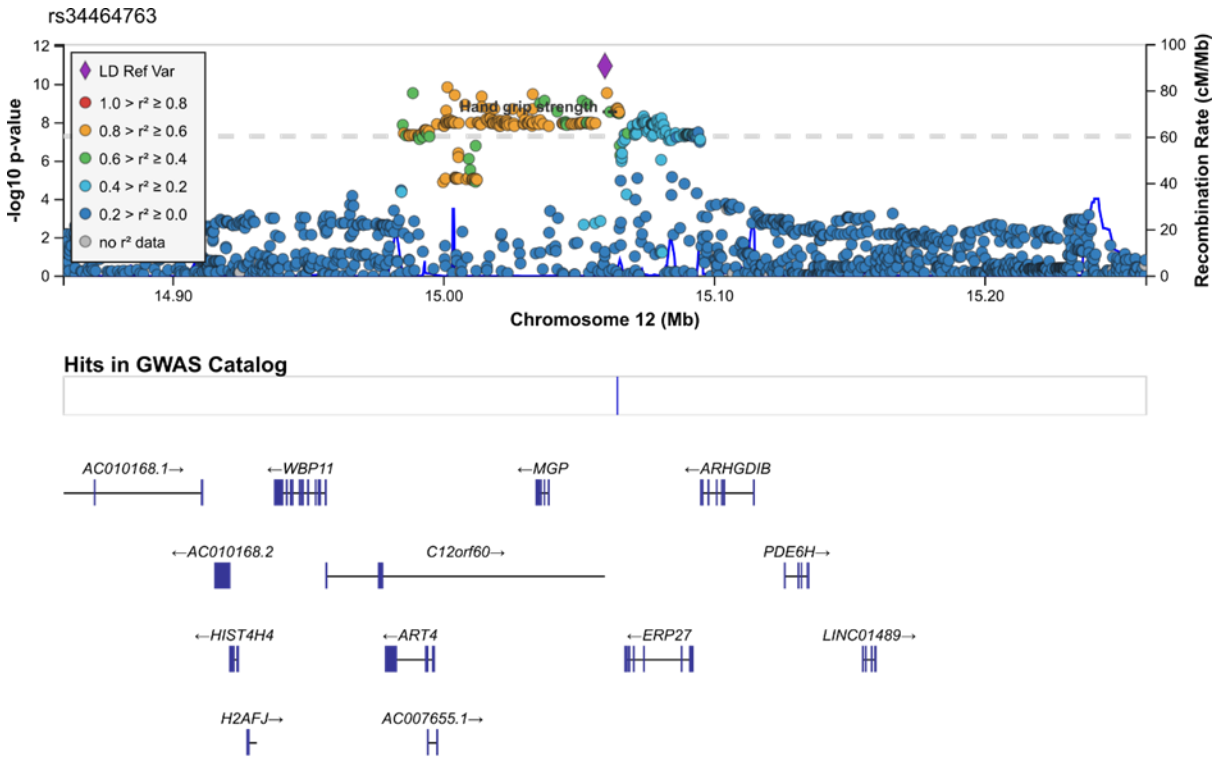


Figure 8-8: rs34464763 (chr12:15032860)

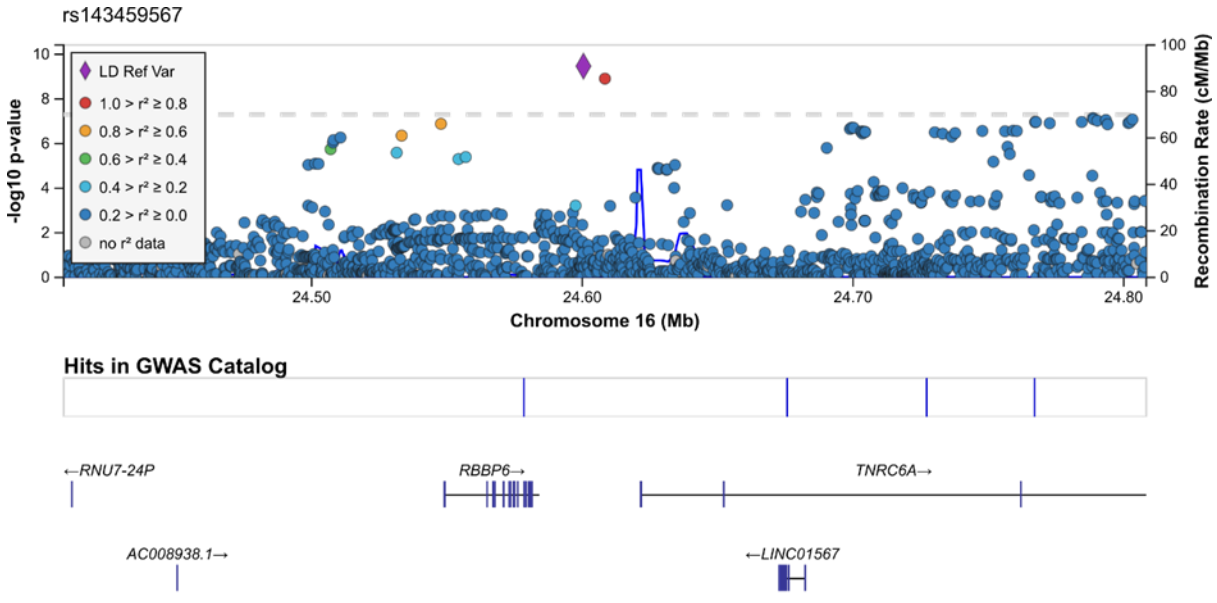


Figure 8-9: rs143459567 (chr16:24600412)

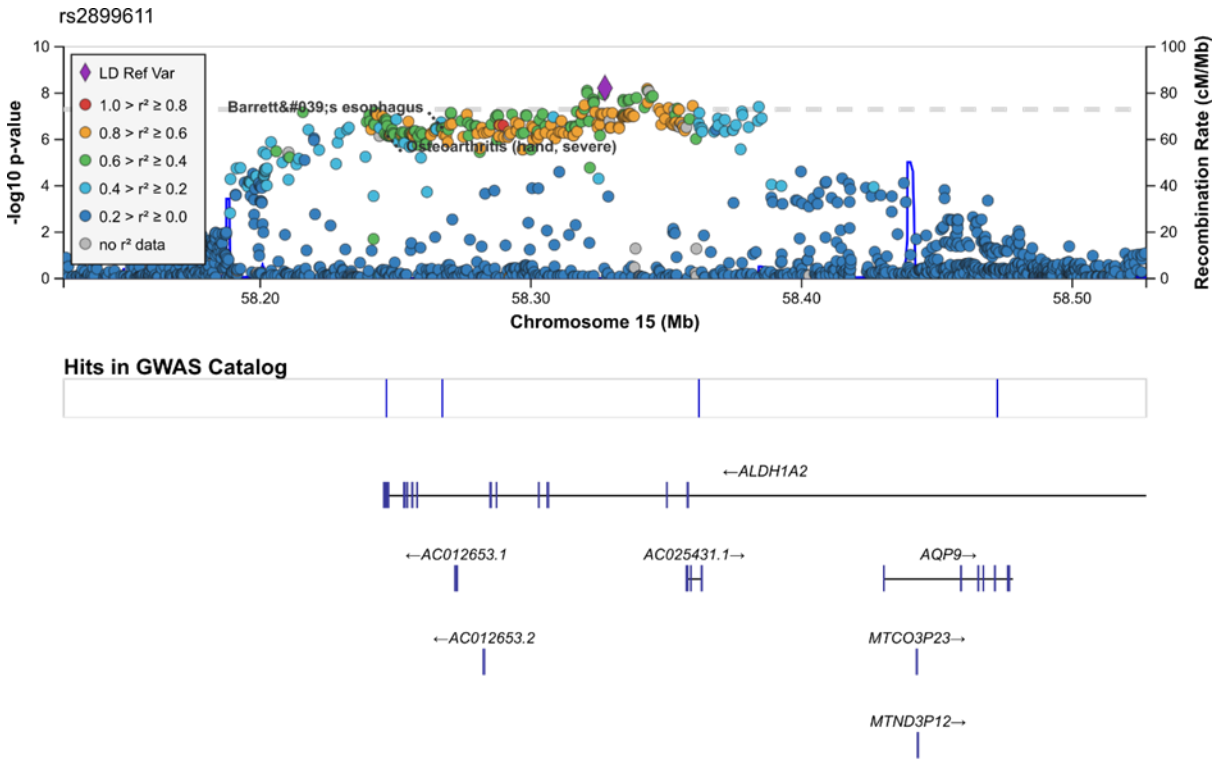


Figure 8-10: rs2899611 (chr15:58327347)

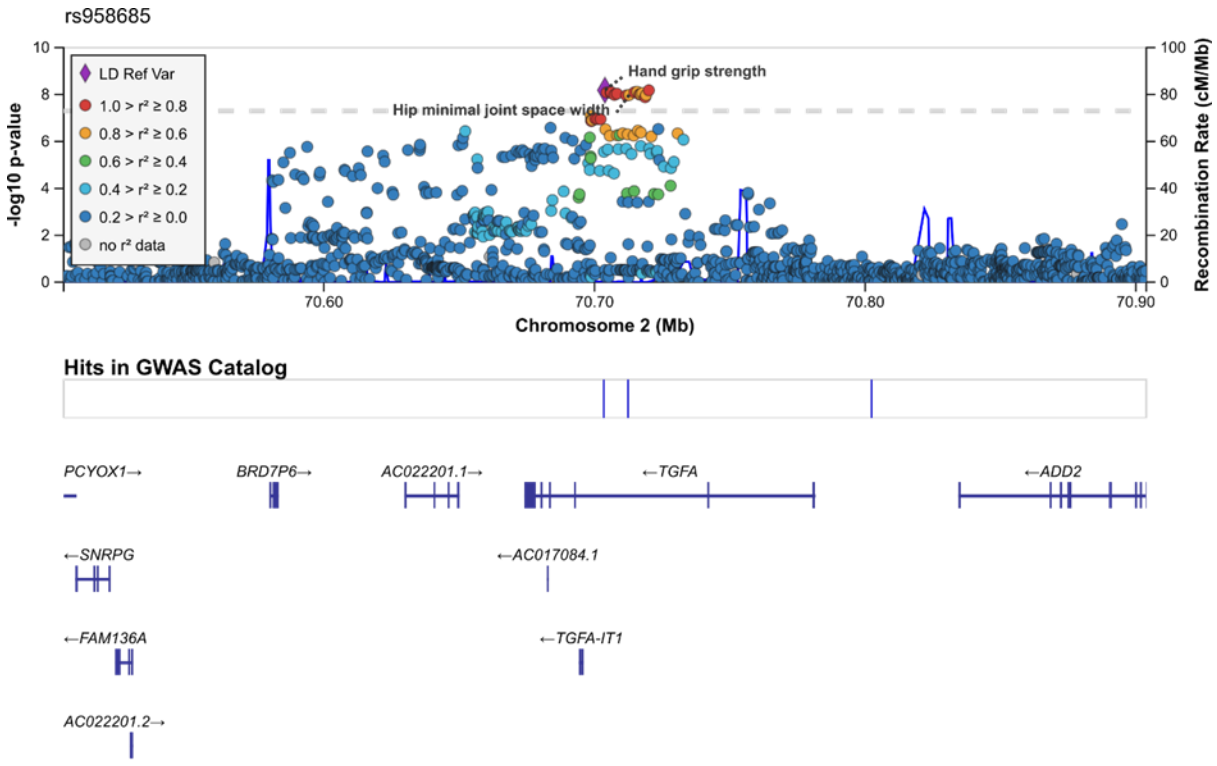


Figure 8-11: rs958685 (chr2:70703847)

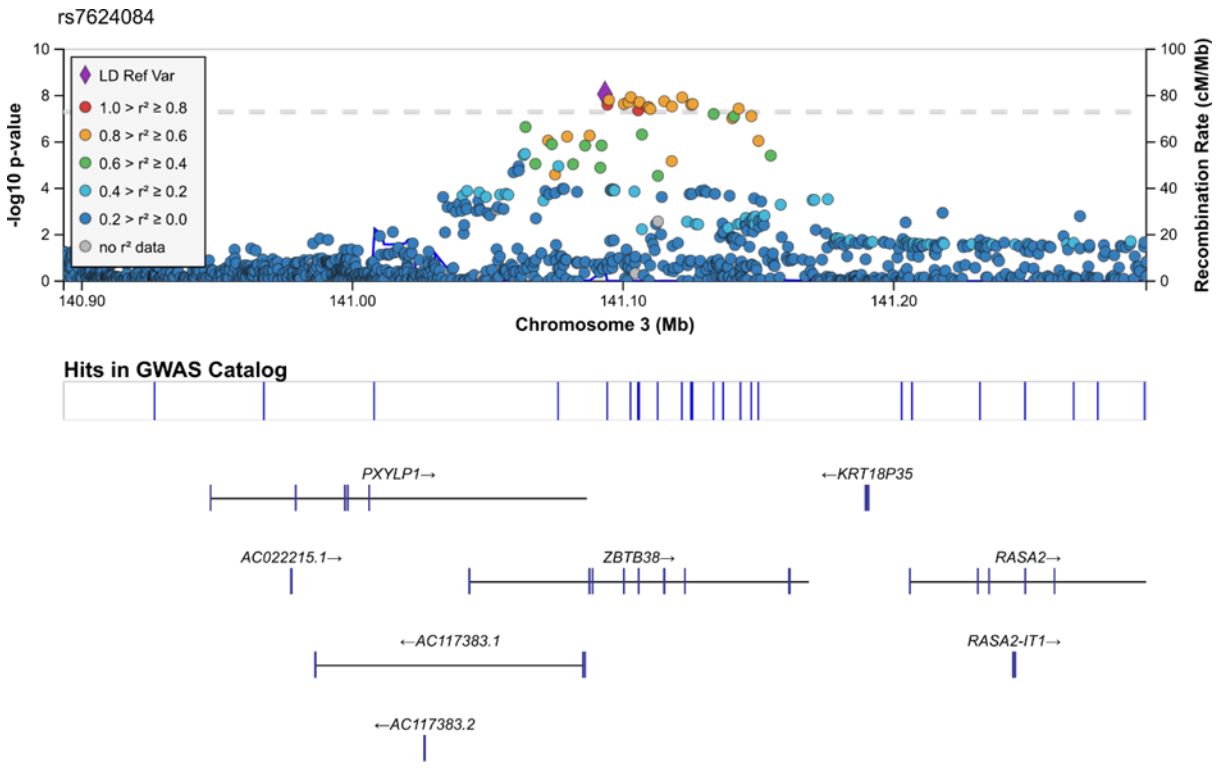


Figure 8-12: rs7624084 (chr3:141093285)

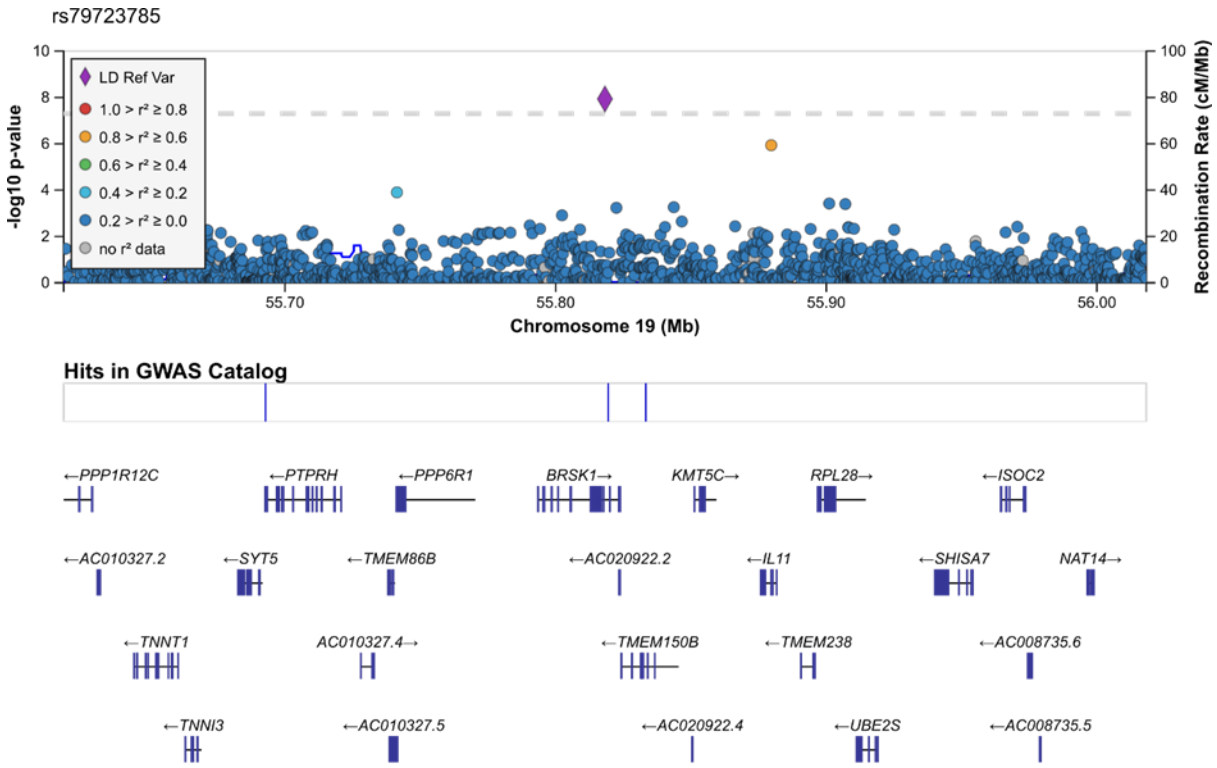


Figure 8-13: rs79723785 (chr19:55818225)

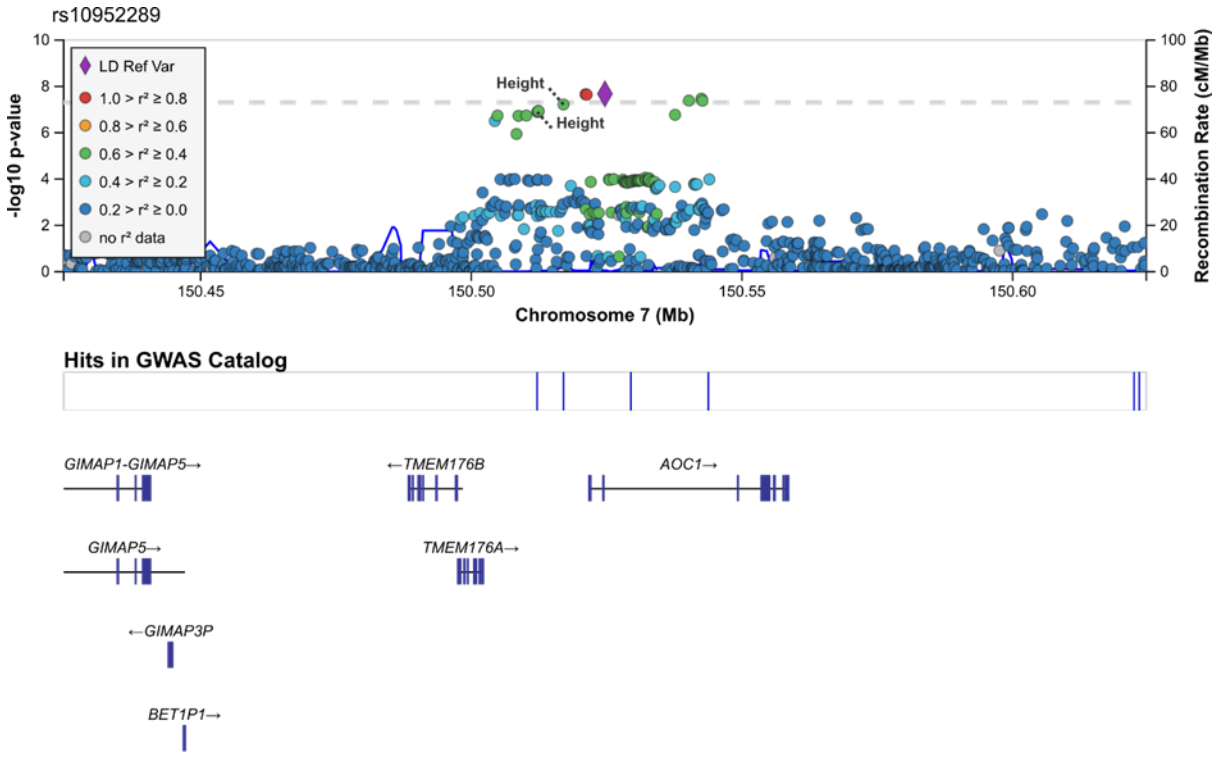


Figure 8-14: rs10952289 (chr7:150524681)

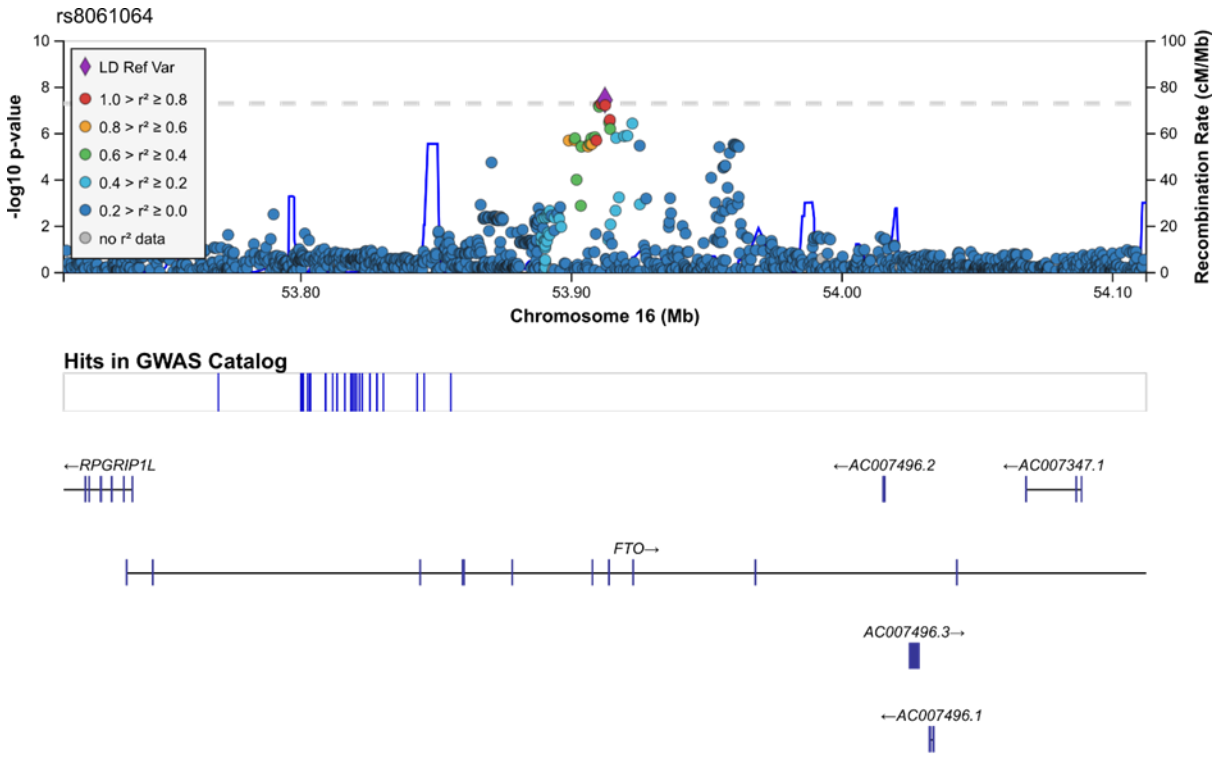


Figure 8-15: rs8061064 (chr16:53912364)

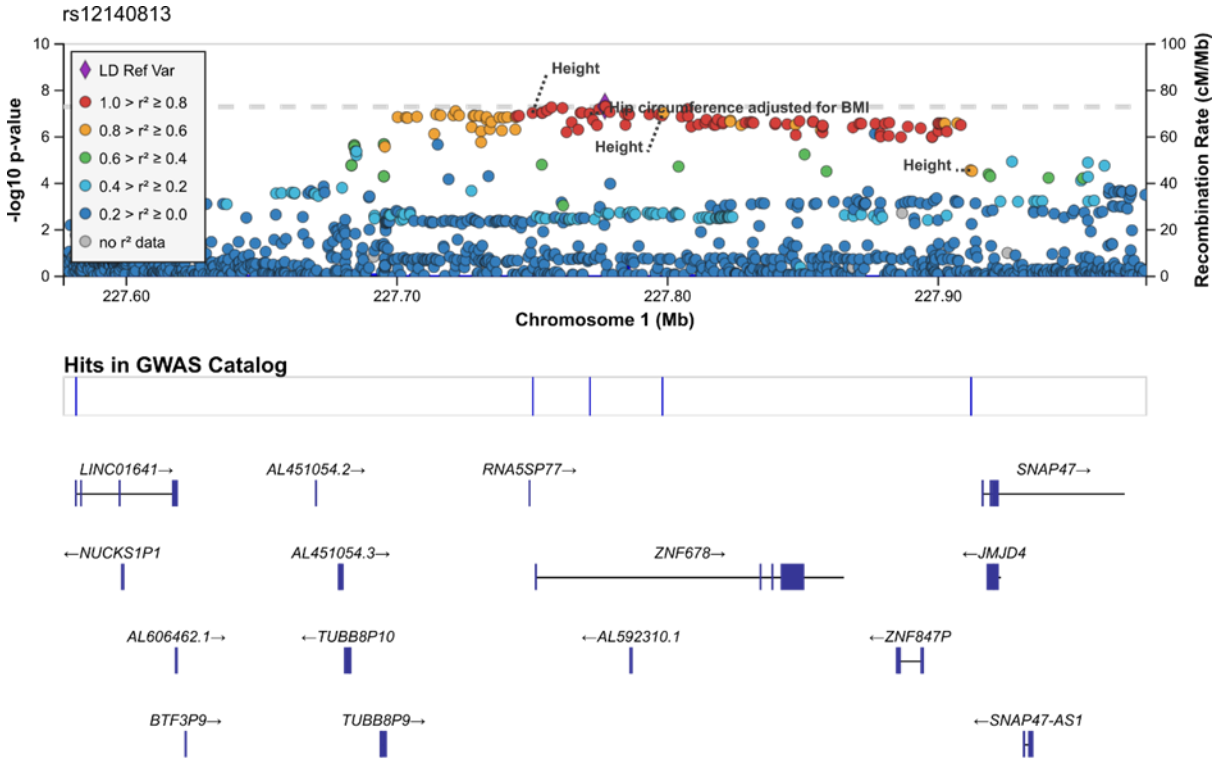


Figure 8-16: rs12140813 (chr1:227776827)

Table 8-1: Summary of cohorts included in the meta-analysis

Study	Number of Females aged >= 60 meeting EWGSOPv.1 low grip criteria (<20kg)	Number of Males aged >= 60 meeting EWGSOPv.1 low grip criteria (<30kg)	Number of Females aged >= 60 meeting FNIH low grip criteria (<16kg)	Number of Males aged >= 60 meeting FNIH low grip criteria (<26kg)	Number of Females Total	Number of Males Total	Number - total
ARIC	650	289	217	134	2,025	1,630	3,655
BASE-II	55	19	5	4	779	752	1,531
BPROOF	328	145	137	64	1,244	1,275	2,519
CHS	601	201	231	88	1,855	1,206	3,061
EPIC-Norfolk	926	503	326	219	3,962	3,546	7,508
FHS	400	142	153	60	1,461	1,245	2,706
HRS	2,380	1,244	1,089	665	6,164	4,650	10,814
InCHIANTI	162	75	90	48	458	361	819
LASA I	121	77	43	37	249	255	504
LASA II	192	85	68	36	632	589	1,221

Long Life Family Study	824	586	446	397	1,788	1,571	3,359
MrOS Gothenburg	0	35	0	10	0	941	941
MrOS Malmo	0	29	0	12	0	891	891
ROSMAP 1	661	198	346	129	1,096	473	1,569
ROSMAP 2	197	48	108	32	266	95	361
Rotterdam Study I	511	234	253	121	853	587	1,440
Rotterdam Study II	273	137	121	58	684	565	1,249
Rotterdam Study III	146	86	41	36	844	640	1,484
SHIP	96	35	43	8	523	513	1,036
TSHA	844	447	525	316	1,218	882	2,100
UK Biobank	24,229	8,807	8,966	3,941	105,597	94,968	200,565
WLS	993	585	393	319	3,770	3,420	7,190
Total	**34,589**	**14,007**	**13,601**	**6,734**	**135,468**	**121,055**	**256,523**
	EWGSOP total=	**48,596**	***FNIH total=***	**20,335**			

Table 8-2: ICD-10 and UK Biobank self-reported codes used in sensitivity analysis

Condition	**UK Biobank self-reported fields**	**ICD-10 codes**	**OPCS codes**	**N**
Osteoarthritis	1465	M15.0; M15.1; M15.2; M15.9; M16.0; M16.1; M17.0; M17.1; M18.0; M18.1; M19.0	O18*; W40*; W41*; W42*; W37*; W38*; W39*; W46*; W47*; W48*; W93*; W94*; W95*	29,380
Rheumatoid arthritis	1464	M05; M06		3,263
Rhizarthrosis		M18; M180; M181; M182; M183; M184; M185; M189		355
Osteoporosis	1309	M80; M81		6,308
Dupuytren's contracture	1544	M720; M7204	T521; T522; T525; T526; T541; T561; T562	1,455

Any autoimmune condition	1234; 1522; 1428; 1464; 1381; 1313; 1382; 1261; 1456; 1463; 1477; 1453; 11222	D69*; D758; D141; D862; E271; E05; E051; E063; E100; E101; E102; E103; E104; E105; E106; E107; E108; E109; G35; G737; G70*; H30*; K900; K510; K512; K513; K514; K515; K518; K519; L40; L400; L401; L402; L403; L404; L405; L408; L409; L12*; L10*; L511; M023; M0230; M0236; M0239; M028; M0281; M0284; M0285; M0286; M0287; M029; M0290; M0293; M0294; M0295; M0296; M0297; M0299; M06*; M320; M321; M328; M329; M45; M350; M30*; M34*; M352		16,951

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