Genetic Associations Between GDF5 and Physical Disability Phenotypes: A PHEWAS Approach

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Background: Declining physical function typically results in increased disabilities, medical institutionalization, health-care costs, and premature mortality. Along with this, low physical function leads to lower quality of life and dependencies. Prior research has identified potential risk factors for physical disability such as lifestyle factors and chronic health risks. However, interventions targeting these risk factors are minimal. This makes it imperative to identify genetic risk factors, which are largely unknown. GDF5 is a novel gene that has been a target in recent genetic studies that identify associations with physical disability.

Methods: PHEWAS technologies are used to identify associations between genetic variants and phenotypes of interest (gait speed, grip strength, FEV1, SPPB, Chair rise, SAVE, and a composite mobility disability score). We have employed PHEWAS analyses accompanied by mixed general linear models that include a kinship matrix to account for the familial relatedness of our sample population. We also controlled for age, sex, visit site, and height. To capture GDF5 and other regulatory genes, we determined gene boundaries using Ensembl genome browser

Results: There were 4440 participants (55% female and 45% male) involved in this analysis, using the Long Life Family Study which is a familial cohort of exceptional longevity. Descriptive results show that the mean age was 69.8. Association analyses showed 52 nominally significant SNPs across all age groups and phenotypes, and 82 nominally significant SNPs across all phenotypes in those aged 60+. We also experienced pleiotropic properties in certain SNPs. For example, RS11814750 is nominally significant with 9 phenotypes that are predictors of physical

disability. Results also revealed a mediation effect with height that brought out significant SNPs that were not associated with phenotypes before height adjustment.

Conclusion: Understanding the genetic component to physical decline and function is necessary to improve quality of life and overall mobility. Results of this PHEWAS analysis will identify candidate SNPs that affect multiple phenotypes related to the physical disability and will also provide the framework for novel therapeutic and prevention measures, which will resolve the public health crisis of limited therapeutics for physical disability.

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Preface

Without the help of my committee, this research would not have been possible. I would like to thank Dr. Santanasto, Dr. Minster, and Dr. Zmuda for all of their hard work and mentorship throughout this entire process. They not only provided me with the resources that I needed to succeed, but also provided their knowledge on this topic so that I could gain a clear understanding on the public health significance. I would also like to thank Dr. Glynn for all of her guidance throughout the previous two years.

I would also like to acknowledge the Long-Life Family Study, both participants and researchers, for providing me with the dataset that made this research possible. Through their years of experience, many genetic studies have been published and a large magnitude of novel results have been documented. Again, thank you all for your hard work and determination.

1.0 Introduction

1.1 Physical Function and Disability

Declining physical function is a risk factor for many adverse health conditions. Degradation of physical function typically results in increased disabilities,[1,2] medical institutionalization,[1] health-care costs,[3,4] and premature mortality.[1,5] Along with this, low physical function leads to lower quality of life, dependency, and can exacerbate or put individuals at risk for certain chronic diseases. It is well known that declining physical function occurs with aging, which poses a public health concern. The population of the United States and most developed and developing countries are rapidly aging. For example, the proportion of those aged 65 and older in the US in 2019 was 16% of the total population.[6] Thus, declining physical function with aging is an enormous public health issue. Further, risk factors for declining physical function range from lifestyle habits and chronic health conditions to body composition changes. However, the biological and genetic influences on physical function with aging remain largely unknown. This master's thesis will summarize what is known to date and aims to identify novel genetic associations with physical function phenotypes.

To study physical function and progression to disability, epidemiological studies employ the Nagi model. Historically, four key stages of disablement have been described, and the Nagi disablement pathway is the most widely used model in research (figure 1).[7] This model represents a sequential pathway that results in physical disability, in which the most downstream stage is disability in activities of daily living (ADLs). The reason that the Nagi model has been the preferred model for epidemiological studies is due to its clear progression, which allows

researchers to study risk factors and consequences at each level. This meaning that the consequences of upstream changes on downstream changes can be clearly conceptualized (ie. effects of physiological changes on functional limitations).

The four stages of the Nagi disablement pathway are as follows – the first stage is pathology which is a biochemical or physiologic disorder that disrupts function, such as Alzheimer's, hypertension, diabetes, or muscle loss.[7] Pathology disruption is generally the result of biological changes at a cellular level. Next is impairment which consists of dysfunctions and structural abnormalities that is caused by pathology. This would be considered the signs and symptoms of pathology. For example, this would consist of pain, memory loss, or decreases in muscle strength.[7] Impairment is a direct result of the pathology disruption. The third stage is functional limitation which is a restriction in performing fundamental activities used in day-to-day life.[7] Functional limitation can be represented by slowed walking speed, difficulty lifting objects, difficulty rising out of a chair, difficulty walking a flight of stairs, decreased lung function or any functional limitation to daily life.[7] The three previously mentioned stages are all upstream of physical disability, the final stage of the disablement pathway. Physical disability itself can be broken into three categories. There is ADL, IADL, and mobility disabilities. ADL disabilities are defined as difficulty performing personal care activities essential for independent living (feeding, changing, toileting, continence, etc.).[7] Those with ADL disabilities often need daily assistance. IADL disabilities are defined as difficulty or inability to perform household activities (shopping, cooking, cleaning, laundry, etc.).[7] iADL disabilities are upstream from ADL disabilities so they are less debilitating. However, those who are diagnosed as IADL often progress into the ADL category. The last category for physical disability is mobility disability which is impairment of gross motor skills and fine motor skills. This is simply an inability or difficulty to walk and engage

in movement. Mobility disability is measured by the ability to walk 400-500m or climb one flight of stairs.[7]

The Nagi model allows clinicians and researchers to formulate hypotheses and studies that identify subclinical factors that are risk factors for progressing through the disablement pathway to physical disability. For example, researchers can measure functional limitations and impairments in order to identify early effects of an intervention aimed at preventing disability or detect "subclinical" changes in physical performance before they manifest as disability. Similarly, the pathway also allows clinicians and researchers to identify those who are at the greatest risk of physical disability. The pathway also provides researchers clear up and down stream effects and outcomes that can be studied in relation to one another and can provides a framework for clear hypothesizes regarding predictors and outcomes – e.g. pathology leading to impairment.

More recently, a newer model has been composed that is a further extension of the Nagi model. The NHATs model encompasses "positive" terms such as capacity and participation. NHATs is a model derived from a National Health and Aging Trends Study. Each participant from the study is being evaluated for late life disability trends.[8] More specifically, NHATs measures physical capacity, activity limitations, participant restrictions, and accommodations.[9] Physical capacity refers to the physical ability of the participant. Activity limitation is the process of having difficulty or needing assistance in mobility tasks, while participant restriction refers to the terminal affect from declining health / function. Accommodation relates to the participants need to be accommodated while completing tasks. Things such as devices to aid in function or actual assistance from an aide. Patients of this study are then classified as fully able, successful accommodation, activity reduction, difficulty even though accommodated, assistance from others.

Much like the Nagi Model, NHATs allows researchers to formulate hypotheses that are directly related to reduction in disability and increases in independency and overall quality of life.[8]

1.1.1 Supplement

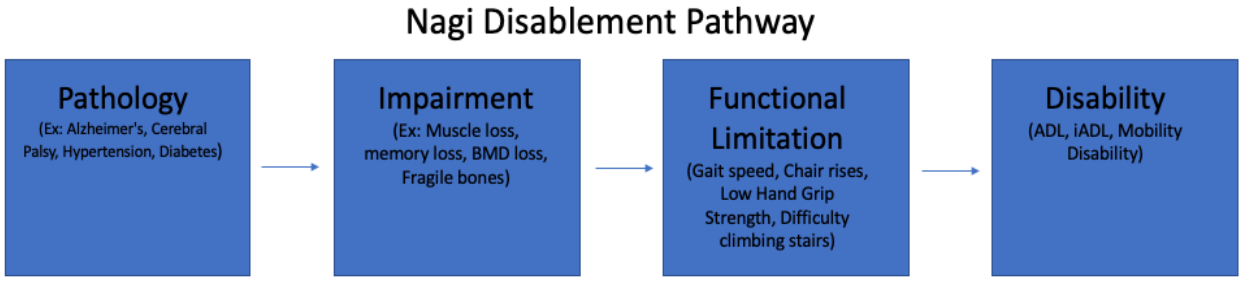

Figure 1

Represents the Nagi Model demonstrating the disablement pathway. Pathology represents interference of normal bodily processes or structure. Impairment consists of all losses or abnormalities, not just active pathology… Includes pain. Functional Limitation is the restriction or lack of ability to perform an activity within ranges considered normal. It is a result of impairment. Disability is the inability or limitation of performing expected roles or activities expected of individuals in a social or physical setting.

1.2 Physical Disability Prevalence / Incidence

Not only does declining physical function lead to many adverse health conditions, but the prevalence and incidence rates are quite high. This, coupled with the rapid aging of the population, makes declining physical function with aging an enormous public health concern. For example, a study done using NHANES data showed that roughly 20% of adults in their sixties are living with ADL and 20% are living with iADL.[10] The prevalence had a minor increase of about 3% (both ADL and iADL) when looking at the 70-79 age group. However, the prevalence rate of ADL in those aged 80-89 increased to roughly 28% and those living with iADL increased to 35%.[10] This is a validated study that identifies the relationship between age and physical disability, however

much more recent data has been published. Another example that uses recent data is in a Swedish study that showed that the incidence rate for ADL in men aged 78-81 was 42.3 per 1000 person years.[11] The ADL in women aged 78-81 was 20.8 per 1000 person years. However, women aged greater than 81 had an ADL incidence of 118.3 per 1000 person years.[11] This study represents the large variation of physical disability incidence by age, and how those in older age groups are at highest risk. Also, this study highlights the sex disparities of physical disability. It shows that women often have a larger prevalence than men. Another study identified 598 participants of Spanish descent aged 65 years or older. They concluded that 34.6% of them were dependent for at least one ADL while 53.5% were represented as IADL.[12] They also identified an increased risk of ADL dependency (Male OR=1.089, Female OR=2.48) for each year of age increase.[12] Both this study and the Swedish study show the large prevalence and incidence rate in the older population. They also identify an increased risk of physical disability with every year of age increase. Along with this, they represent the sex disparities that are present in physical disability.

Another study[13] provided results that had variation based upon sex. The prevalence of physical disability (ADL or IADL) is higher within women compared to men. Pertaining to differences in race, studies have shown that non-Hispanic blacks report a prevalence of roughly 24% compared to whites who report 19.3%.[14,15] Along with this, African Americans have an increased risk of developing health conditions that are considered risk factors for physical disability. This includes things like hypertension, osteo-diseases, and diabetes. The large prevalence rates can be widely attributed to older age, however there are many risk factors involved in physical disability.

1.3 Phenome Wide Association Studies

Phenome wide association studies (PHEWAS) have been made feasible and accessible to much of the research community due to advances in technology and high throughput computing. Much like a GWAS, a PHEWAS can identify associations between genes of interest and clinical phenotypes. More specifically, PHEWAS calculates the relationship between a SNP or genetic variant and a pre-determined set of clinical phenotypes.[16] A clinical phenotype is any observable / measurable characteristic related to a disease or disability. This would be something such as a frailty score to measure physical disability. By using PHEWAS technologies, we can identify SNPs that are associated with physical disability phenotypes of interest. Using this technology, it is possible to identify one or a few SNPS that have pleiotropic properties in which they affect multiple pathways related to phenotypes of interest. This is an important component of a PHEWAS because it allows for novel therapeutics to target one specific gene / SNP that effects various pathways which induces multiple phenotypic changes.

1.4 Physical Function Phenotypes

1.4.1 Hand Grip Strength

Hand grip strength is one of the most widely used measures of muscle function and is well-validated. Not only this, but hand grip strength is an accurate predictor of functional limitation and disability as well as all-cause mortality.[17] The ease of measurement using a dynamometer is what makes this test suitable in a clinical setting. Research has shown many associations between

SNPs of interest and variation in hand grip strength. Within the CASR gene, polymorphisms of the rs1801725 SNP have been associated with increased serum calcium levels which is directly related to lower hand grip strength.[18] Other studies have documented strong associations between muscle function and Vitamin D receptor gene. Polymorphisms in this gene have been known to decrease hand grip strength and thus overall muscle function.[19] Not only do studies identify polymorphisms that result in decreased hand grip strength, but others have documented increases in hand grip strength. A polymorphism in the UCP3 gene has been associated with higher UCP expression and thus increased hand grip strength.[20] Using hand grip strength is one of the most feasible and viable ways to measure physical function.

1.4.2 Gait Speed

Gait speed is another measurement of physical disability, but unlike hand grip strength, it is a measurement of mobility disability. Typically, participants walk 3,4 or 6 meters and are awarded points based upon their speed.[21] This test is an accurate predictor of mobility disability and can represent ADL risk. Although many factors can contribute to low gait speed, genetic studies have shown associations between genetic variants and gait speed. A polymorphism in the IL 18 gene has been known to induce higher IL-18 serum levels which is directly related to lower gait speed.[22] The C polymorphism is related to lower serum levels and increased walk speed. Through meta analyses of multiple GWAS, other researchers have been able to identify over 530 suggestive and 10 significant SNPS that were associated with gait speed.[23] Identifying this association can provide the framework needed for genetic therapies and future therapeutics.

1.4.3 Chair Rise

Chair rises are a measure of physical limitation / function. They are performed by having the participant rise out of a chair as quickly as they can without using the arms for assistance.[21] Studies have shown that poor performance on chair rise tests is a strong predictor of ADL and represents low physical activity levels.[24] Gene studies often choose chair rise as a phenotype when measuring physical disability. For example, six different SNPS located within the CRP, TNF-a, and LTA inflammatory genes had strong interactions with physical activity and physical limitations.[25] Researchers concluded that these SNPS have strong associations with lower extremity performance and thus represent ADL risk. By identifying genetic variants that are associated with chair rise times, researchers will be able to identify a point source for the disability and provide potential therapeutics.

1.4.4 Lung Function

Measurements taken of lung function is another way of determining physical limitations. Lung function is often measured by spirometry, in which the participant blows into a tube that measures the speed and volume of air moving in and out. Although lung function may not seem like a measure of physical disability, it has been associated with various physical tests (walk speed, walk distance, and self-reported physical activity).[26] From a genetic point of view, hundreds of suggestive SNPS have been identified as having a direct relationship with lung function. A GWAS study has identified over 200 genes that have associations with lung function, in which further PHEWAS analysis identified pleiotropic pathways related to lung function and multiple SNPS.[27]

This shows that lung function is an important measure of physical disability due to its effect on multiple physical disability phenotypes.

1.4.5 ¼ Mile Walk

A quarter mile walk test is one of the most widely used performance measures to test mobility disability. Participants are timed for fast paced vs normal paced quarter mile times. Participants with physical limitation typically have a smaller variation between the two times compared to those without physical limitation.[28,29] This is an accurate measure of mobility disability.[29] From a genetic point of view, research suggests that genetic factors may be associated with lower variation in the quarter mile walk test. A polymorphism in the ACE gene has been reported to show increased odds of physical limitation / ADL as measured by the quarter mile walk test.[30] Not only does the ACE gene have an influence quarter mile walk times, but it is also shown to have influence on walking speed and variation in general.[31] The quarter mile walk time is a well validated measure of mobility disability and can also be used as a measure of other physical phenotypes.

1.4.6 SPPB Score

The Short Physical Performance Battery (SPPB) test is a composite of balance, gait speed, and chair rises.[32] This test is well validated and accurately measures physical limitation.[33] Scores are given to participants based upon their performance. A score less than 9 is indicative of a physical limitation.[33] Since this test is a composite, it gives the participants a chance to make up for poor test scores which reduces biases. Genetics play an important role in SPPB score and

specific polymorphisms may induce better or worse scores. The IL-18 gene has shown important roles on physical activity.[22,34] Specifically, polymorphisms in the IL-18 gene result in differences in IL 18 serum levels which dictates physical function. In a test of SPPB, increased serum levels were associated with lower SPPB scores which indicated a physical limitation.[22] Not only this, but meta-analyses show that the C polymorphism in the rs5744256 SNP shows an association with increased SPPB scores and walk times.[34] This conclusion shows the importance of IL-18 gene regulation on SPPB scores, which is a validated measure of physical limitation.

1.4.7 Lean Mass

Lean muscle mass is an accurate measure of lower extremity skeletal strength and is often a predictor of hip fracture and other physical disabilities.[35] Like many other predictors of physical disability, lean muscle mass has been studied in the past and proven to have genetic precursors associated with low mass.[35,36] For example, GWAS analyses have suggested genetic variation of the FADS1, FADS2, and DCHS1 genes is known to have strong associations with increased lean muscle mass.[35] Other studies have shown that genetic variants in the NEB and TNFSF9 genes are known to have associations with lower lean muscle mass and skeletal system development.[36] These studies have provided insight on the genetic component to lean mass and how it is a factor related to physical disability progression.

1.5 Risk Factors of Physical Disability

1.5.1 Lifestyle Risk Factors

There are many risk factors associated with physical disability. Identifying and changing certain lifestyle habits is generally the first line of treatment for physical disability because they can be manipulated without medical intervention. These risk factors constitute as a habit or lifestyle choice that is increasing the odds of physical disablement. For example, an NHANES follow-up study aimed to identify factors related to physical disability. Results of the study showed that not engaging in regular physical activity accounts for variability in physical disability diagnoses.[37] Increased sedentary time and lack of physical activity are two key predictors of physical disability. Along with this, increased BMI has a positive correlation with physical disability. Participants with larger BMI had increased odds of developing a physical disability throughout life.[37] From an environmental point of view, the regression model also identified lack of education, low caloric intake, and low income as statistically significant predictors of physical disability.[37] Another study aimed to determine how BMI impacts moderate to severe physical disability. Results of the study showed that severity of disability had a positive correlation with increasing waist circumference (P-value = 0.03).[38] Similarly, a cross sectional study with 8,681 participants aimed to identify lifestyle risk factors associated with physical disability. They categorized physical disability into three stages; emerging, progressing, and progressed to social restriction. Results of logistic regression showed that increased BMI / obesity was strongly associated with mobility disability across the three stages of disability (Stage 1: OR=1.56, CI=1.34-1.82. Stage 2: OR=3.19, CI=2.38-4.27. Stage 3: OR=2.87, CI=1.90-4.32).[39] They also identified an inverse association between levels of physical activity and severity of all three stages

of disability.[39] The similarities between these studies suggest that lifestyle habits have an important role in physical disability and changes should be made in those struggling with declines in function.

1.5.2 Chronic Health Risk Factors

There are many chronic health conditions that are risk factors related to physical disability. The same NHANES follow-up study from above showed that variability in physical disability scores can be attributed to arthritis and aging.[37] Along with these, a small variability in the scores can be attributed to asthma, cardiovascular disease, history of polio and allergies, hypertension, kidney disease, hip fractures, and chronic pulmonary disease.[37] Having a combination of these conditions often result in a worse state of physical disability. A similar study identified risk factors for ADl and iADl disabilities in a Norwegian population. Like the NHANES results, the Norwegian study had similar conclusions. Using logistic regression, they also identified depression as a major risk factor for ADL and iADL formation.[40] Along with this, many studies have identified hypertension and diabetes as being strongly associated with physical disability.[37,39,41,42] One specific study found a joint association between obesity and hypertension, which shows the impact of an interaction between lifestyle and health risk factors. Results of this study show that those with increased BMI and hypertension have increased risk of ADL disability (OR=1.40, CI=1.05-1.89).[41] They also identified individuals who are hypertensive but have extremely low BMI as being at an increased risk of ADL disability (OR=2.14 CI=1.36-3.36).[41] These studies suggest that chronic health conditions play an important role in the progression from physically capable persons to acquiring a physical disability.

1.5.3 Genetic Risk Factors

Genetic risk factors are often under studied but may play a pivotal role in physical disability progression. Genomic studies have identified genes that are associated with physical disability through the use of GWAS analyses.[23,35,36,43] These studies aim to identify candidate genes or individual SNPs that are associated with a physical disability phenotype. Meaning that results of the analysis will locate which part of the genome is correlated with physical function. GWAS studies are meant to be hypothesis generating in that they may identify novel genes, SNPS or loci that affect physical function. Most genetically induced physical disabilities are caused by variation in genes which are measured by individual SNPS.[18,19,44] These are typically the main targets for this research. For example, a candidate gene study identified a I/D polymorphism of the ACE gene has significant association with men's long jump, a measure of physical function.[45] It is imperative to understand the importance that the genome potentially has on physical function so that the underlying cause of disability can be identified. More on genetic studies will be mentioned in section 1.6.

1.5.4 Treatments

Treatment options for those suffering from physical disability are limited. The most common and effective treatment is simple lifestyle changes such as exercise, diet, and mental health wellness. Research has shown that those suffering with a physical disability often have low physical activity levels.[46,47] Along with this, association analyses have shown that increases in physical activity have beneficiary effects on those with disabilities.[46] The LIFE study tested the hypothesis that long term physical activity programs are more effective than health education in

reducing the risk of mobility disability. Results of the study showed that long term physical activity programs reduced the risk of mobility disability in older adults.[48] Although promising, physical activity alone isn't enough to reverse the detrimental effects of physical disability. The results of the LIFE study showed that the benefit of physical activity in older adults tends to plateau after 2.6 years of engaging in the program. Along with this, older adults and those who are physically disabled may find it hard to engage in a consistent physical regimen. This shows how physical activity alone can be a difficult treatment to not only adopt but maintain over the course of many years.

Research has been attempting to find therapeutics for physical disability as compared to lifestyle changes alone. Vitamin D has been a target of research in past research, but results have not been promising. A meta-analysis aimed to identify the therapeutic benefits of Vitamin D treatment on those suffering from Multiple Sclerosis, a physical disability related disease. Results of the study showed that there is little to no evidence of benefits to engaging in a Vitamin D treatment for those with MS.[49] There were no proven effects of improving disability. Another systematic review identified Vitamin D as having a small significant benefit to sarcopenia / frailty patients but only for those who are deficient in vitamin D.[50] Another treatment option that has undergone extensive research is testosterone treatment. Although the results are slightly more hopeful, much more research is needed to provide concrete results. For instance, a longitudinal RCT followed men for 3 years while monitoring their muscle function. Those who were receiving testosterone treatments had modest but significant increases in muscle mass, power, and stair climbing capabilities.[51] They also mentioned that there is a need for future studies that identify testosterone as a therapeutic for those struggling with a physical disability. This is due to the vagueness in that area of research. These studies shine light on the fact that supplement treatments

may not be the best option for physical disability treatment. However, recent advancements in genetics and high throughput processing have made it feasible to identify strong associations between physical disabilities and biological factors. It is imperative to study the biological and genetic risk factors associated with physical decline in hopes to provide the most effective therapeutic for those at greatest risk.

1.6 Genetic Studies

1.6.1 Candidate Gene Studies

Historically, candidate genes were utilized to study biological candidate genes of interest before it was feasible to generate genome-wide scans. However, results from these studies often result in discovery of significantly associated genes. One of the most widely studied genetic variant for physical disability is within the ACE gene. More specifically, studies have shown that polymorphisms in the rs4646994 SNP are strongly associated with physical disability phenotypes including frailty, long jump, walk speed, mobility, hand grip strength, and adl.[44,45,52,53] One candidate gene study identified the ACE gene as having a strong association in men's long jump results and other muscle related tests.[45] Similarly, they also found strong associations between physical performance and the ACTN3 and PPARA genes.[45]

Other candidate gene studies have identified the APOE gene as having associations with physical function phenotypes.[54–56] However, results of these studies are contradicting. Blazer et. al tested the association between the APOE candidate gene and physical function as the main effect. Results showed that there was no association as the main effect, but there was an

association between the APOE E4 allele interacted with gender.[54] Within women, the E4 allele was able to predict functional decline. Verghese et. al also identified associations with APOE E4 and physical function. Unlike the previous study, they identified associations in declining male gait speed (p=0.04) and increased risk of ADL in men (p=0.007).[55] This once again shows the differences by sex, but results are contradicting. Another candidate gene study identified the SOX6 gene as being strongly associated with wrist BMD.[57] Those carrying the C allele had significantly lower BMD in the wrist compared to those with the T allele.[57] This study recognizes that there are also genetic associations with biological measurements, not just performance measures. Due to the variability in results, it is imperative to provide more candidate gene studies on associations between genetic variation and physical disability phenotypes so that concrete conclusions can be drawn.

1.6.2 Genome Wide Association Studies (GWAS)

As genetic sequencing prices decreased it became possible to generate genetic data at the genome wide level. This led to the advent of the Genome-wide association study (GWASs), which is a hypothesis generating study design where millions of SNPs from across the genome are tested against a phenotype. GWAS studies of physical function have included phenotypes such as muscle mass / strength, hand grip strength, gait speed, and weakness.

One GWAS / replication study focused on lean muscle mass and strength in Caucasian men. Replication studies utilize a discovery sample (N=1627) and then compare results with a replication sample (N=2286). Through the use of GWAS the researchers were able to identify 3 SNPs that had dual associations with Compressive Strength Index and Appendicular Lean Mass, both accurate measures of muscle function.[35] The researchers then performed the study another

time (replication sample) to increase replicability and the results were the same. Other findings have suggested that SNPs in the NEB and RIF1 genes have strong associations with increased skeletal muscle strength.[36] De Mars et. al performed a genome wide scan to identify genes associated with muscle strength. Results of this analysis showed that a small contribution from multiple genes has a greater association with muscle strength compared to one or two important singular genes.[58]

Another popular GWAS chosen phenotype to measure physical function is hand grip strength. A widely popular genomic wide association meta-analysis study (GWAMA) that focuses on 14 cohorts aged 65 and older (CHARGE) utilized hand grip strength as a phenotype. Results of the study have identified two genome wide significant associations (p= 5 x 10^-8) and 39 suggestive associations (p= 5 x 10^-5), thus showing multiple parts of the genome that are significantly associated with variation in hand grip strength.[59] Further, another meta-analysis using the CHARGE cohort identified associations between genomic regions and hand grip strength as a measure of muscle strength. Results of the analyses identified 221 genes that were significantly associated with hand grip strength after adjusting for all chosen covariates.[60] However, some of the identified regions were only significant in micro-cohorts based on sex and age.

Gait speed is another validated phenotype used in GWAS studies examining physical function. Once again, the CHARGE cohort (N=31,478) is being used as a study sample for these GWAMA. Ben-Avraham et. al performed a GWAMA in hopes to discover genetic variation responsible for gait speed. Results of the analysis revealed 69 suggestive genes associated with gait speed.[23] However, strong associations could not be drawn. Further, pathway analysis concluded that gait speed is a polygenic complex trait with associations in five major networks. [23]

These pathways and further eQTL analyses have suggested that the genetic effects on gait speed are derived from synaptic / neural pathways.[23] Another GWAS study utilized the IlSIRENTE cohort that consists of 286 participants aged greater than 80 years. Although this study has a small sample size, the results identified two SNPS, rs928874 (p= 5.61 10^-8) and rs1788355 (p= 5.73 10^-8) as being significantly associated with 4 meter gait speed.[61] However, the study was replicated using a different cohort and the results were not replicated. This shines light on the need for future large scale GWAS studies in hopes to identify concrete conclusions.

One of the most promising GWAS studies on physical disability was performed by Jones et. al 2021.[62] This study used low muscle strength as an indicator of poor health / performance. This phenotype has been related to morbidity and poor health. Researchers used this phenotype in their GWAS study that identified 15 loci that are associated with muscle weakness. The top two hits were HLA-DQA1 (p = 4 x 10^-17), which is involved in arthritis / inflammation, and GDF5 (p = 4 x 10^-14), which has known function in musculoskeletal regulation and cellular development.[62] This makes GDF5 a prime target for future genetic studies. More in-depth research of GDF5 shows that it plays a crucial role in bone / tissue development, neuronal regulation, and also stimulates cytokine responses. Along with this, there is a nearby regulator called GROW1, that also has direct involvement in skeletal formation. The results of this GWAS study were able to identify this important region of the genome that not only has a biological role in physical formation, but also has underlying genetic associations with physical function.

Through the use of this new technology and high throughput sequencing, researchers have been able to identify hundreds of potential genes responsible for physical disability.[23] These studies show the important role that genetics has on physical disability progression. Further research will provide insight for novel therapeutics that may target these genes in which they can

alter multiple pathways that are associated with physical disability phenotypes, thus resulting in better overall health.

1.7 Gaps in Knowledges

Treatment options for physical disabilities are limited. Those that are available such as lifestyle changes (physical activity regimen, diet, etc.) and medications have contradicting results. More specifically, lifestyle changes are often hard to accept and maintain over the duration of a lifetime. The result is a lack of therapeutic options for those with a physical disability. This makes it imperative to study the underlying biological mechanisms that are associated with physical disabilities so that genomic therapeutics can be discovered. Large scale genome wide association studies have identified hundreds of candidate SNPs that are associated with physical disability and function. This provided the framework for future genetic studies. However, to our knowledge, there is little research done using large scale PHEWAS analysis that considers multiple phenotypes and their association with prior proven SNPS of interest that are related to physical disability phenotypes. More specifically, we are using the prior GWAS study from Jones et. al that has identified promising regions within the genome to focus on the GDF5 +/- flanking regions. This region is promising due to it's biological involvement in physical function and known associations. Along with this, we will adjust for height in our model and test it's effect on the associations. Again, to our knowledge this is a novel approach and is leading the way for future studies that investigate this effect. Results of this study would identify candidate SNPs of interest that have proven associations with pleiotropic pathways, thus influencing multiple phenotypes related to

physical disability. Results of this study would fill the gaps in knowledge and provide novel conclusions while using PHEWAS analyses.

2.0 Objective

This study focuses on the genetic contributions to physical function and phenotypes that are in the disablement pathway. The objective of this study is to identify genetic variants within GDF5 and its flanking regions that are associated with a wide range of physical disability phenotypes. To achieve this goal, we employed a targeted PHEWAS approach on a cohort of adults from exceptionally long-lived families (Long Life Family Study). The LLFS has both GWAS data and comprehensive phenotyping. Along with identifying SNPs that are associated with our chosen phenotypes, we also aimed to identify SNPs that exhibit pleiotropy – being associated with more than 1 phenotype. If we can identify a SNP that has influence on multiple phenotypes related to physical disability, then this can indicate an important region of the genome related to physical function. We hypothesize that multiple SNPs of interest within our desired region will have pleiotropic properties related to physical disability phenotypes.

3.0 Methods

3.1 Study Population

For these analyses, genetic and phenotypic data were obtained from the Long Life Family Study (LLFS), which is a familial cohort selected for exceptional longevity. The study population was recruited from three US field centers (Boston MA, New York NY, and Pittsburgh PA) and one in Denmark (Odense, Denmark) field centers. To be eligible, participants and families needed to meet the following criteria: a long lived individual (proband) which was considered to be 90 years old in Denmark and 80 years old in the US, at least one enrolled sibling of the proband, at least one enrolled offspring of either the proband or their sibling(s), and exceptional familial longevity (Family Longevity Selection Score $\geq$7).[63] Once a family was identified, all members of the proband were attempted to be enrolled. Those who agreed signed consent and participated in all study protocols. The baseline visit consisted of phenotyping, blood collection, and DNA extraction. The participants were then invited back for follow-up visits, but these were optional.

3.2 Phenotyping of Physical Disability

All phenotypes were measured at the baseline visit:

Lung Function: FEV1 is a quantitative marker of lung function in which participants are measured on air expiration (in liters) through a spirometer for one second. Three tests are performed to ensure consistency. Lung function was measured with a portable spirometer

(EasyOne, ndd Medical Technologies, Andover, MA) and standardized using American Thoracic Society guidelines.[64,65]

Grip Strength: Grip strength was measured using a JAMAR handheld dynamometer (Sammos Preston Rolyan, Bolingbrook, IL).[63] Participants performed the measure with their dominant hand.

SAVE: To eliminate the ceiling effect of the fried fatigability score and achieve greater differentiation of vigor/frailty status, the Scale of Aging Vigor in Epidemiology was designed.[66,67] SAVE was a composite that included weight change (Unintentional weight change was scored as: 0 (no weight change), 1 (weight gain), 2 (weight loss)), weakness (grip strength), fatigue (questionnaire), physical activity (days walked in prior 2 weeks), and slowness (gait speed), each component scored 0, 1 or 2 using approximate tertiles, and discretely summed from 0 (vigorous) to 10 (frail).[67] LLFS specific values were used for these measures. Each phenotype was measured using the priorly mentioned descriptions. For more details on SAVE, see Sanders et al. 2016.[67]

SPPB: Physical function was measured with SPPB, which includes 3 tests: 4-m gait speed, a balance battery, and 5 repeated chair-rises; each test was scored from 0 to 4, with 4 being best, for a total SPPB score of 0–12.[21,63] **Gait Speed** was measured with two timed usual paced 4-meter walks, and the fastest time was used to calculate gait speed (m/s).[68] **Chair rise** was reported as the time (s) it took to complete 5 repeated chair rises.[63] Gait speed and Chair rise were treated as separate, continuous traits. In order to include those unable to complete the chair-rise test due to a physical problem, in sensitivity analyses, prior statisticians calculated the number of chair-rises/10 s ([5/s to complete]*10), assigning 0 to those unable to complete the task.[32]

Mobility Disability: This phenotype consists of two different components: participants self-reported the ability to walk a quarter mile and / or the ability to walk a flight of steps.

Participants were defined as having mobility disability if they were unable to perform one of or both tasks.

3.3 Genotyping and Gene Boundaries

Genotypic data was generated using Illumina 2.5M Omni SNP array. The Illumina Human Omni 2.5 v1 genotyping array (Illumina Inc., Ca) was used to genotype 2.5 million single-nucleotide polymorphisms. Imputed genetic SNPs were determined based on the reference panel from the NHLBI's TOPMed program,[69] freeze 5b (September 2017) using Minimac4 on the Michigan Imputation Server.[70] Details of the deep-coverage whole genome sequencing methods used in TOPMed are available at the provided references.[71–73] Finally, an MAF cutoff of >0.01 was employed for all SNPs and an r^2 cutoff of ≥.7 for imputed SNPs.

SNPs were pulled within the GDF5 gene and the chosen flanking region which included the GROW1 region, which is an enhancer to GDF5. Gene boundaries were defined based on the Ensembl genome browser (using HGNC Symbol) human genome build GRCh38, and we included all SNPs within a flanking region of +/- 200 kb from the 5"UTR and 3" UTR boundaries. This ensured that the boundaries encompassed the entire gene and potential promotors / enhancers up and downstream of GDF5. By ensuring this extended region, SNPs within GROW1 were able to be captured. Then, an MAF cutoff of >0.01 was employed for all SNPs and an r^2 cutoff of ≥.7 for imputed SNPs.

3.4 Statistical Analysis

For analytical analysis, a phenome wide association study of the GDF5 gene (with flanking regions) was performed. For each association, we used separate mixed general linear model that included a kinship matrix to adjust for familial relatedness and were additionally adjusted for age, sex, and study site. To test for possible mediation via height, we included a model with and without height for each phenotype and compared changes to coefficients and p-value before and after height adjustment. To determine if associations were stronger at older ages, we performed the association analyses in all age groups, and again just in those aged 60+. Prior literature has shown stronger associations in older populations. Nominally significant SNPs were defined as SNPs with a p-value equal to or less than the alpha level of 0.05. A False Discovery Rate (FDR) analysis was utilized to account for multiple comparisons. FDR significant SNPs were defined as SNPs with an FDR p-value equal to or less than the FDR alpha level of 0.05 – which means that ~5% of significant SNPs would be expected false positives.[74]

We employed LD clumping, which takes into account LD between SNPs, and resulted in a final SNP-set that included 1 independent SNP per LD block. The following parameters for clumping were used: clumping region = 246kb, clump r^2 = 0.5, and clump p-value = 0.99. The output included one lead independent SNP per clumping region instead of all SNPs within a specific LD clump.[75] We then annotated our overall association results using LocusZoom plots.[76] An LD heatmap using the new set of SNPs was created (Fig. 5) using the tool, LDMatrix.[87] All analyses were performed on RStudio V4.0.0.

4.0 Results

4.1 Descriptive Results

Mean baseline characteristics are listed in Table 1. There were a total of 4,440 participants with genetic and phenotypic data, 2441 of which were women and 1999 were men. The participants were aged 69.8 ± 15.60 years with a range of 24 – 108 years.

4.1.1 Primary Results: SNP vs. Phenotype Associations

We examined a total of 5,900 SNPs in the locus of interest. Association analyses showed that none were significant after accounting for multiple comparisons (all SNPS FDR P >0.05), with any phenotype. After height adjustment, in all participants, there were 25 SNPs that reached nominal significance (nominal p< 0.05), while 43 SNPs reached nominal significance in those 60+ (Table 3). The phenotype with the most nominally significant SNPs was gait speed adjusted for height in the 60+ population. This phenotype had 12 nominally significant SNPs.

The three phenotypes with the most PHEWAS hits (nominally significant) were: FEV1 in the 60+ group adjusted for height, grip in the 60+ group, and the mobility composite in the 60+ group. These phenotypes were annotated using locus zoom plot. The Manhattan plot for Fev60 adjusted for height is represented in Figure 2 and also shows gene location. Figure 3 represents a Manhattan plot of grip in the 60+ group and shows the top nominally significant SNPs along with gene location. Figure 4 represents the Manhattan plot for the mobility composite in the 60+ group and also shows nearby gene locations.

Table 1 Descriptive Statistics of Cohort and Phenotypes

Characteristic	Values
Men (n)	1999
Women (n)	2441
Men (%)	45%
Women (%)	55%
Mean Age (yrs)	69.8
Mean BMI	27.1
Mean Grip Strength (lb)	29.4 lb
Mean Gait Speed (m/s)	1.03 m/s
Mean SPPB Score	10.05
Mean Chair Rise (s)	11.07 s
Mean SAVE	3.1
Mean FEV (L)	2407.9L
Mean Mob Comp	0.74

Table 2 Significant SNPs ($p < 0.05$) in All Age Groups Amongst Both Models

Phenotype	RS#s
Chair Adjusted for Height	rs180706374
FEV1 Without Height Adjustment	rs2425046, rs73093063, rs118147506, rs1204660
FEV1 Adjusted for Height	rs1204660, rs73093063, rs73109573, rs13037879, rs144799504
Gait Without Height Adjustment	rs112511428, rs2425048, rs34935167, rs35556948, rs118147506, rs151031674, rs142885156
Gait Adjusted for Height	rs112511428, rs34935167, rs35556948, rs151031674, rs78282190

Grip Without Height Adjustment	rs118147506, rs6088788, rs146422112, rs2425050, rs35395621, rs138297660, rs619865, rs55865073, rs666006, rs56259282, rs2425062, rs145452534
Grip Adjusted for Height	rs118107529, rs146422112, rs666006, rs56259282, rs142135093, rs78753171, rs55865073, rs138297660
SPPB Without Height Adjustment	rs151031674, rs35395621, rs180706374, rs144643409, rs35238802, rs78282190, rs224343
SPPB Adjusted for Height	rs151031674, rs35395621, rs144643409, rs180706374, rs35238802, rs78282190

Table 3 Significant SNPs (p < 0.05) in the 60+ Age Group Amongst Both Models

Phenotype	RS#s
Chair Without Height Adjustment	rs8114520, rs34935167
Chair Adjusted for Height	rs8114520, rs34935167, rs118147506
FEV1 Without Height Adjustment	rs2425046, rs118147506, rs73093063, rs1204660, rs34935167, rs71350377, rs17332368, rs2425051, rs3748433
FEV1 Adjusted for Height	rs1204660, rs73093063, rs2425046, rs34935167, rs118147506
Gait Without Height Adjustment	rs34935167, rs117783353, rs139266467, rs35556948, rs151031674, rs79447658, rs2425048, rs118147506
Gait Adjusted for Height	rs34935167, rs139266467, rs151031674, rs35556948, rs79447658, rs117783353, rs1204660, rs78282190, rs144643409, rs35395621, rs224371, rs145452534
Grip Without Height Adjustment	rs118147506, rs62211528, rs8117162, rs146422112, rs73094730, rs619865
Grip Adjusted for Height	rs118147506, rs62211528, rs73094730, rs118107529, rs112321518
Mob Composite Without Height Adjustment	rs17421899, rs112511428, rs62210588, rs941664, rs3748433, rs11696589, rs8120559, rs1204660, rs8114520
Mob Composite Adjusted for Height	rs17421899, rs112511428, rs62210588, rs941664, rs8120559, rs3748433, rs35556948
SAVE Without Height Adjustment	rs139266467, rs180706374, rs35556948
SAVE Adjusted for Height	rs139266467, rs180706374, rs35556948
SPPB Without Height Adjustment	rs117015941, rs151031674, rs35395621, rs224343, rs41290924, rs180706374
SPPB Adjusted for Height	rs151031674, rs117015941, rs35395621, rs41290924, rs180706374, rs17331061, rs7274597, rs224343

Table 4 Number of SNPs Meeting Each p Criterion After Height Adjustment (n=68)

Phenotype	**All Ages, # of SNPs p <0.05**	**60+ Age, # of SNPs p <0.05**	**All Ages, # of SNPs p <0.01**	**60+ Age, # of SNPs p <0.01**	**All Ages, # of SNPs p <0.001**	**60+ Age, # of SNPs p <0.001**
Chair Rise	1	2	0	1	0	0
FEV1	3	3	2	2	0	0
Gait Speed	4	8	1	4	0	0
Grip Strength	7	4	1	1	0	0
Mobility Comp.	0	4	0	3	0	0
SAVE	0	3	0	0	0	0
SPPB	4	8	2	0	0	0

4.2 Results Without Height Adjustment

The non-height adjusted model resulted in 30 nominally significant SNPs in all ages and 43 nominally significant SNPs in 60+. Nominally significant SNPs by phenotype is represented in Tables 2 and 3.

4.3 Height Adjusted Results

Nominally significant SNPs after height adjustment are listed in Tables 2 and 3. After height adjustment, there were 25 nominally significant SNPs in all age groups and 43 nominally

significant SNPs in the 60+ group. After adjusting for height, the all age group had 12 SNPs that were no longer significant across phenotypes but also identified 8 additional SNPs. In the 60+ population, there were 12 SNPs that were no longer significant after height adjustment across phenotypes, but 12 newly identified SNPs were discovered.

4.4 Age Adjusted Results

Based on age, the 60+ group had 43 nominally significant SNPs before height adjustment and 43 after height adjustment. The all age group had 30 nominally significant SNPs before height adjustment and 25 after. These results are listed in Table 2 and Table 3.

4.5 Pleiotropic SNPS

Pleiotropic SNPs were defined by being significant in three or more phenotypes. The most pleiotropic SNP, RS11814750 was nominally significant with nine phenotypes. Looking at Table 5, which represents pleiotropic SNPs, there were a total of 22 phenotypes (height adjusted and non-adjusted) associated with the pleiotropic SNPs in the 60+ group compared to 12 phenotypes in the all age group. Taking the top pleiotropic SNPs, a review of literature was performed using the GWAS catalog. Searching the GWAS catalog, rs1204660 has been statistically significant in two other studies. One that investigated a trait of venous thromboembolism, and the other BMI hip circumference. None of the other top pleiotropic SNPS have publishable data in the GWAS catalog.

4.6 LD Scores

All significant SNPs were annotated to show the LD amongst each other. This is represented in Figure 5. Darker shades of red represent a larger R^2 value while darker shades of blue show higher values of the D' statistic. D' is a scaled version of the D statistic. A D' value of 1 shows that the two SNPs are inherited together 100% of the time, thus high LD. R^2 implies the squared value of the correlation between the two SNPs. An R^2 value of 1 shows that there is high LD / inheritance. The difference between D' and R^2 is that D' will show true correlation / inheritance between the SNPs even if one of the polymorphisms is rare. R^2 values can deflated when one of the alleles are considered rare, regardless of disequilibrium.

Table 5 Pleiotropic SNPs With Three or More Phenotypes

RS	**Phenotypes**
rs8114520	Chair 60 W/ Height, Chair 60, Mobility Composite 60
rs34935167	Chair 60 W/ Height, Chair 60, Fev60, FEV 60 W/ Height, Gait, Gait 60, Gait 60 W/ Height, Gait W/ Height
rs118147506	Chair 60, FEV, FEV 60, FEV 60 W/ Height, GAIT, GAIT 60, Grip, Grip 60, Grip 60 W/ Height
rs180706374	ALL SPPB Phenotypes, Chair W/ Height, SAVE 60, SAVE 60 W/ Height
rs1204660	ALL FEV, Gait 60 W/ Height, Mobility Composite 60
rs35556948	ALL Gait, Mobility Composite 60, SAVE 60, SAVE 60 W/ Height

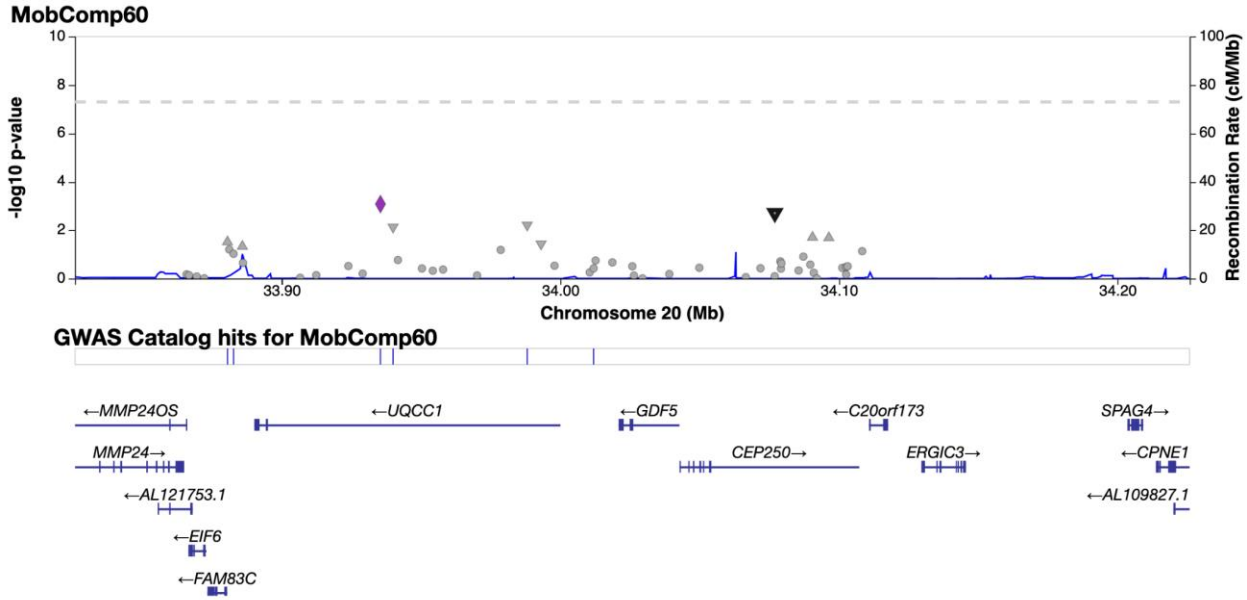

Figure 2

This figure represents an advanced Manhattan plot of the mobility composite phenotype in the 60+ population. Relevant gene locations are listed below the plot.

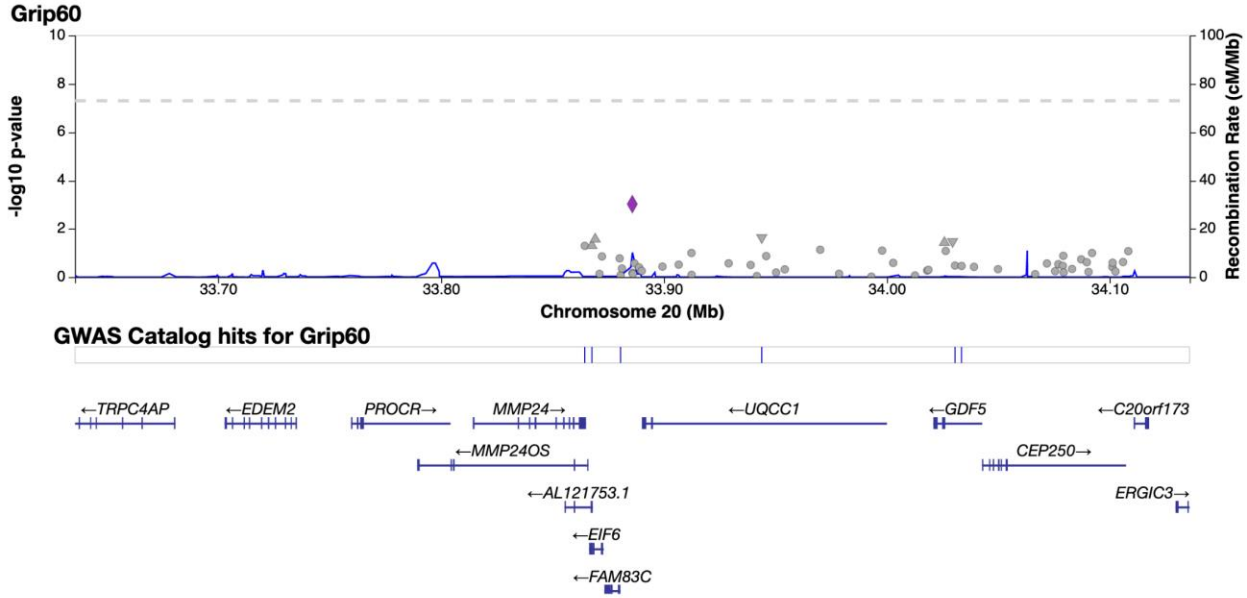

Figure 3

This figure represents an advanced Manhattan plot of the Grip phenotype in the 60+ population. Relevant gene locations are listed below the plot.

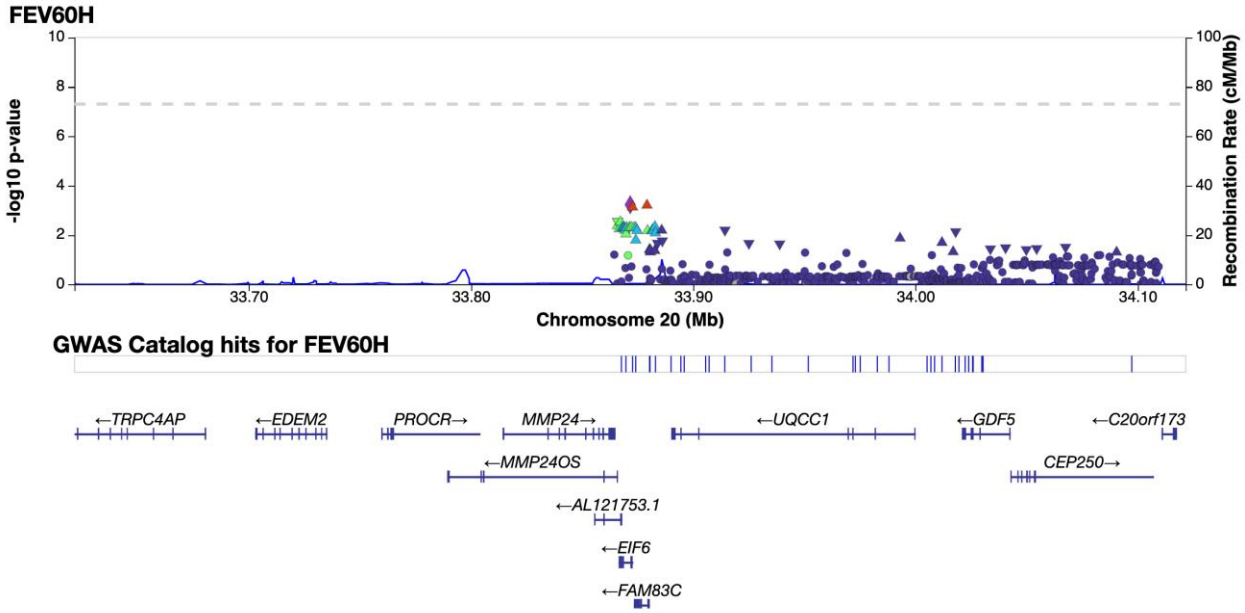

Figure 4

This figure represents an advanced Manhattan plot of the FEV adjusted for height phenotype in the 60+ population. Relevant gene locations are listed below the plot.

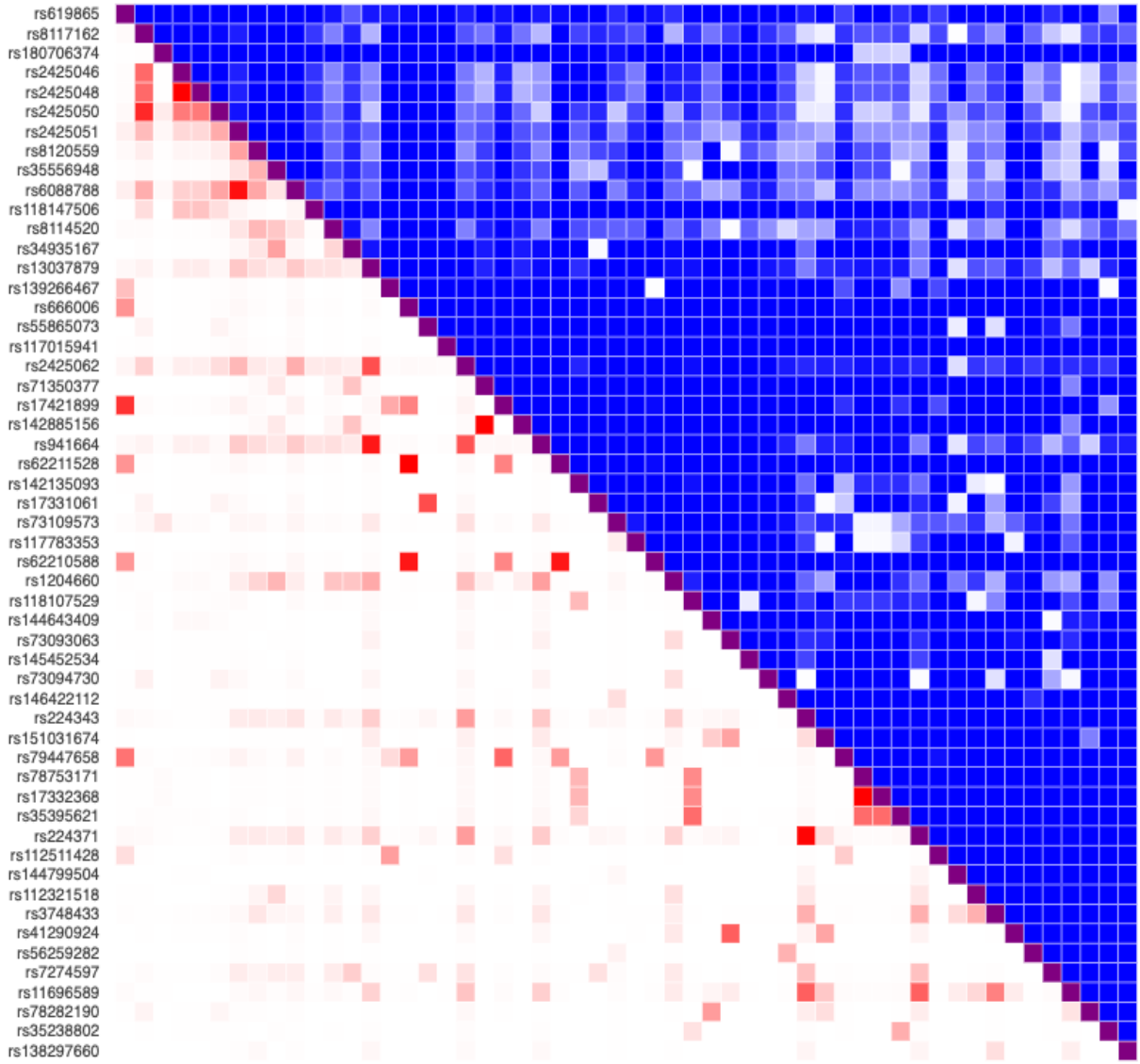

Figure 5

LD heatmap of all nominally significant SNPs. Darker shades of red indicate higher R2 values while darker shades of blue indicate high D'. Annotation performed using the LDMatrix extension and Interactive map at https://analysistools.cancer.gov/LDlink/?tab=ldmatrix.

5.0 Discussion

There were no SNPs in the GDF5 +/- flanking regions associated with physical function phenotypes after adjustment for multiple comparisons. However, there were multiple SNPs within this region that showed pleiotropic properties, as they were nominally significant in more than one phenotype. In fact, there were SNPs that were nominally significant in as many as four different phenotypes. Further, height adjustment affected the association between some SNPs and the outcome of interest. Specifically, we identified SNPs that were associated with a phenotype without height adjustment but were attenuated to non-significance after adjusting for height – and vice versa. These SNPs that were only significant in the non-adjusted model may be acting through pathways associated with height rather than the trait itself. However, after adjusting for height, the GDF5 gene region appears to influence physical function through pathways unrelated to height, as a number of SNPs remained significant after height adjustment. It was also evident that the GROW 1 region may have a major influence on physical function. The three phenotypes with the most PHEWAS hits were annotated using Locus Zoom Plot, and most of the nominally significant SNPs in these three phenotypes were in or near the GROW1 enhancer region, harbored in the UQCC gene. Biologically, UQCC1 and the GROW1 region have mechanisms in musculoskeletal development and osteoarthritis.[77] Therefore, UQCC1 shows promise as a potential candidate gene for physical function. Since most SNPs were in the GROW1 region, and not GDF5, this suggests that the enhancer may have more involvement in physical function compared to GDF5 itself. Previous studies have shown that polymorphisms in GROW1 result in decreased GDF5 expression and lower physical function.[78] However, more genetic analyses targeting GROW1 and UQCC are necessary to elucidate these findings.

There were a set of SNPs that exhibited pleiotropic properties. For example, RS11814750 was associated with 4 phenotypes, in all ages and in those aged 60+. These phenotypes were chair rise, FEV, gait speed, and grip strength, which are widely used measures of physical disability and all are associated with mortality.[17,24,26] This shows promise that rs11814750 can have an important effect on physical function. Indeed, chair rise and gait speed are the two measures that comprise mobility disability, thus, rs11814750 may be particularly important for mobility. According to the GWAS catalog, NHGRI Catalog of Human Genome Wide Association Studies, no prior publications have been made associating this SNP.[79] Another promising SNP with pleiotropic pathways is RS1204660 which is in the UQCC1 gene / GROW1 (2.5kb)[78] region. This SNP was associated with FEV, gait, and the mobility composite. The GWAS catalog shows that this SNP is also associated BMI and venous thromboembolism.[80,81] BMI is a well-known, strong risk factor for physical disability.[82] Again, these SNPs are in the GROW1 region which acts as a major downstream enhancer to GDF5. Biologically, GDF5 and its GROW1 enhancer region are involved in musculoskeletal development. For example, prior research has shown that GDF5 expression initiates cartilage and long bone morphogenesis.[83] Along with this, polymorphisms in the GROW1 region have been known to decrease the effects that this enhancer has on GDF5 function.[78] There is a strong biological premise that mutations in these SNPs could lead to a decrease in physical function.

Age stratification showed varying results within these associations. Specifically, associations were stronger, and more SNPs were significant in in those aged 60+. This result is similar to prior publications that showed physical disability is more prevalent in older age groups and that there are more associations between the SNPs and phenotypes.[6,10,11]

Another main finding was the effect of height on associations between SNPs and phenotypes of interest. There were SNPs that were significant both before and after height adjustment. This suggested that these SNPs may affect physical function via shared pathways with height and through pathways that are unrelated to height. For example, rs34935167 was significant for Gait speed in both non-adjusted and adjusted models which can represent the associations of multiple pathways between height and function. Using model 1 which did not adjust for height, we identified a list of SNPs that were significant with the chosen phenotypes, however, some of these SNPs became non-significant after adjustment for height. Similarly, there were SNPs that were only significant after height adjustment. For example, in the models that contained SSPB and gait as their outcomes, height had an important effect on certain SNPs. These traits contain similar aspects to one another and measure mobility which could be why they had novel SNPs after height adjustment. Referring to figures 2 and 3, height adjustment identified 3 SNPs within these two phenotypes (all ages) that were no longer significant after adjustment. Meaning that the non-adjusted SNPs could be acting through height related pathways and not physical function itself. A potential hypothesis of this action can be explained through physiology. Certain phenotypes such as gait speed can be influenced by the participants height (ie. Longer strides are equivalent to faster times). This is also true for the SPPB score which is a composite with similar components. These traits are similar in the fact that taller height provides a benefit to better scores. This result partly explains the variation in the effect that height adjustment has on the associations. Therefore, the SNPs that are only present in non-adjustment models may not have a "direct" effect on physical function but rather influence physical function via pathways involved with height. Prior literature on this topic is limited, as it is novel to investigate the effect that height has as a mediator between physical disability phenotypes and SNPs. However, a single SNP association study was done that

investigated hand grip strength adjusted for height. After adjustment, they found that hand grip strength was associated with the allele, while non-adjustment had no significant associations.[84] Also, the GWAS catalog shows that some SNPs associated with our phenotypes are also associated with height.[85] From a biological point of view, height does have a physiologic impact on physical function. Longer bones / more height tends to result in longer and stronger muscle fibers. Along with this, evidence shows that there is a direct correlation between bone growth and muscle growth.[86] Putting together the effect we see between the adjusted models and the biological aspects of height; we now realize that the SNPs associated only in the unadjusted models may be acting through other pathways related to height rather than physical function itself.

Although there were no FDR significant associations, we identified nominally significant SNPs that are associated with multiple physical disability phenotypes. We were also able to show that height adjustment identifies SNPs that act on physical function itself rather than related pathways. Adjustment for height identified SNPs that seemed to be associated with phenotypes of interest but were really acting through height related pathways. We also demonstrated the potential importance of the GROW1 enhancer region for physical function – especially in older adults. However, based upon the D' statistic in Figure 5, a lot of the SNPs we identified are in high LD with SNPs from GDF5 regardless of the clumping code that was enacted.[62] Thus, the variants truly driving the effect could not be identified and further research is needed before forming concrete conclusions. The fact that D' seems to show high LD amongst all SNPs while the R^2 values vary can tell us that some of these SNPs are rare / in varying frequencies. However, based upon these results and biological plausibility, variations in the GROW1 enhancer region and GDF5 gene could be important to physical function regardless of the effect of height. There is a

need for further research to determine specific mechanisms, but this sets the groundwork for future genetic association studies that focus on this region.

6.0 Public Health Significance

Multiple studies have shown that basic changes in physical activity can be enough to reduce the risk of physical decline in later years.[33,32] However, these lifestyle interventions are difficult to adopt long-term and for those at highest risk for functional decline. To extend these benefits, it is imperative to elucidate biological mechanisms underlying age-related declines in physical function. Understanding the genetic component to physical decline and function is necessary to improve quality of life and overall mobility. With such a large prevalence[10,11,12] of physical disability within older adults, it is important to not only provide preventative measures but also treatment options. Identifying the core genetic component to physical disabilities will allow for intervention measures to attack disability at its source. Results of this PHEWAS analysis identified potential candidate SNPs that affect multiple phenotypes related to the physical disablement pathway. These results can give rise to novel discoveries within future studies which will provide the framework necessary for novel therapeutics and prevention measures that will counteract effects of physical disabilities from a genetic point of view and thus resolve the public health problem of lack of treatment for physical disability.

Bibliography

1. Cesari M, Kritchevsky SB, Penninx BWHJ, et al. Prognostic value of usual gait speed in well-functioning older people--results from the Health, Aging and Body Composition Study. J Am Geriatr Soc 2005;53(10):1675–80.

2. Guralnik JM, Ferrucci L, Pieper CF, et al. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. J Gerontol A Biol Sci Med Sci 2000;55(4):M221-31.

3. Fried TR, Bradley EH, Williams CS, Tinetti ME. Functional disability and health care expenditures for older persons. Arch Intern Med 2001;161(21):2602–7.

4. Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R. The healthcare costs of sarcopenia in the United States. J Am Geriatr Soc 2004;52(1):80–5.

5. Studenski S, Perera S, Patel K, et al. Gait speed and survival in older adults. JAMA 2011;305(1):50–8.

6. The Administration for Community Living. 2020 Profile of Older Americans. Administration on Aging- U.S. Department of Health and Human Services; 2020.

7. Verbrugge LM, Jette AM. The disablement process. Soc Sci Med 1994;38(1):1–14.

8. Welcome Researchers | NHATS [Internet]. [cited 2022 Feb 3];Available from: https://nhats.org/researcher

9. Freedman VA, Agree EM, Cornman JC, Spillman BC, Kasper JD. Reliability and validity of self-care and mobility accommodations measures in the National Health and Aging Trends Study. Gerontologist 2014;54(6):944–51.

10. Seeman TE, Merkin SS, Crimmins EM, Karlamangla AS. Disability trends among older Americans: National Health And Nutrition Examination Surveys, 1988-1994 and 1999-2004. Am J Public Health 2010;100(1):100–7.

11. Sjölund B-M, Wimo A, Engström M, von Strauss E. Incidence of ADL Disability in Older Persons, Physical Activities as a Protective Factor and the Need for Informal and Formal Care--Results from the SNAC-N Project. PLoS ONE 2015;10(9):e0138901.

12. Millán-Calenti JC, Tubío J, Pita-Fernández S, et al. Prevalence of functional disability in activities of daily living (ADL), instrumental activities of daily living (IADL) and

associated factors, as predictors of morbidity and mortality. Arch Gerentol Geriatr 2010;50(3):306-10.

13. Sex differences in the prevalence of mobility disability in old age:the dynamics of incidence, recovery, and mortality. The Journals of Gerontology Series B: Psychological Sciences and Social Sciences 2000;55(1):S41–50.

14. Santanasto AJ, Miljkovic I, Cvejkus RC, et al. Body composition remodeling and incident mobility limitations in african ancestry men. J Gerontol A Biol Sci Med Sci 2019;74(3):400–5.

15. Mendes de Leon CF, Rajan KB. Psychosocial influences in onset and progression of late life disability. J Gerontol B Psychol Sci Soc Sci 2014;69(2):287–302.

16. Strayer N, Shirey-Rice JK, Shyr Y, Denny JC, Pulley JM, Xu Y. PheWAS-ME: a web-app for interactive exploration of multimorbidity patterns in PheWAS. Bioinformatics 2021;37(12):1778–80.

17. Norman K, Stobäus N, Gonzalez MC, Schulzke J-D, Pirlich M. Hand grip strength: outcome predictor and marker of nutritional status. Clin Nutr 2011;30(2):135–42.

18. Alfred T, Ben-Shlomo Y, Cooper R, et al. Genetic markers of bone and joint health and physical capability in older adults: the HALCyon programme. Bone 2013;52(1):278–85.

19. Bozsodi A, Boja S, Szilagyi A, Somhegyi A, Varga PP, Lazary A. Muscle strength is associated with vitamin D receptor gene variants. J Orthop Res 2016;34(11):2031–7.

20. Crocco P, Montesanto A, Passarino G, Rose G. A common polymorphism in the UCP3 promoter influences hand grip strength in elderly people. Biogerontology 2011;12(3):265–71.

21. Guralnik JM, Simonsick EM, Ferrucci L, et al. A short physical performance battery assessing lower extremity function: Association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol 1994;49(2):M85-94.

22. Frayling TM, Rafiq S, Murray A, et al. An interleukin-18 polymorphism is associated with reduced serum concentrations and better physical functioning in older people. J Gerontol A Biol Sci Med Sci 2007;62(1):73–8.

23. Ben-Avraham D, Karasik D, Verghese J, et al. The complex genetics of gait speed: genome-wide meta-analysis approach. Aging (Albany NY) 2017;9(1):209–46.

24. Bryant MS, Kang GE, Protas EJ. Relation of chair rising ability to activities of daily living and physical activity in Parkinson's disease. Arch Physiother 2020;10(1):22.

25. Liu C-S, Li T-C, Li C-I, et al. Gene-physical activity interactions in lower extremity performance: inflammatory genes CRP, TNF-α, and LTA in community-dwelling elders. Sci Rep 2017;7(1):3585.

26. Simpson CF, Punjabi NM, Wolfenden L, Shardell M, Shade DM, Fried LP. Relationship between lung function and physical performance in disabled older women. J Gerontol A Biol Sci Med Sci 2005;60(3):350–4.

27. Shrine N, Guyatt AL, Erzurumluoglu AM, et al. New genetic signals for lung function highlight pathways and chronic obstructive pulmonary disease associations across multiple ancestries. Nat Genet 2019;51(3):481–93.

28. Lange-Maia BS, Newman AB, Strotmeyer ES, Harris TB, Caserotti P, Glynn NW. Performance on fast- and usual-paced 400-m walk tests in older adults: are they comparable? Aging Clin Exp Res 2015;27(3):309–14.

29. Hardy SE, Kang Y, Studenski SA, Degenholtz HB. Ability to walk 1/4 mile predicts subsequent disability, mortality, and health care costs. J Gen Intern Med 2011;26(2):130–5.

30. Kritchevsky SB, Nicklas BJ, Visser M, et al. Angiotensin-converting enzyme insertion/deletion genotype, exercise, and physical decline. JAMA 2005;294(6):691–8.

31. Buford TW, Hsu F-C, Brinkley TE, et al. Genetic influence on exercise-induced changes in physical function among mobility-limited older adults. Physiol Genomics 2014;46(5):149–58.

32. Santanasto AJ, Glynn NW, Lovato LC, et al. Effect of Physical Activity versus Health Education on Physical Function, Grip Strength and Mobility. J Am Geriatr Soc 2017;65(7):1427–33.

33. Lang IA, Guralnik JM, Melzer D. Physical activity in middle-aged adults reduces risks of functional impairment independent of its effect on weight. J Am Geriatr Soc 2007;55(11):1836–41.

34. Thomas K, Rafiq S, Frayling TM, et al. Interleukin-18 polymorphism and physical functioning in older people: a replication study and meta-analysis. J Gerontol A Biol Sci Med Sci 2009;64(11):1177–82.

35. Han Y, Pei Y, Liu Y, et al. Bivariate genome-wide association study suggests fatty acid desaturase genes and cadherin DCHS2 for variation of both compressive strength index and appendicular lean mass in males. Bone 2012;51(6):1000–7.

36. Yoon KJ, Yi Y, Do JG, Kim H-L, Lee Y-T, Kim H-N. Variants in NEB and RIF1 genes on chr2q23 are associated with skeletal muscle index in Koreans: genome-wide association study. Sci Rep 2021;11(1):2333.

37. Hubert HB, Bloch DA, Fries JF. Risk factors for physical disability in an aging cohort: the NHANES I Epidemiologic Followup Study. J Rheumatol 1993;20(3):480–8.

38. Livne-Margolin M, Tokatly Latzer I, Pinhas-Hamiel O, Harari G, Achiron A. Bodyweight Measures and Lifestyle Habits in Individuals with Multiple Sclerosis and Moderate to Severe Disability. J Clin Med 2021;10(10).

39. Yamada K, Yamaguchi S, Ito YM, Ohe T. Factors associated with mobility decrease leading to disability: a cross-sectional nationwide study in Japan, with results from 8681 adults aged 20-89 years. BMC Geriatr 2021;21(1):651.

40. Storeng SH, Sund ER, Krokstad S. Factors associated with basic and instrumental activities of daily living in elderly participants of a population-based survey: the Nord-Trøndelag Health Study, Norway. BMJ Open 2018;8(3):e018942.

41. Su P, Ding H, Zhang W, et al. Joint Association of Obesity and Hypertension with Disability in the Elderly-- A Community-Based Study of Residents in Shanghai, China. J Nutr Health Aging 2017;21(4):362–9.

42. Lamirault G, Artifoni M, Daniel M, Barber-Chamoux N, Nantes University Hospital Working Group On Hypertension. Resistant hypertension: novel insights. Curr Hypertens Rev 2020;16(1):61–72.

43. Atkins JL, Jylhävä J, Pedersen NL, et al. A genome-wide association study of the frailty index highlights brain pathways in ageing. Aging Cell 2021;20(9):e13459.

44. Chen S, Hao Q, Yang M, et al. Association between Angiotensin-converting enzyme insertion/deletion polymorphisms and frailty among chinese older people. J Am Med Dir Assoc 2015;16(5):438.e1-6.

45. Ahmetov II, Gavrilov DN, Astratenkova IV, et al. The association of ACE, ACTN3 and PPARA gene variants with strength phenotypes in middle school-age children. J Physiol Sci 2013;63(1):79–85.

46. Motl RW, McAuley E. Physical activity, disability, and quality of life in older adults. Phys Med Rehabil Clin N Am 2010;21(2):299–308.

47. Moreira-Silva I, Santos R, Abreu S, Mota J. The effect of a physical activity program on decreasing physical disability indicated by musculoskeletal pain and related symptoms among workers: a pilot study. Int J Occup Saf Ergon 2014;20(1):55–64.

48. Pahor M, Guralnik JM, Ambrosius WT, et al. Effect of structured physical activity on prevention of major mobility disability in older adults: the LIFE study randomized clinical trial. JAMA 2014;311(23):2387–96.

49. Jagannath VA, Filippini G, Di Pietrantonj C, et al. Vitamin D for the management of multiple sclerosis. Cochrane Database Syst Rev 2018;9:CD008422.

50. Pfortmueller CA, Lindner G, Exadaktylos AK. Reducing fall risk in the elderly: risk factors and fall prevention, a systematic review. Minerva Med 2014;105(4):275–81.

51. Storer TW, Basaria S, Traustadottir T, et al. Effects of testosterone supplementation for 3 years on muscle performance and physical function in older men. J Clin Endocrinol Metab 2017;102(2):583–93.

52. Yoshihara A, Tobina T, Yamaga T, et al. Physical function is weakly associated with angiotensin-converting enzyme gene I/D polymorphism in elderly Japanese subjects. Gerontology 2009;55(4):387–92.

53. Seripa D, Paroni G, Matera MG, et al. Angiotensin-converting enzyme (ACE) genotypes and disability in hospitalized older patients. Age (Dordr) 2011;33(3):409–19.

54. Blazer DG, Fillenbaum G, Burchett B. The APOE-E4 allele and the risk of functional decline in a community sample of African American and white older adults. J Gerontol A Biol Sci Med Sci 2001;56(12):M785-9.

55. Verghese J, Holtzer R, Wang C, Katz MJ, Barzilai N, Lipton RB. Role of APOE genotype in gait decline and disability in aging. J Gerontol A Biol Sci Med Sci 2013;68(11):1395–401.

56. Sakurai R, Montero-Odasso M. Apolipoprotein E4 allele and gait performance in mild cognitive impairment: results from the gait and brain study. J Gerontol A Biol Sci Med Sci 2017;72(12):1676–82.

57. Tan L, Liu R, Lei S, et al. A genome-wide association analysis implicates SOX6 as a candidate gene for wrist bone mass. Sci China Life Sci 2010;53(9):1065–72.

58. De Mars G, Windelinckx A, Huygens W, et al. Genome-wide linkage scan for maximum and length-dependent knee muscle strength in young men: significant evidence for linkage at chromosome 14q24.3. J Med Genet 2008;45(5):275–83.

59. Matteini AM, Tanaka T, Karasik D, et al. GWAS analysis of handgrip and lower body strength in older adults in the CHARGE consortium. Aging Cell 2016;15(5):792–800.

60. Pilling LC, Joehanes R, Kacprowski T, et al. Gene transcripts associated with muscle strength: a CHARGE meta-analysis of 7,781 persons. Physiol Genomics 2016;48(1):1–11.

61. Heckerman D, Traynor BJ, Picca A, et al. Genetic variants associated with physical performance and anthropometry in old age: a genome-wide association study in the ilSIRENTE cohort. Sci Rep 2017;7(1):15879.

62. Jones G, Trajanoska K, Santanasto AJ, et al. Genome-wide meta-analysis of muscle weakness identifies 15 susceptibility loci in older men and women. Nat Commun 2021;12(1):654.

63. Santanasto AJ, Cvejkus RK, Wojczynski MK, et al. Circulating Procollagen Type III N-Terminal Peptide and Physical Function in Adults from the Long Life Family Study. J Gerontol A Biol Sci Med Sci 2021;76(7):1273–9.

64. Kulminski AM, Barochia AV, Loika Y, et al. The APOE ε4 allele is associated with a reduction in FEV1/FVC in women: A cross-sectional analysis of the Long Life Family Study. PLoS ONE 2018;13(11):e0206873.

65. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005;26(2):319–38.

66. Sanders JL, Boudreau RM, Fried LP, Walston JD, Harris TB, Newman AB. Measurement of organ structure and function enhances understanding of the physiological basis of frailty: the Cardiovascular Health Study. J Am Geriatr Soc 2011;59(9):1581–8.

67. Sanders JL, Singh J, Minster RL, et al. Association between mortality and heritability of the scale of aging vigor in epidemiology. J Am Geriatr Soc 2016;64(8):1679–83.

68. Renner SW, Qiao Y, Gmelin T, et al. Association of fatigue, inflammation, and physical activity on gait speed: the Long Life Family Study. Aging Clin Exp Res 2022;34(2):367–74.

69. NHLBI Trans-Omics for Precision Medicine WGS-About TOPMed [Internet]. [cited 2022 Jun 20];Available from: https://topmed.nhlbi.nih.gov/

70. Das S, Forer L, Schönherr S, et al. Next-generation genotype imputation service and methods. Nat Genet 2016;48(10):1284–7.

71. Li B, Zhan X, Wing M-K, Anderson P, Kang HM, Abecasis GR. QPLOT: a quality assessment tool for next generation sequencing data. Biomed Res Int 2013;2013:865181.

72. Conomos MP, Reiner AP, Weir BS, Thornton TA. Model-free Estimation of Recent Genetic Relatedness. Am J Hum Genet 2016;98(1):127–48.

73. Jun G, Flickinger M, Hetrick KN, et al. Detecting and estimating contamination of human DNA samples in sequencing and array-based genotype data. Am J Hum Genet 2012;91(5):839–48.

74. False Discovery Rate | Columbia Public Health [Internet]. [cited 2022 Jul 3];Available from: https://www.publichealth.columbia.edu/research/population-health-methods/false-discovery-rate

75. Perform LD clumping on SNP data — ld_clump • ieugwasr [Internet]. [cited 2022 Jul 3]; Available from: https://mrcieu.github.io/ieugwasr/reference/ld_clump.html

76. Boughton AP, Welch RP, Flickinger M, et al. LocusZoom.js: Interactive and embeddable visualization of genetic association study results. Bioinformatics 2021;

77. UQCC1 Gene - GeneCards | UQCC1 Protein | UQCC1 Antibody [Internet]. [cited 2022 May 31]; Available from: https://www.genecards.org/cgi-bin/carddisp.pl?gene=UQCC1

78. Capellini TD, Chen H, Cao J, et al. Ancient selection for derived alleles at a GDF5 enhancer influencing human growth and osteoarthritis risk. Nat Genet 2017;49(8):1202–10.

79. GWAS Catalog [Internet]. [cited 2022 May 27]; Available from: https://www.ebi.ac.uk/gwas/

80. Christakoudi S, Evangelou E, Riboli E, Tsilidis KK. GWAS of allometric body-shape indices in UK Biobank identifies loci suggesting associations with morphogenesis, organogenesis, adrenal cell renewal and cancer. Sci Rep 2021;11(1):10688.

81. Greliche N, Germain M, Lambert J-C, et al. A genome-wide search for common SNP x SNP interactions on the risk of venous thrombosis. BMC Med Genet 2013;14:36.

82. Lv Y-B, Yuan J-Q, Mao C, et al. Association of body mass index with disability in activities of daily living among chinese adults 80 years of age or older. JAMA Netw Open 2018;1(5):e181915.

83. UniProt [Internet]. [cited 2022 Jul 7];Available from: https://www.uniprot.org/uniprot/P43026

84. Shimizu Y, Kawashiri S-Y, Arima K, et al. Association between height-related polymorphism rs17081935 and reduced handgrip strength in relation to status of atherosclerosis: a cross-sectional study. Environ Health Prev Med 2021;26(1):83.

85. Tachmazidou I, Süveges D, Min JL, et al. Whole-Genome Sequencing Coupled to Imputation Discovers Genetic Signals for Anthropometric Traits. Am J Hum Genet 2017;100(6):865–84.

86. Parfitt AM. The attainment of peak bone mass: what is the relationship between muscle growth and bone growth? Bone 2004;34(5):767–70.

87. Nci, Cbiit, Dceg, Machiela. LDlink | An Interactive Web Tool for Exploring Linkage Disequilibrium in Population Groups.