ASSOCIATION OF LEUKOCYTE TELOMERE LENGTH AND GENETIC BIOMARKERS OF LEUKOCYTE TELOMERE LENGTH WITH PERCEIVED PHYSICAL AND MENTAL FATIGABILITY

by

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BS in Biological Sciences, University of Pittsburgh, 2019

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Submitted to the Graduate Faculty of the

Department of Epidemiology

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Public Health

University of Pittsburgh

2020

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

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on

December 7, 2020

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Abstract

Background: Fatigue is a common complaint in older adults, and perceived fatigability is associated with disability and mortality. In the Long Life Family Study (LLFS), perceived physical and mental fatigability are heritable, although genetic biomarkers related to fatigability have not been identified. Genome wide linkage and association analysis has identified multiple loci associated with leukocyte telomere length (LTL), a potential marker of cellular aging.

Objective: The primary objective of this analysis was to determine whether LTL predicts physical and mental fatigability. A secondary objective was to investigate the relationship between genetic biomarkers associated with LTL and fatigability.

Methods: In LLFS, LTL was assayed at baseline (2006-2009) and perceived physical and mental fatigability were measured at Visit 2 (2014-2017) using the validated Pittsburgh Fatigability Scale (PFS, 0-50, higher scores indicating greater fatigability). We performed multivariate linear regression with continuous PFS outcomes and logistic regression with previously established cut-points (≥15 for physical and ≥13 for mental fatigability) to determine whether LTL and SNPs associated with LTL were predictive of fatigability. All models accounted for family structure and were adjusted for field center.

Results: Shorter LTL predicted higher PFS Physical scores (β=2.7, p<0.0001) and greater physical fatigability (OR=1.50, 95%CI [1.22, 1.85], p=0.0001). After adjusting for sex, chronic conditions, and lifestyle factors, the relationship remained significant for continuous scores (β=1.7, p<0.0001) and trended non-significant for the dichotomous outcome (OR=1.38, 95%CI [1.10, 1.73], p=0.057). Shorter LTL predicted higher PFS Mental scores (β=1.7, p=0.0004) and greater mental fatigability (OR=1.35, 95%CI [1.03, 1.78], p=0.032). After full adjustment, the relationship was borderline for continuous scores (β=0.8, p=0.055) and non-significant for greater mental fatigability (OR=1.11, 95%CI [1.21, 1.48], p=0.50). None of the SNPs investigated were associated with fatigability.

Conclusion: LTL predicted physical and mental fatigability, and was more closely related to physical fatigability. Genetic markers examined were not related to fatigability. Additional research to characterize the genetic basis of fatigability and its role in the disability pathway will allow interventions that preserve function in older adults, which will be increasingly important for public health as older adults make up a growing proportion of the overall population.

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Preface

I would like to thank the members of my essay committee, Dr. Kammerer and Dr. Zmuda for their advice and guidance. I would especially like to thank Dr. Glynn, my academic advisor, research mentor, and teacher for over three years.

I would also like to acknowledge all participants, staff, and researchers of the Long Life Family Study for making this work possible, as well as the National Institute on Aging for providing funding.

1.0 INTRODUCTION

1.1 AGING AND FATIGUE

By 2050, the proportion of the global population aged 60 or older is expected to increase to 22%, from 11% in 2016.[1] In the United States, there are expected to be 95 million adults aged 65 or older by 2060, accounting for 23% of the total population.[2] As life expectancy increases and the overall population ages, functional decline and disability related to the aging process are increasingly a focus of public health research and practice. Specifically, identifying early signs of disability and interventions that can prevent the progression to disability is necessary to allow older adults to remain independent and functional as they age.[3]

Fatigue is an important component of the disability pathway. It is a major symptom of many chronic diseases related to the aging process, and a common proximate cause of functional decline and disability.[4] In many cases fatigue in older adults is not explained by an underlying physiological or psychological condition, but still causes difficulty performing activities of daily living, reduces quality of life, and increases risk of negative health outcomes.[3]

1.2 FATIGUE AND FATIGABILITY IN OLDER ADULTS

1.2.1 Definitions and Measurement

Fatigue is a common complaint in older adults. It is a broad concept with varying definitions, but generally refers to an unpleasant feeling of tiredness experienced throughout the whole body, that can have physical, mental, and/or emotional components.[5] Currently, there is no method for measuring fatigue that is widely agreed to be a "gold standard"; different scales have been developed for use in specific sub-populations, such as older adults and cancer patients.[6]

Fatigability is defined as "performance deterioration or perceived effort to perform a standardized task."[7] Although it is related to fatigue, past research has shown that fatigability is a more sensitive measure. This is especially true in older adult populations, in which self-reported measures of fatigue often give misleading results. In epidemiological research, one way of measuring fatigue is by having individuals perform a physical task (such as walking a set distance) and rate their perceived exertion. However, when performing strenuous activities older individuals tend to "self-pace," reducing the energy demand of the task to avoid exceeding a comfortable level of exertion. This results in underestimating susceptibility to fatigue related to performing the activity.[8] Another method of ascertaining level of fatigue is through survey questions that ask about "global fatigue," or tiredness, exhaustion, and other related concepts experienced during daily living (for example the question: "In the past month, on average how often have you felt unusually tired during the day, all (3), most (2), some (1) or none (0) of the time?").[9] This method of assessing fatigue is also flawed because it does not take into account variability in individual's normal level of physical activity. An individual with a high level of physical activity may report experiencing greater fatigue than a sedentary individual due to the

higher energy demand caused by their frequent physical activity, even if the sedentary individual is in fact more susceptible to fatigue. Evaluating physical and mental fatigability avoids these issues by tying perceived fatigue to a task of defined intensity and duration.[8] The comparative benefit of using fatigability measures, as opposed to self-reported fatigue, has been demonstrated by the ability of fatigability measures to predict impending functional decline in a population of older adults in which global fatigue questions were unable to predict functional decline.[9]

Fatigability can be measured as either perceived fatigability, in which individuals rate the level of exertion they experience or would experience in relation to a task of defined intensity, or performance fatigability, in which decreasing performance in a physical activity (for example, walking more slowly) is used to evaluate susceptibility to fatigue. Perceived fatigability can be measured by rating perceived exertion after performing a task, such as a treadmill walk of predefined speed and duration, or by self-reporting the expected exertion that would be associated with a given task.[10] The Pittsburgh Fatigability Scale (PFS) is a validated measure of perceived physical and mental fatigability in which participants are asked to rate the perceived physical and mental fatigue they would experience when performing activities of defined intensity and duration. The PFS Physical and Mental sub-scales have been validated and demonstrate test-retest reliability.[11–14] PFS score cut-points that define greater physical and mental fatigability have previously been established. Greater fatigability, as measured by the PFS, is predictive of mobility decline,[15] worsened neurobiological outcomes,[16] and all-cause mortality.[17]

1.2.2 Prevalence of Fatigue and Fatigability in Older Adults

Fatigue is considered to be one of the most common complaints in older adults, although the exact prevalence of fatigue is difficult to characterize due to the diverse set of methods used for measuring fatigue.[4,8,18–24]

Within the Long Life Family Study, the prevalence of greater perceived physical fatigability is 42.1% among those aged 60 and older. The prevalence of greater perceived physical fatigability is greater with age, ranging from 27.9% among those aged 60-69 to 89.5% among those 90 and older.[25] Higher perceived mental fatigability is less prevalent, experienced by 24.9% of LLFS participants 60 and older. It also increases with age, from 14.5% prevalence in participants 60-69 to 67.2% in those 90 and older.[26] A similar prevalence of higher fatigability was observed among mobility-intact adults aged 60-89 (n = 579) in the Baltimore Longitudinal Study of Aging, with 41.1% of participants experiencing higher perceived physical fatigability and 21.6% experiencing higher perceived mental fatigability.[15] In the Lifestyle Intervention and Independence for Elders (LIFE) Study of sedentary older adults (n = 1,635), the prevalence of higher perceived physical and mental fatigability were both found to be 65.5%.[16] The National Study of Health and Development, a British birth cohort study, collected data on perceived physical fatigability at age 68 for 1,580 adults. The prevalence of higher perceived physical fatigability was 49.4% among women, 37.0% among men, and 43.4% overall.[27]

1.2.3 Aging Outcomes Related to Fatigue and Fatigability

Fatigue can be considered a marker of the physiological decline throughout the aging process, and has been shown to predict functional decline, disability, and mortality in a diverse

set of older adult populations.[28–30] Fatigue is an important component of many chronic diseases including cancer, neurological disorders, and psychiatric conditions; fatigue is commonly studied among populations with these diseases.[6] A high level of fatigue is also associated with an increased hazard of all-cause mortality in the general population.[31]

Perceived physical and mental fatigability are also associated with important outcomes related to the aging process. Fatigability predicts functional decline and loss of mobility, and can therefore be used as a prognostic tool.[10] A continuous measure of perceived physical fatigability has been shown to predict all-cause mortality in adults aged 60 and older[17] and mobility decline in mobility-intact adults aged 60 to 89.[15] Perceived fatigability can also predict future declines in cognitive function, and is longitudinally associated with worsened executive functions.[32]

1.2.4 Heritability of Fatigue and Fatigability

In twin studies, the heritability of fatigue has been estimated to be as low as 6% and as high as 50%.[33,34] One twin study (n = 7,740 individual twins) found that genetic factors may play a larger role in "interfering fatigue" (fatigue that interferes with daily activities) among females (h = 0.26) than males (h = 0.06).[35] However, another twin study (n = 3.740, 1,991 twin pairs) of prolonged and chronic fatigue found the reverse, with a substantially higher estimate of heritability in men (h = 0.51) compared to women (h = 0.18).[36] A large-scale study within the Swedish Twin Registry population (n = 31,406 individual twins) concluded both genetic and environmental factors play a role in chronic fatigue, and did not find any significant differences between sexes.[37] The variability in these results is due in part to differences in how fatigue is defined and measured in each study, suggesting a standardized method for evaluating fatigue is

needed. Furthermore, studies attempting to identify specific genetic biomarkers related to fatigue and chronic fatigue have been inconclusive due to inadequate sample sizes.[38]

Perceived physical and mental fatigability has been shown to be heritable within the Long Life Family Study cohort. Within this population, physical fatigability is estimated to have a higher heritability ($h = 0.26$) than mental fatigability ($h = 0.17$).[26,39] Specific genetic biomarkers related to fatigability have not been identified.

1.3 TELOMERES

1.3.1 Leukocyte Telomere Length

Telomeres are regions at the ends of chromosomes that contain repetitive non-coding sequences of DNA. As cells divide, the total length of the DNA sequence decreases because DNA polymerase is unable to fully copy the lagging strand; telomeres protect coding DNA from being truncated by this process. However, after too many repeated cell divisions, telomeres will disappear completely, and a cell is no longer able to fully replicate coding DNA. Thus, telomeres are thought to be a cellular marker of aging, with shorter telomere lengths indicating a greater cumulative exposure to stresses (such as inflammation) has resulted in a higher number of cellular divisions and a lower potential to continue replicating.[40]

Leukocytes, or white blood cells, are immune cells that assist in the immune response to pathogens. Leukocyte telomere length (LTL) has been studied as a potential marker of the cellular aging process. Shorter LTL is associated with older age, age-related disease, and all-cause mortality.[41] Aging is the strongest known predictor of leukocyte telomere length.[42] These

associations, and the role of telomeres in cell division, support the use of LTL as a cellular marker of the aging process.

1.3.2 Genetics

There is strong evidence that leukocyte telomere length is partially determined by genetic factors.[43] Heritability estimates as high as 78% have been calculated from twin studies.[44] Within the Long Life Family Study, the heritability of LTL has been estimated at 54%.[45] Genome wide association and linkage analyses of the LLFS study population have identified 17 single nucleotide polymorphisms (SNPs) that are potential determinants of LTL.[46] These SNPs are all located within genes that have been previously linked to LTL in other populations.[47–52]

Like fatigability, longevity is known to be partially heritable. The results of twin studies indicate approximately 25% of the variation in human longevity can be attributed to genetic factors; however, the mechanisms by which genetics affect lifespan are mostly unknown.[53] A genome-wide association study of LTL with very large sample size (n = 37,684) identified seven loci that are associated with both LTL and a variety age-related disease including cancer and coronary artery disease.[54] This suggests the genetic determinants of LTL are also related to aging outcomes of interest, but further research is needed to characterize this relationship.

1.4 ASSOCIATIONS OF LEUKOCYTE TELOMERE LENGTH AND FATIGUE/FATIGABILITY

1.4.1 Leukocyte Telomere Length and Fatigue

Fatigability is a novel construct, and its relationship to leukocyte telomere length has not been previously studied. However, existing literature on the closely related concept of fatigue indicates fatigability is likely to be linked to LTL. A Danish twin study (n = 439) found an association between LTL and fatigue in older adults that remained significant after adjusting for age-related diseases and mental health conditions. Telomere shortening is accelerated by oxidative stress and inflammation, which are also known to contribute to a variety of age-related diseases, including cardiovascular disease. The oxidative stress and inflammation pathways could also contribute to overall fatigue, and explain the relationship between leukocyte telomere length and fatigue.[55]

In previous studies, leukocyte telomere length has been associated with a variety of outcomes related to physical fatigue. A study of working middle-aged adults (n = 2,911) found that those who reported severe work-related exhaustion had significantly shorter LTL, on average, than those who did not.[56] Chronic fatigue syndrome (CFS) is a medical condition defined by experiencing fatigue-related symptoms (including exercise intolerance, muscle weakness, and cognitive impairment) for more than six months. Among 639 individuals from the Georgie CFS surveillance study, those who met the case definition for CFS had significantly shorter relative telomere lengths than those who did not.[42] A case-control study of athletes (n = 26) found that participants who reported exercise-associated fatigue had shorter telomere length

in muscular cells, providing further evidence that accelerated shortening of telomeres may be a result of chronic exposure to stress.[57]

Shorter leukocyte telomere length is also related to several medical conditions related to mental (cognitive) fatigue. In a Danish study that compared men whose cognitive performance improved over middle age to those whose cognitive performance declined (n =190), average leukocyte telomere length was significantly shorter among those whose cognitive performance declined.[58] This suggests LTL is related to changes in cognition that occur during the aging process. A meta-analysis of 38 studies (total n = 34,347) concluded that shorter telomere length is associated with depression (both clinically diagnosed and self-reported), as well as severity of depression symptoms among those who are depressed. Of the 38 studies included in this analysis, 33 studied telomere length in leukocytes specifically. When comparing studies of leukocyte telomere length to studies of telomeres from other tissues, only the sub-set analyzing LTL had a significant association with depression outcomes.[59] This indicates that leukocyte telomere length in particular may be useful for studies of cognitive outcomes. Another meta-analysis of 27 studies (total n = 14,827) which included all adult psychiatric disorders as outcomes found a significant effect size for shorter leukocyte telomere length that did not significantly vary by psychiatric disorder, sex, or age.[60] It is unknown whether shorter LTL is associated with neurological conditions because it an indicator of greater cumulative exposure to stressors, or if having shorter telomeres is a direct cause of defects in nerve cells that lead to disease.[40]

These findings provide strong evidence of a relationship between leukocyte telomere length and fatigue, which suggests further insight may be gained by analyzing the relationship between LTL and fatigability, a more sensitive measure of susceptibility to physical and mental

fatigue. Current research shows that both physical and cognitive fatigue-related outcomes are related to leukocyte telomere length. Therefore, both physical and mental fatigability may be related to LTL.

1.4.2 Covariates of Fatigability and Leukocyte Telomere Length

The existence of several covariates known to be associated with both fatigability and LTL is further evidence of a possible relationship between the two. Associations between greater fatigability and older age, being female, having higher BMI, and higher levels of inflammation have been previously reported.[15,25–27] Older age, and higher levels of inflammation are also associated with shorter leukocyte telomere length. Higher BMI is predictive of shorter LTL in some studies, but others have found no relationship.[61] Age is among the strongest predictors of shorter LTL and greater fatigability, consistent with these characteristics being components of the aging process. Sex is the only variable that shows an inconsistent direction of association with LTL and fatigability, as being female is associated with greater fatigability but longer leukocyte telomere length.[61] Further research is needed to understand the mechanistic pathways that connect LTL, fatigability, and related covariates.

1.5 GAPS IN LITERATURE

Leukocyte telomere length is a potential biological marker of the cellular aging process; however, the mechanisms by which LTL affects aging are unclear. There is currently no research on the relationship between LTL and fatigability. Analyzing the relationship between leukocyte

telomere length and physical and mental fatigability will provide further insight into the role of LTL in the aging process. In addition, genetic biomarkers that contribute to varying levels of fatigability as individuals age have not been identified. Investigating whether the genetic loci known to be associated with shorter LTL may also predict greater levels of fatigability is one starting point for a deeper understanding of the genetic basis of fatigability.

The majority of the genetic basis of longevity is still unknown. LTL may be part of a mechanistic pathway by which an individual's genome affects their longevity. The Long Life Family Study is suitable for investigating for the genetic basis of longevity as it includes a large sample of young-old, old-old, and oldest-old adults, and has a family structure.[62]

1.6 PUBLIC HEALTH SIGNIFICANCE

Fatigability is highly prevalent among older adults and associated with disability, loss of function, chronic disease, and mortality; these are all important outcomes in aging populations. Leukocyte telomere length is also associated with aging and aging-related disease. Further insight into the genetic biomarkers and cellular mechanisms that contribute to fatigability will allow researchers and clinicians to identify individuals who have a higher risk of these outcomes and develop targeted interventions to improve health and longevity.

Leukocyte telomere length is a potential cellular biomarker of aging. A deeper understanding of the relationship between LTL and outcomes related to the aging process will allow researchers and clinicians to characterize aging on a cellular and biological level, leading to a more accurate characterization of the aging process than can be gained through chronological measures of age.

2.0 OBJECTIVE

The primary objective of the current research is to determine whether shorter leukocyte telomere length predicts higher PFS Physical and Mental scores and greater physical and mental fatigability in the Long Life Family Study population. The secondary objective is to investigate whether genetic biomarkers associated with leukocyte telomere length are also associated with physical or mental fatigability.

Hypotheses: Individuals with shorter leukocyte telomere length at baseline will have greater physical and mental fatigability scores at Visit 2. Individuals with SNPs associated with shorter leukocyte telomere lengths will have higher physical and mental fatigability scores.

3.0 METHODS

3.1 STUDY POPULATION

The Long Life Family Study (LLFS) is a longitudinal family study of aging and longevity in older adults with field centers in the United States and Denmark. Recruitment was performed by screening families to ensure they met longevity criteria using the family longevity selection score (FLoSS), which has been described previously.[63] At baseline (2006-2009) a total of 4,953 individuals from 539 families were recruited. In-person examinations were performed at baseline, followed by annual telephone follow-ups. A second round of in-person examinations was conducted from 2014-2017. Over 99% of the study population is white.

3.2 FATIGABILITY SCORE ASSESSMENT

The Pittsburgh Fatigability Scale (PFS), a validated measure of perceived fatigability, was introduced during LLFS Visit 2 (2014-2017). Participants rate expected physical and mental fatigue on a scale from 0 (no fatigue) to 5 (extreme fatigue) for 10 different activities, resulting in physical and mental sub-scores ranging from 0-50, with higher scores indicating greater fatigability. For participants missing 1-3 items scores can be imputed. Cut-points dividing scores

into higher and lower fatigability categories have previously been established for the physical and mental sub-scales.[10,15,16]

3.3 TELOMERE ASSAY AND GENOTYPING

In LLFS, blood and saliva sample collection was performed by technicians trained and certified by study personnel according to a central protocol agreed upon by all field centers. Participants who could not be visited in-person completed telephone interviews instead and had blood or saliva samples collected by a lab not affiliated with the study or a physician's office. After collection, samples were shipped to a central laboratory for DNA extraction.[62] The Center for Inherited Disease Research (CIDR) genotyped DNA samples using Infinium Human Omni 2.5 v1 SNP chips (Illumina, San Diego, CA). Quality control was performed by the Division of Statistical Genomics, Washington University in Saint Louis. Briefly, 83.774 SNPs with call rates below 98% and 3,647 SNPs with high rates of Mendelian errors were removed from the dataset, as were 18 individuals with call rates below 97.5%. Additional details can be found elsewhere.[46] Of the 17 SNPs that were identified as being associated with LTL in a previous genome-wide association study, data were available for 15.[46]

Leukocyte telomere length was assayed by performing real-time PCR with primers optimize for telomeres (T) and a single copy reference gene (S). A linear regression formula was then used to calculate LTL in base pairs from the T/S ratio.[46]

3.4 ASCERTAINMENT OF COVARIATES

In LLFS age was verified at baseline by matching participants to birth certificates, early-life census records, or other official documents such as a driver's license.[64] Demographic information including sex and educational history were also collected during Visit 1.[62] At Visit 2, height was measured to the nearest 0.1 cm using a Handi-stat set square (Perspective Enterprises, Portage, MI). Weight was measured to the nearest 0.1 kg using an electronic digital scale (SECA 841, Hanover, MD). These measurements were used to calculate body mass index (BMI) in kg/m^2. Medical history and lifestyle variables were ascertained at baseline, and updated during each annual follow-up and at Visit 2. Participants were asked to self-report a physician diagnosis of cancer (not including skin cancer), diabetes, hypertension, and arthritis. Self-reported history of diabetes and hypertension were confirmed by objective measures of blood pressure and blood glucose performed by centrally trained study staff at Visit 2 and the use of relevant medications.[25] The Framingham Physical Activity Index, which uses the time spent asleep, sedentary, and doing light/moderate/heavy physical activity on a typical day to calculate Metabolic Equivalent of Task (MET) hours/day.[65] Depression symptoms were measured using the Center for Epidemiologic Studies—Depression (CES-D) scale.[66]

3.5 STATISTICAL ANALYSIS

At LLFS Visit 2, complete physical fatigability data was collected for 2,564 participants and complete mental fatigability data was collected for 2,557. Fatigability scores were imputed for participants missing 1-3 items on each subscale (n = 104 physical, 96 mental). This resulted

in 2,668 participants with scores for the physical fatigability sub-scale, 15 of which were missing mental fatigability scores. There were no participants with scores for the mental sub-scale but not the physical sub-scale. The Pittsburgh Fatigability Scale has only been validated in adults 60 and older, so participants younger than 60 at Visit 2 (n = 293) were removed from the analysis, as were those missing leukocyte telomere length data. This resulted in a sample of 2,008 participants, of which 11 were excluded due to quality control issues relating to the physical activity measure, resulting in a final analytical sample of 1,997 (Fig 2).

Descriptive statistics (means and standard deviations for continuous variables and frequencies and proportions for categorical variables), including leukocyte telomere length, the prevalence of selected SNPs, age, sex, lifestyle factors (physical activity, BMI, and education) and age-related chronic conditions (diabetes, hypertension, cancer, depression symptoms, and arthritis) were generated for the two categories of physical and mental fatigability. The statistical significance of the differences between these categories was evaluated using two-sample t-tests for continuous variables and chi-squared tests for categorical variables.

Generalized linear models were created with continuous physical and mental fatigability subscale scores as outcomes to determine whether leukocyte telomere length is a significant predictor of fatigability. All models accounted for family relatedness and were adjusted for field center. Models adjusting for age, sex, lifestyle variables, and chronic conditions were also generated to characterize the effect of these variables on the relationship between LTL and fatigability. Generalized linear models were also used to determine whether candidate SNPs associated with leukocyte telomere length predict fatigability scores. Using the previously established cut-points for higher and lower physical and mental fatigability, logistic regression was used to calculate odds ratios for overall leukocyte telomere length and individual SNPs,

adjusted for covariates and accounting for family structure. For both linear and logistic regression, separate models were run by generation to evaluate whether associations between leukocyte telomere length and fatigability outcomes are consistent within each LLFS generation.

4.0 RESULTS

The age range within the final analytic sample was 60-108 years, with a mean age of 73.7 ± 10.4 years (Table 1). Mean follow-up time between Visit 1 and Visit 2 was 7.98 ± 1.08 years. The sample was 55.4% women and 83.6% had more than a high school education. The overall mean telomere length was 5.35 ± 0.48 kilo base pairs. Average telomere length was 52.0 base pairs shorter in the greater physical fatigability category ($p = 0.01$), 51.3 base pairs shorter in the greater mental fatigability category ($p = 0.03$), and 55.0 base pairs shorter in men than women ($p = 0.01$).

Participants with greater perceived physical fatigability reported lower levels of physical activity, higher prevalence of diabetes, depressive symptoms, and arthritis (all $p < 0.0001$). Self-reported history of cancer diagnosis ($p = 0.0001$) and overweight/obese BMI ($p = 0.03$) were more prevalent in those with greater perceived physical fatigability (Table 1). The associations between chronic disease and lifestyle covariates were similar for greater mental fatigability; however, greater mental fatigability was not significantly associated with cancer history ($p = 0.06$) or overweight/obese BMI ($p = 0.58$). Rates of hypertension did not differ by physical ($p = 0.90$) or mental ($p = 0.15$) fatigability.

Generalized linear models accounting for family structure and adjusted for field center were used to identify covariates significantly associated with leukocyte telomere length and fatigability outcomes (Table 2). Older age ($p < 0.0001$), lower levels of physical activity ($p <$

0.0001), less than high school education ($p = 0.04$), hypertension ($p = 0.03$), and history of cancer diagnosis ($p < 0.0001$) were associated with having shorter leukocyte telomere lengths. Associations between shorter leukocyte telomere length bordering on significance were observed for men ($p = 0.055$) and having higher levels of depression symptoms ($p = 0.56$). Leukocyte telomere length was not significantly associated with BMI ($p = 0.75$), diabetes ($p = 0.15$) or arthritis ($p = 0.18$). In unadjusted Spearman correlations, shorter LTL was correlated with older age ($p <0.0001$), higher PFS Physical scores ($p = 0.0002$) and higher PFS Mental scores ($p = 0.01$) (Table 3).

Shorter leukocyte telomere length at baseline predicted higher PFS Physical scores ($\beta = 2.7$, $p < 0.0001$) at Visit 2 when accounting for family structure and adjusting for field center (Table 4). Adjusting for sex, chronic conditions, and lifestyle factors attenuated the relationship ($\beta = 1.7$, $p < 0.0001$). In logistic models, shorter LTL significantly predicted having greater perceived physical fatigability (OR = 1.50, 95%CI [1.22, 1.85], $p = 0.0001$). This relationship trended non-significant after adjustment (OR = 1.38, 95%CI [1.10, 1.73], $p = 0.057$). In this adjusted model, depressive symptoms, arthritis, physical activity, BMI, and educational history were all highly significant predictors ($p <0.0001$) of greater physical fatigability. In all models that included age, LTL was not associated with perceived physical fatigability.

Shorter LTL also predicted higher PFS Mental scores ($\beta = 1.7$, $p = 0.0004$) (Table 5). This relationship became borderline non-significant after adjusting for sex, chronic conditions, and lifestyle factors ($\beta = 0.8$, $p = 0.055$). Shorter leukocyte telomere length significantly predicted having greater mental fatigability (OR = 1.35, 95%CI [1.03, 1.78], $p = 0.032$); after adjusting for covariates this association was non-significant (OR = 1.11, 95%CI [1.21, 1.48], p =

0.50). When age was included in the models, LTL was not associated with perceived mental fatigability.

Of the 15 SNPs analyzed, 9 had a genotype that was observed in 10 (0.5%) or fewer study participants (Table 6). In models accounting for family structure and adjusted for field center, none of the SNPs investigated were significantly associated with continuous or binary perceived physical and mental fatigability outcomes with a Type I error of 0.05, even before adjusting for multiple comparisons (Table 7).

5.0 DISCUSSION

The results of this analysis indicate that shorter leukocyte telomere length was predictive of perceived physical and mental fatigability when age was not included in the models. None of the SNPs previously found to be associated with leukocyte telomere length were significant predictors of physical or mental fatigability in this sample. This study confirms shorter leukocyte telomere length is strongly associated with older age in a population with a wide age range (60-108 years old). When age was included as a covariate the sign of the beta coefficient for LTL reversed direction, suggesting the inclusion of age was over-adjusting. A difference in LTL that approached statistical significance was observed between sexes, consistent with results from other studies indicating a significant association between sex/gender and LTL.[56,61,67] In previous research the relationship between BMI and LTL has been inconsistent;[61] an association between BMI and LTL was not observed in this analysis.

This is the first study to analyze the relationship between leukocyte telomere length and perceived physical and mental fatigability. The relationship between LTL and fatigability is consistent with LTL being a biological marker of the aging process and fatigability being an outcome related to aging. LTL and fatigability are both associated with a variety of covariates and outcomes related to aging, and further research is needed to understand how these variables interact with the disablement pathway in older adults. One potential explanation for the link

between LTL is fatigability is that shorter leukocyte telomere lengths and increased fatigability are both consequences of accumulated stresses throughout life.

Relationships between shorter LTL and a variety of outcomes related to physical and mental fatigue have been observed in previous research.[42,55–60] In this analysis, LTL was a stronger predictor of physical fatigability than mental fatigability, regardless of whether fatigability was analyzed as a continuous or binary outcome and the other covariates included in regression models. This difference has not been characterized in previous research, as existing measures of fatigue are not capable of producing physical and mental scales that can be directly compared.

Despite having a larger sample size (n = 1,997) than previous studies analyzing the genetic basis of fatigue,[38] SNPs associated with fatigability were not identified in this analysis. However, fatigability is known to be heritable,[26,39] and investigating genetic markers related to fatigability is a potential area for future research. Genome-wide association studies could be used to identify candidate SNPs that may explain differences in fatigability between individuals.

Fatigability is known to be associated with disability, loss of function, and mortality in older adults.[10,15,17,32] Identifying biomarkers that can predict fatigability is therefore useful for public health research and practice. Telomere length is one potential cellular marker of aging; this analysis provides further evidence that telomere length is predictive of important outcomes related to the aging process. Being able to predict future disability in older adults is necessary for successful interventions because disability is usually irreversible and must be prevented and managed before it occurs.

A strength of this study is the wide range of ages among LLFS participants, allowing a more complete investigation of aging among young-old, old-old, and oldest-old individuals. The

large sample size allowed sufficient statistical power for the analyses of LTL and fatigability. It is important to note that (%) of the sample had missing or incomplete PFS scores that could not be imputed. Analysis indicated those with missing data were older and more likely to be women, indicating including those individuals would bias the sample away from the null, as both these factors are associated with shorter LTL and higher fatigability. The fatigability outcome being analyzed—Pittsburgh Fatigability Scale scores—is a validated and reliable measure that provides distinct assessments of physical and mental fatigability. One potential limitation of this study was that leukocyte telomere length was only assayed once, at baseline, and fatigability measures were not introduced until Visit 2. This prevented the analysis from taking into account how changes in LTL over time are associated with changes in fatigability. Longitudinal analyses of telomere length are an important area for future research. If telomere length is truly a biological indicator of aging, faster decreases in telomere length throughout midlife and older age would be expected to predict earlier development of negative health outcomes related to the aging process. Another limitation of this analysis is that it may not be generalizable to other populations, as the selection process for LLFS participants resulted in a study population that is disproportionately white, healthy, long-lived, and well educated compared to the general population (Newman 2011). Many of the SNPs previously identified as being associated with LTL are uncommon, and for 9 of the 15 SNPs analyzed, the homozygote for the minor allele was observed in 10 or fewer study participants. This made it difficult to analyze the contributions of these SNPs to fatigability. Future studies on the relationship between these SNPs and fatigability should be performed with much larger sample sizes to ensure statistical power to detect a difference in fatigability outcomes.

Fatigability is a marker of phenotypic aging and is related to a variety of meaningful outcomes in older adults. This analysis demonstrates that leukocyte telomere length, which is considered a potential biological marker of aging, is predictive of fatigability. Future research is needed to characterize the biological mechanisms that explain this relationship. One possible mechanism is inflammation, which may result in accelerated shortening of telomeres and is known to be related to fatigue and fatigability outcomes.[27,55,68,69] Understanding biological markers that precede declining health and function in older adults may produce targets for interventions that prevent and slow the disability cascade, allowing older adults to remain healthier throughout the aging process. Longevity is known to be heritable, but the mechanisms by which genetics affects longevity are mostly unknown.[53] Identifying genetic biomarkers related to fatigability and other age-related outcomes may allow further research into genetic factors that affect longevity. Understanding the aging process will be an important component of public health research as the overall population continues to shift to older age demographics.

Appendix A Tables

Table 1: Study Population Characteristics (n = 1,997)

Variable	Overall	Lower physical fatigability	Greater physical fatigability	Lower mental fatigability	Greater mental fatigability
LTL (bps)	5347.7 (477.0)	**5369.5 (496.8)**	**5317.5 (446.6)**	**5361.7 (486.3)**	**5310.4 (449.7)**
Physical PFS	14.3 (10.0)	--	--	--	--
Higher physical	836 (41.9%)	--	--	--	--
Mental PFS	8.4 (9.4)	--	--	--	--
Higher mental	491 (24.7%)	--	--	--	--
Age at v2	73.7 (10.4)	**70.2 (7.0)**	**78.6 (12.2)**	**71.4 (8.3)**	**80.6 (12.8)**
BMI	27.3 (5.0)	**26.7 (4.2)**	**28.2 (5.8)**	27.3 (4.8)	27.6 (5.5)
Women	1086 (54.4%)	**584 (50.3%)**	**502 (60.1%)**	799 (53.5%)	283 (57.6%)
Phys activity					
Low	202 (10.2%)	**36 (3.1%)**	**166 (19.9%)**	**85 (5.8%)**	**115 (23.5%)**
Medium	449 (22.6%)	**211 (18.2%)**	**238 (28.6%)**	**322 (21.6%)**	**122 (25.0%)**
High	1340 (67.3%)	**911 (78.7%)**	**429 (51.5%)**	**1082 (72.6%)**	**252 (51.5%)**
Education					
<HS or HS	327 (16.4%)	**127 (11.0%)**	**200 (24.0%)**	**190 (12.7%)**	**134 (27.4%)**
>HS	1667 (83.6%)	**1032 (89.0%)**	**635 (76.0%)**	**1303 (87.3%)**	**355 (72.6%)**
Diabetes	213 (10.7%)	**90 (7.76%)**	**123 (14.7%)**	**145 (9.7%)**	**67 (13.7%)**
HTN	1203 (60.2%)	698 (60.1%)	505 (60.4%)	886 (59.3%)	309 (62.9%)
Cancer	661 (33.1%)	**345 (29.7%)**	**316 (37.8%)**	480 (32.1%)	180 (36.7%)
High CESD	116 (5.8%)	**32 (2.8%)**	**84 (10.1%)**	**49 (3.3%)**	**67 (13.7%)**
Arthritis	915 (45.8%)	**421 (36.3%)**	**494 (59.1%)**	**616 (41.2%)**	**296 (60.29%)**

Mean (sd) shown for continuous variables and n (%) for categorical variables

Bolded values statistically significantly differ between higher/lower fatigability categories (two sample t-test for continuous variables, chi-squared for categorical)

Cut-point = 15 for physical, 13 for mental

Table 2: Associations of Covariates with Physical and Mental Fatigability and Leukocyte Telomere Length

	Physical PFS		Mental PFS		LTL	
	β	p-value	β	p-value	β	p-value
Physical PFS	--	--	--	--	-6.18	<0.0001
Mental PFS	--	--	--	--	-4.19	<0.0001
LTL	-0.0027	<0.0001	-0.0017	0.0004	--	--
Age at v2	0.50	<0.0001	0.42	<0.0001	-12.80	<0.0001
BMI	0.19	<0.0001	0.01	0.82	-0.71	0.75
Gender (ref = men)	1.72	<0.0001	0.94	0.0142	52.97	0.0055
Physical activity	-0.60	<0.0001	-0.45	<0.0001	6.11	<0.0001
Education (ref = HS/<HS)	5.97	<0.0001	5.34	<0.0001	-58.0	<0.0437
Diabetes (ref = no)	2.93	<0.0001	1.48	0.04	-45.73	0.15
HTN (ref = no)	0.78	0.11	0.41	0.38	-67.02	0.003
Cancer (ref = no)	1.96	0.45	10.4	0.44	-82.75	<0.0001
Total CESD	0.83	<0.0001	0.85	<0.0001	-5.19	0.056
Arthritis (ref = no)	4.81	<0.0001	3.44	<0.0001	-27.68	0.18

Associations of covariates with LTL and physical and mental fatigability (adjusted for field center and family structure)

Table 3: Spearman Correlations

	LTL		Age at v2		Total CESD		Total activity		BMI	
	Coeff	p	Coeff	p	Coeff	p	Coeff	p	Coeff	p
Physical PFS	-0.08	0.0002	0.39	<0.0001	0.33	<.0001	-0.46	<.0001	0.11	<0.0001
Mental PFS	-0.06	0.0112	0.27	<0.0001	0.32	<.0001	-0.29	<.0001	0.02	0.3153
LTL	--	--	-0.31	<0.0001	-0.02	0.4281	0.06	0.0080	0.03	0.2849
Age at v2	--	--	--	--	0.12	<.0001	-0.27	<.0001	-0.09	<0.0001
Total CESD	--	--	--	--	--	--	-0.20	<.0001	0.02	0.3309
Total activity	--	--	--	--	--	--	--	--	-0.09	0.0001

Spearman correlations – none of the variables are normally distributed

Table 4: Association of LTL with continuous PFS Physical scores and greater physical fatigability

	PFS Physical continuous scores		Greater physical fatigability (≥15)	
Model*	β	p-value	β	p-value
None (univariate)	2.7	<0.0001	0.4	0.0001
Sex	2.8	<0.0001	0.4	<0.0001
Age	-0.5	0.23	-0.1	0.28
Age and sex	-0.4	0.35	-0.1	0.38
Lifestyle	1.8	<0.0001	0.3	0.0046
Lifestyle, sex	1.9	<0.0001	0.3	0.0021
Lifestyle, sex, age	-0.3	0.42	-0.1	0.52
Chronic	2.1	<0.0001	0.3	0.0018
Chronic, sex	2.2	<0.0001	0.4	0.0010
Chronic, sex, age	-0.2	0.64	-0.1	0.62
Lifestyle, chronic, sex	1.7	<0.0001	0.3	0.0057
Lifestyle, chronic, sex, age	-0.1	0.73	-0.0	0.68

*all models include LTL, account for family structure and are adjusted for field center
Beta coefficient shown are for a one kilo base shorter leukocyte telomere length
Lifestyle variables: physical activity (MET hours/day), BMI (kg/m^2), education
Chronic conditions: diabetes, hypertension, cancer, CES-D score, arthritis

Table 5: Association of LTL with continuous PFS Mental scores and greater mental fatigability

	PFS mental continuous scores		Greater mental fatigability (≥13)	
Model*	β	p-value	β	p-value
None (univariate)	1.7	0.0004	0.3	0.032
Sex	1.7	0.0002	0.3	0.028
Age	-1.0	0.022	-0.3	0.014
Age and sex	-0.9	0.032	-0.3	0.018
Lifestyle	0.9	0.023	0.1	0.32
Lifestyle, sex	1.0	0.018	0.1	0.30
Lifestyle, sex, age	-0.8	0.054	-0.3	0.031
Chronic	1.0	0.015	0.2	0.188
Chronic, sex	1.1	0.012	0.2	0.184
Chronic, sex, age	-0.8	0.061	-0.3	0.033
Lifestyle, chronic, sex	0.8	0.055	0.1	0.50
Lifestyle, chronic, sex, age	-0.7	0.092	-0.3	0.044

*all models include LTL, account for family structure and are adjusted for field center
Beta coefficient shown are for a one kilo base shorter leukocyte telomere length
Lifestyle variables: physical activity (MET hours/day), BMI (kg/m^2), education
Chronic conditions: diabetes, hypertension, cancer, CES-D score, arthritis

Table 6: Locations and frequencies of each SNP in study population (total n = 1.997)

Band	Gene	SNP	n (%) with 0 minor alleles	n (%) with 1 minor allele	n (%) with 2 minor alleles
3q13.2	TMPRSS7	rs16859140	1013 (50.7%)	830 (41.6%)	154 (7.7%)
4q25	DKK2	rs11732697	1881 (94.2%)	111 (5.6%)	5 (0.3%)
4q25	Between DKK2 and PAPSS1	rs7680468	1875 (94.0)	117 (5.9%)	2 (0.1%)
4q25	Between DKK2 and PAPSS1	rs2189194	1874 (93.9%)	117 (5.9%)	5 (0.3%)
6q24.1	AK097143	rs34593685	1794 (89.8%)	199 (10.0%)	4 (0.2%)
8p21.3	LOC100128993	rs76461710	1767 (88.5%)	227 (11.4%)	3 (0.2%)
8p21.3	LOC100128993	rs11787341	1770 (88.7%)	222 (11.1%)	4 (0.2%)
10p13	TRDMT1	rs10904887	551 (27.6%)	1000 (50.1%)	445 (22.3%)
10p13	TRDMT1	rs10904896	559 (28.0%)	999 (50.1%)	438 (21.9%)
10q11.21	Between RASGEF1A and FXYD4	rs10466239	1703 (85.3%)	278 (13.9%)	16 (0.8%)
14q23.2	SYT16	rs4902100	1022 (51.2%)	820 (41.0%)	155 (7.8%)
14q23.2	SYT16	rs2154110	1007 (50.4%)	819 (41.0%)	171 (8.6%)
20q13.2	TSHZ2	rs56230013	1707 (85.5%)	280 (14.0%)	10 (0.5%)
22q12.2	Between UQCR10 and ASCC2	rs6006249	1784 (89.4%)	204 (10.2%)	8 (0.4%)
22q12.2	ASCC2	rs73394838	1785 (89.4%)	204 (10.2%)	8 (0.4%)

Table 7: Associations of SNPs with fatigability outcomes

	PFS Physical scores		Greater physical fatigability (≥15)		PFS Mental scores		Greater mental fatigability (≥13)	
SNP	β	p-value	β	p-value	β	p-value	β	p-value
rs16859140		0.61		0.62		0.34		0.65
1	0.38	0.42	-0.07	0.48	0.52	0.25	-0.07	0.51
2	0.64	0.45	-0.15	0.44	0.87	0.27	-0.14	0.43
rs11732697		0.33		--		0.16		--
1	-0.50	0.59	--	--	-0.58	0.51	--	--
2	-4.41	0.09	--	--	-5.68	0.0009	--	--
rs7680468		0.38		--		0.39		--
1	-0.06	0.94	--	--	-0.25	0.79	--	--
2	-3.27	<0.0001	--	--	-6.86	<0.0001	--	--
rs2189194		0.36		--		0.17		--
1	-0.04	0.96	--	--	-0.25	0.79	--	--
2	-4.37	0.09	--	--	-5.65	0.0009	--	--
rs34593685		0.96		0.98		0.81		0.87
1	0.15	0.82	-0.02	0.89	0.19	0.81	0.09	0.62
2	-0.49	0.87	0.14	0.90	1.28	0.48	-0.12	0.92
rs76461710		0.97		0.92		0.79		0.92
1	0.03	0.97	0.004	0.98	0.22	0.75	0.008	0.96
2	-1.26	0.87	0.50	0.69	-2.45	0.52	-0.62	0.64

rs11787341		0.93		0.96		0.54		0.95
1	0.23	0.73	-0.04	0.80	0.38	0.57	-0.05	0.77
2	0.98	0.87	-0.13	0.90	-3.13	0.29	-0.15	0.91
rs10904887		0.80		0.89		0.43		0.39
1	-0.31	0.56	-0.01	0.91	-0.66	0.21	0.18	0.17
2	-0.03	0.97	-0.06	0.65	-0.25	0.68	0.15	0.29
rs10904896		0.68		0.91		0.23		0.30
1	-0.43	0.42	-0.0006	0.996	-0.86	0.11	0.21	0.11
2	-0.10	0.87	-0.05	0.72	-0.27	0.65	0.14	0.32
rs10466239		0.99		0.97		0.98		0.98
1	0.04	0.94	-0.03	0.84	0.10	0.86	-0.03	0.83
2	-0.28	0.91	-0.07	0.89	0.36	0.90	-0.03	0.95
rs4902100		0.87		0.83		0.65		0.54
1	0.12	0.80	0.06	0.54	0.26	0.59	-0.07	0.55
2	-0.30	0.70	0.03	0.88	0.62	0.41	-0.20	0.28
rs2154110		0.87		0.97		0.60		0.56
1	0.22	0.64	0.02	0.87	0.28	0.56	-0.08	0.52
2	-0.09	0.91	0.04	0.81	0.71	0.36	-0.19	0.32
rs56230013		0.15		0.49		0.10		--
1	0.03	0.96	0.02	0.90	0.46	0.51	--	--
2	-4.72	0.01	0.84	0.28	-3.18	0.01	--	--
rs6006249		0.83		0.73		0.42		0.57
1	0.35	0.59	-0.04	0.79	0.04	0.95	-0.06	0.73
2	-0.56	0.76	0.50	0.48	-3.38	0.20	0.73	0.45
rs73394838		0.83		0.73		0.42		0.57
1	0.35	0.59	-0.04	0.80	0.04	0.95	-0.06	0.73
2	-0.56	0.76	0.50	0.48	-3.38	0.20	0.73	0.45

Reference category for all SNPs is having 0 copies of minor allele, beta coefficients and p-values shown for having 1 and 2 copies of minor allele, Type III Score Test p-value also shown for each SNP
Models with no values shown did not converge

Appendix B Figures

Figure 1: Conceptual Framwork

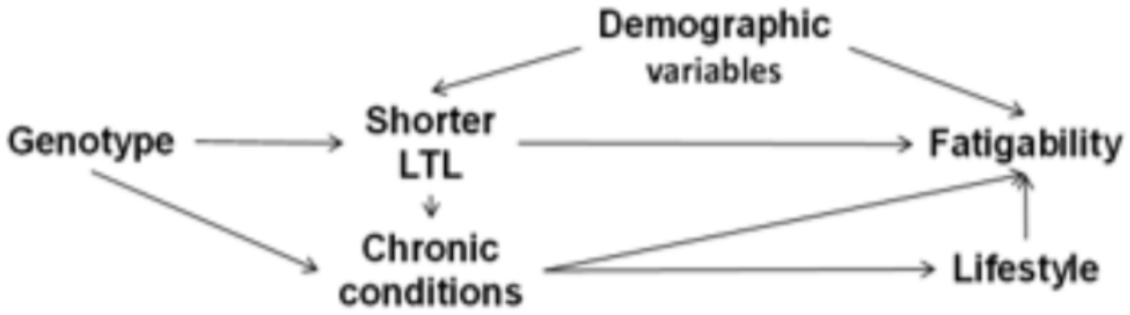
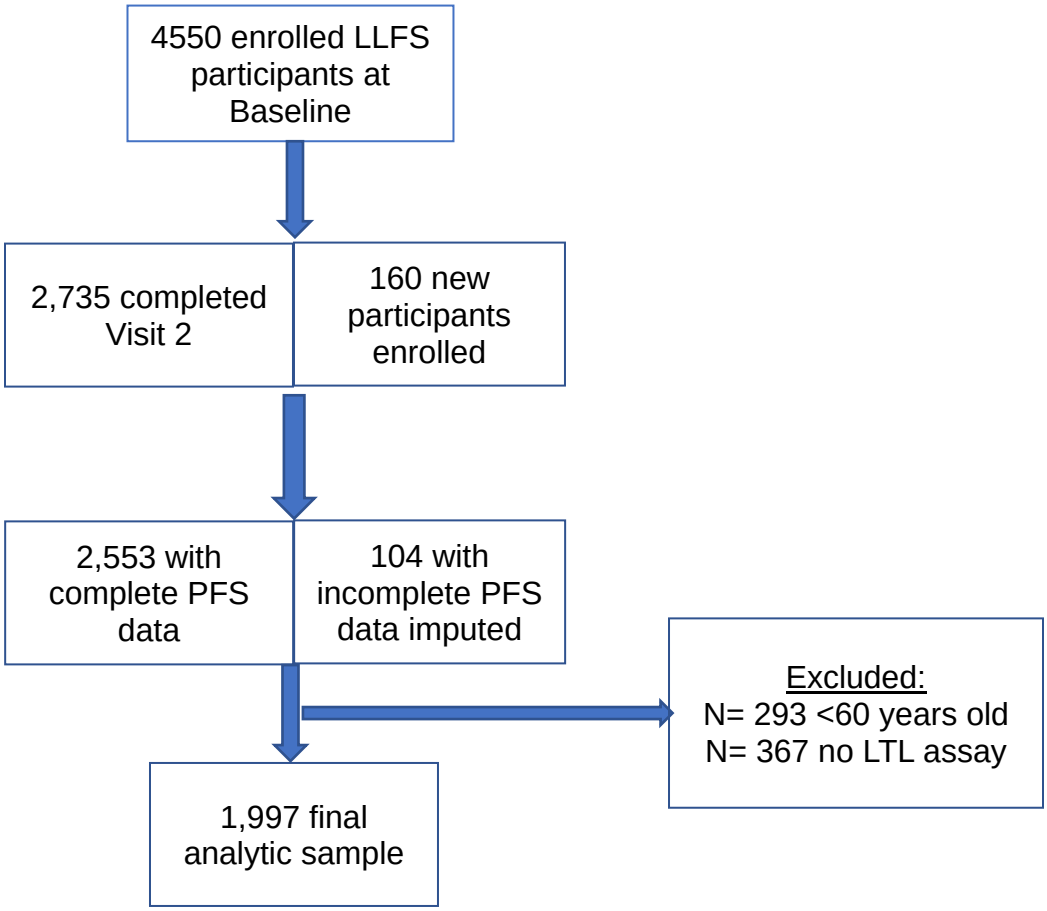


Figure 2: Flow Chart of Analytic Sample



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