

TELOMERES AND BRAIN HEALTH

Written by: Anna Podlasek

Supervisor: Professor Dorothee Auer

Co-supervisor: Professor Rob Dineen

Internal Assessor: Professor Paul Morgan

This thesis will be submitted to the University of Nottingham

for the degree of Master or Research (MRes)

June 2022

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Abstract

Background and aim:

This study looked at the genetic aspects that influence telomere length and their link to healthy brain and cerebrovascular ageing.

Methods:

Using sample data from UK Biobank, we looked into the association between 29 SNPs regulating telomere length and neuroimaging biomarkers of ageing and cerebrovascular disease (hippocampus, white matter hyperintensities [WMH]). The analysis was corrected for technical variables as well as clinical, demographic and lifestyle factors to correct for nuisance variables, including ApoE status due to the well-known association with cardiovascular disease and hippocampal atrophy. We used descriptive statistics and correlation analysis for the baseline assessment. Furthermore, we used variance inflation factor to select variables lacking significant co-linearity and performed linear regression for three dependent imaging-derived phenotypes (log WMHs, right hippocampus, left hippocampus). We used Bonferroni correction on the final models for interpretation.

Results:

The final regression models included technical confounders, genetic data and clinical, and demographic and lifestyle factors. They explained 34.6% of the variation in right hippocampal volume, 40.6% of the left total hippocampal volume, and 36.1% of the variation in log WMHs volume. As an interim step to correct for known associations, we identified the following factors contributing to WMHs load: smoking status, physical activity, diabetes diagnosed by a doctor, number of medications including medications for hypertension, HbA1c, waist to hip, pulse rate, and blood pressure (systolic and diastolic). We have also identified the following contributory factors for hippocampal volume on both sides: smoking status, maternal longevity, waist to hip ratio. Additionally, we identified paternal age and systolic blood

pressure for the left hippocampus, and arterial stiffness, diastolic blood pressure and a number of non-cancerous diseases for the right hippocampus. The overall analysis uncovered that ApoE is the only genetic contributor for all three biomarkers (WMHs, right and left hippocampus), on its own, explaining 0.01% of variability for each. Two SNPs localised on chromosome 10 (rs11191848 and rs9419958) were associated with shorter telomere length and increased WMHs, on its own explaining 0.01% of the variability and 0.03% when combined.

Discussion and limitations:

We refuted the hypothesis on the link between SNPs regulating telomere length and hippocampal volume, which is consistent with the negative meta-analysis by Nielsen et al. looking at the association of direct leucocyte telomere length and hippocampal volume. We identified two SNPs influencing WMHs, out of which rs11191848 was previously reported to be associated with cardiovascular disorders. Limitations of this study were lack of direct telomere length, lack of longitudinal data, and analysis restricted to the Caucasian population between 40 and 85 years.

Conclusions:

ApoE were the only consistent genetic contributor to the neuroimaging biomarkers of brain ageing and cerebrovascular health. There is a link between rs11191848 and rs9419958 contributing to shorter telomere length and increased WMHs load, but no link between hippocampal volumes and SNPs regulating telomere length. Further studies are needed better to understand direct leucocyte telomere length on neuroimaging biomarkers and uncover potential mechanisms for those relationships.

Acknowledgements

I want to thank my supervisors, Prof Dorothee Auer and Prof Robert Dineen, for their support and help with the thesis. In addition, I appreciate the input of my internal assessor, prof Paul Morgan.

I want to express gratitude to Prof Stamatios Sotiropoulos and Alireza Mohammadinezhad Kisomi for granting access to the Uk Biobank data.

Thanks for the statistical and big data discussions to Andrea Venn, Stefan Pszczolkowski, Jessica de Boer, Sieun Lee, Christopher Tench.

I want to express gratitude to my family and friends for their moral support.

Declaration

I declare that this thesis has been prepared by me, and has not been submitted for a higher degree to any other university.

Abbreviations

A – adenine

AD – Alzheimer disease

BMI – body mass index

bp - base-pairs

C – cytosine

CA - cornu ammonis

DG – dentate gyrus

EC - entorhinal cortex

Fig – figure

G – guanine

GM – grey matter

GWAS - genome-wide association meta-analysis

LTL – leukocyte telomere length

MOCA - Montreal Cognitive Assessment

MRI – magnetic resonance imaging

NA - not available

PaS - Parasubiculum

PCA - posterior cerebral artery

PrS – Presubiculum

RBA - relative brain age

SNP – single-nucleotide polymorphism

UK – United Kingdom

UKBB – UK BioBank

S – subiculum

SVD – small vessel disease

T - thymine

Tab – table

TER = TERC - telomerase RNA

TERT - telomerase reverse transcriptase

TL – telomere length

T/S ratio - telomeres to single genes ratio

WMH – white matter hyperintensities

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Chapter 1: Introduction

The world population has tripled, from 2.9 billion in 1950 to 7.8 billion in 2020, and the average life expectancy has risen from 47 to 73 years of age in these seven decades. (Garmany, Yamada and Terzic, 2021) The rate of healthy ageing depends on our genes, lifestyle, and environmental factors.

It is essential to understand the difference between chronological time (life span) and period free from disease (healthspan). People of the same chronological age may exhibit significantly different susceptibilities to age-related diseases and death, likely reflective of differences in their underlying biological ageing processes. (Levine *et al.*, 2018) The health span-lifespan gap is estimated at around nine years. (Garmany, Yamada and Terzic, 2021) The next frontier in ageing research is to maximize healthspan, not only lifespan.

It is well known that better mental health greatly contributes to physical health as well. (Vidaček *et al.*, 2018) In people who live long, there is an accumulation of dysfunctional cells that have shorter telomeres. (Jesus and Blasco, 2012) Therefore, we plan to check for an association between the brain imaging phenotypes and genes regulating telomere length.

Telomeres and ageing

General information

Muller in 1938 and McClintock in 1941 observed for the first time that the natural ends of the linear chromosome behave differently than the unnatural, broken ends of chromosomes created by the X-rays induced damage. (McClintock, 1941; Meynell, 1975; Shay, 2018) They called them telomeres from the Greek words for "telos", meaning end and "meros", meaning part. (Shay, 2018) These early considerations formed the foundation of further research identifying the newly named telomeres as heterochromatin of distinctive structure and function. (Shay, 2018) This discovery was fundamental to suggest a biological mechanism of limited cell replication.

Senescence is understood as biological ageing. In 1961 Hayflick started investigations towards replicative senescence: telomere shortening due to cellular replication, leading to a permanent cell cycle arrest and cell death division in cells with a limited proliferative capacity. That is often referred to as the Hayflick limit. (Hayflick, 1965; Shay, 2018) The hypothesis on the mechanism of telomere shortening was first grounded in the theoretical research work of Olovnikov (Olovnikov, 1973) and confirmed by the experimental work of Harley et al. (Harley, Futcher and Greider, 1990)

Scientists have noticed that all of our chromosomes have similar sequences of DNA at their ends, and whilst not encoding any information, they have a vital function. In 1999 Griffith et al. proved that the double-stranded telomeric repeats are over hanged by a single t-loop, which hides free ends and prevents DNA damage responses. (Griffith *et al.*, 1999; Shay, 2018)

Working on the yeast and human cells, Blackburn and Szostak found that telomeres protect chromosomes from degradation. (Szostak and Blackburn, 1982) Greider and Blackburn identified the enzyme that helps telomeres to protect the chromosomes – telomerase. (Greider and Blackburn, 1985) Telomerase is atypical as it is built of RNA as well as proteins molecules. The

RNA part is complementary to the telomere sequence, which allows the telomeres to be copied efficiently. Without it, the telomere – and therefore chromosome - could not be copied entirely and thus causing shortening with each division. (Greider and Blackburn, 1985) In 2009 the Nobel Prize in Physiology or Medicine was jointly awarded to Greider, Blackburn and Szostak. (Nobelprize.org, 2009)

In later work, telomeres and telomerase were found to play a significant part in the ageing process. (Shammas, 2011a; Dlouha *et al.*, 2014) Telomerase enzyme is prone to mutations, and telomere defects have now been implicated in several diseases. (Shammas, 2011a) Cancer cells have high telomerase activity. This is one of the reasons why they can divide indefinitely. (Shammas, 2011a) Shortened leukocyte telomeres have also been observed in several human diseases (e.g. aplastic anaemia, pulmonary fibrosis, and hepatic disease). (Dlouha *et al.*, 2014) These discoveries have led to research targeting telomeres as therapeutic targets.

Telomeres – structure

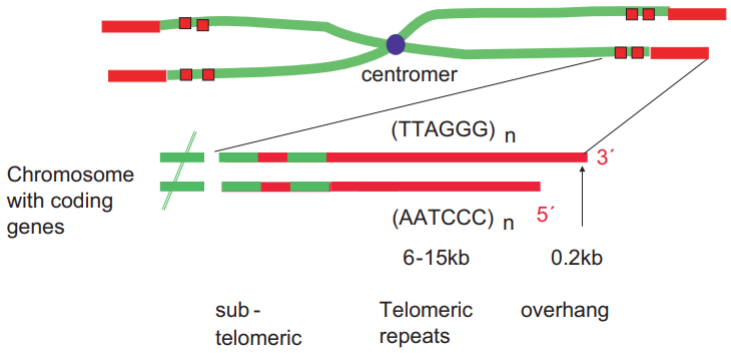


Fig. 1. Simplified DNA telomere structure. After (Saretzki, 2018)

Telomeres are protective structures formed by DNA-protein complexes present at the ends of linear chromosomes. All mammals share the exact

tandem repeat conserved, hexameric DNA sequence (TTAGGG). (Turner, Vasu and Griffin, 2019) It is organised into a unique looped structure to cover the exposed fragments of DNA with a protein shelterin complex, alternatively called telosome, composed of six protein subunits:

1. telomeric repeat binding factor 1 (TRF1),
2. telomeric repeat binding factor 2 (TRF2) –
    a. those are the two main proteins, for which the main function is to bind the shelterin complex to the telomeric DNA, as well as
3. TERF1 interacting nuclear factor 2 (TIN2),
4. TERF2 interacting protein (TERF2IP = RAP1),
5. Shelterin complex subunit and telomerase recruitment factor (ACD = TPP1),
6. Protection of Telomeres 1 (POT1). (De Lange, 2005; Xin, Liu and Songyang, 2008; Victorelli and Passos, 2017; Shay and Wright, 2019; Turner, Vasu and Griffin, 2019).

The looped structure is called a T-loop and is an overhang rich in G (guanine). This loops back to invade the double-stranded telomere tract. It makes loose DNA end to be stored internally within a nucleoprotein structure. (Muñoz-Jordán *et al.*, 2001; De Lange, 2005; Turner, Vasu and Griffin, 2019)

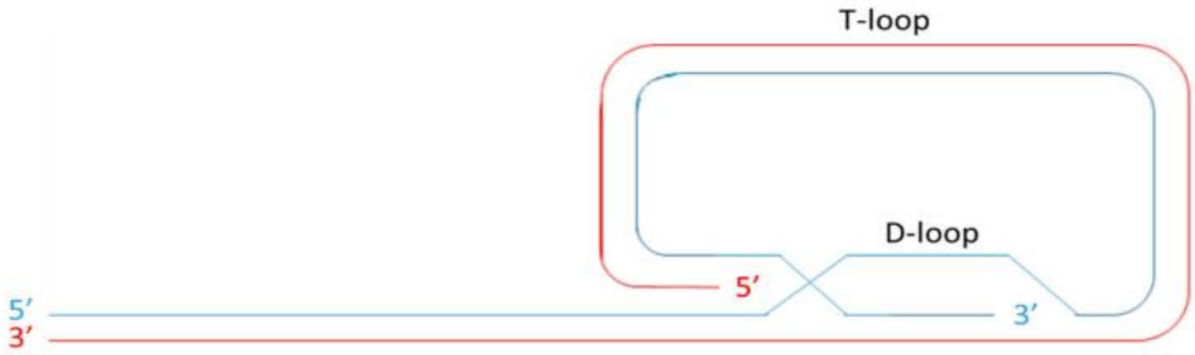


Fig. 2. The telomeric T-loop and the D-loop. After (Turner, Vasu and Griffin, 2019)

Telomeres are the response to the inherited linear chromosomes problems: the end-replication problem and the end-protection problem. (Sfeir, 2012) They cap their DNA strand endings and prevent genetic correction systems from identifying the chromosomal end as a chromosomal break wrongly. (D'Adda Di Fagagna *et al.*, 2003; Smith, Pendlebury and Nandakumar, 2020) During somatic cell replication, telomere length (TL) progressively shortens, as DNA polymerase cannot wholly replicate the 3' end of the DNA strand. (Codd *et al.*, 2013) Therefore, telomeres may be used as a marker of the cell ageing process. (Chan and Blackburn, 2004; Nilsonne *et al.*, 2015)

Telomeres – factors affecting telomere length

The length of telomeres between people can differ already at birth, which can differ between individuals of the same age. (Dlouha *et al.*, 2014) Up to 80% of the length of telomeres can be predicated by the length of telomeres of the parents and fathers telomere length is of greater effect. (Dlouha *et al.*, 2014)

In observational big cohort studies, mean leucocyte telomere length is longer in women than in men. It is associated with age (declining by 20–40 base-pairs (bp) per year). (Codd *et al.*, 2013) An expanding body of evidence suggests a predictable inverse association between telomere length, ageing, age-related diseases and mortality. (Chilton, O'Brien and Charchar, 2017) However, the study on 439 elderly Spanish individuals showed that frailty at baseline was significantly associated with a higher risk of death at follow-up of mean length of 3.5 years (OR: 4.08 [1.97-8.43], $p < 0.001$), but TL did not significantly change the mortality risk (OR: 1.05 [0.94-1.16]).

It is worth noticing that telomere length within different tissues of the same individual is not the same. It is attributed to differences in which particular tissues replicate. A high physiological shortening rate is described in proliferative tissues, for example, the liver. Low physiological shortening rate is described in non-proliferative cells, for example, skeletal muscles. (Butler *et*

al., 1998; Friedrich *et al.*, 2000; Takubo *et al.*, 2002) Dlouha et al. measured relative telomere length (TL) in twelve different human tissues (peripheral blood leukocytes, liver, kidney, heart, spleen, brain, skin, triceps, tongue mucosa, intercostal skeletal muscle, subcutaneous fat, and abdominal fat) from twelve cadavers (age range of 29 weeks of gestation to 88 years old). The highest variability was observed in peripheral leukocytes, and the lowest variability was found in the brain. The leucocyte telomere length was highly correlated with telomere length from intercostal muscles and liver. (Dlouha *et al.*, 2014)

Telomerase

Telomerase was discovered for the first time by Carol Greider, a graduate student in Elizabeth Blackburn's lab. (Greider and Blackburn, 1985) For this discovery, she was also jointly awarded the Nobel Prize in Medicine in 2009. (Greider and Blackburn, 1985; Nobelprize.org, 2009)

Telomerase is a ribonucleic acid-protein complex (RNP) and reverse transcriptase that adds TTAGGG repeats to the 3' of a chromosome. (Zhu, Belcher and Van Der Harst, 2011; Smith, Pendlebury and Nandakumar, 2020) It is composed of

1. a single long non-coding RNA, called telomerase RNA (TER/TERC), serving as a template,
2. dyskerin,
3. associated proteins, including telomerase reverse transcriptase (TERT), serving as a catalytic subunit. (Blackburn and Collins, 2011; Zhou *et al.*, 2011; Sfeir, 2012; Wang and Feigon, 2017)

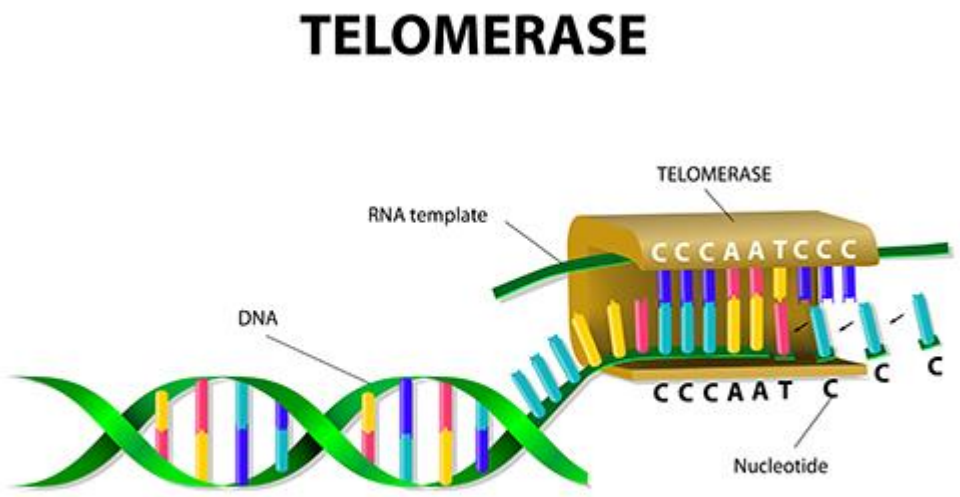


Fig. 3. A schematic depiction of telomerase and its primary function. After (*Telomeres Rule*, no date)

The primary function of telomerase is to maintain the telomere length in proliferative cells. (Zhou *et al.*, 2011; Jafri *et al.*, 2016) Experiments in culture cells proved that cells might be immortalised if telomerase is continuously counteracting telomere shortening. (Kim *et al.*, 1994; Bodnar *et al.*, 1998; Vaziri and Benchimol, 1998) Telomerase in animal models showed neurotrophic, cell survival-enhancing and "antidepressant-like" properties - chronic mild stress leads to a significant decrease in telomerase reverse transcriptase (TERT) level and telomerase activity in the hippocampus. (Zhou *et al.*, 2011; Wolkowitz *et al.*, 2015)

Telomerase activity level is dependent on the type of tissue it is localised within:

- almost undetectable in normal, peripheral somatic cells,
- moderate in germline cells, including male germ cells,
- moderate in stem cells, including neural stem cells in the hippocampal dentate gyrus,

- high in most cancer cells,
- high in stages of embryonic development. (Eriksson *et al.*, 1998; Mattson and Klapper, 2001; Armanios and Blackburn, 2012; Wolkowitz *et al.*, 2015; González-Giraldo *et al.*, 2016; Wang and Feigon, 2017; Liu, Nemes and Zhou, 2018)

Genetic and epigenetic factors modulate telomerase activity. Among the various components of the human telomerase, only the human telomerase RNA component (TERC) and human telomerase reverse transcriptase (TERT) are needed for the reconstitution of telomerase activity. The amount of TERT mRNA showed a positive correlation with telomerase activity and is believed to be the major contributor to the telomerase activity. (Liu *et al.*, 2004) Primary epigenetic mechanisms include methylation modification of DNA and the packaging of DNA into chromatin structure by histone proteins and are actively researched. (Liu *et al.*, 2004)

Ageing

Ageing is a complex process of gradual decline in bodily functions, especially those connected to adaptation. It is a common cause of diseases and death. (Flatt, 2012) There are nine biological hallmarks of ageing: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, mitochondrial dysfunction, cellular senescence, deregulated nutrient sensing, stem cell exhaustion and altered intercellular communication. (Hou *et al.*, 2019)

Many call telomeres a mitotic clock – a measure of each cell's replicative history and future potential representation. (Harley, 1991; Allsopp and Harley, 1995; D'Adda Di Fagagna *et al.*, 2003) It is owed to the fact that telomere shortening may lead to cellular senescence. (Allsopp and Harley, 1995; D'Adda Di Fagagna *et al.*, 2003)

Cellular senescence is not a simple cell death – the senescent cells cannot re-enter the cell division cycle, therefore, cannot further proliferate and

divide, but they are metabolically active. (Shay and Wright, 2005; Cesare and Reddel, 2010) Senescent astrocytes and microglia contribute to neurodegeneration. (Rivera-Torres and José, 2019) Emerging evidence indicates the physiological relevance of cellular senescence that extends beyond embryonic development and tumour suppression into biological processes such as wound healing and tissue repair. (Van Deursen, 2014)

There are multiple hypotheses on the mechanism behind telomere shortening. The end-replication problem is just one of the potential mechanisms for telomeres shortening. (Von Zglinicki, 2002) Exposure to oxidative stress is proposed as a potential pathway. The sequence is rich in guanine, which is especially prone to injury due to oxidative stress – substances like hydrogen peroxide or homocysteine (a well-known risk factor for vascular disease and brain atrophy) exacerbate telomeric length loss. (von Zglinicki *et al.*, 1995; Xu, Neville and Finkel, 2000; Sachdev, 2005)

The telomere shortening rate in the brain is slower than that of other tissues due to the lower rate of cell proliferation in the brain. (Anitha *et al.*, 2019) Their maintenance is necessary to maintain the function of the brain stem cells. (Anitha *et al.*, 2019) Clinically, there is suspicion of a correlation between chromosomal and neural ageing. (Jacobs *et al.*, 2014)Unfortunately, the direct evidence is limited for the human population. (Jacobs *et al.*, 2014) Lukens et al. has investigated 29 participants with Alzheimer's disease (AD). They found a moderate correlation (r = 0.42) between leukocyte telomere length measured from the peripheral blood and human brain tissue examined post-mortem. (Lukens *et al.*, 2009; Nilsonne *et al.*, 2015)

The telomere shortening rate throughout a human life is regulated by endogenous (genetic) and external (nongenetic) factors. (Martínez and Blasco, 2018) In humans, the heritability of leucocyte telomere length is estimated at 64%, and the rate of age-related telomere length attrition was estimated at 28%. (Hjelmborg *et al.*, 2015; Vaiserman and Krasnienkov, 2021)

Other studies estimate telomere length heritability between 44–80%. (Codd *et al.*, 2013)

Pathology

Telomeropathies are a group of disorders caused by mutations in telomere maintenance genes. (Martínez and Blasco, 2018) In animal models, telomerase activity was noticed to be unusually high in certain situations of cell damage (Fu *et al.*, 2002), increased levels of glucocorticosteroids (Haussmann and Heidinger, 2015) and inflammation in human tissues (Gizard *et al.*, 2011; Wolkowitz *et al.*, 2015)

There is consistent scientific evidence linking short telomeres and negative health states such as gastric cancer. In the umbrella review, including 21 meta-analyses, there was suggestive evidence for shorter telomere length in diabetic people and people with Alzheimer's disease than healthy controls. (Samani *et al.*, 2001; Shen *et al.*, 2007; Tentolouris *et al.*, 2007; van der Harst *et al.*, 2007; O'Donnell *et al.*, 2008; Svenson *et al.*, 2008; Mainous *et al.*, 2010; Salpea *et al.*, 2010; Nilsonne *et al.*, 2015; Smith *et al.*, 2019) .

It needs to be kept in mind that interpreting the telomere length and telomerase activity studies in depression and other neuropsychiatric disorders has significant limitations due to the unknown relationship of these peripheral cell ageing markers to corresponding central markers. (Eitan, Hutchison and Mattson, 2014; Lindqvist *et al.*, 2015) A meta-analysis of 38 studies and 34,347 participants showed a significant association between increasing severity of depression and shortened telomere length, with a Cohen's d small effect size of -0.205 ($p<0.0001$, $I^2=42\%$). Further research is needed to assess a potential causality. (Lakens, 2013; Ridout *et al.*, 2016)

The recent study by Liu et al. in the mouse model of depression found that telomeres were shortened in the peripheral blood but were elongated in the prefrontal cortex and amygdala compared with healthy controls. These

findings further illustrate that the telomere length in the peripheral blood may not mirror telomere length in the brain. (Liu *et al.*, 2020)

Genes regulating telomere length

A single-nucleotide polymorphism (SNP) is a variation of DNA sequence variation occurring when one nucleotide A, T, C, G in the genome differs between chromosomes in an individual. (Hart, Johnson and Barton, 2004)

Mean TL shows high heritability, with estimates varying between 44–80%. (Codd *et al.*, 2013) Codd et al. performed a genome-wide meta-analysis of 37,684 individuals from 15 cohorts and identified 7 SNPs in 7 loci associated with mean leucocyte telomer length ($p<5x10-8$). It was followed by replication of selected variants in a further 10,739 individuals from 6 additional cohorts. (Codd *et al.*, 2013)

A research group led by Li et al. conducted GWAS of 78,592 individuals from the European Network for Genetic and Genomic Epidemiology (ENGAGE) study and the European Prospective Investigation into Cancer and Nutrition (EPIC) Cardiovascular Disease (CVD) and InterAct studies. (Li *et al.*, 2020)) They identified six genome-wide significant associations between leucocyte telomere length and loci in or near SENP7, MOB1B, CARMIL1, PRRC2A, TERF2, and RFWD3. Pathak et al. identified further two genetic variants associated with LTL. They are located on chromosome 10, and both map to OBFC1 (also known as an STN1). (Pathak *et al.*, 2020)

In the study by Muskens et al., increased TL estimated genotypically was significantly associated with increased meningioma risk. (Muskens *et al.*, 2019)

Telomere length in the brain tissue

There is a scarcity of studies examining telomere length within the human brain tissue.

Nakamura et al. performed telomere length analysis in a white and grey matter on 72 autopsy samples from Japanese patients aged 0-100. The mean telomere lengths for patients in the 7th decade of life (60-61 years) were less than those of neonates and declined further in the 8th decade of life. The telomere length in further advanced age groups was longer than those in the 8th decade of life. Thus, the 90-100-year age group possessed significantly longer telomeres than the 70s (p=0.029). These results support the hypothesis that telomeres are beneficial for longevity. (Nakamura *et al.*, 2007)

In contrast, the study of brain tissue from 9 infants and 18 adults (18-93 years) compared telomere length between three tissue types within the brain: glial cells of white matter, glial cells of grey matter and neurons. They showed that the telomeres of neurons remain stable throughout life. In contrast, telomeres in white matter glial cells become significantly shorter with age as they are capable of mitosis. (Tomita *et al.*, 2018)

Thomas et al. compared results from 13 AD brain tissue with nine healthy control brain tissue. They found an increased content of telomeres in the brain tissue of patients with AD regardless of gender. (Wikgren et al., 2014; Thomas, O' Callaghan and Fenech, 2008) Lukens et al. conducted a study comparing 29 individuals with sporadic AD with 22 control subjects. They have found no difference in telomere length measured in a cerebellar tissue sample. (Lukens *et al.*, 2009)

Mamdani et al. measured telomere length in five brain regions: the dorsolateral prefrontal cortex, hippocampus, amygdala, nucleus accumbens and substantia nigra. They were derived from 40 samples of post-mortem brain from ten people with depression, ten people with schizophrenia, ten people with bipolar disorder and ten healthy controls. A statistically significant telomere shortening was observed among subjects with depression in the hippocampus (after controlling for age). Considering the difference in mRNA levels of several genes involved in both neuroprotection and stress response, it is hypothesised that there is a hippocampal stress-mediated accelerated cellular ageing in depression. (Mamdani *et al.*, 2015)

van Mierlo et al. conducted TL measurement in grey and white matter brain tissue from the medial frontal gyrus and superior temporal gyrus of post-mortem brain tissue in 9 subjects with schizophrenia and 11 healthy controls. (van Mierlo *et al.*, 2017) They found that TL was significantly longer in the grey matter than white matter in both brain regions for the whole group (no difference between healthy-control, male-female, age-adjusted). (van Mierlo *et al.*, 2017)

Small sample size may cause inconsistencies between studies, and therefore lack of appreciation of intrapersonal variability derived from big cohort studies and lack of control for moderators.

Brain ageing

The brain is the central organ that coordinates all of a human's actions. As the whole body, it is undergoing an ageing process. Depending on the brain health, this process can be expedited. The ageing process is heterogeneous among individuals. The most highlighted feature of cerebral ageing is its atrophy. The brain starts shrinking in early adulthood, and this process accelerates in the 6th decade of life. (Wikgren et al., 2014; Raz and Rodrigue, 2006; Gunning-Dixon *et al.*, 2009) It is believed that decreased synaptic density and neuronal loss and vascular pathology are responsible for those changes on the microscopic level. (Wikgren et al., 2014; Raz and Rodrigue, 2006; Habes *et al.*, 2016)

We are looking in detail into structures connected to neurodegenerative diseases and dementia (hippocampus) and features of cerebrovascular health (white matter hyperintensities as a marker of small vessel ischemia). (Nakamura *et al.*, 2007; Appel *et al.*, 2009)

Anatomy and physiology of hippocampus

The hippocampus is a brain structure particularly sensitive to the ageing processes, and its affection may drive major age-related functional and cognitive impairment. It is an important biomarker for Alzheimer disease (AD) and other dementias. (Appel *et al.*, 2009)

It is a structure embedded in the temporal lobe. (Anand and Dhikav, 2012; Hannula and Duff, 2017). The hippocampus proper has three molecular layers and includes the dentate gyrus (DG), cornu ammonis (CA) fields (CA3, CA2, CA1) and the Subiculum (S). (Hannula and Duff, 2017)

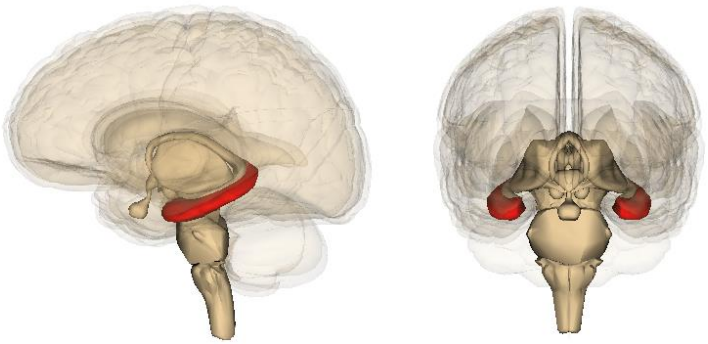


Fig. 4. Location of the hippocampus in the brain. After (The Database Center for Life Science, 2011)

The hippocampus is divided into three segments along is long-axis: hippocampal head, hippocampal body and hippocampal tail. (Woollett, 2010)

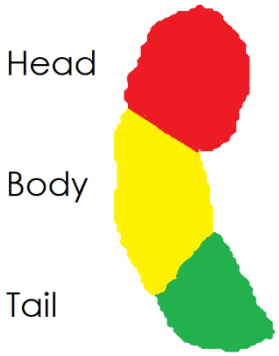


Fig. 5. Three hippocampal subregions are represented on the right hippocampus. Adapted from (Sun *et al.*, 2017)

Nobis et al. worked on brain MRI of almost 20,000 participants of UK Biobank. (Nobis *et al.*, 2019) They derived and reported normative, age- and gender-adjusted hippocampal and total grey matter volume for reference in clinical and research settings. (Nobis *et al.*, 2019)

The hippocampus has been known to play a crucial role in memory formation since the classic HM case published by Scoville and Milner, who lost his ability to form new memory postoperatively. (Scoville and Milner, 1957) In addition to its role in memory creation and maintenance, it receives signals from the olfactory bulb; therefore, it is involved in the sense of smell. (Soudry *et al.*, 2011) By a two way connection with the amygdala, a hippocampus may modulate emotions. (Anand and Dhikav, 2012) Moreover, it may regulate the excitatory function of the hypothalamus. (Anand and Dhikav, 2012)

The dentate gyrus (DG) is one of the brain's unique regions where neurogenesis continues even in adult life. (Bonfanti and Peretto, 2011; Koehl and Abrous, 2011; Anand and Dhikav, 2012; Agrimi *et al.*, 2019) The growing evidence suggests that new neuronal cells may play an important role in functions supported by hippocampus formation, such as memory encoding and mood regulation. (Agrimi *et al.*, 2019) A hippocampal function can be suppressed by chronic stress and its marker - glucocorticosteroids. (Rothman and Mattson, 2010; Anand and Dhikav, 2012)

Hippocampal atrophy

Hippocampal atrophy is typical of the normal ageing process and could be connected to mild memory impairment. In the study of 154 elderly subjects, almost 33% had imaging evidence of hippocampal atrophy. (Golomb *et al.*, 1993) Mega et al. suggest that the right hippocampal head is one of the earliest sites of atrophy in early memory impairment, and the atrophy gradient varies with the disease. (Mega *et al.*, 2002)

The hippocampal atrophy has been extensively studied. (Goukasian *et al.*, 2019) It is very commonly affected in neuropsychiatric disorders, and the relationship with Alzheimer's disease is particularly strong. (Barnes *et al.*, 2009)The rate of its shrinkage may be used as both a diagnostic and prognostic factor. (Frisoni *et al.*, 2010) The hippocampal atrophy was also

studied in schizophrenia, epilepsy, mood disorders, depression, head injury, post-traumatic stress disorders. (Dhikav and Anand, 2007; Bast, 2011; Anand and Dhikav, 2012; Li *et al.*, 2012)

Chronic exposure to oxidative stress, inflammatory cytokines, or glucocorticoids can both speed up the process of shortening telomere and contribute to hippocampal atrophy. (Wolkowitz *et al.*, 2010; Shammas, 2011b; Jacobs *et al.*, 2014) Reactive oxygen species (ROS) are the mechanical driving cause for oxidative stress, and they are produced during ATP synthesis in mitochondria as well as from exogenous sources like UV radiation and pollution. ROS cause oxidative damage to a variety of biomolecules, especially telomeres – a high concentration of guanine make them especially sensitive to oxidative damage. (Reichert and Stier, 2017) Similarly, increased oxidative stress leads to hippocampal atrophy. (Cherbuin *et al.*, 2019) It may suggest common pathways for telomere maintenance and hippocampal integrity. (Wolkowitz *et al.*, 2010; Jacobs *et al.*, 2014) However, it is unknown if inflammatory markers are simple biomarkers for shortened telomeres or a common underlying mechanism.

Association between telomere length and hippocampus

Shorter telomere length is a significant risk factor for many age-related diseases (Shammas, 2011a), and it has been associated with decreased hippocampal volumes. (Palmos *et al.*, 2020) It is still unclear if telomere length is a marker or cause for accelerated hippocampal shrinkage. (Smith et al., 2019)

Different models have been proposed to explain the association between hippocampal volume and LTL. (Nilsonne *et al.*, 2015)

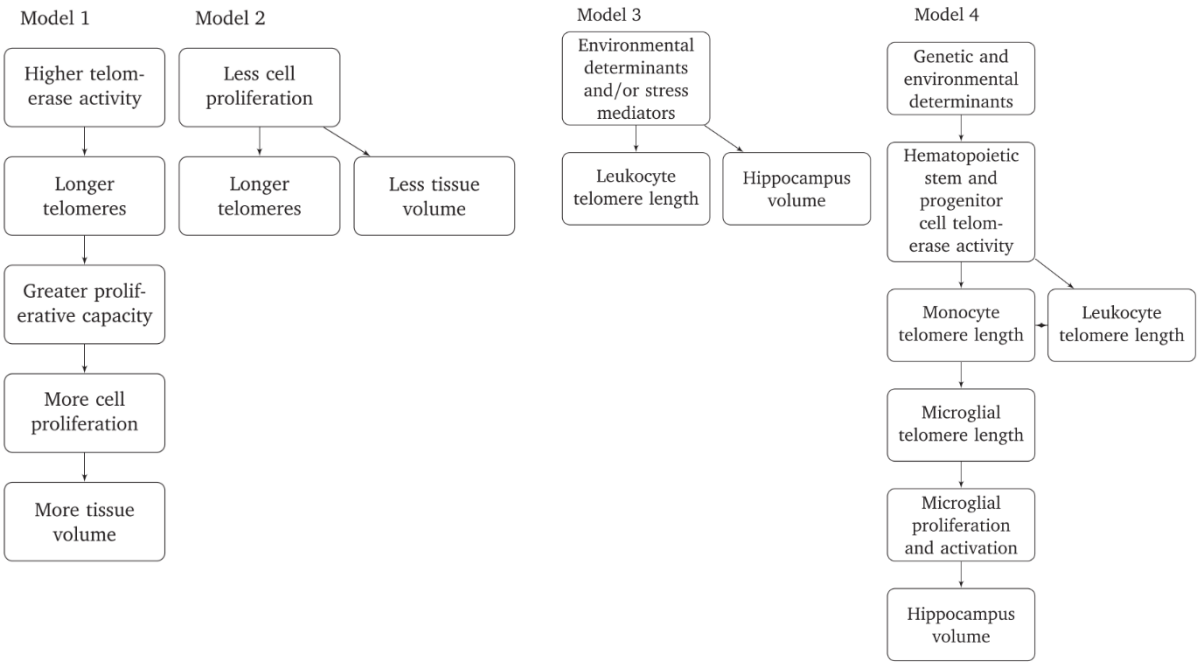


Fig. 6. Four models were proposed for relationships between leukocyte telomere length and hippocampal volume. After (Nilsonne *et al.*, 2015)

Model 1 and model 2 make contradictory assumptions. Model 1 is based on the study by King et al. that assumes that longer telomeres are a marker of higher telomerase activity and high activity of cellular division. (King *et al.*, 2014) In contrast, model 2, based on the study by Wikgren et al. (Wikgren *et al.*, 2012), focuses on telomere length as a marker of fewer cell proliferations. Both models lack the connection between peripheral LTL and brain tissue itself. (Nilsonne *et al.*, 2015) Models 3 and 4 consider external factors, like chronic inflammation and oxidative stress, that may influence the relationship between LTL and the hippocampus. (Nilsonne *et al.*, 2015)

White matter hyperintensities (WMHs)

White matter hyperintensities (WMHs) are a macroscale representation of cerebrovascular ageing, microangiography, small vessel disease and an indicator of poor brain health. (Wardlaw, Valdés Hernández and Muñoz-Maniega, 2015; Veldsman *et al.*, 2020) . They are depicted as hyperintense, brain foci on T2-weighted MRI sequences, out of which 3D fluid-attenuated inversion recovery (FLAIR) is the most sensitive one. (Wikgren et al., 2014; Habes *et al.*, 2016) The burden of WMHs is often raised in the presence of vascular risk factors: hypertension, smoking, diabetes mellitus. (Habes *et al.*, 2016) There is ongoing research on the clinical meaning of WMHs, and they are reported to be associated with cognitive impairment and increase the risk of stroke and dementia. (Wardlaw, Valdés Hernández and Muñoz-Maniega, 2015)

Many mechanisms are hypothesised to be responsible for creating WMHs, mainly ischemic microvascular disease and gliosis. (Wikgren et al., 2014; Raz and Rodrigue, 2006; Gunning-Dixon *et al.*, 2009; Habes *et al.*, 2016) Other proposed mechanisms are dysfunction in the blood-brain barrier, cerebral autoregulation, inflammation and amyloid angiopathy. (Simpson *et al.*, 2007; Gouw *et al.*, 2011; Habes *et al.*, 2016)

Veldsman et al. on the dataset from UK Biobank confirmed that WMHs are considered markers of cerebrovascular burden and are associated with increased risk of vascular cognitive impairment and dementia. They explored the possible importance of their spatial localisation – in periventricular areas or within a deep white matter. (Veldsman *et al.*, 2020) They looked into the modifiable risk factors – up to date hypertension has been considered one of the most significant contributors to WMHs. Veldsman et al. study found that waist-to-hip ratio, diabetes, heavy smoking, and hypercholesterolemia contribute to increased WMH more than hypertension itself. (Veldsman *et al.*, 2020)

Similarly, vascular risk factors (smoking, hypertension, pulse pressure, diabetes, hypercholesterolemia, BMI, waist-hip ratio) were connected to poorer brain

health across grey and white matter in the analysis of 9722 participants of UK Biobank by Cox et al. (Cox *et al.*, 2019)

The first report of the association between WMHs and LTL was published in 2014 by Wikgren et al. (Wikgren *et al.*, 2014) LTL was 371 bp shorter in participants exhibiting subcortical WMHs (p=0.041), and 552 bp shorter participants with periventricular WMHs (p=0.009).

This association was confirmed in the reports by Igari et al. (Igari *et al.*, 2019) and Gampawar et al. (Gampawar, Schmidt and Schmidt, 2020). It is not yet clear whether there is a direct cause-effect relationship between short telomeres and an increase in white matter hyperintensities load or a simple correlation. (Smith et al., 2019)

Telomere length and brain morphology in healthy cohorts

Few cohort studies examined the relationship between telomere length (TL) from blood and brain measurements, indicating a statistically significant correlation with white matter hyperintensities or hippocampus volume. (Wikgren *et al.*, 2012, 2014; King *et al.*, 2014; Staffaroni *et al.*, 2018; Suchy-Dicey *et al.*, 2018; Puhlmann *et al.*, 2019; Rebello *et al.*, 2019), Tab. 1.

The study by King et al. was based on the Dallas Heart Study 2 cohort. (King *et al.*, 2014) The final sample with brain imaging had 1960 participants. Forty-eight brain regions were investigated using FreeSurfer. The strongest association was found for the precuneus, thalamus and then the hippocampus. Leukocyte telomere length was associated with both total cerebral volume and total cortical volume. Only marginal effects were observed after adjusting for vascular risk factors (hypertension, obesity, diabetes mellitus, and smoking status). The final model was adjusted for age, sex, and ethnicity. Overall, King et al. identified LTL as a biomarker associated with brain volume in different regions independent of age. However, additional analyses are required to understand the potential relationship of

regional brain volumes and telomere length with vulnerability to cognitive impairment. (King *et al.*, 2014)

In 2015 Nilsonne et al. published a meta-analysis on LTL and hippocampal volume. They included five studies of 2107 participants. (Grodstein *et al.*, 2008; Wikgren *et al.*, 2012; Jacobs *et al.*, 2014; King *et al.*, 2014; Wolkowitz *et al.*, 2015) A random-effects meta-analysis estimated the statistically insignificant effect to r = 0.12 [95% CI -0.13, 0.37], p=0.35. However, moderate to high risk of bias for included studies and high overall heterogenicity need to be considered whilst interpreting these results. (Nilsonne *et al.*, 2015) Four out of five studies had a relatively low sample size. All the samples included mainly middle-aged women, limiting the association's wider understanding across the bigger population. This meta-analysis does not prove a positive relationship between LTL and hippocampal volume. However, it is not able to disprove the earlier positive findings from the largest included study. (King *et al.*, 2014; Nilsonne *et al.*, 2015) Authors hypothesised that LTL might influence the hippocampus via transmigrating monocytes, which differentiate into microglia in the brain parenchyma. (Nilsonne *et al.*, 2015) The authors tried to understand the possible influence of APOE genotype status on the relationship between LTL and hippocampus; however, in their conclusion, none of the studies showed a piece of positive evidence. (Nilsonne *et al.*, 2015)

As the telomere length may not be the most sensitive marker, some studies looked into the telomerase to TL ratio. It demonstrates a stress-related telomere repair and is evidence of preclinical cognitive decline, where high telomerase values and low telomere length values indicate neurodegeneration. (Jacobs *et al.*, 2014) The study by Jacobs et al. explored the relationship between telomerase activity, TL, and the hippocampus humans' volume to assess whether cellular markers of ageing reflect early age-related structural brain changes. Telomerase to TL ratio studied 47 women with a mean age of 58 years. Nineteen of them were apolipoprotein E (APOE) carriers–ε4, therefore, were had an increased genetic risk of

developing cognitive impairment. Twenty-eight participants included in the control group were non-carrier. (Jacobs *et al.*, 2014) For the ApoE-4 negative group, there was a positive correlation between telomere length and hippocampal volume adjusted for age, education and BMI (for left hippocampus r^2=0.16, p=0.02, for right hippocampus r^2=0.16, p=0.002). The increase of age-adjusted telomerase to TL ratio correlates to a decrease of hippocampal volume (for left hippocampus r^2=0.26, p=0.003, for right hippocampus r^2=0.18, p=0.001). It is worth noticing that the telomerase/TL ratio had more substantial explanatory power on the left. In this study, a telomerase activity on its own did not show a statistically significant relationship with the volume of the hippocampus. (Jacobs *et al.*, 2014) Interestingly, for individuals with the increased risk of dementia understood as carriers of the ApoE-4 allele, the relationship between hippocampal volume, telomere length and telomerase activity was not evident. (Jacobs *et al.*, 2014)

Pathak et al. performed colocalisation analysis(test whether two independent association signals at a locus are consistent with having a shared casual variant) between LTL and 101 brain volume measures to identify causal genomic loci regarding telomere length measures of brain morphology. It was based on 21821 participants from five neuroimaging cohorts: the Human Connectome Project (HCP), the Paediatric imaging, neurocognition and genetics study (PING), the Philadelphia Neurodevelopmental Cohort (PNC), and Alzheimer's Disease Neuroimaging Initiative (ADNI). The European population observed four brain volumes associated with leucocyte telomere length: left postcentral, left caudal middle frontal, right precentral and grey matter. These findings suggest that telomere-based cellular ageing might provide a deeper understanding of multiple biological mechanisms of brain disorders. (Pathak *et al.*, 2020)

The remaining cohort studies had a significantly lower sample size (<500 participants). Staffaroni et al. observed a negative baseline trend between leucocyte TL and fornix mean diffusivity, a marker of episodic memory

deficits. (Douet and Chang, 2015; Staffaroni *et al.*, 2018) There was also a direct association between the extent of TL shortening and hippocampal volume loss, even after controlling for global volume loss. Those results were adjusted for APOe4 status, physical activity, and vascular risk factors (current smoker, heart attack, hypertension, hypercholesterolemia, diabetes). (AM *et al.*, 2018)

Suchey-Dicey et al. showed associations of TL with white matter hyperintensities and ventricular enlargement. The models were adjusted for age, sex, study site, alcohol, smoking, CRP, low-density lipoprotein cholesterol, education, income, BMI. (Suchy-Dicey *et al.*, 2018) Wikgren et al., in their two studies, showed a relationship between shorter TL and subcortical atrophy and white matter hyperintensities (Wikgren *et al.,* 2014) and hippocampal volume. (Wikgren *et al.*, 2012) The data was adjusted for age, sex, and head size.

author, year	population	number of participants	telomeres source	brain feature	results	co-variates
Wikgren 2012	cohort, adults	57	blood	hippocampal volume	yes, in epo3	age, body size
Wikgren 2013	cohort, adults	102	blood	multiregional volume	yes, subcortical atrophy and periventricular WMH	age, sex
Jacobs, 2014	cohort, female adults	47	blood	hippocampal volume	yes, telomerase/TL ratio was a strong predictor of hippocampal volume for APOE-ε4 non-carriers	age, education, BMI
King 2014	cohort, adults	1960	blood	multiregional volume - 48 ROI	yes, multiple associations, including precuneus and hippocampal volumes	age, sex, ethnicity, total intracranial volume, BMI, HTN, diabetes, smoking status, and APOE genotype
Staffaroni 2018	cohort, adults	363	blood	multiparameter, including hippocampal volume	yes, greater TL shortening at FU+ greater hippocampal volume loss, fornix DTI quantification	age, sex, APOE genotype, education, total intracranial volume
Suchy-Dicey 2018	cohort, elderly	69	blood	multiparameter, including hippocampal volume	yes, positive association with hippocampal volume and WMHs	age, sex, study site, alcohol, smoking, CRP, low-density lipoprotein, education income, BMI
Rebello 2019	cohort, kids and adolescents	389	blood	fMRI: posterior cingulate cortex (PCC) and the medialprefrontal cortex (mPFC)	yes with PCC	age, sex, site of acquisition
Puhlmann 2019	cohort, adults	298	blood	cortical thickness	yes, baseline association with praecuneus and PCC,	age, sex, BMI
Pathak, 2020	cohort, adults	21821	blood	multiregional volume - 101 ROI	yes, in European group left postcentral, left caudal middle frontal, right precentral, grey matter were associated with TL	NA

Tab. 1. Summary of studies investigating the association between telomere length and brain imaging features in cohort studies. ROI – a region of interest, LTL – leukocyte telomere length, WMH – white matter hyperintensities, GM – grey matter, HTN – hypertension, fMRI – functional magnetic resonance imaging, CRP – C-reactive protein

To summarise, the research looking at the association between neuroimaging markers of brain ageing and telomere length was reported in healthy cohorts. The telomere length was derived from leucocytes from the peripheral blood. MRI was one of the most common imaging methodologies, with the occasional use of more advanced methods, such as functional MRI. The analysis was most commonly adjusted for age and sex. The association was most commonly found for the hippocampus (number of studies = 4), white matter hyperintensities (number of studies = 2), and precuneus (number of studies = 2). However, as discussed previously, the correlation was not confirmed between hippocampal volume and TL in the meta-analysis. (Nilsonne *et al.*, 2015)

Telomere length and brain morphology in disease

Grodstein et al.. evaluated 29 women (3 with dementia, 7 with mild cognitive impairment, 19 cognitively healthy controls) who underwent measurement of LTL from a peripheral blood sample and 1.5T brain-MRI. Their hippocampal volume was calculated by summing the volumes of both hippocampi. This number was divided by the intracranial volume and consequently multiplied by 1000. (Grodstein *et al.*, 2008) A modified version of the real-time PCR-based telomere assay was used to measure relative average telomere length, and the results were expressed as the telomeres to single genes ratio (T/S ratio). T/S ratio is the relative amount of telomeric DNA (T) to the beta-globin single-copy gene (S), calibrated to a plate reference genomic DNA sample. (Nguyen *et al.*, 2019) The LTL-HC relationship analysis excluded women with dementia to implement results as a possible marker for preclinical changes in hippocampal volume. They found that decreasing telomere length was related to lower hippocampal volume. After adjusting for age and educational attainment, each T/S ratio unit decrease decreased hippocampal volume of 0.25 mL ($p=0.038$). (Grodstein *et al.*, 2008) Moreover, they found that having a telomere length below the median (when compared to above the median), participants had higher odds of dementia

or MCI combined (OR=9.63, 95% CI 1.73–53.65), as well as MCI alone (OR=12.00, 95% CI 1.24–116.5) (Grodstein *et al.*, 2008) In the study of Henje-Blom et al., 54 adolescents with the major depressive disorder had significantly shorter salivary telomere length than 63 participants of matched healthy control group. They were also found to have a considerably smaller right hippocampal volume in the analysis adjusted for age, sex, and total brain volume. Authors also looked into the association between right/left hippocampal volume and TL and found that TL did not significantly predict the left (β=−24.4, $t_{(111)}$=−0.18, P>0.86) or right HV (β=53, $t_{(111)}$=−0.44, P>0.66), when correcting for age, sex, diagnostic group and total brain volume. (Henje Blom *et al.*, 2015)

The study by Wolkowitz et al. examined 25 participants with major depressive disorder and 18 control subjects. (Wolkowitz *et al.*, 2015) They collected information from peripheral blood on leucocytes telomere length and peripheral telomerase activity and performed 3T MRI brain scans out of which hippocampal volumes were extracted. The baseline analysis was adjusted for age and sex. They found a positive correlation between telomerase activity and HC volume (r=0.44, p=0.034) among participants with depression; this effect was not seen among healthy controls. There was no correlation between LTL and brain-imaging features (in both study groups separately, as well as combined). Additionally, the authors noted that plasma IL-6, overnight urinary free cortisol and plasma F2-isoprostanes affect hippocampal volume and markers of peripheral cell ageing (including TL). They confirmed additional exploratory analysis, which showed that those factors did not influence other results. (Wolkowitz *et al.*, 2015)

Studies suggest that schizophrenia is associated with hippocampal atrophy. (Anand and Dhikav, 2012) Shivakumar et al. examined LTL and MRI-brain from 30 patients with schizophrenia who had not yet received their antipsychotic therapy and 60 healthy control subjects. They noticed that male schizophrenia patients had significantly lower relative TL than female patients, and TL in the control group did not differ. Schizophrenia patients showed a

significant TL interaction with the hippocampus (in both hemispheres separately). Men displayed positive association and women negative. (Shivakumar *et al.*, 2018) The impact of sex on TL is consistent with the previous studies and had been previously linked with the longer lifespan among women than men via various proposed mechanisms (protective effect of estrogen to reduce oxidative stress, among others). (Aviv, 2002; Nawrot *et al.*, 2004) Czepielewski et al. looked into TL's association with grey matter (GM) volume in 48 patients with schizophrenia and 64 healthy controls. Again, they found schizophrenia patients to have a significant TL interaction with total GM, which was not seen in the healthy control. (Czepielewski *et al.*, 2018)

author, year	abnormality	number of participants	telomeres source	brain feature	results	co-variates
Grodstein, 2008	females with mild cognitive impairment	29	blood	hippocampal volume	increasing telomere length was strongly related to decreasing hippocampal volume	age, educational attainment, cigarette smoking, history of cardiovascular disease, HTN, high cholesterol type 2 diabetes
Henje Blom, 2015	adolescents with depression	117	saliva	hippocampal volume	no association in analysis adjusted for co-variates	age, sex, diagnostic group and total brain volume
Wolkowitz 2015	depression	43	blood	hippocampal volume	telomerase activity positively correlated with hippocampal volume, no correlation for LTL	age, sex, BMI, lifetime and current tobacco use and alcohol use,
Choi, 2016	obstructive sleep apnea	420	blood	white matter hyperintensities	yes, more severe OSA and shorter telomeres causes more WMH	age, sex, BMI, smoking, drinking, snoring, HTN
Czepielewski, 2017	schizophrenia	112	?blood	grey matter volume (cortical+subcortical)	significant correlation between TL and total GM in patients with schizophrenia	age, duration of schizophrenia
Shivakumar, 2018	schizophrenia	90	blood	hippocampal volume	positive association of TL with hippocampus volume only in male patients with schizophrenia	age, sex, years of education
Powell, 2018 (Powell et al., 2019) (Powell et al., 2019) (Powell et al., 2019) (Powell et al., 2019) (Powell et al., 2019)	mood disorders	112	saliva	fMRI: connectivity	yes, face-related activation in amygdala and cuneus	age, sex
Krysko, 2019	multiple sclerosis	516	blood	brain, grey+white matter volumes	yes, total brain and white matter volume	age, sex, MS duration

Tab. 2. Summary of studies investigating the association between telomere length and brain imaging features in pathology. LTL – leukocyte telomere length, OSA – obstructive sleep apnea, WMH – white matter hyperintensities, GM – grey matter, HTN – hypertension, fMRI – functional magnetic resonance imaging

To summarize, the research looked into the association of telomere length and brain features in the following diseases: multiple sclerosis, mood disorders, schizophrenia, obstructive sleep apnoea, depression, and mild cognitive impairment. Most of the information on telomere length was derived from leucocytes in peripheral blood, except for two studies that analysed saliva samples. In addition, four studies looked in detail at the hippocampal volumes.

Aims, Objectives, Hypothesis

No one has yet looked at the association between the genetic blueprint of telomere length or telomerase activity expressed by single nucleotide polymorphism (SNP) and brain phenotypes. Therefore, it is unknown if this potential association between telomeres and brain measurement may have direct (genetic causality) or indirect effect (for example, via the systemic impact of cardiovascular risk factors).

Our study aims to investigate the association between SNP regulating telomeres length and image-derived phenotypes of the hippocampus (with its subregions: head, body and tail) as a marker of ageing and neurodegenerative disorders and white matter hyperintensities as a marker of cerebrovascular health based on almost 30,000 participants of the UK Biobank dataset.

We hypothesise that telomere maintenance genes are associated with brain imaging markers of ageing and cerebrovascular health. We built models predicting the volumes of neuroimaging biomarkers, considering their correlation and regression analysis to check for the associations with telomere length genetic information and lifestyle risk factors.

The thesis aims to study TL maintenance genes and brain imaging markers indicative of ageing and cerebrovascular health expressed as neuroimaging biomarkers of hippocampus and white matter hyperintensities load.

Chapter 2: Methods

Participant selection and group assignment

UK Biobank is a research resource collecting in-depth health, genetic and imaging information from 533,513 participants. (UK-Biobank, 2022)

Currently, there is available brain imaging of 41,984 UK Biobank participants. The projected final number of imaging datasets for the UK Biobank is 100,000.

We qualified the poor quality of brain scans as lack of data in the UKBB Field ID 25000 "volumetric scaling from T1 head image to standard space". The impaired imaging quality could have represented issue with the equipment (e.g., coil failure, low signal-to-noise ratio, drift, and other hardware performance issues related to MR scanners) and artefacts specific to the participant or scanning session (e.g., excessive head movement) (Alfaro-Almagro *et al.*, 2018; Littlejohns *et al.*, 2020)

To reduce biases in the protocol, we chose to include a subset of participants who, for Freesurfer segmentation, had used both T1 and T2-FLAIR imaging. (UKBB Field ID 25736, T2-FLAIR used (in addition to T1) to run FreeSurfer) (Alfaro-Almagro *et al.*, 2021) Lack of T2-FLAIR sequence means a difference in analysis protocol.

A subset of the UK Biobank cohort of Caucasian ethnic background with available IDPs of brain imaging was used as a primary dataset. We decided to include only the Caucasian population due to (i) genetic variability between ethnicities(Huang, Shu and Cai, 2015) and (ii) known differences in cardiovascular risk factors between ethnicities(Tillin *et al.*, 2014), and (iii) the dominating proportion of white individuals – 503,746 participants which equals to 94.4% of all individuals in the UK Biobank. (Fry *et al.*, 2017; UK-Biobank, 2022) The selection of the subgroup was based on the Caucasian genetic, ethnic background (UKBB Field ID 22006).). This limits the interpretation of the results to the Caucasian cohort.

Finally, we will select a sample of participants with full primary outputs variables, genetic information and confounders.

Inclusion Criteria	Exclusion Criteria
UK Biobank participant with brain imaging and genotyping data	- poor quality of brain imaging data - lack of FLAIR sequence - non-Caucasian - missing data in primary outcomes / genetic information

Tab. 3. Inclusion and exclusion criteria of participants from UK Biobank

Magnetic resonance imaging

The brain imaging was performed in 3 UK centres with the identical, standard Siemens Skyra 3T, with a standard Siemens 32-channel RF receive head coil. (Smith, Alfaro-Almagro and Miller, 2020) Brain imaging covers six modalities (T1, T2 FLAIR, SWI, Resting fMRI, Task fMRI and Diffusion MRI). For the purpose of this analysis, image-derived phenotypes (IDPs) derived from T1-weighted imaging (Resolution: 1x1x1 mm; Field-of-view: 208x256x256 matrix; Duration: 5 minutes; 3D MPRAGE, sagittal) and T2-weighted FLAIR structural imaging (Resolution: 1.05x1x1 mm Field-of-view: 192x256x256 matrix Duration: 6 minutes; 3D SPACE, sagittal) will be used. (Smith, Alfaro-Almagro and Miller, 2020)

Imaging data pre-processing

The pre-processing of brain imaging was performed before IDPs extraction as detailed in UK Biobank Brain Imaging Documentation. (Smith, Alfaro-Almagro and Miller, 2020) No further imaging data pre-processing will be done. An automated processing pipeline for brain image analysis and quality control

was established for UK-Biobank. The images were reconstructed from *k*-space on the scanner computer and saved as DICOM files after the acquisition at the imaging centre, which were further converted to "defaced" NIFTI files available to researchers. During format conversion there was pre-processing (e.g., correcting for head motion and other artefacts) as well as automated quality control that identified issue with the equipment and artefacts specific to the participant or scanning session. The pipeline also automatically generated thousands of image-derived phaenotypes (IDPs), for example regional grey matter volume from T1 scans or volume of WMHs from T2 scans. (Littlejohns *et al.*, 2020)

Feature extraction

For genetic data:

Based on the available literature exploring genetic association with leukocyte telomere length we identified 29 relevant SNPs. (rs9420907, rs10936599, rs10936600, rs2736100, rs2853677, rs8105767, rs2302588, rs2736176). The remaining SNP: rs11125529, rs228595, rs34991172, rs13137667, rs2967374, rs7194734, rs4691895, rs7675998, , rs3219104, rs59294613, rs62053580, rs34978822, rs755017, rs75691080, rs73624724, rs55749605, rs11191848, rs11191849, rs9419958, rs3785074, rs7705526. (Codd *et al.*, 2013; Li*et al.*, 2020; Pathak *et al.*, 2020)

The lack of data available at this stage could decrease our understanding of polygenetic factors influencing telomere length; however, ongoing work on direct leukocyte telomere length measurement for UK Biobank participants could be used for future validation.

For IDPs:

- The volume of the hippocampus in the right and left hemisphere with its subdivision: tail, body, head
- The total volume of white matter hyperintensities (from T1 and FLAIR images)

For demographic and lifestyle factors:

- maternal and paternal longevity (Bertram *et al.*, 1998)
- BMI, waist-to-hip ratio (Lampe *et al.*, 2019)
- handedness, chronotype (Norbury, 2019)
- IPAQ (physical activity category)(Torres *et al.*, 2019)
- qualifications, current employment status (Anderson *et al.*, 2020)
- alcohol drinker status, smoking status (Wolkowitz *et al.*, 2011; Kim *et al.*, 2012; Wilson *et al.*, 2017)

- pulse rate, arterial stiffness, systolic and diastolic blood pressure (Grodstein *et al.*, 2008)
- self-reported number of non-cancer diseases and operations (Shammas, 2011a)
- diagnosis by a doctor: diabetes, vascular heart problem, cancer, PE, DVT (Grodstein *et al.*, 2008)
- blood markers: cholesterol, HbA1c (Grodstein *et al.*, 2008)
- number of medications, medications for cholesterol/hypertension/diabetes (insulin) (Grodstein *et al.*, 2008)

Confounders

For the selection of technical confounders, we followed the recent paper by Alfaro-Almagro et al. on deconfounding UKBB MRI data (Alfaro-Almagro *et al.*, 2021):

- subject-specific confounds: age *(Age at the imaging centre*, UKBB Field ID 21003 - when participants had fur visits reported, visit-3 was the first brain imaging scanning sessions, and visit-4 was a second brain imaging scanning session), *Sex* (genetic sex, UKBB Field ID 22001), head size (*Volumetric scaling from T1 head image to standard space*, UKBB Field ID 25000)
- scanner/acquisition protocol/processing parameters: acquisition site (UKBB Field ID 54), intensity scaling for T1, intensity scaling for T2-FLAIR, T2-FLAIR used (in addition to T1) to run FreeSurfer.
- table position: *Scanner lateral (X) position,* UKBB Field ID 25756, *Scanner transverse (Y) position,* UKBB Field ID 25757; *Scanner longitudinal (Z) position* - UKBB Field ID 25758; *Scanner table position* - UKBB Field ID 25759.

Statistical analyses

All statistical analyses were performed with Statistica 13.1, SPSS 27, Stata SE 17, and R software by the student. The methodology was discussed with external statistician. Basic statistics (mean±SD for continuous data, frequencies for categorical data) will be used to describe the dataset.

For continuous data, the normality of distribution was assessed with the Kolgomorov – S test with Lilliefors correction (to reduce type II error). If required, log transformation of data was conducted to achieve normality of distribution and enable general linear models. Variables of no variance and variables with >20% missing data were deleted from the further analysis.

As the first step of data preparation, the Pearson/Spearman correlation test was conducted as appropriate to check for correlation between independent and dependent values. r>0.7 is interpreted as a significant correlation. (Dancey and Reidy, 2008).

Univariate linear regression models were initially performed to understand the impact of individual independent variables on dependent value. Multiple linear regression was used to assess the dependence of WMHs and right/left whole hippocampal volume (with its subdivision into a head, a body and a tail) on the genetic data and cerebrovascular risk factors while controlling for known confounders (age, sex, head size, scanning session related variables). The models were assessed by R^2 as a widely accepted measure of "goodness of fit". (Logue and Manandhar, 2018)

The first model included all independent variables and was assessed for collinearity by variance inflation factors. The variable with VIF of the value less than 1 or 10 and above will be excluded from further analyses as a clear signal of multicollinearity. (Kim, 2019)

The following linear models will test the inclusion of different independent values sets based (chosen based on the previous steps/models), aiming to achieve maximum adjusted R^2 with a minimum number of independent

variables. Bonferroni correction was used to interpret the statistical significance of the variables from the final model.

Data management plan

Data is stored under the University of Nottingham HPC Augusta directory /gpfs01/share/ukbiobank/msaap24. Output analysis will be stored in the OneDrive folder. There is no planned archiving of data. There is a planned follow-up study when direct leucocyte TL data becomes available (Supplementary Material 1)

Ethics

UK Biobank has approval from the North West Multi-centre Research Ethics Committee, covering the UK – including our analysis. (https://www.ukbiobank.ac.uk/learn-more-about-uk-biobank/about-us/ethics) Each participant signed informed consent.

The access to the UK Biobank data was granted under application number 43822, entitled *Multi-Modal Analysis of the UK Biobank Neuroimaging Data,* Principal Investigator Prof Stamatios Sotiropoulos.

Chapter 3: Results

Genetics influencing telomere length

In June 2021, the pubmed search revealed 330 articles on telomeres and associated SNPs. The title and abstract screen was performed to identity studies reporting SNPs regulating telomere length and telomerase activity. We identified meta-analysis from 2013 which provides the highest level of evidence. (Codd *et al.*, 2013) Afterwards, only two further studies reporting telomere regulating SNPs were identified. (Li *et al.*, 2020; Pathak *et al.*, 2020) Li et al performed GWAS analysis of 78,592 European-descent to identify telomere-regulating SNPs, as well as looked into association between identified loci and 122 diseases in the random sample of 50,000 Uk BioBank participants. (Li *et al.*, 2020) Pathak et al performed genetic colocalization of telomere regulating SNPs with brain morphology in 60,601 participants of European and East-Asian ancestry. (Pathak *et al.*, 2020) In total we identified 29 distinct loci influencing mean TL. The details are shown in Tab. 4.

	Chr	Position	rsid	*Locus*	Ref allele	Risk allele	Risk allele frequency	primary source
1	2	54329370	rs11125529	ACYP2	C	A	0.858	(Codd *et al.*, 2013)
2	11	108105593	rs228595	ATM	G	A	0.42	(Li *et al.*, 2020)
3	6	25480328	rs34991172	CARMIL1	T	G	0.07	(Li *et al.*, 2020)
4	14	73404752	rs2302588	DCAF4	G	C	0.1	(Li *et al.*, 2020)
5	4	71774347	rs13137667	MOB1B	T	C	0.96	(Li *et al.*, 2020)
6	16	80767362	rs2967374	MPHOSPH6	G	A	0.79	(Codd *et al.*, 2013)
7	16	82199980	rs7194734	MPHOSPH6	C	T	0.78	(Li *et al.*, 2020)
8	4	164048199	rs4691895	NAF1	G	C	0.78	(Li *et al.*, 2020)
9	4	164227270	rs7675998	NAF1	A	G	0.217	(Codd *et al.*, 2013)
10	10	105666455	rs9420907	OBFC1	A	C	0.865	(Codd *et al.*, 2013)
11	1	226562621	rs3219104	PARP1	A	C	0.83	(Li *et al.*, 2020)

12	7	124554267	rs59294613	POT1	C	A	0.29	(Li *et al.*, 2020)
13	6	31587561	rs2736176	PRRC2A	G	C	0.31	(Li *et al.*, 2020)
14	16	74680074	rs62053580	RFWD3	A	G	0.17	(Li *et al.*, 2020)
15	20	62291599	rs34978822	RTEL1	C	G	0.02	(Li *et al.*, 2020)
16	20	61892066	rs755017	RTEL1	A	G	0.869	(Codd *et al.*, 2013)
17	20	62269750	rs75691080	RTEL1/STMN3	C	T	0.09	(Li *et al.*, 2020)
18	20	62436398	rs73624724	RTEL1/ZBTB46	T	C	0.13	(Li *et al.*, 2020)
19	3	101232093	rs55749605	SENP7	C	A	0.58	(Li *et al.*, 2020)
20	10	NA	rs11191848	STN1 (OBFC1)	NA	NA	NA	(Pathak *et al.*, 2020)
21	10	NA	rs11191849	STN1 (OBFC1)	NA	NA	NA	(Pathak *et al.*, 2020)
22	10	105675946	rs9419958	STN1 (OBFC1)	T	C	0.86	(Li *et al.*, 2020)
23	3	170974795	rs10936599	TERC	T	C	0.252	(Codd *et al.*, 2013)
24	3	169514585	rs10936600	TERC	A	T	0.24	(Li *et al.*, 2020)
25	16	69406986	rs3785074	TERF2	A	G	0.26	(Li *et al.*, 2020)
26	5	1339516	rs2736100	TERT	A	C	0.514	(Codd *et al.*, 2013)
27	5	1287194	rs2853677	TERT	G	A	0.59	(Li *et al.*, 2020)
28	5	1285974	rs7705526	TERT	C	A	0.33	(Li *et al.*, 2020)
29	19	22215441	rs8105767	ZNF208	A	G	0.3	(Li *et al.*, 2020)
	19	22007281	rs8105767	ZNF208	A	G	0.709	(Codd *et al.*, 2013)

Tab. 4. SNP associated with telomere length. NA - not available, Chr – chromosome, A – adenine, C – cytosine, G – guanine, T - thymine

Description of the dataset

We obtained access to 41,984 brain imaging for UKBB who had concomitant genome analysis. We assessed the poor quality of brain scans as lack of data in the UKBB Field ID 25000 "volumetric scaling from T1 head image to standard space", which decreased the sample size by 2289 subjects to 39,695 participants.

We included only scans that used Freesurfer segmentation analysis, both T1 and T2 imaging from the good quality subset, which excluded 1,723 participants, leaving the subset of 37,973 observations.

As a next step, we excluded participants of the non-Caucasian genetic ethnic group (UKBB Field ID 22016), which further decreased the dataset by 5,731 participants, leaving the subset of 32,242 observations.

Participants with missing data in the primary outcome (WMHs/hippocampal volume), genetic information, and confounders were also excluded, excluding 4,223 observations. The final dataset included 28,019 participants.

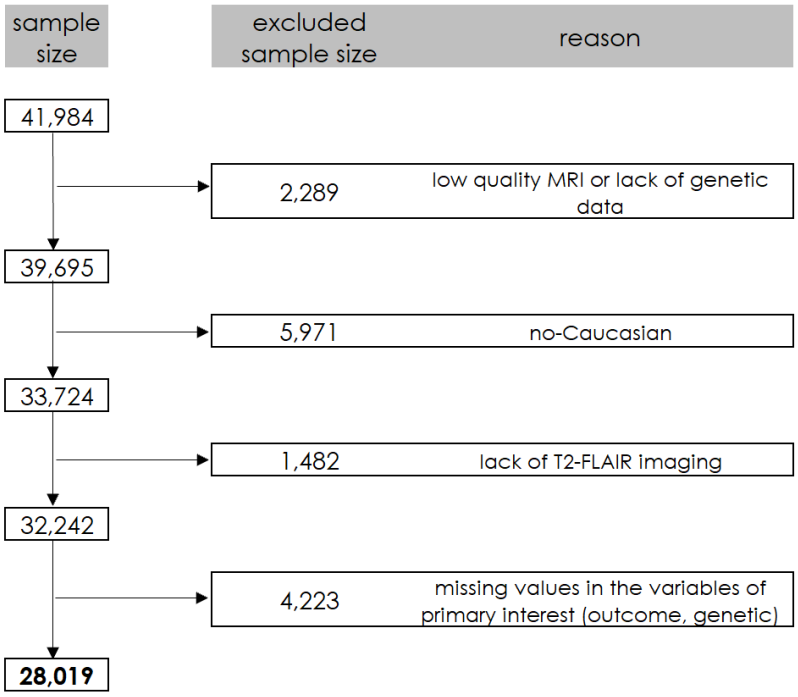


Fig. 7. The flow chart shows the participants selection process:

For all participants, we extracted 77 variables:

- Nine confounders: features deemed as relevant from the clinical practice literature and scanner settings: age at the imaging centre, gender, ApoE4 stats, smoking status, imaging centre, scanner position (lateral, transverse, longitudinal, table), volumetric scaling from T1 head image to standard space,
- 29 genetic SNP,

- 30 lifestyle and demographic factors influencing telomere length, WMHs and hippocampal volume based on the literature search,
- Nine brain IDPs, three used as dependent values (WMHs, right total hippocampal volume, left total hippocampal volume)

After a brief assessment of the first dataset draft of 28,019 participants, we noticed that:

- there is no variability for T1 volumetric scaling; all values represent 6; therefore, this was removed from the confounder list
- ICD 10 coding for migraine, ICD 10 coding for hypertension and headache for 3+ months had more than 20% of missing data, therefore were excluded from further analysis.
- all continuous variables have non-normal distribution as assessed by the Lillieforce normality test ($p<0.05$)

Baseline statistics

Confounders

1. Sex - out of 28,019 participants, 13,201 (47.1%) were male and 14,818 (52.9%) were female.
2. intensity scaling for T2-FLAIR – out of 28,019 imaging sessions, 3 (0.01%) had category 1 intensity scaling for T2-FLAIR, 1 (<0.01%) had category 2 intensity scaling for T2-FLAIR, 6,371 (022.7%) had category 5 intensity scaling for T2-FLAIR, 21,644 (77.2%) had category 6 intensity scaling for T2-FLAIR,
3. imaging centre – out of 28,019 imaging sessions, 17,225 (61.5%) were performed in Cheadle, 7,183 (25.6%) were performed in Newcastle and 3,611 (12.9%) were performed in Reading.

4. age - out of 28,019 participants, the mean age was 63.3 ± 7.5, range 45 – 81.
5. head size (Volumetric scaling from T1 head image to standard space) - out of 28,019 participants, the mean was 1.3 ± 0.12, range 0.8 – 1.8
6. Scanner lateral (X) position - out of 28,019 participants, the mean was 0.68 ± 2.9, range -41.09 – 17.3
7. Scanner transverse (Y) position - out of 28,019 participants, the mean was 65.97 ± 5.9, range 52 – 96
8. Scanner longitudinal (Z) position - out of 28,019 participants, the mean was -27.5 ± 27.2, range -96.7 – 124.5
9. Scanner table position - out of 28,019 participants, the mean was -1,055.1 ± 25.7, range -1197 – -1020

All of the above confounders were added to the baseline regression model.

Genetic baseline information

We identified 29 SNPs regulating leucocyte telomere length (Codd *et al.*, 2013; Li *et al.*, 2020; Pathak *et al.*, 2020). The status of reference allele was established from the literature comparing it to the reference human genome (https://www.internationalgenome.org/home, Codd et al., 2013; Li et al., 2020; Pathak et al., 2020) and the status of minor/major allele for people of the Caucasian genetic background was based on the data from NCBI database od Genotypes and Phenotypes (dbGaP, https://www.ncbi.nlm.nih.gov/gap/)

	hetero		**homo**		**non-carrier**	
SNP	n	%	n	%	n	%
1_rs3219104_C_major	7361	26.3	19955	71.2	703	2.5

2_rs11125529_A_minor	6845	24.4	546	1.9	20628	73.6
3_rs55749605_A_major	13144	46.9	10985	39.2	3890	13.9
3_rs10936599_T_minor	10341	36.9	1695	6.0	15983	57.0
3_rs10936600_T_minor	10335	36.9	1683	6.0	16001	57.1
4_rs13137667_C_major	1447	5.2	26548	94.7	24	0.1
4_rs7675998_G_major	9438	33.7	17201	61.4	1380	4.9
4_rs4691895_C_major	9626	34.4	16940	60.5	1453	5.2
5_rs7705526_A_minor	12266	43.8	2991	10.7	12762	45.5
5_rs2736100_A_minor	13992	49.9	7280	26.0	6747	24.1
5_rs2853677_A_major	13483	48.1	9700	34.6	4836	17.3
6_rs34991172_G_minor	4318	15.4	174	0.6	23527	84.0
6_rs2736176_C_minor	11320	40.4	2285	8.2	14414	51.4
7_rs59294613_A_minor	11546	41.2	2268	8.1	14205	50.7
10_rs11191848_A_minor	13917	49.7	7062	25.2	7040	25.1
10_rs11191849_A_major	13916	49.7	7041	25.1	7062	25.2
10_rs9419958_C_major	6219	22.2	21308	76.0	492	1.8
10_rs9420907_A_major	6226	22.2	21301	76.0	492	1.8
11_rs228595_A_minor	13520	48.3	4685	16.7	9814	35.0
14_rs2302588_C_minor	5122	18.3	285	1.0	22612	80.7
16_rs3785074_G_minor	11500	41.0	2337	8.3	14182	50.6
16_rs62053580_G_minor	7907	28.2	768	2.7	19344	69.0
16_rs7194734_T_major	9905	35.4	16555	59.1	1559	5.6
16_rs2967374_G_major	9860	35.2	16609	59.3	1550	5.5
19_rs8105767_G_minor	11436	40.8	2350	8.4	14233	50.8
20_rs75691080_T_minor	4179	14.9	172	0.6	23668	84.5
20_rs34978822_G_minor	1102	3.9	17	0.1	26900	96.0
20_rs755017_G_minor	6601	23.6	525	1.9	20893	74.6
20_rs73624724_C_minor	6786	24.2	551	2.0	20682	73.8

Tab. 5. The number and percentage of genetic SNP influencing the telomere leucocyte length in the selected UKBB sample. SNP description is as follows: chromosome number _ SNP identifier _ risk allele _ major/minor status of the risk allele. n = number, % = percentage.

Demographic and lifestyle factors

Based on the literature search via electronic databases about factors influencing brain WMHs and hippocampal volume detailed in the tables in the introduction, we identified 30 potential factors that may influence the total volume of WMHs and hippocampal volume.

1. HbA1c – out of 26,872 participants, the mean was 34.96 ± 5.08, range 15.3 – 122.6
2. cholesterol - out of 26,944 participants, the mean was 5.74 ± 1.09, range 1.87 – 12.9
3. waist-to-hip - out of 28,018 participants, the mean was 0.87 ± 0.09, range 0.61 - 1.31
4. BMI - out of 28,019 participants, the mean was 26.49 ± 4.37, range 13.88 – 58.04
5. Pulse rate - out of 27,680 participants, the mean was 68.2 ± 11.4, range 32 – 159
6. Systolic Blood Pressure (SBP) - out of 28,680 participants, the mean was 136 ± 18, range 75 – 231
7. Diastolic Blood Pressure (DBP) – out of 28,637 participants, the mean was 79 ± 10, range 36 – 130
8. Arterial Stiffness (AS) - out of 26,020 participants, the mean was 9.55 ± 3.93, range 1.53 – 330
9. Apo-ε status. – ApoE status was derived from two SNPs: rs429358 and rs7412. Apo-E ε4 allele only (not carriage of ε3/ε3 or ε2/ε2 or ε2/ε3) was

considered a cerebrovascular risk factor based on a substantial body of research for APOE ε4 as a risk factor for cardiovascular disease and sporadic dementia. Based on the number of ε4 alleles, a discrete APOE-ε status covariate was created as 0 (non-carrier of ε4 allele), 1 (heterozygous, i.e. ε3/ε4) and 2 (homozygous, i.e. ε4/ε4)." (Veldsman *et al.*, 2020)

Table 2. APOE Genotype Determination			Rs429358 count of minor allele	Rs7412 count of minor allele
APOE Genotype	*rs429358* Allele	*rs7412* Allele		
ε2/ε2	T	T	0	2
ε3/ε3	T	C	0	0
ε2/ε3	T	CT	0	1
ε2/ε4	CT	CT	1	1
ε3/ε4	CT	C	1	0
ε4/ε4	C	C	2	0

Fig. 8. Representation of the ApoE status determination based on (Koleck *et al.*, 2014)

Out of 28,019 participants, 20,239 (72.2%) are non-carriers, 7,165 (25.6%) are heterozygous, and 615 (2.2%) are homozygous.

10. Father – out of 27,597 participants, 2,427 (8.7%) had an alive father of age below 85 years, 1,404 (5%) had an alive father of age 85 years, and above, 19,665 (70.2%) had a father who was deceased before 85 years, and 4,101 (14.6%) had a father who deceased after 85 years of age.

11. Mother – out of 28,896 participants, 4,465(15.9%) had an alive mother of age below 85 years, 2,967 (10.6%) had an alive mother of age 85 years, and above, 14,344 (51.2%) had a mother who was deceased before 85 years, and 6,120 (21.9%) had a mother who deceased after 85 years of age.

12. Current employment – out of 27,735 participants, 11,370 (40.6%) of participants were working at the time of the scan, and 16,365 (58.4%) were not working – including retirement.
13. Qualifications – out of 26,587 participants, 15,121 (54.0%) were awarded a degree, and 11,466 (41.0%) had an education that did not obtain the degree.
14. Chronotype – out of 27,005 participants, 7,325 (26.1%) were definitely morning person, 9,989 (35.6%) were more morning person, 7.164 (25.6%) were more evening person and 2,527 (9.0%) were definitely an evening person
15. Handedness – out of 28,013 participants, 24,932 (89.0%) were right-handed, 2,649 (9.5%) were left-handed and 432 (1.5%) were ambidextrous
16. IPAQ – out of 28,019 participants, 4,788 (17.1%) had low physical activity, 11,715 (41.8%) had moderate physical activity and 11,516 (41,1%) had high levels of physical activity
17. Smoking status - out of 28,019 participants, 983 (3.5%) are current smokers, 9,543 (34.1%) are previous smokers, and 17,488 (62.4%) of participants have never smoked.
18. Alcohol drinker status – out of 28,018 participants, 26,229 (93.6%) are current drinkers, 933 (3.3%) are previous drinkers, and 856 (3.1%) have never drunk
19. BMI category - out ot 28,019 participants, 204 (0.7%) are underweight, 11,226 (40.1%) have normal BMI, 11,536 (41.2%) are overweight and 5053 (18%) are obese
20. Number of non-cancer illness (self-reported) – out of 28,019 participants, 5,932 (21.2%) reported no non-cancer diseases, 5,993 (21.4%) reported one non-non cancer disease, 4,852 (17.3%) reported

two non-cancer diseases and 11,242 (40.1%) reported three or more non-cancer diseases

21. Number of operations (self-reported) - out of 28,019 participants, 5,235 (18.8%) reported no operations, 7,865 (28.1%) reported one operation, 6,711 (24.0%) reported two operations and 8,208 (29.1%) reported three or more operations

22. Cancer diagnosed by a doctor - out of 28,014 participants, 2,962 (10.6%) were diagnosed with cancer by a doctor, and 25,052 (89.2%) were not

23. Diabetes diagnosed by a doctor – out of 28,017 participants, 1,425 (5.1%) were diagnosed with diabetes by a doctor, and 26,592 (94.9%) were not

24. Vascular/heart problem diagnosed by a doctor – out of 27,999 participants, 5,911 (21.1%) had hypertension diagnosed by a doctor, 242 (0.9%) had angina diagnosed by a doctor, 154 (0.6%) had a MI diagnosed by a doctor, and 116 (0.4%) had a stroke diagnosed by a doctor, whilst 21,576 (77.0%) had none of those diagnoses

25. Deep vein thrombosis (DVT) diagnosed by a doctor – out of 28,016 participants, 271 (1%) had a DVT diagnosed by a doctor, whilst 27,745 (99%) did not

26. Pulmonary embolism (PE) diagnosed by a doctor – out of 28,016 participants, 164 (0.6%) had a PE diagnosed by a doctor, whilst 27,852 (99.4%) did not

27. Number of medications (self-reported) – out of 28,019 participants, 9,050 (32.3%) took zero medications, 5,886 (21.0%) took one medication, 4,592 (16.4%) took two medications and 8,491 (30.3%) took three or more medications

28. Medications for cholesterol – out of 28,013 participants, 2,775 (9.9%) took medications for cholesterol, and 25,238 (90.1%) did not

29. Medications for hypertension – out of 28,013 participants, 6,267 (22.4%) took medications for hypertension, and 6,267 (22.4%) did not
30. Medications for diabetes (insulin) – out of 28,013 participants, 199 (0.7%) took insulin, and 27,814 (99.3%) did not.

Brain IDPs

We identified WMHs and hippocampal volume (left and right) as our primary dependent variables. We obtained data on intracranial volumes and hippocampal subdivision as secondary dependant variables from the UK Biobank dataset, which underwent standardized pipeline image analysis centrally.

1. WMHs - out of 28,019 participants, the mean volume of white matter hyperintensities was 4,987 mm^3 ± 6,529 mm^3, range 9 – 101,414 mm^3. Given the highly right-skewed distribution of WMH load across the population, the data was log-transformed. The Log-WHMs mean was 3.5 ± 0.5, range 0.95 – 5

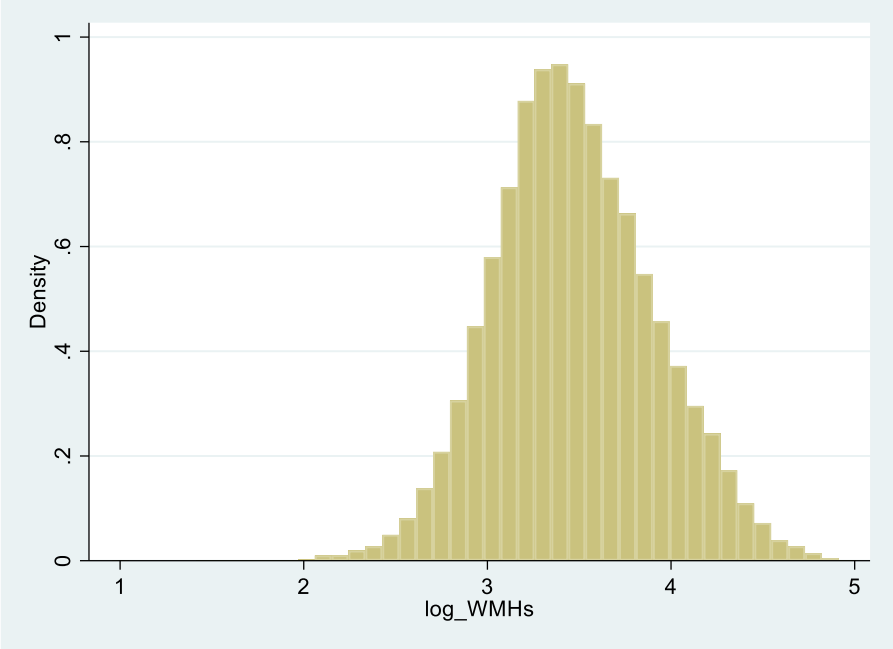


Fig. 9. Histogram of log White Matter Hyperintensities in mm^3

2. The volume of the left hippocampus - 28,019 participants, the mean volume of the left hippocampus was 3,680 mm^3 ± 394 mm^3, range 1.564 – 5,967 mm^3.
3. The volume of the right hippocampus - 28,019 participants, the mean volume of white matter hyperintensities was 3,797 mm^3 ± 413 mm^3, range 1,870 – 6,562 mm^3.

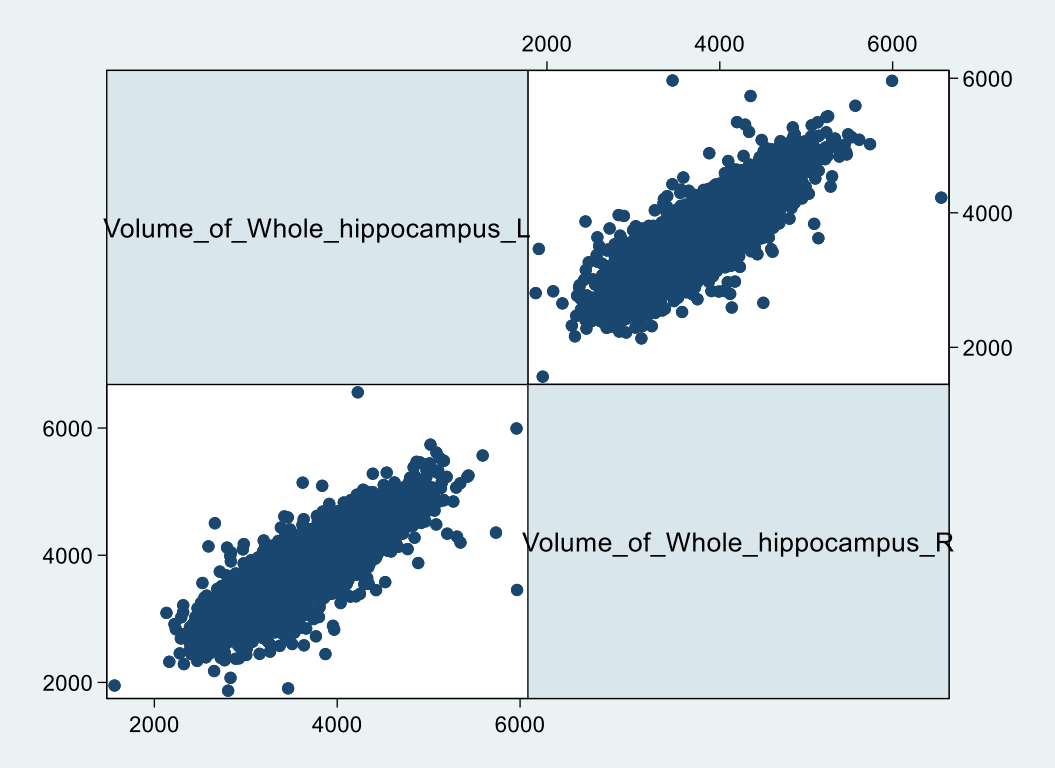


Fig. 10. Scatterplot of the whole volume of right and left hippocampus (mm^3).

Correlation between WMHs and hippocampal volumes

All examined relationships between log WMHs and hippocampal volume and hippocampal subdivision showed a statistically significant minimal negative correlation.

	LEFT r (correlation with log WMHs)	RIGHT r (correlation with log WMHs)
hippocampal volume	-0.1457***	-0.1131***

hippocampal body	-0.1651***	-0.1331***
hippocampal head	-0.1247***	-0.0999***
hippocampal tail	0.0708***	-0.0304***

Tab. 6. Correlation table between white matter hyperintensities and hippocampal volumes. *** - p< 0.001, ** - p<0.01, * - p<0.05

Co-linearity assessment

To comply with variance inflation factors (VIF) assumptions (STAT, n.d.), we had to eliminate x10_rs1191849_A_major and x10_rs9420907 due to low variability within the variable.

Co-linearity was assessed on all remaining variables. Therefore, variables with a high VIF factor (listed in the table) and BMI value (not category) will be removed from further analyses.

	VIF	Df
X3_rs10936600_T_minor	5681.435693	2
X3_rs10936599_T_minor	5681.206231	2
X16_rs2967374_G_major	355.7413	2
X16_rs7194734_T_major	355.353704	2
X20_rs755017_G_minor	234.150984	2
X20_rs73624724_C_minor	232.96963	2
X4_rs7675998_G_major	210.392074	2
X4_rs4691895_C_major	210.332622	2
Age_at_imaging:sex	79.400645	1
sex	78.191303	1

Tab. 7. Variables with a VIF value of 10 and above, which are excluded from further analysis. All the remaining variables achieved VIF <10.

In summary, the following variables were excluded for further analyses due to the high VIF and substantial colinearity: X3_rs10936600_T_minor,

X3_rs10936599_T_minor, X4_rs7675998_G_major, X4_rs4691895_C_major, x10_rs9420907_A_major, x10_rs1191849_A_major, X16_rs7194734_T_major, X16_rs2967374_G_major, X20_rs755017_G_minor, X20_rs73624724_C_minor, leaving 19 genetic variables containing SNPs. The concerned variables were removed, since the presence of multicollinearity implies that the information that this variable provides about the response is redundant in the presence of the other variables. (James *et al.*, 2014) It also helps to avoid unreliability in the interpretation of correlation coefficients and instability of estimates. (Salkind, 2012)

GLM regression modelling for right hippocampus

correlation assessment

We compared all continuous variables with the dependant variable of the total volume of the right hippocampus by Spearman rank correlation. All correlations were statistically significant ($p<0.05$), except for one confounder – a lateral table position. None of the correlations is significant, so no variable was excluded due to the high correlation with the dependent variable. Two variables display moderate correlation, but as they are confounders, they are not considered for exclusion (Age at imaging, r = -0.33; head size, r = -0.54). The detailed correlation coefficients are displayed in the table.

Variable	Spearman Rank Order Correlations
	Volume_of_Whole_hippocampus_R
Volume_of_Whole_hippocampus_R	1.000000
waist_to_hip	0.167830*
BMI	0.072794*
DBP_automated	0.056802*
Arterial_Stiffness	0.050059*
Scanner_table_position	0.037588*

Intensity.scaling.for.T2_FLAIR	0.021271*
Scanner_lateral_positino	0.004405
SBP_automated	-0.037861*
Scanner_transverse_position	-0.054226*
Pulse_rate_automated	-0.062237*
Cholesterol	-0.063107*
Scanner_longitudinal_position	-0.076421*
log_WMHs	-0.105655*
WMHs	-0.105655*
HbA1c	-0.109392*
Age_at_imaging	-0.331752*
Volumetric_scaling_from_T1_head_image_to_standard_space	-0.538414*

Tab. 13. Spearman correlation between right hippocampus and mediating variables or variables of interest. * - $p<0.05$

model 00

The baseline model was built solely on confounders. The adjusted R^2 was 0.4326 F-statistic: 1315 on 13 and 22401 DF, p-value: < 2.2e-16.

univariate analysis

We performed a series of univariate linear regression analyses, which looked at the change in adjusted R2 when a single variable was added to the model_00 based on confounders. None of the variables improved the model by more than 1%. (Watała C., 2002)

	right hippocampus	change by 1%
confounders	0.4325844	
X1_rs3219104_C_major	0.4325839	No
X2_rs11125529_A_minor	0.4325505	No
X3_rs55749605_A_major	0.4326692	No
X4_rs13137667_C_major	0.4326174	No
X5_rs7705526_A_minor	0.4327018	No
X5_rs2736100_A_minor	0.4327258	No
X5_rs2853677_A_major	0.4327052	No
X6_rs34991172_G_minor	0.4325358	No
X6_rs2736176_C_minor	0.4325424	No
X7_rs59294613_A_minor	0.432737	No
X10_rs11191848_A_minor	0.4326025	No
X10_rs9419958_C_major	0.4325504	No
X11_rs228595_A_minor	0.4325566	No
X14_rs2302588_C_minor	0.4325773	No
X16_rs3785074_G_minor	0.4325553	No
X16_rs62053580_G_minor	0.4325497	No
X19_rs8105767_G_minor	0.4325614	No
X20_rs75691080_T_minor	0.4325424	No
X20_rs34978822_G_minor	0.4325423	No
ApoE4.status	0.4330551	No
Smoking_status	0.4336226	No
father	0.4318816	No
mother	0.4337256	No
current_employment	0.4316782	No
qualifications	0.4280955	No
chronotype	0.4344869	No

Handedness	0.4326333	No
IPAQ	0.4326081	No
Alcohol_drinker_status	0.4325605	No
BMI_cat	0.4325743	No
no_of_noncancer_illnesses.self.	0.4328001	No
no_of_operations.self.	0.4326135	No
Cancer_diagnosed_by_doctor.	0.4325732	No
Diabetes_diagnosed_by_doctor	0.4328436	No
Vascular.heart_problems_diagnosed_by_doctor._	0.4330173	No
DVT_diagnosed_by_doctor	0.4325708	No
PE_diagnosed_by_doctor	0.4325999	No
no_of_medications	0.4329548	No
med_cholesterol	0.4324553	No
med_HTN	0.432642	No
med_insulin	0.4325383	No
HbA1c	0.4311404	No
Cholesterol	0.4318778	No
waist_to_hip	0.4330813	No
BMI	0.4326235	No
Pulse_rate_automated	0.4319006	No
DBP_automated	0.4319544	No
SBP_automated	0.4319451	No
Arterial_Stiffness	0.4334402	No

Tab. 14. Summary of R^2 of the univariate models predicting right hippocampal volume.

model 01

We built the baseline linear regression model, including all pre-specified confounders (9 and one interaction) and the remaining extracted variables (47 variables) – a total of 57 variables.

The adjusted R^2 for the model as 0.4323, F-statistic: 124.6 on 102 and 16456 DF, p-value:<2.20E-16

Out of all variables, only coefficients for the following ones were statistically significant:

Coefficient	Estimate	Std.	Error	t-value	Pr(>|t|)
X3_rs55749605_A_majorhomo	11.100	5.055	2.196	0.028	*
X4_rs13137667_C_majorhomo	25.300	10.770	2.349	0.019	*
X4_rs13137667_C_majornon-carrier	185.800	84.070	2.210	0.027	*
ApoE4.statushomo	-42.520	16.630	-2.557	0.011	*
Smoking_statusnever	50.760	13.000	3.906	0.000	***
Smoking_statusprevious	33.520	13.300	2.520	0.012	*
fatherD_<85	26.580	9.616	2.764	0.006	**
fatherD_85+	25.780	11.320	2.276	0.023	*
motherA_85+	38.740	10.090	3.840	0.000	***
motherD_<85	18.630	8.195	2.273	0.023	*
motherD_85+	24.040	9.634	2.495	0.013	*
waist_to_hip	-137.500	41.390	-3.323	0.001	***
DBP_automated	-0.793	0.323	-2.454	0.014	*
SBP_automated	0.534	0.183	2.918	0.004	**
Arterial_Stiffness	1.629	0.665	2.449	0.014	*

Tab. 15. Statistically significant beta-coefficients from the model 01 predicting right hippocampal volume, * - <0.05, ** - p<0.01, *** - p<0.001

model 02

We built the baseline linear regression model including all pre-specified confounders (9 and one interaction) and the remaining extracted variables chosen based on the previous model (10 variables) – a total of 20 variables. The p-value after Bonferroni correction is 0.05/20=0.003

The adjusted R^2 for the model as 0.3462, F-statistic: 493.3 on 31 and 19694 DF, p-value: <2.20E-16

Out of all variables, only coefficients for the following ones were statistically significant:

Coefficient	Estimate	SE	t	P	stat sig
3_rs55749605	9.542	4.625	2.063	0.039	*
4_rs13137667	164.409	78.130	2.104	0.035	*
ApoE4.statushomo	**-62.960**	**15.292**	**-4.117**	**0.000**	***
Smoking_statusnever	**49.188**	**11.899**	**4.134**	**0.000**	***
Smoking_statusprevious	30.715	12.174	2.523	0.012	*
fatherD_<85	**28.082**	**8.936**	**3.142**	**0.002**	**
fatherD_85+	24.675	10.508	2.348	0.019	*
motherA_85+	**39.768**	**9.349**	**4.254**	**0.000**	***
motherD_<85	**22.177**	**7.594**	**2.920**	**0.003**	**
motherD_85+	**27.377**	**8.876**	**3.085**	**0.002**	**
waist_to_hip	**-150.901**	**33.843**	**-4.459**	**0.000**	***
DBP_automated	-0.761	0.283	-2.686	0.007	**
SBP_automated	**0.526**	**0.164**	**3.203**	**0.001**	***
Arterial_Stiffness	1.263	0.518	2.439	0.015	*

Tab. 16. Statistically significant beta-coefficients from model 02 predicting right hippocampal volume. In bold are those which remained significant after the Bonferroni correction for multiple comparisons, p<0.003

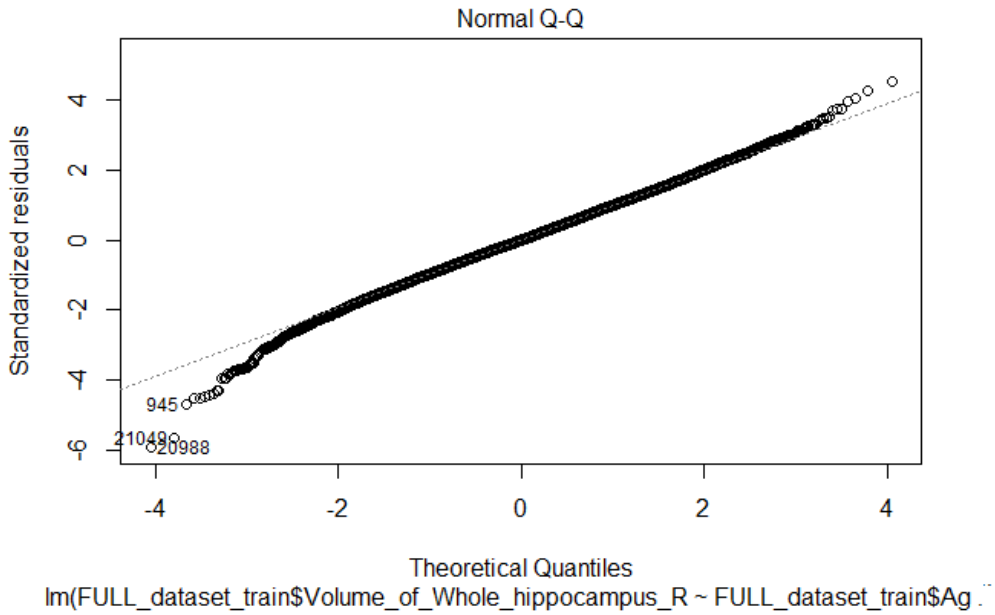


Fig. 12. QQ-plot of model 2 predicting right hippocampal volume.

Based on the regression modelling for the right hippocampal volume, we can summarize that from our dataset, the 34.62% variance in the right hippocampal volume is driven by the scanner and scanning protocols, ApoE4 status, smoking status, maternal and paternal age, waist to hip ratio, and systolic blood pressure. We looked separately into unadjusted regression analysis of ApoE status - it is explaining 0.01% variance of right hippocampal volume.

GLM regression modelling for left hippocampus

correlation assessment

We compared all continuous variables with the dependant variable of the total volume of the right hippocampus by Spearman rank correlation. All correlations were statistically significant ($p<0.05$). None of the correlations is significant, so no variable was excluded due to the high correlation with the dependent variable. Two variables display moderate correlation, but as they

are confounders, they are not considered for exclusion (Age at imaging, r = -0.36; head size, r = -0.51). The detailed correlation coefficients are displayed in Tab. 17.

Variable	Spearman Rank Order Correlations MD pairwise deleted Volume_of_Whole_hippocampus_L
waist_to_hip	0.157275*
BMI	0.074054*
DBP_automated	0.054714*
Arterial_Stiffness	0.051383*
Scanner_table_position	0.017231*
Scanner_lateral_position	0.014753*
SBP_automated	-0.049679*
Scanner_longitudinal_position	-0.049911*
Pulse_rate_automated	-0.058175*
Scanner_transverse_position	-0.059976*
Cholesterol	-0.066001*
HbA1c	-0.116485*
Age_at_imaging	-0.360324*
Volumetric_scaling_from_T1_head_image_to_standard_space	-0.509646*

Tab. 17. Spearman correlation between left hippocampus and mediating variables or variables of interest., * - <0.05

model 00

The baseline model was built solely on confounders. The adjusted R^2 was 0.4026, F-statistic: 1163 on 13 and 22401 DF, p-value: < 2.2e-16

univariate analysis

We performed a series of univariate linear regression analyses, which looked at the change in adjusted R2 when a single variable was added to the model_00 based on confounders. None of the variables improved the model by more than 1%. (Watała C., 2002)

	left hippocampus	**change by 1%**
confounders	0.426063	
1_rs3219104	0.4248344	no
2_rs11125529	0.4248411	no
3_rs55749605	0.425056	no
4_rs13137667	0.4249479	no
5_rs7705526	0.4249568	no
5_rs2736100	0.4249971	no
5_rs2853677	0.424973	no
6_rs34991172	0.4249234	no
6_rs2736176	0.4248543	no
7_rs59294613	0.4249436	no
10_rs11191848	0.4249685	no
10_rs9419958	0.4248311	no
11_rs228595	0.4248616	no
14_rs2302588	0.4248325	no
16_rs3785074	0.4248833	no
16_rs62053580	0.4248372	no
19_rs8105767	0.4248349	no
20_rs75691080	0.4248323	no
20_rs34978822	0.4248892	no
ApoE4.status	0.4255851	no

Smoking_status	0.4255242	no
father	0.4249993	no
mother	0.4262073	no
current_employment	0.4243162	no
qualifications	0.4216878	no
chronotype	0.4270394	no
Handedness	0.4249053	no
IPAQ	0.424926	no
Alcohol_drinker_status	0.4248521	no
BMI_cat	0.4248202	no
no_of_noncancer_illnesses.self.	0.4252546	no
no_of_operations.self.	0.4249532	no
Cancer_diagnosed_by_doctor.	0.4248968	no
Diabetes_diagnosed_by_doctor	0.4253454	no
Vascular.heart_problems_diagnosed_by_doctor._	0.4256087	no
DVT_diagnosed_by_doctor	0.4248192	no
PE_diagnosed_by_doctor	0.424841	no
no_of_medications	0.4253779	no
med_cholesterol	0.424871	no
med_HTN	0.4250921	no
med_insulin	0.4249119	no
HbA1c	0.4244417	no
Cholesterol	0.4244022	no
waist_to_hip	0.425331	no
BMI	0.4248625	no
Pulse_rate_automated	0.4240664	no
DBP_automated	0.4241252	no
SBP_automated	0.4240547	no

Arterial_Stiffness	0.4248728	no

Tab. 18. Summary of R^2 of univariate models predicting left hippocampal volume.

model 01

We built the baseline linear regression model, including all pre-specified confounders (9 and one interaction) and the remaining extracted variables (47 variables) – a total of 57 variables.

The adjusted R^2 for the model as 0.4037, F-statistic: 110.9 on 102 and 16456 DF, p-value:<2.20E-16.

Out of all variables, only coefficients for the following ones were statistically significant:

Coefficients:	Estimate	SE	t	p	stat sig
6_rs34991172	-8.073	3.468	-2.328	0.020	*
10_rs11191848	-6.729	3.198	-2.104	0.035	*
ApoE4.statushomo	-24.241	8.972	-2.702	0.007	**
ApoE4.statusnon-carrier	8.053	2.926	2.752	0.006	**
Smoking_statusnever	28.897	7.010	4.122	0.000	***
Smoking_statusprevious	19.960	7.175	2.782	0.005	**
fatherA_85+	15.649	7.173	2.182	0.029	*
fatherD_<85	13.302	5.187	2.565	0.010	*
fatherD_85+	12.903	6.109	2.112	0.035	*
motherA_85+	22.390	5.442	4.114	0.000	***
motherD_<85	10.050	4.421	2.273	0.023	*
motherD_85+	14.296	5.197	2.751	0.006	**
no_of_noncancer_illnesses.self.2	-11.092	4.454	-2.490	0.013	*
no_of_operations.self.2	9.108	4.005	2.274	0.023	*

no_of_operations.self.3+	9.693	4.003	2.421	0.015	*
waist_to_hip	-54.079	22.325	-2.422	0.015	*
DBP_automated	-0.572	0.174	-3.281	0.001	**
SBP_automated	0.309	0.099	3.125	0.002	**
Arterial_Stiffness	1.365	0.359	3.805	0.000	***

Tab. 19. Statistically significant beta-coefficients from the model 01 predicting left hippocampal volume., * - <0.05, ** - p<0.01, *** - p<0.001

model 02

We built the baseline linear regression model including all pre-specified confounders (9 and one interaction) and the remaining extracted variables chosen based on the previous model (17 variables) – a total of 27 variables. After the Bonferroni correction, the p-value for statistical significance is 0.05/27=0.002

The adjusted R^2 for the model was 0.4058, F-statistic: 365 on 37 and 19688 DF, p-value: <2.20E-16

Out of all variables, only coefficients for the following ones were statistically significant:

Coefficients	**Estimate**	**SE**	**t**	**p**	**stat sig**
6_rs34991172	-7.81371	3.18632	-2.452	0.014204	*
ApoE4.statushomo	**-33.6406**	**8.27343**	**-4.066**	**4.80E-05**	***
ApoE4.statusnon-carrier	7.60121	2.67411	2.843	0.00448	**
Smoking_statusnever	**28.63753**	**6.43465**	**4.451**	**8.61E-06**	***
Smoking_statusprevious	**19.14772**	**6.58406**	**2.908**	**0.003639**	**
fatherA_85+	16.44029	6.70197	2.453	0.014174	*
fatherD_<85	12.94564	4.83322	2.678	0.007402	**

fatherD_85+	13.26482	5.68296s	2.334	0.019598	*
motherA_85+	**23.67984**	**5.05608**	**4.683**	**2.84E-06**	***
motherD_<85	12.00176	4.10686	2.922	0.003478	**
motherD_85+	**17.17407**	**4.79981**	**3.578**	**0.000347**	***
no_of_noncancer_illnesses.self.2	**-12.7177**	**3.86974**	**-3.286**	**0.001016**	**
no_of_noncancer_illnesses.self.3+	**-11.5081**	**3.34031**	**-3.445**	**0.000572**	***
no_of_operations.self.2	7.63294	3.67407	2.078	0.037767	*
no_of_operations.self.3+	9.01316	3.62727	2.485	0.01297	*
waist_to_hip	**-68.6848**	**18.35821**	**-3.741**	**0.000184**	***
DBP_automated	**-0.49829**	**0.15316**	**-3.253**	**0.001142**	**
SBP_automated	0.25582	0.08874	2.883	0.003945	**
Arterial_Stiffness	**1.03171**	**0.28014**	**3.683**	**0.000231**	***

Tab. 20. Statistically significant beta-coefficients from model 02 predicting left hippocampal volume. In bold are those which remained significant after the Bonferroni correction for multiple comparisons, p<0.002

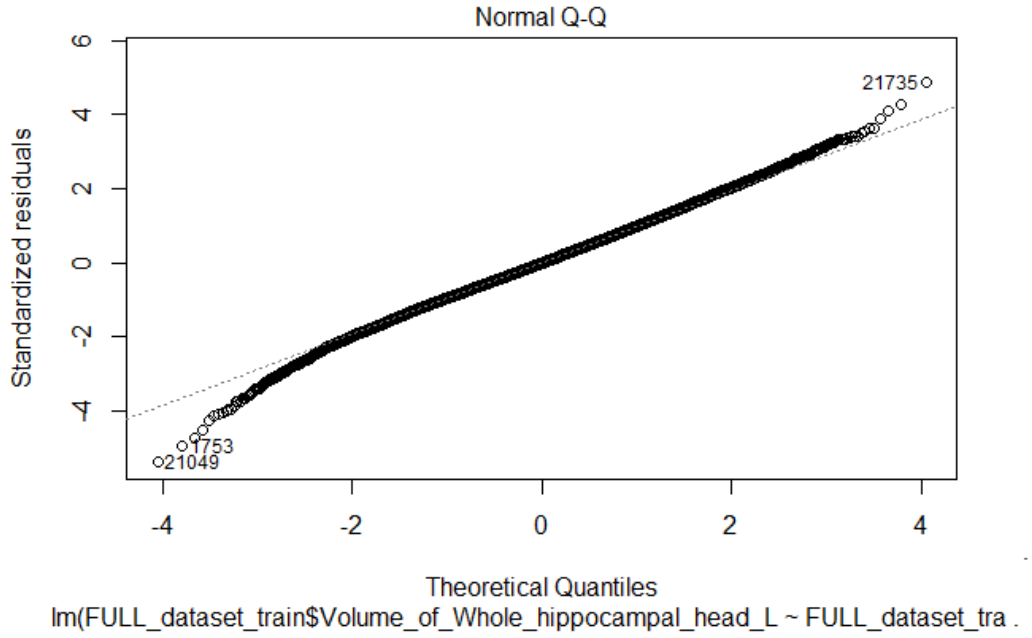


Fig. 13. QQ-plot of model 2 predicting left hippocampal volume.

Based on the regression modelling for the left hippocampal volume, we can summarize that from our dataset, the 40.58% variance in the left hippocampal volume is driven by the scanner and scanning, ApoE4 status, smoking status, maternal longevity, waist to hip ratio, diastolic blood pressure, and arterial stiffness. Additionally, it is influenced by a number of non-cancer illnesses. We looked separately into unadjusted regression analysis of ApoE status - it is explaining 0.01% variance of left hippocampal volume.

GLM regression modelling for hippocampal subdivision

We run a multivariate linear regression model for hippocampal subdivision (head, body, tail). We included in the models 9 pre-specified co-variates, 8 covariates that achieved statistical significance in the regression model for the whole hippocampal volume on both sides (maternal age, paternal age, ApoE status, smoking status, wait-to-hip ratio, arterial stiffness, systolic blood pressure and diastolic blood pressure), as well as all 29 SNPs regulating telomere length.

	LH		RH		LH_head		RH_head		LH_body		RH_body		LH_tail		RH_tail	
	beta/p	**SE**	**beta/p**	**SE**	**beta/p**	**SE**	**beta/p**	**SE**	**beta/p**	**SE**	**beta/p**	**SE**	**beta/p**	**SE**	**beta/p**	**SE**
log_WMHs	-0.057***	5.371	-0.039***	5.409	-0.048***	2.935	-0.035***	2.969	-0.071***	2.076	-0.053***	2.094	-0.027***	1.125	-0.005	1.105
volumetric scaling	-0.536***	20.40	-0.566***	20.55	-0.524***	11.15	-0.559***	11.28	-0.483***	7.887	-0.507***	7.955	-0.424***	4.272	-0.441***	4.198
intensity scaling	0.010	6.765	0.008	6.812	0.009	3.696	0.002	3.739	0.011	2.615	0.014	2.637	0.006	1.416	0.012	1.392
scanner position - lateral	0.014**	0.664	0.001	0.668	0.004	0.363	0.006	0.367	0.025***	0.257	-0.008	0.259	0.013*	0.139	0.005	0.137
scanner position - transverse	-0.023***	0.401	-0.026***	0.404	-0.014*	0.219	-0.020**	0.222	-0.026***	0.155	-0.037***	0.156	-0.031***	0.0840	-0.013*	0.0826
scanner position - longitudinal	-0.096***	0.160	-0.116***	0.161	-0.063***	0.0876	-0.095***	0.0886	-0.097***	0.0620	-0.116***	0.0625	-0.144***	0.0336	-0.124***	0.0330
scanner table position	-0.052***	0.176	-0.050***	0.178	-0.024*	0.0964	-0.031*	0.0975	-0.059***	0.0682	-0.060***	0.0688	-0.094***	0.0369	-0.066***	0.0363
age	-0.370***	0.334	-0.355***	0.336	-0.350***	0.182	-0.342***	0.184	-0.367***	0.129	-0.353***	0.130	-0.264***	0.0699	-0.234***	0.0687
sex	0.024**	5.932	0.013	5.974	0.039***	3.241	0.003	3.279	-0.011	2.293	-0.004	2.313	0.039***	1.242	0.070***	1.221
imging centre	-0.007	3.420	-0.004	3.444	-0.006	1.868	-0.003	1.890	-0.014*	1.322	-0.008	1.333	0.008	0.716	0.003	0.704
ApoE	0.016**	2.185	0.010*	2.200	0.015**	1.194	0.009	1.208	0.015**	0.845	0.009	0.852	0.012*	0.458	0.013*	0.450
smoking	-0.011*	3.627	-0.010*	3.653	-0.010*	1.982	-0.011*	2.005	-0.010*	1.402	-0.007	1.414	-0.009	0.759	-0.011*	0.746
paternal age	0.013*	2.364	0.019***	2.380	0.012*	1.291	0.017***	1.307	0.013*	0.914	0.020***	0.922	0.009	0.495	0.011	0.486
maternal age	-0.005	1.955	-0.006	1.968	-0.006	1.068	-0.005	1.080	-0.006	0.756	-0.008	0.762	0.001	0.409	-0.005	0.402
wait-to-hip	-0.030***	30.06	-0.031***	30.27	-0.029***	16.42	-0.027***	16.62	-0.024***	11.62	-0.025***	11.72	-0.029***	6.294	-0.040***	6.185
diastolic BP	-0.015*	0.252	-0.015*	0.254	-0.022***	0.138	-0.021**	0.139	-0.009	0.0975	-0.010	0.0983	0.003	0.0528	-0.001	0.0519
systolic BP	0.028***	0.145	0.028***	0.146	0.031***	0.0791	0.031***	0.0801	0.025***	0.0560	0.024***	0.0565	0.013	0.0303	0.016*	0.0298
arterial stifness	0.015**	0.485	0.012*	0.489	0.018***	0.265	0.015**	0.268	0.009	0.188	0.007	0.189	0.010	0.102	0.009	0.0999

rs3219104	0.002	3.951	0.004	3.979	0.001	2.159	0.002	2.184	0.005	1.527	0.008	1.540	-0.000	0.827	0.002	0.813
rs11125529	0.004	2.215	0.002	2.231	0.002	1.210	-0.000	1.224	0.007	0.856	0.003	0.864	0.004	0.464	0.007	0.456
rs10936599	-0.003	2.688	-0.000	2.707	-0.006	1.469	-0.002	1.486	0.001	1.039	0.003	1.048	-0.000	0.563	0.000	0.553
rs10936600	0.059	17.23	0.066	17.35	0.098*	9.414	0.116**	9.523	0.023	6.660	0.023	6.717	-0.012	3.607	-0.026	3.545
rs55749605	-0.058	17.23	-0.063	17.35	-0.095*	9.413	-0.114**	9.523	-0.021	6.659	-0.020	6.716	0.007	3.607	0.031	3.545
rs13137667	0.008	8.491	0.008	8.551	0.006	4.639	0.009	4.693	0.009	3.282	0.008	3.310	0.007	1.778	0.005	1.747
rs4691895	0.018	11.31	0.015	11.39	0.026	6.180	0.027	6.252	0.004	4.372	0.005	4.409	0.012	2.368	-0.004	2.327
rs7675998	-0.020	11.19	-0.021	11.27	-0.027	6.112	-0.029	6.183	-0.009	4.324	-0.013	4.361	-0.013	2.342	-0.007	2.302
rs2736100	-0.011*	2.025	-0.008	2.039	-0.009	1.107	-0.008	1.119	-0.010*	0.783	-0.008	0.790	-0.012*	0.424	-0.006	0.417
rs2853677	0.006	2.607	0.006	2.626	-0.002	1.425	-0.001	1.441	0.012*	1.008	0.008	1.017	0.017**	0.546	0.024***	0.536
rs7705526	-0.006	2.849	-0.001	2.869	-0.003	1.557	0.002	1.575	-0.009	1.101	-0.000	1.111	-0.006	0.597	-0.009	0.586
rs2736176	-0.007	2.619	-0.002	2.638	-0.013**	1.431	-0.007	1.448	-0.004	1.012	-0.000	1.021	0.009	0.548	0.009	0.539
rs34991172	0.003	2.000	-0.004	2.014	0.000	1.093	-0.009	1.105	0.003	0.773	-0.002	0.780	0.009	0.419	0.010	0.411
rs59294613	0.009	1.992	0.010*	2.006	0.008	1.088	0.008	1.101	0.009	0.770	0.010*	0.777	0.008	0.417	0.010	0.410
rs11191848	0.004	3.009	0.004	3.031	0.002	1.644	0.003	1.663	0.005	1.163	0.002	1.173	0.007	0.630	0.012	0.619
rs11191849	-0.011	3.071	-0.009	3.093	-0.008	1.678	-0.006	1.697	-0.014*	1.187	-0.010	1.197	-0.011	0.643	-0.015*	0.632
rs9419958	0.033	121.9	0.128	122.7	0.087	66.59	0.158	67.36	-0.075	47.11	0.066	47.51	0.075	25.52	0.108	25.07
rs9420907	-0.037	121.8	-0.132	122.7	-0.093	66.55	-0.166	67.33	0.072	47.08	-0.064	47.49	-0.076	25.50	-0.109	25.06
rs228595	-0.002	2.106	0.001	2.121	0.001	1.150	0.002	1.164	-0.004	0.814	-0.001	0.821	-0.005	0.441	-0.001	0.433
rs2302588	-0.002	2.450	0.005	2.468	-0.005	1.339	0.004	1.354	-0.002	0.947	0.005	0.955	0.006	0.513	0.006	0.504
rs2967374	-0.003	1.996	-0.002	2.010	-0.006	1.091	-0.003	1.103	-0.001	0.772	-0.002	0.778	0.002	0.418	0.002	0.411
rs3785074	0.001	2.116	0.002	2.131	0.000	1.156	0.004	1.170	0.003	0.818	0.001	0.825	-0.000	0.443	-0.002	0.435

rs62053580	0.008	12.93	-0.007	13.02	0.036	7.065	0.024	7.147	-0.011	4.998	-0.032	5.041	-0.040	2.707	-0.046*	2.660
rs7194734	-0.006	12.96	0.011	13.05	-0.033	7.079	-0.019	7.162	0.009	5.008	0.036	5.051	0.044*	2.713	0.047*	2.666
rs8105767	-0.001	1.997	-0.001	2.011	-0.005	1.091	-0.004	1.104	0.003	0.772	0.003	0.779	0.002	0.418	-0.000	0.411
rs34978822	0.005	2.678	0.006	2.697	0.001	1.463	0.006	1.480	0.007	1.035	0.006	1.044	0.007	0.561	0.002	0.551
rs73624724	0.006	4.836	0.001	4.870	0.005	2.642	-0.002	2.673	0.007	1.869	0.006	1.885	0.006	1.013	-0.001	0.995
rs755017	0.016	9.508	0.030	9.575	-0.004	5.195	0.015	5.256	0.038	3.675	0.047*	3.707	0.020	1.991	0.025	1.956
rs75691080	-0.016	9.408	-0.029	9.474	0.004	5.140	-0.015	5.200	-0.039	3.637	-0.043*	3.668	-0.020	1.970	-0.028	1.936
N	24650		24650		24650		24650		24650		24650		24650		24650	
R-sq	0.427		0.437		0.404		0.413		0.362		0.372		0.263		0.277	

Tab. 21. Summary of the regression models for hippocampal subdivision. Beta coefficients, their significance levels, standard errors are presented for each variable. The number of valid observation, and R sq value is presented for each regression model.

Based on the regression modelling for the hippocampal subdivisions, we can summarize that the hippocampal subdivision can be predicted in 27.7-43.7% from our dataset. The models perform best for the total hippocampal volume, the head, the body, and the tail. There are no single SNPs that consequently influence hippocampal volume across the subdivision. Considering variance explained by TL maintenance genes, the bilateral head volume is associated with 2 SNPs (rs10936600, rs55749605), and the bilateral tail volume is mainly driven by 2 SNPs (rs2853677, rs7194734). No single SNPs are influencing both hippocampal bodies.

GLM regression modelling for log WMHs

Correlation assessment

We compared all continuous variables with the dependant variable of log WMHs by Spearman rank correlation. All correlations were statistically significant ($p<0.05$), but only one was moderate (log WMHs ~ Age at the imaging centre, $r = 0.508775$). None of the correlations is strong or very strong, so no variable was excluded due to high correlation to the dependant variable. The detailed correlation coefficients are displayed in Tab. 8,

Variable	Spearman Rank Order Correlations
	log_WMHs
Age_at_imaging	0.508775*
SBP_automated	0.259154*
Intensity.scaling.for.T2_FLAIR	0.206030*
waist_to_hip	0.189646*
HbA1c	0.173197*
Scanner_table_position	0.171818*
Scanner_transverse_position	0.112218*
BMI	0.110104*
DBP_automated	0.086981*
Arterial_Stiffness	0.081171*
Cholesterol	0.049221*
Scanner_lateral_position	0.048657*
Pulse_rate_automated	0.037171*
Volumetric_scaling_from_T1_head_image_to_standard_space	-0.159631*
Scanner_longitudinal_position	-0.286912*

Tab. 8. Spearman correlation between log WMHs and mediating variables or variables of interest. * - p<0.05

model 00

The baseline model was built solely on confounders. The adjusted R^2 was 0.3275812. F-statistic: 841 on 13 and 22401 DF, p-value: < 2.2e-16

univariate analysis

We performed a series of univariate linear regression analyses, which looked at the change in adjusted R2 by 1% (Watała C., 2002) when a single variable was added to the model_00 based on confounders.

	log WMHs	**change of R^2 by 1%**
confounders	0.3275812	
1_rs3219104	0.3276162	no
2_rs11125529	0.3276912	no
3_rs55749605	0.3275252	no
4_rs13137667	0.3275392	no
5_rs7705526	0.3275507	no
5_rs2736100	0.3275402	no
5_rs2853677	0.3275315	no
6_rs34991172	0.3278177	no
6_rs2736176	0.3275344	no
7_rs59294613	0.327587	no
10_rs11191848	0.3277511	no
10_rs9419958	0.3277558	no
11_rs228595	0.3276293	no
14_rs2302588	0.3275499	no

16_rs3785074	0.3276617	no
16_rs62053580	0.3276021	no
19_rs8105767	0.3275702	no
20_rs75691080	0.3275746	no
20_rs34978822	0.3275342	no
ApoE4.status	0.3279781	no
Smoking_status	0.3297942	no
father	0.3283381	no
mother	0.3287119	no
current_employment	0.327336	no
qualifications	0.327356	no
chronotype	0.3294827	no
Handedness	0.3274861	no
IPAQ	0.3281093	no
Alcohol_drinker_status	0.327553	no
BMI_cat	0.3328839	yes
no_of_noncancer_illnesses.self.	0.3291301	no
no_of_operations.self.	0.3275044	no
Cancer_diagnosed_by_doctor.	0.3275475	no
Diabetes_diagnosed_by_doctor	0.3308998	yes
Vascular.heart_problems_diagnosed_by_doctor._	0.3398918	yes
DVT_diagnosed_by_doctor	0.3275021	no
PE_diagnosed_by_doctor	0.3274707	no
no_of_medications	0.3343882	yes
med_cholesterol	0.3275518	no
med_HTN	0.3398388	yes
med_insulin	0.3280993	no
HbA1c	0.3322474	yes

Cholesterol	0.3276751	no
waist_to_hip	0.3323592	yes
BMI	0.3329727	yes
Pulse_rate_automated	0.3325906	yes
DBP_automated	0.3355754	yes
SBP_automated	0.3362524	yes
Arterial_Stiffness	0.3308201	no

Tab. 9. Summary of R^2 of univariate models predicting log WMHs.

Several variables (BMI, diabetes diagnosed by a doctor, vascular heart disease diagnosed by a doctor, number of medications, medications for hypertension, HbA1c, wait-to-hip, pulse rate, SBP, DBP) improved the model by more than 1%.

model 01

We built the baseline linear regression model, including all pre-specified confounders (9 and one interaction) and the remaining extracted variables (47 variables) – a total of 57 variables.

The adjusted R^2 for the model as 0.3663, F-statistic: 94.83 on 102 and 16456 DF, p-value: <2.20E-16

Out of all variables, only coefficients for the following ones were statistically significant:

Coefficient	Estimate	Std.	Error	t-value	Pr(>|t|)
1_rs3219104	-0.016	0.006	-2.518	0.012	*
2_rs11125529	0.064	0.020	3.247	0.001	**
6_rs34991172	0.016	0.007	2.115	0.034	*

10_rs11191848	0.017	0.007	2.519	0.012	*
10_rs11191848	-0.023	0.007	-3.402	0.001	***
10_rs9419958	-0.026	0.007	-3.738	0.000	***
11_rs228595	0.014	0.006	2.288	0.022	*
ApoE4.statusnon-carrier	-0.021	0.006	-3.317	0.001	***
Smoking_statusnever	-0.079	0.015	-5.251	0.000	***
Smoking_statusprevious	-0.059	0.015	-3.823	0.000	***
IPAQmoderate	-0.015	0.006	-2.439	0.015	
motherA_85+	-0.033	0.012	-2.817	0.005	**
Diabetes_diagnosed_by_doctoryes	0.045	0.016	2.850	0.004	**
no_of_medications1	0.017	0.008	2.127	0.033	*
no_of_medications3+	0.024	0.009	2.698	0.007	**
med_cholesterolyes	0.024	0.010	2.314	0.021	*
med_HTNyes	0.066	0.010	6.711	0.000	***
HbA1c	0.001	0.001	1.985	0.047	*
waist_to_hip	0.180	0.048	3.738	0.000	***
Pulse_rate_automated	0.002	0.000	6.437	0.000	***
DBP_automated	0.001	0.000	3.792	0.000	***
SBP_automated	0.001	0.000	5.863	0.000	***

Tab. 10. Statistically significant beta-coefficients from the model 01 predicting log WMHs., * - <0.05, ** - p<0.01, *** - p<0.001

model 02

We built the baseline multivariate linear regression model including all pre-specified con-founders (9 and one interaction) and the remaining extracted variables chosen based on the previous model (17 variables) – a total of 27 variables.

The adjusted R^2 for the model as 0.3608, F-statistic: 265.8 on 45 and 21067 DF, p-value: <2.20E-16. Out of all variables for this multivariate model, only coefficients for the following ones were statistically significant:

Coefficients:	Estimate	SE	t	p	stat sig
2_rs11125529	0.047	0.018	2.666	0.008	**
6_rs34991172	0.019	0.007	2.787	0.005	**
10_rs11191848	0.014	0.006	2.333	0.020	*
10_rs11191848	-0.020	0.006	-3.315	0.001	***
10_rs9419958	-0.022	0.006	-3.615	0.000	***
11_rs228595	0.012	0.005	2.184	0.029	*
ApoE4. (status non-carrier)	-0.021	0.006	-3.767	0.000	***
Smoking_statusnever	-0.088	0.013	-6.574	0.000	***
Smoking_statusprevious	-0.064	0.014	-4.682	0.000	***
motherA_85+	-0.026	0.010	-2.650	0.008	**
IPAQmoderate	-0.018	0.005	-3.373	0.001	***
Diabetes_diagnosed_by_doctoryes	0.047	0.013	3.552	0.000	***
no_of_medications1	0.015	0.007	2.234	0.025	*
no_of_medications3+	0.032	0.007	4.386	0.000	***
med_cholesterolyes	0.024	0.009	2.692	0.007	**
med_HTNyes	0.086	0.007	12.035		***
HbA1c	0.002	0.001	3.135	0.002	**
waist_to_hip	0.193	0.039	4.907	0.000	***
Pulse_rate_automated	0.002	0.000	7.804	0.000	***
DBP_automated	0.002	0.000	4.807	0.000	***
SBP_automated	0.001	0.000	7.230	0.000	***

Tab. 11. Statistically significant beta-coefficients from the model 02 predicting log WMHs.. * - <0.05, ** - p<0.01, *** - p<0.001

model 03

We built the baseline linear regression model including all pre-specified confounders (9 and one interaction) and the remaining extracted variables chosen based on the previous model (16 variables) – a total of 26 variables. The p-value after Bonferroni correction is 0.05/26=0.002

The adjusted R^2 for the model as 0.3608, F-statistic: 278.1 on 43 and 21069 DF, p-value: <2.20E-16. Out of all variables, only coefficients for the following ones were statistically significant:

	Estimate	**SE**	**t**	**p**	**stat sig**
2_rs11125529	0.047	0.018	2.666	0.008	**
6_rs34991172	0.019	0.007	2.794	0.005	**
10_rs11191848	0.014	0.006	2.317	0.020	*
10_rs11191848	**-0.020**	**0.006**	**-3.326**	**0.001**	***
10_rs9419958	**-0.022**	**0.006**	**-3.597**	**0.000**	***
11_rs228595	0.012	0.005	2.164	0.031	*
ApoE4.statusnon-carrier	**-0.021**	**0.006**	**-3.765**	**0.000**	***
Smoking_statusnever	**-0.088**	**0.013**	**-6.565**	**0.000**	***
Smoking_statusprevious	**-0.064**	**0.014**	**-4.669**	**0.000**	***
motherA_85+	-0.027	0.010	-2.696	0.007	**
IPAQmoderate	**-0.018**	**0.005**	**-3.361**	**0.001**	***
Diabetes_diagnosed_by_doctoryes	**0.047**	**0.013**	**3.562**	**0.000**	***
no_of_medications1	0.015	0.007	2.231	0.026	*
no_of_medications3+	**0.032**	**0.007**	**4.399**	**0.000**	***
med_cholesterolyes	0.024	0.009	2.682	0.007	**
med_HTNyes	**0.086**	**0.007**	**12.019**	**0.000**	***
HbA1c	**0.002**	**0.001**	**3.129**	**0.002**	***
waist_to_hip	**0.193**	**0.039**	**4.893**	**0.000**	***
Pulse_rate_automated	**0.002**	**0.000**	**7.831**	**0.000**	***
DBP_automated	**0.002**	**0.000**	**4.803**	**0.000**	***
SBP_automated	**0.001**	**0.000**	**7.215**	**0.000**	***

Tab. 12. Statistically significant beta-coefficients from model 03 predicting log WMHs. In bold are those which remained significant after the Bonferroni correction for multiple comparisons, p<0.002

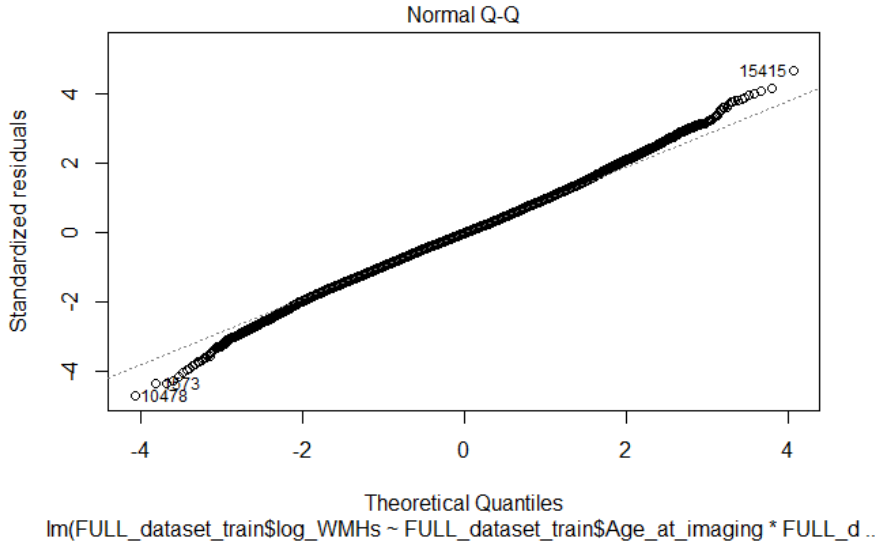


Fig. 11. QQ-plot of model 3 predicting log-WMHs

Based on the regression modelling for log-WMHs, we can summarize that from our dataset, the 36.08% variance in the WMHs volume is driven by the scanner and scanning protocols, 2 SNPs (rs11191848, rs9419958), ApoE4 status, smoking status, maternal longevity, physical activity, diabetes, blood pressure (systolic and diastolic), heart rate, diabetes and its control measured by HbA1c levels, a number of medications including medications for hypertension and waist-to-hip ratio. We looked separately into unadjusted regression analysis of 2 selected SNPs and ApoE status; rs11191848 is explaining 0.01% variance of log WMHs, rs9419958 is explaining 0.01% variance of log WMHs, combined rs11191848 and rs9419958 are explaining 0.03% variance of log WMHs, and ApoE status is explaining 0.01% variance of log WMHs.

Chapter 4: Discussion

Our study is the first one looking into an indirect link between genetic factors influencing telomerase activity and telomere length as a marker of cellular senescence and brain health expressed as hippocampal volume as a marker of brain ageing and neurodegeneration and the white matter hyperintensity load as a marker of cerebrovascular disease.

Brain biomarkers and lifestyle factors – correlation

As a part of our analysis, we looked into the correlation between ageing brain biomarkers – white matter hyperintensities and hippocampal volumes (right and left). We looked into their associations with 30 literature-based lifestyle factors ranging from co-morbidities (diabetes, hypertension, dyslipidaemia) to maternal/paternal longevity, vascular risk factors (smoking, alcohol consumption, increased BMI ad markers of visceral obesity)

Correlation between white matter hyperintensities and hippocampus

We had found a weak correlation between WMHs and hippocampal volume ($r = -0.1457$, $p<0.05$ for the left hippocampus and $r = -0.1131$, $p<0.05$ for the right hippocampus). This stands in agreement with the previous research. Caillaud et al. examined a cohort of 126 participants, including 67 participants with subjective cognitive decline, 29 participants with mild cognitive impairment and 30 cognitively healthy controls. Their study confirmed the moderate correlation between the right hippocampus and Face-Name memory in participants with subjective cognitive changes ($r = 0.534$, $p = 0.033$), as well as the relation between the left hippocampus and the delayed word recall in participants with mild cognitive impairment ($r = 0.878$, $p = 0.002$), and the relation between WMHs and executive functions among participants with subjective cognitive decline ($r = -0.261$, $p = 0.035$)

and healthy controls (r = 0.529, p = 0.006). (Caillaud *et al.*, 2020) Interestingly, the Framingham offspring study examined 1,049 participants with neurocognitive tests and neuroimaging. The same procedure was repeated at 6.5 years follow up among 561 participants. They have shown that both – increase in white matter hyperintensities and a decrease in hippocampal volume bear increased risk of conversion from healthy cognition into mild cognitive impairment. (Bangen *et al.*, 2018)

White matter hyperintensities and lifestyle factors

We looked into the correlation between white matter hyperintensities and lifestyle factors. There is a positive moderate correlation with age at imaging ($r = 0.508$, $p<0.05$), and weak correlation with SBP ($r = 0.259$, $p<0.05$), waist to hip ratio ($r = 0.189$, $p<0.05$), HBA1c levels (($r = 0.173$, $p<0.05$)) and BMI ($r = 0.110$, $p<0.05$), as well as scanner position (for table position $r = 0.508$, $p<0.05$; for transverse position $r = 0.508$, $p<0.05$, for longitudinal position $r = 0.287$, $p<0.05$). Similar findings were reported previously. White matter hyperintensities are one of the most prevalent findings on brain MRI. Meta-analysis on rate and risk factors of white matter hyperintensities showed that hypertension (OR 1.72, 95% CI 1.19 to 2.46) was associated with WMH growth. Moreover, they showed that yearly increase in WHMs load is variable between 0.6cc and 2.50 cc. (Brown, Low and Markus, 2021) LIFE-adult study of 1,825 participants aged 20-82 years. High waist-to-hip ratio contributed to the increased amount of WMHs within the deep white matter - mean ß = 0.0043 [0.0008 SE], 95% confidence interval, [0.00427, 0.0043]. On the other hand, higher systolic blood pressure was associated with WMH in periventricular white matter regions. The authors hypothesised that the visceral adiposity increases the white matter hyperintensities in the deep white matter via inflammatory pathways, particularly interleukin-6. They have confirmed it with mediation analysis. (Lampe *et al.*, 2019)

Hippocampus and lifestyle factors

We looked into the correlation between hippocampal volume and lifestyle factors. For the right one, there is a weak correlation with waist-to-hip ratio ($r = 0.168$, $p<0.05$) and HbA1c ($r = -0.109$, $p<0.05$), and a moderate correlation with the age at imaging ($r = -0.538$, $p<0.05$) For the left one, there is a weak correlation with waist-to-hip ratio ($r = 0.157$, $p<0.05$) and HbA1c ($r = -0.116$, $p<0.05$), and a moderate correlation with the age at imaging ($r = -0.360$, $p<0.05$). Summarizing this information, it seems that hippocampal volume is decreasing with increasing years and HbA1c value, as a marker of diabetes. On the other hand, the increased waist-to-hip ratio as a marker of visceral obesity goes in parallel to the increased hippocampal volume.

The moderate correlation of hippocampal volume to ageing is well known. A recent study by Nobis et al. created hippocampal volume nomograms, which show a clear downwards trend in time. It has been derived from 19,700 scans of healthy participants from UK BioBank. (Nobis *et al.*, 2019) This relationship is also reported in the state of disease, especially in Alzheimer disease. (Fiford *et al.*, 2018; Dicks *et al.*, 2019)

There are multiple reports linking diabetes and hippocampal atrophy. (Roberts et al., 2014; Hirabayashi et al., 2016) A longer duration increases the atrophy rate. This is as hippocampal atrophy is likely to occur due to chronic and longstanding exposure to glucose toxicity, abnormal insulin metabolism, microvascular disease in diabetic patients. (Hirabayashi et al., 2016) Interestingly, our results show that not diabetes itself, but high HbA1c values – that are mean blood sugar levels from the last three months – are more correlated to the hippocampal volume. This could mean that poor disease control negatively influences brain biomarkers rather than having a disease itself.

We have found that hippocampal volume is not correlated with BMI as a marker of general obesity itself but has a weak correlation with a waist-to-hip ratio as a marker of central obesity. Similar findings were reported by Hamer

et al., showing a weak negative correlation between hippocampal volume and waist-to-hip ratio (r = -0.024, p = 0.017) (Hamer and Batty, 2019)

Brain biomarkers – regression analysis of genetics and lifestyle factors

All regression models in our analysis were adjusted for patient-specific factors (age, sex, and interaction between age and sex, head size) and scanning session-specific factors (scanning location, scanning protocol, head and table position). The final regression models explain 40.58% of the variation in left total hippocampal volume, 34.62% of the variation in right total hippocampal volume, and 36.08% of the variation in WMHs volume.

ApoE status (homozygous ε4 allele)is the most consistent genetic contributor to poorer brain health (higher white matter hyperintensities load and smaller hippocampal volumes. The relationship between ApoE status and hippocampal volume is well described in the literature, especially among patients with mild cognitive impairment and Alzheimer disease. (Fleisher et al., 2005; Fiford et al., 2017) The meta-analysis of 14 studies showed that APOE ε4 carrier status is associated with decreased hippocampal volume (pooled SMD: −0.47; 95% CI −0.82 to −0.13; p=0.007). The authors also suggested that the biological basis for the underlying mechanism of APOE ε4 as a risk factor for developing AD is unknown yet. (Liu et al., 2015) A similar relationship is described in the literature for a carrier of e4 allele and increased WMHs load. For example, the ALFA study conducted by Rojas et al. examined 575 participants; among those 74 were *APOE-ε4* homozygotes, 220 heterozygotes and 281 noncarriers. Each participant underwent MRI and had their white matter hyperintensity load assessed by the Fazekas scale, where Fazekas < 2 was deemed normal, and Fazekas ≥2 was deemed pathological. After correcting the analysis for cardiovascular and sociodemographic factors. *APOE-ε4* homozygotes bear a significantly higher risk (OR 3.432; 95% CI [1.297–9.082]; *p* = 0.013) of having a pathological level of white matter hyperintensities interpreted via Fazeka scale of 2 or more.. (Rojas *et al.*, 2018)

We identified the following genetic and lifestyle associations:

- log-WMHs with ApoE; non-carrier for A minor allele at rs11191848 on chromosome 10, locus STN1/OBFC1; homozygous for C significant risk allele at rs9419958 on chromosome 10, locus STN1/OBFC1; smoking status, physical activity, diabetes diagnosed by a doctor, number of medication, medications for hypertension, HbA1c, waist to hip ratio, pulse rate, DBP, SBP
- right total hippocampal volume with ApoE, smoking status, paternal longevity, mother longevity, waist to hip ratio, SBP
- left total hippocampal volume with ApoE, smoking status, maternal longevity, waist to hip ratio, DBP, arterial stiffness, number of non-cancer illnesses

We have found non-carrier status for A minor allele at rs11191848 and homozygous status for C major risk allele at rs9419958, both localised on chromosome 10, locus STN1/OBFC1 are connected to shorter telomere length and increased white matter hyperintensity volume. Previous studies described OBFC1 to be associated with cerebrovascular risk factors (Maubaret *et al.*, 2013; Madrid *et al.*, 2016) Maubaret et al. conducted a study on 2353 participants of European ancestry analysing four SNPs at three loci BICD1 (rs2630578 GγC), 18q12.2 (rs2162440 GγT), and OBFC1 (rs10786775 CγG, rs11591710 AγC). Only minor allele G of OBFC1 (rs10786775) was proven to be associated with longer TL (β=0.029, P=0.04). A 2-SNP OBFC1 haplotype was associated with a higher risk of coronary heart disease, but not diabetes mellitus. (Maubaret *et al.*, 2013) Madrid et al. studied 105,055 individuals (17235 with ischemic heart disease) in Copenhagen, Denmark and Coronary ARtery Disease Genome-wide Replication and Meta-analysis (CARDIoGRAM) consortium dataset comprising 184 967 participants (60 837 cases of ischemic heart disease). They found that a 200-bp–shorter telomere length was associated with a multivariable-adjusted hazard ratio for ischemic heart

disease of 1.02 (95% CI, 1.01–1.03). Moreover, the odds ratios for ischemic heart disease were 1.05 (1.03– 1.08) for OBCF1. (Madrid *et al.*, 2016)

Moreover, STN1/OBFC1 gene, which influences WMHs load, is reported to

- be associated with Coats plus syndrome - Cerebroretinal Microangiopathy with Calcifications and Cysts (Acharya *et al.*, 2021)
- be associated with laryngeal carcinoma in the Chinese Han male population (Han *et al.*, 2019)
- be associated with pulmonary phenotypes - primarily pulmonary fibrosis, sometimes emphysema and rarely pulmonary vascular disease. (Levy *et al.*, 2010; Hoffman *et al.*, 2018)

None of the studies proposed the mechanism underlying those associations. Genotype-phenotype relations are weak, suggesting that patients' environmental factors and genetic background determine pulmonary disease phenotypes to a large degree. (Hoffman *et al.*, 2018)

We have found that non-carrier status for G minor allele at rs43991172 on chromosome 6, locus CARMIL1, may be associated with increased white matter hyperintensities burden and decreased left hippocampal volume. However, this was not statistically significant after the Bonferroni correction. CARMIL family proteins have a function in cell migration. (Liang *et al.*, 2009) To assess the effect of CARMIL-1 knockdown on cell migration, a scratch wound-healing assay was performed with HT-1080 cells and time-lapse video microscopy. The recordings showed decreases in lamellipodia, ruffling, and macropinosomes, compared with control cells. It led to prolonged wound gap filling. (Liang *et al.*, 2009) This gene was not previously described to be associated with white matter hyperintensities, hippocampal volumes and fibrosis.

We have found homozygous status for A minor risk allele at rs11125529 on chromosome 2, and locus ACYP2 might contribute to the increased white matter hyperintensities load and is associated with shorter telomeres. Again, it

lost statistical significance after the Bonferroni correction. ACYP2 gene has been reported to

- increase the stroke risk (Liang *et al.*, 2017),
- be associated with multiple cancers (in the Chinese population):
    - glioma(M. Li *et al.*, 2020)
    - liver cancer (Chen *et al.*, 2017; Zhao, Liu, *et al.*, 2019)
    - renal cancer (Wang *et al.*, 2019)
    - gastrointestinal cancer (Duan *et al.*, 2019)
    - laryngeal squamous cell cancer (Zhao, Niu, *et al.*, 2019)
- increase the risk of cisplatin-induced ototoxicity (Thiesen *et al.*, 2017)
- be associated with non-cancer illness: immunoglobulin A nephropathy (Drogemoller *et al.*, 2018; Jin *et al.*, 2019)

The previous literature did not clarify the underlying mechanisms between ACYP2 gene expression and shortened telomeres. Similarly to CARMIL-1, neither association nor hypothesis on potential mechanism was previously described in relation to white matter hyperintensities, hippocampal volumes or fibrosis.

Persyn et al. performed a genetic study of three MRI markers of cerebral small vessel disease in UKBB imaging data

- white matter hyperintensities (N = 42,310)
- fractional anisotropy (N = 17,663)
- mean diffusivity (N = 17,467)

Across the three traits, they identified 31 loci, of which 21 were previously unreported. They performed a transcriptome-wide association study to identify associations with gene expression, which allowed to identify 66 associated genes across the three traits. (Persyn *et al.*, 2020) The table 22 summarizes a top SNP influencing WMHs they identified.

SNP	chromosome	locus	p
rs12120143	1	DENND1B	p=6.45 × 10−09
rs7566761	2	AC098824.6a	p=7.62 × 10−13
rs7596872	2	EFEMP1	p=2.06 × 10−20
rs17576323	2	AC007319.1	p=3.15 × 10−08
rs72934505	2	ICA1L, WDR12, CARF, NBEAL1, CYP20A1	p=4.31 × 10−13
rs830179	3	KLHL24	p=4.67 × 10−09
rs17148926	5	CTC-441N14.4	p=4.07 × 10−09
rs275350	6	PLEKHG1	p=8.83 × 10−17
rs3215395	7	ZAN	p=2.18 × 10−08
rs4630220	10	SH3PXD2A	p=1.21 × 10−14
rs11838776	13	COL4A2	p=7.90 × 10−11
rs11160570	14	EVL, DEGS2	p=6.10 × 10−13
rs12906662	15	MTFMT, SLC51B	p=6.42 × 10−09
rs17616633	16	RP11-437L7.1a	p=7.33 × 10−11
rs12928520	16	C16orf95	p=1.26 × 10−13
rs6587216	17	EPN2	p=8.01 × 10−09
rs8071429	17	DCAKD, NMT1	p=2.61 × 10−16
rs7214628	17	WBP2, TRIM47, TRIM65	p=4.99 × 10−36
rs429358	19	APOE	p=1.15 × 10−09

Tab. 22. SNPs influencing WMHs load identified by Persyn et al.

There is no overlap between our results and the loci identified by Persyn et al. – except for ApoE status. This could be because we looked from the genetic influencers of telomere length and telomerase activity – it is possible that as they may contribute in a small fraction to the overall WHMs burden, they are not the main driver for them. Other genetic contributors and mediators need to be further investigated, and a hypothesis of underlying mechanism formed. Similarly to us, they have found a correlation of white matter hyperintensities with common cardiovascular risk factors (diabetes, hypertension, high wait-to-hip ratio). (Persyn *et al.*, 2020)

Limitations

The main limitation of this study is that it has not addressed directly measured leucocyte telomere length but looked into genetic machinery reported in the literature to regulate telomerase activity and, therefore, telomere length.

Moreover, we have looked into the correlation and association between genetic and lifestyle factors with neuroimaging biomarkers of brain ageing and cerebrovascular health. However, unfortunately, no data enabled us to perform detailed biomolecular analysis to understand any potential underlying mechanisms.

It also remains unclear if telomere length is simply a biomarker of disease or if it plays a causal role in disease processes. (Smith *et al.*, 2019) There is no longitudinal data available within the Uk Biobank to check for this association in time.

We have looked only at the Caucasian subpopulation of the UK Biobank that stands for the majority of the dataset (94.4%, due to genetic variability and low sample size of participants of different genetic backgrounds). Therefore, our results lack worldwide generalisability.

Our population was between 40 and 85 years of age; therefore, the relationships between neuroimaging biomarkers and genetic and lifestyle factors are not well understood among young, middle-aged and elderly patients.

There is a risk of brain imaging selection bias as we excluded from the analysis imaging of the poor quality. In some cases there is a risk that the reduced quality of images might have been secondary to ageing or lack of cooperation as a marker of cerebrovascular health.

Clinical relevance and future work

We hope to provide further data supporting a genetic blueprint for ageing and contribute to the model cumulative effect of the susceptibility of accelerated ageing and risk factor from lifestyle.

As data on direct leucocyte-telomere length became available from UK Biobank in 2021, we have planned further studies linking the direct telomere length and neuroimaging biomarkers to understand their relationship better. The description of the association should lead to further research into the underlying mechanism – and if uncovered, a new therapeutic target aiming at the pathways contributing to decreased cerebrovascular health and ageing.

Background of further work

It is hypothesised that leukocyte TL is a marker of biological ageing, particularly of the ageing brain. (Wikgren et al., 2014). Among 909 participants 909 from the Austrian Stroke Prevention Study, longer LTL was associated with larger WMH load ($\beta = 0.03$, $p = 0.04$) (Gampawar, Schmidt and Schmidt, 2020). Short telomers are also suggested to be a biomarker of the risk and prognosis of ischemic stroke. (Tian et al., 2019)

The mechanisms underlying small vessel disease are not yet fully understood. (Khan et al., 2007) It is a major contributor to dementia, cognitive decline, gait impairment, mood disturbance and stroke. (ter Telgte et al., 2018) Increased age, hypertension, and current smoking are well-known risk factors for the disease. (Dijk et al., 2008)

Therefore we plan to assess its relationship among participants participating in UK Biobank Study. We hypothesise that participants with shorter telomere lengths have a higher burden of white matter hyperintensities.

Materials of further work

1. **Participant selection** from UK Biobank – from all participants with concomitant brain imaging and blood test after which LTL was calculated, we will exclude:
    a. scans with poor quality (no volumetric scaling information, ID 25000)
    b. non-Caucasians (was based on the Caucasian genetic, ethnic background (UKBB Field ID 22006); due to (i) genetic variability between ethnicities(Huang, Shu and Cai, 2015) and (ii) known differences in cardiovascular risk factors between ethnicities(Tillin *et al.*, 2014), (iii) the dominating proportion of white individuals.(Fry *et al.*, 2017) (iv) non-Caucasian from UK Biobank have longer LTL (Codd *et al.*, 2021))
    c. without FLAIR imaging
    d. patients with stroke and cancer

2. **Outcome variable**
    a. WMHs adjusted for head size (*Volumetric scaling from T1 head image to standard space*, UKBB Field ID 25000)

3. **Variables (explanatory/mediating)**
    a. LTL expressed as Z-adjusted log from adjusted for 9 procedural variables T/S ratio

4. **Technical variables of no interest** - for the selection of technical confounders of no interest, we followed the recent paper by Alfaro-Almagro et al. on deconfounding UKBB MRI data (Alfaro-Almagro *et al.*, 2021):
    a. subject-specific confounds: age *(Age at the imaging centre*, UKBB Field ID 21003 - when participants had 4 visits reported, visit-3 was the first brain imaging scanning sessions, and visit-4 was a

second brain imaging scanning session), *Sex* (genetic sex, UKBB Field ID 22001)

b. scanner / acquisition protocol / processing parameters: acquisition site (UKBB Field ID 54), intensity scaling for T1, intensity scaling for T2-FLAIR, T2-FLAIR used (in addition to T1) to run FreeSurfer.

c. table position: *Scanner lateral (X) position,* UKBB Field ID 25756, *Scanner transverse (Y) position,* UKBB Field ID 25757; *Scanner longitudinal (Z) position* - UKBB Field ID 25758; *Scanner table position* - UKBB Field ID 25759.

5. Biological variables of no interest

a. affecting WMHs: cardiovascular risk factors (hypertension, waist-to-hip ratio, diabetes, heavy smoking, hypercholesterolemia and homozygous APOE ε4 status) (Veldsman *et al.*, 2020)

b. affecting LTL: WCC as age, ethnicity, sex and white cell count explain ~5.5% of LTL variance(Codd *et al.*, 2021)

Methods of further work

6. Statistical analysis plan

a. descriptive statistics: LTL, WMHs, co-variates

b. linear regression model for WMHs

i. assessment of linearity with scatterplots and correlation (Pearson for 2 continuous variables, Spearman rank for mixed types of variables)

ii. assessment of VIF for co-linearity and removal of the variables with VIF <0.1 or VIF>10

iii. univariate regression analysis

iv. multivariate regression analysis

Chapter 5: Conclusions

We have observed a weak correlation between WMHs and hippocampal volumes. The final regression models explain

- 36.1% of the variation in WMHs volume, when adjusting for ApoE; non-carrier for A minor allele at rs11191848 on chromosome 10, locus STN1/OBFC1; homozygous for C significant risk allele at rs9419958 on chromosome 10, locus STN1/OBFC1; smoking status, physical activity, diabetes diagnosed by a doctor, number of medication, medications for hypertension, HbA1c, waist to hip ratio, pulse rate, DBP, SBP and technical confounders
- 43.6% of the variation in right total hippocampal volume, when adjusting for ApoE, smoking status, paternal longevity, mother longevity, waist to hip ratio, SBP
- 40.6% of the variation in right total hippocampal volume, when adjusting for ApoE, smoking status, maternal longevity, waist to hip ratio, DBP, arterial stiffness, number of non-cancer illnesses

The overall analysis uncovered that ApoE is the only consistent genetic contributor for all three biomarkers (WMHs, right and left hippocampus), on its own, explaining 0.01% of variability for each. The final models refute our hypothesis that SNPs regulating TL were associated with markers of brain ageing, specifically right or left hippocampal volume Two SNPs regulating TL localised on chromosome 10 (rs11191848 and rs9419958) are associated with increased WMHs. rs11191848 has been previously reported to be a biomarker of cardiovascular diseases, whilst there is no clinical significance described for rs9419958 other than influencing leucocyte telomere length.

Further studies are needed to better understand direct leucocyte telomere length on neuroimaging biomarkers and uncover potential mechanisms for those relationships.

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