Cognitive and Epigenetic Predictors of Healthcare Utilization in the Canadian Longitudinal Study on Aging: Factor- and Indicator-level Examinations

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Background: Healthcare utilization among older adults is a valuable indicator of health status and provides a mechanism linking illness to healthcare costs. Cognitive function and epigenetic age—as indicators of nervous system and cellular integrity at a biological level—are both correlated with age and may be important predictors of healthcare utilization. It is unclear how strongly the two predictor categories are associated, however, and it is not known whether they are best understood as correlated processes under a general "systemic resilience" (SR) construct, or as separate factors with independent influence on healthcare utilization. In the present research study, I examine indicator-level and factor-level predictors of healthcare utilization, with a focus on cognition and epigenetics. In doing so, the factor-level and indicator-level associations with healthcare utilization are evaluated, with an eye toward testing the validity of a superordinate SR construct.

Objectives and hypotheses: This study examined the factor structure of SR as a higher-order construct, encompassing epigenetic age and cognitive function. It compared factor-level associations with healthcare utilization (emergency department [ED] visits and hospitalizations) to those observed at the indicator-level, while evaluating age and sex as potential moderators. *A priori* hypotheses were that a higher-order model would best fit the data, the associations would be stronger for women (vs. men) and older (vs. middle-aged) adults, and significant pathways would emerge at both the factor and indicator levels.

Methods: Data were drawn from the Canadian Longitudinal Study on Aging (CLSA) Comprehensive Cohort *(n*=30,097; age range=45-85 at enrolment), focusing on the subsample who completed epigenetic assays (*n*=1,478). Structural equation modelling (SEM) was used to evaluate competing structural configurations and predict healthcare utilization. Age and sex moderation were examined using multi-group analysis. Logistic regressions were used to examine indicator-level associations.

Results: A correlated two-factor model representing epigenetic age and cognitive function as distinct but related constructs, rather than as components of a higher-order SR model or a single unified factor was selected as the most appropriate model based on fit indices and parsimony. Cognitive function emerged as a predictor of hospitalizations at the factor-level (b -0.254; 95% CI; -0.461, -0.046). Supplemental analyses suggested no significant sex moderation, while evidence for age moderation was inconclusive. At the indicator-level, analyses suggested the mental alternation test (MAT) and intrinsic epigenetic age acceleration (IEAA) were reliable predictors of ED visits. Similarly, the animal fluency test (AFT) was a predictor of hospitalizations.

Conclusions: Cognitive function and epigenetic age may be best considered as correlated, but fundamentally independent processes in older adults. Among factor-level predictors of healthcare utilization outcomes, cognitive function was reliable but not epigenetic age. At the indicator level, mental flexibility and cell-intrinsic aging were predictive of select facets of healthcare utilization. These findings suggest that both cognitive and epigenetic markers have some value in predicting future healthcare costs among older adults, but that systemic resilience may be less useful at the whole organism level.

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List of Abbreviations

AAD	Age Acceleration Difference
AAR	Age Acceleration Residual
AFT	Animal Fluency Test
AIC	Akaike Information Criterion
BIC	Bayesian Information Criterion
CFA	Confirmatory Factor Analysis
CFI	Comparative Fit Index
CI	Confidence Interval
CLSA	Canadian Longitudinal Study on Aging
CpG	Cytosine-Guanine Dinucleotide
CRT	Choice Reaction Time
DNA	Deoxyribonucleic Acid
DNAm	DNA methylation
EAA	Epigenetic Age Acceleration
EBPM	Event-Based Prospective Memory
ED	Emergency Department
EDTA	Ethylenediaminetetraacetic acid
EEAA	Extrinsic Epigenetic Age Acceleration
FAS	Controlled Oral Word Association Test
FIML	Full Information Maximum Likelihood
GDP	Gross Domestic Product
IEAA	Intrinsic Epigenetic Age Acceleration
MAT	Mental Alternation Test
MCQ	Maintaining Contact Questionnaire
MLR	Robust Maximum Likelihood
MRI	Magnetic Resonance Imaging
OR	Odds Ratio
PCB	Polychlorinated Biphenyl
PET	Positron Emission Tomography
PMT	Prospective Memory Test
REY	Rey Auditory Verbal Learning Test
RMSEA	Root Mean Square Error of Approximation
SEM	Structural Equation Modelling
SR	Systemic Resilience
SRMR	Standardized Root Mean Square Residual
STP	Stroop Test
TBPM	Time-Based Prospective Memory
WHO	World Health Organization
WLSMV	Weighted Least Square Mean and Variance

1.0 Introduction

Population aging refers to the increase in the proportion of people aged 60 years or over and a concomitant decrease in the proportion of people aged 15 years or younger (1–4). In Canada, population aging mirrors global patterns, with an expected increase in the proportion of adults aged 65 years or over from 19% in 2022 to 23% in 2030 (5). This trend raises considerable concern for healthcare (1). An older population brings about unique health challenges, such as an increase in the prevalence of chronic diseases, including Alzheimer's disease, heart disease, stroke, and diabetes (2). Taken together, these factors contribute to decreased population health and increased resource pressure on healthcare systems, underscoring the importance of understanding the processes that shape aging trajectories (2).

Crucially, not all individuals within a population will experience the same aging trajectories (6). In theory, individuals can experience different levels of functioning, disease burden, and mortality risk, even at the same chronological age (6,7). While some older adults maintain independence and are cognitively intact well into later life, others experience early morbidity and rapid decline. Importantly, this kind of heterogeneity in aging increases with age, emphasizing the individuality of each older adult (6). Such variability has important implications for clinical practice, reinforcing the need for comprehensive assessment and specialized care (6).

Multiple factors can contribute to heterogeneity in aging outcomes, including demographic and socioeconomic variables, such as education, lifestyle, income, and occupation, which interact with biological factors to shape health trajectories (8). For instance, individuals with higher incomes may have greater access to healthy foods, healthcare resources, and health-promoting environments. These social, ecological, and demographic factors may also influence

individuals at the cellular level, thereby impacting disease risk. Two important metrics that can capture the consequences of changes in cellular function are biological clocks and cognitive performance measures. Biological clocks provide a direct measure of aging processes, while cognitive performance measures reflect the output of the central nervous system, the integrity of which is a reliable indicator of the aging process (9).

1.1 Biological Aging

Biological aging refers to changes at the molecular, cellular, and organ levels over time, which together contribute to clinically meaningful differences between older adults (9). These differences may manifest as functional deterioration, diminished sensory or cognitive abilities, or other impairments (10). This may be through metabolic pathways influenced by lifestyle factors (e.g., substance use, physical activity diet) or through indirect means that can weaken biological repair functions, such as through chronic stress exposure (9). Environmental and social/cultural contexts can shape aging processes as well, including through exposure to environmental toxins and availability of nutrition and health care services (9). Over time, these exposures accumulate and contribute to varied aging trajectories and outcomes across individuals.

Research into factors underlying variation in health outcomes has led to the development of several constructs relevant to aging, including multimorbidity and disability (6). Multimorbidity refers to the coexistence of multiple diseases in the same person, and is associated with increased vulnerability and age-related homeostatic dysregulation (3). Disability can be defined as physical, mental, and emotional limitations or difficulties that can interfere with an individual's ability to perform daily activities (11). Although these constructs are

clinically useful, they focus primarily on disease burden and functional impairment, rather than on adaptive processes or recovery potential.

1.2 Resilience

One concept that been increasingly gaining recognition in aging research and reflects heterogeneity in aging is resilience, defined as the ability to maintain or recover function following exposure to health-related stressors or transient perturbations (12). Although resilience has been studied in psychological and social domains, its conceptualization at the level of the whole organism remains limited. A systemic perspective on resilience recognizes that biological systems do not operate independently, but dynamically and interdependently with other systems to respond to health insults. This perspective helps explain why individuals with similar disease burdens or risk profiles might experience divergent health trajectories. Systemic resilience (SR) extends the concept of resilience by referring to the integrated capacity of multiple systems, biological and cognitive, to collectively maintain or restore function in the face of health stressors or insults. Accordingly, SR can offer a unifying framework for studying aging as a dynamic and adaptive process, rather than as just an accumulation of deficits or impairments.

1.3 Epigenetic Clocks

Advances in biomedical research have made it possible to examine biomarkers that capture biological processes that may underlie variability in SR. These biomarkers can be used to predict morbidity, mortality, and functional impairment(13–15). Where chronological age is used to describe the period elapsed since birth, biological aging is a measure of an individual's health over the lifespan, capturing genetics, metabolic, and environmental changes over time (16). These measures can provide insight into why individuals of the same chronological age

experience different outcomes. Within the field of biological aging, the most robust and widely used biomarkers are epigenetic clocks, built on methylation levels of cytosine-guanine dinucleotide (CpG) sites (16). Epigenetic clocks are affected by environmental and life experiences, which can capture influences beyond genetic variation alone (16). This sensitivity to environmental influence makes epigenetic measures particularly compelling and well-suited to studying heterogeneity in health outcomes.

1.4 Cognitive Function

Cognitive function refers to the core mental processes that allow individuals to acquire knowledge and process information through thought, experience, and the senses (17). Cognitive function is subdivided into several domains, including executive function, memory and learning, attention, motor function, and language (18). Preserving these processes is essential for maintaining healthy aging. Cognitive impairment is a major contributor to poorer outcomes in life. Declines in cognitive function can compromise one's ability to carry out basic and instrumental activities of daily life, including medication management, financial decision-making, and meal preparation, thereby reducing quality of life and increasing dependency (19).

Cognitive function, similar to biological aging, is highly heterogeneous across individuals. Numerous factors contribute to the preservation of cognitive abilities, such as physical activity and social connection, while others, such as sleep disturbances and stress, can negatively affect cognitive function (18). Furthermore, cognitive decline may not be uniform across all domains (20). For example, it is possible for an individual to have excellent long-term memory, but impaired executive function (20). This variability can be partly explained by the concept of cognitive reserve, where lifetime experiences (e.g., education, employment, and

engagement in mentally stimulating activities) and genetic factors can influence the brain's reaction to age-related neuropathology (21). Higher cognitive reserve may delay or ameliorate the expected clinical impact of minor or major neurocognitive disorders (22,23).

1.5 Epigenetic Age, Cognitive Function, and SR

Prior studies have shown that both chronological and epigenetic age are associated with cognitive performance (24–26). However, it remains unclear whether cognitive performance and epigenetic age can be jointly conceptualized as markers of a superordinate construct, such as SR. The primary objective of this thesis was to utilize data from the Canadian Longitudinal Study on Aging (CLSA) and examine whether cognitive performance and epigenetic age jointly represent SR. This was done using confirmatory factor analysis (CFA) to evaluate the fit of competing explanatory models where: 1) cognitive and epigenetic indicators form two latent factors under a higher-order SR construct, 2) cognitive and epigenetic indicators form two correlated factors without a higher order SR construct, or 3) both indicators (cognitive and epigenetic) form a single latent construct (SR) without intermediate-level clustering.

Following CFA, structural equation modelling (SEM) was used to assess which model, from the three mentioned above, best fit the data. A secondary objective was to examine the moderating effects of age and sex. The best-fitting model was then used to examine associations between latent constructs and healthcare utilization, as a relevant health outcome for middle-aged and older adults in the CLSA. By combining cognitive and biological measures, this thesis seeks to advance the understanding of how multiple systems can contribute to SR and to clarify the processes that contribute to heterogeneity in health outcomes that are relevant to middle-aged and older adults, i.e., healthcare utilization. Healthcare utilization was chosen for study because it can capture real-world consequences of health and is especially important for older adults, who

disproportionately use healthcare services (27). Examining types of healthcare utilization can underscore the real-world impacts of biological and epigenetic factors.

2.0 Background

2.1 Resilience

As a concept within systems biology, resilience is the ability of a complex organism to absorb, resist, or recover from health insults. The latter may manifest on a moment-to-moment, day-to-day or year-to-year time scale. There is a long history of considering "resilient" qualities of systems in health care, social sciences and life sciences research (28–36). For instance, a healthcare system is resilient if it can effectively deal with an influx of patients and still offer high-quality care; a psychological system is resilient if an individual can cope with a transient stressor without decompensation; and a biological system is resilient if it can adapt to the introduction of a pathogen without losing integral functioning (28–36). Resilience is also a central concept in other fields, such as in engineering and agriculture, underscoring its importance and relevance across many domains of inquiry (37,38).

Importantly, when applied to any system, the capacity for resilience is not static, but dynamic. Resilience continually evolves and changes in response to intersections between social, psychological, and biological domains of an individual's life (39). SR also applies to multiple systems in the human body. Multiple interconnected systems can contribute to one's overall capacity to recover or maintain function in the face of health insults. This aligns with the recognition that physiological systems are deeply interconnected. For example, the neuroendocrine, immune, metabolic, and cardiovascular systems are all involved in maintaining homeostasis and coordinating energy balance, such that stress or damage to one system can be buffered or supplemented by another (40).

This raises the question of whether resilience can be meaningfully conceptualized at the level of the whole organism, integrating the combined characteristics of multiple systems rather

than each system in isolation. The concept of SR has been proposed to capture this multisystemic perspective. The thesis was undertaken to explore whether cognitive and biological systems contribute to SR. Although it is possible to include multiple systems, SR can represent a higher-order construct that reflects the combined influence of these systems on health outcomes. Therefore, by capturing not only the individual contributions of these systems, but also their interactions and joint contributions, SR can potentially offer greater predictive value than individual system-specific measures alone.

In this thesis, SR is conceptualized as a higher-order latent construct, reflecting integrated adaptive capacity across multiple biological and cognitive systems, and serving as the central framework for examining heterogeneity in health outcomes (41,42). Differences in SR could potentially explain why some people age healthier and live longer, while others suffer from disease or experience steep loss of function later in life. SR is not to be confused with existing measures of morbidity and mortality, which focus on accumulation of deficits and impairment. Individuals may exhibit divergent health trajectories, regardless of their morbidity; SR may explain some of this divergence.

2.2 Aging

Aging is a complex process involving progressive loss of integrity in cells, tissues, and organs. When accelerated, these losses can lead to decreased functional ability and greater susceptibility to disease (44,45). Thanks to social, medical, and economic advances, people throughout most of the world live longer and healthier lives (46,47). According to the World Health Organization (WHO), by 2050, one in five people will be over the age of 60 years (48). While this can bring about many socioeconomic, political, and cultural benefits, population aging has also led to an increase in the prevalence of complex health conditions and geriatric

syndromes (43–46). However, ‘old’ age is not the sole predictor of these outcomes and does not explain why variability exists among older adults of the same age (51). Adverse health outcomes affect not only the individual, but can also strain healthcare systems, emphasizing the societal importance of understanding the underlying processes of aging outcomes (52).

Current research suggests that the interaction between intrinsic (cellular, genetic, epigenetic) and extrinsic (lifestyle, environment) factors drives the pace and heterogeneity of aging. The cellular and molecular mechanisms of aging outcomes are thought to be related to an accumulation of physiological damage over time, which some label as the “hallmarks of aging”(45). Individuals most exposed to health hazards will accumulate a greater amount of damage to physiological systems and experience accelerated aging, ultimately bringing about the risk of earlier mortality (14). This damage can be described as genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled autophagy, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis (53). These items are grouped into primary, antagonistic, and integrative hallmarks, each reflecting different stages of biological dysregulation. The primary items – genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis – are considered to be the root causes of cellular aging and are associated with many age-related diseases (54). The secondary/antagonistic items – deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence – serve protective or adaptive roles, but can become deleterious if activated chronically (54). The integrative items—stem cell exhaustion, chronic inflammation, and altered intercellular communication—emerge from accumulated cellular damage and failure of compensatory mechanisms, reflecting the systemic decline driven by the other items (54). Importantly, these

processes are interconnected and mutually reinforcing (54). Collectively, the aforementioned items represent plausible cellular, molecular, and systemic processes through which poorer health outcomes may arise.

2.3 Biological Age and Epigenetic Aging

Biological aging can be conceptualized as a lack of SR. Biological aging differs from chronological aging, which simply reflects how long someone has been alive. Biological aging can vary widely for each individual of similar chronological age, and can be influenced by genetics, environmental exposures, and health behaviour patterns (55,56).

Biological age involves a progressive, non-linear pathway for individuals, reflecting variability in aging patterns (57). Therefore, although chronological age is the strongest risk factor for many chronic diseases, biological age explains variation in disease risk within age groups, reflecting the degree of physiological deterioration of an individual on cellular and molecular levels (56,58). Unlike chronological age, biological age is fluid and fluctuating, indicating that it is possible to repair biological aging, which can be an important determinant of successful aging and longevity (59). For instance, interventions such as caloric restriction of food intake and physical activity have been shown to slow or even partially reverse biological aging markers in the brain (60).

While the full scope of biological aging is still not fully understood, three broad components are thought to contribute to age-related biological decline (43). The first component is depletion of exhaustible body reserves, such as immune resources, which limits the ability of the body to respond to stressors and recover. The second component is an age-related slowdown of physiological processes and responses due to declines in the rate of metabolism, proliferation, information processing, and other factors that can lead to a prolonged recovery time. Finally, the

last components are the inherently imperfect mechanisms of cell repair and tissue washing, which result in an accumulation of damage over time, increasing dysregulation of body systems and increasing allostatic load (43).

Because biological age captures the cumulative impact of biological and environmental stressors, it can serve as a more integrative marker of functional decline than any individual biomarker alone.

2.4 Epigenetics

Epigenetics refers to changes in gene expression that can occur without altering the deoxyribonucleic acid (DNA) sequence itself, providing a partial picture of how life experiences can be biologically embedded over time (61). These changes can influence how genes are turned on or off by affecting how easily cellular machinery can access and read DNA, thereby regulating gene activity (62).

Epigenetic alterations are associated with the other hallmarks of aging and can provide a proxy measure of biological aging. There are three primary epigenetic mechanisms that have been identified; histone modification, DNA methylation (DNAm), and non-coding RNA activity (62). This study focuses primarily on DNAm-based measures of biological aging.

DNAm is a mechanism by which specific cytosine-guanine dinucleotide (CpG) sites can be modified via transfer of a methyl group, which affects gene regulation (63,64). Methylated CpG sites can recruit gene suppressor proteins, reducing interactions between DNA and transcription factors, and can drive the formation of heterochromatin, preventing transcription or leading to gene silencing (62). Being able to alter DNAm patterns is a key component of cell differentiation, specification, and maturation (65). These changes can occur in response to environmental, social, and behavioural exposures, including diet, hormones, stress, drugs and

exposure to chemicals such as Polychlorinated Biphenyls (PCB)s, lead, and BPA (Bisphenol A) (14,65). Because epigenetic changes can be stable and heritable, as well as malleable and dynamic, they are informative markers of life-course exposures and ongoing biological and environmental processes (65).

This loss or gain of methylation is the basis of many diseases, including cancer. Hypomethylation of repetitive elements has been suggested to be deleterious to cells, through the insertion of mutations and by introducing instability later on through deletions and genomic rearrangements (66). Epigenetic mechanisms also play an important role in aging because they are associated with a decondensation of chromatin, a generalized lower level of methylation, and hypermethylation at specific loci (67). As individuals age, there is an associated decline in repetitive element methylation in normal tissues, which has been associated with risk factors for cancer and neurological and cardiovascular diseases, thereby indicating that DNAm plays a significant role in many human age-related diseases (66).

Biological aging can be measured using epigenetic clocks, which use DNAm values from all sources of DNA in the genome across the lifespan (68). The first generation of these clocks identifies methylation patterns that vary by chronological age (63). Therefore, if an individual has an older epigenetic age than their chronological age, it could be inferred that their biological age is older, as well (63). These first-generation clocks include the "Hannum clock," which is based on 71 CpGs in leukocytes in the blood, and the "Horvath clock," which is based on 353 CpGs and is consistent across different cell types and tissues (69). The second generation of epigenetic clocks investigate associations between biomarkers and time-to-event data to predict mortality risk (69). These clocks include PhenoAge, which is based on 513 DNA methylation

sites, and GrimAge, which was developed using DNAm-based markers of smoking and plasma proteins linked to morbidity and mortality (71,72). Finally, the third generation includes the DunedinPACE (Pace of Aging Calculated from the Epigenome), which was developed by tracking the rate of physiological change in 19 biomarkers over 20 years among individuals of the same chronological age, then identifying DNAm patterns that captured individual differences in rate (62). This clock was designed to reflect individual differences in age-related decline (63).

Another important measure related to epigenetic aging is epigenetic age acceleration (EAA), which refers to the discrepancy between an individual's chronological age and their epigenetic age (73). A faster, or positive, age acceleration signifies individuals who are aging faster biologically, while an inverse, or slower, age acceleration signifies slower biological aging (64,74). One aspect to note is that there are known sex differences in biological aging. Interestingly, when examining EAA, men are shown to have higher epigenetic age and EAA compared to women at all ages, which also holds true across different tissues (75,76). Men also have shorter lifespans, which may be attributed to a higher level of proinflammatory markers. These markers are linked to higher prevalence of cardiovascular diseases and metabolic disorders (67).

Previous studies have consistently shown that older epigenetic age and faster EAA are linked with poorer aging outcomes independent of chronological age. Faster EAA has been associated with the development of many chronic diseases, including cancer, cardiovascular disease, and dementia, as well as a shorter lifespan independent of other predictors of aging and death, such as diabetes, education, and cardiovascular disease (68,77,78). For example, a study that looked at the association between EAA and longevity in women found that increased EAA

measured by the Hannum, Horvath, Pheno, and Grim clocks were all associated with lower odds of survival to age 90 years in those with intact mobility (64). Evidence from a multi-country study further supported these findings: individuals with faster EAA showed reduced mobility, more cognitive deficits, and subsequent mortality, indicating that epigenetics are capable of capturing a fundamental aging process (79). Together, these findings suggest that having a younger epigenetic age and slower EAA may be indicative of greater resilience and better health outcomes. Given that epigenetic age can capture systemic biological dysregulation, examining its relationship with cognitive performance can provide insight into cross-system SR.

2.5 Cognitive Function

Cognitive function can be divided into specific domains, such as executive function, memory, processing speed, attention, language, and visuospatial abilities (80). Executive function encompasses the processes allowing for control over other cognitive abilities so that cognitive resources can be used to solve problems and plan for the future; memory refers to the mechanism responsible for encoding, storing, and retrieving information for later use; processing speed refers to the measure of time required to process and respond to information; attention refers to the ability to concentrate and focus on specific stimuli; language encompasses comprehension, naming, reading, writing, and repetition; visuospatial abilities involve the ability to understand space in two and three dimensions; and (81,82). Many changes in these processes occur across the lifespan, with marked improvements from childhood to early adulthood, followed by variability and potential decline later in life, depending on individual and contextual factors (83).

Although the negative influence of age on cognition is greater for individuals over 50 years, there is general decline across many cognitive faculties, including episodic memory, spatial visualization, and processing speed as one ages (26,84,85). In particular, processing speed and working memory, the system that temporarily holds and manipulates information necessary for cognitive tasks (e.g., reasoning, learning, and comprehension), are the cognitive domains most sensitive to aging (26,85,86).

Changes in cognitive functioning—and overt cognitive performance—can be partially explained by structural changes that the brain undergoes during the aging process (87). Aging is often accompanied by reductions in brain volume, synaptic spines and numbers of synapses, which together account for reductions in gray matter (80,87). White matter also shows significant deterioration, to an even greater extent than gray matter (80). Neuron-level changes can contribute to reductions in specific cognitive abilities, with studies indicating that gray matter volume, cortical thickness, prefrontal volume, and myelination all contribute to age-related declines in various cognitive abilities (80,87,88). There is also an increase in plaques and neurofibrillary tangles, a reduction in neurotransmitters including acetylcholine and serotonin and their binding sites, and a decrease in neurotrophic factors (e.g., brain-derived neurotrophic factor [BDNF]) (60,89). These changes have implications for mood, memory, cognitive impairment, and motor function (60,89).

The frontal lobe, responsible for working and episodic memory, organization, planning, abstraction, and sequencing, is most affected by age, followed by the temporal lobe, responsible for learning, spatial and episodic memory, and facial recognition (89). The parietal lobe is next, responsible for the primary somatosensory cortical functions and functions of the secondary cortices, and finally, the occipital lobe, responsible for primary visual cortex tasks (89).

Similar to biological age, age-related cognitive decline is not entirely fixed and varies across individuals. Several studies in animals have shown that environmental enrichment, a setting where animals can experience enhanced cognitive and social interactions in addition to sensory and motor abilities, can reduce changes in cortical thickness, dendritic branching, spine density, neurogenesis, and gliogenesis, improving performance on tasks (60). In humans, while cognitive decline is generally not reversible, aspects of brain aging may be modifiable. Evidence suggests that successful brain aging is possible with the right combination of healthy habits, which typically include diet, physical and mental exercise, maintaining an active social life, and not smoking (60).

Importantly, cognitive function varies between individuals, as well as within domains (20). A large degree of variability exists in the onset, trajectory, and severity of these declines, with greater inter- and intra-individual variability introduced in midlife onward (90). On one end of the spectrum are individuals with dramatic cognitive deterioration early on (90). On the other end are the "SuperAgers," individuals over the age of 80 years with cognitive abilities (performance on episodic memory tasks) that are commensurate with individuals 20-30 years younger (90). These differences may be due to many different biological, psychological, health-related, environmental, and lifestyle factors and mechanisms (20). One possible explanation for this is differential brain activation in older adults, characterized by greater bilateral activation for tasks that typically only involve unilateral activation (20). While some speculate this is a compensatory mechanism, others suggest it represents inefficient cognitive processing (20). Another explanation is cognitive reserve, which proposes that experiences, such as education, occupation, social connection, and genetic factors, may allow individuals to maintain cognitive function despite age-related or pathological brain changes (21–23). For instance, evidence

suggests that greater cognitive reserve can reduce the risk of Alzheimer's disease or attenuate the impact of Alzheimer's pathology on cognitive decline over time in those with the disease (91,92).

Some findings show that low cognitive performance can predict functional decline, suggesting that cognitive function may indeed be a marker for SR (35,93). A study that examined executive function at baseline and at 6-year follow-up found that individuals with executive function impairments had the highest proportion of daily living challenges, be they functional or instrumental, compared to other groups (93). These individuals also had increased risk of mortality, indicating the importance of baseline function as a predictor of further decline (93). Another study that examined baseline episodic memory in cognitively intact older adults found that it was the best predictor of 4-year changes in episodic memory, compared to demographic factors, amyloid positron emission topography (PET) and structural magnetic resonance imaging (MRI) measures, and genetic variables (94). Similar results can be observed in older adults with dementia, where poor neuropsychological test results at diagnosis can be linked to more rapid decline (95). This body of literature suggests that cognitive performance at baseline may predict morbidity and mortality later on (95).

2.6 Epigenetic Age and Cognitive Function

Understanding the factors underlying SR and its determinants may aid in comprehending both the aging process and susceptibility to disease (96). It has been previously established that epigenetic processes are vital for brain function and may be associated with worse cognitive performance (96,97). Some studies show that approximately 30% of variance in general cognitive function can be explained by epigenetic changes, which can be quantified by blood-based biomarkers (98,99). Importantly, blood-based DNAm patterns have been shown to

correlate with brain methylation patterns, supporting their use as proxies for central nervous system processes (100). Methylation at specific sites in the brain can be associated with loss of volume, brain disorders, decreased cognitive efficiency, increased emotional reactivity, and more (96). For example, methylation in genes related to synaptic transmission, neurotrophic signaling, and glucocorticoid receptor sensitivity have been linked to hippocampal atrophy and a dysregulated stress response (100–103). A specific example of epigenetics and a brain disease would be Alzheimer's disease. Recent genome-wide analyses indicate that genes associated with neuroplasticity are less expressed in the brain of those with Alzheimer's disease, while those involved in the immune response and inflammation are more expressed (104).

Epigenetic processes also support long-term memory formation. Therapies such as histone deacetylase inhibitors, aimed at defective epigenetic components, can potentially modulate cognitive impairment and decline, providing evidence for epigenetic processes in brain function (96). Animal models have demonstrated that manipulating enzymes involved in DNAm can restore synaptic plasticity and improve performance on memory tasks, underscoring the potential for reversing epigenetically mediated deficits (105,106). Therefore, it seems that epigenetic dysfunction is correlated with impairments in synaptic plasticity (104).

Furthermore, because DNAm patterns can be influenced by the environment, epigenetics has potential to explain variability in cognitive function that cannot be accounted for by differences in DNA sequences alone (96). This includes physical, social, and economic influences, and the interplay between these factors (107). A study that examined 33 physical and 40 social exposomal factors across 34 countries found that adverse physical/environmental exposures, such as lack of green space and poor water quality, were associated with accelerated structural aging of the brain, particularly in limbic subcortical, and cerebellar regions (107).

Furthermore, adverse social exposures, including poverty and reduced gross domestic product (GDP), were associated with accelerated functional aging in the frontotemporal and limbic networks (107). These exposures, when taken together, explained up to 15.5 times more variance in brain aging than individual predictors, indicating the profound effect of synergistic interactions that can be captured through biological mechanisms (107).

Finally, the role of DNAm in memory, learning, and synaptic plasticity has been shown in several studies, suggesting that epigenetic modifications are associated with cognitive performance (108,109). A previous study looking at middle-aged adults found that EAA was associated both cross-sectionally and prospectively with lower performance on multiple cognitive tests, including the Stroop test, the Rey Auditory Verbal Learning Test, and the Digital Symbol Substitution Test (110). This association was stronger for women, who may experience faster cognitive decline than men (110,111). These findings are in contrast to another study that examined epigenetic age and cognitive decline among urban-dwelling adults aged 30-64 years at baseline, where there was a greater association between EAA and cognitive decline among men on visual memory tests and attention/processing speed tests (25). A third study that examined EAA in older adults found that EAA was associated with sex-specific cognitive decline later in life. In women, EAA was associated with poorer memory, while in men, EAA was associated with slower processing speed and an increased risk of dementia (112). These findings, then, support analysis of sex effects on the structure of the SR construct and its validity indicators, as well as an examination of interrelationships between epigenetic and cognitive factors more broadly.

2.7 Current Gaps in Research

A relatively large proportion of existing research on epigenetic aging and cognitive function looks at direct relationships between cognitive function and epigenetic age, and their associations with outcomes separately, rather than as indicators of a higher-order construct (25,45,64,84,97,110,112–116). This has meant that shared variance among indicators within each category, and the presence of superordinate constructs (such as SR) has remained largely unexplored. Most research in epigenetic age and cognitive function also focuses on older adults, with fewer studies examining middle-aged adults or the moderating effects of age. Investigating middle-aged to older populations is particularly important, as it allows for the examination of whether associations between biological and cognitive indicators differ across age groups. Looking at the moderating effects of age on this association in a broader population can also provide valuable insights into the strength of association and how it differs across age groups.

The CLSA dataset is ideal for this study because it is a large, population-based sample of middle-aged and older Canadians, includes relevant measures of cognitive function and epigenetic age, and contains information on a large variety of confounders and effect modifiers. Using this dataset, the understanding of associations between biological markers of age, cognitive function, as well as their potential relationship with SR, can be explored within a Canadian context among middle-aged and older adults, thereby providing more insights into factors affecting the evolving health outcomes of Canadians.

In addition, limited attention has been given to the potential moderating effects of sex when looking at epigenetic age and cognitive function. Although women may experience faster age-related declines in cognitive function than men, men likely have more accelerated epigenetic aging than women, suggesting that men and women are differentially affected by EAA (117,118). Several studies also report EAA and greater epigenetic age are more strongly

associated with poorer cognitive outcomes in women (101), perhaps due to biological phenotypes, hormonal influences, and the number of chronic diseases (112). One explanation could be the hormone estrogen, which provides anti-inflammatory, neuroprotective effects and can enhance cognitive function (67,112). Therefore, during menopause, when estrogen levels fall, these protective effects may be compromised, leading to osteoporosis, cardiovascular diseases, and poorer cognitive performance (60). This is further supported by the fact that EAA is associated with earlier onset menopause, indicating that DNAm plays an important role in the onset of menopause (75). Women are also most susceptible to developing autoimmune diseases, which can negatively impact their health and contribute to inflammation (67). Other ways that sex might influence epigenetic mechanisms could include the Y chromosome containing different epigenetic modifiers, genomic imprinting, somatic sex-specific expression of Y-linked transcriptional regulator genes in males, female-specific expression of X-linked transcriptional regulators that are paternally imprinted, increased expression of X-linked transcriptional regulators that escape X-inactivation, sequestration of transcriptional regulators by sex chromosomes, and sex hormones and their receptors (67).

This study aims to address these gaps by examining whether epigenetic age and cognitive function can be used to operationalize SR, potentially providing greater predictive value than either measure alone. In health research, healthcare utilization is commonly used as a marker of underlying health status and functional decline (121). Within this study, a breakdown in SR may manifest as increased healthcare utilization, particularly through higher rates of emergency department (ED) visits and hospitalizations. Viewing healthcare utilization through these two metrics allows for a focus on acute, clinically significant events rather than elective or routine care, thereby making these two outcomes critical for evaluating the validity of the SR construct.

Importantly, these outcomes were selected as they capture downstream consequences of deterioration in overall health status and organism-level failure of adaptive capacity across multiple systems, rather than routine symptom-level complaints. Therefore, within SR, these outcomes can provide observable, whole-system-level manifestations of underlying deterioration in health regulation and functioning at a high level of clinical significance.

By combining biological age markers and cognitive performance measures, this thesis evaluates whether these domains can jointly represent SR and whether SR itself helps explain heterogeneity in healthcare utilization outcomes within a Canadian context.

3.0 Research Objectives and Hypotheses

The thesis has three major objectives:

1) To examine the factor structure of SR as a higher-order construct encompassing epigenetic age and cognitive function;

2) To examine sex and age moderators of these models; and

3) To compare factor- and indicator-level associations with health care utilization outcomes (ED visits and hospitalizations).

It is hypothesized that a higher-order structural model involving cognitive function and epigenetic aging as latent constructs under a superordinate SR construct will provide a better fit to the data compared to both a two-factor model which treats them as separate, correlated constructs, and a single-factor model which unifies them into one construct. It is expected that the optimal factor structure will hold for both men and women and middle-aged and older adults, but the strength of association may be stronger for women and older adults. Further, it is anticipated that there will be associations between these domains, at both the factor and indicator level, with healthcare utilization outcomes. Specifically, greater SR, younger epigenetic age, and better cognitive performance will be associated with fewer ED visits and hospitalizations.

4.0 Methods

4.1 Data Access and Ethics

The CLSA approved a data access application for this thesis project (# 2501025). The University of Waterloo's Office of Research Ethics also approved an ethics application for this project (# 47477). All CLSA participants provided informed consent prior to enrollment, and the study was conducted in a manner consistent with the declaration of Helsinki. Data were downloaded from CLSA servers to a secure server at the University of Waterloo. All institutional rules and regulations for data access and management were observed.

4.2 Sample

4.2.1 Data Source

The CLSA is a longitudinal, population-based, panel study that recruited 51,338 middle-aged and older adults in Canada from ages 45-85 years at enrollment, with follow-up every three years (122). The CLSA was established to explore the needs of the aging population and inform health policies and promote healthy aging practices. The CLSA collects physical, psychosocial, social, and environmental health data from its participants (123). The 51,338 participants were separately recruited into two cohorts differentiated by sample frames, as well as by data collection methods: (1) the "Tracking" Cohort, comprising 21,241 participants randomly selected from all ten provinces; and (2) the "Comprehensive" Cohort, comprising 30,097 participants randomly selected from within 25-50 kilometres of 11 data collection sites in seven provinces. Sampling was stratified by age group, sex, province, and data collection site, and later by education to increase representation from low education groups. Participants were recruited through four sampling frames: Statistics Canada's Canadian Community Health Survey-Healthy Aging (CCHS-HA) (Tracking Cohort only), the registries of provincial health care systems, the

Quebec Longitudinal Study on Nutrition and Aging (NuAge) (Comprehensive Cohort only), and random digit dialing (RDD) of landline telephones (122,124–126).

In the Tracking Cohort, data are collected via computer-assisted telephone interviews (122). In the Comprehensive Cohort, participants provide data through computer-assisted in-home interviews and via additional questionnaires, tests, physical measurements, and biological specimens at the data collection sites. Recruitment and collection of baseline data began in 2010 and was completed in 2015 (122,127).

Given the magnitude of the study and challenges in participant retention, a Maintaining Contact Questionnaire (MCQ) was administered by telephone to all participants approximately 18 months following baseline data collection by telephone, to maintain awareness of the CLSA and its importance to participants, to document changes in contact information, to initiate tracing mechanisms for participants who could not be contacted, and to gather additional health-related information, such as healthcare service utilization, physical activity, and nutritional risk (Comprehensive n=28,789, Tracking n=19,052) (128–130). This questionnaire was phased out after baseline and questions that were part of the MCQ were moved to the main questionnaires for follow-up 1.

Individuals who resided in the three territories, on federal First Nations reserves or settlements, who were full-time members of the Canadian Armed Forces, who were institutionalized, who could not speak English or French, who could not physically participate independently, and who showed obvious signs of cognitive impairment were excluded (122).

4.2.2. Analytic Sample

Of the 30,097 participants in the Comprehensive cohort, 27,120 participants provided blood samples at baseline (131). A visual depiction of the derivation of the sample is shown in Figure 1. All sample selections for epigenetic analysis were made to reflect the distribution of the Comprehensive Cohort in terms of age, sex, and data collection site (132). The Tracking Cohort was not included in this thesis because the CLSA did not collect epigenetic data from these participants.

Alphanumeric data collected at baseline from the CLSA Comprehensive Cohort were the sources of demographic and cognitive information, and the baseline epigenetic module from the Comprehensive Cohort was used for epigenetic data. The MCQ was used for healthcare utilization data, specifically ED visits and hospitalizations. Of the individuals with complete epigenetic data (n=1,478), 266 had missing data. Therefore, a complete case analysis (CCA) was conducted using the 1,212 individuals without missing data, and a sensitivity analysis was done for the full 1,478 participants using full-information maximum likelihood (FIML) to impute missing data. In the indicator-level analysis, a further subset of n=1,172 with complete data was used.

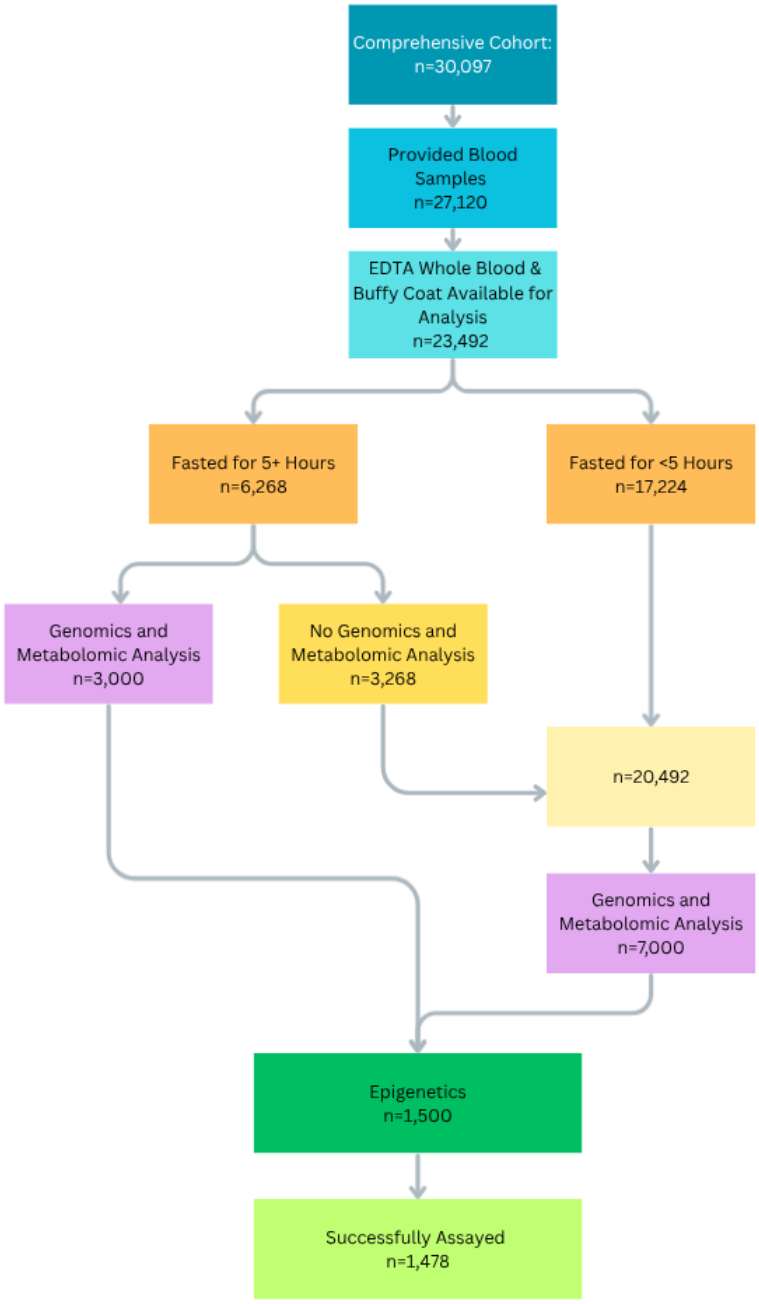


Figure 1: Derivation of the full epigenetic analytic sample (n=1,478) from the CLSA Comprehensive Cohort n=30,097)

4.3 Measures:

Measures included variables divided into cognitive, epigenetic, and health service utilization (ED visits and hospitalizations) variables. Details for each group of variables are provided below and in Tables A1 and A2 in Appendix A.

4.3.1 Cognitive Variables

There are eight cognitive tasks that the CLSA administers to participants in the Comprehensive Cohort that collectively assess various facets of cognitive ability, including memory, executive function, and psychomotor speed (122,133). Test administration is conducted in French or English, and includes modified versions of several neuropsychological tests (134). These tests include the modified Rey Auditory Verbal Learning Test immediate recall (REYI) and 5-minute delayed recall (REYII), the Mental Alternation Test (MAT), the Controlled Oral Word Association Test (FAS), the Animal Fluency Test (AFT), the Prospective Memory Test (PMT), the modified Victoria Stroop test (STP), and the Choice Reaction Time task (CRT) (134–144,144–149).

Raw scores on the REYI, FAS, MAT, and AFT at baseline were used as separate indicators of the cognitive function construct in the final models. These four tests and the process underlining their selection as indicators will be explained next. The modified REY immediate recall (REYI) and 5-minute delayed recall (REYII) present participants with 15 words to test both learning and retention (scores range from 0-15) (134,150). This test was modified to keep within the time constraints of the CLSA interviews and omitted an interference test and reduced the number of recall administrations from five to two (134). For immediate recall, participants listened to a recording of 15 words and were immediately asked to repeat as many as they could within 90 seconds (151). Five minutes later, in the delayed recall phase, they were required to

repeat as many words as possible within 60 seconds, without hearing the list again (151). The MAT measures processing speed and mental flexibility. Participants had 30 seconds to alternate between the numbers 1-26 and the matching letters of the alphabet, e.g., 1-A, 2-B, etc. The FAS assesses phonological fluency, requiring participants to name as many words as possible, beginning with a given letter (F, A, and S). Scoring on this test depends on the number of admissible words provided for each letter. The AFT measures verbal fluency, requiring participants to name as many animals as possible in 60 seconds, with scoring dependent on strict (only taxonomically distinct animals that differ at species level receive a point) or lenient criteria. Imaginary animals, the same animal at different life stages, variations, and repetitions are not scored. In this study, the strict criterion was used to provide a more conservative measurement of verbal fluency (134,152).

4.3.1.1 Cognitive Variables Excluded from Analysis

The CRT, STP, EBPM, TBPM, and REYII were excluded from the analyses described in Section 4.6 below. A description of these tests can be found in Appendix A. The CRT was removed due to concerns about the standardization of the testing procedures across data collection sites, such as where participants should place their hands on the touch screen, which could affect the reliability and validity of the data (153). The sensitivity of the touch screens also varied between sites. Preliminary analyses in the thesis found the STP had the lowest item-total correlation of all the cognitive tests (raw.r = .25, Std.r = .50) and was therefore dropped from analysis. Turning to the TBPM and EBPM, several transformations, including, logarithmic, square root, cube-root, and Yeo-Johnson, were undertaken during the assumption-checking phase of data analysis. The distributions of both tests' scores remained highly left-skewed, leading to their removal from the study. Finally, REYII was not included as an indicator of the

latent cognition construct because it caused suppression of factor loadings among the other indicators to below the selected minimum values of .50. Compared with the remaining indicators, which assess facets of functions ascribed to the frontal lobe, the REYII is likely to introduce demands that engage hippocampal function (154). This functional heterogeneity would have reduced the quality of the measure of the latent cognitive factor.

4.3.2 Epigenetic Variables

The CLSA provides six measures of epigenetic aging, calculated using the Horvath DNAmAge clock calculator: DNAmAge, Age Acceleration Difference (AAD), Age Acceleration Residual (AAR), Intrinsic EAA (IEAA), Extrinsic EAA (EEAA), and Hannum epigenetic age (132). Hannum age, EEAA, and AAR at baseline were used as separate indicators of the epigenetic age construct in the final models.

AAR is the residual from regressing DNAmAge onto chronological age, is typically used as the universal measure of EAA, and is expressed in years. EEAA represents an estimate of epigenetic age in years that is attributable to age-related changes in blood cell type composition and is more relevant to aging of the immune system. Finally, Hannum epigenetic age is calculated using the Hannum clock in years (132).

4.3.2.1 Epigenetic Variables Excluded from Analysis

AAD, DNAmAge, and IEAA were ultimately excluded from the analyses described in Section 4.6 below. A description of these variables can be found in Appendix A. AAD was removed because it was calculated as the difference between DNAmAge and chronological age, making it redundant to keep in the model. When examining initial correlation matrices, IEAA and AAR were found to have a very high correlation *(r* = .92). Because AAR is typically used as the universal measure of age acceleration, IEAA was removed from the model (132). DNAmAge

was removed from the final model because it resulted in instability in factor loadings in the CFA (a factor loading over 1.00, suggesting that over 100% of variation in the indicator can be explained by the latent construct, which is not possible) and non-convergence of the model.

4.3.3 Healthcare Utilization Outcome Variables

Healthcare utilization variables included ED visits and hospitalizations, modelled as categorical variables (yes/no in the last 12 months). These outcomes were selected because they represent clinically relevant indicators of acute healthcare utilization and health system burden in aging populations (155–157). Rates of ED visits and hospitalizations increase substantially with age due to greater prevalence of multimorbidity, functional decline, and complex care needs (27). Older adults account for a disproportionately high number of ED visits and hospitalizations, and these rates continue to increase alongside population aging (157). For example, in Canada from 2023-2024, individuals over 65 years of age comprised 25% of all ED visits, despite only representing 19% of the population (158). Similarly, in 2024, older adults constituted approximately half of the hospital inpatient population in Canada (159). These trends have important implications for healthcare expenditures; in 2023, older adults accounted for approximately 47% of Canadian healthcare spending, with this proportion increasing steadily (160).

Identifying predictors of ED visits and hospitalization is therefore a major priority in aging and healthcare research, particularly for informing prevention strategies and resource allocation. Analyzing these outcome variables can provide evidence to inform health policy. These variables were also measured ~18 months post-baseline in the MCQ, as shown in the figure below, allowing for a prospective design and temporality between baseline predictors and subsequent healthcare service utilization.

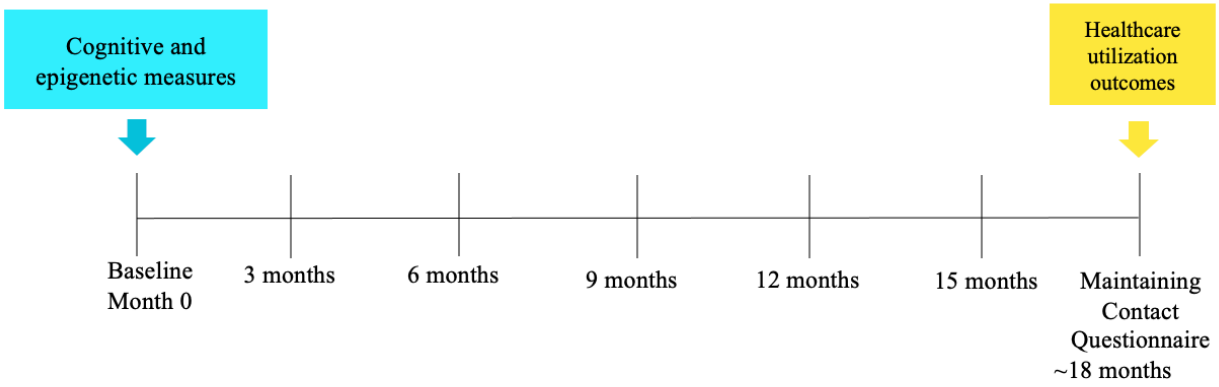


Figure 2: Timeline for data collection of baseline epigenetic, cognitive, and healthcare utilization variables

4.3.4 Moderators

Sex and age at baseline were used as moderators of the association between epigenetic age and cognitive function. Sex at birth was reported as male or female and age was grouped into middle aged (45-64 years) and older adults (aged 65 years or over) to examine differences between middle-aged and older adults.

4.4 DNA Methylation Analysis

The method used for DNAm profiling in the CLSA is explained in detail elsewhere (132). However, to summarize, the CLSA includes DNAm data across 865,918 total genomic sites (862,927 CpG probes, 2,932 CHH probes, and 59 SNP probes) from peripheral blood mononuclear cell (PBMC) samples. DNA was extracted from frozen PBMC samples using a QIA symphony workstation and then bisulfite conversions were carried out using Zymo EZ DNAm kits. From there, DNAm was measured using the Illumina Infinium MethylationEPIC BeadChip microarrays by scanning using Illumina Iscan Control Software. Finally, the raw

DNAm values were transformed into beta values in GenomeStudio, falling within a range of 0-1, representing methylation percentage (132).

4.5 Covariates

Covariates were selected based on previous literature involving studies examining associations between epigenetic age and cognition (14,26,112,115,116,161,162). Covariates were separated into two general categories: demographic/socioeconomic factors and health status. Although these covariates may change with time, only the baseline values were used in the analysis.

4.5.1 Demographic/Socioeconomic Variables

Demographic/socioeconomic factors included province, total household income, education, and language of administration (French or English). Total household income and education were included as proxies of socioeconomic status, which are known to be associated with cognitive performance and biological aging (145,163). Total household income was categorized based on total annual household income: less than $20,000, from $20,000 to under $50,000, from $50,000 to under $100,000, from $100,000 to under $150,000, and $150000 or more. Education was measured by category: less than high school, high school diploma, some post-secondary education, and post-secondary diploma. Language of administration was included because previous studies indicated that language can affect cognitive test scores independent of actual cognitive capability (105, 121). Following CLSA recommendations, province and education were also included as covariates to account for the study's stratified recruitment (164).

4.5.2 Health Status

The CLSA evaluated chronic conditions using self-reports. Participants were asked to report whether a doctor ever told them they had any of the following conditions, including cardiovascular problems, neurological problems, gastrointestinal problems, vision problems, cancer, diabetes, osteoporosis, osteoarthritis, hyperthyroidism, hypothyroidism, mood disorders, among others. Number of chronic conditions was treated as a continuous variable for the purposes of this study. The variable was included because it reflects accumulated physiological stress and disease exposure across systems, which can influence both epigenetic aging, cognition, and other health outcomes (29,38,39,102,103).

4.6 Data Analysis

4.6.1 Descriptive Analysis

Baseline descriptive statistics were computed to summarize sample characteristics for the analytic sample of 1,478, as well as the CCA sample of 1,212 individuals. Distributions for continuous variables (e.g., epigenetic age and cognitive test scores), were assessed using histograms and QQ plots and presented using means and standard deviations if normally distributed, or medians and interquartile ranges if non-normally distributed. Categorical variables were summarized with frequencies and percentages. To depict the general pattern of associations among variables, a correlation matrix for variables included in the final analysis was computed using Pearson correlations for continuous variables, tetrachoric correlations for dichotomous variables, and biserial correlations for continuous-dichotomous pairs (along with heatmaps).

4.6.2 Assumptions

As a prelude to conducting the confirmatory factor analyses (CFAs) (see Section 4.6.3 below), various assumptions were tested. First, normality of all indicators was examined using QQ-plots and histograms, along with skewness (>2) and kurtosis (>7). Variables that were not normally distributed were transformed using square-root, cube-root, logarithmic, and Yeo-Johnson transformations. Outliers were reviewed using Mahalanobis distance; CFA models were run without outliers to assess whether outlier removal would affect model fit (167,168). If the fit indices after removal of outliers were not improved, then outliers were retained, as observations are not recommended to be discarded without strong evidence that they resulted from errors or other anomalies (169).

Next, correlations between indicators within each construct (cognitive function and epigenetic age) were examined to assess for potential multicollinearity and redundancy. If the correlation between two indicators was high (> 0.9), one was selectively removed from the model and the other retained, based on theoretical relevance. This helped to reduce redundancy among indicators, as including indicators that are highly correlated with one another can lead to inflation of standard errors, instability in the results, or analysis failure due to linear dependence (168).

To check for reliability, Cronbach's alpha was generated to ensure all indicators consistently measured their respective underlying constructs. A value of 0.70 or higher was considered acceptable (170). Item-total correlations were also examined, to measure strength of association between an indicator and the overall factor score. Item-total correlations of ≥ 0.30 were considered acceptable (171). Any indicators with lower item-total correlations were removed.

4.6.3 Confirmatory Factor Analysis

CFA is used to test whether observed variables (indicators), load onto hypothesized latent constructs. Each latent construct represents an underlying concept or characteristic that cannot be directly measured but is inferred from the shared variance among its constituent variables (e.g., the cognitive test scores). CFA was used to evaluate whether the epigenetic age and cognitive function indicators were best represented as two separate and related constructs, as components of a higher-order SR construct, or as a single unified construct. In the models, epigenetic age was modeled as a latent construct composed of AAR, EEAA, and Hannum epigenetic age, and cognitive function was modeled as a latent construct composed of REYI, FAS, AFT, and MAT. All variables were standardized using z-scores ($\mu=0$; $\sigma=1$) and CFAs for cognitive function and epigenetics were first run separately prior to constructing the larger models.

When running the models, the lambda (λ) of the indicator with the highest factor loading was set to 1.00 as is recommended, as latent variables do not have an inherent scale (172). This approach sets the scale of the latent variable based on a reference indicator, allowing all other loadings to be freely and stably estimated relative to this reference.

Although the CLSA has derived variables available, such as variables providing combined scores for different mixes of individual cognitive tests, these variables were not used in this study (173). The purpose of this study was to examine the factor structure of SR and its associated variables, which includes how each variable loads onto the latent constructs. Therefore, to gain a better understanding of the exact structure of each construct, each cognitive test variable was kept in its natural form for inclusion in the CFA.

Three competing models were tested to evaluate how epigenetic age and cognitive function are best represented. Each model tested a different hypothesis about the relationship

between cognitive function, epigenetic age, and SR. In the first model, the two domains were specified as separate but related constructs, allowing their association to be freely estimated. This suggests that cognitive function and epigenetic age are considered distinct domains that contribute separately to health outcomes, rather than through an underlying common pathway. In the second model, both domains were specified as lower-order factors reflecting a single higher-order construct of SR, meaning that cognitive function and epigenetic age are distinct, but their shared variance can be explained by an overarching construct (SR). In the third model, all indicators loaded directly onto a single latent SR construct, implying that cognitive function and epigenetic age represent one unified dimension without separate cognitive and epigenetic domains.

A comparison between the fit of the three models was undertaken, with consideration for parsimony. Indicators that were found to demonstrate weak loading ($\lambda < 0.50$) onto the latent constructs were removed from the model to improve model fit. Main analyses were conducted using the `lavaan`, `semTools`, `semPlot`, `tidySEM`, and `psych` packages in R version 4.5.2 (The R Foundation for Statistical Computing, Vienna, Austria) (174–179). The hypothetical models can be viewed in Figures 2, 3, and 4 below.

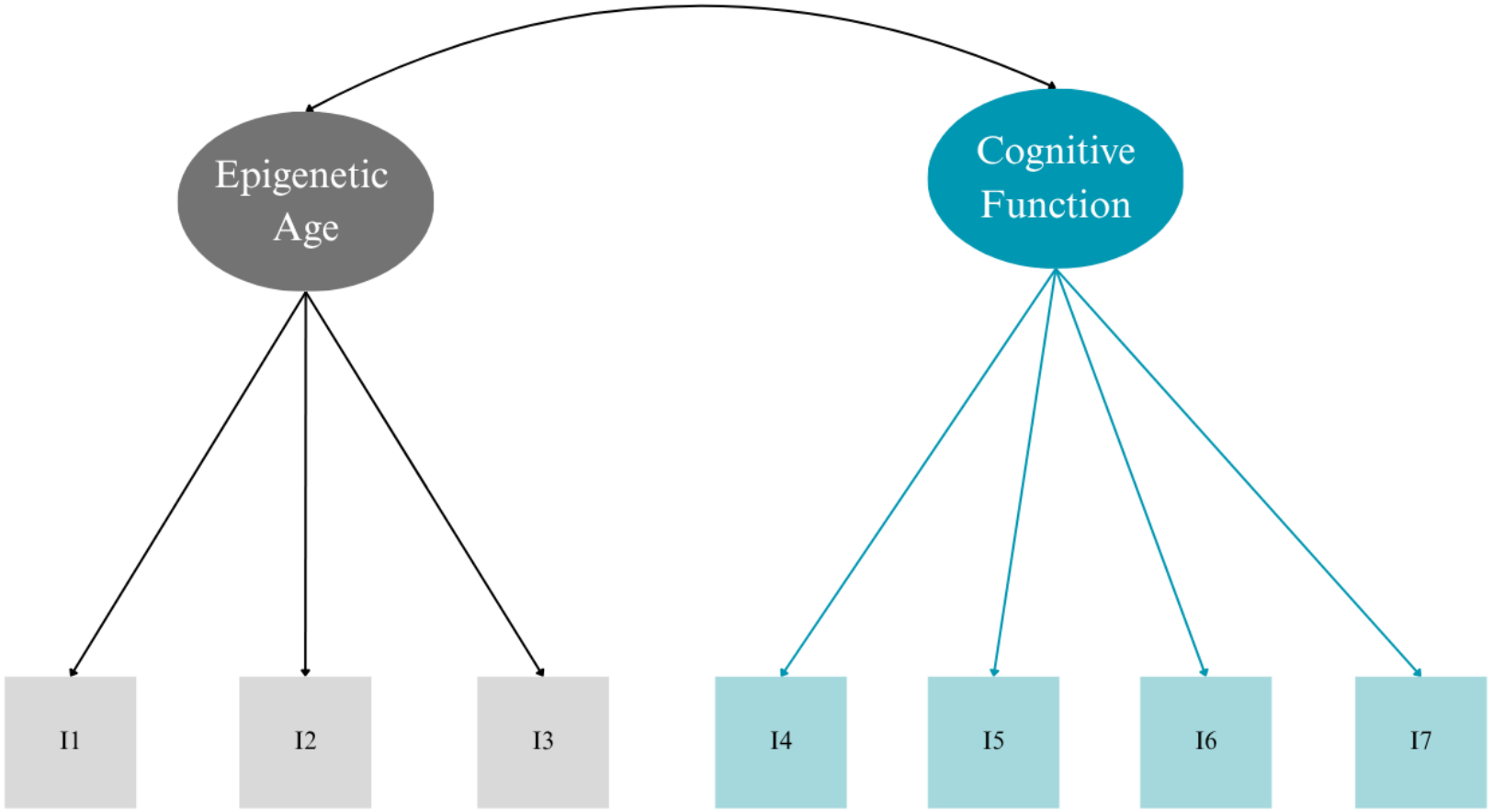


Figure 3: Hypothesized two-factor correlated model with epigenetic age and cognitive function as inter-correlated but distinct latent constructs

Note: I=indicator. Solid arrows from the latent construct to indicators represent measurement paths; curved double-headed arrow represents the correlation between latent constructs.

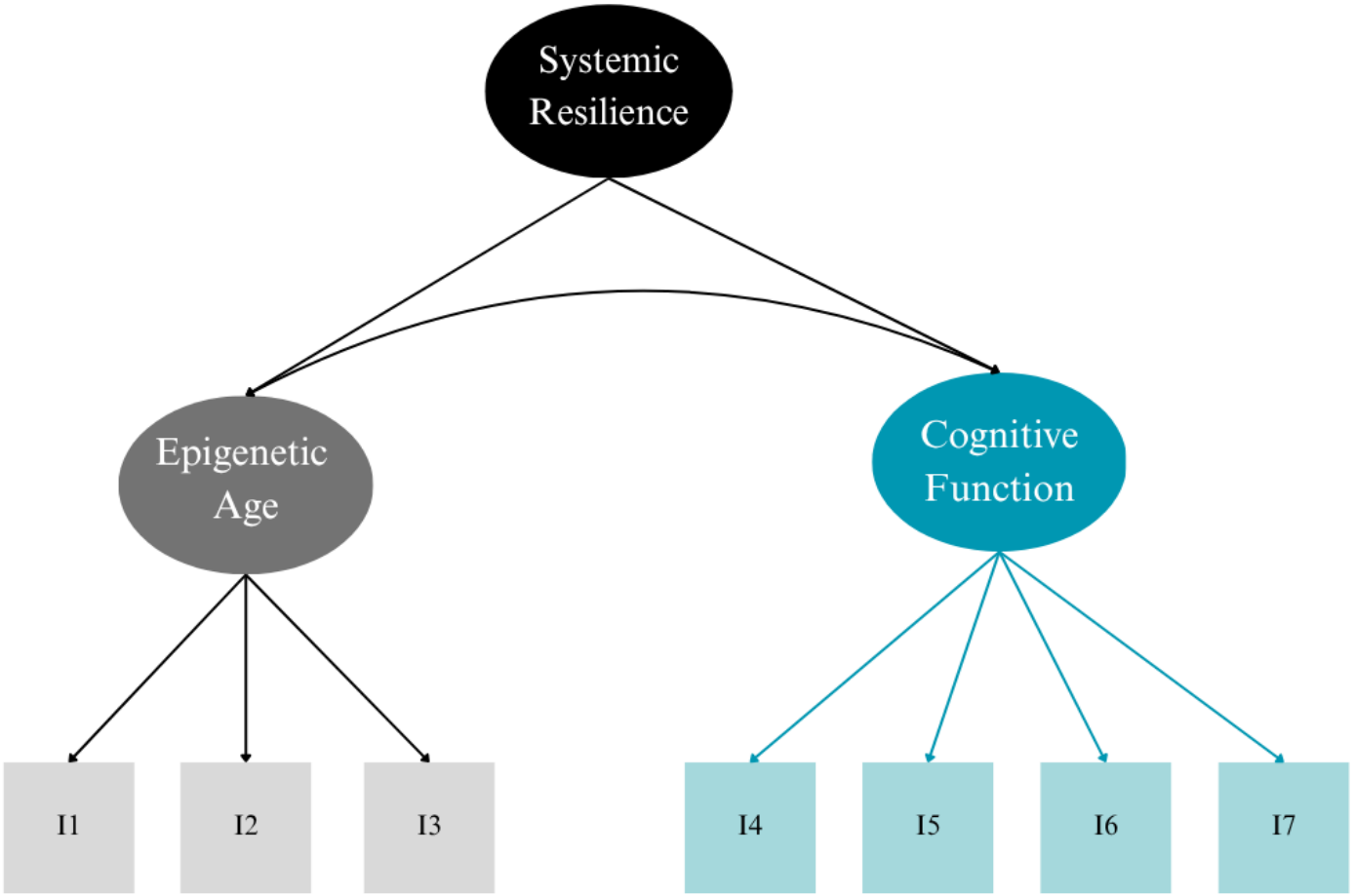


Figure 4: Hypothesized higher-order hypothetical model with a superordinate factor representing SR

Note: I=indicator. Solid arrows from the latent construct to indicators represent measurement paths; curved double-headed arrow represents the correlation between latent constructs.

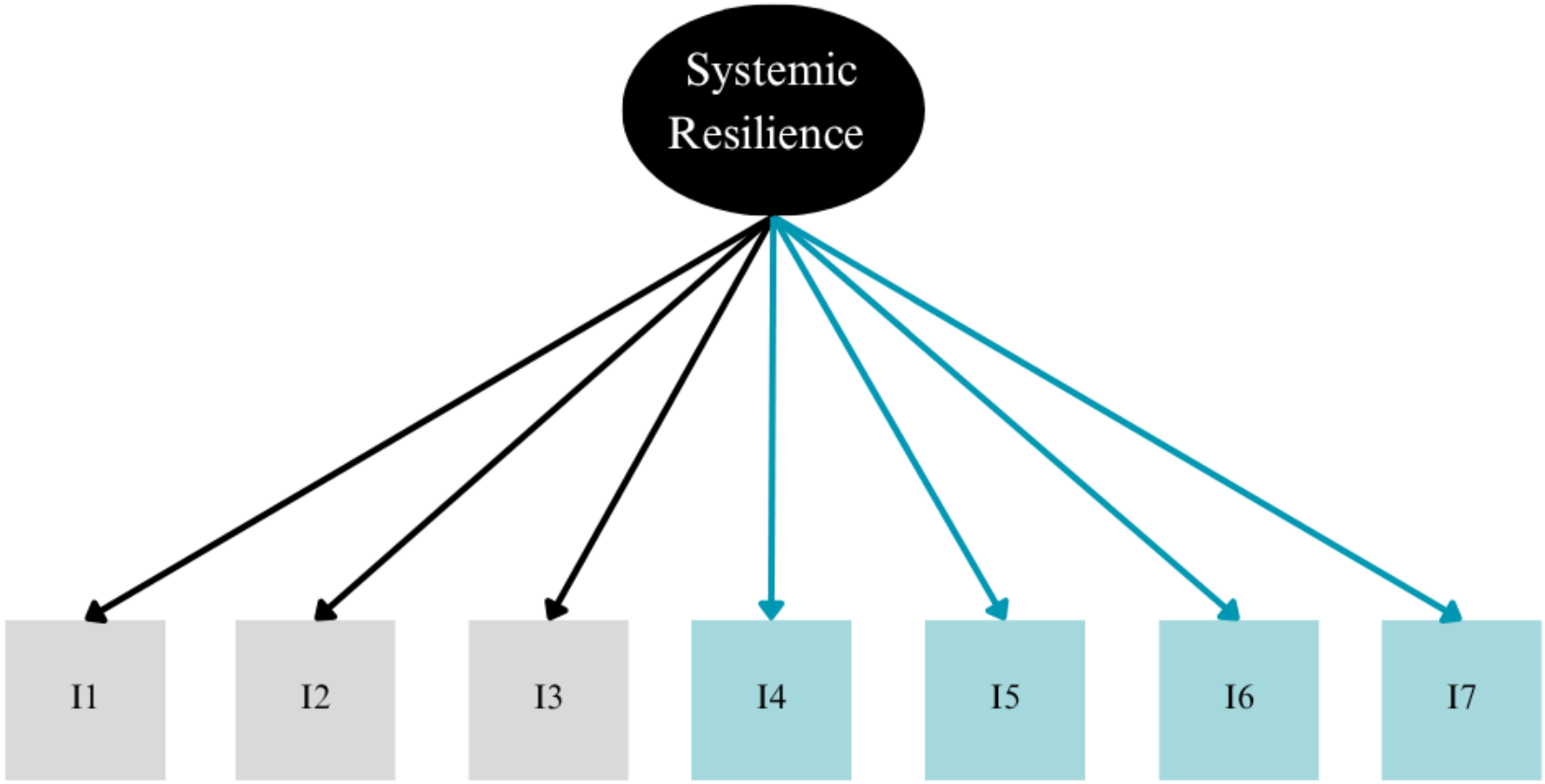


Figure 5: Hypothesized single-factor model with indicators directly loading onto SR

Note: I=indicator. Solid arrows from the latent construct to indicators represent measurement paths.

Models were estimated using robust maximum likelihood (MLR) as a conservative approach to account for potential deviations from normality (e.g., skewness and other non-normality in the data), which is frequently appropriate for some cognitive and biological variables (180,181). Goodness of fit was assessed using multiple indices: the root mean square error of approximation (RMSEA) (values ≤ 0.08 is good), typically reported with a 90% confidence interval (CI); the comparative fit index (CFI) (≥ 0.90 is good); the standardized root mean squared residual (< 0.08 is good); the Akaike information criterion (AIC), and the Bayesian information criterion (BIC) (lower AIC and BIC values indicate better model fit) (182,183). A difference of 10 in AIC and BIC provides strong evidence in favor of the lower-scoring model, meaning that competing models with a $\Delta>10$ fail to offer additional explanatory power to justify their added complexity (180,181). Robust or scaled values for the fit indices were reported when available.

4.6.4 Sensitivity Analyses

4.6.4.1 Full-Information Maximum Likelihood of Missing Data for Measurement Models

Full information maximum likelihood (FIML) is commonly used when using MLR. FIML operates under the assumption that data are missing at random (MAR) and utilizes all available information by estimating model parameters directly from incomplete data without direct imputation of missing values (185,186). It does so by computing a likelihood function for each individual based on their observed values, with the overall likelihood obtained by summing the individual log-likelihoods across all cases (187).

A model using FIML with the full sample was run and results were compared to results from the main analysis using CCA to assess the potential impact of missing data on study results.

4.7 Validation using SEM

To further explore the construct validity of the best fitting model, the predictive validity was examined. The best fitting model was used as a predictor of healthcare utilization, which included ED visits and hospitalization. Both of these outcomes were measured at ~18 months post-baseline, meaning that this analysis was effectively prospective in nature, allowing the establishment of some degree of temporality between baseline predictors and subsequent healthcare service utilization.

In the structural model, ED visits and hospitalizations were regressed onto the latent constructs specified in the final measurement model, which varied depending on whether a two-factor, higher-order, or single-factor structure was retained. The corresponding structural models based on each measurement specification are shown in Figures 5, 6, and 7 below.

Rather than using MLR for the SEM analysis, the weighted least square mean and variance (WLSMV) estimator was used, as it is a robust estimator most appropriate for data that involve categorical variables. WLSMV estimates associations with an underlying tendency or propensity of an outcome rather than the observed outcome directly (188). Due to the WLSMV's limited ability to deal with missingness, the CCA sample of 1,212 individuals who have complete data on all the variables of interest was used in this analysis. The sample of 1,212 meets the recommended minimum sample size of 200-500 for SEM using WLSMV (189). Province, income, education, language, and comorbidity counts were used as covariates and specified with paths to each latent construct and the outcomes. Three alternative models were examined to determine how to operationalize income and education, given that these variables may not satisfy linear or interval-scaling assumptions: a composite score, separate continuous variables, and dummy-coded categorical variables. The final specification was selected based on

model fit indices and interpretability and was used in all subsequent analyses. Province was dichotomized as high-population (BC, ON, and QB) versus low-population (all others). Moderation by sex and age were examined after measurement invariance (configural, metric, and scalar) was tested, which evaluates whether constructs are measured equivalently across groups of the moderators (190). If measurement invariance fails, then group differences in structural effects might not reflect true moderation. From there, moderation analyses were performed by examining an unconstrained model versus a constrained model, where regression paths were forced to be equal across age or sex groups to examine whether fit changed depending on group. A worsening of fit would indicate that there was moderation, while no evidence of worsening would indicate that effects were similar across groups. Equal group sizes are ideal for multi-group analysis, and although there are no strict sample size requirements, a minimum that has been noted is 100 cases per group, with larger samples recommended for more complex models (172). Fit indices were reported for all models, and for structural paths, unstandardized regression coefficients (b) with 95% CIs (CI_b) were reported for assessing statistical significance, while standardized regression coefficients (β) were reported to facilitate interpretation of effect sizes (168).

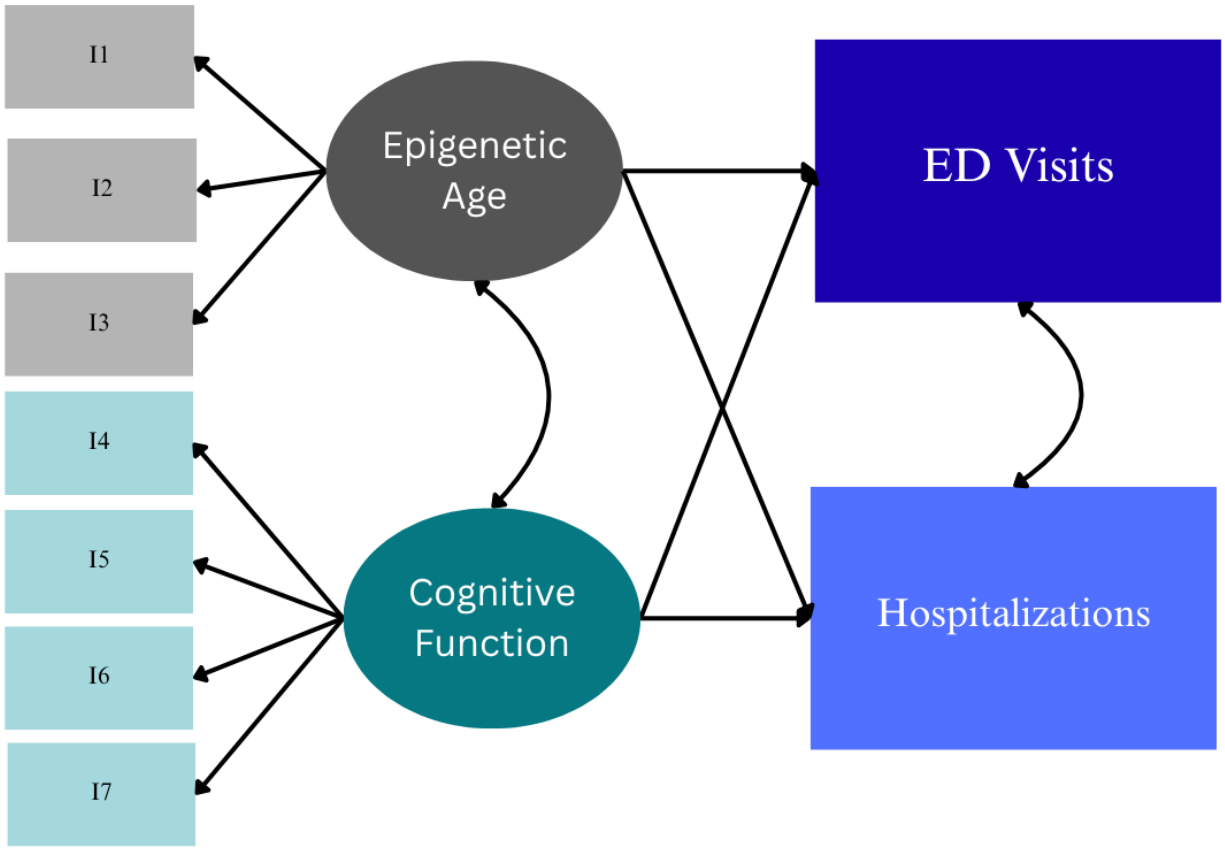


Figure 6: Hypothesized validation model for higher-order model

Note: I=indicator; ED=emergency department. Solid arrows from the latent construct to indicators represent measurement paths; curved double-headed arrows represent correlations; solid arrows from the latent construct to outcomes and other latent constructs represent structural paths.

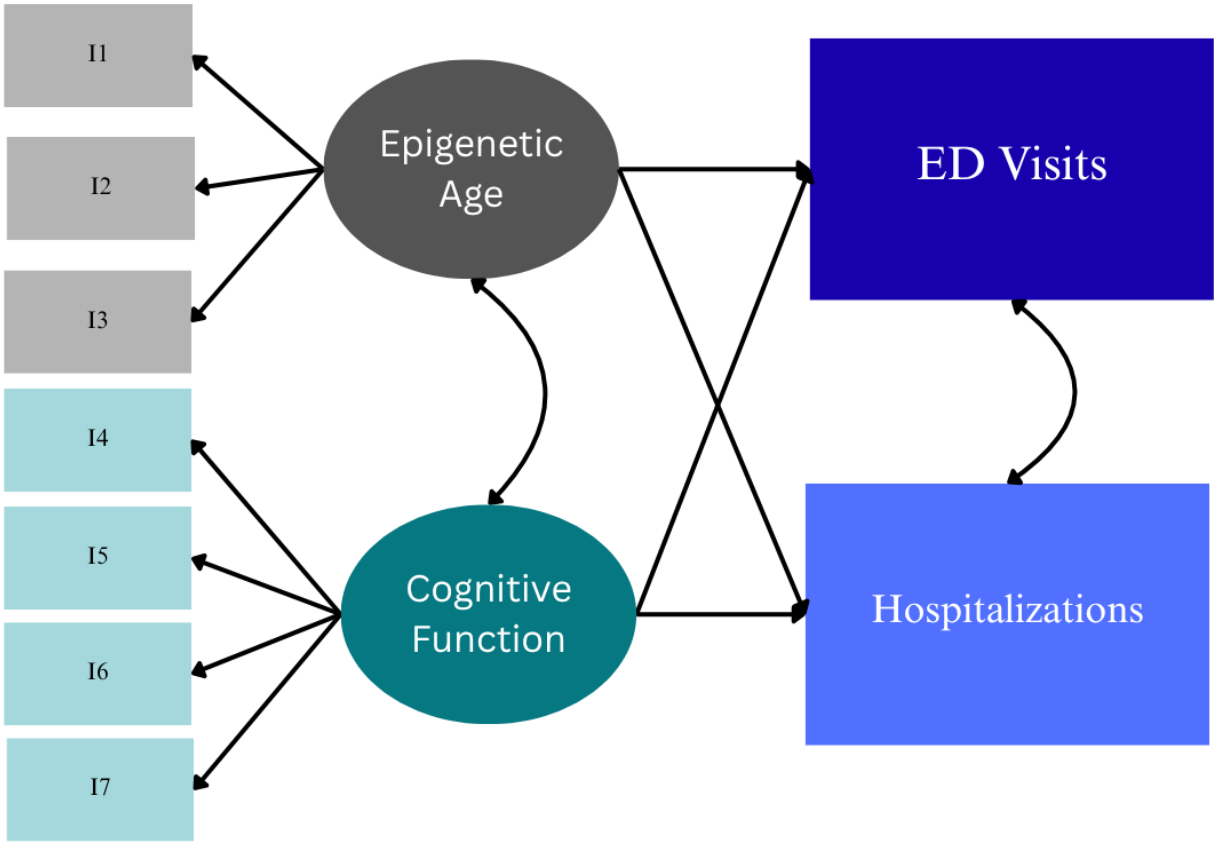


Figure 7: Hypothesized validation model for two-factor model

Note: I=indicator; ED=emergency department. Solid arrows from the latent construct to indicators represent measurement paths; curved double-headed arrows represent correlations; solid arrows from the latent construct to outcomes represent structural paths.

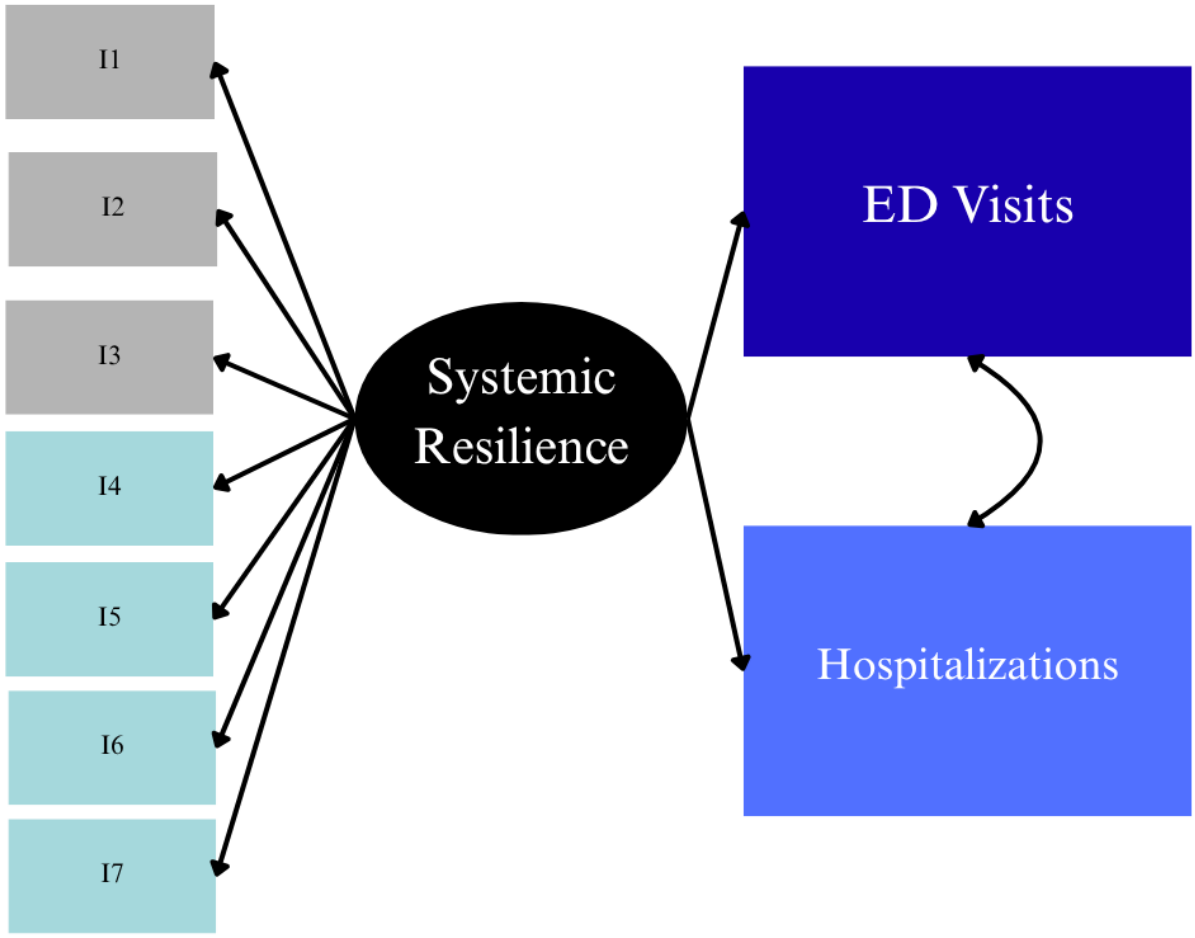


Figure 8: Hypothesized validation model for single-factor model

Note: I=indicator; ED=emergency department. Solid arrows from the latent construct to indicators represent measurement paths; curved double-headed arrows represent correlations; solid arrows from the latent construct to outcomes represent structural paths.

4.8 Indicator-Level Analysis

Additional analyses were run using logistic regression to further examine associations between epigenetic and cognitive indicators (individually) and health service utilization, while probing for potential interactions with age. These analyses were performed to complement the results from the SEM by assessing whether associations observed at the latent construct level were consistent at the level of the individual indicators, including indicators that were not used in the final models to investigate their associations with outcomes. Additionally, a correlation matrix and heatmap involving all epigenetic and cognitive indicators was generated following methods previously described in Section 4.6.1. This approach also allowed for clearer interpretation of specific predictor-outcome relationships and the examination of interactions between predictors with age, as moderation by age could not be reliably assessed in the SEM models due to measurement invariance limitations.

Separate models were run for each outcome indicator (i.e., ED visits and hospitalizations). Assumptions such as linearity for predictors were examined prior to running analyses by testing a quadratic term for each predictor in the logistic regression model. All other assumptions were met, including independent observations. Continuous predictors and covariates were standardized using z-scores ($\mu=0$; $\sigma=1$) prior to analysis. Binary covariates were retained in their original form. Sex was included as a covariate. For each indicator, an interaction model including the indicator, age, their interaction (indicator x age), and covariates was first estimated. If the interaction with age was not significant, age was included as a covariate. All models were estimated using the binomial distribution with a logit link function. Results were reported as odds ratios (Ors) and 95% CIs. The sample used involved individuals with complete data on all epigenetic and cognitive variables, outcomes, and covariates (n=1,172).

5.0 Results

5.1 Descriptive Analysis

Table 1 shows the sample characteristics of the 1,478 participants with complete epigenetic data at baseline in the FIML sample and the sample characteristics of the 1,212 participants included in the CCA sample. In the CCA sample, participants aged 55-64 years comprised the largest proportion of the sample (32.9%), while those aged 75+ years comprised the least (17.7%). The number of males and females was similar, with slightly more females than males (50.1%: 49.9%). English was the predominant language of administration (80.4%), and most participants came from Ontario, Quebec, or British Columbia (63.9%). More than three-quarters of participants had a post-secondary degree/diploma and over half had an annual total household income between $20,000 and $100,000 (58.2%). The majority of participants were married or living with a partner (64.4%) and over half of participants reported having three or more chronic conditions. Most participants reported regular alcohol consumption (73%), and the majority did not smoke within the past 30 days (60.2%). In the FIML sample, results were mostly similar, except for the proportion of males to females, where there were slightly more males than females (51%: 49%).

Table 1: Characteristics of FIML sample and CCA sample

Variable	n = 1,478 (FIML)	n = 1,212 (CCA)
Sex		
Male	747 (51%)	605 (49.9%)
Female	731 (49%)	607 (50.1%)
Age		
45-54	376 (25%)	316 (26.1%)
55-64	479 (32%)	399 (32.9%)
65-74	360 (24%)	290 (23.9%)
75+	263 (18%)	207 (17.7%)
Language of administration		
English	1,201 (81%)	974 (80.4%)
French	277 (19%)	238 (19.6%)
Education		
Less than high school	84 (5.7%)	65 (5.4%)
High school diploma	145 (9.8%)	108 (8.9%)
Some post-secondary education	143 (9.7%)	107 (8.8%)
Post-secondary degree/diploma	1,106 (75%)	932 (76.9%)
Province		
Alberta	144 (9.7%)	96 (7.9%)
British Columbia	310 (21%)	269 (22.2%)
Manitoba	156 (11%)	127 (10.5%)
Newfoundland and Labrador	112 (7.6%)	91 (7.5%)
Nova Scotia	152 (10%)	123 (10.1%)
Ontario	309 (21%)	255 (21.0%)
Quebec	295 (20%)	251 (20.7%)
Total annual household income		
Less than $20,000	94 (6.4%)	78 (6.4%)
From $20,000 to under $50,000	364 (24.6%)	314 (25.9%)
From $50,000 to under $100,000	458 (31%)	392 (32.3%)
From $100,000 to under	248 (17%)	220 (18.2%)
$150,000 $150,000 or more	235 (16.8%)	208 (17.2%)
Missing	79 (5.3%)	NA
Marital status		
Single	149 (10%)	127 (10.5%)
Married/common-law	941 (64%)	780 (64.4%)
Widowed	147 (9.9%)	102 (8.4%)
Divorced	180 (12%)	157 (10.5%)
Separated	61 (4.1%)	46 (3.8%)
Chronic conditions		
0	156 (10.6%)	131 (10.8%)
1	258 (17.5%)	219 (18.1%)
2	256 (17.3%)	213 (17.6%)
3+	808 (54.7%)	649 (53.5%)

Smoking status		
Non-user	868 (84%)	730 (60.2%)
Occasional user	27 (2.6%)	23 (1.9%)
Daily user	140 (14%)	105 (8.7%)
Missing	443 (23%)	354 (30.1%)
Alcohol consumption		
Non-drinker	184 (13%)	140 (11.6%)
Occasional drinker	199 (14%)	162 (13.4%)
Regular drinker	1,058 (73%)	885 (73.0%)
Missing	37 (2.4%)	25 (2.0%)

Note: Data are presented as n (%). FIML= full information maximum likelihood; CCA= complete case analysis.

5.1.1. Epigenetic Age

Epigenetic age measures were approximately normally distributed in both samples. Descriptive statistics for epigenetic variables used in the final model are reported in Table 2 below.

Table 2: Epigenetic age of FIML sample and CCA sample

Epigenetic Age	**n = 1,478 (FIML)**	**n = 1,212 (CCA)**
Hannum Age	63.25 ± 12.32	62.91 ± 12.23
Age Acceleration Residual	0.00 ± 4.46	-.05 ± 4.47
Extrinsic Epigenetic Age Acceleration	0.00 ± 6.87	-.04 ± 6.87

Note: Data are presented as mean ± standard deviation (SD). FIML= full information maximum likelihood; CCA= complete case analysis

5.1.2. Cognitive Function

Table 3 below presents baseline performance on cognitive tests included in the final model. Due to skewness in several measures, medians and interquartile ranges are also reported.

Table 3: Cognitive function raw scores and ranges of FIML sample and CCA sample

Cognitive Function Variables	FIML Mean ± SD	FIML Range	CCA Mean ± SD	CCA Range
Controlled Oral Word Association Test	38.75 ± 12.78	6–83	39.3 ± 12.61	6–83
Rey Auditory Verbal Learning Test 1	5.78 ± 1.94	0–13	5.84 ± 1.93	0–13
Mental Alternation Test	26.3 ± 8.71	0–51	26.7 ± 8.65	0–51
Animal Fluency Test	19.43 ± 5.87	4–40	19.78 ± 5.82	5–40

Note: FIML= full information maximum likelihood; CCA= complete case analysis; SD= standard deviation; IQR= interquartile range

5.1.3 Correlation Matrix

All cognitive measures were significantly associated with Hannum Age, but not the other epigenetic indicators. ED visits were significantly associated with most cognitive tests (MAT, REYI, FAS) and hospitalization was significantly associated with all cognitive measures and Hannum age, although all these associations were more modest. The heatmap can be seen below.

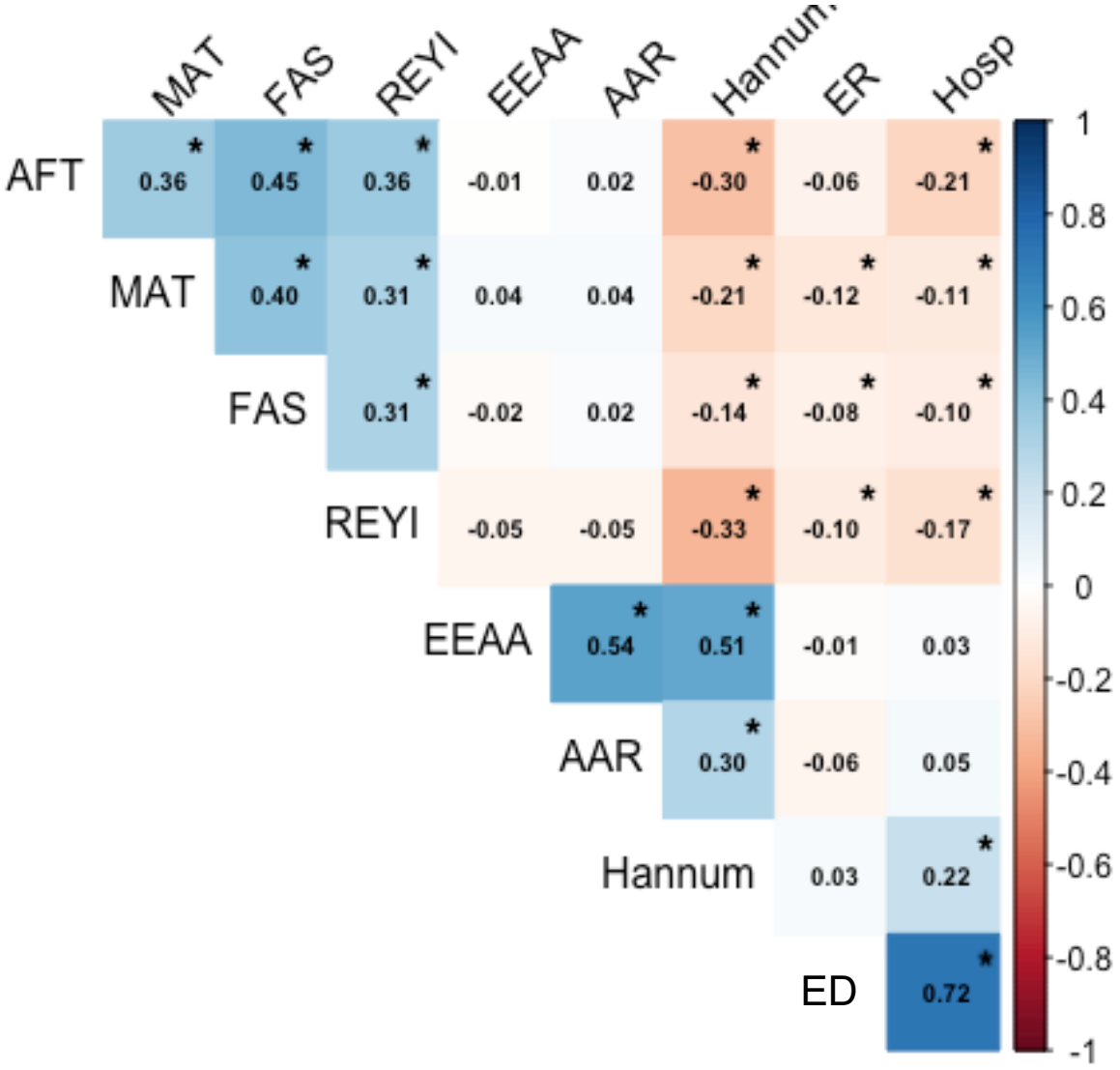


Figure 9: Hybrid heatmap for correlations between variables with Pearson correlations for continuous variables, tetrachoric correlations for dichotomous variables, and biserial correlations for continuous-dichotomous pairs

Note: Significant correlations marked with an asterisk. AFT=animal fluency test; MAT=mental alternation test; FAS=controlled oral word association test; REYI=Rey auditory verbal learning test trial 1; EEAA=extrinsic epigenetic age acceleration; AAR=age acceleration residual; ED=emergency department visits.

5.2 Assumptions and Initial Screening

All final epigenetic variables were found to be normally distributed. No significant skewness or kurtosis was indicated, as shown in Tables B1 and B2 in Appendix B. For the final cognitive variables, all were also normally distributed. Outliers were not determined to be a major concern as model fits were not improved after excluding them, and correlation matrices did not indicate the possibility of multicollinearity (see Appendix C for model fit indices with outliers removed). Cronbach's alpha for epigenetic and cognitive variables were acceptable, as were item-total correlations (these can be viewed in Appendix D). For epigenetics, the raw Cronbach's value was .78, while the standardized value was .80. For cognitive function, the raw Cronbach's value was .61, and the standardized value was .71.

5.3 CFA

The final epigenetics construct was indicated by AAR, EEAA, and Hannum age, and the cognitive function construct by REYI, MAT, AFT and FAS. Each CFA was initially run independently and the models for epigenetics and cognitive function are presented below.

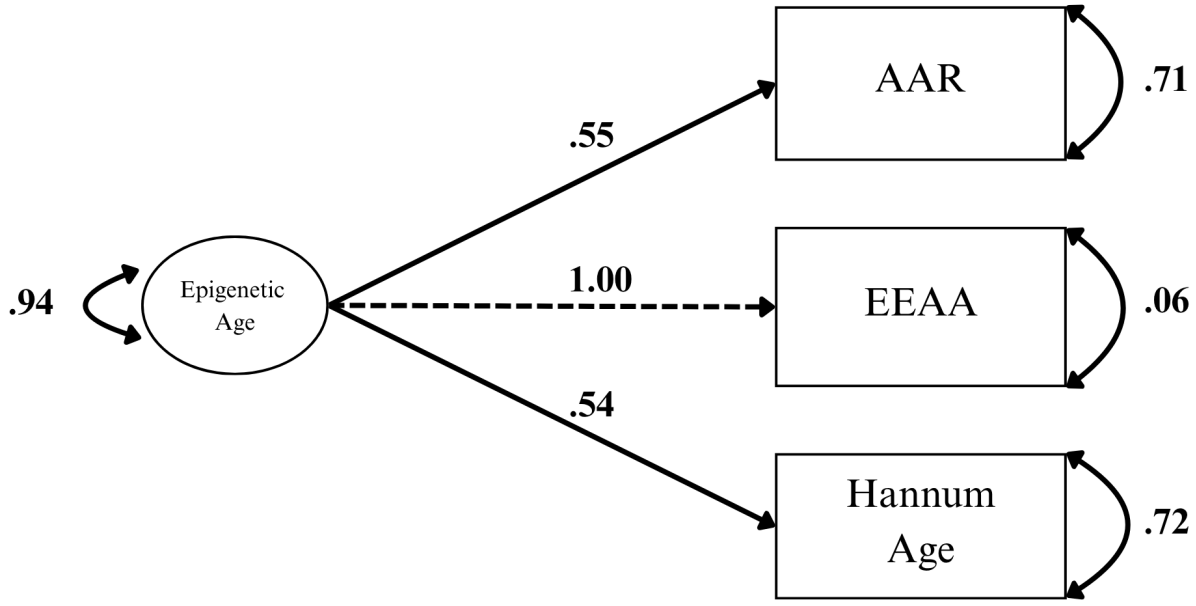


Figure 10: Epigenetics single construct CFA with final indicators

Note: AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration. Solid arrows from the latent construct to indicators represent measurement paths; dashed arrow represents measurement path fixed to 1.00; self-looping double-headed arrows represent variances.

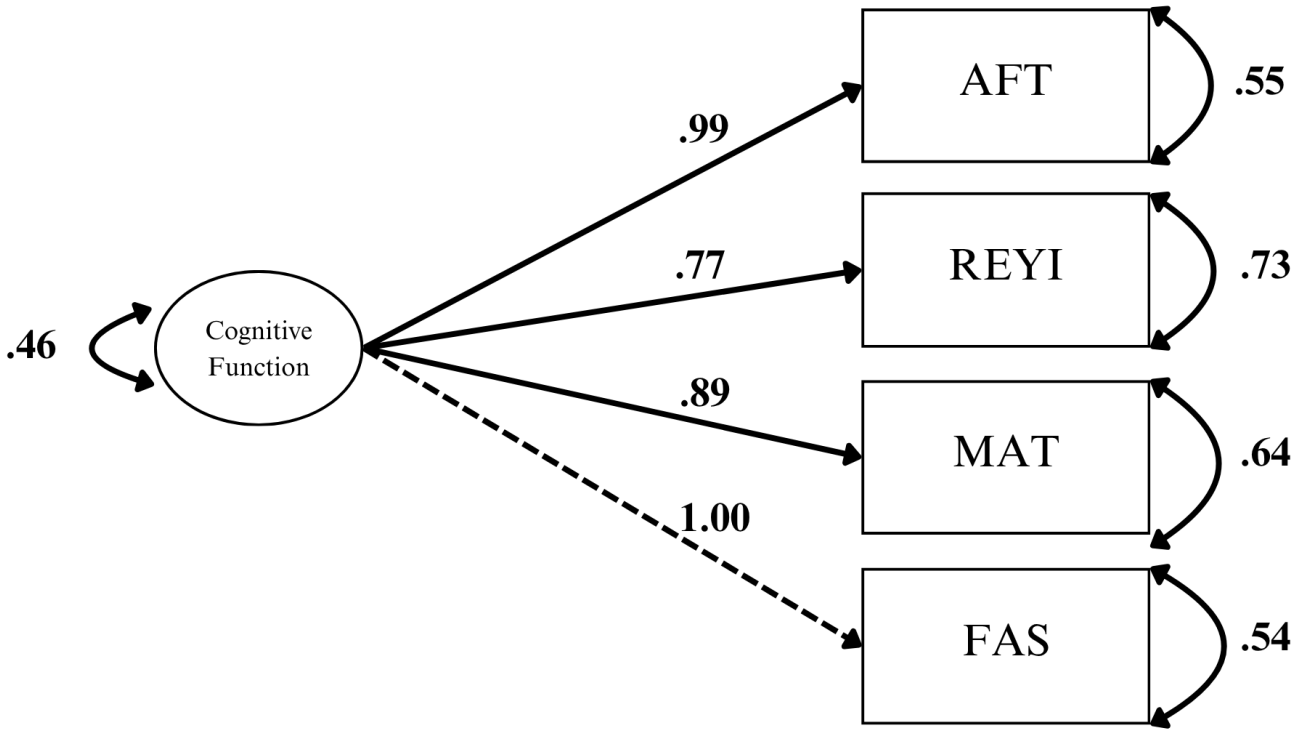


Figure 11: Cognition single construct CFA with final indicators

Note: AFT=animal fluency test; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test. Solid arrows from the latent construct to indicators represent measurement paths; dashed arrow represents measurement path fixed to 1.00; self-looping double-headed arrows represent variances.

These models were then merged into a two-factor model, a higher-order model, and a single factor model. These models are shown below.

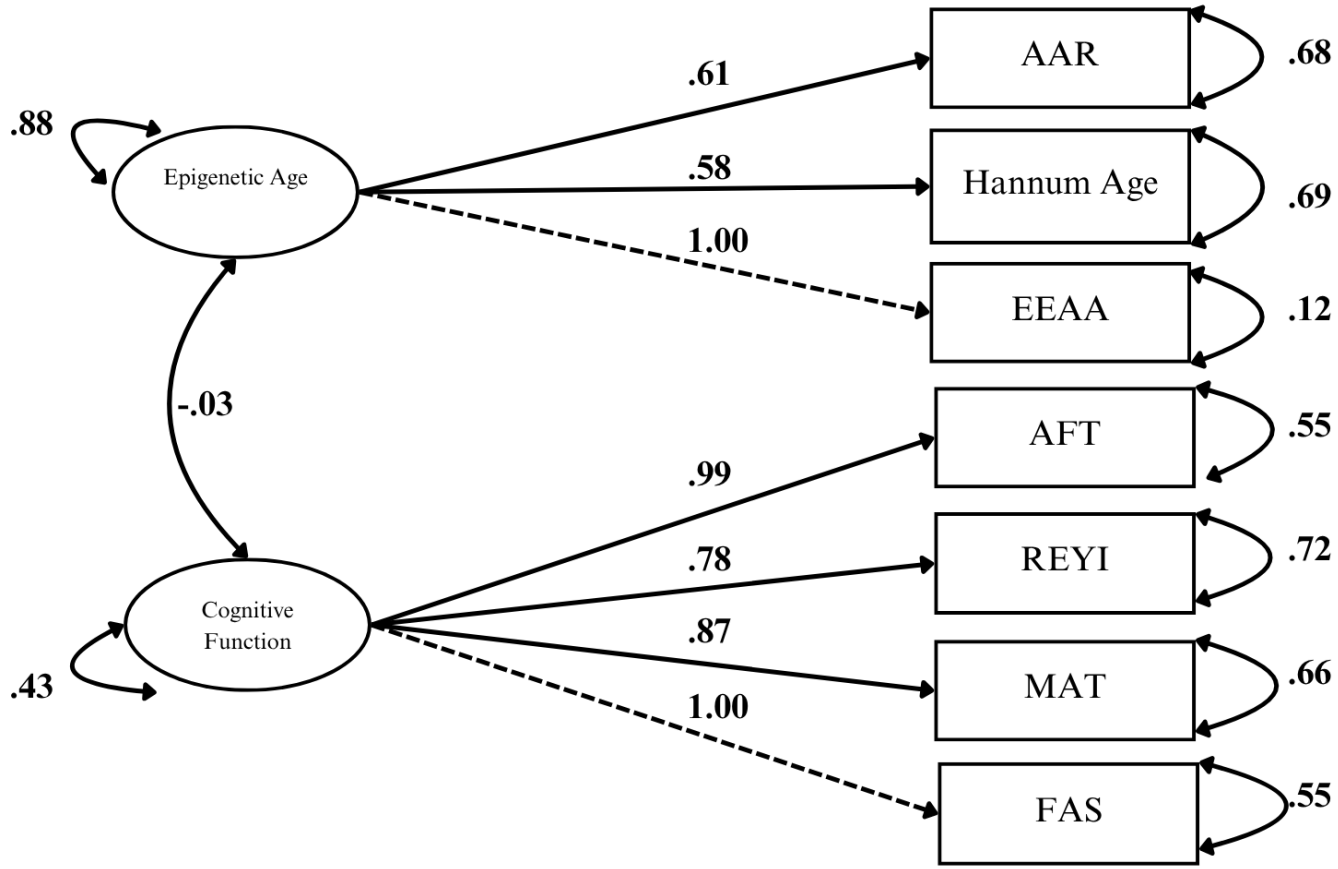


Figure 12: Two-factor CFA model for CCA sample

Note: Fit Indices: Scaled CFI= .839, Scaled RMSEA (90% CI) = 0.135 (0.121, 0.148), SRMR= 0.084, AIC=22454.67, BIC= 22566.87. CCA=complete case analysis; AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test. Solid arrows from the latent construct to indicators represent measurement paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances; curved double-headed arrow represents correlations.

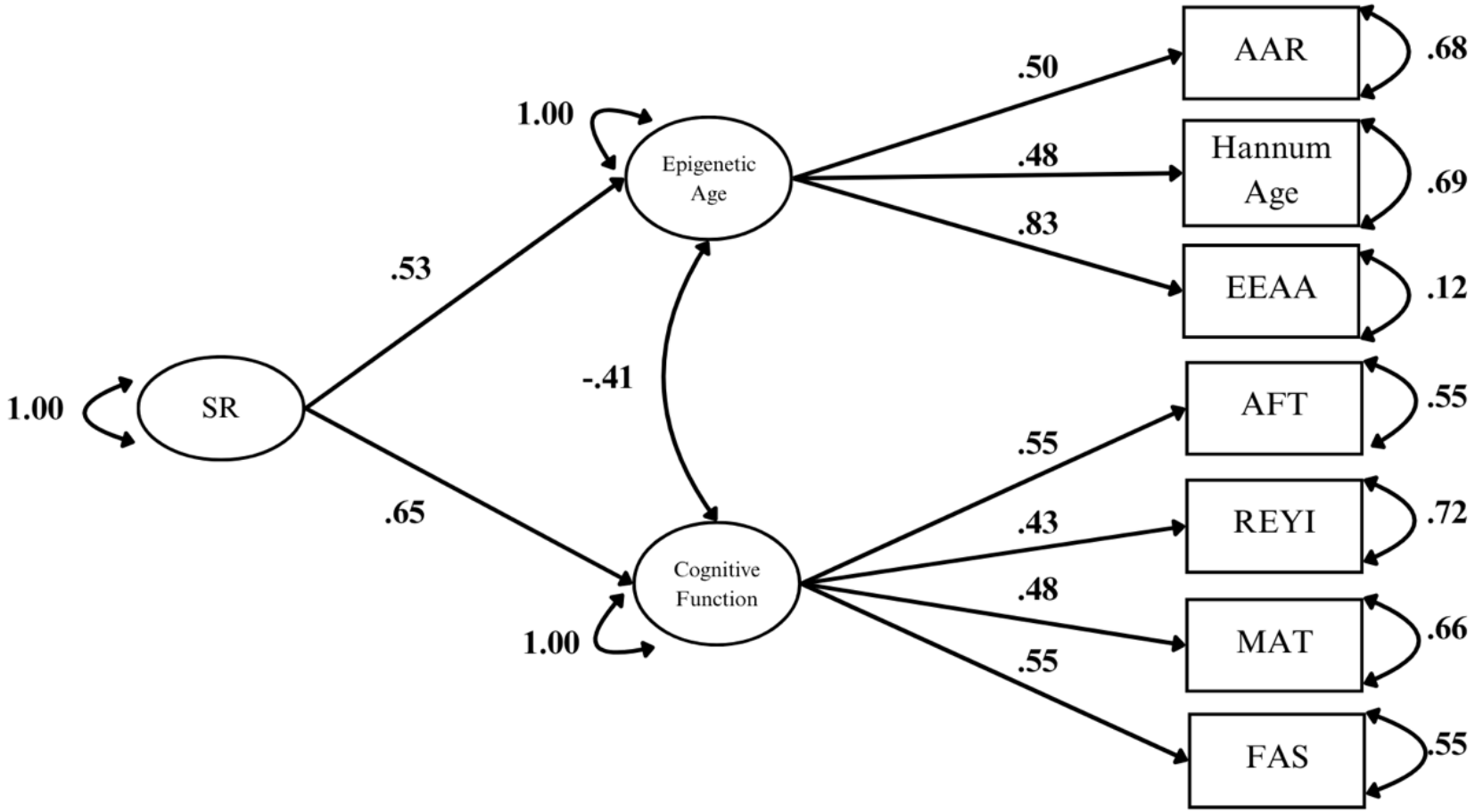


Figure 13: Higher-order CFA model for CCA sample

Note: Fit Indices: Scaled CFI= .864, Scaled RMSEA (90% CI) = 0.135 (0.121, 0.148), SRMR= 0.084, AIC=22458.67, BIC= 22581.07. CCA=complete case analysis; AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test; SR=systemic resilience. Solid arrows from the latent construct to indicators represent measurement paths; solid arrows between latent constructs represent structural paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances; curved double-headed arrow represents correlations.

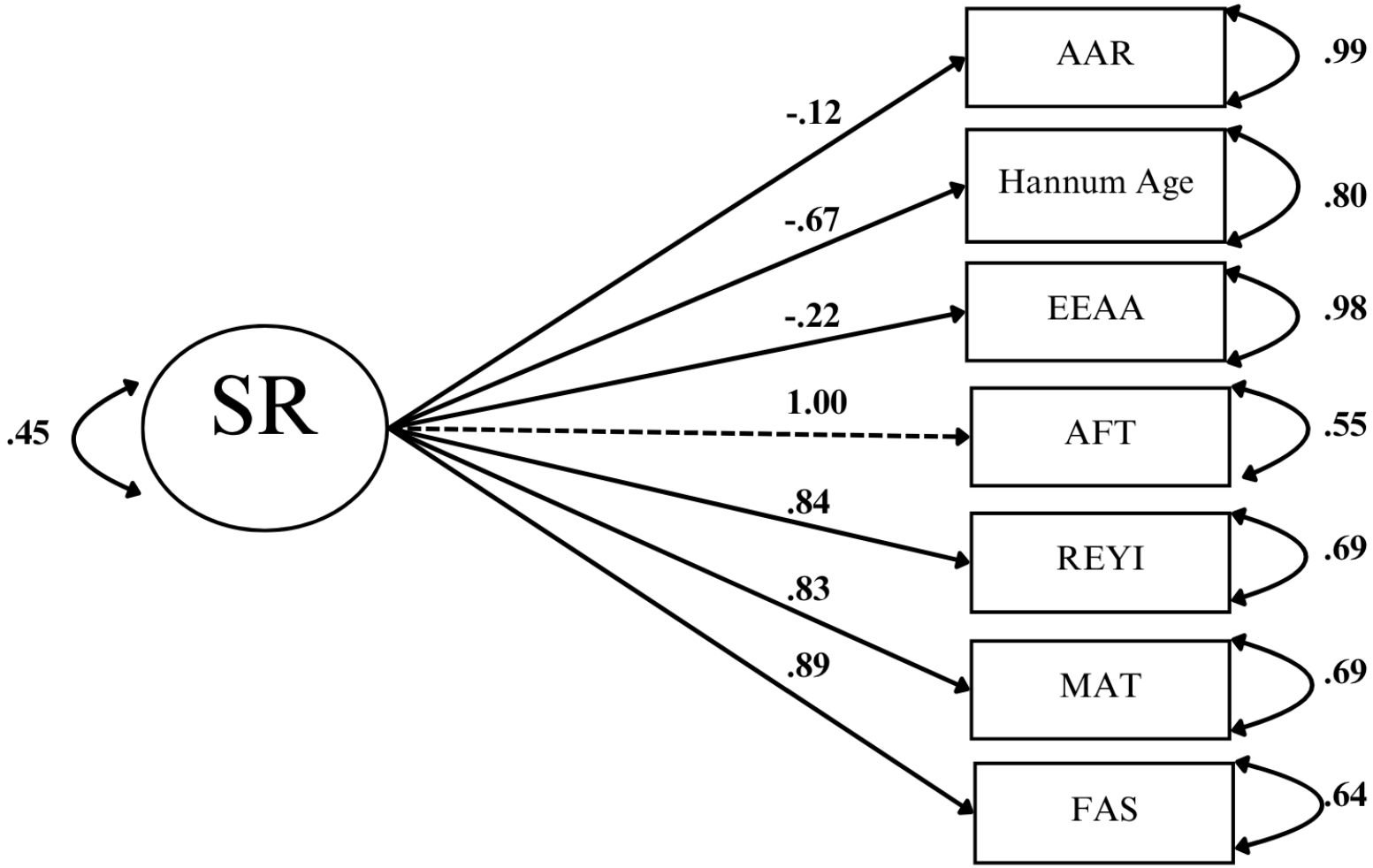


Figure 14: Single-factor CFA model for CCA sample

Note: Fit Indices: Scaled CFI= .411, Scaled RMSEA (90% CI) = 0.248 (0.234, 0.261), SRMR= 0.133, AIC=23079.49, BIC= 23186.59. CCA=complete case analysis; AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test; SR=systemic resilience. Solid arrows from the latent construct to indicators represent measurement paths; dashed arrow represents measurement path fixed to 1.00; self-looping double-headed arrows represent variances.

These models meet the requirement of having at least three indicators per latent construct, as well as the recommended sample size guidelines for CFA (191,192). A common rule of thumb suggests a ratio of approximately 20 observations per estimated model parameter (168). In the two-factor correlated model, there are 8 parameters; in the higher-order model, there are 10; and in the single-construct model, there are 7 parameters. Given that the sample size for the analysis is 1,212 participants, this comfortably exceeds the recommended minimum sample size for all models.

5.3.1 Model Fit

Based on the fit indices above, overall model fit was acceptable, but sub-optimal across some specifications. For example, CFI values for all models were below commonly accepted thresholds (>0.90). However, SRMR values for the higher-order and two-factor models were within commonly accepted thresholds (0.084 for both). Looking at the relative fit indices, AIC and BIC were lower for the two-factor model, with the BIC difference exceeding 10, indicating strong support for the two-factor structure over the higher-order model. Likewise, when considering parsimony, the two-factor model was preferred as it provided comparable fit to the higher-order model without adding another factor. Therefore, the two-factor model was retained as the best-performing model among those tested and was used in subsequent analyses.

5.3.2 Sensitivity Analyses

To examine the impact of missingness and the use of CCA, the three measurement models were re-estimated using FIML, with 1,478 individuals. The models from this FIML are presented in Appendix E. A summary table that displays model fit indices for these models compared to fit indices for the CCA model is shown below.

Table 4: Fit indices for FIML and CCA higher-order, two-factor, and single-factor models

Model	CFI	RMSEA (90% CI)	SRMR	AIC	BIC
Complete-Case Analysis (CCA)					
Higher-order	.864	0.135 (0.121, 0.148)	0.084	22458.67	22581.07
Two-factor	.839	0.135 (0.121, 0.148)	0.084	22454.67	22566.87
Single-factor	.411	0.248 (0.234, 0.261)	0.133	23079.49	23186.59
Full Information Maximum Likelihood (FIML)					
Higher-order	.872	0.129 (0.117, 0.141)	0.084	26909.23	27036.39
Two-factor	.848	0.129 (0.117, 0.141)	0.084	26905.23	27021.79
Single-factor	.455	0.235 (0.223, 0.247)	0.131	27646.09	27757.36

Note: CFI = comparative fit index; RMSEA = root mean square error of approximation (90% CI in parentheses); SRMR = standardised root mean square residual; AIC = Akaike information criterion; BIC = Bayesian information criterion; CCA = complete-case analysis; FIML= full-information maximum likelihood; CI= confidence interval

Consistent with the CCA models, model fit was equivocal, as some fit indices remained acceptable, but sub-optimal across other specifications. For instance, CFI values were again below commonly accepted thresholds (<0.90 for all) while SRMR values for the high-order and two-factor models were within commonly accepted thresholds (0.084 for both). Therefore, although the CFI was slightly higher for the higher-order model, it was still below threshold. Looking at relative fit indices, AIC and BIC were still lower for the two-factor model, indicating superior fit. The BIC difference exceeded 10 in the FIML models as well, indicating strong support for the two-factor structure over the higher-order model. When considering parsimony, the two-factor model was preferred over the higher-order model because it yielded similar fit indices with less complexity. These findings support the use of the two-factor structure, as results were consistent across both CCA and FIML samples.

5.4 Validation Study

The simplified version of the final model from the validation study can be seen below and includes adjustment for covariates (chronic condition load, education, income, province, and

language). This model includes income and education coded as dummy variables, as doing so demonstrated the most favourable model fit indices relative to alternative specifications. Other models tested are provided in Appendix F, along with their fit indices. A full version of the model with all covariates can be found in Appendix F as well.

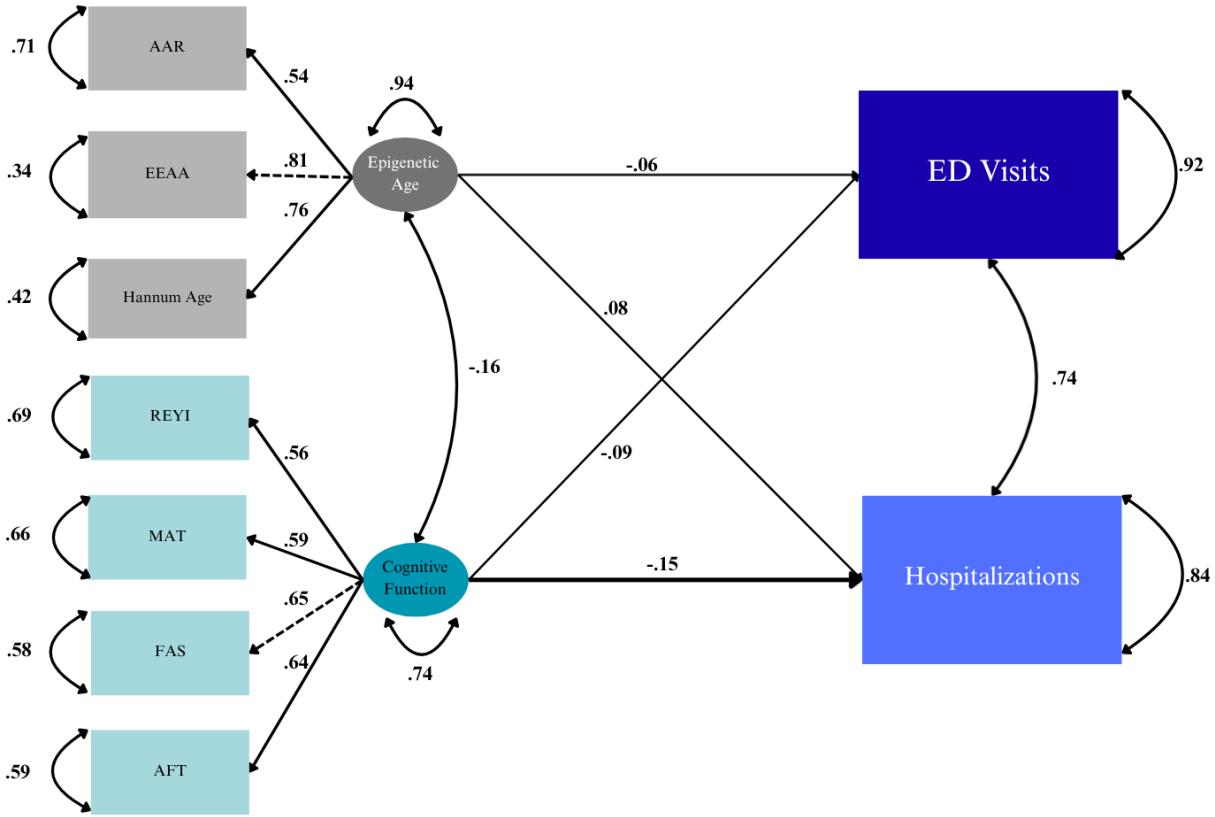


Figure 15: Validation model adjusted for covariates (education, income, chronic condition load, province, and language)

Note: Significant pathways bolded. AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test. Solid arrows from the latent construct to indicators represent measurement paths; solid arrows between latent constructs and outcomes represent structural paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances; curved double-headed arrows represent correlations.

The overall fit of this model is acceptable for most indices (CFI=.816; RMSEA= 0.055, 90% CI= 0.050, 0.061) and very good for others (SRMR=0.05). Table 5 provides factor loadings and correlations between constructs.

Table 5: Factor loadings and correlations between constructs in the final validation model

Indicator	**Standardized Factor Loadings**	
	Epigenetic Age	Cognitive Function
Age Acceleration Residual	.54	-
Extrinsic Epigenetic Age Acceleration	.81	-
Hannum Age	.76	-
Rey Auditory Verbal Learning Test 1	-	.56
Mental Alternation Test	-	.59
Controlled Oral Word Association Test	-	.65
Animal Fluency Test	-	.64
Factor correlations		
Cognitive Function	**-.16**	

Note: Significant correlation is bolded.

All indicators loaded significantly onto their respective latent constructs in the expected directions ($p < 0.001$). For epigenetic age, moderate to strong standardized factor loadings were observed (.54 to .81). The greatest factor loadings for epigenetics were for Hannum Age ($\lambda = .76$) and EEAA ($\lambda = .81$), indicating that these indicators contribute most strongly to the latent construct. For cognitive function, all indicators loaded moderately onto the latent construct (.56 to .65). The latent correlation between epigenetic age and cognitive function was negative ($r = -.16$), suggesting that higher epigenetic age and faster acceleration is associated with slightly lower cognitive function. This association was significant ($p < 0.05$).

Table 6 provides regression coefficients and 95% CIs for pathways from the latent constructs to the outcomes. A table with full regression outputs can be found in Appendix G.

Table 6: Regression path coefficients for validation model with 95% CIs

Outcomes and predictors	Regression coefficient estimate (b)	95% CI_b	Standardized coefficient (β)
ED Visits			
Epigenetic Age	-0.081	(-0.198, 0.036)	-.064
Cognitive Function	-0.144	(-0.326, 0.037)	-.091
Hospitalizations			
Epigenetic Age	0.099	(-0.051, 0.249)	.076
Cognitive Function	**-0.254**	**(-0.461, -0.046)**	**-.154**

Note: Significant pathways are bolded. CI= confidence interval; ED=emergency department visits.

For ED visits, neither epigenetic age (95% CI_b= -0.198, 0.036) nor cognitive function (95% CI_b= -0.326, 0.037) were found to be significant predictors, indicating that within this model and this sample, there was no evidence that these constructs were associated with ED visit frequency. In contrast, cognitive function was a significant predictor of hospitalizations (95% CI= -0.461, -0.046). The standardized coefficient was β= -.154, meaning that a one standard deviation increase in cognitive function was associated with a .154 standard deviation decrease in the underlying latent propensity of hospitalizations. In other words, higher cognitive function (i.e., higher scores on cognitive tests) was slightly associated with fewer hospitalizations.

5.4.1 Moderation Analyses

Full regression outputs can be seen in Appendix H.

5.4.1.1 Moderation by Sex

Measurement invariance across sex was assessed using multi-group analysis. The number of males and females was similar, with 605 males and 607 females. Fit indices for the configural model, metric, scalar, and the constrained models are presented in Table 7 below.

Table 7: Fit indices for configural, metric, scalar, and constrained models

Model	CFI	RMSEA	90% CI RMSEA	SRMR
Configural	.820	0.054	(0.047, 0.060)	0.057
Metric	.820	0.053	(0.047, 0.059)	0.056
Scalar	.825	0.051	(0.045, 0.057)	0.056
Constrained	.805	0.048	(0.042, 0.053)	0.057

Note: CFI= comparative fit index; RMSEA= root mean square error of approximation; CI= confidence interval; SRMR= standardized root mean squared residual

Measurement invariance testing indicated that the proposed factor structure operated similarly across males and females. Constraining factor loadings to equality (metric invariance) did not significantly reduce model fit ($\Delta\chi^2$=3.95, p=0.624), supporting metric invariance. Similarly, constraining item intercepts (scalar invariance) also did not significantly reduce model fit ($\Delta\chi^2$=6.46, p=0.264), providing support for scalar invariance. These results indicate that the measurement model operates equivalently across groups, and thus moderation by age can be examined.

Following establishment of measurement invariance, a multi-group structural model was estimated in which all regression paths were constrained to be equal across groups. Comparison of this model with the freely estimated configural model revealed a significant deterioration in model fit ($\Delta\chi^2$=104.94, p = < 0.001), suggesting that constraining structural paths greatly reduced model fit. This provides evidence of potential differences in structural relationships between males and females. Moderation results are further detailed in Table 8 and Figures 15-16.

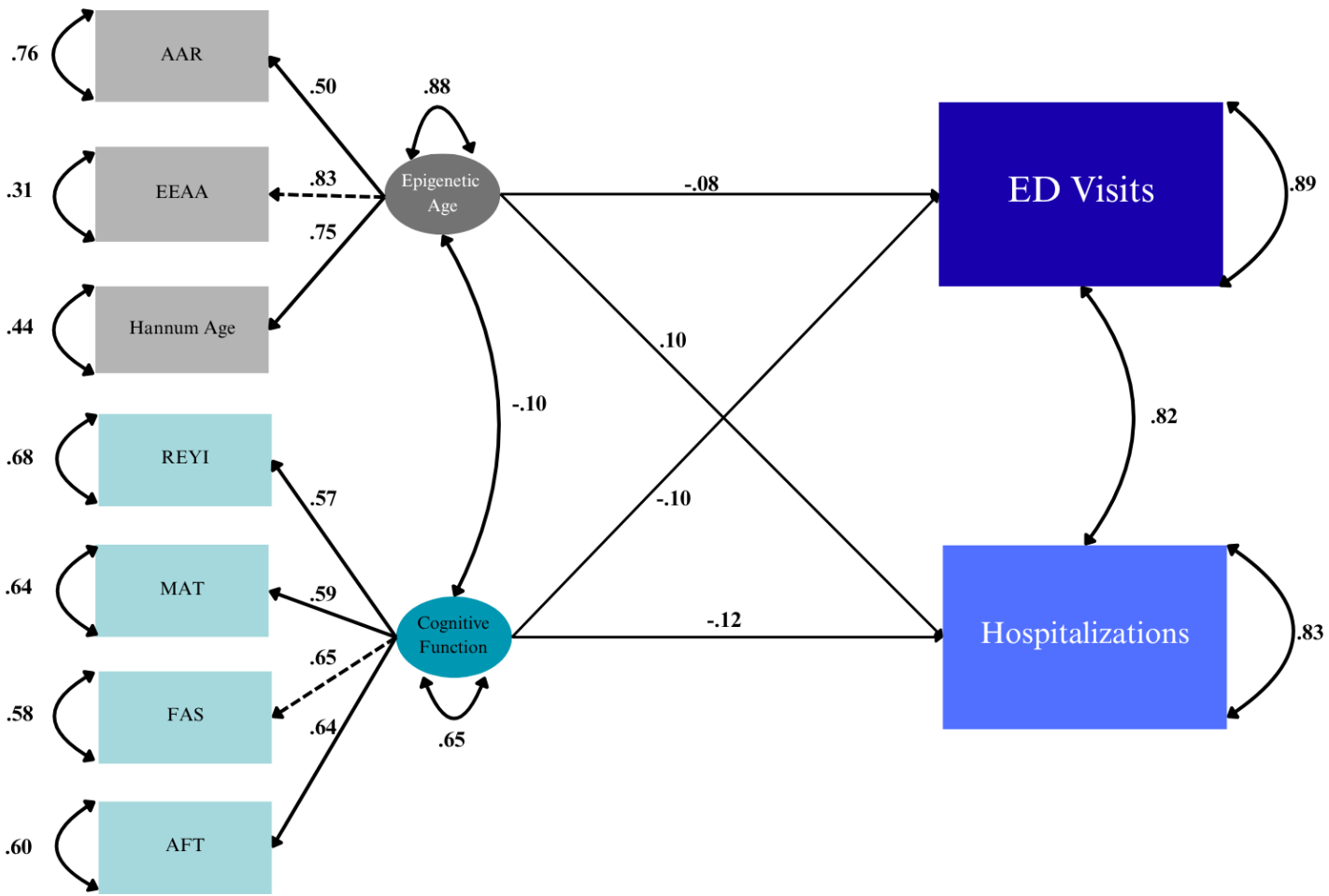


Figure 16: Moderation by sex model for males with covariates adjusted.

Note: AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test. Solid arrows from the latent construct to indicators represent measurement paths; solid arrows between latent constructs and outcomes represent structural paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances; curved double-headed arrows represent correlations.

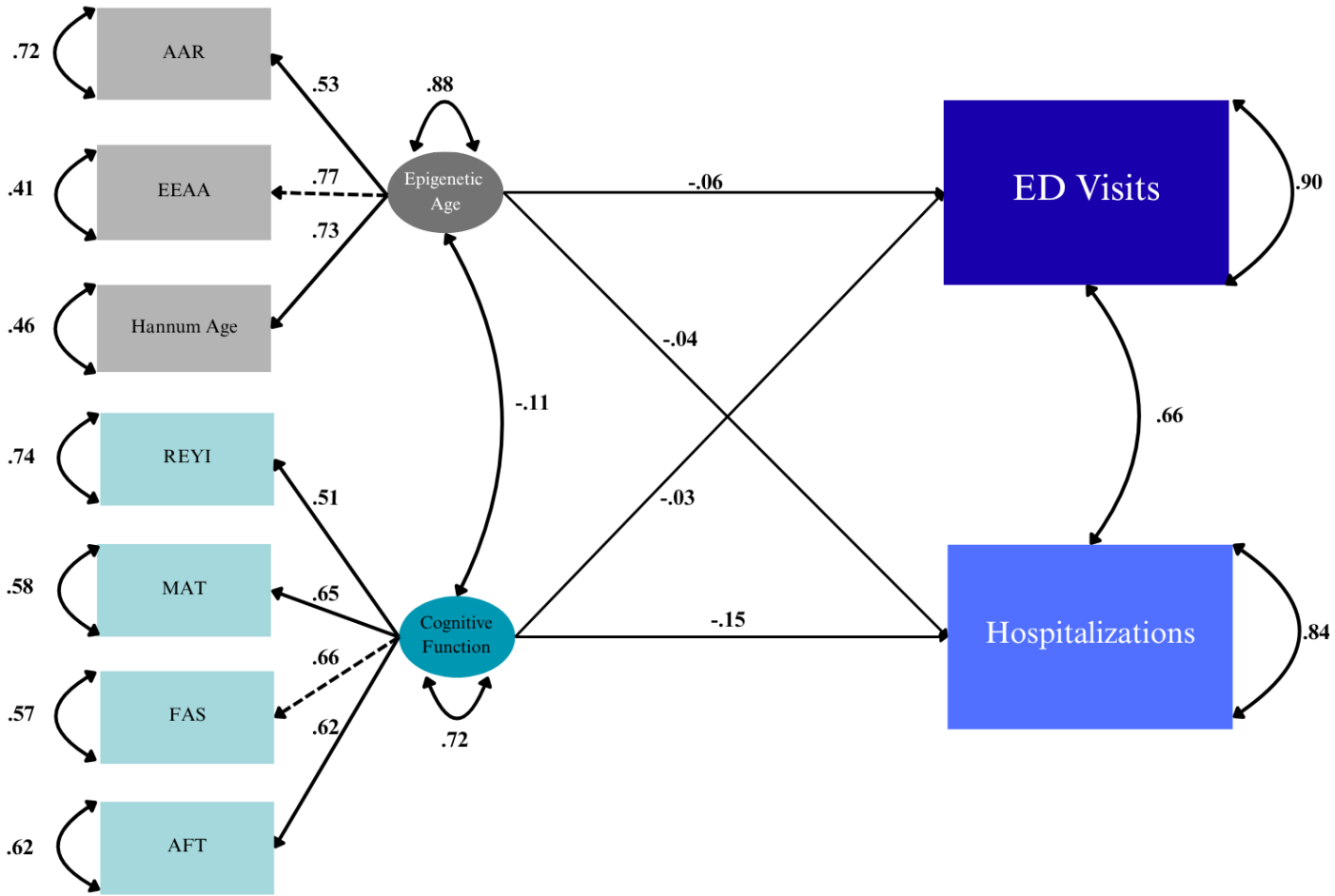


Figure 17: Moderation by sex model for females with covariates adjusted

Note: AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test. Solid arrows from the latent construct to indicators represent measurement paths; solid arrows between latent constructs and outcomes represent structural paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances; curved double-headed arrows represent correlations.

Table 8: Regression coefficients and 95% CIs for moderation by sex

Group and Outcome	Regression coefficient estimate (b)	95% CI_b	Standardized coefficient (β)
Males and ED Visits			
Epigenetic Age	-0.103	(-0.274, 0.067)	-.078
Cognitive Function	-0.154	(-0.424, 0.117)	-.097
Females and ED Visits			
Epigenetic Age	-0.087	(-0.268, 0.094)	-.064
Cognitive Function	-0.046	(-0.305, 0.213)	-.028
Males and Hospitalizations			
Epigenetic Age	0.134	(-0.082, 0.349)	.099
Cognitive Function	-0.192	(-0.491, 0.107)	-.118
Females and Hospitalizations			
Epigenetic Age	-0.059	(-0.292, 0.173)	-.042
Cognitive Function	-0.245	(-0.530, 0.039)	-.146

Note: *Significant pathways are bolded. CI= confidence interval; ED=emergency department visits.

Although constraining paths significantly worsened overall model fit, suggesting some moderation by sex, examination of the primary associations of interest revealed no significant sex-based moderation, suggesting that moderation by sex might have been significant in other pathways in the model. Neither epigenetic age nor cognitive function significantly predicted outcomes across groups and effect sizes were generally small and comparable across sexes. For hospitalizations, cognitive function showed a consistent negative association across both groups, indicating that higher cognitive function was associated with fewer hospitalizations. Epigenetic age was not a significant predictor of hospitalizations in either group, and effects were small and inconsistent in direction.

Overall, there were slightly stronger associations among males for both outcomes except for the association between cognitive function and hospitalization, where the association was slightly stronger among females, although this association was not significant either.

5.4.1.2 Moderation by Age

The ratio of middle-aged to older adults was 715:497, which may have reduced sensitivity to detect more subtle age-related effects. Fit indices for the configural model, metric, scalar, and constrained models can be seen in Table 9 below.

Table 9: Fit indices for configural, metric, scalar, and constrained models

Model	CFI	RMSEA	90% CI RMSEA	SRMR
Configural	.924	0.036	(0.029, 0.043))	0.043
Metric	.917	0.037	(0.030, 0.044)	0.040
Scalar	.903	0.039	(0.032, 0.046)	0.040
Constrained	.939	0.028	(0.021, 0.035)	0.041

Note: CFI= comparative fit index; RMSEA= root mean square error of approximation; CI= confidence interval; SRMR= standardized root mean squared residual

From these fit indices, the proposed factor structure was supported at the configural level, suggesting that this structure was appropriate across age groups. However, constraining factor loadings to equality (metric invariance) significantly worsened model fit ($\Delta\chi^2$ =16.89, p≤ 0.005), indicating a lack of support for metric invariance. Similarly, constraining item intercepts to equality (scalar invariance) also significantly worsened model fit ($\Delta\chi^2$=41.40, *p*≤ 0.001), providing evidence against scalar invariance across age groups.

As measurement invariance was not established at the metric and scalar levels, comparisons of structural paths across age groups should be interpreted with caution, as differences observed may be partially influenced by measurement non-equivalence. Therefore, the following results are strictly exploratory: a multi-group comparison between the freely (configural) model and the constrained model indicated no significant difference in model fit ($\Delta\chi^2$=53.09, *p*= 0.164), suggesting that there is no strong evidence of moderation by age group. Moderation models adjusted for covariates can be seen in Figures 17 and 18, and regression outputs for the moderation analysis can be seen in Table 10 below.

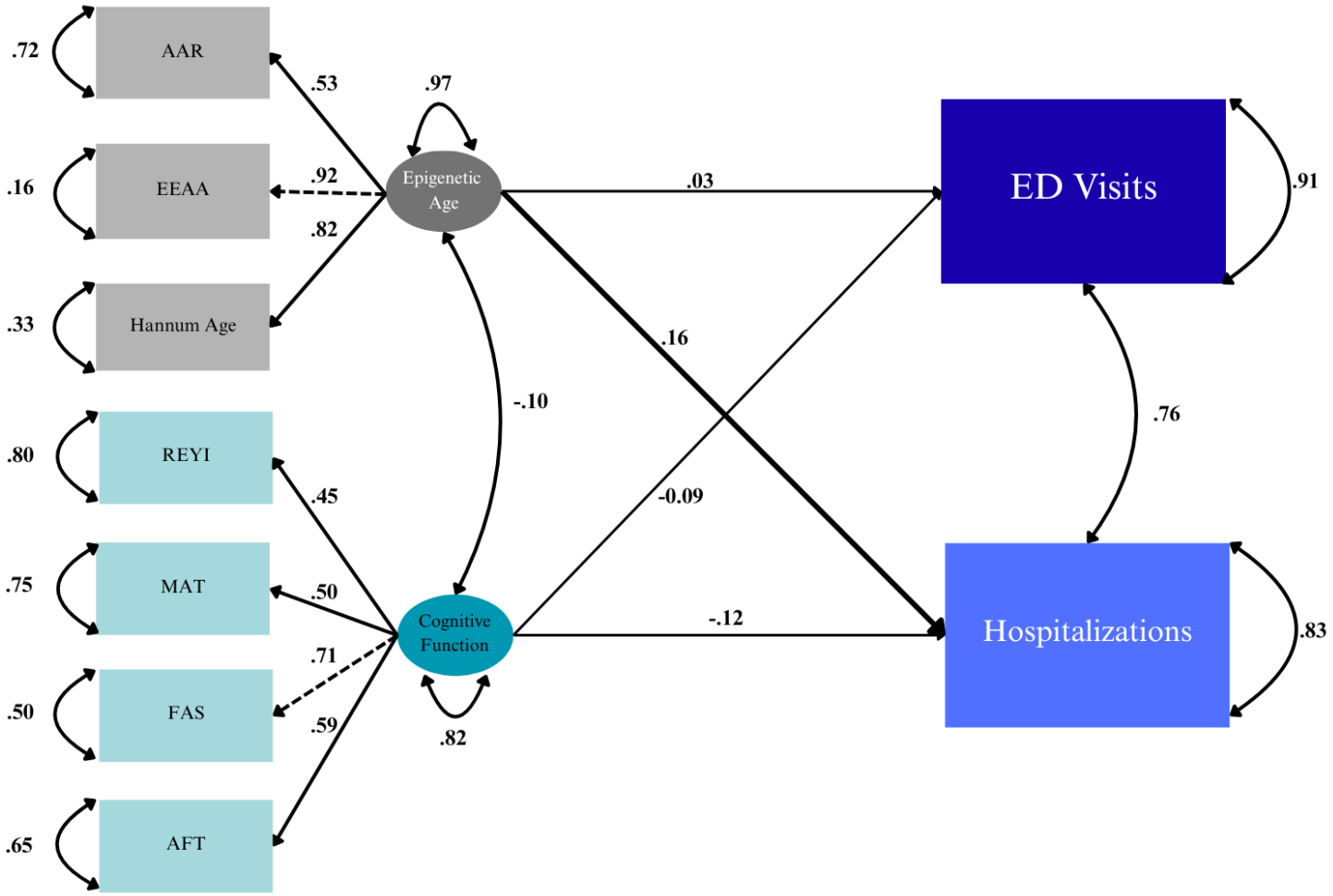


Figure 18: Moderation model for middle-aged adults adjusted for covariates.

Note: AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test. Solid arrows from the latent construct to indicators represent measurement paths; solid arrows between latent constructs and outcomes represent structural paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances; curved double-headed arrows represent correlations. Significant paths bolded.

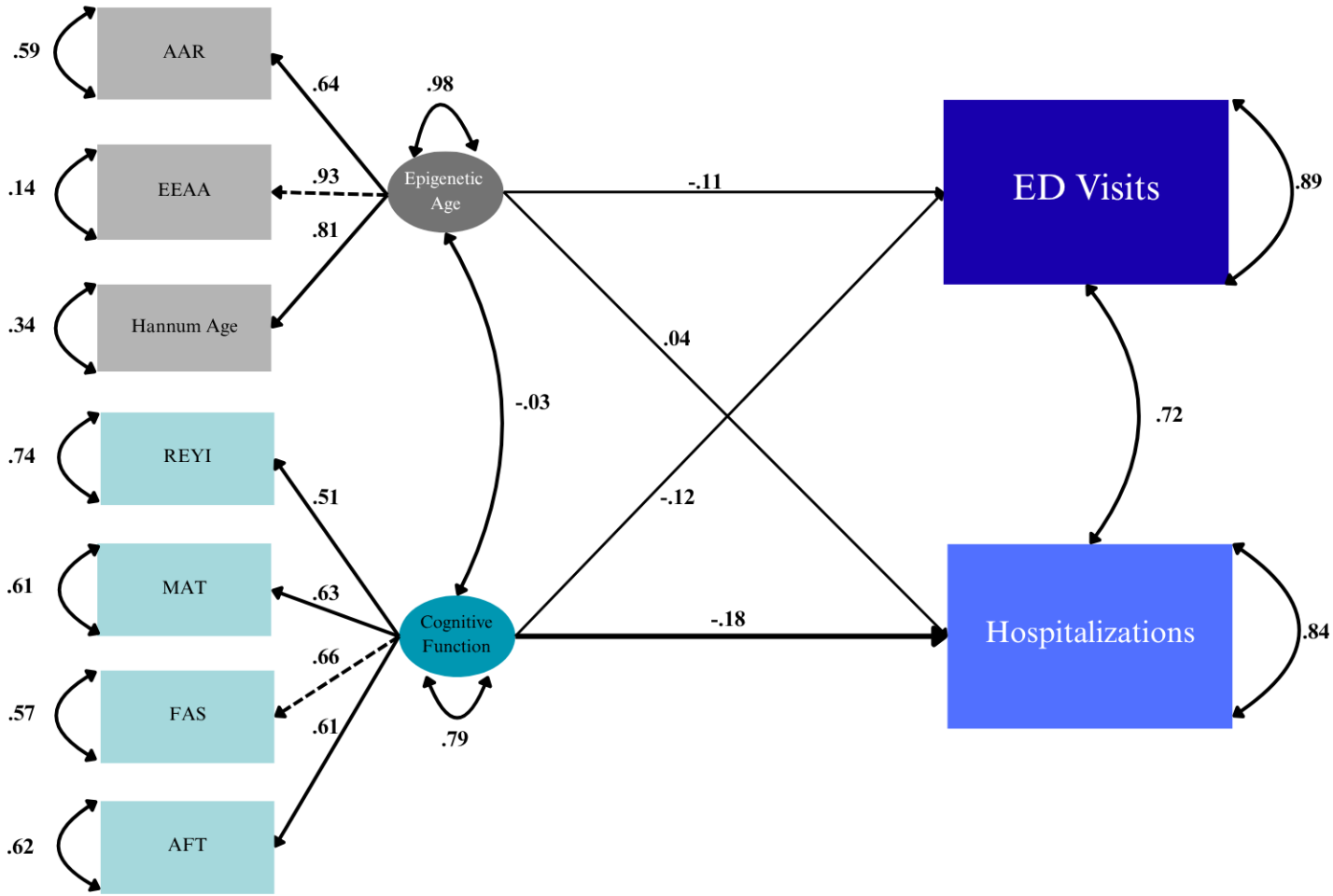


Figure 19: Moderation model for older adults adjusted for covariates

Note: AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test. Solid arrows from the latent construct to indicators represent measurement paths; solid arrows between latent constructs and outcomes represent structural paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances; curved double-headed arrows represent correlations. Significant paths bolded.

Table 10: Regression coefficients and 95% CIs for moderation by age group

Group and Outcome	Regression coefficient estimate (b)	95% CI_b	Standardized coefficient (β)
Middle-aged Adults and ED Visits			
Epigenetic Age	0.035	(-0.095, 0.164)	.030
Cognitive Function	-0.135	(-0.338, -0.068)	-.091
Older Adults and ED Visits			
Epigenetic Age	-0.121	(-0.260, 0.020)	-.109
Cognitive Function	-0.200	(-0.474, 0.075)	-.123
Middle-aged Adults and Hospitalizations			
Epigenetic Age	**0.195**	**(0.027, 0.363)**	**.164**
Cognitive Function	-0.187	(-0.431, 0.057)	-.124
Older Adults And Hospitalizations			
Epigenetic Age	0.042	(-0.126, 0.209)	.037
Cognitive Function	**-0.291**	**(-0.581,-0.001)**	**-.175**

Note: Significant pathways are bolded. CI= confidence interval; ED=emergency department visits

Given the lack of measurement invariance, these findings should be considered exploratory and interpreted with caution. Overall, regression results indicated some variation in significant predictors across age groups. Among middle-aged adults, older epigenetic age was a significant, albeit weak, predictor of more hospitalizations (β=.164; b=0.195; 95% CI_b=0.027, 0.363), such that a one standard deviation increase in epigenetic age was associated with a .164 increase in the underlying propensity for hospitalizations. In contrast, among older adults, higher cognitive function was a weak, but significant, predictor of fewer hospitalizations (β=-.175; b=-0.291, CI_b=-0.581, -0.001), such that a one standard deviation increase in cognitive function was associated with a .175 decrease in the underlying propensity for hospitalization. For ED visits, neither cognitive function nor epigenetic age were significant predictors.

5.5 Indicator-Level Analysis

From the full correlation matrix (Figure 20), in terms of zero order associations, several epigenetic variables were significantly associated with hospitalization, including Hannum, AAD,

and DNAmAge. Likewise, AAD predicted ED visits. Among cognitive indicators, all but FAS, STP and EBPM were significantly associated with hospitalization, while MAT, REYI, and REYII were significantly associated with ED visits.

Some epigenetic and cognitive measures were correlated with each other. Hannum, AAD, and DNAmAge were significantly correlated with all cognitive indicators. On the other hand, AAR, IEAA, and EEAA were not correlated with cognitive measures. ED visits and hospitalizations were significantly associated with one other, as well. A heatmap with all indicators is shown below. A heatmap showing bivariate associations between variables and age can be found in Appendix I.

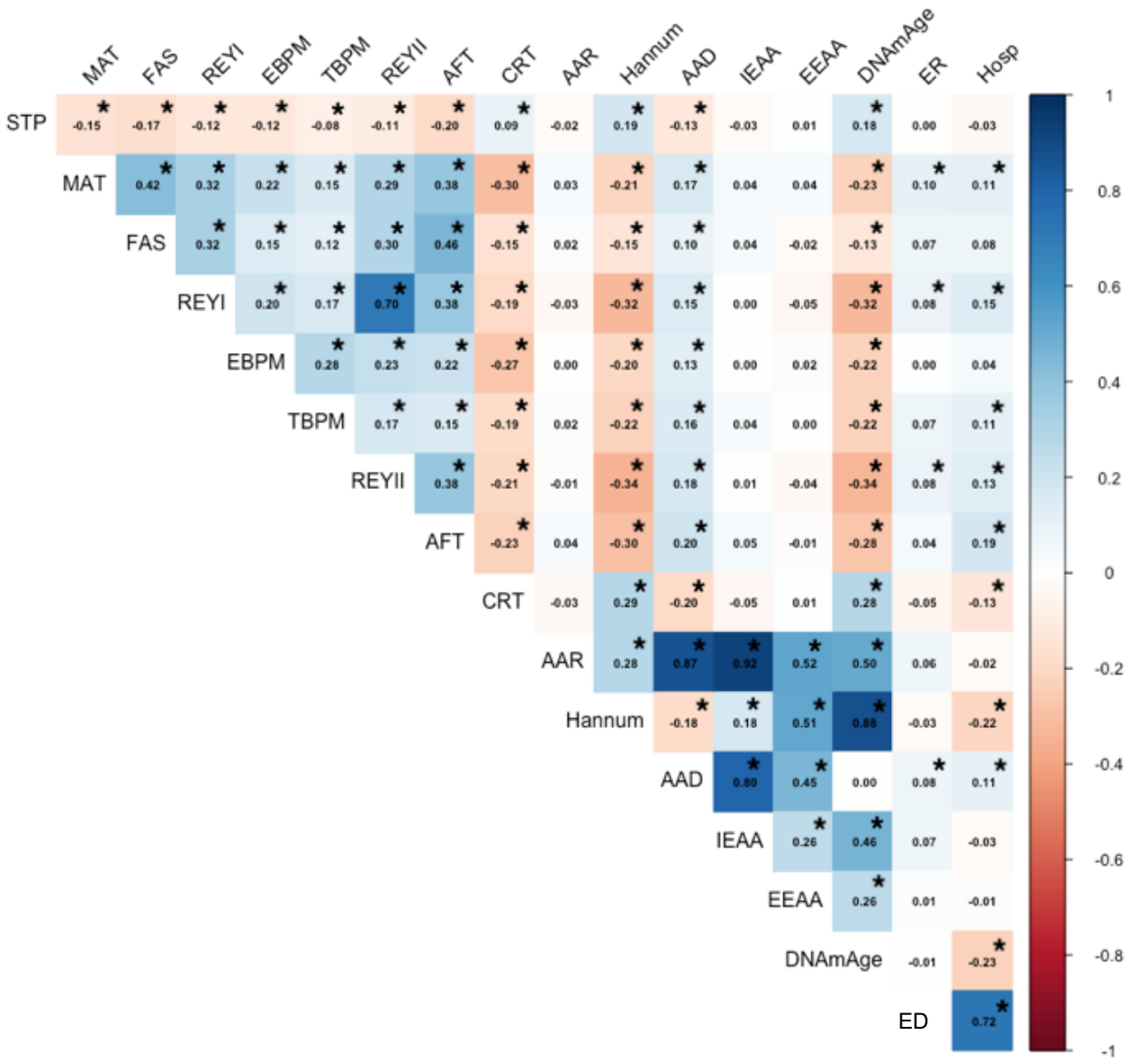


Figure 20: Hybrid heatmap for correlations between all epigenetic and cognitive variables with Pearson's correlations for continuous variables, tetrachoric correlations for dichotomous variables, and biserial correlations for continuous-dichotomous pairs

Note: Significant values marked with an asterisk*. STP=Stroop test; MAT=mental alternation test; FAS=controlled oral word association test; REYI=Rey auditory verbal learning test trial 1; EBPM=event-based prospective memory test; TBPM=time-based prospective memory test; REYII= Rey auditory verbal learning test trial 2; AFT=animal fluency test; CRT=choice reaction test; AAR=age acceleration residual; AAD=age acceleration difference; IEAA=intrinsic epigenetic age acceleration; EEAA=extrinsic epigenetic age acceleration; ED=emergency department visits.

No quadratic terms were statistically significant, indicating non-violation of the linearity of the logit assumption. This suggests the relationship between each predictor and the log-odds of both outcomes is adequately linear. In the interaction models, no interactions between age and predictors were found to be significant; therefore, age was included as a covariate. A full table with ORs and 95% CIs can be found in Appendix J, Table 1. Table J2 contains the ORs and 95% CIs following the removal of chronic conditions from the model, to assess impact of controlling for chronic conditions.

The results suggested that among the cognitive and epigenetic variables, only the AFT was a significant predictor of hospitalizations (OR=0.79, 95% CI= 0.61, 1.00): higher AFT scores were associated with lower odds of hospitalization. Specifically, each one standard deviation increase in AFT scores was associated with a 21% reduction in the odds of hospitalization. The MAT was significantly associated with ED visits (OR=0.84, 95% CI= 0.72, 0.98): higher MAT scores were associated with a lower odds of ED visits. Specifically, each one standard deviation increase in MAT scores was associated with a 16% reduction in the odds of visiting the ED. The IEAA was also a significant predictor for ED visits (OR=0.86, 95% CI=0.74, 1.00): higher IEAA was associated with lower odds of ED visits. Specifically, each one standard deviation increase in IEAA was associated with a 14% reduction in the odds of visiting the ED. These associations were not statistically significant after Hochberg correction for multiple comparisons; given the tertiary nature of these analyses, findings are considered exploratory.

6.0 Discussion

6.1 Summary of Study Findings

A primary objective of the current investigation was to examine the factor structure of the SR construct, with references to its proposed indicators, epigenetic age and cognitive function. Secondary and tertiary objectives included examining associations between latent constructs (epigenetic age and cognitive function)/indicators and two healthcare service utilization variables – ED visits and hospitalizations – and the moderating effects of age and sex. The secondary and tertiary objectives were approached using factor-level and indicator-level analyses. It was hypothesized that a higher-order model with SR as an overarching construct would best fit the data, but this was not directly supported in this study. Although differences in fit did not provide clear evidence against the higher-order factor model, results from the main analysis indicated that an uncorrelated two-factor structure, representing cognitive function and epigenetic age, provided the best relative fit. This structure was consistent across both FIML and CCA, supporting the robustness of the model.

When examining the final validation model, epigenetic age and cognitive function were found to be distinct, but weakly correlated constructs, with a small negative association suggesting that greater epigenetic age and faster EAA were associated with slightly lower cognitive performance. It was also hypothesized that greater SR and its subcomponents would be associated with healthcare service utilization in theoretically meaningful ways. Consistent with this hypothesis, cognitive function was significantly associated with hospitalizations, such that better cognitive performance was linked to fewer hospital admissions. However, neither epigenetic age nor cognitive function were found to be significant predictors of ED visits.

Finally, it was hypothesized that the strength of association between predictors and outcomes would be stronger for women and older adults. For sex, moderation analyses suggested no meaningful differences between males and females.

Furthermore, age moderation could not be fully interpreted due to lack of measurement invariance, limiting comparability across groups. Lack of measurement invariance suggests that constructs are measured differentially across groups. Thus, differences observed may not true differences due to group membership, but rather due to differences in how the items are interpreted or function in each group. Nevertheless, exploratory analyses suggested that in middle-aged adults, epigenetic age was a significant predictor of hospitalizations, whereas in older adults, cognitive function was a significant predictor of hospitalizations.

Indicator-level analyses suggested that there were associations between specific cognitive and epigenetic indicators and healthcare utilization. The AFT was a significant predictor of hospitalizations, while the MAT and IEAA were significant predictors of ED visits.

In summary, the findings suggest that, at the factor level, epigenetic age and cognitive function may be relatively distinct constructs and cognitive processes may play a more consistent role in predicting health outcomes at both the factor and indicator levels. However, at the indicator level, there was evidence for more predictive power in relation to certain epigenetic measures, particularly IEAA. This suggests that the predictive power of individual clocks and individual epigenetic measures may exceed the general predictive power of the superordinate factors for which they form indicators, at least for the variables used in this study.

6.2 Interpretation of Findings in Relation to SR

This study conceptualized SR as a higher-order latent construct reflecting shared variance across biological aging and cognitive function domains. In the higher-order model, a small-to-

moderate association was observed between epigenetic age and cognitive function, suggesting potential shared variance that could reflect a higher-order model. This provides partial support for the theoretical premise that SR may reflect integration across physiological systems, rather than a single biological pathway.

Although the two-factor and higher-order models demonstrated comparable fit, the two-factor model was ultimately selected as the more appropriate model based on relative fit indices and model parsimony. In this model, epigenetic aging and cognitive function were better represented as distinct, but related, constructs rather than indicators of a unified latent construct. Therefore, within the CLSA, evidence did not emerge to support SR as a single construct defined solely by epigenetic aging and cognitive function.

However, these findings do not necessarily invalidate the SR framework. Instead, they suggest SR is inherently multidimensional and cannot be fully captured using only epigenetic age and cognitive function. Additional domains, such as inflammatory markers (e.g., C-reactive protein, interleukin-6) and endocrine regulation (e.g., cortisol) could contribute to a more comprehensive, higher-order construct of SR. For instance, although an association exists between inflammatory biomarkers and epigenetic age, some research suggests they may function as partially independent markers of aging and mortality (193). Epigenetic age might only represent one component of SR and other biomarkers may be required to obtain a more comprehensive characterization of SR. Nonetheless, the addition of many more domains might increase overlap with existing constructs, such as allostatic load, reducing construct distinctiveness (194). This highlights a tension between comprehensiveness and conceptual specificity, where overly broad operationalization might risk SR becoming indistinguishable from general physiological dysregulation constructs.

Alternatively, the thesis' findings could reflect limitations in the analytic sample, such as relatively low prevalence of ED visits/hospitalizations, or restricted range of cognitive/epigenetic/healthcare utilization measures. Overall, the current findings suggest incomplete empirical support for conceiving SR as a construct solely composed of epigenetic age and cognitive function.

6.3 Epigenetic Age and Cognitive Function as Distinct and Related Systems

The weak, negative association between epigenetic age and cognitive function indicates that these two domains are related yet fundamentally distinct. Epigenetic age reflected systemic biological dysregulation across multiple tissues, while cognitive function reflected integrated neural functioning and brain-specific structural and functional integrity.

The observed association between epigenetic age and cognitive function—although relatively modest in magnitude—suggests of the possibility of shared mechanisms underlying the two. Both inflammatory processes and stress-related pathways are possibilities. Inflammation from chronic stress can increase the presence of inflammatory markers in the brain, contributing to cognitive impairment and variation in epigenetic aging (195,196) This aligns with previous research reporting negative associations between various measures of epigenetic age and cognitive function (25,26,112,115).

However, the small magnitude of the observed association between epigenetic age and cognitive function reinforces the idea that these constructs may capture different dimensions of health rather than a single unified process. Notably, although there is a large body of literature supporting the association between the two, results remain mixed, with some studies finding no significant association (119). For instance, one study examining IEAA found no relation between epigenetic age and cognitive function in midlife (119). This suggests that the relationship

between epigenetic age and cognitive function may be context-dependent across samples or populations, with age, sample characteristics, and measurement approaches all affecting the magnitude of the association.

6.4 Cognition, Epigenetics, and Healthcare Utilization Outcomes

The finding that better cognitive function is linked to reduced hospitalizations is consistent with prior evidence linking baseline cognitive performance to adverse health outcomes, increased healthcare utilization, and institutionalization (197–199). Several studies have shown that poorer cognitive function is associated with greater hospitalization risk in older adults without dementia (199). Poorer cognitive function has also been linked to longer lengths of hospital stay, and poorer prognoses in general, which may be due to greater comorbidity and more severe illness (199). For instance, a study looking at the impact of mild cognitive impairment on healthcare utilization and costs found that older adults with lower scores on reaction time, prospective memory, and fluid intelligence tests incurred higher healthcare utilization and costs compared to those with higher scores (200). Similar results were found in another study reporting patients with moderate to severe cognitive impairment were more likely to be hospitalized and to visit the ED (201).

A possible explanation for these findings is that individuals with poorer cognitive function may be more clinically complex and more ill, as well as less functionally independent, which is why they are more likely to be hospitalized. Additionally, cognitive impairment has been shown to be associated with increased risks for other chronic conditions and mortality, which are linked to increased healthcare utilization (202,203).

Turning to epigenetic age, the lack of association with hospitalizations runs contrary to findings linking accelerated aging and older epigenetic age to increased risk of hospitalization

(204). Several explanations could account for these discrepancies. First, epigenetic aging effects may emerge over longer time horizons than captured in the present study's relatively short follow-up period. Second, differences in epigenetic and outcome measures could limit direct comparability across studies. For example, first-generation clocks (used in this study) are primarily trained to predict chronological age, rather than health outcomes, which may limit their ability to detect associations with healthcare utilization (205). Third, adjustment for chronic disease burden may have attenuated associations, as epigenetic age may partially operate through accumulated disease burden processes already captured in the model. Although these are all plausible, the second explanation is considered the most likely.

It is important to note that although epigenetic clocks are used widely as biomarkers of biological age, their interpretation remain subject to ongoing debate. Recent studies highlight that although they are robust predictors of chronological age and are associated with many health outcomes, they may not directly capture causal mechanisms of aging (206,207). Instead, they may reflect correlated biological processes, including changes in cell composition and systemic physiological variation (206,207). Furthermore, the computational construction of epigenetic clocks introduces added complexity, as different clocks vary in their training targets and predictive ability, limiting comparability (206). These considerations suggest that epigenetic age should be interpreted as an informative, indirect correlate of biological aging rather than a definitive measure of underlying aging mechanisms.

6.5 Moderation by Sex and Age

Sex moderation analyses suggested limited and inconsistent differences in associations across groups. Although some effects appeared slightly stronger in males, overall, the effects were small and non-significant.

Conversely, age moderation analyses suggested that the structures of epigenetic age and cognitive function may not be directly comparable across groups, as evidenced by a lack of measurement invariance. This limits interpretation of the observed group differences, suggesting that measurement structures may function differently across the lifespan. Despite this, exploratory analyses revealed some variation in effect patterns across groups. For instance, among middle-aged adults, epigenetic age was a significant predictor of hospitalizations, whereas, among older adults, cognitive function was a significant predictor of hospitalizations. For ED visits, neither cognitive function nor epigenetic age were significant predictors. A possible explanation for this finding is that ED use is influenced by a broad range of factors beyond epigenetic age and cognitive function. ED visits can be triggered by a wider range of acute, situational, and environmental circumstances, which could make them more sensitive to external influences and less directly related to the biological and cognitive processes examined in this study (209).

6.6 Findings from Indicator-level Analysis Examining Indicator Associations with Outcomes

Overall findings from the supplemental logistic regression analyses suggested that AFT, MAT and IEAA were significant predictors of healthcare utilization. Specifically, higher AFT scores were associated with lower odds of hospitalization, while higher MAT scores and greater IEAA were associated with lower odds of ED visits. Additionally, no significant interactions between age and predictors were observed, suggesting that associations did not differ significantly by age.

Differences in predictive abilities across cognitive measures may reflect the degree to which each test captures functionally relevant aspects of cognition. The AFT involves both language and executive functioning and is clinically useful for distinguishing individuals with mild cognitive impairment and dementia from those experiencing normal aging (208). Similarly, the MAT evaluates cognitive flexibility and executive functioning, and has shown promise as a measure of cognitive decline in older adulthood (209). Cognitive impairment has previously been associated with higher rates of ED visits and hospitalizations (210). Executive functioning may also be more strongly associated with health outcomes than the other cognitive domains. For example, a study examining executive function, memory, and Medicare expenditures among women found that impairments in executive function were associated with higher healthcare expenditures and hospital use (213). Another study examining attention, executive function, and memory as predictors of mortality among adults with systolic heart failure found that impairment in executive function, but not memory, was associated with mortality risk (211) Therefore, the REYI and FAS may more specifically assess cognitive processes such as memory and verbal fluency, with executive function to a lesser extent. The AFT and MAT, in contrast, may be more strongly tied to the mental flexibility facet of executive function and broader cognitive changes that influence health outcomes and subsequent healthcare utilization, and may demonstrate greater predictive ability than the other tests.

In terms of IEAA, higher IEAA was associated with fewer ED visits. One explanation for the current findings with respect to epigenetic predictors could be that CLSA participants comprise a healthier subsample of persons, meaning that they may have better health outcomes regardless of their IEAA. It is also possible for overadjustment to have occurred due to adjusting for chronic condition count. Indeed, when chronic condition count was removed from the logistic

regression models, the association between IEAA and ED visits was no longer significant, as shown in Table 2 in Appendix J (OR=0.89, 95% CI=0.77, 1.03). This suggests that adjusting for chronic conditions may inadvertently distort the true effect of epigenetic aging on healthcare utilization. As for reasons why other epigenetic indicators were not significantly associated with the outcomes, it is possible that EEAA, which is influenced by immune cell changes, may fluctuate temporarily and did not capture increased healthcare utilization in the analytic sample. Furthermore, Hannum age and DNAmAge are first-generation clocks, which were developed for predicting chronological age, rather than health outcomes (205). Therefore, they may be weaker predictors, and so AAD and AAR, which are derived from DNAmAge, may also not predict healthcare use.

6.7 Strengths

This study has several notable strengths. First, the use of the CLSA provided access to a large, population-based Canadian dataset with a rich set of relevant cognitive, biological, demographic, and health variables. The wide age range of participants also allowed for examination of the moderating effects of age across mid- to later-life adulthood.

Second, the use of a sensitivity analysis for validation of the measurement model strengthened the robustness of the findings by demonstrating consistency across results obtained using CCA and FIML.

Third, the application of CFA and comparison of several competing models (single-factor model, two-factor model, and higher-order model) strengthened the validity of the measurement approach by testing multiple possible model structures.

Fourth, this study incorporated a comprehensive set of indicators for both epigenetic age and cognitive function, rather than relying on pre-derived composite variables. A key advantage

of SEM is its ability to construct latent variables from observed indicators and account for measurement error, allowing for a clearer, unattenuated assessment of structural relationships. In this study, all available indicators were retained and evaluated individually, enabling a more detailed assessment of construct structure and the contribution of each indicator. This approach enhanced construct validity and provided greater transparency in how latent variables were formed.

Fifth, the latent epigenetic construct was composed of indicators that captured different, but complementary, components of epigenetic age, improving construct coverage and validity. Hannum age reflects one of the most widely validated blood-based epigenetic clocks, while EEAA incorporated immune-related changes and age-related shifts in blood cell composition. AAR captures deviation from chronological age integrating DNAmAge, another well-established epigenetic clock. Each indicator is derived differently and is sensitive to somewhat different biological pathways, and their shared variance is likely to reflect a core underlying construct, improving robustness.

Sixth, conducting a bottom-up, indicator-level analysis in addition to a top-down, factor-level analysis allowed for thorough examination of all variables, including those excluded from the main models, and their associations with healthcare utilization. This approach strengthened the study by providing a more comprehensive assessment of predictors. Moreover, examining interactions with age allowed detection of potential effects of age that could not be assessed in the main SEM due to measurement invariance constraints.

Finally, the inclusion of a prospective validation component is an important strength. By examining associations between the baseline latent constructs and healthcare utilization

measured approximately 18 months later, the study introduces a temporal dimension that strengthens the interpretation of associations, moving beyond purely cross-sectional analysis.

6.8 **Limitations**

First, due to self-selection, participants in the CLSA tended to be wealthier, more educated, Canadian-born, and healthier than the general population (122). The CLSA also excluded individuals with overt cognitive impairment at baseline, which meant the sample was cognitively healthier than the target population. This resulted in a cognitively healthier and more homogeneous sample, with less variation in cognitive performance and healthcare utilization. As a result, observed effect sizes may be conservative, and moderation effects may be more difficult to detect within this sample. Furthermore, because the analytic sample was a subset of the full Comprehensive Cohort (n = 1,478 to n = 1,212 and n=1,172), these results may not be generalizable to the full sample (n = 30,097). Therefore, the results of this thesis may only apply to individuals who resemble the analytic sample.

Second, the use of self-reported measures introduces the potential for misclassification of some study variables. For example, reporting of chronic conditions requires participants to indicate whether or not a physician ever told them they have a specific condition. This was not verified with other data sources (e.g., chart review), which means that the accuracy of participants' self-reporting was unascertainable. Hospitalizations and ED visits were also based on self-report without additional verification. However, these two outcomes were assessed as part of a larger MCQ, without any direct link to epigenetic or cognition measures, so any misreporting is unlikely to be related to exposure. Thus, misclassification would likely be nondifferential and any bias in results would likely be toward the null.

Third, in the sensitivity analysis, FIML was used to handle missing data, which assumes data are MAR. This assumption could not be definitively verified. To the extent missingness deviated from MAR, the calculated regression coefficients in the FIML analysis would not validly estimate the true associations.

Moreover, two broad cognitive domains were sampled in the thesis: memory and executive functioning. SEM of cognition could have involved multiple domains under a higher-order cognition factor with at least three indicators each to provide a more comprehensive look at cognitive function. In the present study, this criterion was only met for executive functioning. In addition, the thesis only included four cognitive tests, which meant the cognitive function domain may have been more constrained than optimal, limiting the breadth of measurement of cognition and potentially attenuating associations with outcomes. The same can be said for the epigenetic variables. For example, different measures of epigenetic age could be combined into one epigenetic age factor, and different measures of age acceleration could be combined into an acceleration factor. These two components could then be combined to form a higher-order construct of epigenetic aging.

Furthermore, functional or structural magnetic resonance imaging data were not collected in the Comprehensive Cohort. Structural imaging can visualize anatomical properties of the brain, while functional imaging can discern activity in areas of the brain at a given time, for example during cognitive task performance (214). Together, these measures can give more insight into changes associated with aging and differences between age and sex groups. However, this limitation does not directly preclude addressing the study's main objectives, and

future work integrating imaging data could provide additional insight into age-related brain-level changes.

Finally, conclusions are restricted to the specific epigenetic clocks and healthcare utilization outcomes used in this study. It is possible that different clocks, such as second- or third- generation epigenetic clocks, may match or outperform cognitive indicators in predicting healthcare use. Such clocks may also behave differently within the structural models, including those containing a superordinate construct, such as SR.

6.9 Implications and Future Directions

Epigenetic aging and cognitive function are two potential markers of SR and may predict healthier aging outcomes. Men and women may experience different biological events that influence the association between cognition and epigenetic age. In the same way, the association between epigenetic aging and cognitive function may also differ across the lifespan, such that older adults are most susceptible to its effects. Therefore, accounting for these factors could reveal the role they play and aid in identifying those who are at greater risk of vulnerability, and ensure valuable resources are distributed to them accordingly to prevent further decline.

There is currently limited research that examines the association between these factors and the effects of age and sex as moderators in a broader population, especially using CLSA data, which has the potential to provide further understanding of health outcomes of Canadians across a wide age range.

From a clinical perspective, the strong association between cognitive function and hospitalization suggests that cognitive measures may serve as more proximal indicators of

healthcare utilization. This highlights the value of incorporating cognitive screening into risk reduction approaches for healthcare utilization and functional decline. In contrast, epigenetic aging markers may be more useful for long-term risk trajectories rather than short-term healthcare outcomes.

Several avenues for future research emerge from this study. First, the SR structure should be evaluated across other datasets, as the current study was limited to a sample of 1,478 middle-aged and older adults. Although findings from this study did not support a superordinate factor model as superior to a two-factor model reflecting cognitive function and epigenetics, the same may not be the case in other samples. Future research should examine the validity of SR across the lifespan, including in children, adolescents, and young adults. In older samples, multimorbidity and other related health factors may confound associations between epigenetic and cognitive domains, whereas these influences may be reduced in younger populations. It could also be that further refinement of the SR construct can be considered. Currently, only epigenetic age and cognitive function are used to operationalize SR, but adding additional domains, such as inflammatory markers, could provide a more comprehensive representation of SR, although additions should be made with caution to ensure model parsimony and no overfitting.

Second, longitudinal modelling over the span of many years to decades could further examine the associations between epigenetic age, cognitive function, and other health outcomes, such as mortality and institutionalization. The current study only examined associations between predictors and healthcare utilization within an 18-month follow-up period, which may be too short to capture real changes. This can also help clarify whether these associations may fluctuate

or remain stable across the lifespan, and whether epigenetic age may become a significant predictor over a longer period of time. Associations between epigenetic age and cognition are also still purely cross-sectional in this study, as epigenetic data were only available at baseline. Therefore, future studies that examine temporal and bidirectional aspects of this association could be beneficial.

Third, additional investigation may be needed to fully understand the moderating effects of age. The lack of invariance observed in this study regarding age moderation suggests that epigenetic age and cognitive function may not operate equivalently across the lifespan. Further studies should examine this research in a wider age range, with a larger sample size.

Fourth, given the findings from the indicator-level analysis, more work could examine associations between individual epigenetic indicators and health outcomes. This should include using a larger longitudinal dataset with more epigenetic variables, including second and third generation clocks as well, which were not available in the CLSA.

Finally, future studies should continue to explore heterogeneity in outcomes across sex and how socioeconomic status might play a role. Although no significant differences were observed in the variables of interest, more pronounced differences were observed in socioeconomic covariates. In particular, education and income demonstrated stronger associations with hospitalization outcomes among females. This may reflect sex-specific differences in healthcare utilization patterns, as females might exhibit greater hospitalization rates for certain conditions (215). Another plausible interpretation is that individuals with higher socioeconomic status are more likely to be diagnosed and admitted and have access to healthcare. Alternatively, these findings may also reflect differential pathways through which

socioeconomic factors influence health and healthcare use in females compared to males. These findings suggest that social determinants play an important role in shaping cognitive function and epigenetic age, which might affect health outcomes as well. Expanding these analyses can help clarify the exact role that socioeconomic status plays and if other differences due to sex may emerge.

7.0 Conclusion

Although the concept of system-level resilience has been studied in other disciplines, its operationalization within health research remains underdeveloped. In particular, the factor structure of SR has not been explored yet using epigenetic and cognitive function data from the CLSA. This thesis therefore represents one of the first empirical investigations into SR using these domains and evaluates both its measurement structure and its associations with health outcomes. The findings indicated that a two-factor model representing epigenetic age and cognitive function provided the best relative fit, suggesting that these constructs are better represented as distinct, but related, domains as opposed to a single unified latent factor. Overall, cognitive function emerged as a more consistent predictor of healthcare utilization at the factor-level, specifically hospitalizations, whereas associations involving epigenetic age were weaker and less consistent. However, these relationships varied across subgroups, with some evidence suggesting potential differences by sex. At the indicator-level, MAR, AFT, and IEAA emerged as significant predictors of healthcare utilization. Taken together, this study provides an avenue for the use of epigenetic age and cognitive function as predictors of healthcare utilization. Future research should build on these findings by examining SR in larger and more diverse populations, incorporating additional indicators for epigenetic age and cognitive function, as well other health outcomes. Longitudinal study designs will be especially important for clarifying temporal relationships and assessing how SR-related constructs evolve over time. Further work is also needed to better understand potential moderation by age and sex.

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Appendix A-Predictor Variables

Table A 1: Description of cognitive test variables

Cognitive measures	Scoring	Brief description of test
Rey Auditory Verbal Learning Test (REY) Trial 1 Immediate And REY 5-minute delayed recall	0-15 on each	This test presents participants with 15 words to assess.
Animal Fluency (AF)	Dependent on strict or lenient scoring	This test requires participants to name as many animals as possible in 60 seconds.
Mental Alternation Test (MAT)	0-52	Participants have 30 seconds to alternate between the numbers 1-26 and letters of the alphabet.
Controlled Oral Word Association Test (FAS)	Dependent on number of admissible words per letter.	Participants must name as many words as possible beginning with the first letter.
Stroop Test (Victoria)	Interference ratio	This test has three parts: a) dot, to first name the colour of dots printed on a

	(time to complete the last card / time required to complete the first card)	card, b) word, to name the ink colour of non-colour words on a card, and c) colour, to name the ink colour for colour words that do not correspond with the ink colour.
Prospective Memory Test (PMT)	0-18 total depending on intention to perform, accuracy of response, and need for reminders.	This test contains event (EBPM) and time-based prospective memory (TBPM) tasks that are cued after 15- or 30-minute delays.
Choice Reaction Time (CRT)	Mean response time	Participants must touch a box with a white box on a computer screen as quickly as possible once it appears in one of two black boxes.

Table A 2: Description of epigenetic variables

Epigenetic Age Measure	Description
DNAmAge	The absolute DNAm age calculated using the Horvath clock in biological years.
Age Acceleration Difference (AAD)	The difference between chronological age and DNAmAge.
Age Acceleration Residual (AAR)	Typically used as the universal measure of EAA. Calculated as the residual from regressing DNAmAge onto chronological age.
Intrinsic Epigenetic Age Acceleration (IEAA)	An estimate of epigenetic age that is attributable to intrinsic changes within cells and accounts for both chronological age and blood cell type proportions.
Extrinsic Epigenetic Age Acceleration (EEAA)	An estimate of epigenetic age that is attributable to age-related changes in blood cell type composition and is more relevant to aging of the immune system.
Hannum Age	The epigenetic age calculated using the Hannum clock.

Appendix B-Skewness and Kurtosis

Table B 1: Kurtosis/skew table for epigenetic variables

Variable	Skew	Kurtosis	Skew flag*	Kurtosis flag[a]
Intrinsic Epigenetic Age Acceleration	0.13	2.49	FALSE	FALSE
Extrinsic Epigenetic Age Acceleration	0.28	0.69	FALSE	FALSE
DNAmAge	0.32	-0.05	FALSE	FALSE
Hannum Age	0.22	-0.31	FALSE	FALSE
Age Acceleration Difference	0.03	0.67	FALSE	FALSE
Age Acceleration Residual	0.49	1.83	FALSE	FALSE

*Skew flag=FALSE if skew<2

[a]Kurtosis flag=FALSE if kurtosis<7

Table B 2: Kurtosis/skew table for cognitive variables

Variable	Skew	Kurtosis	Skew flag*	Kurtosis flag[a]
Choice Reaction Time	2.78	17.20	TRUE	TRUE
Mental Alternation Test	-0.4	0.39	FALSE	FALSE
Stroop Test	7.70	125.11	TRUE	TRUE
Controlled Oral Word Association Test	0.26	0.06	FALSE	FALSE
Rey Auditory Verbal Learning Test 1	0.25	0.10	FALSE	FALSE
Rey Auditory Verbal Learning Test 2	0.23	-0.11	FALSE	FALSE
Time-Based Prospective Memory Test	-3.63	16.03	TRUE	TRUE
Event-Based Prospective Memory Test	-2.47	5.88	TRUE	FALSE
Animal Fluency Test	0.32	-0.02	FALSE	FALSE

*Skew flag=TRUE if skew>2

[a]Kurtosis flag=TRUE if kurtosis>7

Appendix C-Model Fit Indices After Outlier Removal

Table C 1: Fit indices for higher-order, two-factor, and single-factor models after outlier removal

Model	CFI	RMSEA (90% CI)	SRMR	AIC	BIC
Complete-Case Analysis (CCA)					
Higher-order	.863	0.134 (0.120, 0.148)	0.084	22362.98	22485.28
Two-factor	.839	0.134 (0.120, 0.148)	0.084	22358.98	22471.09
Single-factor	.420	0.244 (0.231, 0.258)	0.133	22977.11	23084.13

Note: CFI = comparative fit index; RMSEA = root mean square error of approximation (90% CI in parentheses); SRMR = standardised root mean square residual; AIC = Akaike information criterion; BIC = Bayesian information criterion; CCA = complete-case analysis; FIML = full-information maximum likelihood; CI= confidence interval

Appendix D-Item-total correlations for epigenetic and cognitive indicators

Table D 1: Item-total correlations for epigenetic and cognitive indicators used in the final models

Variable	Raw.r	Standardized.r
Extrinsic Epigenetic Age Acceleration	.668	.729
Hannum Age	.933	.849
Age Acceleration Residual	.599	.732
Controlled Oral Word Association Test	.800	.753
Animal Fluency Test	.691	.757
Rey Auditory Verbal Learning Test Trial 1	.485	.690
Mental alternation Test	.740	.727

Appendix E-FIML CFA Models

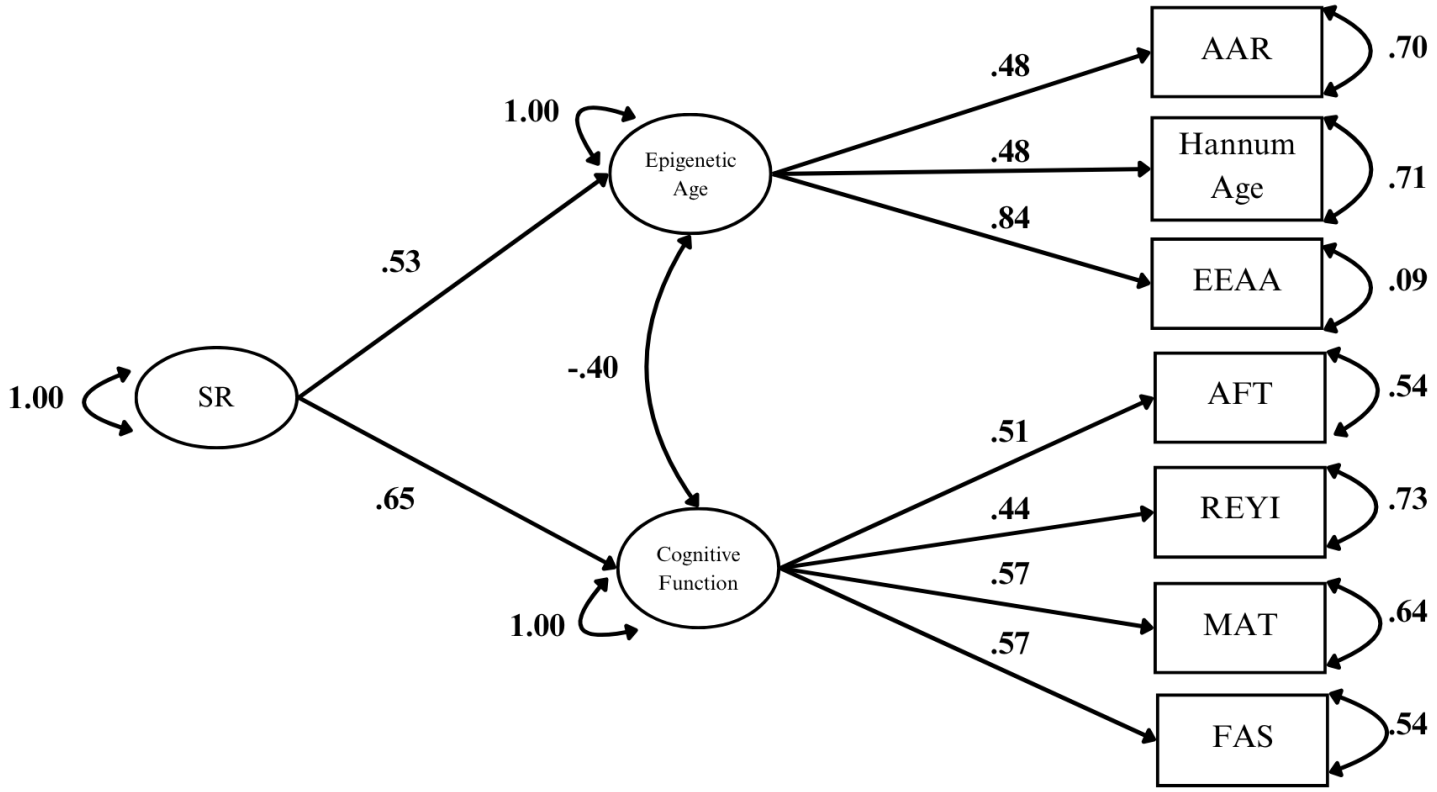


Figure E 1: Higher-order model with a superordinate factor representing SR for FIML sample

Note: Fit indices: Scaled CFI= .872, Scaled RMSEA (90% CI) = 0.129 (0.117, 0.141), SRMR= 0.084, AIC= 26909.23, BIC= 27036.39. AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test; SR=systemic resilience; FIML=full information maximum likelihood. Solid arrows from the latent construct to indicators represent measurement paths; solid arrows between latent constructs represent structural paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances; curved double-headed arrow represents correlations.

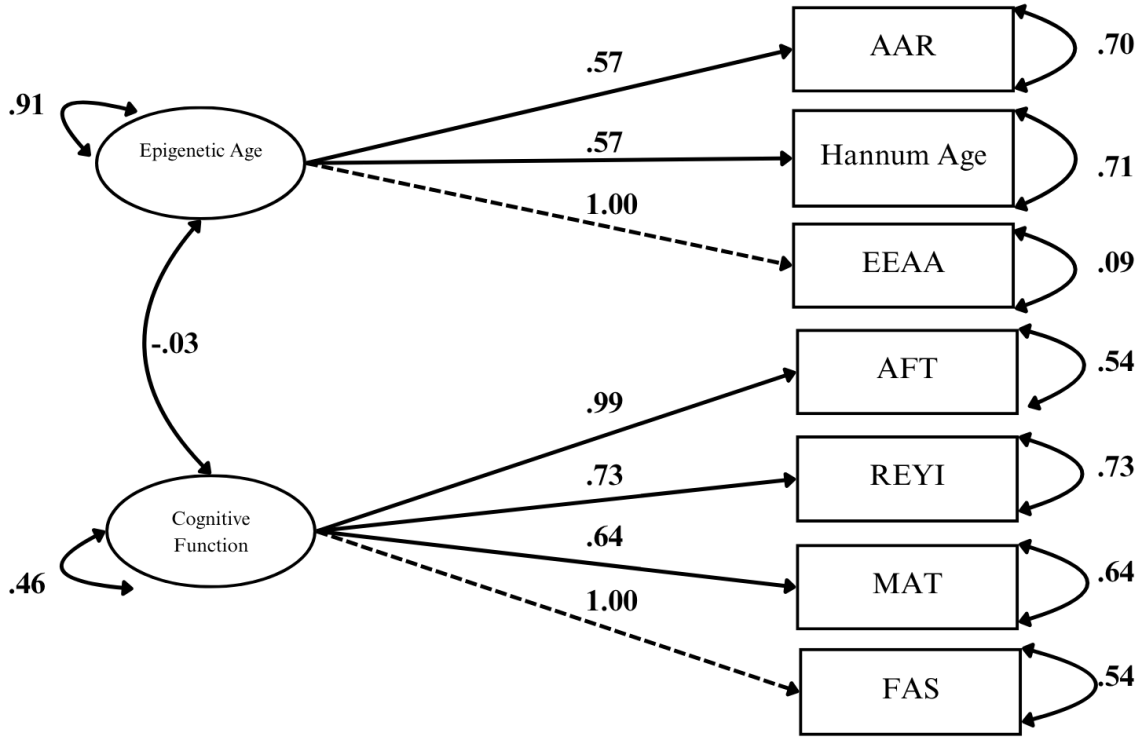


Figure E 2: Two-factor correlated model with epigenetic age and cognitive function as latent constructs for FIML sample

Note: Fit indices: Scaled CFI= .848, Scaled RMSEA (90% CI) = 0.129 (0.117, 0.141), SRMR= 0.084, AIC= 26905.23, BIC= 27021.79. AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test; SR=systemic resilience; FIML=full information maximum likelihood. Solid arrows from the latent construct to indicators represent measurement paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances; curved double-headed arrow represents correlations.

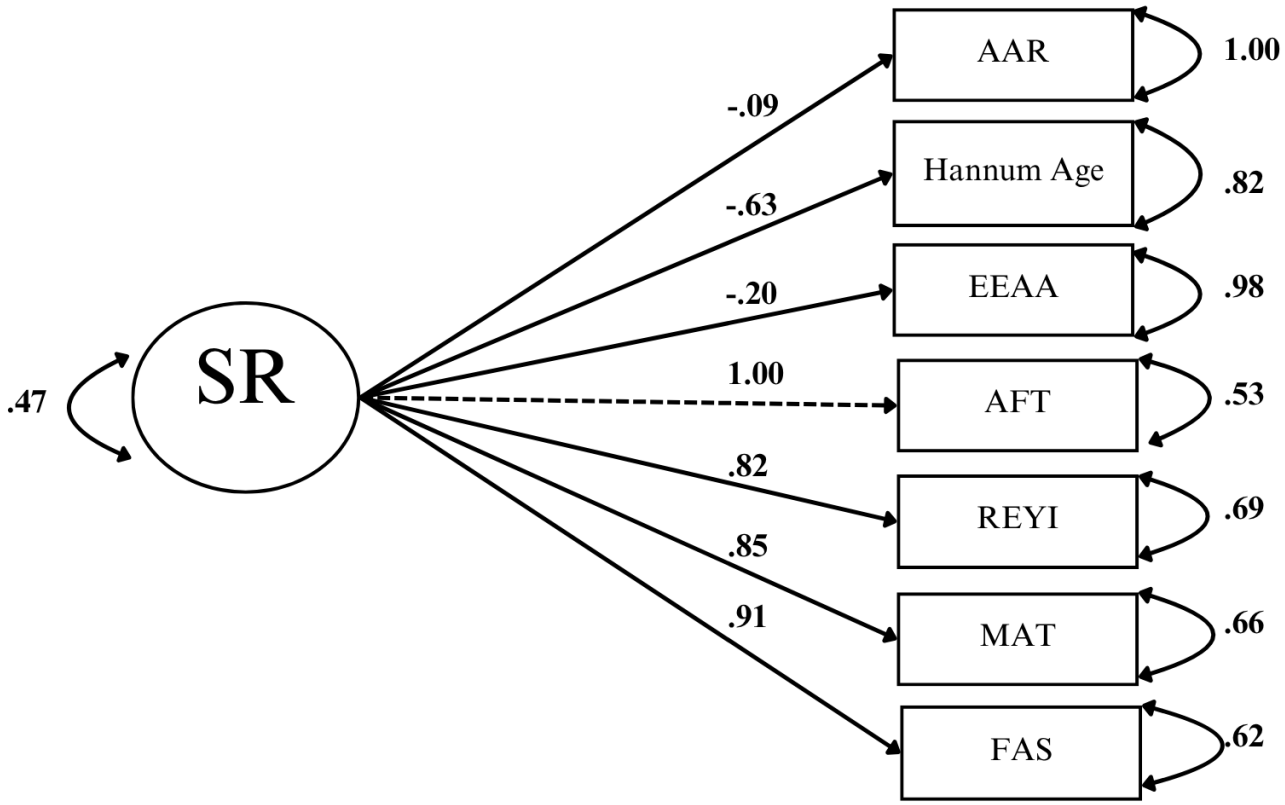


Figure E 3: Single-factor model with all indicators loading onto SR for FIML sample

Note: Fit indices: Scaled CFI=. 455, Scaled RMSEA (90% CI) = 0.235 (0.223, 0.247), SRMR= 0.131, AIC= 27646.09, BIC= 27757.36. AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REYI=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test; SR=systemic resilience; FIML=full information maximum likelihood. Solid arrows from the latent construct to indicators represent measurement paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances.

Appendix F-SEM Validation Models

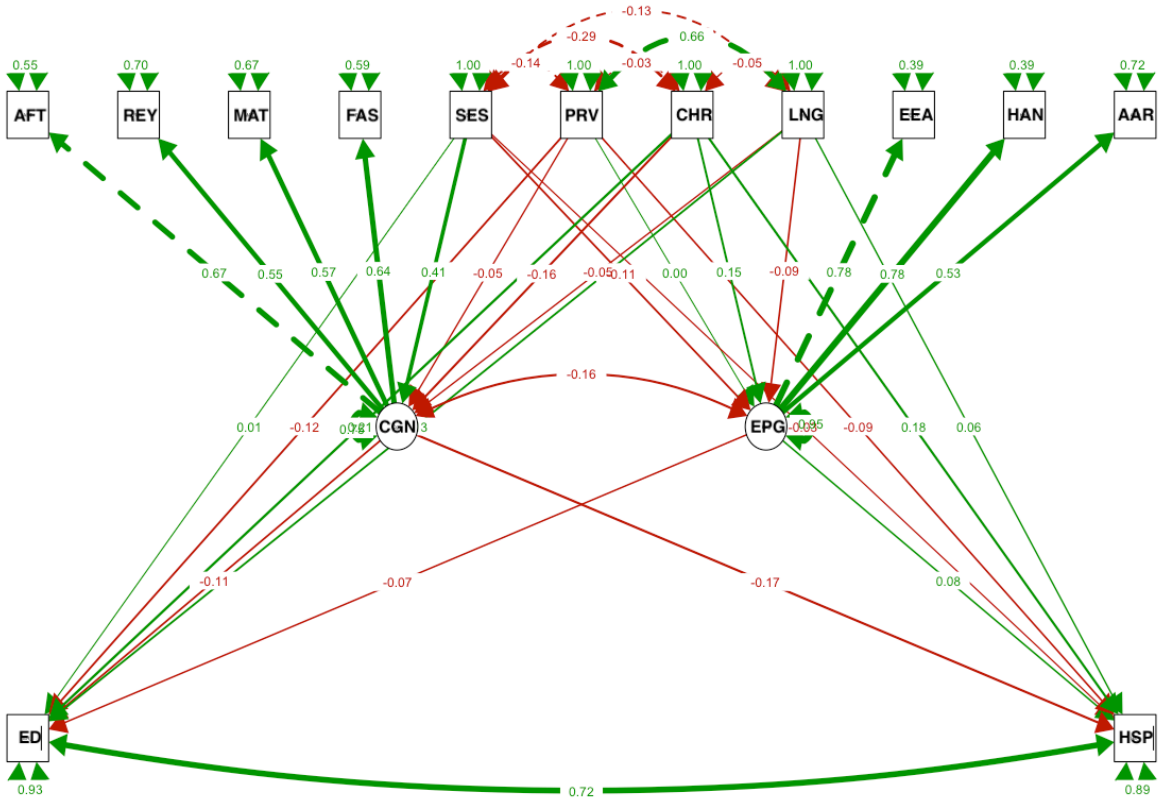


Figure F 1: Validation model with composite score created from income and education

Note : Fit indices : CFI =.773 ; SRMR=0.055 ; RMSEA=0.079, 90% CI= (0.072, 0.087). AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REY=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test; SR=systemic resilience; FIML=full information maximum likelihood; SES=socioeconomic status; PRV=province; CHR=chronic condition count; LNG=language; HAN=Hannum age; ED=emergency department visits; HSP=hospitalizations. Solid arrows from the latent construct to indicators represent measurement paths; curved double-headed arrows represent correlations; solid arrows from latent constructs to outcomes represent structural paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances.

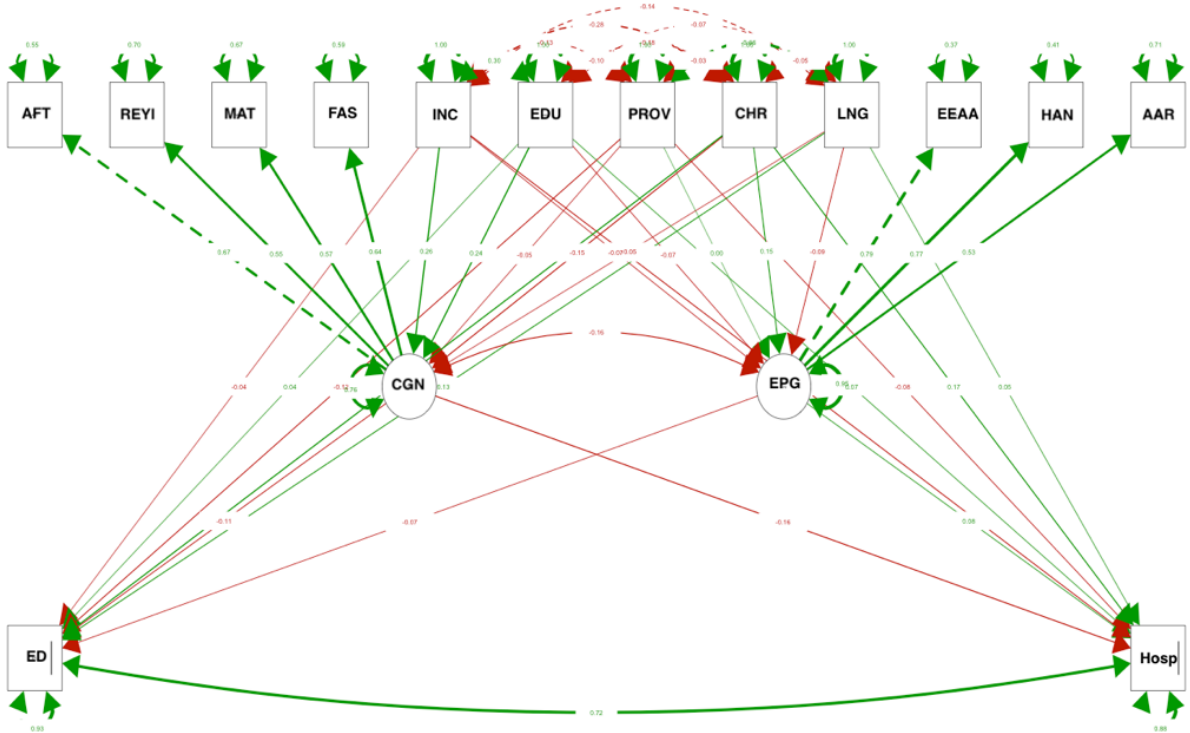


Figure F 2: Validation model with income and education modelled as separate covariates

Note: Fit indices : CFI =.779 ; SRMR=0.054 ; RMSEA=0.074, 90% CI= (0.067, 0.081). AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REY=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test; SR=systemic resilience; FIML=full information maximum likelihood; EDU=education; INC=annual total household income; PRV=province; CHR=chronic condition count; LNG=language; HAN=Hannum age; ED=emergency department visits; HSP=hospitalizations. Solid arrows from the latent construct to indicators represent measurement paths; curved double-headed arrows represent correlations; solid arrows from latent constructs to outcomes represent structural paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances.

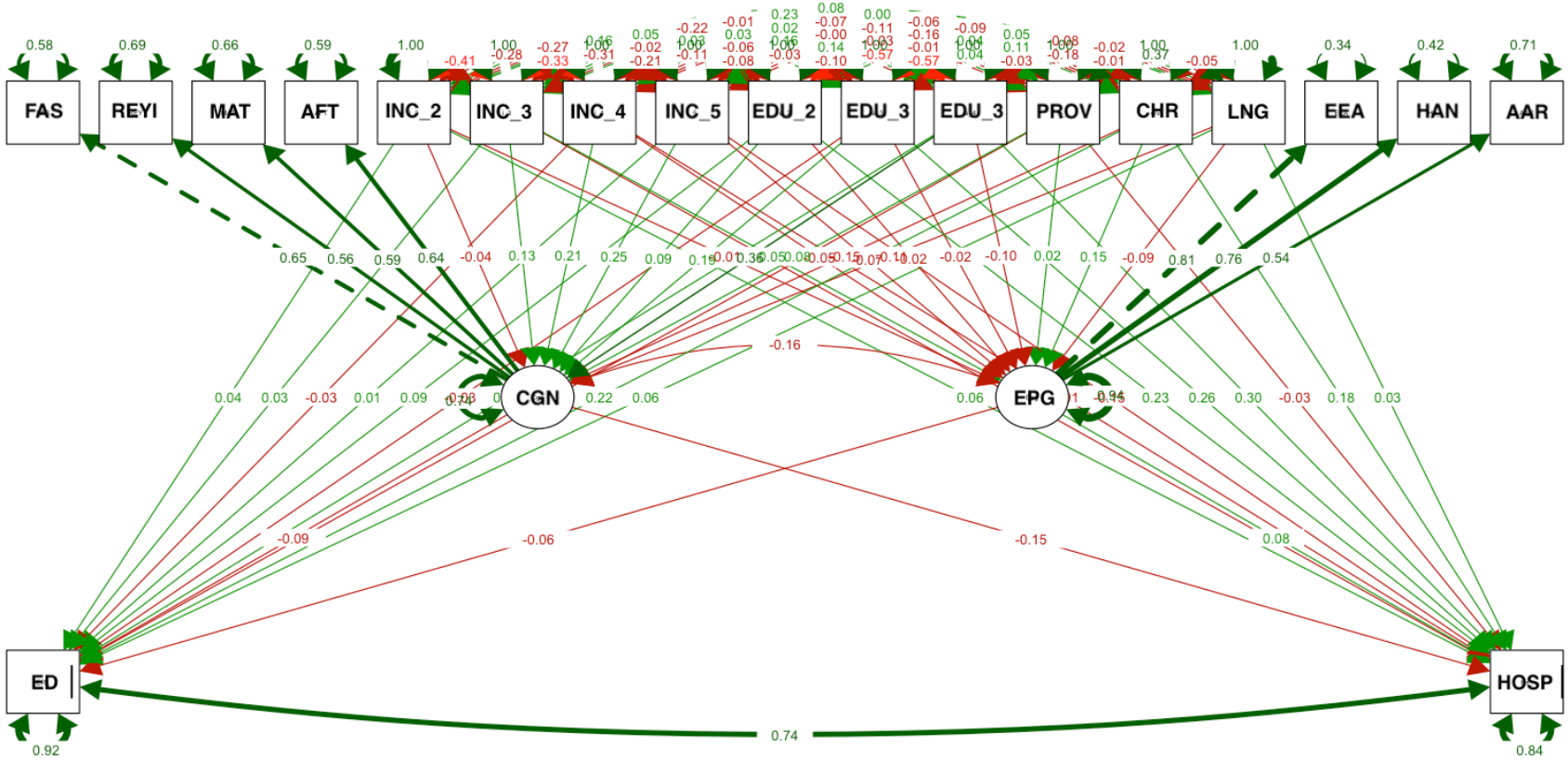


Figure F 3: Full final validation model with all covariates displayed

Note: Fit indices: CFI=.816; RMSEA=0.055, SRMR=0.051; 90% CI= (0.05, 0.061). AAR=age acceleration residual; EEAA=extrinsic epigenetic age acceleration; REY=Rey auditory verbal learning test trial 1; MAT=mental alternation test; FAS=controlled oral word association test; AFT=animal fluency test; SR=systemic resilience; FIML=full information maximum likelihood; EDU=education; INC=annual total household income; PRV=province; CHR=chronic condition count; LNG=language; HAN=Hannum age; ED=emergency department visits; HSP=hospitalizations. Solid arrows from the latent construct to indicators represent measurement paths; curved double-headed arrows represent correlations; solid arrows from latent constructs to outcomes represent structural paths; dashed arrows represent measurement paths fixed to 1.00; self-looping double-headed arrows represent variances.

Appendix G-Full Regression Output for Validation Model

Table G 1: Full regression output for validation model with regression coefficients and 95% CIs

Predictors	Regression coefficient estimate (b)	95% CI_b	Standardized coefficients (β)
ED Visits			
Epigenetic Age	-0.181	(-0.198, 0.036)	-.064
Cognitive Function	-0.144	(-0.326, 0.037)	-.091
Income (Ref: <$20k)			
$20k-$50k	0.086	(-0.279, 0.451)	.036
$50k-$100k	0.071	(-0.299, 0.441)	.032
$100k-$150k	-0.078	(-0.484, 0.329)	-.029
$150k+	0.014	(-0.396, 0.424)	.005
Education (Ref: < high school)			
High school diploma	0.345	(-0.085, 0.775)	.095
Some post-secondary education	-0.124	(-0.592, 0.344)	-.034
Post-secondary degree/diploma	0.266	(-0.110, 0.643)	.108
Province (Ref: low population provinces)			
High population provinces (ON, QB, BC)	-0.103	(-0.287, 0.081)	-.048
Chronic conditions	**0.086**	**(0.054, 0.118)**	**.221**
Language (Ref: English)			
French	0.163	(-0.059, 0.386)	.062
Hospitalizations			
Epigenetic Age	0.099	(-0.051, 0.249)	.076
Cognitive Function	**-0.254**	**(-0.461, -0.046)**	**-.154**
Income (Ref: <$20k)			
$20k-$50k	0.147	(-0.292, 0.587)	.060
$50k-$100k	-0.018	(-0.465, 0.430)	-.008
$100k-$150k	-0.020	(-0.524, 0.484)	-.007
$150k+	-0.412	(-0.976, 0.152)	-.145
Education (Ref: < high school)			
High school diploma	**0.861**	**(0.271, 1.452)**	**.229**

Some post-secondary education	**0.995**	**(0.392, 1.598)**	**.263**
Post-secondary degree/diploma	**0.766**	**(0.220, 1.311)**	**.301**
Province (Ref: low population provinces)			
High population provinces (ON, QB, BC)	-0.071	(-0.311, 0.170)	-.032
Chronic conditions	**0.071**	**(0.032, 0.110)**	**.176**
Language (Ref: English)			
French	0.073	(-0.227, 0.373)	.027
Cognitive Function			
Income (Ref: <$20k)			
$20k-$50k	-0.061	(-0.232, 0.111)	-.041
$50k-$100k	**0.178**	**(0.010, 0.346)**	**.128**
$100k-$150k	**0.362**	**(0.182, 0.542)**	**.214**
$150k+	**0.431**	**(0.241, 0.622)**	**.249**
Education (Ref: < high school)			
High school diploma	0.217	(-0.020, 0.454)	.095
Some post-secondary education	**0.443**	**(0.203, 0.683)**	**.193**
Post-secondary degree/diploma	**0.554**	**(0.343, 0.765)**	**.358**
Province (Ref: low population provinces)			
High population provinces (ON, QB, BC)	**0.115**	**(0.025, 0.206)**	**.085**
Chronic conditions	**-0.037**	**(-0.054, -0.021)**	**-.152**
Language (Ref: English)			
French	**-0.182**	**(-0.295, -0.068)**	**-.111**

Epigenetic Age			
Income (Ref: <$20k)			
$20k-$50k	-0.018	(-0.230, 0.193)	-.010
$50k-$100k	0.083	(-.132, 0.298)	.047
$100k-$150k	-0.101	(-0.333, 0.131)	-.047
$150k+	-0.143	(-0.382, 0.010)	-.066
Education (Ref: < high school)			
High school diploma	-0.071	(-0.340, 0.198)	-.025
Some post-secondary education	-0.059	(-0.332, 0.214)	-.020
Post-secondary degree/diploma	-0.200	(-0.429, 0.028)	-.103
Province (Ref: low population provinces)			
High population provinces (ON, QB, BC)	0.037	(-0.080, 0.154)	.022
Chronic conditions	**0.045**	**(0.025, 0.066)**	**.147**
Language (Ref: English)			
French	**-0.195**	**(-0.336, -0.542)**	**-.095**

Note: Cognitive function and epigenetic age are latent constructs. Significant pathways are bolded. ON=Ontario; QB=Quebec; BC=British Columbia

Appendix H-Full Regression Outputs for Moderation Analyses

Table H 1: Full regression output for sex moderation models color-coded based on group

Predictors	Regression coefficient estimate (b) (95% CI)	Standardized coefficient (β)
ED Visits		
Epigenetic Age	-0.103 (-0.274, 0.067)	-.078
	-0.087 (-0.268, 0.094)	-.064
Cognitive Function	-0.154 (-0.424, 0.117)	-.097
	-0.046 (-0.305, 0.213)	-.028
Income (Ref: <$20k)		
$20k-$50k	0.308 (-0.325, 0.942)	.117
	-0.066 (-0.551, 0.419)	-.029
$50k-$100k	0.153 (-0.458, 0.745)	.067
	-0.026 (-0.527, 0.475)	-.012
$100k-$150k	-0.054 (-0.696, 0.587)	-.021
	-0.099 (-0.668, 0.471)	-.033
$150k+	-0.001 (-0.648, 0.646)	-.000
	0.020 (-0.564, 0.604)	.006
Education (Ref: < high school)		
High school diploma	0.539 (-0.216, 1.294)	.141
	0.281 (-0.273, 0.834)	.079
Some post-secondary education	0.539 (-0.251, 1.328)	.131
	-0.552 (-1.169, 0.065)	-.162
Post-secondary degree/diploma	**0.706 (0.067, 1.346)**	**.264**
	-0.030 (-0.522, 0.462)	-.013
Province (Ref: low population provinces)		
High population provinces (ON, QB, BC)	0.000 (-0.268, 0.268)	.000
	-0.227 (-0.493 0.039)	-.103

Chronic conditions	**0.109 (0.057, 0.160)**	**.253**
	0.084 (0.038, 0.130)	**.225**
Language (Ref: English)		
French	0.107 (-0.225, 0.439)	.039
	0.237 (-0.070, 0.544)	.093
Hospitalizations		
Epigenetic Age	0.134 (-0.082, 0.349)	.099
	-0.059 (-0.292, 0.173)	-.042
Cognitive Function	-0.192 (-0.491, 0.107)	-.118
	-0.245 (-0.530, 0.039)	-.146
Income (Ref: <$20k)		
$20k-$50k	0.054 (-0.596, 0.705)	.020
	0.332 (-0.361, 1.024)	.143
$50k-$100k	-0.061 (-0.700, 0.578)	-.026
	0.049 (-0.664, 0.762)	.021
$100k-$150k	-0.137 (-0.826, 0.552)	-.052
	0.071 (-0.739, 0.881)	.023
$150k+	-0.552 (-1.299, 0.195)	-.209
	-0.277 (-1.199, 0.646)	-.086
Education (Ref: < high school)		
High school diploma	0.451 (-0.443, 1.345)	.115
	1.123 (0.215, 2.031)	**.306**
Some post-secondary education	0.840 (-0.143, 1.822)	.199
	1.102 (0.207, 1.997)	**.313**
Post-secondary degree/diploma	0.422 (-0.443, 1.286)	.153
	0.968 (0.143, 1.794)	**.398**
Province (Ref: low population provinces)		
High population provinces (ON, QB, BC)	-0.156 (-0.497, 0.186)	-.069
	0.034 (-0.349, 0.417)	.015
Chronic conditions	**0.071 (0.008, 0.134)**	**.161**
	0.084 (0.030, 0.138)	**.219**
Language (Ref: English)	0.142 (-0.331, 0.615)	.050
French	0.013 (-0.423, 0.449)	.005

Cognitive Function		
Income (Ref: <$20k)		
$20k-$50k	**-0.570 (-0.838, -0.301)**	**-.344**
	0.306 (0.087, 0.524)	**.221**
$50k-$100k	-0.140 (-0.396, 0.116)	-.097
	0.455 (0.238, 0.673)	**.334**
$100k-$150k	0.145 (-0.117, 0.407)	.090
	0.526 (0.282, 0.770)	**.288**
$150k+	0.170 (-0.100, 0.441)	.105
	0.687 (0.420, 0.953)	**.357**
Education (Ref: < high school)		
High school diploma	-0.079 (-0.419, 0.261)	-.033
	0.381 (0.066, 0.697)	**.174**
Some post-secondary education	0.058 (-0.289, 0.405)	.022
	0.621 (0.315, 0.928)	**.296**
Post-secondary degree/diploma	0.208 (-0.090, 0.506)	.123
	0.752 (0.483, 1.022)	**.519**
Province (Ref: low population provinces)		
High population provinces (ON, QB, BC)	**0.160 (0.035, 0.285)**	**.123**
	0.078 (-0.050, 0.206)	.058
Chronic conditions	**-0.061 (-0.085, -0.037)**	**.116**
	-0.031 (-0.053, -0.009)	**-.135**
Language (Ref: English)		
French	**-0.324 (-0.490, -0.157)**	**-.225**
	-0.059 (-0.206, -0.088)	-.038

Epigenetic Age		
Income (Ref: <$20k)		
$20k-$50k	-0.217 (-0.527, 0.093)	-.109
	0.232 (-0.032, 0.496)	.140
$50k-$100k	-0.045 (-0.356, 0.266)	-.026
	0.207 (-0.065, 0.479)	.127
$100k-$150k	**-0.354 (-0.673, -0.034)**	**-.183**
	-0.002 (-0.312, 0.308)	-.001
$150k+	**-0.352 (-0.679, -0.026)**	**-.181**
	-0.136 (-0.474, 0.201)	-.059
Education (Ref: < high school)		
High school diploma	0.196 (-0.185, 0.577)	.067
	-0.357 (-0.696, -0.017)	**-.137**
Some post-secondary education	0.017 (-0.375, 0.409)	.006
	-0.107 (-0.435, 0.221)	-.043
Post-secondary degree/diploma	-0.032 (-0.352, 0.287)	-.016
	-0.394 (-0.672, -0.116)	**-.228**
Province (Ref: low population provinces)		
High population provinces (ON, QB, BC)	0.039 (-0.119, 0.197)	.024
	0.050 (-0.106, 0.205)	.031
Chronic conditions	**0.082 (0.051, 0.112)**	**.251**
	0.046 (0.020, 0.072)	**.168**
Language (Ref: English)		
French	-0.170 (-0.361, 0.021)	-.082
	-0.166 (-0.353, 0.021)	-.089

Note: Cognitive function and epigenetic age are latent constructs. Significant pathways are bolded ($p<0.05$). Color coded based on group (blue for males, orange for females). ON=Ontario; QB=Quebec; BC=British Columbia

Table H 2: Full regression output for age moderation models color-coded based on group

Predictors	Regression coefficient estimate (b) (95% CI)	Standardized coefficient (β)
ED Visits		
Epigenetic Age	0.035 (-0.095, 0.164)	.030
	-0.121 (-0.260, 0.019)	-.109
Cognitive Function	-0.135 (-0.338, 0.068)	-.091
	-0.200 (-0.474, 0.075)	-.123
Income (Ref: <$20k)		
$20k-$50k	0.202 (-0.331, 0.736)	.074
	0.061 (-0.473, 0.596)	.028
$50k-$100k	0.098 (-0.431, 0.627)	.043
	0.139 (-0.414, 0.692)	.063
$100k-$150k	0.029 (-0.525, 0.583)	.012
	-0.183 (-0.840, 0.473)	-.057
$150k+	0.054 (-0.501, 0.608)	.022
	0.155 (-0.515, 0.826)	.041
Education (Ref: < high school)		
High school diploma	0.119 (-0.634, 0.876)	.028
	0.440 (-0.107, 0.987)	.139
Some post-secondary education	-0.158 (-0.954, 0.639)	-.037
	-0.153 (-0.755, 0.450)	-.048
Post-secondary degree/diploma	0.077 (-0.592, 0.745)	.027
	0.336 (-0.138, 0.811)	.152
Province (Ref: low population provinces)		
High population provinces (ON, QB, BC)	-0.063 (-0.310, 0.184)	-.029
	-0.155 (-0.440, 0.129)	-.071
Chronic conditions	**0.117 (0.068, 0.166)**	**.243**
	0.079 (0.035, 0.124)	**.215**
Language (Ref: English)		

French	0.197 (-0.092, 0.487)	.077
	0.127 (-0.227, 0.481)	.047
Hospitalizations		
Epigenetic Age	**0.195 (0.027, 0.363)**	**.164**
	0.042 (-0.126, 0.209)	.037
Cognitive Function	-0.187 (-0.431, 0.057)	-.124
	-0.291 (-0.581, -0.001)	**-.175**
Income (Ref: <$20k)		
$20k-$50k	0.132 (-0.525, 0.789)	.047
	0.210 (-0.441, 0.861)	.095
$50k-$100k	0.078 (0.605, 0.761)	.034
	-0.029 (-0.724, 0.666)	-.013
$100k-$150k	0.200 (-0.494, 0.894)	.078
	-0.225 (-0.104, 0.587)	-.069
$150k+	-0.174 (0.905, 0.557)	-.069
	-0.792 (-0.186, 2.804)	-.205
Education (Ref: < high school)		
High school diploma	0.633 (-0.462, 1.728)	.145
	0.957 (1.535, 1.760)	**.295**
Some post-secondary education	0.999 (-0.060, 2.059)	.229
	0.945 (0.077, 1.814)	**.289**
Post-secondary degree/diploma	0.462 (-0.541, 1.466)	.156
	0.940 (1.551, 1.724)	**.415**
Province (Ref: low population provinces)		
High population provinces (ON, QB, BC)	-0.181 (-0.546, 0.183)	-.081
	0.051 (-0.295, 0.397)	.023
Chronic conditions	**0.115 (0.052, 0.178)**	**.234**
	0.055 (0.002, 0.109)	**.145**
Language (Ref: English)	0.193 (-0.270, 0.656)	.073
French	-0.036 (-0.502, 0.430)	-.013
Cognitive Function		
Income (Ref: <$20k)		
$20k-$50k	-0.224 (-0.520, 0.071)	-.122

	0.107 (-0.127, 0.340)	.080
$50k-$100k	0.173 (-0.108, 0.453)	.113
	0.248 (0.011, 0.486)	**.183**
$100k-$150k	**0.290 (0.002, 0.578)**	**.171**
	0.497 (0.220, 0.774)	**.253**
$150k+	**0.404 (0.108, 0.700)**	**.241**
	0.356 (0.036, 0.675)	**.153**
Education (Ref: < high school)		
High school diploma	0.165 (-0.320, 0.649)	.057
	0.255 (0.039, 0.550)	.131
Some post-secondary education	0.393 (-0.081, 0.867)	.136
	0.488 (0.173, 0.802)	**.248**
Post-secondary degree/diploma	**0.571 (0.142, 1.00)**	**.293**
	0.521 (0.257, 0.785)	**.383**
Province (Ref: low population provinces)		
High population provinces (ON, QB, BC)	**0.149 (0.014, 0.284)**	**.101**
	0.122 (-0.021, 0.265)	.091
Chronic conditions	0.006 (-0.023, 0.035)	.018
	-0.025 (-0.050 .0.001)	**-.111**
Language (Ref: English)		
French	**-0.215 (-0.383, -0.046)**	**-.123**
	-0.254 (-0.443, -0.064)	**-.151**

Epigenetic Age		
Income (Ref: <$20k)		
$20k-$50k	-0.108 (-0.418, 0.202)	-.046
	0.010 (-0.359, 0.378)	.005
$50k-$100k	-0.051 (-0.346, 0.245)	-.026
	0.231 (-0.152, 0.614)	.116
$100k-$150k	-0.193 (-0.500, 0.114)	-.089
	0.141 (-0.305, 0.587)	.049
$150k+	-0.255 (-0.565, 0.055)	-.120
	0.330 (-0.132, 0.792)	.097
Education (Ref: < high school)		
High school diploma	**-0.669 (-1.176, -0.162)**	**-.183**
	0.232 (-0.162, 0.626)	.081
Some post-secondary education	**-0.558 (-1.066, -0.050)**	**-.153**
	0.168 (-0.225, 0.561)	.058
Post-secondary degree/diploma	**-0.639 (-1.088, -0.189)**	**-.258**
	0.075 (-0.256, 0.406)	.038
Province (Ref: low population provinces)		
High population provinces (ON, QB, BC)	0.032 (-0.130, 0.194)	.017
	0.018 (-0.184, 0.221)	.009
Chronic conditions	0.009 (-0.028, 0.047)	.023
	0.019 (-0.013, 0.052)	.058
Language (Ref: English)		
French	**-0.225 (-0.416, -0.035)**	**-.102**
	-0.053 (-0.314, 0.208)	-.022

Note: Cognitive function and epigenetic age are latent constructs. Significant pathways are bolded ($p<0.05$). Color coded based on group (blue for middle-aged, orange for older adults). ON=Ontario; QB=Quebec; BC=British Columbia

Appendix I-Age Correlations Heatmap

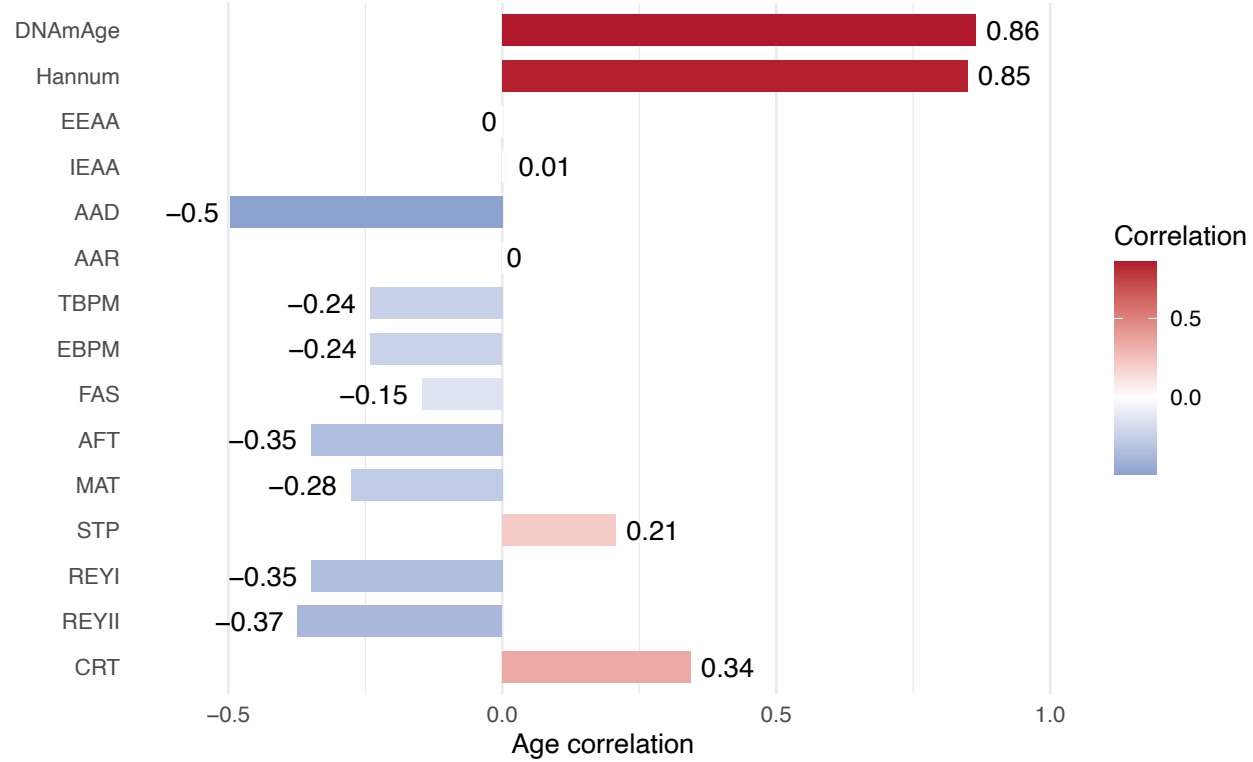


Figure I 1: Heatmap showing bivariate correlations between all indicator-level predictors and age

Note: STP=Stroop test; MAT=mental alternation test; FAS=controlled oral word association test; REYI=Rey auditory verbal learning test trial 1; EBPM=event-based prospective memory test; TBPM=time-based prospective memory test; REYII= Rey auditory verbal learning test trial 2; AFT=animal fluency test; CRT=choice reaction test; AAR=age acceleration residual; AAD=age acceleration difference; IEAA=intrinsic epigenetic age acceleration; EEAA=extrinsic epigenetic age acceleration.

Appendix J- Logistic Regression Models (Indicator-Level)

Table J 1: ORs and 95% CIs for fully adjusted logistic regression model

Outcome	Predictor	OR	95% CI	p-value	Adjusted p-value
Hospitalization	Age Acceleration Residual	1.00	(0.82, 1.24)	0.932	0.962
	Age Acceleration Difference	1.01	(0.79, 1.28)	0.932	0.962
	Intrinsic Epigenetic Age Acceleration	1.05	(0.85, 1.29)	0.651	0.962
	Extrinsic Epigenetic Age Acceleration	0.98	(0.79, 1.22)	0.947	0.962
	Hannum Age	0.96	(0.64, 1.44)	0.847	0.962
	DNAmAge	1.02	(0.67, 1.53)	0.932	0.962
	Choice Reaction Time	1.04	(0.83, 1.27)	0.700	0.900
	Rey Auditory Verbal Learning Test 2	0.98	(0.77, 1.25)	0.871	0.900
	Rey Auditory Verbal Learning Test 1	0.93	(0.73, 1.18)	0.560	0.900
	Stroop Test	0.89	(0.67, 1.12)	0.393	0.900
	Mental Alternation Test	0.96	(0.78, 1.20)	0.749	0.900
	Animal Fluency Test	**0.79**	**(0.61, 1.00)**	**0.052**	**0.468**
	Controlled Oral Word Association Test	0.94	(0.75, 1.18)	0.617	0.900
	Event-Based Prospective Memory	0.95	(0.79, 1.18)	0.656	0.900
	Time-Based Prospective Memory	0.99	(0.81, 1.24)	0.900	0.900
Emergency Department Visits	Age Acceleration Residual	0.86	(0.74, 1.00)	0.054	0.162
	Age Acceleration Difference	0.84	(0.70, 1.00)	0.054	0.162
	Intrinsic Epigenetic Age Acceleration	**0.86**	**(0.74, 1.00)**	**0.051**	0.162
	Extrinsic Epigenetic Age Acceleration	0.95	(0.82, 1.11)	0.526	0.526

Hannum Age	0.90	(0.67, 1.19)	0.454	0.526
DNAmAge	0.74	(0.55, 1.00)	0.054	0.162
Choice Reaction Time	1.06	(0.90, 1.24)	0.473	0.965
Rey Auditory Verbal Learning Test 2	0.88	(0.75, 1.04)	0.128	0.965
Rey Auditory Verbal Learning Test 1	0.90	(0.76, 1.06)	0.196	0.965
Stroop Test	0.94	(0.79, 1.09)	0.479	0.965
Mental Alternation Test	**0.84**	**(0.72, 0.98)**	**0.022**	**0.198**
Animal Fluency Test	0.96	(0.82, 1.13)	0.662	0.965
Controlled Oral Word Association Test	0.96	(0.82, 1.12)	0.578	0.965
Event-Based Prospective Memory	1.00	(0.86, 1.17)	0.965	0.965
Time-Based Prospective Memory	0.95	(0.81, 1.12)	0.507	0.965

Note: CI= confidence interval; OR=odds ratio; significant pathways bolded

Table J 2: ORs and 95% CIs for logistic regression model with all covariates except for chronic condition load

Outcome	Predictor	OR	95% CI
Hospitalization	Age Acceleration Residual	1.04	(0.85, 1.27)
	Age Acceleration Difference	1.05	(0.83, 1.32)
	Intrinsic Epigenetic Age Acceleration	1.08	(0.88, 1.31)
	Extrinsic Epigenetic Age Acceleration	1.01	(0.81, 1.25)
	Hannum Age	1.01	(0.68, 1.51)
	DNAmAge	1.09	(0.73, 1.62)
	Choice Reaction Time	1.03	(0.83, 1.26)

	Rey Auditory Verbal Learning Test 2	0.95	(0.75, 1.21)
	Rey Auditory Verbal Learning Test 1	0.90	(0.71, 1.14)
	Stroop Test	0.92	(0.70, 1.14)
	Mental Alternation Test	0.94	(0.76, 1.17)
	Animal Fluency Test	**0.78**	**(0.61, 0.99)**
	Controlled Oral Word Association Test	0.94	(0.75, 1.17)
	Event-Based Prospective Memory	0.97	(0.80, 1.20)
	Time-Based Prospective Memory	0.96	(0.79, 1.19)
Emergency Department Visits	Age Acceleration Residual	0.89	(0.77, 1.03)
	Age Acceleration Difference	0.88	(0.74, 1.04)
	Intrinsic Epigenetic Age Acceleration	0.89	(0.77, 1.03)
	Extrinsic Epigenetic Age Acceleration	0.97	(0.83, 1.12)
	Hannum Age	0.92	(0.70, 1.22)
	DNAmAge	0.79	(0.59, 1.06)
	Choice Reaction Time	1.05	(0.89, 1.22)
	Rey Auditory Verbal Learning Test 2	0.87	(0.74, 1.02)
	Rey Auditory Verbal Learning Test 1	0.88	(0.74, 1.03)
	Stroop Test	0.97	(0.82, 1.12)
	Mental Alternation Test	**0.83**	**(0.71, 0.96)**
	Animal Fluency Test	0.96	(0.82, 1.13)
	Controlled Oral Word Association Test	0.95	(0.81, 1.11)

Event-Based Prospective Memory	1.00	(0.87, 1.18)
Time-Based Prospective Memory	0.92	(0.79, 1.08)

Note: CI= confidence interval; OR=odds ratio; significant pathways bolded