From the Department of Medical Epidemiology and Biostatistics
Karolinska Institutet, Stockholm, Sweden

GENETICS AND BIOMARKERS OF FRAILTY: TOWARDS INDIVIDUALIZED MANAGEMENT OF THE FRAILTY SYNDROME

MAK, Ka Long Jonathan

麥家朗

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Genetics and biomarkers of frailty: Towards individualized management of the frailty syndrome

Thesis for Doctoral Degree (Ph.D.)

By

MAK, Ka Long Jonathan

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Principal Supervisor:
Docent Juulia Jylhävä
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Co-supervisors:
Docent Sara Hägg
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Docent Ralf Kuja-Halkola
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Prof. Chandra Reynolds
University of Colorado Boulder
Institute for Behavioral Genetics

Opponent:
Prof. Emer. Kaisu Pitkälä
University of Helsinki
Department of General Practice and Primary Health Care

Examination Board:
Docent Chengxuan Qiu
Karolinska Institutet
Department of Neurobiology, Care Sciences and Society
Division of Aging Research Center

Docent Serhiy Dekhtyar
Karolinska Institutet
Department of Neurobiology, Care Sciences and Society
Division of Aging Research Center

Prof. Valgeir Thorvaldsson
University of Gothenburg
Department of Psychology

To Mum, Dad, and Yannis, whose unwavering support and belief in me have been instrumental in shaping who I am today

To Forever, whose love and encouragement have made every step of this journey worthwhile

Popular science summary of the thesis

Aging affects people differently. Some become sick and disabled early on, while others remain healthy well into their golden years. This variability in the rate of aging underlies the concept of "frailty" – a state of increased vulnerability resulting from the depletion of our body's in-built physiological reserves during aging. Compared to robust older adults, frail individuals are more prone to negative health events like falls, hospitalizations, and early death. To pave the way for more personalized patient care, it is imperative to understand the mechanisms behind frailty and create standardized tools for early detection.

Quantitative genetics: estimating the heritability of frailty

Quantitative genetic analysis is a powerful statistical method that leverages the varying genetic relatedness between individuals, such as identical twins (sharing 100% of genes) and non-identical twins (sharing roughly 50% of genes), to study the contributions of genetic and environmental factors to individual differences observed in a trait. This method is especially useful when exploring the "heritability" of complex traits – the proportion of variation in the trait explained by genetic differences between individuals. Through advanced statistical models, we can also evaluate how factors like sex, age, and environment affect genetic influences on frailty.

In **Study I**, we worked with data from 42,994 Swedish twins and found that around half of the variation in frailty is influenced by genetic factors, and the other half is attributable to unique environmental factors specific to each individual. We also discovered a higher heritability of frailty in women compared to men, as well as in underweight and obese groups. In our subsequent investigation in **Study II**, which involved 2,496 Swedish twins monitored over 27 years, we found that while genetic influences on frailty remained relatively stable as individuals aged, unique environmental influences notably increased, indicating that factors like lifestyle behaviors, injuries, and diseases gain importance over genes in shaping frailty during late life. These findings shed light on the interplay between genes and the environment in influencing frailty throughout a person's lifespan and provide the foundation for further research into the specific genetic markers associated with frailty.

Epigenetic & metabolic biomarkers: uncovering frailty mechanisms

Biomarkers are measurable indicators of biological processes and can range from simple measurements like blood pressure to complex molecules detected in laboratory tests. Identifying novel biomarkers of frailty, such as epigenetic and metabolic biomarkers, can provide valuable insights into the biological mechanisms driving frailty.

In **Study III**, we focused on a type of epigenetic biomarker called DNA methylation, which refers to the addition of chemical tags at specific DNA locations (known as CpG sites) that can switch genes "on" and "off" without changing the DNA sequence. In two datasets comprising Swedish and Danish participants, we found that frailty correlates with DNA methylation of several CpG sites that may involve in cancer and neuronal pathways. In **Study IV**, we analyzed 200 metabolic biomarkers across large population datasets from the UK, Sweden, and Finland. We observed strong links between frailty and 34 of these biomarkers, such as amino acids and cholesterols. By leveraging genetic data to study causal relationships, we further demonstrated that higher levels of glycoprotein acetyls, an inflammation marker, could potentially cause a higher risk of frailty. This suggests chronic inflammation, a long-lasting state of immune system reaction, may play an important role in frailty development.

Electronic frailty index: a promising tool for frailty screening

Despite the impact of frailty on individuals' health, routine assessment and standardized measurement of frailty are currently lacking in clinical settings, particularly in Sweden. Some geriatric clinics in Stockholm have started using the Clinical Frailty Scale, but this method demands additional time and resources, which may not always be feasible in time-pressed clinical environments. An alternative is to generate an automated frailty score from routinely collected electronic health records, eliminating the need for extra data collection.

In **Study V**, we developed an "electronic frailty index" using disease, functioning, and laboratory data from the electronic health records of 13,188 geriatric patients in Stockholm. Our results showed that this index predicts mortality outcomes more accurately than existing frailty measures. By potentially integrating it into the Swedish health system, the electronic frailty index holds great promise for risk stratification and informing clinical decisions.

論文科普摘要 Popular science summary (in Chinese)

每個人的衰老過程都不盡相同，有些人可能在中年就患上慢性病或出現殘疾，有些人則可能在晚年仍能保持健康。「衰弱」（frailty）是一個反映我們在衰老過程中整體生理功能下降的概念，與健康人士相比，衰弱人士一般有較高的健康風險，例如更加容易跌倒、生病住院、甚至死亡等等。面對日漸加劇的人口老化問題，預防或延緩長者出現衰弱狀況變得尤為重要。為此，我們需要進一步了解衰弱的機制和病理，以期能在社區中實行早期篩檢和預防措施。

定量遺傳學：計算衰弱的遺傳度

定量遺傳分析（quantitative genetics）是統計學中利用親緣關係來計算遺傳度（heritability）的方法，而遺傳度是指某一特徵在人與人之間的差異有多大比例是由遺傳因素所決定。由於同卵雙胞胎的基因完全相同，而異卵雙胞胎的基因則只有 50% 相同，我們可以透過比較衰弱在兩者之間的相似度，計算出衰弱有多大程度是分別由遺傳和環境因素所影響。此外，我們更可以利用統計模型來估算性別、年齡及其他環境因素如何改變遺傳因素對衰弱的影響。我們在**研究一**利用了來自 42,994 名瑞典雙胞胎的數據，計算出衰弱在人群中的差異大約有一半是由遺傳因素所決定的，而另一半則由個人環境因素所影響。此外，衰弱在女性、體重過輕及肥胖人士中都有較高的遺傳度。在隨後的**研究二**中，我們對 2,496 名瑞典雙胞胎進行了長達 27 年的追蹤研究，發現隨著年齡增長，遺傳因素對衰弱的影響沒有太大改變，而環境因素的影響則顯著增加。該研究揭示出衰弱在老年時有較大程度是由生活習慣、傷患等環境因素所影響，這些結果更為進一步研究衰弱的基因分子奠定了基礎。

表觀遺傳及代謝生物標記：了解衰弱的機制

「生物標記」（biomarker）一般是指可用作測量和反映生物過程的指標，例如血壓或血液內的化學物質。而研究衰弱的生物標記，例如表觀遺傳（epigenetic）和代謝（metabolic）標記，有助於我們了解它的病理生理機制。表觀遺傳是指在不改變 DNA 序列的情況下影響基因表達的途徑，例如「DNA 甲基化」（DNA methylation），即細胞在 DNA 特定位置上（名為 CpG 位點）添加或刪除化學標記來控制基因表達的抑制或激活。在**研究三**中，我們利用了來自北歐的兩個研究數據，分析了「DNA 甲基化」與衰弱的關聯。結果發現，衰弱與涉及癌症和神經系統的 CpG 位點相關。在**研究四**中，我們分析了英國、瑞典和芬蘭三個大型研究數據中的 200 個代謝生物標記，發現衰弱與其中 34 個標記物（例如氨基酸和膽固醇）具有很

強的關聯。此外，我們利用基因數據估算了代謝生物標記與衰弱之間的因果關係，發現一種炎症標記 ——「乙酰基糖蛋白」（glycoprotein acetyls），能導致較高的衰弱風險。該研究結果反映出慢性炎症（即一種持續的免疫系統反應）可能是導致衰弱發生的重要機制。

電子衰弱指數：自動化的衰弱檢測工具

儘管衰弱會對老年人的健康造成極大的危害，但目前瑞典的臨床醫療中仍缺乏對衰弱的統一評估標準。近年，斯德哥爾摩一些老年護理醫院已經開始使用「臨床衰弱量表」（Clinical Frailty Scale）作為檢測衰弱的工具，但由於醫護人員需要花額外時間為病人進行評估，導致只有一部分醫院有足夠資源開展衰弱檢測。另一種可行的替代方法是從已有的電子健康記錄中生成衰弱評分，從而無需收集額外數據也能為病人進行實時的衰弱評估。我們在**研究五**利用了 13,188 名斯德哥爾摩老年病人的醫療記錄，在結合病人的疾病、身體機能和血液檢查數據後，開發了一個「電子衰弱指數」，並發現該指數比現有的衰弱測量工具能更加準確地預測未來的死亡風險。如果將來能把「電子衰弱指數」成功整合到瑞典的醫療系統，通過該指數反映老年病人的衰弱程度，便可更有效地為病人進行風險分層，以助醫護人員及早為病人提供適切的治療。

Abstract

Frailty is an age-related, dynamic state of multisystem physiological decline and is a strong predictor of disability and mortality. To move towards an individualized management of frailty, a better understanding of its biological underpinnings and an early identification of frail older adults are necessary. The overarching aim of this thesis was to unravel the genetic, epigenetic, and metabolic determinants of frailty and to develop an automated frailty assessment tool for the Swedish health system.

In **Study I,** we calculated a frailty index (FI) for 42,994 Swedish twins and assessed sex differences in the genetic and environmental contributions to the FI. Overall, we observed a higher heritability of the FI in women (52%) than in men (45%). Moreover, the correlations between FI and its two main risk factors, body mass index and education, were mainly attributable to genetic factors and environmental factors shared within twin pairs, respectively, suggesting that different mechanisms may underlie these associations.

In **Study II**, we examined genetic and environmental influences on the FI trajectories in 2,496 twins followed up to 27 years. A bilinear latent growth curve model best fit the data, indicating a four-to-five times faster FI increase after age 75. While genetic influences were relatively stable across age, individual-specific environmental influences increased substantially after age 75 especially in men, amplifying the overall FI variance in late life.

In **Study III**, we performed an epigenome-wide analysis in 526 Swedish twins and identified 171 CpG sites associated with the FI at a false discovery rate of <0.05. Many of the identified sites have previously been associated with chronological age and age-related diseases. We further validated five of these sites in an independent sample of 304 Danish twins, which are mapped to genes that may involve in cancer and neurological pathways.

In **Study IV**, we explored the associations of 168 metabolomic and 32 clinical biomarkers with two measures of frailty using observational and Mendelian randomization analyses. In three population-based studies comprising >100,000 individuals, we identified 34 biomarkers independently and robustly associated with the FI. Specifically, we highlighted a putative causal effect of glycoprotein acetyls, an inflammatory biomarker, on frailty.

In **Study V**, we developed an electronic frailty index (eFI) using electronic health records from 18,225 patients admitted to nine geriatric clinics in Stockholm. Among the assessed frailty and comorbidity measures, the eFI had the best discriminative ability for mortality.

In summary, this thesis provides novel insights into the biological mechanisms of frailty, suggesting that both genetic and environmental factors play important roles in frailty development, with chronic inflammation as the key underlying mechanism. Moreover, our developed Swedish eFI is a promising tool that can potentially be incorporated in the Swedish health system to guide clinical decisions.

List of scientific papers

I. **Mak JKL**, Reynolds CA, Hägg S, Li X, Ericsson M, Pedersen NL, Jylhävä J, Kuja-Halkola R. Sex differences in genetic and environmental influences on frailty and its relation to body mass index and education. *Aging (Albany NY)*. 2021;13(13):16990–17023.

II. **Mak JKL**, Kuja-Halkola R, Bai G, Hassing LB, Pedersen NL, Hägg S, Jylhävä J, Reynolds CA. Genetic and environmental influences on longitudinal frailty trajectories from adulthood into old age. *The Journals of Gerontology: Series A*. 2023;78(2):333–341.

III. **Mak JKL**, Skovgaard AC, Nygaard M, Kananen L, Reynolds CA, Wang Y, Kuja-Halkola R, Karlsson IK, Pedersen NL, Hägg S, Soerensen M, Jylhävä J. Epigenome-wide analysis of frailty: results from two European twin cohorts. (*Submitted*).

IV. **Mak JKL**, Kananen L, Qin C, Kuja-Halkola R, Tang B, Lin J, Wang Y, Jääskeläinen T, Koskinen S, Lu Y, Magnusson PKE, Hägg S, Jylhävä J. Unraveling the metabolic underpinnings of frailty using multicohort observational and Mendelian randomization analyses. *Aging Cell*. 2023;22(8):e13868.

V. **Mak JKL**, Hägg S, Eriksdotter M, Annetorp M, Kuja-Halkola R, Kananen L, Boström AM, Kivipelto M, Metzner C, Bäck Jerlardtz V, Engström M, Johnson P, Lundberg LG, Åkesson E, Sühl Öberg C, Olsson M, Cederholm T, Jylhävä J, Religa D. Development of an electronic frailty index for hospitalized older adults in Sweden. *The Journals of Gerontology: Series A*. 2022;77(11):2311–2319.

Related papers not included in the thesis

- **Mak JKL**, Kuja-Halkola R, Wang Y, Hägg S, Jylhävä J. Frailty and comorbidity in predicting community COVID-19 mortality in the U.K. Biobank: The effect of sampling. *Journal of the American Geriatrics Society*. 2021;69(5):1128–1139.
- **Mak JKL**, Eriksdotter M, Annetorp M, Kuja-Halkola R, Kananen L, Boström AM, Kivipelto M, Metzner C, Bäck Jerlardtz V, Engström M, Johnson P, Lundberg LG, Åkesson E, Sühl Öberg C, Olsson M, Cederholm T, Hägg S, Religa D, Jylhävä J. Two years with COVID-19: the electronic frailty index identifies high-risk patients in the Stockholm GeroCovid study. *Gerontology*. 2023;69(4):396–405.
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- **Mak JKL**, Religa D, Jylhävä J. Automated frailty scores: towards clinical implementation. *Aging (Albany NY).* 2023;15(11):4571–4573.
- **Mak JKL*,** McMurran CE*, Hägg S. Clinical biomarker-based biological ageing and future risk of neurological disorders in the UK Biobank. (*Submitted*). *Contributed equally
- **Mak JKL**, Karlsson IK, Tang B, Wang Y, Pedersen NL, Hägg S, Jylhävä J, Reynolds CA. Temporal dynamics of epigenetic aging and frailty from midlife to old age. (*Submitted*).
- Kananen L, Hong X, Annetorp M, **Mak JKL**, Jylhävä J, Eriksdotter M, Hägg S, Religa D. Health progression for Covid-19 survivors hospitalized in geriatric clinics in Sweden. *PLoS One*. 2023;18(3):e0283344.
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- Bai G, Wang Y, **Mak JKL**, Ericsson M, Hägg S, Jylhävä J. Is frailty different in younger adults compared to old? Prevalence, characteristics and risk factors of early-life and late-life frailty in samples from Sweden and UK. *Gerontology*. 2023. Online ahead of print.

Contents

List of abbreviations

A	Additive genetic factors
AIC	Akaike information criterion
AUC	Area under the receiver operating characteristic curve
BMI	Body mass index
C	Common/shared environmental factors
CCI	Charlson Comorbidity Index
CFS	Clinical Frailty Scale
CGA	Comprehensive geriatric assessment
CI	Confidence interval
CpG	Cytosine-phosphate-Guanine
CRP	C-reactive protein
D	Dominance genetic factors
DMR	Differentially methylated region
DZ	Dizygotic
E	Unique/non-shared environmental factors
eFI	Electronic frailty index
EHR	Electronic health record
EWAS	Epigenome-wide association study
FDR	False discovery rate
FI	Frailty index
FP	Frailty phenotype
GlycA	Glycoprotein acetyls
GWAS	Genome-wide association study
H	Total genetic factors/broad-sense heritability
HbA1c	Glycated hemoglobin
HFRS	Hospital Frailty Risk Score
HR	Hazard ratio
ICD	International Classification of Diseases

IPT	In-person testing
IV	Instrumental variable
IVW	Inverse variance weighted
LASSO	Least absolute shrinkage and selection operator
LDL	Low-density lipoprotein
LSADT	Longitudinal Study of Aging Danish Twins
MR	Mendelian randomization
MR-PRESSO	MR-pleiotropy residual sum and outlier
MZ	Monozygotic
NMR	Nuclear magnetic resonance
OCTO-Twin	Origins of Variance in the Oldest-Old: Octogenarian Twins
OR	Odds ratio
Q	Questionnaire
RCT	Randomized controlled trial
SALT	Screening Across the Lifespan Twin Study
SATSA	Swedish Adoption/Twin Study on Aging
SD	Standard deviation
SNP	Single nucleotide polymorphism

1 Introduction

As life expectancy rises and fertility rates drop globally, the world is witnessing a rapid growth of the older population. This demographic shift presents substantial challenges for healthcare and social systems.[1] Among these challenges, frailty stands out as a particularly problematic aspect of population aging,[2] affecting around 20% of older adults.[3] Research has consistently shown a strong association between frailty and a variety of adverse outcomes beyond chronological age, such as premature mortality, loss of activities of daily living, hospitalization, physical limitations, and falls.[4] Over the past two decades, a range of frailty scales have been developed and proven to be valuable tools in understanding the heterogeneity in aging and guiding clinical decisions for care of older adults.[5] As frailty is potentially reversible until reaching a critical point of no return,[6] early identification and intervention are crucial to delaying, and even preventing disability and morbidity in old age.

Due to the complex and multifactorial nature of frailty, unraveling its underlying causes has been challenging.[7] However, by employing twin and longitudinal study designs, we can gain new insights into how genes and the environment influence frailty across the lifespan.[8] Additionally, the recent advancements in high-throughput "omics" analysis, including epigenomics and metabolomics, coupled with machine learning and causal inference methods, present new opportunities to explore the biomarkers and molecular mechanisms of frailty.[9] On the other hand, how frailty can be used and integrated in clinical practice remains unclear. The currently available frailty assessments often require additional time and resources; therefore, there is a need to develop an automated, efficient frailty screening tool, which is tailored to each country and with good predictive performance for adverse outcomes, to facilitate the clinical application of frailty.

This thesis consists of five studies. The first four studies focus on the biological mechanisms contributing to frailty, where we investigated the heritability, and the epigenetic and metabolic biomarkers of frailty using data from several population-based cohorts. In the fifth study, we developed a frailty screening tool that could help physicians identify high-risk older adults within the Swedish health system. Throughout the thesis, we also highlight potential sex differences and longitudinal changes in frailty.

2 Literature review

2.1 Biological aging

As we age, our bodies experience a gradual decline across various biological levels, from molecular and cellular processes (e.g., telomere attrition, epigenetic alterations, cellular senescence)[10] to physiological changes in different organ systems.[11] This cumulative degradation ultimately leads to an increased susceptibility to diseases, disability, and mortality.[12] However, it is important to note that individuals of the same age can exhibit different health statuses and levels of vulnerability to adverse outcomes.[13,14] This heterogeneity in aging has led to the development of biological age measures, including telomere length, epigenetic ages, clinical biomarker-based algorithms, and frailty, which aim to quantify the impact of biological aging on health.[15,16] These measures usually capture age-related changes at different biological scales.[17] Specifically, frailty can be considered as a system-level measure of aging that reflects an overall functional decline and can be used as a clinical tool to identify individuals at risk of adverse outcomes.[5]

2.2 Definition of frailty

Frailty is commonly defined as a syndrome, or more generally, as a clinical state of reduced physiological reserve and increased vulnerability to stressors, caused by an age-related decline in functioning across multiple organ systems.[2,18] Taking a holistic view in terms of a complex dynamical system, frailty can be conceptualized as a phenomenon that emerges when various interconnecting and interacting physiological systems fail to maintain homeostasis.[19] As illustrated in **Figure 1**, when facing minor stressors (e.g., infection, surgery), robust individuals often experience a small decline in functioning and are able to return to homeostasis within a relatively short period of time.[2] In contrast, frail individuals whose physiological reserves have already been depleted are more vulnerable to adverse outcomes when facing minor stressors, and are unable to return to their baseline homeostasis.[2] Frailty has been described as one of the best predictors of mortality among the existing biological age measures.[17,20] Its association with all-cause and cause-specific mortality has been demonstrated in different populations and settings,[21–24] and this association appears to be independent of shared familial factors such as genetic and childhood environmental factors.[22] Other than mortality, frailty has also been shown to predict a wide range of negative health outcomes such as falls,[25] fractures,[26] disabilities,[27] cardiovascular diseases,[28,29] cognitive impairment and dementia,[30,31] psychiatric illnesses,[32] worse quality of life,[33] hospitalizations,[34] severe COVID-19 infection,[35] and increased healthcare costs.[36,37]

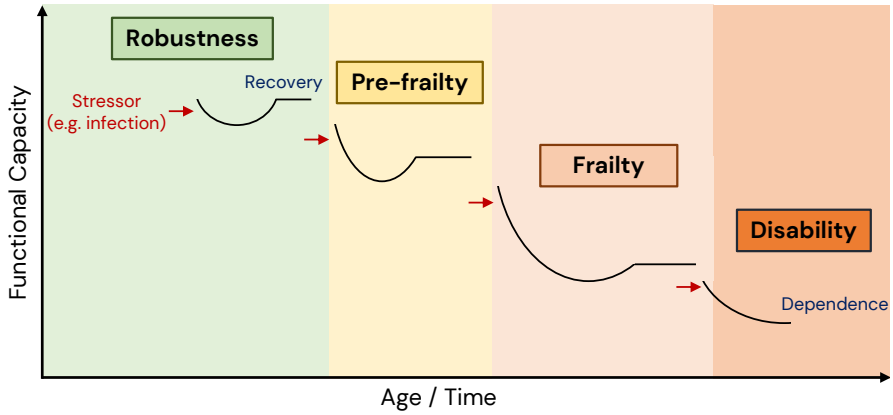

Figure 1. Transition from robustness through frailty to disability. This figure is adapted from Dent et al. *The Journal of Nutrition, Health & Aging* 2019,[18] under the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

Although there is now a widespread agreement on the theoretical definition of frailty,[38] there is still no consensus on how to best measure frailty due to the independent work performed by different frailty researchers, the complex etiology of frailty, and the difficulties in distinguishing frailty from other similar concepts.[39] Depending on the operational approach, frailty can be viewed as a syndrome[40] or an age-related state,[41,42] and can be either a distinct or overlapping concept with disability and multimorbidity.[43] Other closely-related concepts such as resilience (which emphasizes on coping and recovery to return to the basal state) and intrinsic capacity (which emphasizes on functional reserves) may also be complementary with the concept of frailty (which emphasizes on failure to maintain homeostasis).[44] In recent years, there has also been increasing research on the subtypes of frailty, such as cognitive frailty (i.e., the coexistence of physical frailty and cognitive impairment),[45] social frailty (i.e., the continuum of being at risk of losing resources to fulfill basic social needs),[46] and oral frailty (i.e., the decline in oral function together with physical and cognitive impairments).[47] Nevertheless, more work is still needed to elucidate how these additional constructs can be applied for clinical use.[48]

To date, the two most widely adopted and validated operational approaches to frailty are the syndrome of phenotypic frailty proposed by Fried et al. in 2001 (commonly referred to as the physical frailty phenotype [FP]),[40] and the accumulation of deficit model proposed by Rockwood et al. in the same year (i.e., the frailty index [FI]).[41] Despite carrying the same name of "frailty", these two models have notable conceptual differences. The FP sees frailty as a clinical syndrome with distinct pathophysiology from multimorbidity and disability and is defined based on the presence of three or more out of five components: exhaustion, slow walking speed, weak grip strength, unintentional weight loss, and low physical activity (**Table 1**).[40] The FI, on the other hand, considers frailty as a more general

age-related state of increased vulnerability proportional to the number of health deficits accumulated in an individual, with a score ranging from 0–1 (**Table 1**).[41,42] The deficits can be any age-associated diseases, signs, symptoms, disabilities, and abnormal laboratory test values; and when at least 30 items from a range of physiological systems are included, the FI often shows good predictive accuracy for adverse outcomes.[42] Compared to the FP, the FI does not differentiate frailty with multimorbidity and disability since these items can be included in the FI calculation. Meanwhile, the FI captures better the multidimensional nature of frailty. Since it is often used as a continuous score, it also has better discrimination at the lower and middle ends of the frailty continuum and is more suitable for younger adults.[49]

Table 1. Comparison of the commonly used frailty measures.[39,50]

Measure	No. of items	Components	Setting		Requirement		
			Community	Clinical	Time (min)	Equipment	Personnel training
FP[40]	5	Exhaustion, slowness, weakness, weight loss, low activity levels	Yes	Yes	<10	Yes	Yes
FI[41]	≥30	Deficits such as diseases, signs, symptoms, disabilities	Yes	Yes	<20	No	Yes
eFI[51]	≥30	Same as FI, with variables derived from EHRs	Yes	Yes	0[a]	No	No
CFS[52]	1	Clinical evaluation, graded from 1–9	No	Yes	<5	No	Yes
HFRS[53]	109	Frailty-related ICD-10 codes	No	Yes	0[a]	No	No
EFS[54]	9	Cognition, health, hospitalization, social support, nutrition, mood, function, continence, medication	No	Yes	<10	No	Yes
FRAIL[55]	5	Fatigue, resistance, ambulation, illness, weight loss	Yes	Yes	<5	No	No
TFI[56]	15	Self-reported Items related to physical, psychological, and social domains	Yes	No	<10	No	No
GFI[57]	15	Self-reported items related to physical, cognitive, polypharmacy, and psychosocial health	Yes	No	<10	No	No
PRIMSA-7[58]	7	Self-reported items related to age, sex, social support, activities of daily living	Yes	No	<10	No	No

CFS, Clinical Frailty Scale; *eFI*, electronic frailty index; *EFS*, Edmonton Frailty Scale; *EHR*, electronic health records; *FI*, frailty index; *FP*, frailty phenotype; *GFI*, Groningen Frailty Indicator; *HFRS*, Hospital Frailty Risk Score; *ICD*, International Classification of Diseases; *TFI*, Tilburg Frailty Indicator

[a] The eFI and HFRS were derived from routinely collected electronic health records and therefore do not require additional time for data collection

Other than the FP and FI, many other frailty measures have been developed in attempt to simplify frailty measurement or for use in specific settings. Examples include the electronic frailty index (eFI),[51] Clinical Frailty Scale (CFS),[52] Hospital Frailty Risk Score (HFRS)[53], Edmonton Frailty Scale[54], FRAIL (Fatigue, Resistance, Ambulation, Illness, Loss of Weight) scale,[55] Tilburg Frailty Indicator,[56] Groningen Frailty Indicator,[57] and PRISMA-7.[58] **Table 1** summarizes the use of the common frailty measures. There are several important differences between these measures. Firstly, while most tools are suitable for population-level screening in community-dwelling adults, some of them are developed specifically for clinical practice (e.g., CFS, HFRS).[39] Secondly, these measures vary from short screening tools (e.g., CFS, FRAIL) to more sophisticated and comprehensive clinical assessment (e.g., FI).[39] Thirdly, these measures usually identify related but distinct groups of frail individuals due to the different domains of frailty being captured (e.g., FP captures physical aspects, while FI is more multidimensional).[59,60] Therefore, the choice of the instrument often depends on the purpose of performing frailty assessment, the target population, and the data availability.[39,50,61] Notwithstanding these differences, most of the frailty measures are proven to be useful tools in predicting adverse outcomes, with the greatest predictive accuracy usually observed for the FI[59] or the FP.[60] They also exhibit similar characteristics, such as a higher prevalence with advancing age[62], a generally higher frailty score in women than in men,[63] and a dynamic nature with the possibility to be reversed.[64,65]

2.3 Epidemiology of frailty

The prevalence of frailty varies largely by the study population and the frailty measure used, making it difficult to obtain an accurate global estimate.[3,62,66,67] For community-dwelling adults, a meta-analysis in 2012 revealed an overall frailty prevalence of 11% in 21 high-income countries.[62] Later studies suggested a prevalence of 17% in low- and middle-income countries,[66] and 20% in countries in Latin America and the Caribbean.[67] Likewise, a recent study by O'Caoimh et al. reported a large variation of frailty prevalence across geographic locations, from the lowest of 8% in Europe to the highest of 22% in Africa; there was also a higher prevalence of frailty measured by the FI (24%) than the FP (12%) or the CFS (17%).[3] Furthermore, the prevalence of frailty is generally higher outside community settings, which was ~50% in nursing homes,[68,69] and 10–37% in general surgical patients.[70]

Age and female sex are often considered as risk factors for frailty. Although frailty can also affect younger adults, the prevalence of frailty as defined by the FI increases dramatically with advancing age, from 2% among those aged <30 years to ~20% among those ≥65 years and over 40% among those ≥85 years.[3,49] However, the existing evidence is mainly from cross-sectional studies; knowledge on the longitudinal progression and trajectories of frailty with age is still limited.[71] While some studies observed an accelerated increase in frailty at older compared to younger ages,[72,73] others found a similar rate of increase in frailty over age.[74] The causes of the sex differences in frailty are also largely unknown.

Previous studies have reported a "sex-frailty paradox", in which women usually have higher levels of frailty but men are more vulnerable to death at any given level of frailty.[75] Some possible reasons for this apparent sex difference could be (i) a lower physiological reserve in men than in women; (ii) differences in the nature of deficits accumulated in men and women; and (iii) differences in psychosocial factors such as health-seeking behavior and social support.[75,76] Besides, compared to a self-reported frailty measure, the sex difference seems to be less obvious or even appears in opposite direction (i.e., higher frailty level in men) when using performance-based[77] or laboratory item-based frailty measures.[78] All these uncertainties highlight the importance of taking longitudinal changes and sex differences into consideration when performing frailty research.

Apart from age and sex, several sociodemographic (e.g., ethnic background, low education), physical (e.g., underweight, obesity), psychosocial (e.g., poor social support, depressive symptoms), and lifestyle factors (e.g., smoking, unhealthy diet) have been considered as environmental risk factors for frailty.[72,79] In particular, a recent Mendelian randomization (MR) study suggested that genetic predispositions to higher body mass index (BMI) and lower educational attainment had the strongest association with frailty among various modifiable risk factors.[80] However, more studies are needed to understand the mechanisms underlying these associations. Moreover, the biological risk factors of frailty (e.g., genetics and biomarkers) are still largely unexplored; an improved knowledge on its biological mechanisms would be fundamental in the development of preventive strategies for frailty.[81]

2.4 Biology of frailty

2.4.1 Heritability of frailty

Genetic and environmental factors, in combination with epigenetic changes, determine the complex physiological manifestations during aging and frailty development.[2,7] Many age-related traits, including frailty, show large variability in the population especially at older ages.[82] Twin studies provide a valuable framework for dissecting this variability into genetic (i.e., heritability) and environmental components, given that monozygotic (MZ) twins share 100% of their segregating alleles, while dizygotic (DZ) twins share on average 50%.[8] Since most aging traits are highly polygenic and genome-wide association studies (GWASs) can primarily detect common variants with moderate effect sizes, calculating the heritability of frailty using a twin study design is particularly informative for studying the complete range of genetic influences, including dominant genetic influences that may not be captured by GWAS.[83,84] Extended twin models also enable the exploration of gene-environment interaction,[85] longitudinal changes in genetic and environmental influences over time,[86] as well as quantitative and qualitative sex differences.[87] Quantitative sex differences refer to the same genetic factors affecting the trait in men and women to

different degrees, while qualitative sex differences involve different sources of genetic factors influencing the trait in men and women.[87] Although twin studies may be subject to violations of the equal environment (i.e., MZ and DZ cotwins are treated similarly) and random mating assumptions, previous research has indicated that these violations have minimal impacts on heritability estimates.[88]

Only a few prior studies have assessed the heritability of frailty. Using data from 3,719 Danish twins, Dato et al. estimated a heritability of 43%, although frailty in this study was defined using a "cluster analysis approach" which may not be generalizable to other settings.[89] Two other studies including the same cohort of ~3,700 UK female twins found a heritability of 30–45% for the FI and 25% for the FP.[90,91] Also, the FI and the FP showed strong genetic (r=0.57) and environmental correlations (r=0.44),[91] suggesting that these two measures share their genetic and environmental etiologies to a large extent and thus capture a largely similar construct.[91] Nevertheless, since these two studies included only women and were cross-sectional,[90,91] it is unclear whether the heritability of frailty may differ in men and women and may change across age.

2.4.2 Biomarkers of frailty

Dysregulation in various physiological systems, such as the endocrine system, immune system, brain, and skeletal muscles, have been linked to frailty.[2] Many of the physiological systems are often interconnected, and these interacting systems can jointly increase the risk of frailty.[92] With the complex pathophysiology of frailty, studying biomarkers of frailty would be important for understanding mechanisms and improving diagnosis.[93] Biomarkers are generally defined as any objective medical signs or indications that can be measured accurately and reproducibly, and can range from genetic/metabolic data to clinical markers measured in laboratory tests, as well as functional/physiological measurements.[94] Several clinical biomarkers have consistently been shown to be associated with frailty, such as those related to inflammation (e.g., C-reactive protein [CRP], tumor necrosis factor alpha, interleukin 6), hormones (e.g., insulin-like growth factor 1, vitamin D), metabolism (e.g., albumin, glucose), and immune system (e.g., white blood cell count, CD4+/CD8+ cell ratio).[93,95,96] Incorporating biomarkers into frailty measures may also help in identifying pre-clinical frailty. Several studies have suggested that an FI including laboratory test measures/clinical biomarkers (e.g., CRP, fasting blood glucose, cholesterol, hemoglobin, blood pressure) can be used to identify older adults at risk of mortality.[78,97,98] Similarly, Mitnitski et al. created an FI by combining circulating biomarkers, including those related to inflammation (e.g., tumor necrosis factor alpha, interleukin 6), immune system (e.g., CD4+ and CD8+ T cells), cellular aging (e.g., telomere length, DNA damage), genetics (e.g., *APOE* genotype), and epigenetics (e.g., DNA methylation).[99] Importantly, all these studies suggested that a biomarker-based FI could complement a clinical deficit-based FI in predicting mortality, especially in those individuals who were not yet clinically frail.[97,99]

With the recent advances in statistical methods and computational tools for analyzing "omics" data, personalized medicine is emerging as an approach that uses the individual's genetic and biomarker information for prevention and management of diseases.[9] Some of the most common omics data types include genomics, epigenomics, transcriptomics, proteomics, and metabolomics.[9] However, the omics biomarkers of frailty is still a relatively new area of research, and the current knowledge is mainly limited to the genomics, epigenomics, and metabolomics of frailty (**Table 2**).[100,101]

Table 2. Potential omics biomarkers of frailty.

Omics	Technology	Frailty measure	Biomarkers	Refs.
Genomics	Genome-wide genotyping	FI	Genetic loci associated with BMI, cardiovascular diseases, smoking, HLA proteins, depression, neuroticism	80,102
		FP	Genetic loci associated with BMI, lipids, coronary artery disease, hypertension, diabetes, cancer	102,103
Epigenomics	DNA methylation microarrays	FI	CpGs associated with multiple diseases such as amyotrophic lateral sclerosis, Huntington's disease, cancers, neurodegenerative disorders	104
		FP	A CpG (cg18314882) associated with oncogenesis and regulation of intracellular lipids	105
Metabolomics	Liquid chromatography-mass spectrometry	FI	Metabolites related to the carnitine shuttle and vitamin E pathways	106
		FP	Metabolites involved in glycolysis and tricarboxylic cycle, and neurotransmitters	107

The first GWAS of frailty was conducted in 2018, which utilized two cohorts from the US and the UK with relatively small sample sizes and identified two FI-associated single nucleotide polymorphisms (SNPs) in the *KBTBD12* and *GRIN2B* genes.[108] Later, a large GWAS meta-analysis incorporating data from the UK Biobank and the Swedish TwinGene study revealed 14 genetic loci associated with the FI, of which 13 have previously been linked to various disease risk factors (e.g., BMI and smoking initiation) and mental health conditions (e.g., depression and neuroticism).[80] Another recent GWAS focusing on the FP within the UK Biobank identified 37 loci that were previously linked to BMI, lipids, cardiovascular diseases, and cancers.[103] Notably, these studies estimated the SNP-based heritability of frailty to be only around 6–11%,[80,103] which is considerably lower than the 25–45% observed in twin studies.[90,91] This discrepancy could be due to the rare and non-additive genetic influences that were not detected in the GWAS analyses.[83]

DNA methylation, mostly occurs in cytosine-phosphate-guanine (CpG) sites, is the most common epigenetic mechanism involving in the regulation of gene expression.[109] It is affected by both genetic and environmental factors and can change across the life course.[109] As changes in methylation levels are linked to both aging[10] and age-onset pathologies (e.g., cancer, Alzheimer's disease),[109] they could similarly be associated with frailty. With a 7-year follow-up, Bellizzi et al. found a significantly decreased global DNA methylation level in individuals with a worsening frailty status defined using cluster analysis,[110] although no significant association was observed in another cross-sectional study which used the FP to define frailty.[111] There have also been a few epigenome-wide association studies (EWAS) of frailty that have identified several frailty-associated CpG sites.[104,105,112,113] However, these studies were mostly cross-sectional in nature and did not assess whether the identified CpGs may be associated with frailty longitudinally over age. There has also been no CpG that is consistently associated with frailty across different populations. On the other hand, some studies have shown an association between DNA methylation-based epigenetic ages and frailty,[114] although these correlations seem to be primarily explained by chronological age.[17] Another recent study also showed that epigenetic age measures was associated with the FI cross-sectionally, but not with changes in frailty longitudinally, highlighting the importance of using longitudinal data to examine the relationship between epigenetic factors and frailty.[115]

Metabolomics is the study of small molecules involving in biochemical reactions in the body (e.g., amino acids, carbohydrates, fatty acids, vitamins). It is the downstream output of gene-environment interactions and is often indicative of one's current health status.[9] Associated technologies, such as nuclear magnetic resonance (NMR) and liquid chromatography–mass spectrometry, allow quantification of a large variety of metabolites, and abnormal metabolite levels often represent presence of diseases.[9] Metabolomics has been increasingly used in recent years to study mechanisms and identify novel drug targets for several diseases such as cancer, diabetes, and Alzheimer's disease.[116] In the few metabolomics analyses of frailty to date, metabolites involving in energy producing pathways seemed to have the strongest association with frailty.[100,101,117] For instance, a study by Westbrook et al. showed that glycolytic and tricarboxylic cycle intermediates, as well as neurotransmitters (e.g., N-acetyl-aspartyl-glutamate, glutamate, γ-aminobutyric acid) were elevated in frail individuals defined by the FP.[107] Using non-targeted metabolomics in combination with MR analysis, Rattray et al. revealed that metabolites related to the carnitine shuttle and vitamin E pathways were significantly associated with the FI.[106] Another study using the Edmonton Frailty Scale also identified several metabolites related to muscle and nitrogen metabolism (e.g., tryptophan, isoleucine, leucine, arginine, hippurate), as well as antioxidation (e.g., ergothioneine, acetyl-carnosine, urate).[118] However, most of these studies had relatively small sample sizes and were mostly unable to establish causal relationships between metabolites and frailty due to potential confounding by

genetic and environmental factors. More large-scale metabolomics studies, in combination with other omics-based technologies (e.g., MR analysis[119]) and methods to deal with the high-dimensional data,[120] are necessary to identify metabolites that can be applied in clinical practice and used as the potential targets for frailty prevention and treatment.[106]

2.5 Frailty in clinical practice

2.5.1 Frailty assessment

With the aging population and the adverse clinical outcomes associated with frailty,[121,122] a routine frailty assessment could be beneficial for both the patient (e.g., guiding clinical decisions) and the health system (e.g., allocation of resources).[123] Frailty screening can also serve as an entry point for identifying older adults who would benefit most from a detailed assessment of their underlying causes of heightened vulnerability, i.e., the comprehensive geriatric assessment (CGA).[124] The CGA is known as the gold standard for caring of hospitalized frail older adults.[124] It is a geriatrician-led, multidisciplinary, and multidimensional assessment of the overall health of older adults, including medical, cognitive, psychological, functional, and social aspects, subsequently leading to a more individualized plan for treatment and follow-up.[125] Performing CGA has been shown to increase the likelihood of being alive and discharged to home following a hospital admission.[126,127] However, due to the high demand of time and expertise for performing CGA, a simple, quick, and validated frailty screening tool would be valuable for resource allocation.

The CFS is one of the most frequently used frailty measures in clinical settings. It is a quick screening tool based on clinical evaluation on several domains such as diseases, functioning, and cognition,[52] and often has a high accuracy and feasibility.[128] Nevertheless, as it requires in-person evaluation by physicians or trained nurses, the CFS could potentially lead to interrater bias and may not be always feasible in settings that are lacking time and resources for frailty assessment.[129–131]

To reduce the burden of performing a bedside frailty assessment, several automated frailty scores based on healthcare databases (e.g., electronic health records [EHRs] or administrative claims data) have been proposed in recent years for population-level frailty screening.[132] One example is the HFRS developed by Gilbert et al. in 2018, which is calculated based on the *International Classification of Diseases, Tenth Revision* (ICD-10) codes and can identify frail older patients in hospital settings.[53] As it is easy to be calculated and implemented, there has been a growing interest in adopting the HFRS in different patient groups.[133–135] However, a limitation of the HFRS is that it is more similar to a comorbidity measure and could miss out some aspects of the frailty concept such as weakness, activities of daily living, and polypharmacy.[53]

Another example is the eFI developed by Clegg et al. in 2016, which comprised 36 items derived from the UK primary care Read codes and was one of the first eFI models that has adopted the Rockwood FI model into clinical practice.[51] This eFI has been incorporated in the frailty management guidelines by the National Health Service in England.[136] Other than the UK eFI, similar models have been created in other countries such as the US,[137,138] Canada,[139] Australia,[140] the Netherlands,[141] and China.[142] Items included in these frailty scores are usually clinical knowledge-driven (e.g., combining information on diagnoses, functional abilities, and laboratory measures[138]) or data-driven using machine learning methods (e.g., using the FI[137,139] or the FP[143,144] as the reference standard). Although these tools were mostly developed for primary care settings, accumulating evidence has also shown their utility in predicting adverse outcomes in hospital settings.[142,145–147] Meanwhile, there has been no such eFI tool developed in Sweden or other Nordic countries thus far. To aid in resource allocation and risk stratification, there is an increasing need to test whether an eFI model can be adopted to the Swedish context. Importantly, an ideal frailty assessment tool should be available in real time, require minimal time and resources for data collection, capture the multidimensional concept of frailty rather than merely reflect a proxy of related entity (e.g., multimorbidity, disability), and could be applicable in different settings and patient groups.[123,148] Such a tool would have a great potential of paving the way towards a unified frailty assessment and improving patients care in clinical settings.

2.5.2 Frailty management

The ultimate goal of performing frailty assessment is to prevent progression and reduce severity of frailty. To date, most randomized controlled trials (RCTs) use resistance exercise training in combination with protein supplementation to improve physical performance of frail older adults.[149–151] Although these interventions are generally effective and are easy to implement, they focus mainly on physical frailty and sarcopenia.[149–151] It is also essential to consider the multidimensionality of frailty and apply a more proactive, person-centered approach for frailty prevention and treatment.[152] The "Sarcopenia and Physical Frailty in Older People: Multicomponent Treatment Strategies" project was the first long-term, multi-center RCT that aimed at preventing mobility disability in community-dwelling frail older adults.[153] Compared to previous RCTs, this study used a more person-tailored approach of structured physical activity and nutritional counselling, allowing more flexibility in meeting diverse needs of the participants.[153] During a mean follow-up of 26.4 months, those assigned to the multicomponent intervention group had a significantly reduced incidence of mobility disability compared to the control group who received a healthy aging lifestyle education program.[154] On the other hand, other individually-tailored interventions, such as those based on CGAs, showed inconclusive evidence on their effectiveness in reducing frailty.[155] For future development of personalized medicine, understanding the molecular mechanisms of frailty would be of great importance.[50]

3 Research aims

The overarching aim of this thesis is to pave the way towards an individualized management of frailty, through enhancing our understanding of the heritability and the omics biomarkers of frailty, and developing an eFI to identify high-risk older patients in Sweden (**Figure 2**). Specifically, the five included studies aim to:

I. Investigate sex differences in the genetic and environmental influences on frailty, and the gene-environment interplay of frailty with BMI and education.

II. Examine genetic and environmental influences on the longitudinal trajectories of frailty from adulthood to late-life.

III. Explore the associations between frailty and genome-wide DNA methylation levels of CpG sites.

IV. Identify metabolic biomarkers that are strongly and independently associated with frailty and examine their potential causal relationships.

V. Develop an eFI for hospitalized older adults in Sweden and assess its associations with mortality, readmission, and length of hospital stay.

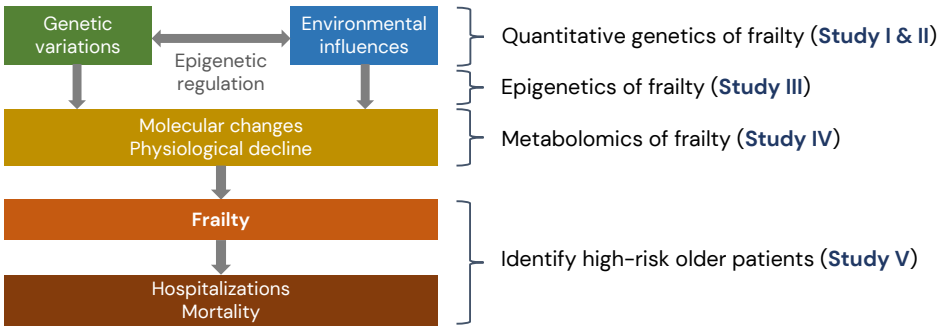

Figure 2. Overview of the study aims in relation to the potential mechanisms of frailty.

4 Materials and methods

4.1 Data sources

This thesis used several population-based data sources, as outlined in **Figure 3**. These sources include four sub-studies from the Swedish Twin Registry: (i) the Screening Across the Lifespan Twin Study (SALT; used in the heritability analysis in **Study I**),[156] (ii) the Swedish Adoption/Twin Study on Aging (SATSA; used in the longitudinal twin modeling in **Study II** & EWAS analysis **Study III**),[157] (iii) the Origins of Variance in the Oldest-Old: Octogenarian Twins (OCTO-Twin; used in the longitudinal twin modeling in **Study II**),[158] and (iv) TwinGene (used as a replication cohort in the observational analysis in **Study IV**).[159] A sub-study from the Danish Twin Registry, the Longitudinal Study of Aging Danish Twins (LSADT),[160,161] was used as a replication cohort in **Study III**. The UK Biobank (as a discovery cohort)[162] and the Finnish Health 2000 Survey (as a replication cohort)[163] were used in the observational analysis in **Study IV**. Finally, EHR data from geriatric clinics in Stockholm were used for creating the eFI in **Study V**.

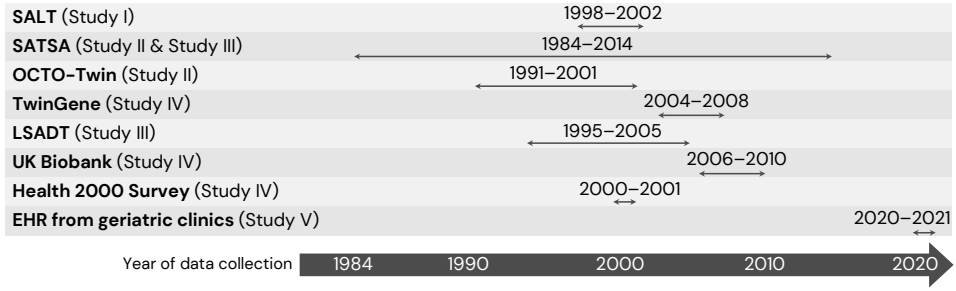

Figure 3. Timeline of the included cohorts

4.1.1 Screening Across the Lifespan Twin Study (SALT)

SALT is a cross-sectional study conducted between 1998 and 2002, including 44,919 same-sex and opposite-sex twins born in 1958 or earlier from the Swedish Twin Registry.[156] The response rate was 65% for those born in 1886–1925 and 74% for those born in 1926–1958.[164] Participants completed a comprehensive telephone interview survey and provided information on demographics, health conditions, medication use, and lifestyle factors.[156] Zygosity was determined primarily through questions about intra-pair similarities during childhood, which has shown to be >95% accurate when validated against DNA testing.[165] In **Study I**, after excluding those with missing data on frailty and zygosity, 42,994 participants remained for the analysis, including 4,788 MZ males (1,820 complete pairs), 5,997 MZ females (2,438 complete pairs), 7,640 DZ same-sex males (2,633 complete

pairs), 8,808 DZ same-sex females (3,279 complete pairs), and 15,761 DZ opposite-sex twins (5,791 complete pairs).

4.1.2 Swedish Adoption/Twin Study on Aging (SATSA)

SATSA is a longitudinal study that has collected data from reared together and reared apart same-sex twins over nine mailed questionnaire (Q) and 10 in-person testing (IPT) waves between 1984 and 2014.[157] The questionnaires covered demographics, health status, and lifestyle behaviors, while the IPTs included cognitive tests, physical and functional health examinations, and blood sampling. All SATSA twins were invited to the Q waves, while the IPT waves utilized a cohort-sequential design wherein individuals aged older than 50 years were invited to the IPTs.[157] The Q waves preceded the IPT waves by ~18 months, and the assessment types (Q or IPT) were generally conducted at 3-year intervals, with a break after Q4 due to a lapse in funding.[157] While both members of twin pairs were invited to participate in SATSA, individual participation was also welcomed even if a co-twin was unable or chose not to participate. **Study II** used data from 1,842 SATSA participants (9,534 repeated measurements over 15 waves) who had information on frailty.[72,166] For the EWAS analysis in **Study III**, we additionally excluded individuals with missing data on whole blood DNA methylation, resulting in a sample size of 526 individuals (1,331 repeated measurements).

4.1.3 Origins of Variance in the Oldest-Old: Octogenarian Twins (OCTO-Twin)

OCTO-Twin is a longitudinal study of oldest-old twins that has recruited 351 complete twin pairs aged over 79 years at baseline, with 5 IPT waves at 2-year intervals from 1991 to 2001.[158,166] The IPTs included cognitive tests and physical and functional health examinations. **Study II** included 654 OCTO-Twin participants (2,063 repeated measurements) with data on frailty.

4.1.4 TwinGene

TwinGene is a follow-up study of SALT conducted between 2004 and 2008, which has collected blood samples and information on chronic diseases and mediation use from 12,648 older twins who had previously participated in SALT.[159] The replication analysis in **Study IV** included 11,025 TwinGene participants who had complete data on frailty and metabolic biomarkers.

4.1.5 Longitudinal Study of Aging Danish Twins (LSADT)

LSADT is part of the Danish Twin Registry and is a longitudinal study of same-sex twins aged ≥70 years initiated in 1995.[160,161] Participants provided information on demographics, health status, and lifestyle factors in six waves at 2-year intervals up to 2005.[160,161] Whole

blood samples were collected from 689 same-sex twins in 1997.[161] The replication analysis in **Study III** included 304 participants with complete data on frailty and DNA methylation measured in 1997.

4.1.6 UK Biobank

The UK Biobank served as the discovery cohort for investigating the metabolomic and clinical biomarkers associated with frailty in the observational analysis in **Study IV**. It is a cross-sectional, population-based study that has recruited over 500,000 adults aged 37–73 years between 2006 and 2010.[162] During baseline assessment, participants completed a touch-screen questionnaire, provided biological samples, and underwent physical measurements in one of the 22 centers throughout England, Wales, and Scotland.[162] After excluding those who had withdrawn from the UK Biobank, were self-reported as non-white ethnicity, and had missing data on frailty and biomarkers, the observational analysis included 90,573 and 67,488 participants who had complete data on the 168 metabolomic biomarkers and 32 clinical biomarkers, respectively. We also used genetic data from the UK Biobank in the MR analyses in **Study IV** (described below in § 4.3.4).

4.1.7 Health 2000 Survey

The Finnish Health 2000 Survey is a cross-sectional, nationally representative survey conducted between 2000 and 2001, including 8,028 Finns aged 30 years of older.[163] The survey incorporated self-administered questionnaires, interviews, health examinations, and laboratory measurements, and the participation rate in the health examination was 85%.[163] The replication analysis in **Study IV** included 6,073 individuals after excluding those with missing data on frailty and biomarkers.

4.1.8 Electronic health records from geriatric clinics

In **Study V**, we conducted a retrospective cohort study using EHR data from nine geriatric clinics in the Stockholm area. The data included patients with unplanned admissions between March 1, 2020, and June 17, 2021, for any causes except COVID-19. The geriatric clinics specialize in inpatient geriatric care and are either standalone geriatric hospitals or part of larger emergency hospitals. They typically admit older patients who have reduced physical and/or cognitive function, have multimorbidity, and require geriatric medical care and/or rehabilitation. Patients without discharge information or with a length of stay <24 hours were excluded. Most patients had only one admission during the study period (73.0%), and for those with multiple admissions, we used only data from their first available admission. In total, we included 13,188 patients who had sufficient data for calculation of the eFI in the analysis.

4.2 Measurements

4.2.1 Frailty

Frailty was defined using the FI in **Studies I–IV**. We used a 44-item FI in SALT,[22] a 42-item FI in SATSA,[167] a 41-item FI in OCTO-Twin,[166] a 43-item FI in LSADT, a 49-item FI in the UK Biobank,[168] and a 38-item FI in the Health 2000 Survey. The FIs in all cohorts were constructed using similar deficit items based on the deficit accumulation model,[42] where a wide range of self-reported items, such as diseases, signs, symptoms, and disabilities, that were available within the cohort were incorporated in the corresponding FI. Each participant's frailty items were then summed up and divided by the total number of items considered.[42] For example, an individual who has nine deficit points out of 45 items would receive an FI of 9/45 = 0.2. For all cohorts, participants who had over 20% missing data across the deficit items were excluded. Imputation was used in SALT,[22] SATSA,[167] and OCTO-Twin[166] to replace missing values of deficit items. A "total-varying" FI was used in the other cohorts, such that each individual could have a different denominator when calculating the FI, depending on the number of non-missing items per person. The FI was primarily used as a continuous score (ranging from 0–1) in the analyses. In **Study III**, we also considered the FI as a categorical variable based on the previously used cut-off points: non-frail (FI ≤0.1), prefrail (0.1< FI ≤0.21), and frail (FI >0.21).[49]

In **Study IV**, we calculated the FP in the UK Biobank as a secondary outcome. Based on the five frailty criteria proposed by Fried et al.,[40] a modified FP was previously constructed in the UK Biobank,[169,170] where exhaustion, slowness, weight loss, and low physical activity were assessed by self-reported questionnaire items, and weakness was determined by the grip strength measured at baseline. The number of frailty criteria present in an individual was summed up to create the FP score (ranging from 0–5).

In **Study V**, we developed an eFI using routinely collected EHR data, and compared it against the CFS, HFRS, and the Charlson Comorbidity Index (CCI; as a measure of comorbidity). Our eFI was constructed based on a US eFI model,[138] and it comprised 48 items in three categories (**Box 1**): (i) disease diagnoses based on ICD-10 codes; (ii) functioning and other health indicators; and (iii) laboratory/anthropometric measures. Following the deficit accumulation model,[42] we calculated the eFI as the sum of deficit items divided by the total number of non-missing items in each patient. We considered a patient as having sufficient data for calculation of the eFI if she/he had data on ≥30 deficit items and had at least half of the functioning and/or lab measures available. The eFI was categorized into four groups: fit (eFI ≤0.15), mild frailty (0.15< eFI ≤0.2), moderate frailty (0.2< eFI ≤0.25), and severe frailty (eFI >0.25). The CFS was scored by a physician or trained nurse at admission (ranging from 1–9) and categorized into three groups (1–3, 4–5, and 6–9). The HFRS was calculated based on 109 weighted ICD-10 code items and was categorized into low-risk (<5), intermediate-risk (5–15), and high-risk (>15) groups.[53] The CCI was computed based on ICD-10 codes using an algorithm adapted for the Swedish context.[171]

Box 1. List of the 48 items included in the Swedish eFI.

Disease diagnoses based on ICD-10 codes	Functioning and other health indicators
Anemia	Activity limitation
Asthma	Cognitive impairment
Atrial fibrillation	Falls
Cancer	Food intake status
Chronic pain	General condition
Congestive heart failure	Incontinence
Coronary atherosclerosis and other heart disease	Mobility
Dementia	Oral health
Depression	Sensory impairment
Diabetes	Weight loss
Dizziness or vertigo	
Dyspnea	**Laboratory/anthropometric measures**
Fragility fracture	C-reactive protein
Hypertension	Creatinine
Hypotension/syncope	Glucose
Liver disease	Hemoglobin
Myocardial infarction	Obesity
Osteoporosis	Potassium
Parkinsonism and tremor	Pulse
Peptic ulcer	Sodium
Peripheral vascular disease	Underweight
Pulmonary disease	
Renal disease	
Rheumatoid arthritis or osteoarthritis	
Skin ulcer	
Stroke or transient ischemic attack	
Thyroid disease	
Urinary system disease	
Valvular disease	

4.2.2 DNA methylation

In SATSA, whole blood DNA methylation was measured either by the Illumina's Infinium HumanMethylation450K or MethylationEPIC array,[172,173] where methylation levels of CpGs were quantified as β values (ranging from 0–1, representing percentage of methylation).[174] During data pre-processing, samples were excluded if they showed poor correlation with genetic controls or if the predicted sex based on signal ratio from sex chromosomes was incorrect. Probes were excluded if they overlapped with a SNP, had a detection *p*-value over 0.05, or resided on sex chromosomes. The methylation data were normalized using the "dasen" method from the *wateRmelon* R package,[175] corrected for batch effects using the "ComBat" method from the *sva* R package,[176] and adjusted for cellular compositions using the Houseman method[177] based on a blood cell reference panel.[178] In LSADT, DNA methylation was measured by the Infinium HumanMethylation450K array, and similar data pre-processing steps had been performed as previously described.[161,179] In **Study III**, for the EWAS analysis in SATSA, we included 245,545 CpGs that passed quality control on both DNA methylation arrays and had <15% difference in the mean β values between the two arrays; the CpGs identified from the EWAS were then selected for replication in LSADT.

4.2.3 Metabolic biomarkers

In the UK Biobank, TwinGene, and Health 2000 Survey, circulating metabolomic biomarkers were measured using the Nightingale's high-throughput NMR metabolomics platform. During the initial release of the NMR metabolomics data in the UK Biobank,[180,181] data on 168 metabolomic biomarkers were available for a random subset of 118,461 non-fasting baseline EDTA plasma samples. The metabolomic biomarkers include clinically validated biomarkers such as cholesterols, fatty acids, amino acids, inflammation markers, as well as emerging biomarkers like lipoprotein subclasses. The same 168 biomarkers were available and measured from fasting serum samples in TwinGene and the Health 2000 Survey. Samples that failed quality control (i.e., labeled as "high lactate", "high pyruvate", "low glucose", or "low protein") were excluded from the analysis. We additionally investigated 32 clinical biomarkers obtained from serum and urine samples, including risk factors for diseases (e.g., low-density lipoprotein [LDL] cholesterol and triglyceride for cardiovascular diseases), diagnostic measures (e.g., glycated hemoglobin [HbA1c] for diabetes), and other markers (e.g., creatinine for renal function). To facilitate the comparison of effect sizes, all the 200 biomarkers were standardized to mean=0 and standard deviation (SD)=1 before the analysis in **Study IV**.

4.2.4 Other health outcomes

In **Study I**, we assessed BMI and education for their bivariate associations with the FI in SALT. BMI was calculated based on self-reported weight and height at baseline, and education was defined as the self-reported number of years of education completed.

In **Study V**, we analyzed the associations of frailty and comorbidity measures with in-hospital mortality, 30-day mortality, and 6-month mortality as the primary outcomes, where the dates of death were obtained from the Swedish Population Register. Additionally, 30-day readmission to any of the nine included geriatric clinics, as well as the length of stay were used as the secondary outcomes.

4.3 Statistical analysis

A summary of the methods used in each study is provided in **Table 3**. All the analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria).

Table 3. Summary of the methods used in each study.

Study	Aim	Data sources	Exposures	Outcomes	Covariates	Statistical methods
I	To estimate sex differences in the genetic and environmental influences on frailty and its relation to BMI and education	• SALT (*n*=42,994)	-	• FI • BMI • Education	Age	• Twin-based structural equation models (including univariate, bivariate, and moderation models)
II	To examine genetic and environmental influences on the longitudinal frailty trajectories	• SATSA (*n*=1,842) • OCTO-Twin (*n*=654)	-	• FI	Study (OCTO-Twin vs. SATSA)	• Latent growth curve models • Twin-based structural equation models
III	To explore the associations between frailty and DNA methylation levels of CpGs	• SATSA (*n*=526) • LSADT (*n*=310)	• FI	• Methylation levels of 245,545 CpG sites	Age, sex, smoking, BMI, methylation array (EPIC vs. 450K)	• Generalized estimating equations • Linear mixed-effects models • DMR analysis
IV	To investigate the effects of metabolic biomarkers on frailty	• UK Biobank (*n*=90,573) • TwinGene (*n*=11,025) • Health 2000 Survey (*n*=6,073)	• 168 NMR metabolomic biomarkers • 32 clinical biomarkers	• FI • FP	Age, sex, baseline assessment center, BMI, smoking, alcohol, education, deprivation	• Linear regression • LASSO regression • Two-sample MR • Co-twin control analysis
V	To develop and validate an eFI for hospitalized geriatric patients in Stockholm	• EHRs from nine geriatric clinics in Stockholm (*n*=13,188)	• eFI • CFS • HFRS • CCI	• In-hospital mortality • 30-day mortality • 6-month mortality • 30-day readmission • Length of stay	Age, sex, clinics	• Logistic regression • Cox proportional-hazards models • Linear regression

BMI, body mass index; *CCI*, Charlson comorbidity index; *CFS*, Clinical Frailty Scale; *DMR*, differentially methylated region; *eFI*, electronic frailty index; *EHR*, electronic health record; *FI*, frailty index; *FP*, frailty phenotype; *HFRS*, Hospital Frailty Risk Score; *LASSO*, least absolute *shrinkage* and selection operator; *LSADT*, Longitudinal Study of Aging Danish Twins; *MR*, Mendelian randomization; *NMR*, nuclear magnetic resonance; *OCTO-Twin*, Origins of Variance in the Oldest-Old: Octogenarian Twins; *SALT*, Screening Across the Lifespan Twin Study; *SATSA*, Swedish Adoption/Twin Study of Aging

4.3.1 Quantitative genetic analysis

Quantitative genetics is the study of the relative contributions of genetic (heritability) and environmental factors to the variation of a trait in a population. In the classical twin design, this can be achieved by contrasting the observed phenotypic similarity with the genetic similarity between MZ and DZ twins, who share 100% and ~50% of their segregating genes, respectively.[182] If the trait is heritable, MZ twins would show a larger intraclass (within-twin-pair) correlation for the trait compared to DZ twins. In **Studies I** & **II**, by fitting twin-based structural equation models (using the full information maximum-likelihood modeling in the R package *OpenMx*), we decomposed variances and covariances of traits into their genetic and environmental components:

- *Additive genetic factors* (*A*), which represent the sum of allelic effects at multiple loci that influence the trait, and is referred to as the "narrow-sense heritability". It correlates 100% in MZ twins and 50% in DZ twins.
- *Dominance genetic factors* (*D*), which represent interactions between alleles within the same locus. It correlates 100% in MZ twins and 25% in DZ twins. The combined influences of *A* and *D* are referred to as the "broad-sense heritability" (*H*).
- *Common/shared environmental factors* (*C*), which represent environment shared by twins within a pair (e.g., family environment). It correlates 100% in both MZ twins and DZ twins.
- *Unique/non-shared environmental factors* (*E*), which represent environmental influences unique to each individual and include measurement error as well. It is uncorrelated in both MZ twins and DZ twins.

Of note, *C* increases the similarity of DZ twins, but *D* decreases their similarity relative to MZ twins. In the classical twin model that includes only two pairs of relatives, there is insufficient information to estimate both *C* and *D* simultaneously, along with *A* and *E*. Therefore, either an *ACE* or *ADE* model was fitted at one time, and these two models were compared against the *AE* model to assess if the *C* or *D* parameters could be removed from the models without a significant loss in model fit. Goodness of fit of the models were compared using likelihood ratio tests, where $p<0.05$ indicate a worse fit of the observed data. The models with the lowest Akaike information criterion (AIC) were considered as the best-fitting (most parsimonious) models.[183]

In **Study I**, we first fitted *univariate* twin models (which focus on one trait at a time) in SALT to estimate the variance components of the FI, while allowing for quantitative (different magnitude of heritability in men and women) and qualitative sex differences (different genetic sources in men and women, modeled by multiplying a genetic correlation parameter to the expected genetic covariance of opposite-sex twin pairs). Extending the univariate model, we fitted *bivariate* twin models (which focus on two traits at a time) using the Cholesky decomposition method with a "correlated factor model" solution,[182] to estimate the genetic and environmental contributions to the variances and covariances

of the FI with BMI and education (**Figure 4**). From the bivariate models, we calculated the proportions of the phenotypic correlation explained by genetic ("bivariate heritability") and environmental factors. Lastly, moderation models were fitted to test if genetic and environmental influences on the FI are moderated by different levels of BMI and education. Moderation may occur on the variance that is unique to the FI or on the covariance between FI and the moderator. Thus, we fitted a series of full bivariate moderation models[85] and extended univariate moderation models[184] to examine these possible moderating effects. All the univariate, bivariate, and moderation models were adjusted for age by regressing it out of the means of the FI, BMI, and education.

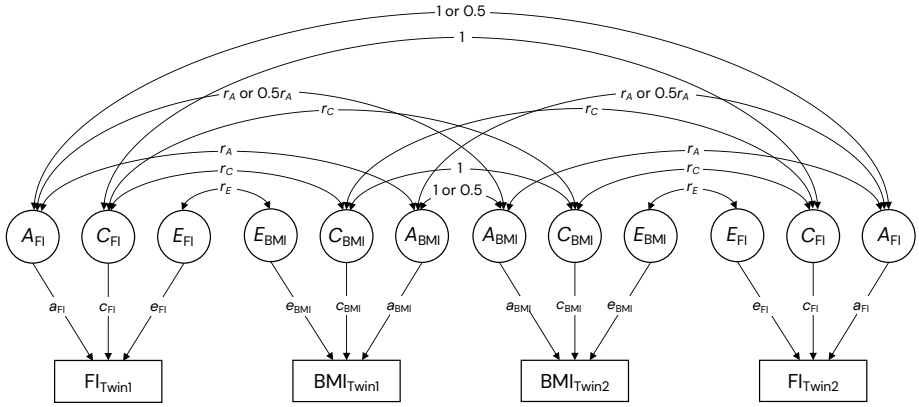

Figure 4. Path diagram of a bivariate *ACE* model for FI and BMI. Squares represent measured traits (FI and BMI), and circles represent latent variance components (*A*, *C*, and *E*). Path coefficients (*a*, *c*, and *e*) are estimated in the model. Double-headed arrows represent correlations between variance components. A similar model was fitted for FI and education. The bivariate *ACE* model was then compared with the *ADE* and *AE* models to determine the best-fitting model. Quantitative sex differences were allowed in the models, where variance components and etiological correlations were estimated separately in men and women.

In **Study II**, after identifying the best-fitting phenotypic growth model of the FI in SATSA and OCTO-Twin (described below in § 4.3.2), we extended it to a biometric model[185] fitted within the structural equation modeling framework to study genetic and environmental influences on the longitudinal FI trajectories over age (**Figure 5**). Similar to the bivariate models in **Study I**, we used the Cholesky decomposition method to decompose variances of the latent growth variables (i.e., an intercept at age 75 years and two slopes), as well as the covariances between these variables into genetic and environmental sources. Using this approach, we could not only estimate the heritability of the growth parameters themselves, but also the expected genetic and environmental variances of the FI at any given age, so that we can examine if the heritability of the FI may change across the lifespan.[185,186]

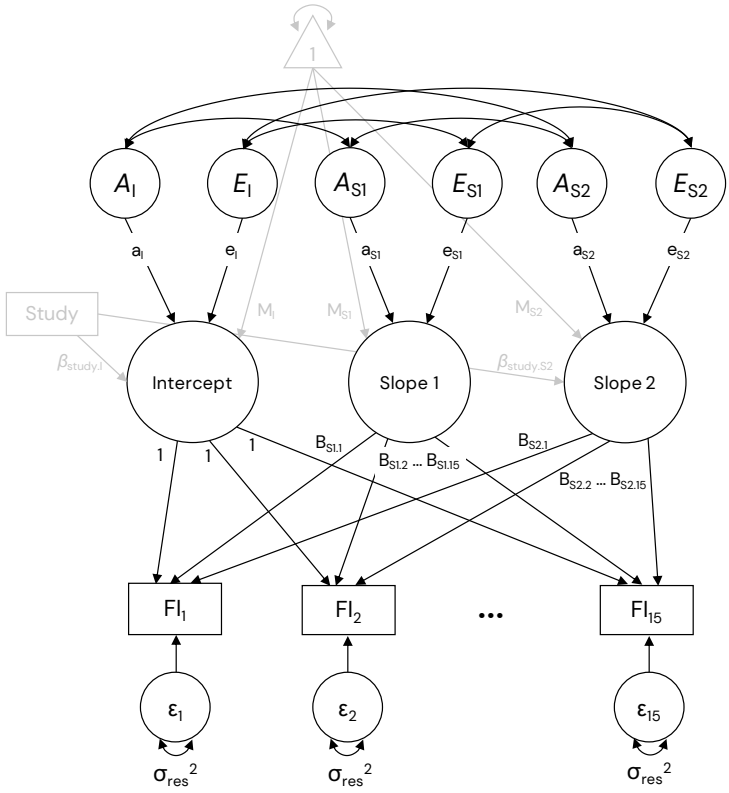

Figure 5. Path diagram of an *AE* bilinear growth model of the FI. This figure is adapted from Mak et al. *The Journals of Gerontology: Series A* 2023.[187] For simplicity, only one twin is shown (path diagram for the co-twin is identical). The best-fitting growth model consists of three latent (circles) factors: intercept at 75 years, slope <75 years ("slope 1"), and slope >75 years ("slope 2"). The upper half of the diagram shows the biometric decomposition of variation about the intercept, slope 1, and slope 2. Double-headed arrows indicate additive genetic and unique environmental correlations. The lower half of the diagram shows the phenotypic model. FI_1 to FI_{15} represents the measured (squares) variables of FI from wave 1 to wave 15. $B_{S1.1}$ to $B_{S1.15}$ and $B_{S2.1}$ to $B_{S2.15}$ represent the age-based coefficient of slope 1 and slope 2 respectively. ε_0 to ε_{15} represent residual errors, and σ_{res}^2 represents residual variance (i.e., variation not accounted for by the growth model). M_I, M_{S1}, and M_{S2} represent the mean intercept, mean slope 1, and mean slope 2, respectively. $\beta_{study.I}$ and $\beta_{study.S1}$ represent the regression coefficients of study (i.e., OCTO-Twin vs. SATSA) on intercept and slope 2, respectively.

4.3.2 Latent growth curve modeling

In **Study II,** we fitted age-based latent growth curve models within the multilevel modeling framework in SATSA and OCTO-Twin to describe the longitudinal trajectories of the FI,[188] where chronological age (in years) was used as the underlying timescale. These models allowed for estimation of both fixed effects (representing the average trajectory of the FI in the sample) and random effects (representing the variation around the mean trajectory). We considered random effects in three levels: FI measurements (level 1) within individuals (level 2), who were nested within twin pairs (level 3).

A series of unconditional models (without including any covariates) were first fitted to compare different functional forms, including linear, quadratic, and bilinear two-slope models. We found that a bilinear two-slope model with a knot point at age 75 best described the longitudinal trajectories of the FI (which is illustrated in the lower half of **Figure 5**). This model consisted of an intercept representing the mean FI at 75 years, a slope <75 years ("slope 1") representing the average annual change in FI before 75 years, and a slope >75 years ("slope 2") representing the average annual change in FI after 75 years. The random effects of this model included variances and covariances of the intercept, slope 1, and slope 2 at the individual and twin pair levels. A residual variance (constrained to be equal for each measurement occasion) was also included, indicating the unreliable variance not accounted for by the growth model. After identifying the best functional form, we extended the model to include time-invariant covariates, including study (OCTO-Twin vs. SATSA) and birth cohort (born ≥1926 vs. <1926), and examined if the inclusion of these covariates improved the model fit. For all the growth models, fixed and random effects parameters, except for the regression coefficients of the time-invariant covariates, were estimated separately for men and women. Also, the models were fitted separately in SATSA and in the full sample (i.e., SATSA and OCTO-Twin combined) to determine whether including a selected sample of oldest-old twins from OCTO-Twin would influence the results.

Subsequently, the best-fitting latent growth curve model was extended to a biometric model to study genetic and environmental influences on the FI trajectories as described above in § 4.3.1.

4.3.3 Regression analysis

Regression models were used in **Studies III**–**V** to examine the associations between exposure and outcome variables while adjusting for measured confounding factors. In general, we estimated β-coefficients for continuous outcomes using linear regression models, odds ratios (ORs) for binary outcomes using logistic regression models, and hazard ratios (HRs) for time-to-event outcomes using Cox models. Additionally, generalized estimating equations, or mixed models that incorporated random effects were used when analyzing twin and longitudinal data to account for the correlated observations.[189]

Specifically, in **Study III**, we conducted an EWAS to explore the cross-sectional associations between the FI (independent variable) and DNA methylation levels of 245,545 CpG sites (dependent variables) at baseline of SATSA, using generalized estimating equations with cluster-robust standard errors to account for twin relatedness. The FI was considered both as a continuous (per 10% increase) and as a categorical variable (frail vs. non-frail; and prefrail vs. non-frail) in the models. The CpG sites associated with the FI at a false discovery rate (FDR)[190] of <0.05 were considered as statistically significant and were then

analyzed for their longitudinal associations with the FI in SATSA and replicated in LSADT. The longitudinal analysis was performed using linear mixed-effects models with random effects at the individual and twin pair levels to account for the correlation between repeated measurements of DNA methylation and the FI. The replication analysis in LSADT was performed using generalized estimating equations with cluster-robust standard errors. All the models were adjusted for age, sex, smoking, BMI, and DNA methylation array (450K vs. EPIC array). In addition to the EWAS analysis on individual CpG sites, we also conducted a differential methylated region (DMR) analysis using *dmrff* in R,[191] which essentially combines summary statistics of nearby CpGs.[191,192] For the DMR analysis, a Bonferroni-adjusted $p<0.05$ was considered as statistically significant.

In the first part of the analysis in **Study IV** (i.e., observational analysis), we investigated the associations between 200 metabolic biomarkers (including 168 metabolomic biomarkers and 32 clinical biomarkers as independent variables) and the FI (dependent variable) in the UK Biobank using linear regression models, adjusted for age, sex, baseline assessment center, BMI, smoking, alcohol, education, and deprivation. Biomarkers associated with the FI at a Bonferroni-corrected *p*-value threshold of 0.0025 (i.e., 0.05/200) were considered as statistically significant. We also performed a sensitivity analysis using the FP score as a secondary outcome, and conducted subgroups analyses stratified by age at baseline, sex, and ethnicity. Additionally, we employed a penalized linear regression model – the least absolute shrinkage and selection operator (LASSO)[193] – to select the metabolites that were most strongly and independently associated with the FI while mutually adjusting for each other and also adjusting for age and sex. LASSO is a feature selection tool that is particularly useful when dealing with high-dimensional data such as NMR metabolomics.[120] It constrains the sum of the absolute values of the regression coefficients, resulting in a sparse model that contains only the most informative variables contributed to the variance of the FI.[193] The biomarkers that were statistically significantly associated with the FI in the linear regression models and selected by the LASSO model were then replicated for their associations with the FI in TwinGene and the Health 2000 Survey. The replication analysis was performed using linear regression models adjusted for age, sex, BMI, smoking, education, and alcohol. The models in TwinGene additionally accounted for twin relatedness using cluster-robust standard errors. The estimates from the replication cohorts were then meta-analyzed using a random-effects model;[194] we considered those with $p<0.05$ as the "replicated biomarkers", which were then brought forward to the MR analyses to investigate potential causal relationships (described below in § 4.3.4).

In **Study V,** we assessed the relationships between frailty and comorbidity measures (eFI, CFS, HFRS, CCI) and various outcomes in geriatric patients, including in-hospital mortality, 30-day readmission, 30-day mortality, 6-month mortality, and the length of stay. We used logistic regression models for in-hospital mortality and 30-day readmission, Cox models for 30-day and 6-month mortality, and linear regression models for the length of

stay. All models were adjusted for age and sex, and additionally accounted for the clustering of patients in geriatric clinics using stratified Cox models or conditional generalized estimating equations. The diagnostic performance of the logistic regression models was assessed using the area under the receiver operating characteristic curve (AUC), while the Harrell's C-statistics were used for the Cox models.[195]

4.3.4 Mendelian randomization (MR) analysis

In the second part of the analysis in **Study IV**, we performed two-sample MR analyses to examine potential causal effects of the metabolic biomarkers identified from observational analyses on the FI and FP scores. MR is a causal inference method that uses genetic variants as instrumental variables (IVs) to assess the effect of an exposure on an outcome, which helps to overcome the limitations of confounding and reverse causation that are often encountered in observational studies (**Figure 6**).[196] To provide valid causal inference, genetic variants used as IVs should fulfill three assumptions: (i) they should be robustly associated with the exposure (relevance assumption), (ii) they should be independent of any confounders (independence assumption), and (iii) they should only affect the outcome through the exposure but not though other pathways (exclusion restriction assumption).[196]

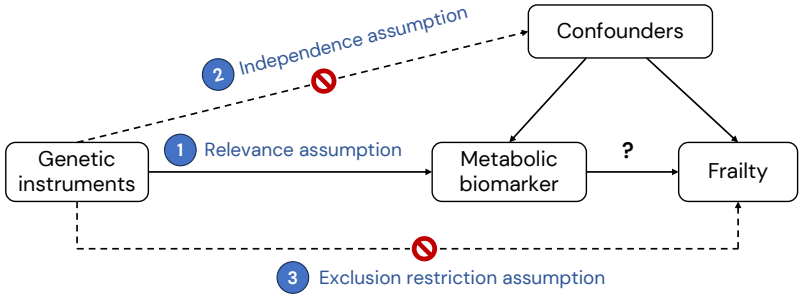

Figure 6. Design and the three main assumptions of the Mendelian randomization analyses for metabolic biomarkers and frailty.

The SNPs associated with the exposures (biomarkers) were selected from the largest available GWASs conducted in European populations, including the UK Biobank (*n*=115,078, for NMR metabolomic biomarkers),[197] the Meta-Analyses of Glucose and Insulin-related traits Consortium (*n*=123,665, for HbA1c),[198] the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (*n*=204,402, for CRP),[199] and the Global Lipids Genetics Consortium (*n*=187,365, for total cholesterol, LDL-cholesterol, and triglycerides).[200] For other clinical biomarkers, we performed a GWAS in a randomly selected 50% of the UK Biobank sample. SNPs were selected as IVs if they were associated with the biomarker of

interest at a genome-wide significance level ($p<5\times10^{-8}$) and not in linkage disequilibrium with other SNPs ($r^2<0.001$ within a clumping window of 10,000 kb). *F*-statistics were used to assess the instrument strength, where SNPs with an *F*-statistic of >10 are typically considered as strong instruments.[196] To obtain summary statistics for SNP-outcome (frailty) associations, we performed GWAS analysis for the FI and FP in UK Biobank subsamples that did not overlap with the exposure GWASs, to avoid an overfitting bias and an inflated false positive rate in two-sample MR.[201]

The primary approach used in the MR analysis was the multiplicative random-effects inverse variance weighted (IVW)-MR method, which provides unbiased estimates if all the IVs are valid or if the overall pleiotropy is balanced to be zero.[202] To correct for multiple testing, we considered an FDR-corrected *p*-value threshold of 0.011 as statistically significant. Other MR methods that relax assumptions on horizontal pleiotropy, including MR-Egger,[203] weighted median,[204] weighted mode,[205] and MR-pleiotropy residual sum and outlier (MR-PRESSO)[206] were used as sensitivity analyses. Besides, as many of the IVs were associated with more than one metabolomic biomarker, we performed a sensitivity analysis by excluding these potentially pleiotropic SNPs (i.e., using only SNPs that were not associated with other metabolomic biomarkers at genome-wide significance as the IVs). Furthermore, to examine whether any observed association may be driven by the individual deficit items included in the FI, we repeated the MR analysis using 11 modified FIs that were stripped of items from each category as the outcome variables (i.e., FIs removing cancers, cardiometabolic, cranial, gastrointestinal, immunological, infirmity, mental wellbeing, musculoskeletal, pain, respiratory, and sensory items).

4.3.5 Co-twin control analysis

In **Study IV**, we additionally conducted a co-twin control analysis in TwinGene to investigate the associations of GlycA and creatinine (which were the biomarkers that showed putative causal effects from MR) with the FI while controlling for unmeasured family-constant confounders such as genetic or shared environmental factors. The co-twin control method compares the population-level estimates with the within-pair estimates in MZ twins and DZ twins and can test whether an association is independent of familial influences (i.e., whether it is in line with a causal hypothesis).[207] In cases where the association may be attributed to shared genetic factors (pleiotropy), we would expect an attenuation of the association within MZ twin pairs, whereas the estimated association in DZ twin pairs would be expected to lay between the population-level and MZ estimates. Conversely, in cases where the association may be attributed to shared environmental factors, a similar attenuation of the association would be expected in both MZ and DZ twins. The within-twin-pair estimates were obtained using conditional generalized estimating equations, adjusted for age, sex, BMI, smoking, alcohol, and education.

4.4 Ethical considerations

For the first four studies involving cohorts from the Swedish Twin Registry (SALT, SATSA, OCTO-Twin), the Danish Twin Registry (LSADT), the Finnish Health 2000 Survey, and the UK Biobank, informed consents were obtained from all the participants prior to data collection. The participants were informed of the overall aims of the study, potential risks, contact person, and the procedures of the data collection. It was emphasized that participation is voluntary, and they can withdraw from the study freely at any time without giving any reason. Specifically, participants who have requested to withdraw from the UK Biobank cohort were excluded from the analysis in **Study IV**. For **Study V** that involved retrospective data extracted from EHRs, informed consent is not required; however, ethical approval is also needed and had been obtained before the start of the study. **Studies I–IV** were approved by the Regional Ethics Review Board in Stockholm (Dnr 2015/1729-31/5 and 2016/1888-31/1), and **Study V** was approved by the Swedish Ethical Review Authority (Dnr 2021-02096).

Moreover, since we are working with sensitive data (e.g., health status, biomarker data), it is crucial to protect the integrity and privacy of the individuals and process the data in compliance with the General Data Protection Regulation (GDPR).[208] All the data used in this thesis were pseudonymized and the researchers do not have access to the keys for linking to or identifying the individuals. Besides, the data are stored in secure departmental servers and only the researchers involving in the studies have access to the data, thus with minimal chance of data breach.

In accordance with the ethical principal of beneficence, this research will potentially benefit to the general public through an increased knowledge on how we can provide improved care for frail older adults. There should also be minimal harm to the participants both physically and mentally. Nevertheless, one potential physical harm could be the pain induced during collection of blood samples. Another important ethical aspect regarding frailty research is stigmatization. Being labeled as "frail" may cause negative feelings by older adults.[209] Therefore, when applying the results to clinical practice, it is crucial to understand the perspectives and feelings of older adults on the term "frailty" and avoid stereotyping.[210,211]

Finally, when disseminating the results, it is necessary to maintain transparency and honesty. For all studies, instead of focusing on significant *p*-values, we always attempted to use the most appropriate statistical methods and present all the observed results. The potential limitations of the studies are also discussed thoroughly in the papers and in section 6 of the thesis.

5 Results

5.1 Study I

5.1.1 Sex differences in heritability of frailty

The study sample consisted of 23,054 women and 19,940 men from SALT, with an overall mean age of 58.8 years and a median FI of 0.108. Women had a higher median FI (0.119 vs. 0.097) and were on average older than men (59.2 vs. 58.4 years). The FI correlated positively with BMI (r=0.13) and negatively with years of education (r=-0.09). For the FI, BMI, and education, the intraclass correlations for MZ twins were greater than that for DZ twins, suggesting genetic influences on all the three traits. We first fitted univariate sex-limitation models to investigate quantitative and qualitative sex differences in the heritability of the FI. As shown in **Table 4**, the best-fitting univariate model for the FI was an *ADE* model with only quantitative sex differences, suggesting that the magnitude of heritability differed between men and women, but there was no evidence that different genetic factors influence frailty in men and women. Specifically, the broad-sense heritability of the FI was estimated to be 45% (95% confidence interval [CI]: 41–48%) in men and 52% (50–55%) in women, with the rest of the variation explained by unique environmental factors.

Table 4. Model fitting results and parameter estimates from univariate sex-limitation models of the FI. This table is adapted from Mak et al. *Aging* 2021.[212]

Model	Model fit statistics				Parameter estimates for men and women				
	AIC	ΔLL	Δdf	*p*	*A*	*D/C*	*H*	*E*	r_{fm}
Saturated	19953	-	-	-	-	-	-	-	-
ADE full sex-limitation	19940	19.1	16	0.264	M: 7% F: 41%	M: 38% F: 11%	M: 44% F: 52%	M: 56% F: 48%	0.69
ADE quantitative sex-limitation	**19939**	**19.7**	**17**	**0.288**	**M: 0% F: 41%**	**M: 44% F: 11%**	**M: 45% F: 52%**	**M: 55% F: 48%**	**1.00**
ADE no sex difference	19949	32.1	18	0.021	M: 0% F: 44%	M: 49% F: 4%	M: 49% F: 49%	M: 51% F: 51%	1.00
ACE full sex-limitation	19961	40.4	16	0.001	M: 41% F: 51%	M: 0% F: 0%	M: 41% F: 51%	M: 59% F: 49%	0.76
AE full sex-limitation	19957	40.4	18	0.002	M: 41% F: 51%	M: 0% F: 0%	M: 41% F: 51%	M: 59% F: 49%	0.76

AIC, Akaike's Information Criterion; *LL*, log-likelihood; *df*, degrees of freedom; *A*, additive genetic factors; *D*, dominance genetic factors; *C*, common environmental factors; *H*, total genetic factors/ broad-sense heritability; *E*, unique environmental factors; r_{fm}, genetic correlation between men and women, estimated using opposite-sex twins. M and F represents parameter estimates for men and women respectively. *p*-values were obtained from likelihood ratio tests comparing with the saturated model (i.e., a model that fully describes the observed data), where $p<0.05$ was considered as a significantly reduced model fit. All models were adjusted for age. Best-fitting model is shown in bold.

5.1.2 Genetic and environmental overlap with BMI and education

Bivariate twin modeling was then applied to examine the overlap of genetic and environmental variances of the FI with BMI and education. The best-fitting bivariate model for the FI and BMI was an *ADE* model, with an estimated "bivariate heritability" of 81% for men and 87% for women, indicating that a substantial part of the correlation between these two traits could be explained by genetic factors in common to both (**Figure 7**). In contrast, the best-fitting bivariate model for FI and education was an *ACE* model, where common environmental factors contributed 65% and 74% to the correlation between these two traits in men and women, respectively (**Figure 7**).

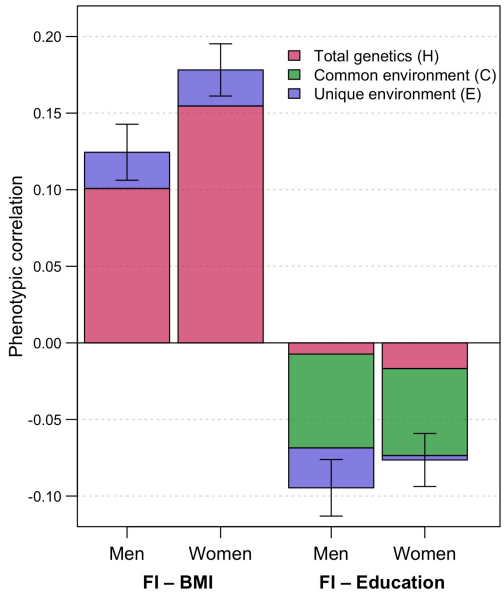

Figure 7. Proportion of correlations of FI with BMI and education explained by genetic and environmental factors. This figure is adapted from Mak et al. *Aging* 2021.[212]

5.1.3 Moderation by BMI and education

We further fitted moderation models to examine if genetic and environmental influences on the FI vary by levels of BMI and education. **Figure 8** illustrates the variance components of the FI over BMI and education estimated from the best-fitting moderation models (i.e., a full *ADE* bivariate moderation model for FI and BMI, and an extended *ADE* univariate moderation model for FI and education). Overall, we found that the heritability of the FI (as indicated by the red color in the lower panels of **Figure 8**) tended to be greater at low and high BMI levels, but it did not seem to vary across education years. These patterns of moderation were similar in men and women.

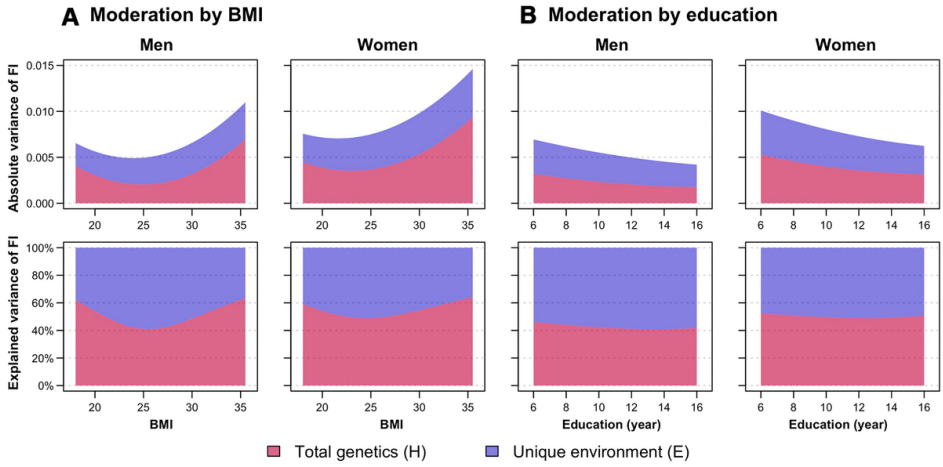

Figure 8. Moderation analysis of FI by **(A)** BMI and **(B)** education, stratified by sex. This figure is reproduced from Mak et al. *Aging* 2021.[212]

5.2 Study II

5.2.1 Frailty trajectories from adulthood into old age

We examined longitudinal trajectories of the FI in 1,842 younger and older adults from SATSA (mean baseline age 62.1 years; 58.3% women) and 654 oldest-old adults from OCTO-Twin (mean baseline age 83.4; 66.2% women). The median FI at baseline was higher in OCTO-Twin than in SATSA (0.195 vs. 0.080). Participants contributed to a maximum of 15 waves, with 71.9% of the participants in SATSA and 60.1% in OCTO-Twin having at least three FI measurements available. Age-based latent growth curve models were first fitted to characterize the sex-specific FI trajectories, separately in SATSA and in the full sample (i.e., SATSA and OCTO-Twin combined data). The best-fitting model was a bilinear growth model with an intercept at age 75, which indicated that in SATSA, the mean FI at age 75 was higher in women than in men (0.1265 vs. 0.1046), and the slope rates increased 4–5 times in both sexes after age 75 (**Table 5**). In the full sample of 2,496 twins, including "study" as a covariate in the model improved the model fit, indicating that OCTO-Twin participants had, on average, a 0.0817 higher FI at age 75 and a 0.0069 lower rate of FI increase compared to SATSA participants (**Table 5**). The variances of the intercept were larger than those of the slopes, suggesting that individual differences in the mean FI trajectory were primarily carried by the intercept. In SATSA, intraclass correlations for the intercept were around 0.3 for both sexes, while slope 1 was much more correlated within twin pairs in women (0.29) than in men (0.04). These correlations were similar in the full sample. However, correlations for slope 2 were lower in the full sample than in SATSA, indicating less similarity in the rates of change in FI among older twins from OCTO-Twin.

Table 5. Parameter estimates from the best-fitting latent growth curve models of the FI. This table is reproduced from Mak et al. *The Journals of Gerontology: Series A* 2023.[187]

	SATSA (*n* = 1,842)		Full sample (*n* = 2,496)	
	Men	**Women**	**Men**	**Women**
Fixed effects (means)				
Intercept at 75 years	10.46*	12.65*	10.38*	12.88*
OCTO-Twin (ref. SATSA)	-		8.17*	
Slope 1 (<75 years)	0.14*	0.21*	0.14*	0.22*
Slope 2 (>75 years)	0.76*	0.85*	0.75*	0.78*
OCTO-Twin (ref. SATSA)	-		-0.69*	
Random effects (variances and correlations)				
Level 1: observations				
Residual variance	13.46	16.07	15.11	17.57
Level 2: individual level				
Variance of intercept	34.12	55.29	38.16	63.68
Variance of slope 1	0.03	0.04	0.03	0.04
Variance of slope 2	0.63	0.49	0.60	0.54
Correlation between intercept and slope 1	0.70	0.86	0.67	0.86
Correlation between intercept and slope 2	0.05	0.004	-0.57	-0.38
Correlation between slope 1 and slope 2	0.08	0.17	-0.29	-0.03
Level 3: twin pair level				
Variance of intercept	13.83	30.50	16.17	34.87
Variance of slope 1	0.001	0.01	0.001	0.02
Variance of slope 2	0.16	0.18	0.06	0.09
Correlation between intercept and slope 1	0.32	0.83	0.76	0.90
Correlation between intercept and slope 2	0.03	-0.21	-0.17	-0.38
Correlation between slope 1 and slope 2	0.38	-0.18	-0.08	-0.22
Intraclass (twin) correlations				
Intercept at 75 years	0.29	0.36	0.30	0.35
Slope 1 (<75 years)	0.04	0.29	0.03	0.30
Slope 2 (>75 years)	0.20	0.27	0.09	0.14

The best-fitting model was a bilinear two-slope latent growth curve model with an inflection point (intercept) at age 75. Slope 1 represents change of the FI until age 75, and slope 2 represents change of the FI from age 75 onwards. The FI used in the models was multiplied by 100 (as a percentage of deficit from 0–100%). The full sample represents the SATSA and OCTO-Twin combined data. Intraclass correlations indicate the extent to which the intercept, slope 1, and slope 2 correlate within twin pairs. * Fixed effects parameters with $p<0.05$.

5.2.2 Genetic and environmental influences on frailty trajectories

We then extended the phenotypic models to biometric models, which decomposed random effects of the latent growth variables (i.e., variances and covariances of the intercept, slope 1, and slope 2) into their genetic and environmental etiologies. The best-fitting biometric model was an *AE* model, which estimated the heritability of the intercept, slope 1, and slope 2 in the full sample to be 55%, 45%, and 18% in women, and 42%, 3%, and 26% in men, respectively. From this model, we also calculated the expected changes in the

variance components of the FI across age (**Figure 9**). Overall, there was a substantial increase in the total FI variance after age 75. In men, *A* remained similar across age, while *E* increased sharply after 75 years. In women, both *A* and *E* increased with age, but the increase was larger for the latter in late life. These results were largely similar when removing the OCTO-Twin participants from the analysis.

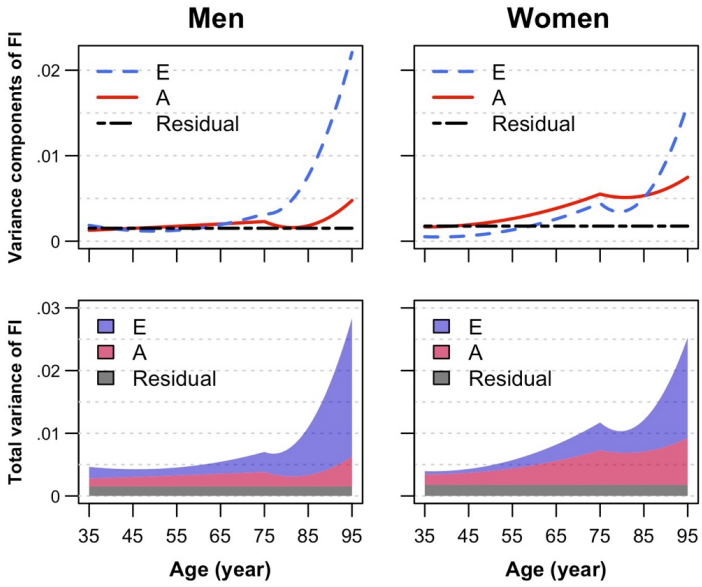

Figure 9. Expected changes in FI variance with age in the full sample, stratified by sex. This figure is reproduced from Mak et al. *The Journals of Gerontology: Series A* 2023.[187]

5.3 Study III

5.3.1 Epigenome-wide analysis of frailty

A total of 526 SATSA participants (mean age at baseline 68.3 years; 58.7% women) and 304 LSADT participants (mean age at baseline 78.5 years; 69.4% women) were included in the discovery and replication cohort, respectively. The median FI was similar in SATSA (0.077) and in LSADT (0.081). A cross-sectional EWAS was first conducted using baseline data from SATSA to identify FI-associated CpGs. Of the 245,545 CpGs that passed quality control, 29 and 162 were statistically significantly (FDR <0.05) associated with the continuous FI and the categorical FI comparing frail vs. non-frail participants, respectively (**Figure 10**). None of the CpGs were associated with prefrailty at FDR <0.05. **Table 6** lists the 20 CpGs that were significantly associated with both the continuous and categorical FI, where the top 5 were cg04309480 (*LRRN2*), cg00155846 (*OLFM1*), cg01369033 (-), cg20624041 (*LIPT2*), and cg27638713 (*MCM3*). Other than the individual CpG sites, we

also identified a DMR in the *PACRG* gene in chromosome 6 that was associated with both the continuous and categorical FI at a Bonferroni-adjusted $p<0.05$.

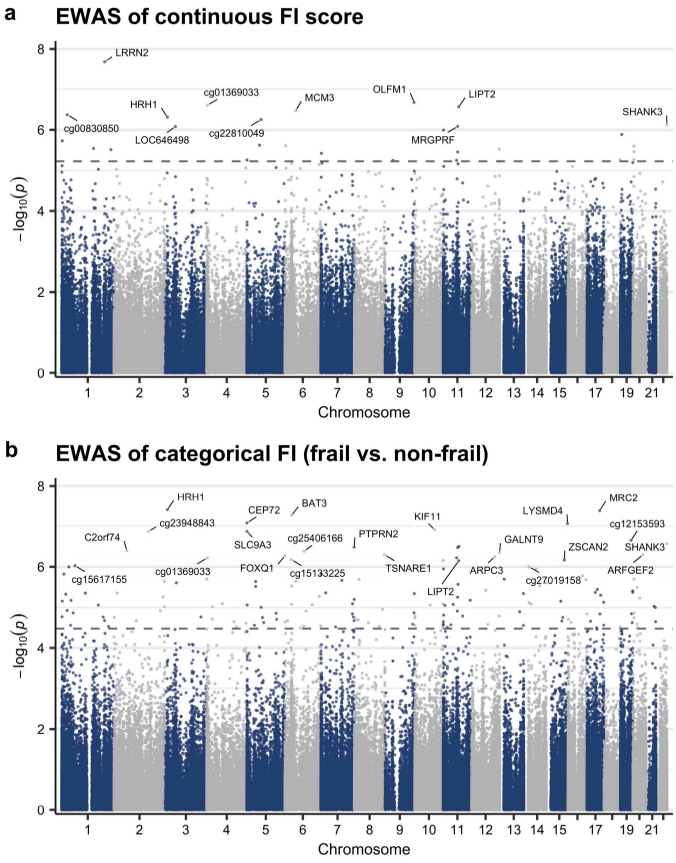

Figure 10. Manhattan plot for the cross-sectional epigenome-wide associations with (a) continuous FI score (per standard deviation increase) and (b) categorical FI (frail vs. non-frail) in SATSA.

To characterize the significant CpG sites, we performed gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis,[213] although we did not identify any significant terms after applying the FDR adjustment. In addition, we queried the EWAS Catalog (http://ewascatalog.org/) and the GWAS Catalog (https://www.ebi.ac.uk/gwas/; both accessed on June 9, 2023) to search for previously reported traits that were associated with our identified CpG sites. Many of the CpGs have previously been shown to be associated with chronological age (135 sites), rheumatoid arthritis (18 sites), clear cell renal carcinoma (15 sites), and pancreatic ductal adenocarcinoma (7 sites). Also, many of the genes which our identified CpGs mapped to have been associated with BMI, educational attainment, and cognitive function.

Table 6. Top CpGs associated with the FI at baseline in SATSA.

CpGs	Gene	Chr	Position	Continuous FI (per 10% increase)			Categorical FI (frail vs. non-frail)		
				β	*p*	FDR	*β*	*p*	FDR
cg04309480	*LRRN2*	1	204655678	0.010	2.1×10^{-8}	0.005	0.022	2.1×10^{-5}	0.040
cg00155846	*OLFM1*	9	138011566	0.012	2.1×10^{-7}	0.017	0.028	1.3×10^{-5}	0.033
cg01369033	-	4	3292386	0.007	2.5×10^{-7}	0.017	0.016	6.0×10^{-7}	0.007
cg20624041	*LIPT2*	11	74204975	-0.004	2.7×10^{-7}	0.017	-0.011	7.1×10^{-7}	0.007
cg27638713	*MCM3*	6	52149159	-0.004	3.4×10^{-7}	0.017	-0.011	2.3×10^{-6}	0.012
cg00830850	-	1	26672649	0.011	4.3×10^{-7}	0.017	0.025	1.2×10^{-5}	0.031
cg14458903	*HRH1*	3	11203475	0.005	4.9×10^{-7}	0.017	0.013	3.9×10^{-8}	0.004
cg22810049	-	5	67953707	0.011	5.6×10^{-7}	0.017	0.025	9.8×10^{-6}	0.027
cg20098420	*SHANK3*	22	51155589	0.012	7.6×10^{-7}	0.019	0.030	2.7×10^{-7}	0.006
cg21936959	*MRGPRF*	11	68782049	0.006	8.3×10^{-7}	0.019	0.016	3.4×10^{-7}	0.006
cg23595571	*TOLLIP*	11	1313100	0.012	1.0×10^{-6}	0.021	0.035	1.1×10^{-6}	0.009
cg09867208	*PRDM16*	1	3351390	0.009	1.9×10^{-6}	0.033	0.022	6.9×10^{-6}	0.023
cg16763089	*LOC149837*	20	5485284	-0.029	2.5×10^{-6}	0.036	-0.076	2.0×10^{-6}	0.012
cg10369955	*GALNT9*	12	132865474	0.004	3.0×10^{-6}	0.038	0.011	4.5×10^{-7}	0.007
cg03287299	*LOC149837*	20	5485245	-0.020	3.5×10^{-6}	0.039	-0.051	1.8×10^{-5}	0.037
cg06173857	*GNA12*	7	2855704	0.003	3.8×10^{-6}	0.040	0.007	1.5×10^{-6}	0.011
cg20295248	*LOC149837*	20	5485270	-0.029	5.3×10^{-6}	0.049	-0.073	1.4×10^{-5}	0.033
cg06954658	*SALL3*	18	76740093	-0.005	5.5×10^{-6}	0.049	-0.010	3.0×10^{-5}	0.048
cg08917022	*CEP72*	5	623259	0.012	5.5×10^{-6}	0.049	0.033	8.3×10^{-8}	0.004
cg01256440	*FLRT1*	11	63886459	0.008	5.6×10^{-6}	0.049	0.023	6.0×10^{-7}	0.007

Chr, chromosome; *FDR*, false discovery rate; *FI*, frailty index. Listed are the 20 CpGs significantly associated with both the continuous and categorical FI at FDR <0.05 from the cross-sectional EWAS in SATSA.

5.3.2 Replication of the identified CpGs

For the 171 CpGs that were significantly associated with either the continuous or categorical FI in the cross-sectional EWAS, we performed a longitudinal analysis using all available measurements in SATSA and a replication analysis in an independent sample of LSADT participants. Consistent directions of associations were observed when comparing the cross-sectional and longitudinal estimates in SATSA. In LSADT, five out of the 171 CpG sites were associated with the FI at $p<0.05$ and were directionally consistent with the estimates in SATSA, including cg04309480 (*LRRN2*), cg20624041 (*LIPT2*), cg21936959 (*MRGPRF*), cg10850119 (*FBXO4*), and cg06897860 (-).

Lastly, we performed a literature search and identified 80 CpGs that were previously reported to be associated with frailty.[104,105,113,214] We investigated their associations with the FI in SATSA and LSADT, and found only one of these CpGs (cg00252813 in the *GAPDH* gene) showed consistent associations with the FI in both SATSA and LSADT at $p<0.05$.

5.4 Study IV

5.4.1 Identification of frailty-associated metabolic biomarkers

In the observational analyses, two subsamples from the UK Biobank were used as the discovery cohorts, which comprised 90,573 (mean age 56.8 years; 54.4% women; mean FI 0.123) and 67,488 participants (mean age 57.5 years; 39.1% women; mean FI 0.130) who had complete data on the 168 NMR metabolomic biomarkers and 32 clinical biomarkers, respectively. Linear regression models were first used to assess associations between each of the 200 metabolic biomarkers and the FI, adjusting for age, sex, baseline assessment center, BMI, smoking, alcohol, education, and deprivation. A total of 164 biomarkers were statistically significantly associated with the FI after the Bonferroni correction for multiple testing. Particularly, glycoprotein acetyls (GlycA) showed the strongest positive association among the metabolomic biomarkers, with each SD increase corresponding to a 0.56% higher FI (**Figure 11**). Meanwhile, many of the lipids and lipoproteins showed negative associations with the FI. Largely similar results were observed when using FP as the outcome and in subgroups by age, sex, and ethnicity. Further, due to the high intercorrelation between the metabolic biomarkers, we employed the LASSO procedure and identified 77 biomarkers that exhibited strong and independent associations with the FI.

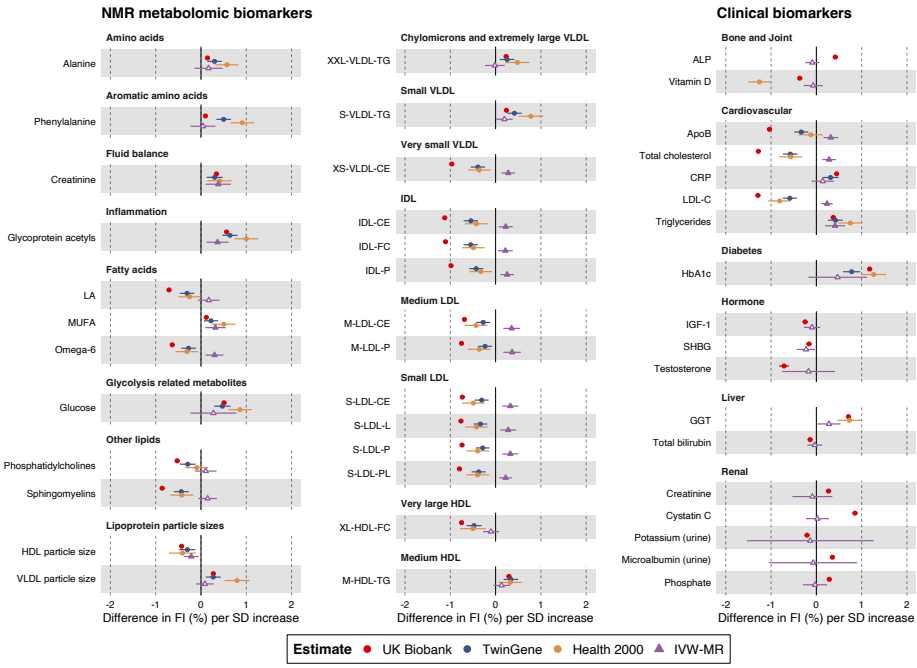

Figure 11. Observational and MR effect estimates of selected metabolic biomarkers on FI. This figure is reproduced from Mak et al. *Aging Cell* 2023.[215] Effect sizes represent changes in FI (%) per SD increase in biomarker level, except the IVW-MR estimates for CRP and HbA1c, which are per log mg/L increase and per % increase, respectively. For IVW-MR estimates, filled triangles represent $p<0.011$ (FDR-corrected threshold).

Based on the results from the linear regression and LASSO models, 41 metabolomic and 18 clinical biomarkers were selected for replication in 11,025 TwinGene (mean age 58.3 years; 55% women; mean FI 0.121) and 6,073 Health 2000 participants (mean age 52.5 years; 55% women; mean FI 0.177). Meta-analysis of the biomarker-FI associations in these two cohorts revealed that 34 out of the 49 available biomarkers had significant associations with the FI at $p<0.05$. These replicated biomarkers included metabolomic biomarkers from various domains, including amino acids (e.g., alanine), fluid balance (e.g., creatinine), inflammation (GlycA), fatty acids (e.g., linoleic acid, monounsaturated fatty acids), and lipoprotein subclasses. Additionally, clinical biomarkers such as LDL-cholesterol, CRP, and HbA1c were also significantly associated with the FI (**Figure 11**).

5.4.2 Causal inference using Mendelian randomization

Two-sample MR analyses were then performed to investigate causal relationships between 44 selected biomarkers (34 replicated and 10 unavailable in TwinGene and Health 2000) and the FI and FP scores. We selected IVs from the largest available GWASs, and all of them had an estimated *F*-statistics of >10. Using the IVW-MR method, we identified 19 significant associations with the FI at an FDR-corrected threshold of $p<0.011$ (**Figure 11**). Specifically, several of these MR estimates were consistent with the observational estimates. For example, each SD increase in the genetically predicted levels of GlycA and creatinine was associated with a 0.37% and 0.38% increase in the FI, respectively (**Figure 12**). However, lipids traits such as apolipoprotein B, total cholesterol, LDL-cholesterol, and lipoprotein subclasses generally had a negative association with the FI in the observational analysis but a positive association in the IVW-MR analysis (**Figure 11**). None of the 44 biomarkers were statistically significantly associated with the FP score.

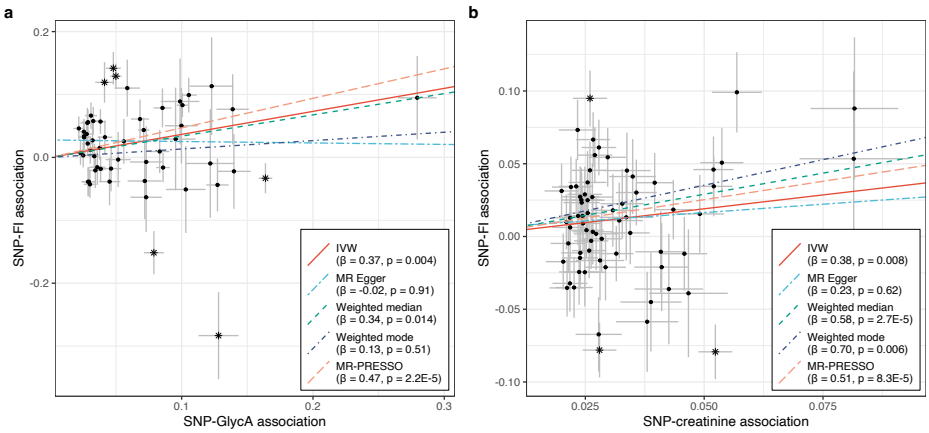

Figure 12. MR scatter plots for the effects of **(a)** glycoprotein acetyls and **(b)** creatinine on FI. This figure is reproduced from Mak et al. *Aging Cell* 2023.[215]

When using other MR methods including MR-Egger, weighted median, weighted mode, and MR-PRESSO, the estimates for most biomarkers remained consistent, although there was evidence of directional pleiotropy for GlycA, monounsaturated fatty acids, and total lipids in small LDL (p<0.05 for MR-Egger intercept). In the sensitivity analysis of removing potentially pleiotropic SNPs for each biomarker, the MR estimates for GlycA and creatinine remained robust, but estimates for most lipids and lipoproteins were attenuated. We further repeated the MR analysis using 11 stripped FIs as the outcomes. When cardiometabolic items such as heart failure, stroke, and diabetes were removed from the FI, the MR estimates for monounsaturated fatty acids, omega-6, cholesterols, and lipoprotein subclasses were attenuated to null, indicating that their effects on the FI may be mediated by cardiometabolic diseases. The MR estimates for GlycA and creatinine remained statistically significant across all the stripped FIs.

Finally, for creatinine and GlycA that exhibited potential causal effects on the FI, we additionally performed subgroup analysis in the UK Biobank and co-twin control analysis in TwinGene to examine if their observational associations are influenced by their related traits (kidney disease for creatinine;[216] CRP & LDL-cholesterol for GlycA[217]) or confounded by shared familial factors. Interestingly, while the GlycA-FI association remained robust across all subgroups, the creatinine-FI association was attenuated to null in participants without chronic kidney disease, indicating that the association may be confounded/mediated by kidney disease. In the co-twin control analysis, we observed a slight attenuation of the GlycA-FI association within DZ pairs, and an even greater, but incomplete attenuation within MZ pairs, indicating potential genetic confounding (**Figure 13**). The population-level and within-pair estimates for the creatinine-FI association, however, were mostly statistically nonsignificant, limiting us to conclude the extent of familial confounding.

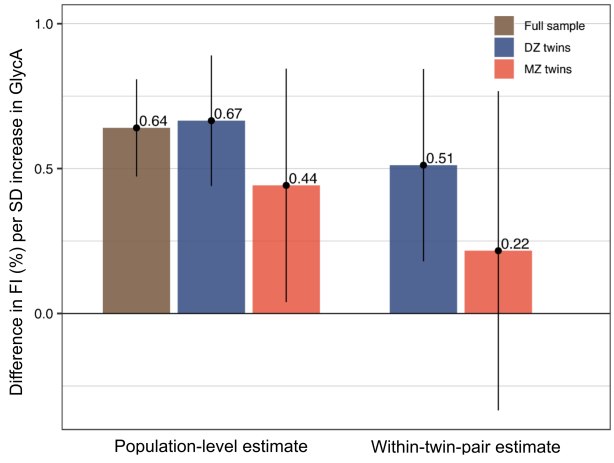

Figure 13. Population-level and within-twin-pair estimates for the association between GlycA and FI in the full sample, DZ twins (2762 pairs), and MZ twins (1132 pairs) in TwinGene. This figure is adapted from Mak et al. *Aging Cell* 2023.[215]

5.5 Study V

5.5.1 Swedish eFI for hospitalized older adults

Among the 13,188 patients who had sufficient data for calculation of the eFI, the mean age was 83.1 years, and 60.2% were women. Fragility fracture, congestive heart failure, dementia, stroke/transient ischemic attack, and urinary system disease were the most common causes of admission. The overall in-hospital mortality rate was 1.4% and the median length of stay was 6.7 days. The eFI had a median of 0.181 and an approximately normal distribution (**Figure 14**). The proportions of patients categorized as fit, mildly frail, moderately frail, and severely frail were 29.3%, 33.1%, 24.4%, and 13.2%, respectively. Men had significantly higher frailty scores than women based on the eFI and HFRS ($p<0.05$ from chi-squared tests), but not the CFS. The eFI showed moderate correlations with the CFS (Spearman's correlation 0.420), and weaker correlations with the HFRS (0.289) and CCI (0.368).

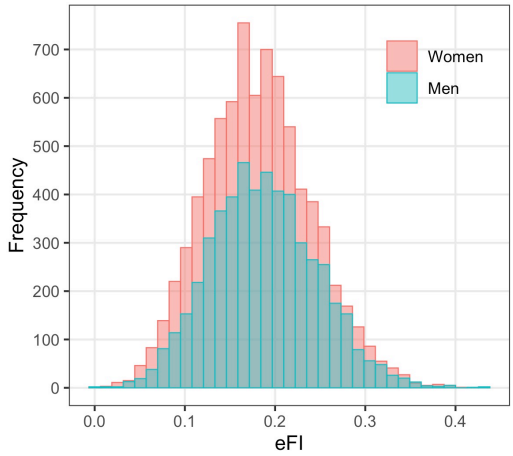

Figure 14. Distribution of the eFI stratified by sex (n=13,188).

5.5.2 Associations between eFI and adverse health outcomes

The eFI was strongly associated with in-hospital mortality (OR per 0.1 increase: 5.07, 95% CI: 4.23–6.09), 30-day mortality (HR: 3.26, 95% CI: 2.90–3.67), and 6-month mortality (HR: 2.66, 95% CI: 2.47–2.85) after adjusting for age and sex and accounting for clustering by the geriatric clinics (**Table 7**). Similar positive associations were observed for the CFS, HFRS, and CCI (**Table 7**). Notably, among all the frailty and comorbidity measures, the eFI had the highest discriminative ability for in-hospital mortality (AUC: 0.813), 30-day mortality (Harrell's C: 0.733), and 6-month mortality (Harrell's C: 0.707). We also found a statistically significant association between the eFI and a longer length of stay. However, the eFI, as well as the CFS, HFRS, and CCI, all had a relatively poor discrimination for 30-day readmission (all AUCs <0.6).

Table 7. Associations between frailty and comorbidity measures and mortality outcomes (n = 13,188). This table is reproduced from Mak et al. *The Journals of Gerontology: Series A* 2022.[218]

Model	**In-hospital mortality**, OR (95% CI)		**30-d mortality**, HR (95% CI)		**6-mo mortality**, HR (95% CI)	
	Model 1[a]	Model 2[a]	Model 1[a]	Model 2[a]	Model 1[a]	Model 2[a]
eFI						
Continuous, per 0.03 increase	1.65 (1.54, 1.78)*	1.63 (1.54, 1.72)*	1.43 (1.38, 1.48)*	1.43 (1.38, 1.48)*	1.34 (1.31, 1.37)*	1.34 (1.31, 1.37)*
Continuous, per 0.1 increase	5.34 (4.20, 6.82)*	5.07 (4.23, 6.09)*	3.28 (2.91, 3.69)*	3.26 (2.90, 3.67)*	2.70 (2.52, 2.90)*	2.66 (2.47, 2.85)*
Categorical						
Fit (≤0.15)	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
Mild frailty (>0.15–0.2)	4.60 (1.94, 13.5)*	4.64 (2.5, 8.8)*	2.60 (1.90, 3.55)*	2.63 (1.93, 3.60)*	1.91 (1.63, 2.22)*	1.94 (1.66, 2.26)*
Moderate frailty (>0.2–0.25)	12.5 (5.51, 35.7)*	12.1 (5.3, 27.7)*	4.79 (3.54, 6.49)*	4.83 (3.57, 6.54)*	3.42 (2.95, 3.97)*	3.45 (2.97, 4.01)*
Severe frailty (>0.25)	32.8 (14.7, 93.3)*	31.5 (14.3, 69.5)*	9.81 (7.27, 13.2)*	9.87 (7.31, 13.3)*	5.81 (4.99, 6.77)*	5.78 (4.96, 6.74)*
CFS (n = 4,945)						
Continuous, per point increase	1.63 (1.34, 2.00)*	1.86 (1.29, 2.69)*	1.57 (1.42, 1.74)*	1.78 (1.60, 1.98)*	1.44 (1.36, 1.52)*	1.60 (1.51, 1.70)*
Categorical						
1–3	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
4–5	4.33 (1.26, 27.1)*	4.19 (1.37, 12.9)*	3.74 (1.88, 7.46)*	3.99 (2.00, 7.98)*	1.98 (1.45, 2.72)*	2.10 (1.53, 2.89)*
6–9	7.22 (2.21, 44.4)*	9.74 (3.44, 27.6)*	6.11 (3.12, 12.0)*	8.42 (4.24, 16.7)*	3.68 (2.72, 4.97)*	4.86 (3.56, 6.64)*
HFRS						
Continuous, per point increase	1.06 (1.02, 1.11)*	1.04 (1.01, 1.07)*	1.04 (1.02, 1.07)*	1.04 (1.02, 1.06)*	1.04 (1.02, 1.05)*	1.03 (1.02, 1.05)*
Categorical						
Low risk (<5)	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
Intermediate risk (5–15)	1.63 (1.19, 2.20)*	1.48 (1.00, 2.18)	1.26 (1.08, 1.48)*	1.23 (1.04 1.44)*	1.22 (1.11, 1.34)*	1.20 (1.09, 1.32)*
High risk (>15)	Not estimable	Not estimable	1.21 (0.45, 3.25)	1.04 (0.39, 2.80)	0.70 (0.33, 1.48)	0.64 (0.30, 1.35)
CCI						
Continuous, per point increase	1.40 (1.31, 1.50)*	1.41 (1.33, 1.50)*	1.34 (1.29, 1.39)*	1.34 (1.30, 1.39)*	1.33 (1.31, 1.36)*	1.33 (1.30, 1.36)*

[a] Model 1 was adjusted for age and sex; Model 2 was additionally accounted for clustering of patients in the nine geriatric clinics. * p<0.05.

6 Discussion

6.1 Genetic and environmental influences on frailty

In the first two studies, we employed a twin design to investigate the interplay between genetics and the environment in relation to frailty. Our results showed that variations in the FI are attributable to a combination of genetic factors, including additive and dominance effects, as well as unique environmental factors. In **Study I,** adjusting for age, we estimated that the broad-sense heritability of the FI was 52% in women and 45% in men. Similar findings were seen in **Study II**, where the FI heritability at age 75 was 55% in women and 42% in men. These estimates are in line with previous twin studies on frailty, which reported heritabilities in the range of 25–45%.[90,91] Intriguingly, the largest GWASs on frailty to date have only found a SNP-based heritability of 6–11%.[80,103] Our **Study I**, with a large sample size and robust statistical power, demonstrated a significant contribution of dominance genetic factors to the FI, particularly pronounced in men. Thus, it is conceivable that the "missing heritability" in frailty can in part be explained by the non-additive genetic influences,[219] as well as rare variants,[220] that GWAS typically overlook. Since the results from twin studies imply that genetics may play a pivotal role in the development of frailty, it requires further research to identify the specific genetic factors involved.

One novel aspect of **Study I** is its exploration of sex differences in the heritability of frailty. Prior research consistently highlighted a sex-specific pattern in frailty, where women often exhibit a higher FI but a lower mortality risk at any given FI score or age compared to men, and the underlying reasons for this discrepancy remain elusive.[63] Our findings highlight that the FI heritability was statistically significantly higher in women; however, there was no indication that distinct sets of genetic factors are at play between the sexes in influencing the FI. This pattern of higher heritability in women aligns with that found for psychological and neurological traits.[221–223] Given the connection between frailty genetics and neurological pathways,[80] the heightened heritability of the FI in women might suggest a greater genetic susceptibility to frailty among women. Alternatively, the lower heritability in men could be attributable to their tendency to report health problems with less precision,[224] leading to an increased unique environmental variance (that also captures measurement errors) relative to genetic variance in a questionnaire-based FI.

Regarding environmental influences, both **Studies I** & **II** indicate a negligible impact of environmental influences shared within twin pairs, such as childhood experience or family environment, on frailty. Instead, a substantial portion of its variability can be explained by environmental factors unique to each individual. **Study II** further suggests that not only does frailty levels increase significantly in late life, but the unique environmental variance, particularly in men, also becomes more pronounced. It is challenging to pinpoint specific environmental factors as the primary drivers of frailty, due to the multidimensional nature

of the condition, which is associated with various physical, social, behavioral, and psychological factors.[72,79] However, these results emphasize that frailty is not solely determined by genetics, but is also greatly influenced by the environment, especially in late life, which can possibly be delayed or even reversed through adopting a healthy lifestyle including exercise, nutrition supplementation, and social participation.[149,225]

Since BMI and education are often regarded as the two most prominent modifiable risk factors for frailty,[80] we delved deeper into the genetic and environmental influences on frailty in relation to these two factors to shed light on the underlying mechanisms. For frailty and BMI, we found that their association was primarily explained by shared genetic factors, probably stemming from common mechanisms related to energy metabolism, inflammation, and synaptic pathways.[80,226,227] Furthermore, we observed that the FI heritability increased at both low and high BMI levels, mirroring the U-shaped association between BMI and frailty reported in the literature.[228–230] This suggests that underweight and obese individuals may face an elevated risk of frailty due to a more pronounced expression of genetic susceptibilities. In contrast, when examining the association between frailty and education, we found that it was primarily influenced by environmental factors shared within twin pairs. Moreover, the FI heritability did not seem to be modified by education levels. These results suggest a potential pathway that an improved family environment may contribute to higher educational attainment, subsequently reducing frailty by enhancing health literacy and promoting health-seeking behaviors.[231–233]

6.2 DNA methylation and frailty

Transitioning from our investigation of the genetic and environmental contributions to frailty, **Study III** studied the epigenetics of frailty as a possible mechanism for gene-environment interactions. As an exploratory analysis, we first performed an EWAS in SATSA and unveiled 171 differentially methylated CpG sites significantly linked to the FI in a cross-sectional context. Many of these associations were directionally consistent in the longitudinal analysis across age. Upon look-up in the EWAS Catalog, we noticed a substantial number of these CpGs had prior associations with chronological age and age-related traits, such as rheumatoid arthritis and carcinoma. Among these, we highlighted five specific sites that were replicated in the LSADT cohort, including cg04309480, cg20624041, cg21936959, cg10850119, and cg06897860. These sites are mapped to several genes (*LRRN2*, *LIPT2*, *MRGPRF*, and *FBXO4*) that have been linked to cancer development[234–237] and neuronal function.[238,239] Other than the individual CpG sites, we also revealed a DMR of the FI within the *PACRG* gene, which is known for its involvement in immune signaling.[240]

In the literature, there have only been a few prior EWASs on frailty.[104,105,113,214] Similar to our findings, a recent analysis in the German population also suggested a potential link between the FI and CpG sites implicated in cancer development, neurodegenerative

disorders, and other age-related diseases.[104] Notably, within the list of the 80 frailty-associated CpGs previously reported in the literature,[104,105,113,214] we were able to validate only one site in our Swedish and Danish samples at $p<0.05$. Similarly, among the 171 CpGs identified in SATSA, we successfully replicated only five sites in LSADT (5/171 ≈ 2.9%), which is somewhat lower than expected. While this could be due to our relatively modest sample sizes or differences in characteristics of SATSA and LSADT participants, recent research has shown similarly low replication rates (usually <5%) in the EWAS results for various frailty-related diseases, such as chronic obstructive pulmonary disease, ischemic heart disease, and stroke.[241] The generally low replicability in EWASs may arise from differences in the statistical models and covariate strategies employed in different studies,[241] or the inherent low reliability in the majority of CpG sites.[242,243] Thus, it appears that the current evidence does not support a consistent and robust association between CpG sites and frailty across different populations. Nevertheless, these results, taken collectively, at least offer some hints that frailty may share common genetic and epigenetic pathways associated with cancer and neurological disorders. This also aligns with our earlier research which suggested a potential link between frailty and cancers through shared genetic factors.[244] To further advance our understanding in the epigenetics of frailty, it is important for more extensive studies with larger sample sizes to confirm our findings, and identify the potential epigenetic biomarkers of frailty. For instance, in an unpublished study, we have preliminary results indicating that epigenetic age measures combining information from age-related CpG sites,[243] particularly the DunedinPACE clock,[245] may be dynamically linked to an increased FI across age and could serve as a more robust epigenetic biomarker of frailty.

6.3 Metabolic biomarkers of frailty

Further down the "omics" layers, **Study IV** focused on the metabolomics of frailty, where we explored the relationships between 168 NMR-based metabolomic biomarkers and 32 clinical biomarkers with frailty. These blood biomarkers reflect the downstream output of the interactions between various biological processes (*e.g.*, genetics, epigenetics, transcriptomics, and proteomics) and environmental factors, thereby providing important insights into the biological mechanisms underlying frailty development.[117] By employing multivariable linear regression models in a large cohort of up to 90,573 UK Biobank participants, we showed that 164 out of the 200 metabolic biomarkers were statistically significantly associated with the FI, even after adjusting for sociodemographic and lifestyle factors and applying the stringent Bonferroni correction. Given the high collinearity among the biomarkers, we also applied the LASSO feature selection method, narrowing down the list to 59 biomarkers with the strongest associations with the FI. To validate our findings, we replicated the analysis on independent samples from TwinGene and Health

2000 participants, confirming 34 of the identified biomarkers. Our results align with previous studies, demonstrating, for instance, a positive association between CRP and frailty,[246] and a negative association between LDL-cholesterol and frailty.[247] The large number of significant associations observed across different cohorts also underscores the multifaceted nature of frailty, intertwined with numerous physiological systems.[2]

To further mitigate confounding and reverse causation that may be present in the observational results,[196] we performed a series of MR analyses and identified 19 biomarkers that exhibited significant associations with the FI. Among these potential causal biomarkers, 16 were lipids and lipoproteins, including apolipoprotein B, total cholesterol, LDL-cholesterol, lipoprotein subclasses, triglycerides, and omega-6. Interestingly, while our observational analyses predominantly revealed inverse associations between these lipid traits and frailty, the MR results suggested that they were generally associated with an increased FI. This seeming contradiction could be explained by uncontrolled confounding factors in the observational analyses, or potential nonlinear association between lipids and frailty that we did not examine.[248] It could also be due to the different interpretations between the observational and MR estimates, where the former, especially in cross-sectional studies, typically reflect associations over a shorter period of time, while the latter reflect effects over a lifetime.[196] Notably, lipids and lipoproteins have consistently been implicated in the development of cardiovascular diseases and diabetes.[249] In a sensitivity analysis where we removed cardiometabolic items from the FI, the causal estimates of these biomarkers attenuated, implying that their impact on the FI could be mediated by cardiometabolic diseases. Similarly, we also observed a potential causal effect of creatinine, a biomarker indicative of kidney function,[250] on frailty. However, subgroup analysis revealed that this association was significant solely in individuals with chronic kidney disease, suggesting that the link between creatinine and the FI may be driven by kidney diseases, considering the association between frailty and kidney function.[251]

Meanwhile, we identified a robust, positive association between GlycA and frailty in both observational and MR analyses, even after accounting for individual deficit items in the FI and other related traits such as CRP and LDL-cholesterol.[217] GlycA is a novel inflammation marker that reflects the concentration and glycosylation of acute-phase proteins during inflammatory states, and is a more sensitive marker than CRP in capturing low-grade inflammation.[217,252] Although the MR-Egger and co-twin control results suggested potential pleiotropic effects in the GlycA-FI association, possibly due to the overlapping signal of GlycA with lipoproteins and triglycerides,[217] our findings suggest that GlycA may at least capture part of the inflammatory response that is causally linked to frailty. There has been a growing body of literature emphasizing chronic, low-grade inflammation as a key mechanism contributing to frailty and the aging process (i.e., "inflammaging"), which could arise from senescence in the immune system during aging.[95,96,253–255] Compared to previous studies that have predominantly examined the association between inflammation and frailty in cross-sectional settings,[255] our results provide additional evidence supporting a

causal relationship between chronic inflammation and frailty, and highlight the potential utility of GlycA as a biomarker for the identification and monitoring of frailty.

6.4 Electronic frailty index for the Swedish health system

To move towards an individualized management of frailty, the first and crucial step is to enhance the assessment of frailty. Traditional frailty assessments often require in-person evaluations, making them not always practical in clinical settings.[39] In response to this challenge, there has been a growing interest in developing frailty measures based on the routinely collected EHR or health administrative data, such as the HFRS and the eFI which has already been adopted in some countries.[132] Considering Sweden's aging population, it is crucial to assess the potential of incorporating a similar measure into the Swedish EHR system to aid in identifying high-risk patients during routine clinical practice. In **Study V**, we calculated an eFI that adheres to the Rockwood deficit accumulation model[42] and a US eFI model developed by Pajewski and colleagues.[138] This approach is highly generalizable, which can theoretically incorporate any age-associated deficit items found in the EHRs and provide a good predictive ability for mortality when the deficit items cover a wide range of physiological systems.[42,256] Specifically, our Swedish eFI includes disease items, functional assessments, and laboratory measures, thereby capturing not only multimorbidity, but also other functional aspects of frailty. This was confirmed by its moderate correlation with the CFS, but weaker correlations with the ICD-code-based CCI and HFRS.

Importantly, we found that our Swedish eFI outperformed the existing frailty and comorbidity scales (CFS, HFRS, and CCI) in predicting in-hospital mortality, achieving an AUC of 0.813 when combined with age and sex. It also performed better in predicting 30-day and 6-month mortality compared to the CFS and HFRS and was associated with a longer length of hospital stay. Hence, when this eFI is incorporated in the EHR system as an automated and standardized frailty screening tool, it could have substantial implications for frailty management within the Swedish healthcare system.[256] For instance, it could complement the CFS and assist clinicians in identifying individuals at risk of frailty at an earlier stage, facilitating timely interventions. It could also serve as a tool for monitoring patients' health status over time and enable more efficient communications between different care providers. Furthermore, the eFI holds potential for use in research contexts, such as studying the time trends of frailty over time in the population.

6.5 Methodological considerations

The major strengths of this thesis include the rigorous study designs and analytical methods, applied across multiple large population-based cohorts to address the diverse

research questions related to genetics and biomarkers of frailty. In particular, **Studies I** & **II** incorporated advanced twin methods, including sex-limitation models and biometric latent growth curve models,[86,87] to investigate, for the first time, the sex differences and longitudinal changes in the heritability of frailty. In **Study III**, apart from the EWAS analysis, we conducted both longitudinal and replication analyses to examine the robustness of the identified CpGs over time and across different populations. **Study IV** applied a combination of observational, MR, and co-twin control methods to minimize confounding and enhance causal inference for the identified metabolite-frailty associations.[196,207] **Study V** employed a retrospective cohort study design within the EHR data to develop an eFI for the older Swedish population.

However, it is important to note that the validity of our findings, especially for the twin and MR results, relies on the underlying assumptions of these methods. For twin studies, the fundamental assumptions include an equal environmental similarity for MZ and DZ twins and a random mating in the population. Violations of these assumptions may occur, for instance, due to differential treatment of MZ and DZ twins[88] or assortative mating,[257] although previous studies have shown that heritability estimates remain robust even when these assumptions are violated.[88] For the MR analysis, we tested the relevance assumption using the *F*-statistics as a test of instrument strength, with no violations found.[196] As for the independence and exclusion restriction assumptions, which are generally untestable, we performed several sensitivity analyses to mitigate the impact of horizontal pleiotropy in our MR analysis.[196]

As in any epidemiological study, several potential biases should also be considered.[258] Selection bias, which refers to the situation when the study sample does not represent the target population,[258] is a primary concern. It can occur due to healthy selection, which may be present in most of our cohorts in **Studies I–IV** since participation is voluntary. For example, it has been shown that participants from the UK Biobank are less likely to smoke and have fewer health problems compared to the general population.[259] Healthy selection is particularly relevant in aging research because older participants must have been healthy and survived to a certain age to participate in the study.[260] In **Study II**, we intentionally included a sample of oldest-old twins from OCTO-Twin to test whether the results are affected by potential selection bias. While OCTO-Twin participants demonstrated a significantly slower rate of FI increase compared to SATSA participants, potentially indicating a lower mortality risk in late life,[261] the overall results remained consistent even with the inclusion of these oldest-old twins. In longitudinal studies, selection bias can also arise if losses to follow-up are nonrandom, although we have tried to account for this in the models in **Study II** using a full-information maximum likelihood modeling approach.

On the other hand, the FIs used in **Studies I–IV** were all based on self-reported data, which may have led to inaccurate reporting and measurement error,[77] thereby inflating the unique environmental variance component in **Studies I** & **II**, especially in men.[224] Similarly,

any measurement error in the FI and biomarker levels in **Studies III** & **IV** could have resulted in non-differential misclassification bias, potentially underestimating their associations.[258] In **Study V**, readmissions to hospitals other than the included geriatric clinics were not captured, likely leading to misclassification of the 30-day readmission outcome variable and contributing to the generally low AUCs observed when predicting readmission using frailty and comorbidity measures.

Finally, regarding the generalizability of our results, although we always attempted to replicate our findings in independent samples where data are available, our conclusions were based on samples of European ancestry and may not be applicable to other populations. In **Studies I–III**, our results were also based on twin samples and may not be fully generalizable to the general population, although studies have shown that twins are highly comparable to singletons even at old ages.[262] Additionally, due to data availability, most of our studies focused on the FI as the measure of frailty. While the FI has been widely validated and adopted, our findings may not be generalizable to other physical frailty measures, such as the FP, which differs from the FI in certain aspects.[60] This was also evident in **Study IV** where we could not find any significant associations between the metabolic biomarkers and the FP in the MR analysis. Hence, it would be beneficial for further research on the genetics and biomarkers of other measures of frailty to obtain a comprehensive understanding on the mechanisms underlying frailty and aging.

7 Conclusions

In summary, the present thesis investigated the interplay between biological and environmental factors that influence frailty, and developed an eFI tailored for integration into the Swedish health system. In the five studies, we showed that:

I. Both genetic and individual-specific environmental factors contribute to a large proportion of the variation in frailty. The heritability of frailty is higher in women (52%) than in men (45%), although it appears to be the same genetic factors influencing frailty in both sexes. Different mechanisms seem to underpin the associations of frailty with BMI and education, which are primarily explained by genetic factors and environmental factors shared within twin pairs, respectively.

II. Frailty increases with age, and its rate of increase and variability become much higher after age 75 in both men and women. Most of the amplification in frailty variability in late-life is due to individual-specific environmental factors, while genetic influences on frailty remain relatively stable over age.

III. Frailty is associated with DNA methylation in several CpG sites that may involve in cancer development and neurological pathways. Nonetheless, further studies are warranted to corroborate and expand upon these findings.

IV. Frailty exhibits significant associations with a substantial portion of the blood metabolome. However, it is important to consider the potential influence of reverse causation, confounding, or mediation through other diseases in these associations. Notably, the inflammation marker GlycA appears to be causally linked to a higher degree of frailty, suggesting its potential utility as a biomarker of frailty.

V. An eFI constructed based on routinely collected disease diagnoses, functioning items, and laboratory measures has good predictive performance for mortality outcomes, which can potentially be incorporated in the Swedish EHR system for frailty screening in the population.

8 Points of perspective

The rapid expansion of the frailty literature over the past two decades, especially during the COVID-19 pandemic,[35] has greatly improved our understanding of the utility and importance of the frailty concept in studying the heterogeneity of aging and predicting adverse outcomes in older adults. Contributing to the literature, this thesis brings new knowledge into the biology of frailty and demonstrates the potential of leveraging routine EHR data to facilitate frailty screening. Despite these advancements, the exact mechanisms underlying this complex syndrome are still far from fully understood. Moving forward, it is essential for future research to continue identifying specific molecular biomarkers of frailty, which could potentially be applied in clinical practice for early detection and development of individualized treatment strategies for frail individuals. Below are some suggested directions for future research based on our findings:

- The largest GWAS of the FI to date has only reported a SNP-based heritability of 11%,[80] which is much lower than our estimated twin-based heritability of ~50%. To address the missing heritability of frailty,[220] more large-scale studies based on whole-genome or whole-exome sequencing in collaborative efforts are warranted to identify the rare variants that may contribute to frailty development.
- Our studies predominantly focused on white populations, underscoring the need for more multiethnic investigations to elucidate the biology of frailty and aging across different populations.
- Given the complexity of frailty, it seems implausible for a single biomarker to capture the multifaceted mechanisms that occur across numerous physiological systems. As such, future research should prioritize multi-omics and longitudinal studies, incorporating both spatial and temporal scales in the analysis. This could enable a deeper understanding of the multiple interacting aging processes that likely underpin frailty development.[12]
- Our research highlighted chronic inflammation as a pivotal mechanism underlying frailty. However, results remain inconclusive regarding the benefits of targeting inflammation to alleviate frailty.[263,264] Hence, more longitudinal and interventional studies are required to develop the preventive strategies for frailty. The potential of GlycA as a biomarker for identifying and monitoring frail patients also warrants further investigation.
- Although our eFI demonstrated good predictive performance for mortality outcomes, its efficacy in guiding clinical decisions and improving patient outcomes in real-world settings remains unknown and needs to be investigated in future studies.

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