BIOMARKERS OF FRAILTY

Explorative research with a multi-domain approach

Liset Rietman

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(met een samenvatting in het Nederlands)

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General introduction

Introduction

Older people who are frail are at higher risk of negative health outcomes. To prevent frailty, we need to better understand the underlying mechanisms and identify people at risk of becoming frail at an early stage. The aim of this thesis is therefore to identify (bio)markers (based on questionnaire data, anthropometric markers, biochemical markers, and genetic data) of frailty, which could be indicative of the underlying processes that cause frailty and/or could help to detect people at risk. This chapter provides a brief overview of the concept of frailty, instruments to measure frailty, epidemiology of frailty, and the purpose and outline of this thesis.

The ageing population

Life expectancy rises and, as a result, populations are ageing in many countries worldwide (1). The proportion of older people in the Netherlands will rise sharply in the coming 20 years (**Figure 1.1**), with a doubling of the proportion of 80+ years (the 'oldest old') from over 800.000 in 2020 up to 1.6 million in 2040 (2).

Currently, the remaining life expectancy in the Netherlands for a 65-year old man is 19 years. Twelve of these years will be in good or excellent self-perceived health. For a 65-years old woman life expectancy is 21 years with 13 years in good or excellent self-perceived health (3). Ageing is frequently accompanied by a decline in functioning in one or more domains (*e.g.* physical, cognitive, psychological, social). Fortunately, most people are resilient and have intrinsic capacities or resources that enable them to stay functionally independent (4). However, sometimes these intrinsic capacities are not sufficient and functional limitations in one or more domains (*i.e.* frailty) arise.

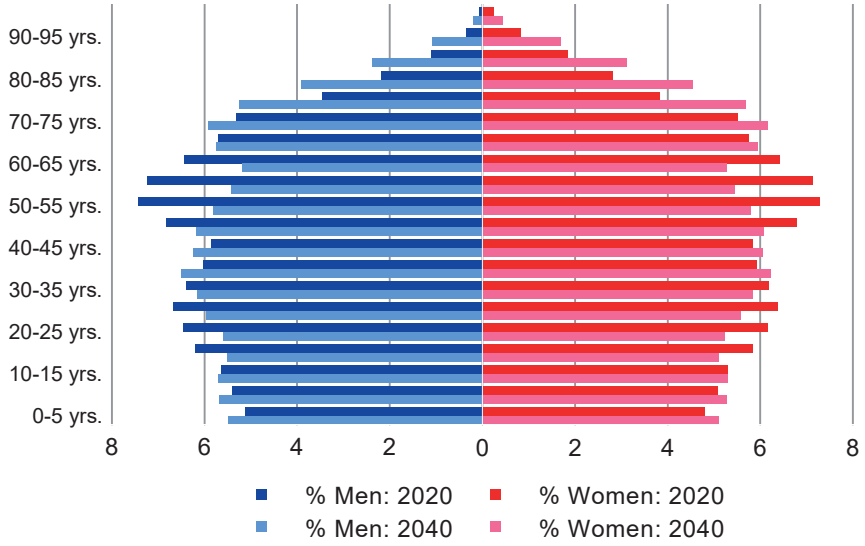

Figure 1.1. Dutch population pyramid showing the age composition in the Netherlands in 2020 and 2040 stratified by sex (2).

The concept of Frailty

The concept of 'Frailty' already exists for several decades. The Federal Council on Aging (USA) was the first using a definition for frailty in 1978. They defined frail people as "persons, usually but not always, over the age of 75, who because of an accumulation of various continuing problems often require one or several special services in order to cope with daily life" (5). Thereafter, more frailty definitions were published (6). Frailty is an age–associated syndrome and can be described as a state of increased vulnerability to adverse health outcomes (7) when exposed to stressors caused by the cumulative decline in one or more domains of functioning (8–11). Adverse health outcomes include injuries, such as fractures through falls, but also disability, hospitalisation, institutionalisation, and mortality (12–15). The difference between a non–frail and frail older individual is the response to a sudden change in health status (8) (**Figure 1.2**). When a non–frail older individual suffers a minor stressor, such as an infection, his/her health deteriorates slightly but then quickly returns to baseline. However, when a frail older person contracts the same infection, his/her health deteriorates much more, improves slowly and does not return to baseline, which can eventually lead to disability and, in turn, to dependency. An important characteristic of frailty is that it is reversible, thus frail

older people can return to a non-frail state when frailty is identified in an early stage (16–20). Hence, early identification of frailty is important to prevent progression and to be able to return to a non-frail state.

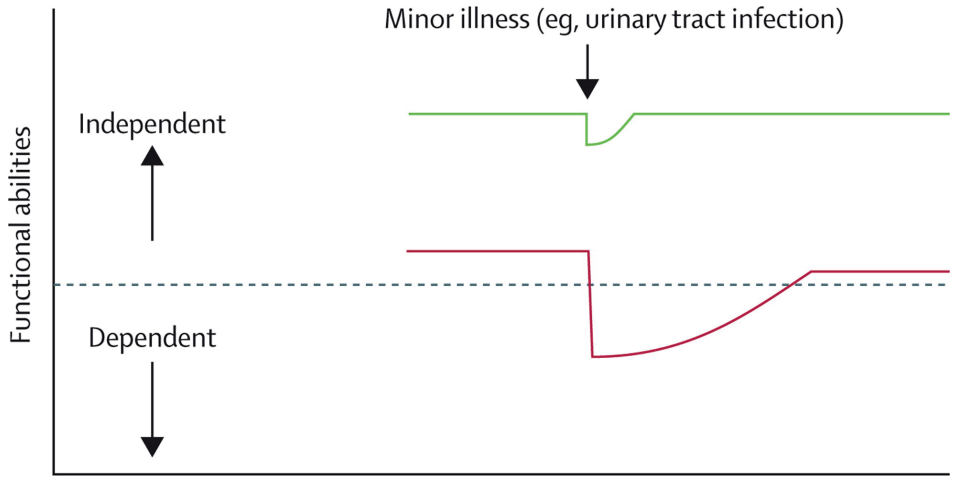

Figure 1.2. Visualisation of the difference in health between frail and non-frail people. The change in health status in non-frail older people (green line) compared to frail older people (red line) after a minor illness (reprinted from Clegg *et al.*, 2013 (8) with permission from Elsevier).

Measuring frailty

Over the years, many frailty instruments have been developed, of which 67 are used in research (21). Three frailty instruments that are often used are: 1) the Frailty Phenotype focusing on physical frailty (9), 2) the Frailty Index that sums the accumulation of deficits (22), 3) the Tilburg Frailty Indicator using a multidimensional approach to identify frail older people (23). Of these three instruments, the Frailty Phenotype and the Frailty Index are used in both national and international research. The Tilburg Frailty Index (originally a questionnaire in Dutch) is widely used in national research, but is also gaining ground in international research as more and more translations become available.

Frailty Phenotype

The Frailty Phenotype (FP) defines physical frailty based on five physical criteria (9). These criteria are unintentional weight loss, exhaustion, low physical activity,

slow walking pace and low grip strength. This instrument focuses on one domain of functioning, *i.e.* the physical domain. Physical frailty, comorbidity, and disability are three terms that are often used interchangeably (24). Comorbidity is defined as the presence of one or more conditions in addition to the presence of a primary disease (25). Disability can be defined as "a difficulty in functioning at the body, person, or societal levels, in one or more life domains, as experienced by an individual with a health condition in interaction with contextual factors" (26). It was shown that these three concepts are in fact three distinct clinical conditions (24).

Frailty Index

The Frailty Index (FI) is based on the principle of the accumulation of deficits (22). From this point of view, frailty can be seen as a multifactorial and dynamic age-associated syndrome that is accompanied by physiological changes in multiple body systems (27). These physiological changes do not always lead to diseases. The FI counts the number of deficits which not only includes diseases, but also symptoms, signs, disabilities, and laboratory measurements in multiple domains of functioning resulting in a score ranging from 0 up to 1, where a higher scores means a higher level of frailty (27). Hence, the FI includes comorbidity in its measure, while it has been shown that physical frailty, based on the FP, and comorbidity are distinct conditions (24).

Tilburg Frailty Indicator

Over time, the focus on physical frailty moved towards other domains such as the cognitive, psychological and social domains. The cause for this shift was that initially, frailty was defined in biomedical terms, while over the years it was recognised that psychosocial factors could also play a role (28). In response to this shift, new frailty instruments were developed. The Tilburg Frailty Indicator (TFI) (23), the Edmonton Frail Scale (29), and the Groningen Frailty Indicator (30) are all frailty instruments that include measurements for multiple domains. Each of these instruments has its own domain classification. The TFI is a questionnaire based frailty instrument and measures three frailty domains, *i.e.* the physical, psychological, and social domains.

Frailty domains studied in this thesis

In this thesis, the TFI is used as basis for measuring frailty. As mentioned, the TFI distinguishes three frailty domains: the physical, psychological and social frailty domains. Cognitive functioning is included in the psychological domain (23). In

this thesis however, the psychological and cognitive frailty domains are considered different domains of functioning. Therefore, these domains were treated as separate frailty domains (**Figure 2.1** (see **Chapter 2**)). Hence, in this thesis we study four domains of frailty which are the physical, cognitive, psychological, and social frailty domains.

Epidemiology of frailty

Prevalence

In the Netherlands in 2011, about 25% of the people of 65 years and older living at home were frail based on the TFI (31). Moreover, in nursing homes almost 75% of the people of 65+ years were frail (31). From 2020 up to 2030, it is expected that the number of frail people of 65+ years in the Netherlands will increase by 25% from about 800,000 up to 1 million. However, it is also shown that during the period 2010–2030, among the Dutch population a decrease in the proportion of people with a low educational level and an increase in the proportion of people with an intermediate or high educational level is expected (31). As a higher level of education leads to a lower risk of being frail, the change in the distribution of educational levels among the Dutch population may lead to a slower increase in the number of frail people than expected from demographic projections alone (31).

Determinants

With respect to characteristics of frail versus non–frail groups, numerous studies using different frailty instruments have shown that the prevalence of frailty increases with age (32, 33). Moreover, frail people are more often women, have a low educational level, an unhealthy lifestyle, poorer health, and have higher comorbidity and disability rates (9, 19, 34–36). In addition to differences in these sociodemographic and lifestyle factors in frail compared to non–frail people, differences on a biological level have been observed. Several studies found that the levels of inflammatory biomarkers, such as interleuking–6 (IL–6) and C–reactive protein (CRP), are higher in physically and overall frail people compared to non–frail people (37, 38). Based on these findings, it is hypothesised that frail people have chronic low–grade inflammation (8, 39). A recent review concluded that results regarding inflammatory biomarkers are still contradictory and other (bio) markers (such as nutritional, endocrine, haematological and genetic markers) are not yet studied thoroughly (40). Therefore, further investigation into the association of these (bio)markers with frailty is needed. In this review it was also shown that

the potential influence of genetics on frailty is gradually receiving more attention (40). For example in studies focusing on the association of single nucleotide polymorphisms (SNPs) with the FP (41) or with the FI (42).

Different study designs

Both cross-sectional and longitudinal study designs are used to study (bio) markers of frailty. Cross-sectional study designs can be used to study (bio) markers levels, while longitudinal study designs can be used to study (bio)marker trajectories. Studying (bio)marker trajectories over the life course and before the onset of frailty is important since these studies could provide insight in the development of frailty. This is based on the hypothesis that people who become frail would have more unfavourable (bio)markers trajectories than people who do not become frail. Further, cross-sectional and longitudinal studies can lead to different results. For example, the inflammatory biomarker CRP has been linked longitudinally to physical frailty (43), cognitive decline (44), and risk of dementia (45, 46). In addition, Soysal *et al.* (38) showed that higher CRP and IL-6 levels are cross-sectionally associated to physical frailty, but not longitudinally. Hence, it is important to study both levels and trajectories of (bio)markers.

Aim of this thesis

The aim of this thesis is to identify (bio)markers for different domains of frailty (*i.e.* physical, cognitive, psychological, social). Frailty among older people leads to a higher risk of negative health outcomes. To prevent or even reverse frailty, we need a better understanding of the underlying mechanisms of frailty. Identifying (bio)markers could contribute to our knowledge about these mechanisms. Furthermore, (bio)markers could be a valuable tool for early detection of those at higher risk of frailty. The (bio)markers that are studied in this thesis consist of markers (*e.g.* self-reported health, body mass index), biomarkers (*e.g.* c-reactive protein, urea), and genetic markers, *i.e.* single-nucleotide polymorphisms (SNPs) for different frailty domains. For this explorative research we use data of two cohort studies, multiple research designs, and various analysis techniques.

In this thesis, a multi-domain approach is used to study frailty because in our view frailty is not purely a biomedical syndrome, *i.e.* related to the physical domain, but also relates to other domains of functioning, such as the cognitive, psychological, and social domains. In addition, since it was shown that frailty and comorbidity are distinct concepts, we use a multi-domain approach instead of the FI, based

on the accumulation of deficits, where comorbidity is included as one of the deficits. Moreover, we study the domains independently because we expect that the associations between (bio)markers and the different frailty domains will not be uniform. The major focus in frailty research has been on the physical domain. The cognitive domain has been studied less, but is important too, for example for healthy ageing and to remain self–reliant. Therefore, two chapters are dedicated to cognitive functioning.

The Doetinchem Cohort Study and MARK–AGE

In this thesis, data of two independent cohort studies are used to study the differences in (bio)marker levels and in (bio)markers trajectories between frail and non–frail older people: the Doetinchem Cohort Study (DCS) and the European study to establish biomarkers of human Ageing (MARK–AGE). In **Chapters 2**, **3**, **5**, and **6** data of the DCS was used. The DCS is a longitudinal population–based cohort study starting in 1987–1991 (round 1) examining 7769 men and women aged 20–59 years living in Doetinchem, a town in the Netherlands, with follow–up examinations every five years (42, 47, 48) (**Figure 1.3**). **Chapter 4** was based on data of MARK–AGE, which is a consortium aiming to study biomarkers of human ageing. MARK–AGE, as previously described (49), is a cross–sectional study and includes multiple European populations.

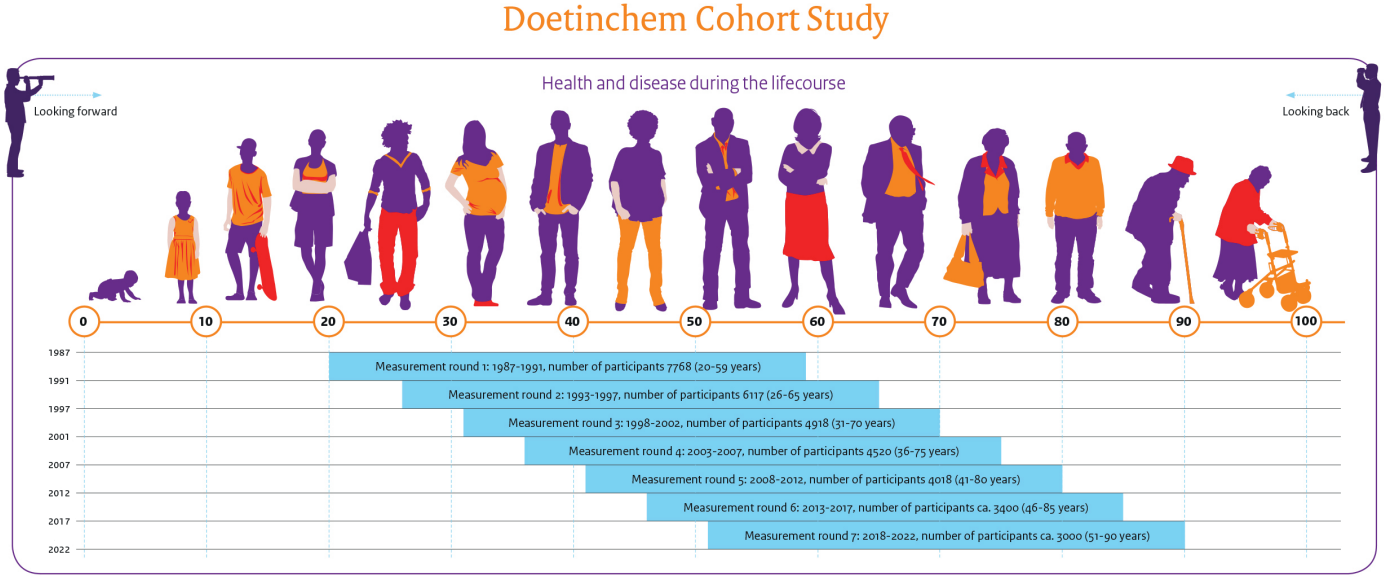

Figure 1.3. Overview of the Doetinchem Cohort Study (DCS). The DCS is a Dutch longitudinal population-based cohort study starting in 1987-1991 (round 1) examining 7769 men and women aged 20-59 years living in Doetinchem with follow-up examinations every five years.

Outline of this thesis

The chapters within this thesis are divided into two parts. In **Part I (Chapters 2–4)**, (bio)markers for four domains of frailty, *i.e.* physical, cognitive, psychological, and social, are studied cross–sectionally. In **Chapter 2**, we explore how sociodemographic and lifestyle factors, life events and health are associated with frailty using a multi–domain approach of frailty in the DCS. In **Chapter 3**, we report on the association between BMI and physical, cognitive, psychological and social frailty in the DCS. In **Chapter 4**, we study over 300 (bio)markers for three frailty domains, i.e. the physical, cognitive and psychological domains, in MARK–AGE. As mentioned above, two chapters are dedicated to cognitive functioning. Therefore, **Part II** (**Chapters 5** and **6**) of this thesis focuses on identifying (bio)markers for cognitive frailty and general cognitive function using longitudinal data of the DCS. In **Chapter 5**, we explore (bio)marker trajectories in adults who became cognitively frail compared to age– and sex–matched controls who did not become cognitively frail over a 15–year follow–up. In **Chapter 6**, we study which SNPs are associated with general cognitive function at baseline and which SNPs are associated with a decline in general cognitive function over a 20–year follow–up. In **Chapter 7**, the general discussion, the main findings are summarised and reviewed. Further, conceptual and methodological aspects are discussed, as well as the implications of these findings for public health and future research.

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Part I

Exploring (bio)markers for the four frailty domains cross-sectionally

2

A four-domain approach of frailty explored in the Doetinchem Cohort Study

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Abstract

Background

Accumulation of problems in physical, psychological, cognitive, or social functioning is characteristic for frail individuals. Using a four-domain approach of frailty, this study explored how socio-demographic and lifestyle factors, life events and health are associated with frailty.

Methods

The study sample included 4019 men and women (aged 40-81 years) examined during the fifth round (2008-2012) of the Doetinchem Cohort Study. Four domains of frailty were considered: physical (≥4 of 8 criteria: unintentional weight loss, exhaustion, strength, perceived health, walking, balance, hearing and vision impairments), psychological (2 criteria: depressive symptoms, mental health), cognitive (<10th percentile on global cognitive functioning), and social frailty (≥2 of 3 criteria: loneliness, social support, social participation). Logistic regression was used to study the cross-sectional association of socio-demographic factors, lifestyle, life events and chronic diseases with frailty domains.

Results

About 17% of the population was frail on one or more domains. Overlap between the frailty domains was limited since 82% of the frail population was frail on one domain only. Low educated respondents were at higher risk of being psychologically and socially frail. Having multiple diseases was associated with a higher risk of being physically and psychologically frail. Being physically active was consistently associated with a lower risk of frailty on each of the four domains. Short or long sleep duration was associated with a higher risk of being physically, psychologically, and socially frail.

Conclusions

Socio-demographic factors, lifestyle and multimorbidity contributed differently to the four frailty domains. It is important to consider multiple frailty domains since this helps to identify different groups of frail people, and as such to provide tailored care and support. Lifestyle factors including physical activity, smoking and sleep duration were associated with multiple domains of frailty.

Introduction

With ageing, changes occur in physical, psychological, cognitive, and social functioning. Accumulation of problems in one or more of these domains of functioning is characteristic for frail people. Originally, frailty was mainly focused on the physical problems that older people encounter, such as in Fried's popular 'phenotype of frailty' (1). Broader definitions of frailty, looking beyond physical functioning, have now been put forward (2–4) one of which is the definition by Gobbens *et al.* (5). According to them, frailty is 'a dynamic state affecting an individual who experiences losses in one or more domains of human functioning (physical, psychological, social) caused by the influence of a range of variables and which increases the risk of adverse outcomes'. A multidimensional approach to frailty is coherent with the interdisciplinary diagnostic process used in the Comprehensive Geriatric Assessment for frail older people, which also examines physical, mental (including both psychological and cognitive functioning), and social functioning (6, 7).

Frailty often leads to restrictions in mobility and reduced self-reliance, and a greater risk of clinically significant adverse outcomes such as hospitalisation, institutionalisation and mortality (1, 4, 8–10). In several European countries it is government policy to stimulate older people to participate in society and to live at home for as long as possible (11, 12). Primary prevention of frailty is therefore needed, directed at both delaying the onset of frailty and slowing down the frailty process as prevention of frailty might eventually lead to prevention or postponement of hospitalisation and institutionalisation of elderly people. Insight in factors that are associated with the presence of frailty is a first step to assist the identification of potentially vulnerable groups. For the physically frail, a series of socio-demographic, lifestyle, and health-related factors have been shown to be associated with frailty, such as age, female sex, cardiovascular diseases, multimorbidity, BMI, and smoking (13). As part of a broad frailty definition, little is known about factors associated with the psychological and social domains of frailty. Especially, the association between lifestyle factors and frailty has rarely been studied (13).

Recently, the concept of cognitive frailty has been proposed (14). Since there is increasing support for the idea of cognitive frailty being a separate frailty domain (15, 16), a four-domain approach of frailty was adopted for the current study

including the physical, cognitive, psychological and social domains of frailty. It was recently shown that the overlap between these frailty domains was limited, which implicates that the domains largely entail distinct populations and frailty prevention may target multiple frailty domains (17).

In this study, we explored how socio-demographic factors, lifestyle factors, life events, biological risk factors and chronic diseases were associated with physical, psychological, cognitive, and social frailty in a population-based study of men and women aged 40–81 years.

Methods

Study population

Data of men and women aged 40–81 years participating in the Doetinchem Cohort Study were used for the current study. The Doetinchem Cohort Study is an ongoing population-based cohort study aimed to study the impact of (changes in) lifestyle and biological risk factors on various aspects of health and wellbeing of men and women, aged 20–59 years at baseline, from the Netherlands. A total of 12405 participants (response rate 62%) were first examined in 1987–1991 (round 1). Of those, a random sample of 7768 participants was re-invited to be examined in 1993–1997 (round 2, n=6113), 1998–2002 (round 3, n=4916), 2003–2007 (round 4, n=4520) and 2008–2012 (round 5, n=4019). The response rates for all follow-up measurements varied between 75% and 80%. For the current study, we used data from the fifth examination round. Written informed consent was obtained from all participants. The Medical Ethics Committees of the Netherlands Organization of Applied Scientific Research and the University of Utrecht approved the study. Full details of the study have been reported elsewhere (18).

Conceptual model of frailty

The integral conceptual model of frailty which includes the physical, psychological, and social domains of frailty was the basis of our study (19). In the original model, the psychological domain included feelings of anxiety and depression, a decline in coping, and a decline in cognitive functioning. For the current study, the conceptual model was extended with a fourth domain, being cognitive frailty (**Figure 2.1**) (14). The rationale for adding a fourth domain was that limitations in functioning due to anxious and depressive feelings (*i.e.* the psychological domain) are considered to be fundamentally different from limitations in functioning due to *e.g.* memory problems (*i.e.* cognitive functioning). In addition, we noticed that cognitive functioning was previously not consistently positioned in one domain of frailty; it belonged either to the physical or the psychological domain (1, 7, 19). Furthermore, we added several potential determinants of frailty to the model, including health care and support, and providing informal care (**Figure 2.1**). Inadequate formal care or support for chronic diseases or acute disorders may lead to a strong deterioration of physical, cognitive, psychological functioning and increase the risk to become frail. Informal caregiving is also related to negative health outcomes (20).

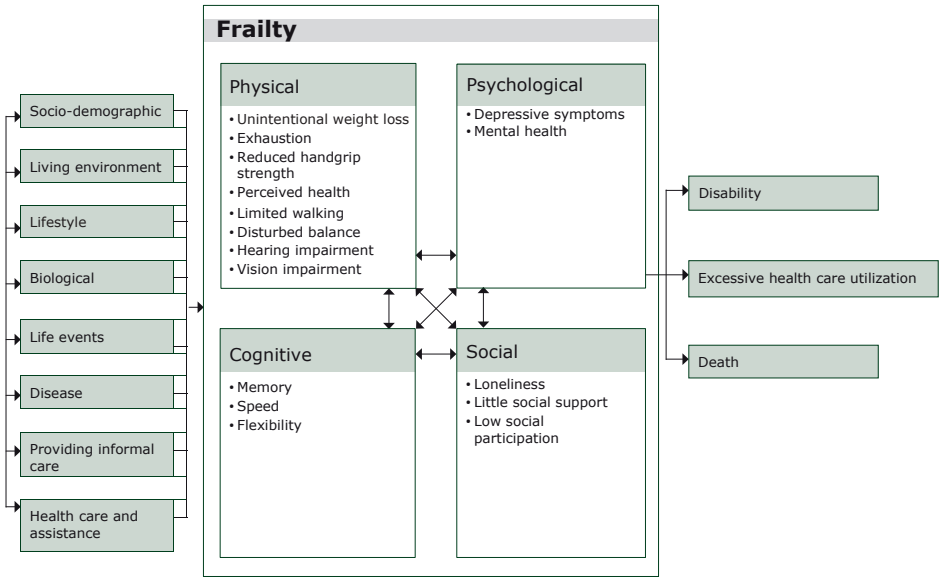

Figure 2.1. Adapted version of the integral conceptual model of frailty, based on Gobbens (19).

Operationalisation of frailty domains

The frailty criteria per domain were based on the Tilburg Frailty Indicator (TFI, **Table 2.1**) (21). The original TFI was not included in our questionnaires. For each item on the TFI we identified the best possible proxy in our questionnaires and data-collection. A detailed description of the criteria can be found in the **Supplementary Methods**. Participants were considered to be physically frail if they fulfilled ≥4 of 8 frailty criteria described by Gobbens (21, 22). Participants were considered to be cognitively frail when scoring <10th percentile on a global cognitive functioning score based on memory, speed, and flexibility. Cognitive scores were adjusted for level of education and number of tests performed during follow-up. Psychological frailty was defined as fulfilling both criteria for depression (23) and for poor mental health (24). Social frailty was defined as meeting ≥2 of 3 criteria using the Loneliness scale (25), Social Support List-12 (26) and a questionnaire about social participation from the Dutch Municipal Health Services Elderly Monitor (27). Overall frailty was defined as all participants being frail on one or more domains.

Table 2.1. Overview of the criteria used to operationalise physical, cognitive, psychological, and social frailty.

Domains	Criteria	Cut-off	Based on
Physical frailty	– Unintentional weight loss – Exhaustion – Low handgrip strength – Perceived health – Limited in walking – Disturbed balance – Hearing impairment – Vision impairment	≥4 criteria	– Unintentional weight loss: >5% weight loss between round 4 and 5 and not being on a diet – Exhaustion: 2 questions of the Center for Epidemiologic Studies Depression scale (CES-D) (23) – Handgrip strength: dynamometer, sex-specific cut-off stratified for BMI (1) – Perceived health: one question of 36-Item Short-Form Health Survey (SF-36) (24, 28) – Self-reported 100 m walking – Tandem Stand Balance Test – 3 questions regarding hearing – 3 questions regarding vision
Cognitive frailty	– Low global cognitive functioning	<10th percentile	– Global cognitive functioning score based on tests for memory, speed and flexibility (29): ○ 15 Words Verbal Learning Test ○ Stroop Colour-Word Test ○ Word Fluency Test ○ Letter Digit Substitution Test
Psychological frailty	– Depressive symptoms – Mental health	≥2 criteria	– Center for Epidemiologic Studies Depression scale (CES-D) (23) – Mental Health Inventory 5 (MHI-5) (24, 30)
Social frailty	– Loneliness – Low social support – Limited social participation	≥2 criteria	– Loneliness Scale (25, 31) – Social Support List-12 (SSL-12) (26) – Questionnaire Dutch Elderly Monitor (27)

Other measurements

Socio-demographic factors

Level of education was categorised into low (intermediate secondary education or less), intermediate (intermediate vocational and higher secondary education) and high (higher vocational education or university). Work status was defined as having a paid job (including salaried employment and self-employed) or being unemployed. Household composition was defined as living alone or not living alone (living with a partner, with children, with parents or other adults). Being married also included registered partnership.

Lifestyle

For defining smoking status, we distinguished current smokers and non-smokers. To establish whether or not people had a healthy diet, the World Health Organisation's dietary recommendations for the prevention of chronic disease were applied (32). Score on the healthy diet indicator ranged from 0 to 9 and was based on the sum of the number of nutrients (out of a group of seven nutrients) and the number of products from two food groups for which intake was within the recommended range (33). Being physically active was defined as adherence to the Dutch physical activity guideline, which recommends 30 minutes of moderate to vigorous physical activity per day on at least 5 days per week (34). The average sleep duration per 24-hour period was assessed in four categories: 5 hours or less, 6 hours, 7 or 8 hours, and 9 hours or more. Alcohol consumption was assessed in four categories: never, not anymore, <1 glass a week, and ≥1 glass a week (35).

Life events

We determined recent life events (*i.e.* widowhood, divorce) by evaluating potential changes in marital status between round 4 and round 5. Adults who were married in round 4 and became a widow/widower in round 5 were categorised as being widowed; those who were married in round 4 and were divorced in round 5 were categorised as being divorced.

Biological risk factors and chronic disease

BMI was calculated based on measured body weight and height and categorised into normal weight <25 kg/m^2, overweight 25–29.9 kg/m^2, and obesity ≥30 kg/m^2 (36). Multimorbidity was defined as having two or more chronic diseases (37) out of the following five self-reported diseases: diabetes, cancer, myocardial infarction, cerebrovascular accident, and chronic respiratory symptoms.

Statistical analyses

Descriptive analyses were carried out for the total study population and for the physically, cognitively, psychologically and socially frail separately. For each frailty domain, we used logistic regression models to explore the factors associated with frailty. Odds ratios and 95% confidence intervals of two multivariable models are shown. The first model was adjusted for socio-demographic factors (model 1) and the second model was adjusted for all socio-demographic factors, lifestyle, life events, biological risk factors and chronic diseases (model 2). All analyses were carried out in SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

In our population aged 40–81 years, 2.7% was physically frail, 6.3% was psychologically frail, 7.7% was cognitively frail, and 4.1% was socially frail (**Table 2.2**). Women were more frequently physically and psychologically frail (63.6% and 68.7%) than men (36.4% and 31.3% respectively), whereas men were more often cognitively frail than women (68.8% *vs.* 31.2%). The mean age of those with physical, cognitive and social frailty was higher compared to that of the total study population. Each of the domains of frailty showed a higher percentage of respondents with a low educational level compared to the total study population.

17.1% of the population was frail on one or more of the domains. Of the frail population, 81.5% was frail on one of the domains, 15.2% was frail on two domains, 2.9% was frail on three domains, and 0.4% was frail on all four domains. The greatest overlap was observed for physical and psychological frailty and for social and psychological frailty (one third of the physically and socially frail being also psychologically frail) (**Table 2.2**).

Table 2.2. Characteristics of the study population and persons being physically, psychologically, cognitively, and socially frail.

	Study population (N=4019)	Physically frail (N=110; 2.7%)	Psychologically frail (N=252; 6.3%)	Cognitively frail (N=311; 7.7%)	Socially frail (N=166; 4.1%)
Socio-demographic					
Women	2118 (52.7%)	70 (63.6%)	173 (68.7%)	97 (31.2%)	81 (48.8%)
Age, yr	59.9 (SD 9.6)	68.7 (SD 9.1)	59.3 (SD 9.9)	68.8 (SD 8.0)	63.0 (SD 10.4)
Level of education					
Low	1657 (41.2%)	76 (69.1%)	142 (56.3%)	154 (49.5%)	94 (56.6%)
Intermediate	1320 (32.9%)	14 (12.7%)	67 (26.6%)	85 (27.3%)	47 (28.3%)
High	1042 (25.9%)	20 (18.2%)	43 (17.1%)	72 (23.2%)	25 (15.1%)
Married	3211 (80.3%)	63 (57.3%)	151 (60.4%)	232 (75.3%)	108 (65.1%)
Living alone	555 (13.9%)	38 (34.6%)	63 (25.2%)	68 (22.2%)	43 (25.9%)
Paid job	2024 (50.5%)	7 (6.4%)	113 (44.8%)	52 (16.8%)	59 (35.8%)
Lifestyle					
Current smoking	682 (17.1%)	30 (27.8%)	77 (30.7%)	51 (16.6%)	34 (20.7%)
Healthy diet, score (0–7)	2.9 (SD 1.2)	2.6 (SD 1.1)	2.9 (SD 1.1)	2.8 (SD 1.2)	2.9 (SD 1.2)
Physically active	3112 (77.5%)	46 (41.8%)	170 (67.5%)	212 (68.4%)	108 (65.1%)
Sleep duration					
≤5 hr	163 (4.1%)	12 (11.0%)	29 (11.5%)	15 (4.9%)	17 (10.3%)
6 hr	703 (17.6%)	24 (22.0%)	71 (28.3%)	52 (16.8%)	36 (21.7%)
7 or 8 hr	2880 (72.2%)	56 (51.4%)	127 (50.6%)	208 (67.3%)	95 (57.2%)
≥9 hr	245 (6.1%)	17 (15.6%)	24 (9.6%)	34 (11.0%)	18 (10.8%)
Alcohol consumption					
Never	399 (10.0%)	28 (25.5%)	40 (15.9%)	39 (12.6%)	21 (12.7%)
Not anymore	124 (3.1%)	11 (10.0%)	16 (6.3%)	15 (4.8%)	9 (5.5%)
Low (<1 glass/wk)	849 (21.2%)	23 (20.9%)	61 (24.2%)	54 (17.4%)	39 (23.6%)
Frequent (≥1 glasses/wk)	2630 (65.7%)	48 (43.6%)	135 (53.6%)	202 (65.2%)	96 (58.2%)
Life events					
Widowed	74 (2.0%)	4 (4.0%)	12 (5.3%)	7 (2.5%)	4 (2.6%)
Divorced	66 (1.7%)	1 (1.0%)	10 (4.4%)	0 (0.0%)	7 (4.6%)
Biological risk factors and chronic disease					
BMI, kg/m^2	26.8 (SD 4.2)	30.0 (SD 6.9)	27.4 (SD 5.0)	28.1 (SD 4.4)	27.1 (SD 4.7)
Multimorbidity[a]	320 (8.0%)	43 (39.1%)	40 (15.9%)	61 (19.6%)	26 (15.7%)
Frailty					
Physically frail	110 (2.7%)	–	34 (13.5%)	26 (8.4%)	19 (11.5%)
Psychologically frail	252 (6.3%)	34 (31.2%)	–	31 (10.0%)	52 (31.3%)
Cognitively frail	311 (7.7%)	26 (23.6%)	31 (12.3%)	–	20 (12.0%)
Socially frail	166 (4.1%)	19 (17.3%)	52 (20.6%)	20 (6.5%)	–

Abbreviations: BMI, body mass index; SD, standard deviation.

Frequencies (percentage) or means (SD) are presented.

[a] Multimorbidity was defined as having two or more conditions out of diabetes, cancer, myocardial infarction, cerebrovascular accident, and chronic respiratory symptoms.

Factors associated with physical, psychological, cognitive, and social frailty
An intermediate level of education, a paid job, a healthy diet, being physically active, and frequent alcohol consumption were associated with a lower risk of being *physically frail*, whereas, being 70 to 81 years, current smoking, a short sleep duration, and multimorbidity were associated with a higher risk of being physically frail (**Table 2.3**). Having a paid job showed the strongest negative association with being physically frail (Odds Ratio (OR) 0.16 (95% Confidence Interval (CI) 0.07–0.41)).

A higher risk of being *psychologically frail* was observed for the following factors: female sex, low educational level, current smoking, short and long sleep duration, and multimorbidity. Being married and being physically active were associated with a lower risk of being psychologically frail. A short sleep duration (≤5 hours: 4.25 (95% CI 2.58–6.98); 6 hours: 2.39 (95% CI 1.72 3.34)) and a long sleep duration (≥9 hours: 2.11 (95% CI 1.24–3.59)) were consistently associated with psychological frailty.

Being 70 to 81 years (0.26 95% CI 0.19–0.36) was the only factor associated with a higher risk of being *cognitively frail*. Being female, a paid job, a healthy diet, and being physically active were associated with a lower risk of being cognitively frail. A low educational level and short sleep duration were associated with a higher risk of being *socially frail*. Being a female, being married, and being physically active were associated with a lower risk of being socially frail.

Overall, physical activity was consistently associated with a lower risk of being frail on all four domains. Short sleep duration was consistently associated with three out of the four frailty domains. Living alone, life events, and overweight or obesity were not associated with any of the domains of frailty in the multivariable model.

Table 2.3. Results of the logistic regression analyses on the associated factors with physical, cognitive, psychological, and social frailty.

	Physically frail		Psychologically frail		Cognitively frail		Socially frail	
	Model 1[d] OR (95% CI)	Model 2[e] OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Socio-demographic								
Women	1.10 (0.71 1.68)		**1.67 (1.26 2.21)**	**1.90 (1.37 2.63)**	**0.29 (0.22 0.39)**	**0.26 (0.19 0.36)**	**0.69 (0.49 0.95)**	**0.59 (0.41 0.85)**
Age								
40–49 yr[a]	1	1	1		–	–	1	
50–59 yr[a]	1.61 (0.53 4.92)	1.93 (0.53 7.04)	1.02 (0.70 1.49)		1	1	1.10 (0.65 1.86)	
60–69 yr	1.23 (0.40 3.76)	1.79 (0.48 6.62)	0.70 (0.44 1.10)		1.35 (0.89 2.06)	1.28 (0.82 2.00)	0.97 (0.53 1.78)	
70–81 yr	**3.00 (1.00 9.07)**	**3.89 (1.05 14.42)**	**0.50 (0.29 0.86)**		**3.91 (2.50 6.12)**	**3.32 (2.06 5.35)**	1.46 (0.76 2.80)	
Level of education								
Low	1.58 (0.94 2.66)	0.88 (0.49 1.57)	**1.88 (1.31 2.71)**	**1.52 (1.03 2.24)**	**1.49 (1.08 2.06)**		**2.24 (1.42 3.54)**	**2.06 (1.27 3.34)**
Intermediate	0.60 (0.30 1.22)	**0.42 (0.21 0.95)**	1.20 (0.80 1.78)	0.93 (0.60 1.43)	1.15 (0.81 1.64)		1.56 (0.95 2.56)	1.31 (0.77 2.23)
High	1	1	1	1	1		1	1
Married	**0.36 (0.18 0.72)**		**0.30 (0.21 0.45)**	**0.34 (0.22 0.55)**	0.74 (0.42 1.31)		**0.48 (0.28 0.82)**	**0.47 (0.26 0.87)**
Living alone	0.80 (0.38 1.70)		0.82 (0.52 1.30)		1.13 (0.62 2.07)		1.04 (0.57 1.91)	
Paid job	**0.11 (0.04 0.26)**	**0.16 (0.07 0.41)**	**0.69 (0.49 0.98)**		**0.42 (0.28 0.64)**	**0.36 (0.23 0.56)**	0.66 (0.42 1.04)	
Lifestyle								
Current smoking	**2.55 (1.60 4.07)**	**3.18 (1.87 5.42)**	**1.93 (1.43 2.59)**	**1.92 (1.37 2.67)**	1.53 (1.08 2.17)		1.25 (0.84 1.86)	
Healthy diet	**0.79 (0.67 0.94)**	**0.78 (0.64 0.94)**	1.06 (0.95 1.18)		**0.87 (0.78 0.97)**	**0.88 (0.79 0.99)**	1.00 (0.88 1.14)	
Physically active	**0.22 (0.14 0.32)**	**0.32 (0.20 0.51)**	**0.62 (0.47 0.82)**	**0.71 (0.52 0.98)**	**0.50 (0.38 0.66)**	**0.60 (0.40 0.82)**	**0.58 (0.42 0.82)**	**0.60 (0.41 0.86)**
Sleep duration								
≤5 hr	**2.97 (1.51 5.84)**	**2.44 (1.10 5.43)**	**4.14 (2.62 6.55)**	**4.25 (2.58 6.98)**	1.36 (0.75 2.46)		**2.89 (1.66 5.05)**	**3.11 (1.73 5.60)**
6 hr	**1.84 (1.11 3.05)**	1.54 (0.86 2.75)	**2.36 (1.73 3.22)**	**2.39 (1.72 3.34)**	1.20 (0.85 1.69)		**1.55 (1.04 2.31)**	1.38 (0.89 2.14)
7 or 8 hr	1	1	1	1	1		1	1
≥9 hr	**2.36 (1.32 4.21)**	1.66 (0.86 3.22)	**2.42 (1.50 3.91)**	**2.11 (1.24 3.59)**	1.40 (0.90 2.15)		**1.93 (1.13 3.30)**	1.71 (0.96 3.05)

(Table 2.3 continues)

	Physically frail		Psychologically frail		Cognitively frail		Socially frail	
	Model 1[d] OR (95% CI)	Model 2[e] OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Alcohol consumption								
Never	1	1	1		1		1	
Not anymore	1.38 (0.62 3.06)	1.46 (0.59 3.60)	1.61 (0.84 3.06)		0.86 (0.43 1.73)		1.14 (0.48 2.68)	
Low (<1 glass/wk)	**0.54 (0.30 0.97)**	0.55 (0.27 1.12)	0.70 (0.46 1.08)		**0.60 (0.37 0.96)**		0.97 (0.56 1.70)	
Frequent (≥1 glasses/wk)	**0.40 (0.24 0.68)**	**0.53 (0.29 0.98)**	**0.65 (0.44 0.96)**		**0.60 (0.40 0.91)**		0.78 (0.47 1.31)	
Life events								
Widowed	0.51 (0.17 1.54)		1.48 (0.74 2.97)		0.60 (0.25 1.42)		0.51 (0.17 1.48)	
Divorced[b]	0.76 (0.10 5.95)		1.30 (0.62 2.74)		–		2.15 (0.89 5.17)	
Biological risk factors and chronic disease								
BMI								
Normal (<25 kg/m^2)	1		1		1		1	
Overweight (25–30 kg/m^2)	0.67 (0.39 1.14)		1.25 (0.92 1.71)		1.29 (0.94 1.76)		0.78 (0.54 1.12)	
Obese (≥30 kg/m^2)	**2.12 (1.29 3.47)**		1.24 (0.86 1.80)		**1.80 (1.26 2.59)**		0.72 (0.46 1.14)	
Multimorbidity[c]	**4.47 (2.95 6.79)**	**3.81 (2.32 6.26)**	**2.22 (1.55 3.18)**	**1.83 (1.16 2.87)**	**1.71 (1.25 2.34)**		1.30 (0.84 2.02)	

Abbreviations: BMI, body mass index; OR, odds ratio; 95% CI, 95% confidence interval.
Odds ratios and 95% confidence intervals are presented in this table. Bold values indicate significance ($p<0.05$).
[a] For cognitively frail: age categories are 45–59 yr, 60–69 yr, 70–81 yr, 45–59 yr is the reference category.
[b] For cognitively frail: no respondents were divorced.
[c] Multimorbidity was defined as having two or more conditions out of diabetes, cancer, myocardial infarction, cerebrovascular accident, and chronic respiratory symptoms.
[d] Model 1 are multivariate models adjusted for socio-demographic variables: sex, age, level of education, marital status, living situation, job status.
[e] Model 2 are multivariate models adjusted for socio-demographic variables, lifestyle, life events, biological risk factors, and chronic disease (all variables in the table). For model 2 only statistically significant odds ratios are presented to increase readability of the table.

Frailty on one or more domains

A higher age, a low level of education, current smoking, short and long sleep duration, and multimorbidity were associated with a higher risk to be frail on one or more of the four domains (**Supplementary Table 2.1**). Being a female, being married, having a paid job, having a healthy diet, being physically active, and consuming alcohol (≥1 glasses/wk) were associated with a lower risk to be frail on one or more domains.

Discussion

This study suggests that each of the different frailty domains all had a specific combination of associated factors. Most socio-demographic factors and lifestyle were associated with being frail on each of the domains. Being physically active was consistently associated with a lower risk of being frail on each of the four domains. A short or long sleep duration was associated with a higher risk of being physically, psychologically, and socially frail. Other factors associated with one or more domains of frailty were female sex, high age, a low educational level, being married, a paid job, current smoking, a healthy diet, and multimorbidity.

Drawing on the integral conceptual model of frailty, we observed a prevalence of 17.1% among men and women of 40 to 81 years who lived independently. The proportion of frail persons in a population is dependent on the definition of frailty used (38) and on characteristics of the study population. The prevalence of frailty that we observed is relatively low compared to recent other studies based on the integral conceptual model of frailty (21, 22, 39), which could be explained by the large age range of our population and the absence of persons over 81 years of age. The majority of studies directed to frailty have focused solely on people over the age of 65, despite emerging evidence suggesting that frailty begins much earlier than that (40, 41). Our findings show that frailty may already exist at a relative young age and therefore extend the findings of previous studies.

The relationship between socio-demographic factors and physical frailty has been described in the literature (13, 42). Like age, sex also contributed differently to each of the frailty domains: being female was associated with a higher risk of being psychologically frail and a lower risk of being cognitively and socially frail. A recent review showed socio-economic status to be inversely associated with physical frailty (13). Our findings support a higher risk of being psychologically and socially frail for people with low education. Briefly, socio-demographic factors are important for frailty but their impact varied for each of the domains of frailty.

Lifestyle factors in relation to multiple domains of frailty have not (yet) been studied extensively. An unhealthy lifestyle was previously found to be associated with a higher risk of being physically and socially frail (43–45), and psychologically frail (including cognitive frailty) (43, 44). However, in these studies lifestyle was assessed by a single item in a self-report questionnaire. Such assessment of

lifestyle precludes unambiguous interpretation, because it remains unclear which lifestyle factors participants had in mind when answering the question and what aspect they considered to be unhealthy (44). Our findings provide novel insight into the specific lifestyle factors (physical activity, smoking, diet, alcohol consumption, sleep) associated with the different domains of frailty. Physical activity was significantly associated with all domains of frailty in our study. A previous study of Strawbridge also considered a broad range of risk factors including lifestyle, in relation to a multidimensional definition of frailty (46). Being physically inactive, either at one instant or at several measurements over a period of 29 years, was associated with a higher risk of being frail. Other studies confirmed the associations of physical activity with physical frailty and cognitive decline (47, 48), as far as we know no studies included the domains of psychological and social frailty. Our results are in line with earlier studies showing that current smoking was associated with a higher risk of being physically frail (49), and a healthy diet was associated with a lower risk of being physically and cognitively frail (50, 51). A new insight based on our findings is that short sleep duration was associated with a higher risk to be physically, psychologically and socially frail, and long sleep duration was associated with a higher risk to be psychologically frail. Sleep deprivation contributes to a number of molecular, immune and neural changes that play a role in the development of health problems (52). Previously, sleep quality and sleep disturbances, but not sleep duration, were reported to be associated with physical frailty (53). More detailed studies are needed to understand the relation between sleep and each of the frailty domains (54).

In addition to socio-demographic factors and lifestyle, we studied life events, multimorbidity and overweight. Life events were not associated with any of the frailty domains in our study. Other studies reported life events to be associated with a higher risk of being psychologically frail (43–45). Overweight and obesity were not associated with frailty in our multivariate models, which is comparable to the findings of Strawbridge (46). Some studies that adjusted for socio-demographic variables and smoking, but not for other lifestyle factors, did report an association between obesity and (physical) frailty (55, 56). These inconsistent results regarding the relationship between life events and overweight with frailty can be explained by methodological differences such as the definition of life events. Finally, multimorbidity was associated with a higher risk to be physically and psychologically frail, which is in line with other studies (43–46). Fried illustrated that frailty is distinct but overlapping with comorbidity, with almost 70% of the frail

persons also having two or more diseases (8).

In general, there is no consensus about a definition of frailty (38, 57) as shown by the different approaches described in the literature. Besides the well-known Frailty Phenotype approach (1), the Frailty Index (FI) is another dominant approach in frailty research (58). The FI involves the accumulation of diseases, symptoms, signs, disabilities or any deficiency in health with age (59). Although different domains of human functioning are incorporated in the FI, it differs from our approach to frailty because it considers frailty as much broader than functioning alone. The incorporation of social functioning in the concept of frailty is an area of discussion. During the development of the integral conceptual model of frailty a group of frailty experts agreed upon including social functioning in this model (19). The social domain cannot be left out because it is relevant to an integrated view of human beings (3, 60), the relationship with adverse outcomes is demonstrated (61, 62), and 'social relationships' and 'social support' are viewed as determinants of frailty (46, 63, 64). Two other reasons to consider social functioning as part of a multidimensional definition of frailty are (1) social functioning is regarded as separate health domain in the Comprehensive Geriatric Assessment and therefore viewed as relevant in clinical practice (7), and (2) in a qualitative study the majority of interviewed elderly persons reported to consider reduced social functioning as an important component of frailty (65).

The Doetinchem Cohort Study is a unique cohort for studying frailty because of the relatively wide age range of the participants and the ability to define multiple frailty domains due to the wide array of collected variables. The original TFI scale is based on self-report, but we were able to combine self-reported and objectively measured variables to define frailty. Since the population has a wide age range and includes middle-aged adults, we used similar or more stringent cut-off points than applied earlier by van Campen (22). A sensitivity analysis for cognitive frailty with a lower cut-off (<7.5%) confirmed the findings of cognitive frailty defined by 10th percentile, except for alcohol consumption, obesity and multimorbidity. Since data required to define social frailty (as well as some of the indicators for physical and psychological frailty) were measured for the first time in the most recent completed round of the Doetinchem Cohort Study, longitudinal analyses were not possible. As such, all analyses were cross-sectional and causal inferences cannot be made. To illustrate this, we take the example of having a paid job. We found that having a paid job was strongly associated with a lower risk

to be physically frail. This may suggest a protective effect of having a paid job for being physically frail. However, it may also imply that frail persons have stopped working because of limitations in work functioning. Future studies are needed to address the prospective association between a various range of factors and the development of physical, psychological, cognitive, and social frailty. Another limitation is the measurement of life events. Other studies have shown that especially death of a loved one was associated with frailty (43). The questionnaire used for the Doetinchem Cohort study did not assess death of a loved one. We therefore decided to use the variable 'being widowed' as a proxy. We should however, acknowledge that the life events being widowed and death of a loved one may partly overlap but are not the same. This may explain the lack of association with psychological frailty. Although the response rates for the examination rounds varies between 75% and 80%, we cannot exclude selective response that may have caused an underrepresentation of severely frail participants, in particular of physically and cognitively frail individuals. To minimize a healthy cohort effect, home visits were offered if participants were not able to get to the municipal health services where the examinations were carried out. This way, frail participants could still participate in the study. However, due to potential selective response the observed associations may be underestimated.

The present study suggests that frailty, in particular psychological frailty, may already be present at a relatively young age. Socio-demographic factors, lifestyle and multimorbidity contributed differently to each of the frailty domains. This highlights the relevance of a multidimensional approach to frailty as operationalised in the integral conceptual model of frailty. Understanding which groups of older adults are at risk of being frail on each domain may help to prevent frailty and to identify frail individuals in an early stage. Identification of frail individuals is an important step to preclude the development of undesirable outcomes, to provide adequate healthcare and support, and to effectively prevent and delay the development of frailty by health professionals. Since we know little about factors associated with the psychological, cognitive and social domains of frailty, our findings add to available literature and are relevant for clinical practice (38). We found that lower educated adults were at higher risk of being frail, and therefore preventive strategies should be directed at this group. Furthermore, our findings suggest that lifestyle factors, specifically physical activity and sleep, are associated with the presence of frailty. As lifestyle is potentially modifiable, interventions directed to improve lifestyle may provide new opportunities for the prevention of frailty in the future. But first

longitudinal research should be conducted to better examine how lifestyle affects the development of frailty and its dynamic course.

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Supplementary Methods

Physical frailty

Participants were considered physically frail when they fulfilled the specified requirements for ≥4 of the following 8 criteria: exhaustion, unintentional weight loss, low handgrip strength, perceived health, walking, balance, poor hearing, and poor vision (**Table 2.1**) (1). Unintentional weight loss was defined as weight loss >5% between round 4 and 5 and the participant reporting not to be on a diet. Presence of exhaustion was assessed using the following two statements of the Center for Epidemiologic Studies Depression scale (CES-D) (2): "I felt that everything I did was an effort" and "I could not get going"(2). Participants answering a 'moderate amount of the time (3-4 days)', or 'most of the time' to either of these questions were considered to be exhausted. Handgrip strength was measured using a dynamometer (Jamar, Sammons Preston Rolyan). Cut-off points for handgrip strength in kg were stratified by sex and BMI. Cut-off points for men were ≤29.0 kg for BMI ≤24.0, ≤30.0 kg for BMI 24.1-26.0, ≤30 kg for BMI 26.1-28.0, and ≤32.0 kg for BMI >28.0. Cut-off points for women were ≤17.0 kg for BMI ≤23.0, ≤17.3 kg for BMI 23.1-26.0, ≤18.0 kg for BMI 26.1-29.0, and ≤21.0 kg for BMI >29.0. The cut-off points used for exhaustion and handgrip strength were based on Fried (3). Limitations in walking due to health were assessed with a question regarding 100 meter walking. Poor perceived self-reported health were those with a self-reported health of less than good, on a scale with the following response categories: poor, fair, good, very good, excellent. The question was taken from the 36-Item Short-Form Health Survey (SF-36) (4, 5). The Tandem Stand Balance Test was used to test balance in participants ≥60 years old. Failing or refusing to do the test, or not being able to keep balance >10 seconds, was considered a positive score for this criterion (being <60 years counted as a negative score). Hearing impairment (present or absent) was also assessed by self-report, with questions on hearing in general, and experienced difficulties in group conversations. Vision impairment (present or absent) was assessed in a similar manner.

Cognitive frailty

Participants were considered cognitively frail when scoring <10th percentile on global cognitive functioning (**Table 2.1**). Global cognitive functioning was assessed with a neuropsychological test battery. Memory function, information processing speed and cognitive flexibility were tested using 15-words Verbal Learning Test, the Stroop Colour-Word Test, the Word Fluency Test and the Letter Digit

Substitution Test. Nooyens *et al.* describe the cognitive tests in more detail (6). The cognitive tests were performed among participants ≥45 years old. Cognitive scores were adjusted for level of education and number of tests performed during follow-up.

Psychological frailty

Psychological frailty was defined as fulfilling both criteria for depression and criteria for general mental health (**Table 2.1**). Depressive symptoms were assessed with the CES-D. Those with a CES-D score of ≥16 (out of range of 0-60) were defined to have a high risk of depression. Mental health status was measured with the Mental Health Inventory-5 (MHI-5) (5). Scores of five questions on a six-point scale were transformed into a total score ranging from 0 to 100 and a cut-off point of ≤60 was used to indicate poor mental health status (7). Coping was no criterion for the psychological domain because it was not available.

Social frailty

Social frailty was defined as meeting ≥2 of 3 criteria using the Loneliness scale, Social Support List-12 and a questionnaire about social participation from the Dutch Municipal Health Services Elderly Monitor (**Table 2.1**). A cut-off point of ≥9 on the Loneliness scale (11 items with a score 0 or 1) was used to indicate loneliness (8, 9). A score of <24 (out of 48) on the 12-item Social Support List was considered to indicate low social support (10). Finally, participants in the lowest decile on The Dutch Municipal Health Services Elderly Monitor (11), a questionnaire regarding social participation used in the Dutch public health care for elderly people (15 items with a maximum score of 65), were considered to have limited social participation. Living alone was not included in the definition of the social domain of frailty because we studied living alone as a determinant of all frailty domains.

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Supplementary Table 2.1. Results of the logistic regression analyses on the associated factors with frailty on any of the domains.

	Frail on one or more domains	
	Model 1[b] OR (95% CI)	Model 2[c] OR (95% CI)
Socio-demographic		
Women	**0.67 (0.56 0.81)**	**0.62 (0.51 0.77)**
Age		
40–49 yr	1	1
50–59 yr	**1.49 (1.09 2.05)**	**1.70 (1.20 2.41)**
60–69 yr	1.39 (0.98 1.98)	**1.74 (1.17 2.58)**
70–81 yr	**2.99 (2.05 4.35)**	**3.70 (2.42 5.66)**
Level of education		
Low	**1.62 (1.29 2.04)**	**1.29 (1.01 1.65)**
Intermediate	1.20 (0.94 1.54)	1.03 (0.79 1.34)
High	1	1
Married	**0.42 (0.31 0.57)**	**0.41 (0.28 0.58)**
Living alone	0.78 (0.55 1.12)	0.71 (0.47 1.06)
Paid job	**0.50 (0.39 0.65)**	**0.58 (0.44 0.77)**
Lifestyle		
Current smoking	**1.69 (1.36 2.10)**	**1.60 (1.26 2.04)**
Healthy diet	**0.93 (0.86 1.00)**	**0.93 (0.86 1.00)**
Physically active	**0.52 (0.43 0.63)**	**0.62 (0.50 0.76)**
Sleep duration		
≤5 hr	**2.56 (1.77 3.69)**	**2.30 (1.55 3.43)**
6 hr	**1.68 (1.35 2.10)**	**1.53 (1.20 1.94)**
7 or 8 hr	1	1
≥9 hr	**2.02 (1.49 2.76)**	**1.76 (1.26 2.46)**
Alcohol consumption		
Never	1	1
Not anymore	1.07 (0.66 1.73)	1.20 (0.71 2.02)
Low (<1 glass/wk)	0.74 (0.54 1.00)	0.86 (0.61 1.21)
Frequent (≥1 glasses/wk)	**0.59 (0.45 0.78)**	**0.71 (0.52 0.97)**
Life events		
Widowed	0.84 (0.48 1.47)	1.12 (0.63 2.00)
Divorced	1.01 (0.51 1.98)	1.08 (0.54 2.17)
Biological risk factors and chronic disease		
BMI		
Normal (<25 kg/m^2)	1	1
Overweight (25–30 kg/m^2)	1.09 (0.89 1.34)	1.01 (0.81 1.26)
Obese (≥30 kg/m^2)	**1.35 (1.06 1.71)**	1.01 (0.77 1.33)
Multimorbidity[a]	**2.38 (1.84 3.09)**	**1.89 (1.42 2.52)**

Abbreviations: BMI, body mass index; OR, odds ratio; 95% CI, 95% confidence interval.
Odds ratios and 95% confidence intervals are presented in this table.
[a] Multimorbidity was defined as having two or more out of diabetes, cancer, myocardial infarction, cerebrovascular accident, and chronic respiratory symptoms.
[b] Model 1 are multivariate models adjusted for socio-demographic variables: sex, age, level of education, marital status, living situation, job status.
[c] Model 2 are multivariate models adjusted for socio-demographic variables, lifestyle, life events, biological risk factors, and chronic disease (all variables in the table).

3

The association between BMI and different frailty domains: A U-shaped curve?

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Abstract

Background

Previous studies showed a U-shaped association between BMI and (physical) frailty. We studied the association between BMI and physical, cognitive, psychological, and social frailty. Furthermore, the overlap between and prevalence of these frailty domains was examined.

Methods

The Doetinchem Cohort Study is a longitudinal population-based study starting in 1987-1991 examining men and women aged 20-59 with follow-up examinations every 5 yrs. For the current analyses, we used data from round 5 (2008-2012) with 4019 participants aged 41-81 yrs. Physical frailty was defined as having ≥2 of 4 frailty criteria from the Frailty Phenotype (unintentional weight loss, exhaustion, physical activity, handgrip strength). Cognitive frailty was defined as the <10th percentile on global cognitive functioning (based on memory, speed, flexibility). Psychological frailty was defined as having 2 out of 2 criteria (depression, mental health). Social frailty was defined as having ≥2 of 3 criteria (loneliness, social support, social participation). BMI was divided into four classes. Analyses were adjusted for sex, age, level of education, and smoking.

Results

A U-shaped association was observed between BMI and physical frailty, a small linear association for BMI and cognitive frailty and no association between BMI and psychological and social frailty. The four frailty domains showed only a small proportion of overlap. The prevalence of physical, cognitive and social frailty increased with age, whereas psychological frailty did not.

Conclusions

We confirm that not only underweight but also obesity is associated with physical frailty. Obesity also seems to be associated with cognitive frailty. Further, frailty prevention should focus on multiple domains and target individuals at a younger age (<65yrs).

Introduction

Frailty can be described as the result of the accumulation of deficits (1) in physical (2), psychological or social functioning (3). This accumulation may lead to an increased vulnerability. One event, for example breaking a hip through falling, can have a major impact on frail individuals. The recovery process can take a long time and sometimes these individuals do not recover at all. In other words, frail individuals become relatively easily imbalanced and have difficulty returning to their balanced state (4, 5). Frailty also increases the risk for negative health outcomes (6, 7) including falls, disability, hospitalisation, institutionalisation, and mortality (2, 8–10). To prevent the development of these undesirable outcomes a first step is to identify frail individuals, preferably in an early stage. For this purpose, different frailty instruments have been developed over the years. The Frailty Phenotype for example, as first described by Fried (2), refers to physical frailty. The Frailty Index of Rockwood (11) is based on a broader definition of frailty and is built on the principle of the accumulation of deficits; it contains items regarding the presence of diseases, the ability to perform everyday activities, and physical and neurologic signs. Over time, the focus has shifted to other frailty domains, such as psychological and social frailty. In response to this development new frailty instruments were established. The Tilburg Frailty Indicator (12), the Edmonton Frail Scale (13), and the Groningen Frailty Indicator (14) are all instruments that include measurements for multiple frailty domains, which are aggregated into an overall score. In addition, frailty domains are also being studied individually. Currently, in particular the concept of cognitive frailty receives much attention (15–18).

One important indicator for frailty is underweight or unintentional weight loss. The association between having a low body mass index (BMI) and frailty is acknowledged and is for example included in Fried's definition of the Frailty Phenotype as well as in other definitions. However, recent studies also found an association between obesity and frailty. An important finding, because the prevalence of overweight and obesity among the elderly is increasing (19). In fact, the results seem to indicate that there is a U-shaped association between BMI and (physical) frailty (20–22), so both ends of the weight continuum are of concern.

Here we report on the association between BMI and physical, cognitive, psychological and social frailty in the Doetinchem Cohort Study. We investigated whether having a low or high BMI is associated with a higher risk of physical frailty

only, or if this U–shaped association also applies to cognitive, psychological, and/or social frailty. In addition, to gain more insight in these domains we studied the overlap between and prevalence of the different frailty domains.

Methods

Participants and study design

The Doetinchem Cohort Study is an ongoing longitudinal population-based cohort study, which started in 1987-1991. The study design is described by Verschuren *et al.* (23). Briefly, the Doetinchem Cohort Study was designed to study the influence of lifestyle and biological risk factors on health over the life course. At baseline (round 1) men and women aged 20-59 years and living in Doetinchem, a provincial town in the Netherlands, were examined. From the 12439 participants who participated in the first round, a random sample of 7769 persons was re-invited for a follow-up study, to be reexamined every 5 years for 25 years. Those who were invited in round 2 (1993-1998) were invited again, excluding those who emigrated or actively withdrew from the study. The response rates varied between 75% and 80%, resulting in 4019 participants for round 5. For round 5, the study was approved according to the guidelines of the Helsinki Declaration by the Medical Ethics Committee of the University Medical Center Utrecht. Written informed consent was obtained from all participants during each examination round. For the current analyses, we used data from the fifth examination round (2008-2012) (n=4019). First, we excluded 20 participants with missing data for BMI (n=3999). Next, there were 619 missings regarding cognition data because cognitive function was tested only among participants ≥45 yrs. Finally, there were 8 missings for psychological frailty and 5 missings for social frailty. As a result, the analyses for physical frailty included 3999 participants, for cognitive frailty 3380 participants, for psychological frailty 3991 participants, and for social frailty 3994 participants.

Measurements

The study protocol consisted of questionnaires and physical, functional and biological measurements. Trained personnel performed all measurements in a standardised way.

Socio-demographic factors

Level of education was measured as the highest level reached during follow-up and categorised into low (intermediate secondary education or less), intermediate (intermediate vocational and higher secondary education) and high (higher vocational education or university).

Lifestyle
Smoking status was categorised into current smoker, former smoker and non–smoker. Being physically active was defined as adherence to the Dutch physical activity guideline, which recommends 30 minutes of moderate to vigorous physical activity per day on at least 5 days per week (24).

Body composition measurements
Body weight was measured to the nearest 100 g on calibrated scales and height to the nearest 0.5 cm. BMI was calculated and categorised into underweight < 20 kg/m^2, normal weight 20–24.9 kg/m^2, overweight 25–29.9 kg/m^2, and obesity ≥ 30 kg/m^2.

Disease
Multimorbidity was defined as having two or more chronic diseases based on self–report. The following diseases were asked via self–report and included: diabetes, cancer, myocardial infarction, cerebrovascular accident, and chronic non–specific lung diseases.

Frailty criteria
An overview of the frailty criteria per domain are listed in **Table 3.1**. We used the Frailty Phenotype to define physical frailty (Fried), because this is a validated and widely used instrument. The cognitive, psychological, and social frailty domains are based on the Tilburg Frailty Indicator (TFI) (12) and the corresponding conceptual model of Gobbens (25). In the TFI, cognitive functioning is part of the psychological frailty domain. There is increasing support for the idea of cognitive frailty being a separate frailty domain (15, 16). Consequently, we constructed separate cognitive and psychological frailty domains, which are based on the TFI. A detailed description of the criteria can be found in the **Supplementary Methods**. Briefly, participants were considered to be physically frail (Fried) if they fulfilled ≥2 of 4 frailty criteria described by Fried (unintentional weight loss, exhaustion, physical activity, and handgrip strength) (2). Participants were considered cognitively frail when scoring <10th percentile on a global cognitive functioning score based on memory, speed, and flexibility. Cognitive scores were adjusted for level of education and number of tests performed during follow–up. Psychological frailty was defined as fulfilling both criteria for depression (26) and for general mental health (27). Social frailty was defined as meeting ≥2 of 3 criteria using the Loneliness scale (28), Social Support List–12 (29) and a questionnaire about

social participation from the Dutch Municipal Health Services Elderly Monitor (30). Participants were considered to be physically frail (Gobbens) if they fulfilled ≥4 of 8 frailty criteria described by Gobbens (12). Physical frailty (Gobbens) was used for the sensitivity analysis.

Our cut-off point for physical frailty (Fried) and physical frailty (Gobbens) deviate from the cut-off points described by these authors. Due to data unavailability we use 4 instead of 5 criteria for physical frailty (Fried) and adjusted the cut-off point so the prevalence is similar to the prevalence described per age group by Fried et al (2). We adjusted the cut-off point for physical frailty (Gobbens) from ≥3 of 8 criteria to ≥4 of 8 frailty criteria, so the prevalence is similar to the prevalence described by Fried.

Table 3.1. Overview criteria per frailty domain.

Domains	Criteria	Cut-off	Based on
Physical frailty (Fried) (2)	– unintentional weight loss – exhaustion – low physical activity – reduced handgrip strength	≥ 2 criteria	– unintentional weight loss: > 5% weight loss between round 4 and 5 and not being on a diet – low physical activity: meeting all of the following three criteria: 1) < 10th percentile of a physical activity score 2) <25th percentile of walking hours per week during the last 12 months 3) failing to meet the Dutch physical activity guideline – exhaustion: 2 statements of the Center for Epidemiologic Studies Depression scale (26) – handgrip strength: dynamometer
Physical frailty (Gobbens) (12)	– unintentional weight loss – exhaustion – reduced handgrip strength – perceived health – limited in walking – disturbed balance – hearing impairment – vision impairment	≥ 4 criteria	– unintentional weight loss (see physical frailty (Fried)) – exhaustion (see physical frailty (Fried)) – handgrip strength (see physical frailty (Fried)) – perceived health: one question of 36-Item Short-Form Health Survey (27, 31) – 1 question on 100 m walking – Tandem Stand Balance Test – 3 questions regarding hearing – 3 questions regarding vision
Cognitive frailty	– reduced global cognitive functioning	< 10th percentile	– global cognitive functioning score based on tests for memory, speed and flexibility: ○ 15 Words Verbal Learning Test (32) ○ Stroop Colour–Word Test (33) ○ Word Fluency Test (34) ○ Letter Digit Substitution Test (35)
Psychological frailty	– depressive symptoms – mental health	= 2 criteria	– Centre for Epidemiologic Studies Depression scale – Mental Health Inventory 5 (27)
Social frailty	– loneliness – little social support – low social participation	≥ 2 criteria	– Loneliness Scale (28) – Social Support List-12 (29) – Questionnaire Dutch Elderly Monitor (30)

Note: This table shows the criteria used per frailty domain. Physical frailty (Fried), cognitive frailty, psychological frailty, and social frailty were used to study the association between BMI and frailty. Physical frailty (Fried) and physical frailty (Gobbens) were used for the sensitivity analysis.

Statistical analyses

Descriptive analyses were carried out for the non-frail population and for the physical, cognitive, psychological and social frail groups separately. For each frailty domain, the association between BMI and frailty was studied using a logistic regression model with classes of BMI as the independent variable. As mentioned above, BMI was categorised into four classes: underweight, normal weight, overweight, and obese. In the logistic regression model, we adjusted for socio-demographic characteristics (sex, age, and level of education) and smoking status, because these are considered to be potential confounders for frailty (1, 21, 36, 37). Age was considered as a continuous variable. Smoking status for the logistic regression model was categorised into smoking and non-smoking. The overlap between the different frailty domains was studied with frequency tables. In order to assess the effect of using different definitions of physical frailty, we performed a sensitivity analysis comparing physical frailty as defined by Fried (2) based on four criteria and physical frailty as defined by Gobbens (12) entailing eight criteria. We calculated the inter-rater agreement regarding the two definitions for physical frailty using Cohen's Kappa coefficient. All analyses were carried out in SAS 9.3 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Population characteristics

Characteristics of the non–frail population and the frail populations stratified by the different frailty domains are presented in **Table 3.2**. The mean age of the psychologically frail population was similar to the mean age of the non–frail population. In the other three frailty domains, the mean age was higher compared to the non–frail population. Psychological frailty was more common among women (68.9%) than men (31.1%). In contrast, cognitive frailty was more common among men (67.4%). Compared to the non–frail population, a low education, and current smoking were more prevalent, mean BMI was higher, and multimorbidity was higher in the frail population for all four domains.

Table 3.2. Characteristics table of the non-frail and the frail populations.

	Non-frail (n=2747)	Physically frail (Fried) (n=150)	Cognitively frail (n=310)	Psychologically frail (n=248)	Socially frail (n=162)
Socio-demographic					
sex (men) (%)	1274 (46.4)	58 (38.7)	209 (67.4)	77 (31.1)	84 (51.9)
age (yrs) (SD)	60.2 (8.8)	63.8 (11.1)	70.2 (6.8)	59.2 (9.8)	63.0 (10.4)
low level of education (%)	1091 (39.7)	77 (51.3)	154 (49.7)	141 (56.9)	92 (56.8)
Body composition					
BMI[a] (kg/m^2) (SD)	26.7 (4.0)	28.1 (5.9)	28.0 (4.3)	27.4 (5.0)	27.1 (4.7)
underweight (%)	52 (1.9)	8 (5.3)	–	9 (3.6)	6 (3.7)
normal weight (%)	950 (34.6)	41 (27.3)	75 (24.2)	69 (27.8)	54 (33.3)
overweight (%)	1247 (45.4)	53 (35.3)	156 (50.3)	114 (46.0)	71 (43.8)
obese (%)	498 (18.1)	48 (32.0)	79 (25.5)	56 (22.6)	31 (19.1)
Lifestyle					
current smoker (%)	410 (15.0)	39 (26.4)	55 (18.0)	77 (31.2)	34 (21.3)
physical activity[b] (%)	2220 (80.9)	61 (40.7)	223 (72.2)	169 (68.2)	107 (66.1)
Health and disease					
poor self-reported health (%)	312 (11.4)	72 (48.0)	85 (27.5)	116 (47.0)	53 (32.7)
multimorbidity[c] (%)	183 (6.7)	32 (21.3)	56 (18.2)	38 (15.3)	24 (14.8)

Abbreviation: BMI, body mass index.

[a] BMI: underweight (< 20 kg/m^2), normal weight (20-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obesity (≥ 30 kg/m^2).

[b] physical activity: meet Dutch physical activity guideline (30 minutes of moderate to vigorous physical activity per day on at least 5 days per week).

[c] multimorbidity: having two or more out of diabetes, cancer, myocardial infarction, cerebrovascular accident, and chronic respiratory symptoms.

Legend: N (%) or mean (SD).

Note: Summing up the total numbers of participants for the different frailty domains (n=150 for physical, n=310 for cognitive, n=248 for psychological, n=162 for social) leads to an overestimation of the frail population, because participants can be frail for one, or more domains. In total, 703 participants are frail for one or more domains.

BMI and frailty

Unadjusted proportions showed a U-shaped association between BMI and physical and psychological frailty (**Figure 3.1**). A linear association was found between BMI (starting at the BMI class of 20–24.9 kg/m^2) and cognitive frailty. There was no association between BMI and social frailty.

After adjustment for sex, age, level of education and smoking status, the U-shaped association between BMI and physical frailty remained. Prevalence of physical frailty was 8.2% in underweight participants, 2.9% in normal weight participants, 2.6% in overweight participants and 5.0% in obese participants (**Figure 3.2**). After adjustment for the confounders mentioned above, the association between BMI and cognitive frailty attenuated, but was still linear. However, after adjustment no association was observed between BMI and psychological and social frailty.

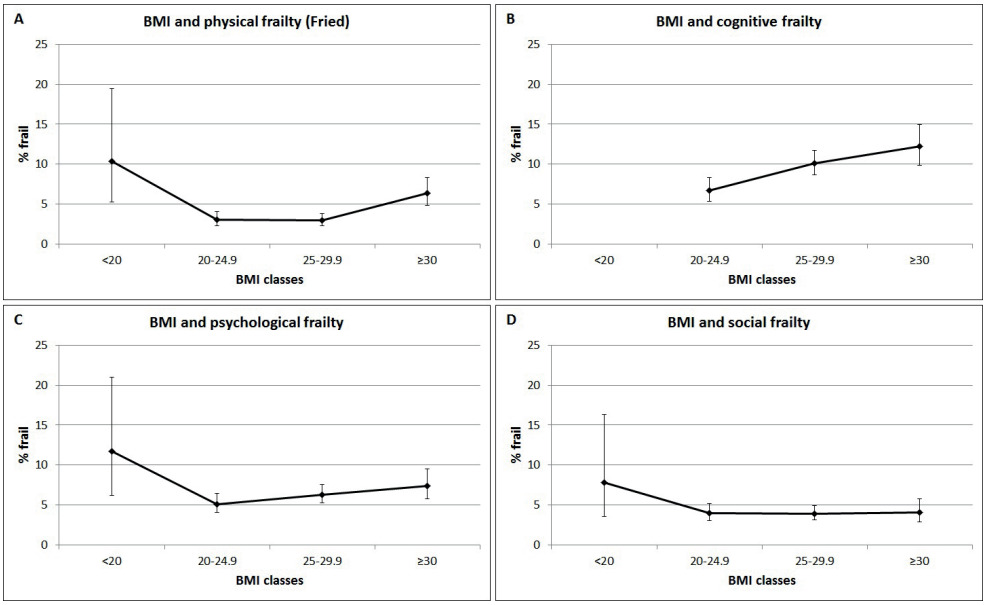

Figure 3.1. The association between BMI and frailty (unadjusted proportions). Physical frailty (Fried) **(A)**, cognitive frailty **(B)**, psychological frailty **(C)**, and social frailty **(D)**. *Note:* There were no participants in the lowest BMI class (BMI <20) for cognitive frailty.

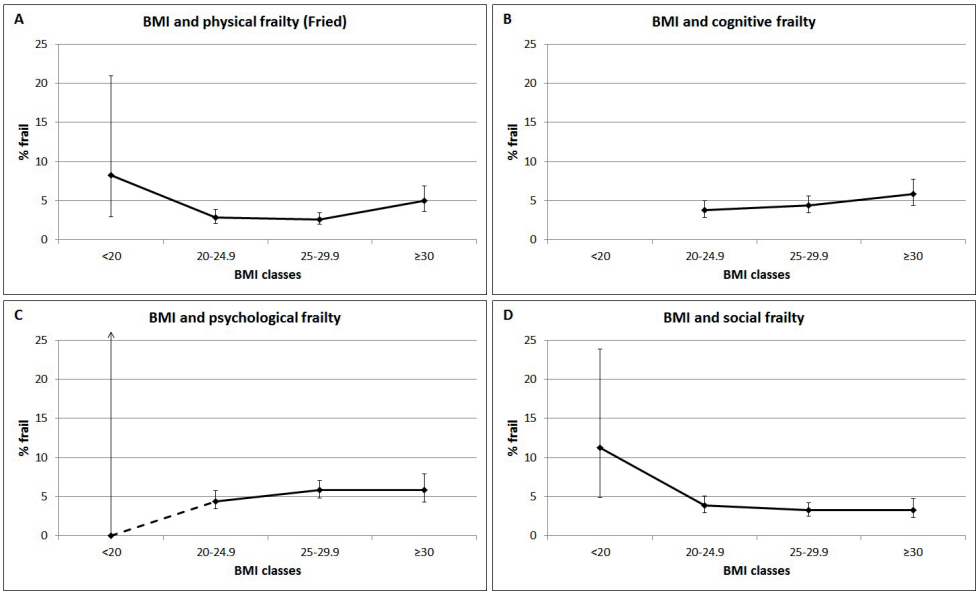

Figure 3.2. The association between BMI and frailty (adjusted proportions). Physical frailty (Fried) **(A)**, cognitive frailty **(B)**, psychological frailty **(C)**, and social frailty **(D)**. *Note:* Proportions were adjusted for sex, age, level of education, and smoking. There were no participants in the lowest BMI class (BMI <20) for cognitive frailty and there were few participants in the lowest BMI class for psychological frailty.

Sensitivity analysis

In order to assess the effect of using different definitions of physical frailty, we performed a sensitivity analyses where we compared the association between BMI and physical frailty defined by Fried (2) with physical frailty defined by Gobbens (12). The prevalence of physical frailty (Fried) was 3.8% (n=150) and the prevalence of physical frailty (Gobbens) was 2.7% (n=108). The sensitivity analysis showed a similar U-shaped association between BMI and physical frailty (Gobbens) for both the unadjusted and adjusted proportions (**Figure 3.3 A** and **B**). The Kappa agreement regarding the two physical frailty instruments was moderate with 42% (with n=56 being physically frail according to both instruments).

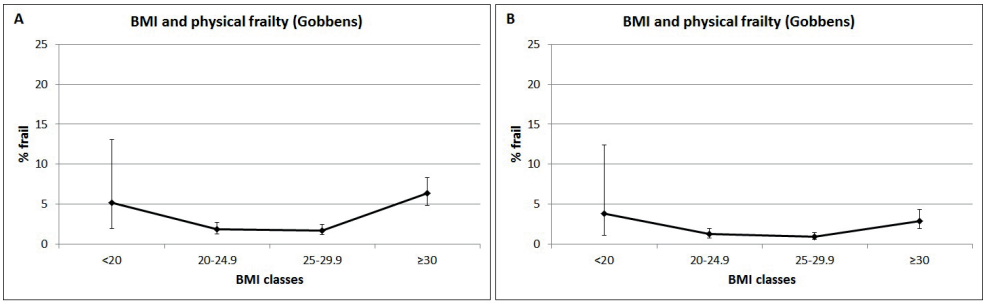

Figure 3.3. The association between BMI and physical frailty (Gobbens). Unadjusted proportions **(A)** and adjusted proportions for sex, age, level of education, and smoking **(B)**.

Overlap of frailty domains

A total of 150 (3.8%) participants were physically frail (Fried), 310 (9.2%) were cognitively frail, 248 (6.2%) were psychologically frail, and 162 (4.1%) socially frail. Only one participant was frail for all four domains. Limited overlap was observed between the different frailty domains (**Figure 3.4**). The percentage overlap between pairs of two domains ranged from 4.9% to 12.0%. The lowest overlap was observed between the cognitive and the social domain with 4.9% participants meeting the criteria for (at least) these two domains. The highest overlap was observed between the psychological and the social domain with 12.0% participants meeting the criteria for (at least) these two domains. The percentages were obtained by dividing the number of participants who were frail for both these two domains by the sum of individuals within these two domains.

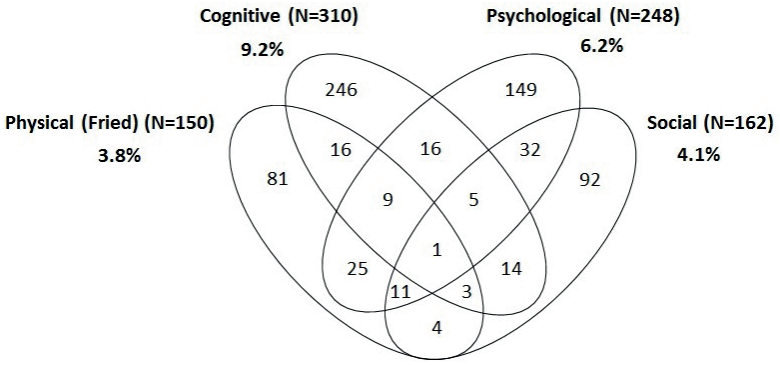

Figure 3.4. Venn diagram showing the prevalence and overlap between the different frailty domains.

Prevalence per age group

When studying the prevalence in five-year age groups, the prevalence increased with age for physical, cognitive, and social frailty (**Figure 3.5**). However, psychological frailty was most common in the lowest age group of 41–44 years old.

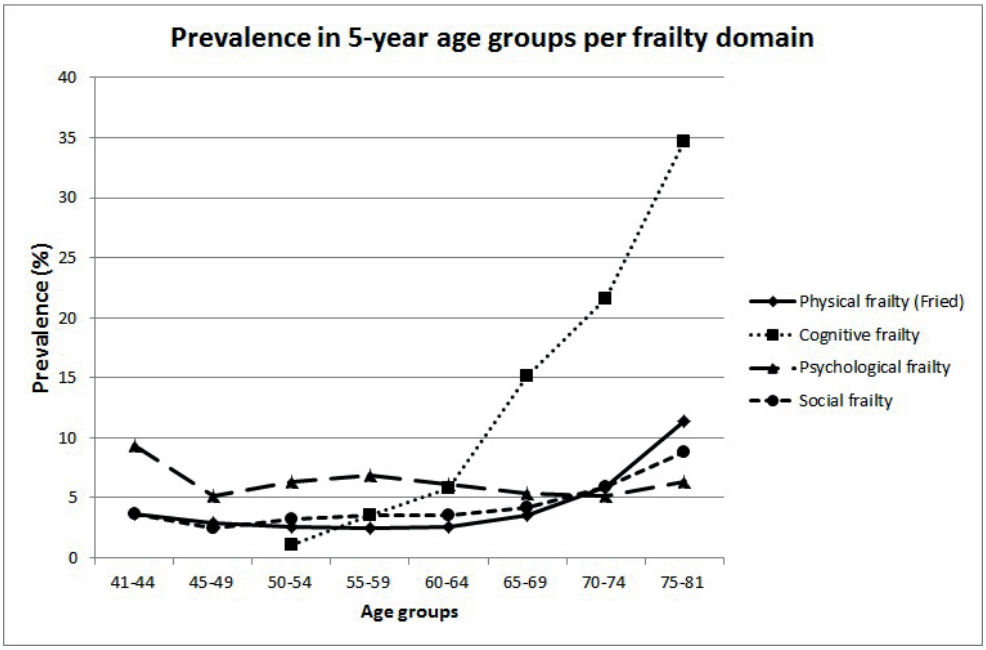

Figure 3.5. Prevalence in five-year age groups per frailty domain.
Note: Cognitive testing was done in participants ≥ 45 yrs.

Discussion

In this study, we found a U-shaped association between BMI and physical frailty, a small linear association between BMI and cognitive frailty and no association between BMI and psychological and social frailty. Further, the different frailty domains (physical, cognitive, psychological, and social) showed only a small proportion of overlap. Finally, the prevalence of physical, cognitive and social frailty increased with age, whereas the prevalence of psychological frailty did not.

Our findings regarding the association between BMI and physical frailty (Fried) are in line with previously published results (20, 21). The studies of Blaum, Hubbard, and our study, all showed that there is a U-shaped association between BMI and physical frailty based on the Frailty Phenotype. Physical frailty as defined by Gobbens seems to identify a population that is in general older and less healthy than the physically frail population as defined by Fried (**Supplementary Table 3.1**). Nevertheless, the association between BMI and physical frailty (Gobbens) showed a similar U-shaped curve. Thus, the association between BMI and physical frailty as defined by Fried is similar in different populations. Further, the association between BMI and physical frailty is similar when using different instruments for defining physical frailty. It is not yet clear why both underweight and obesity are associated with physical frailty. Possibly these individuals have a shared characteristic which could be the signal or outcome of a similar (underlying) biological mechanism, resulting in a U-shaped association between BMI and physical frailty.

Our results regarding the associations between BMI and psychological and social frailty are in line with the results reported by Gobbens *et al.* (12). They showed that there is a statistically significant association between BMI and physical frailty and no statistically significant association between BMI and psychological and social frailty. The association between BMI and cognitive frailty has, to the best of our knowledge, not yet been studied.

Our study has several strengths and limitations. We studied frailty in the Doetinchem Cohort Study, a large population-based study with a high response rate, which gave us amongst others a better understanding of the age-distribution of the different frailty domains. The fact that we studied the association between BMI and four separate frailty domains is in our view a strength, because it gives us more insight in the differences between the frailty domains. On the one hand,

the operationalisation of these domains could be considered a limitation, because they are based on validated instruments, but they are not an exact copy of these instruments. On the other hand, for some domains such as the cognitive frailty domain we used all available data to define frailty (3 cognitive tests) instead of a single question, which was used in the original frailty instrument (TFI). For physical frailty, the criteria from the Frailty Phenotype were used and for psychological, and social frailty the criteria from the TFI were used. In contrast to the TFI, we considered psychological and cognitive frailty as two separate domains because psychological processes differ considerably from cognitive processes. In the literature on frailty, cognitive frailty is also increasingly being recognized as a separate domain (15, 16). For cognitive frailty, we used well-known and validated cognitive functioning tests. Unfortunately, we did not have data on (self)-reported dementia, which is recommend to take into consideration according to the definition for cognitive frailty that is currently being developed (15, 16). However, due to selection bias (participants with declining cognitive function tend to refuse to participate in the cognitive functioning tests) we assume that the available cognitive data are from participants without dementia.

The associations between BMI and the different frailty domains were studied cross-sectionally, prohibiting causal inference. In an additional analysis, we also studied the association between BMI measured in round 2 (15 years earlier) and frailty in round 5. The associations between BMI (round 2) and the different frailty domains (round 5) are similar to the association we found in the cross-sectional analyses (see **Supplementary Figure 1** and **2**). In these additional analyses, the number of cases in the lowest BMI class is limited and interpretation of the results for this BMI class is difficult.

A different limitation is the BMI cut-off points used in this study. Due to the age range of our population, we could not use BMI cut-off points that are specific for elderly individuals. Currently, it is suggested that a BMI <23 would be a suitable cut-off point for underweight in elderly (38). In addition, the cut-off point regarding obesity in elderly is also under discussion and it is suggested that the value should be higher than a BMI of 30. However, the age-range of the participants in the Doetinchem Cohort Study is between 41 and 81 years old. Therefore, we decided to only adjust the cut-off point for underweight from <18.5 to <20, which is in line with other studies (21, 39) and to keep the other cut-off points according to the WHO recommendations.

To the best of our knowledge, there is only one other paper describing the overlap between different frailty domains. Garre-Olmo (40) described the overlap between the physical, mental, and social frailty domains in men and women aged 75 and over, with the mental frailty domain including both psychological and cognitive measurements. In their study, 1.9% of the individuals were found to be frail for all three domains. In our study, 0.1% of the individuals were frail for the physical, cognitive, psychological, and social domain. This difference could be explained by the fact that our population is much younger, or by the fact that they have three frailty domains while we have four frailty domains. Both the study of Garre-Olmo *et al.* and our study seem to show limited overlap between the different frailty domains, which could have important implications regarding frailty research and prevention. Prevention that focusses on a specific frailty domain could be more beneficial than prevention focusing on overall frailty. For example, prevention for physical frailty will most likely focus on physical activity and nutrition, while prevention for social frailty will be directed towards improving someone's social network. Furthermore, the age at which prevention should start could also differ per frailty domain where prevention for psychological frailty should start at an earlier age than prevention for cognitive frailty. Because the scope of the prevention strategies will differ quite a lot, it will be more efficient to develop a prevention strategy per frailty domain.

The Doetinchem Cohort Study is a unique cohort for studying the development of frailty because of the relative young age of the participants and the ability to define multiple frailty domains due to the wide array of collected variables. Compared to other studies, the prevalence of frailty is lower which could indeed be explained by the relatively young population. Frailty is often studied in populations where the participants are ≥65 yrs. Because of the age distribution of our population, we were able to extend the results of previous studies and show that several domains (physical, psychological, social) of frailty were already present from age 41 onwards. This suggests that frailty may already develop at a relatively young age indicating that identification and prevention should start prior to age 65. In our study, psychological frailty was present at a relatively low age (highest prevalence in age group 41–49) while physical, cognitive, and social frailty were present at a higher age (highest prevalence in age group 70–81). In addition, the prevalence for physical, cognitive, and social frailty seems to increase with age, while the prevalence for psychological frailty does not seem to be age related. Whether the prevalence for physical, cognitive, and social frailty will keep increasing with age in

the Doetinchem Cohort Study will be revealed in the coming years.

In conclusion, we found a U-shaped association only between BMI and physical frailty. A small linear association was observed between BMI and cognitive frailty, and there was no association between BMI and psychological and social frailty. The prevalence of physical, cognitive and social frailty increased with age, whereas the prevalence of psychological frailty did not.

We confirm that both underweight and obesity are associated with physical frailty. Obesity also seems to be associated with cognitive frailty. Although we cannot draw any causal inferences from this study, we do think that maintenance of a healthy body weight throughout the life course is important. Furthermore, the limited overlap between the different frailty domains is a first indication that the domains entail distinct populations. Therefore, we suggest to target prevention on multiple frailty domains (e.g. physical, cognitive, psychological, and social frailty) rather than on one domain only. Finally, when taking the age-distribution into account, prevention for frailty should start at a younger age (<65 yrs) and prevention for psychological frailty may start even earlier.

Acknowledgement
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Supplementary Methods

Physical frailty (Fried)

Participants were considered to be physically frail if they fulfilled ≥ 2 of 4 frailty criteria described by Fried (1) (**Table 3.1**): unintentional weight loss, exhaustion, low physical activity and reduced handgrip strength. Unintentional weight loss was defined as weight loss > 5% between round 4 and 5 and the participant reporting not to be on a diet. Presence of exhaustion was assessed using the following two statements of the Center for Epidemiologic Studies Depression scale (2) : "I felt that everything I did was an effort" and "I could not get going". Participants answering a 'moderate amount of the time (3–4 days)', or 'most of the time' to either of these questions were considered to be exhausted. Low physical activity was defined as meeting all of the following three criteria: 1) <10th percentile of a physical activity score consisting of low (<4 metabolic equivalents), middle (4–6.5 metabolic equivalents), and high (>6.5 metabolic equivalents) physical activities and taking into account the time spent on each activity; 2) <25th percentile of walking hours per week during the last 12 months; 3) failing to meet the Dutch physical activity guideline. Handgrip strength was measured using a dynamometer (Jamar, Sammons Preston Rolyan). Cut-off points for handgrip strength in kg were stratified by sex and BMI. Cut-off points for men were ≤29.0 kg for BMI ≤24.0, ≤30.0 kg for BMI 24.1–26.0, ≤30 kg for BMI 26.1–28.0, and ≤32.0 kg for BMI >28.0. Cut-off points for women were ≤17.0 kg for BMI ≤23.0, ≤17.3 kg for BMI 23.1–26.0, ≤18.0 kg for BMI 26.1–29.0, and ≤21.0 kg for BMI >29.0. The cut-off points used for exhaustion and handgrip strength were identical to Fried (1).

Physical frailty (Gobbens)

Participants were considered physically frail according to Gobbens (3) when they fulfilled the specified requirements for ≥4 of the following 8 criteria: exhaustion, unintentional weight loss, reduced handgrip strength, perceived health, walking, balance, poor hearing, and poor vision (**Table 3.1**). Exhaustion, unintentional weight loss, and reduced handgrip strength were measured as described above for physical frailty (Fried) (1). Limitations in walking due to health were assessed with a question regarding 100 meter walking. Poor perceived self-reported health were those with a self-reported health of less than good, on a scale with the following response categories: poor, fair, good, very good, excellent. The question was taken from the 36-Item Short-Form Health Survey (4, 5). The Tandem Stand Balance Test was used to test balance in participants ≥60 years old. Failing or refusing to do the test, or not being able to keep balance >10 seconds, was

considered a positive score for this criterium (being <60 years counted as a negative score). Hearing impairment (present or absent) was also assessed by self–report, with questions on hearing in general, and experienced difficulties in group conversations. Vision impairment (present or absent) was assessed in a similar manner.

Cognitive frailty

Participants were considered cognitively frail when scoring <10th percentile on global cognitive functioning (**Table 3.1**). Global cognitive functioning was assessed with a neuropsychological test battery. Memory function, information processing speed and cognitive flexibility were tested using 15 Words Verbal Learning Test (6), the Stroop Color–Word Test (7), the Word Fluency Test (8) and the Letter Digit Substitution Test (9). Nooyens *et al.* (10) describe the cognitive tests in more detail. The cognitive tests were performed among participants ≥45 years old. Participant <45 years old were defined as not being cognitively frail. Cognitive scores were adjusted for level of education and number of tests performed during follow–up.

Psychological frailty

Psychological frailty was defined as fulfilling both criteria for depression and criteria for general mental health (**Table 3.1**). Depressive symptoms were assessed with 20 questions of the Center for Epidemiologic Studies Depression scale. Those with a score of ≥16 (out of range of 0–60) were defined to have a high risk of depression. Mental health status was measured with the Mental Health Inventory 5 (5). Scores of five questions on a six–point scale were transformed into a total score (11) ranging from 0 to 100 and a cut–off point of 60 was used to indicate a poor (≤60) mental health status (12).

Social frailty

Social frailty was defined as meeting ≥2 of 3 criteria using the Loneliness scale, Social Support List–12 and a questionnaire about social participation from the Dutch Municipal Health Services Elderly Monitor (**Table 3.1**). A cut–off point of ≥ 9 on the Loneliness scale (11 items with a score 0 or 1) was used to indicate loneliness (13, 14). A score of <24 (out of 48) on the 12 item Social Support List was considered to indicate low social support (15). Finally, Participants in the lowest decile on The Dutch Municipal Health Services Elderly Monitor (11), a questionnaire regarding social participation used in the Dutch public health care for elderly people (15 items with a maximum score of 65), were considered to have little social participation.

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Supplementary Table 3.1. Characteristics table for physical frailty (Gobbens).

	Physically frail (Gobbens) (n=108)
Socio-demographic	
sex (men) (%)	40 (37.0%)
age (yrs) (SD)	68.7 (9.1)
low level of education (%)	74 (68.5%)
Body composition	
BMI[a] (kg/m^2) (SD)	30.0 (6.9)
underweight (%)	4 (3.7%)
normal weight (%)	25 (23.2%)
overweight (%)	31 (28.7%)
obese (%)	48 (44.4%)
Lifestyle	
current smoker (%)	30 (28.3%)
physical activity[b] (%)	46 (42.6%)
Health and disease	
poor self-reported health (%)	90 (83.3%)
multimorbidity[c] (%)	42 (39.3%)

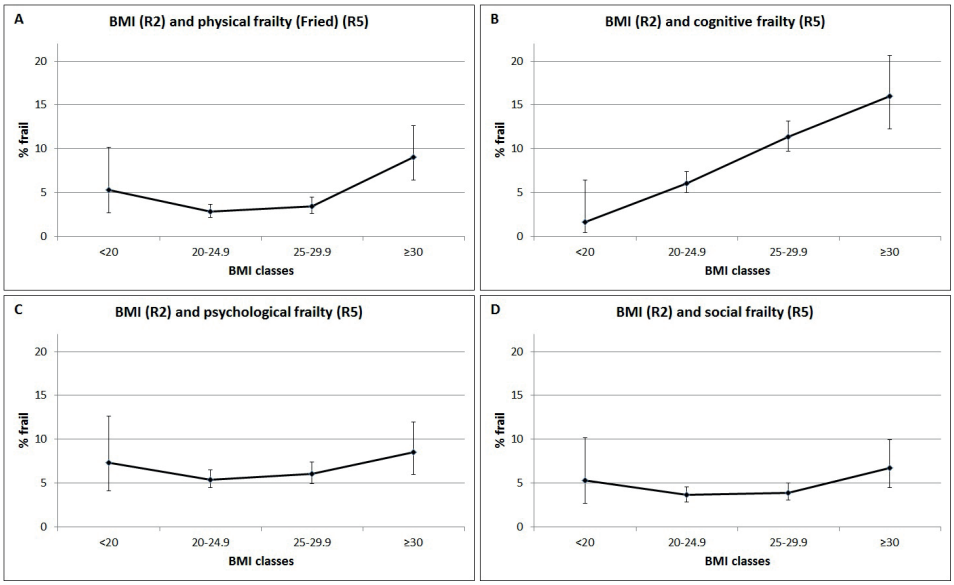
Abbreviation: BMI, body mass index.

[a] BMI: underweight (< 20 kg/m^2), normal weight (20–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obesity (≥ 30 kg/m^2).

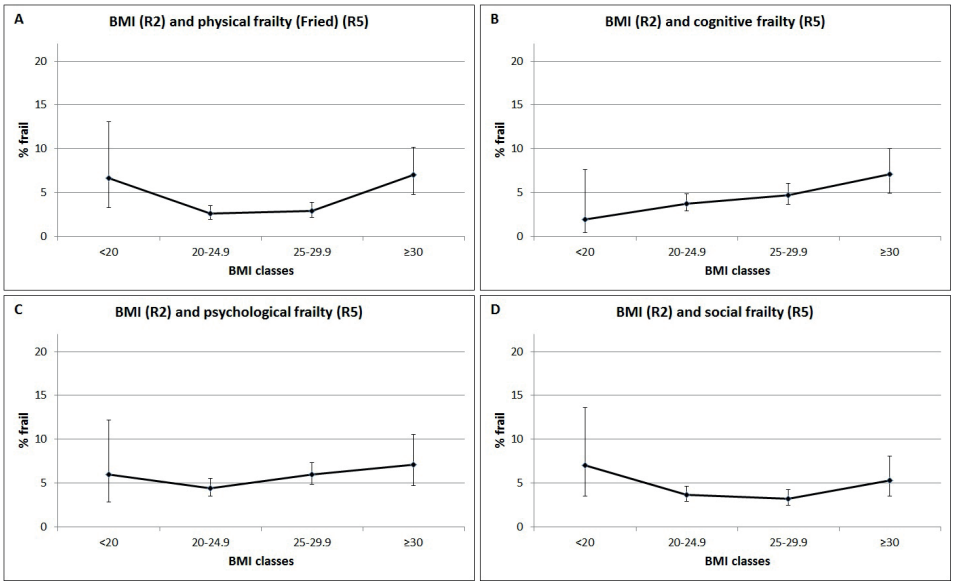
[b] physical activity: meet Dutch physical activity guideline (30 minutes of moderate to vigorous physical activity per day on at least 5 days per week).

[c] multimorbidity: having two or more out of diabetes, cancer, myocardial infarction, cerebrovascular accident, and chronic respiratory symptoms.

Legend: N (%) or mean (SD)



Supplementary Figure 3.1. The association between BMI (round 2) and frailty (round 5) (unadjusted proportions). Physical frailty (Fried) **(A)**, cognitive frailty **(B)**, psychological frailty **(C)**, and social frailty **(D)**. *Note:* In Round 2 the participants were 15 years younger than in Round 5.



Supplementary Figure 3.2. The association between BMI (round 2) and frailty (round 5) (adjusted proportions). Physical frailty (Fried) **(A)**, cognitive frailty **(B)**, psychological frailty **(C)**, and social frailty **(D)**. *Note:* In Round 2 the participants were 15 years younger than in Round 5.

4

Antioxidants linked with physical, cognitive and psychological frailty: Analysis of candidate biomarkers and markers derived from the MARK-AGE Study

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Abstract

Background

Frailty among elderly people leads to an increased risk for negative health outcomes. To prevent frailty, we need a better understanding of the underlying mechanisms and early detection of individuals at risk. Both may be served by identifying candidate (bio)markers, *i.e.* biomarkers and markers, for the physical, cognitive, and psychological frailty domains.

Methods

We used univariate (Rank–ANOVA) and multivariate (elastic net) approaches on the RASIG study population (age range: 35–74 years, n=2220) of the MARK-AGE Study to study up to 331 (bio)markers between individuals with and without frailty for each domain. Biomarkers and markers identified by both approaches were studied further regarding their association with frailty using logistic regression.

Results

Univariately, we found lower levels of antioxidants, including β-cryptoxanthin and zeaxanthin, in those who were physically, cognitively or psychologically frail. Additionally, self-reported health was worse in these three frail groups. Multivariately, we observed lower levels of β-cryptoxanthin and zeaxanthin in the cognitively frail. Levels of these carotenoids were inversely associated with the risk of being cognitively frail after adjusting for confounders.

Conclusions

Antioxidants and self-reported health are potential (bio)markers to detect persons at risk of becoming frail. The biomarkers identified may indicate the involvement of inflammation in frailty, especially for physical and cognitive frailty.

Introduction

Frailty is an age-associated syndrome and can be described as the accumulation of deficits in physical, cognitive, psychological or social functioning, leading to an increased risk for negative health outcomes (1) such as injuries including fractures through falls, disability, hospitalisation, institutionalisation, and mortality (2–5). To prevent or if possible reverse frailty, we need a better understanding of the underlying mechanisms of frailty. Identifying biomarkers could contribute to our knowledge about these mechanisms. In addition, the mechanisms behind frailty could help target preventive interventions. Furthermore, (bio)markers, *i.e.* biomarkers and markers, could be a valuable tool for early detection of those at increased risk of frailty. Evidence is accumulating that chronic low-grade inflammation may be involved in the development of frailty (6–9). Hubbard *et al.* (10) found that several inflammatory biomarkers such as C-reactive protein (CRP) and interleukin-6 (IL-6) were associated with two frailty definitions. Besides inflammation and the related oxidative stress process, several other biological processes have been linked to frailty such as haematological processes, immunosenescence, and other forms of cellular ageing (11–13).

Over the years, many frailty instruments have been developed with the focus on 'physical frailty', or the 'accumulation of deficits', or a 'multidimensional' approach to frailty with little agreement between instruments (14). With respect to biomarker research, most studies examined biomarkers for physical frailty, or for the accumulation of deficits. Only few studied associations between biomarkers and specific frailty domains, such as the association between brain-derived neurotrophic factor (BDNF) and cognitive frailty (15). Our aim is to identify (bio)markers for three specific frailty domains, *i.e.* the *physical*, *cognitive* and *psychological* domains. The rationale of this approach is based on our previous study showing limited overlap between different frailty domains suggestive of distinct sub-groups (16). Moreover, this approach may provide additional evidence on whether the underlying mechanisms per domain differ or not.

Methods

Participants and study design

The 'European study to establish biomarkers of human Ageing' (MARK–AGE) is a consortium aiming to study biomarkers of human ageing. The MARK–AGE Study, as previously described (17), is a cross–sectional study and includes multiple populations. For the present analyses we used the 'Randomly recruited Age–Stratified Individuals from the General population' (RASIG) study population within the age–range of 35–74 years (n=2220) in accordance to the MARK–AGE study design. We excluded 2 participants with a body mass index (BMI) >55 kg/m^2 and we excluded the Finnish participants (n=70) because this is a population isolate (18). There were 184 missing observations for the cognition data. As a result, the analyses for *physical* and *psychological* frailty each included 2128 participants, whilst the analysis for *cognitive* frailty comprised 1944 participants.

Measurements

Below we will briefly describe the different measurements that are relevant for the present analyses. The standard operating procedures, including collection of biological material, have been previously described (19).

Upon written informed consent the following information was obtained with a standardised questionnaire: socio–demographic factors (sex, age, level of education, marital status), lifestyle factors (smoking status, nutrition, physical activity), health status (self–reported health, falls, hospitalisation), mood (ZUNG depression scale), and mental health (Mental Health Inventory–5). Weight and height were measured to calculate the BMI. Further, waist and hip circumference, blood pressure, heart rate at rest, and handgrip strength were measured. Immediate and delayed memory function was tested using the 15–Picture Word Learning test (20). Cognitive flexibility was measured with the Stroop test (21) and cognitive speed was tested using the Digit Symbol Substitution test (22). Using the biological material (plasma, serum, peripheral blood mononuclear cells, and urine), an extensive set of biomarkers was measured such as DNA–based, immunological, and oxidative stress biomarkers. The complete list of biomarkers has been described earlier (23).

Frailty criteria

We used a multidimensional approach to study frailty. *Physical* frailty was defined according to the Frailty Phenotype (2). Participants were considered physically frail if they fulfilled at least 2 out of 4 frailty criteria described by Fried: unintentional weight loss, exhaustion, low physical activity and reduced handgrip strength (**Supplementary Methods**). Because gait speed was not available, we used 4 instead of 5 criteria for physical frailty and we adapted the cut-off point for physical frailty accordingly.

The other frailty domains were based on the Tilburg Frailty Indicator (24). Our operationalisation of these domains has been previously described (16). Participants were considered *cognitively* frail when scoring below the 10th percentile on global cognitive functioning. Global cognitive functioning was based on the scores of participants on the 15-Picture Word Learning test, the Stroop test, and Digit Symbol Substitution test. These scores were transformed into z-scores and combined into one global cognitive functioning score that was adjusted for level of education.

Psychological frailty was defined as fulfilling 2 out of 2 criteria: depressive symptoms and poor mental health. Depressive symptoms were assessed with 20 questions of the Zung self-rating depression scale (ZUNG) (25). Those with a score of >50 (out of a range of 0-100) were considered to have depressive symptoms. Mental health status was measured with the Mental Health Inventory-5 (26). Scores of five questions on a six-point scale were transformed into a total score ranging from 0 to 100. A score of 60 or below indicated reduced mental health (27).

Statistical analyses

The MARK-AGE dataset as of November 2015 was used for the statistical analyses. Prior to the analyses we made a pre-selection by removing (bio)markers that were measured only in a fraction of the cohort, or that were unlikely to be related to one of the frailty domains (mainly questionnaire data), or for which batch corrected data was available resulting in a list with 371 (bio)markers (**Supplementary Table 4.1**; including details on missing observations per frailty domain).

Descriptive analyses were carried out for the non-frail, *physically* frail, *cognitively* frail, and *psychologically* frail groups separately.

Following Saccenti *et al.* (28), we performed two complementary analyses, univariate and multivariate analyses, to identify candidate (bio)markers for three frailty domains, both having their advantages and disadvantages (**Supplementary Methods**). With the univariate analyses, we tested the null hypothesis that the frail group had the same (bio)marker level as the non–frail group. This was tested for each frailty domain and each (bio)marker using Rank–ANOVA, a non–parametric version of ANOVA, while adjusting for sex and age. Since Rank–ANOVA does not accept all character variables, 331 of the 371 (bio)markers were studied with this approach. To control for false discoveries, we used the Benjamini–Hochberg correction for multiple testing (29). Because of biological differences between men and women, analyses were repeated in a sensitivity analysis where we stratified for sex. The analyses were carried out in SAS 9.3 and SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

Our multivariate approach was based on machine learning techniques. We used a variable selection procedure based on Stability Selection (30) in combination with elastic net (31) to identify candidate (bio)markers. Since this procedure can only be applied on observations without missing values (most (bio)markers were not measured for all individuals), we trimmed the candidate (bio)markers–list in such a way as to maximize the sum of the fraction of (bio)markers (*i.e.* covariates) and the fraction of frail individuals. This resulted in 55 frail cases and 222 (bio) markers for *physical* frailty, 141 frail cases and 275 (bio)markers for *cognitive* frailty, and 117 frail cases and 219 (bio)markers for *psychological* frailty. Next, skewed (bio)markers were transformed using the Yeo–Johnson power transformation (32). Since our dataset was imbalanced (*i.e.* low number of frail cases and high number of non–frail controls) and we needed to adjust for sex and age, we used a form of undersampling (33, 34) prior to the Stability Selection procedure. In our approach, we sampled controls in such a way that within each age and sex stratum (age in five–year categories) the number of cases and controls were identical. As undersampling leads to extra sampling variability, we repeated our approach 10,000 times. In each iteration, undersampling was used to create a balanced dataset, the variable selection procedure was applied, and a list of variable selection probabilities was obtained. A final ranking of variable selection probabilities was obtained by averaging the selection probabilities over all iterations. Biomarkers or markers with an average selection probability of >0.5 were used for further analyses, in which the risk of being frail was calculated by subtracting the average value of the 10^{th} percentile for a certain (bio)marker from

the 90th percentile from that (bio)marker, *i.e.* the delta. Given the low number of cases, a sensitivity analyses with stratification for sex was not possible for the multivariate analyses. The multivariate approach was carried out in R 3.3.2.

Biomarkers or markers identified by both approaches were studied as covariates in a binomial logistic regression model to obtain effect sizes of each (bio)marker. There we adjusted for confounders based on literature (35) being sex, age, level of education, BMI, smoking status and season of blood collection. For cognitive frailty, we adjusted for depressive symptoms in addition. Technical details on the statistical analysis can be found in the **Supplementary Methods**.

Results

Population characteristics

Characteristics of the groups with and without frailty stratified by the different frailty domains are presented in the baseline table (**Table 4.1**). The *psychologically* frail group was younger than the *physically* and *cognitively* frail groups. *Psychological* frailty was more common among women, while *cognitive* frailty was more common among men. The *cognitively* frail group had a higher systolic and diastolic blood pressure than the *physically* and *psychologically* frail groups. All three frail groups had a lower educational level and higher BMI than the non–frail group. In addition, the *physically*, *cognitively*, and *psychologically* frail groups showed limited overlap (**Figure 4.1**).

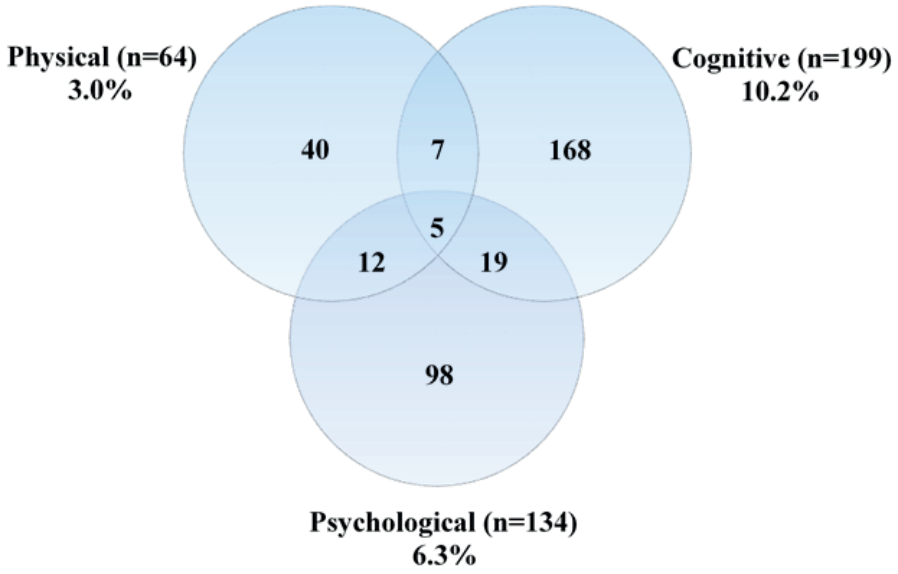

Figure 4.1. Venn diagram showing the prevalence and overlapping individuals between the different frailty domains (*i.e.* the physically, cognitively and psychologically frail groups).

Table 4.1. Characteristics table of the non-frail and the frail groups.

	Non-frail[a] N=1628	Physically frail N=64	Cognitively frail N=199	Psychologically frail N=134
Socio-demographic factors				
Women, % (n)	51.4 (837)	50.0 (32)	37.7 (75)	70.9 (95)
Age (years), mean (SD),	53.4 (11.0)	62.8 (10.6)	64.3 (8.5)	57.8 (10.5)
Low level of education, % (n)	8.7 (141)	20.6 (13)	11.6 (23)	24.6 (33)
Marital status widowhood, % (n)	5.0 (82)	10.9 (7)	11.1 (22)	11.2 (15)
Anthropometric data				
BMI (kg/m^2), mean (SD)	25.7 (4.2)	28.4 (5.3)	27.5 (4.3)	26.8 (4.7)
Waist circumference, mean (SD)	90.7 (13.8)	94.8 (12.4)	96.0 (11.3)	91.9 (13.6)
Systolic blood pressure, mean (SD)	132.7 (19.0)	138.5 (21.8)	142.2 (20.3)	130.7 (22.2)
Diastolic blood pressure, mean (SD)	80.7 (10.7)	82.4 (11.3)	84.5 (12.4)	78.8 (11.0)
Lifestyle factors				
Low physical activity[b], % (n)	10.3 (54)	58.0 (29)	19.0 (29)	20.0 (12)
Current smoker, % (n)	17.6 (287)	25.0 (16)	16.6 (33)	26.1 (35)
Low fruit consumption[c], % (n)	21.5 (350)	20.3 (13)	17.6 (35)	32.8 (44)
Low vegetable consumption[c], % (n)	16.9 (275)	21.9 (14)	13.1 (26)	21.6 (29)
Low vitamin supplement use[c], % (n)	85.6 (1394)	81.3 (52)	81.4 (162)	85.1 (114)
Health and disease				
Poor/fair self-reported health, % (n)	5.7 (93)	51.6 (33)	27.1 (54)	44.8 (60)
Falls[d], % (n)	8.4 (137)	12.5 (8)	10.6 (21)	19.4 (26)
Hospitalisation[e], % (n)	11.0 (179)	31.3 (20)	13.1 (26)	16.4 (22)
Season of blood collection				
Winter, % (n)	19.4 (314)	15.6 (10)	24.1 (48)	18.7 (25)
Spring, % (n)	34.6 (561)	37.5 (24)	35.2 (70)	40.3 (54)
Summer, % (n)	24.0 (389)	21.9 (14)	14.1 (28)	20.9 (28)
Fall, % (n)	22.0 (357)	25.0 (16)	26.6 (53)	20.2 (27)

(Table 4.1 continues)

	Non-frail[a] N=1628	Physically frail N=64	Cognitively frail N=199	Psychologically frail N=134
Biomarkers[f]				
α-Carotene (µmol/l), median (IQR)	0.15 (0.09–0.25)	0.10 (0.05–0.16)	0.12 (0.07–0.21)	0.12 (0.07–0.19)
β-Carotene (µmol/l), median (IQR)	0.58 (0.37–0.88)	0.39 (0.26–0.55)	0.48 (0.30–0.77)	0.52 (0.30–0.84)
β-Cryptoxanthin (µmol/l), median (IQR)	0.22 (0.12–0.38)	0.15 (0.07–0.29)	0.15 (0.07–0.37)	0.19 (0.10–0.38)
Zeaxanthin (µmol/l), median (IQR)	0.044 (0.028–0.065)	0.040 (0.023–0.063)	0.036 (0.022–0.054)	0.039 (0.025–0.058)
α-Tocopherol (µmol/l), median (IQR)	27.8 (23.6–32.6)	27.6 (23.0–33.7)	26.6 (22.5–32.1)	27.6 (23.3–32.4)
Selenium (ppb), median (IQR)	110 (97–124)	99 (88–113)	107 (92–121)	108 (97–119)
SeAlbSeP (ppb)[g], median (IQR)	88 (76–99)	78 (68–89)	85 (71–98)	86 (76–95)
CunotCp (ppb)[h], median (IQR)	169 (146–195)	167 (151–192)	161 (143–188)	170 (137–201)
Monocytes (10^6/l), median (IQR)	442 (360–554)	526 (450–640)	499 (420–615)	470 (382–592)
CMV (U/l), median (IQR)	19.1 (1.8–60.2)	50.6 (4.7–100.2)	36.5 (9.9–96.7)	36.3 (3.4–82.4)
Guanidinoacetate 3.97s (area (a.u.))[i], median (IQR)	8.4 (5.7–12.2)	7.1 (4.7–11.3)	7.6 (5.3–11.5)	8.4 (5.7–12.4)
Cholesterol (mmol/l), median (IQR)	5.5 (4.9–6.2)	5.3 (4.6–6.0)	5.3 (4.7–6.0)	5.5 (4.8–6.0)

Abbreviation: BMI, body mass index; IQR, interquartile range; CMV, cytomegalovirus antibodies.

[a] Participants in the non-frail group were not frail for any frailty domain.

[b] Physical activity was not measured in participants <60 yrs. We defined participants <60 years as being physically active. Participants were considered low physically active if they did not participate in housework every day.

[c] Low fruit consumption, low vegetable consumption, and low vitamin supplement use were defined by combining the consumption frequencies of '1–3 times a week', '1–3 times a month', and 'never'.

[d] The number of falls was defined as one or more falls in the last 12 months.

[e] Hospitalisation was defined as being hospitalised (with an overnight stay) within the last 12 months.

[f] Selected biomarkers based on findings of our subsequent analyses; the median and IQR of untransformed biomarkers.

[g] SeAlbSeP (ppb): Plasma Selenium eluting with retention time of Albumin or Selenoprotein P (absolute value in ppb).

[h] CunotCp (ppb): Plasma Copper not bound to Ceruloplasmin (absolute value in ppb).

[i] Relative Guanidinoacetate 3.97s concentration

Prevalence

A total of 64 (3.0%) participants were *physically* frail, 199 (10.2%) were (by definition) *cognitively* frail, and 134 (6.3%) were *psychologically* frail (**Figure 4.1**). When studying the prevalence in five-year age groups per frailty domain, the prevalence of *physical* and *cognitive* frailty rapidly increased from age 60 onwards, while the prevalence of *psychological* frailty did not show a pronounced age-related increase (**Figure 4.2a**). Stratified by sex, **Figure 4.2b** confirms the sex-specific prevalence for *psychological* and *cognitive* frailty across all age groups. Furthermore, it shows similar slopes for men and women within frailty domains.

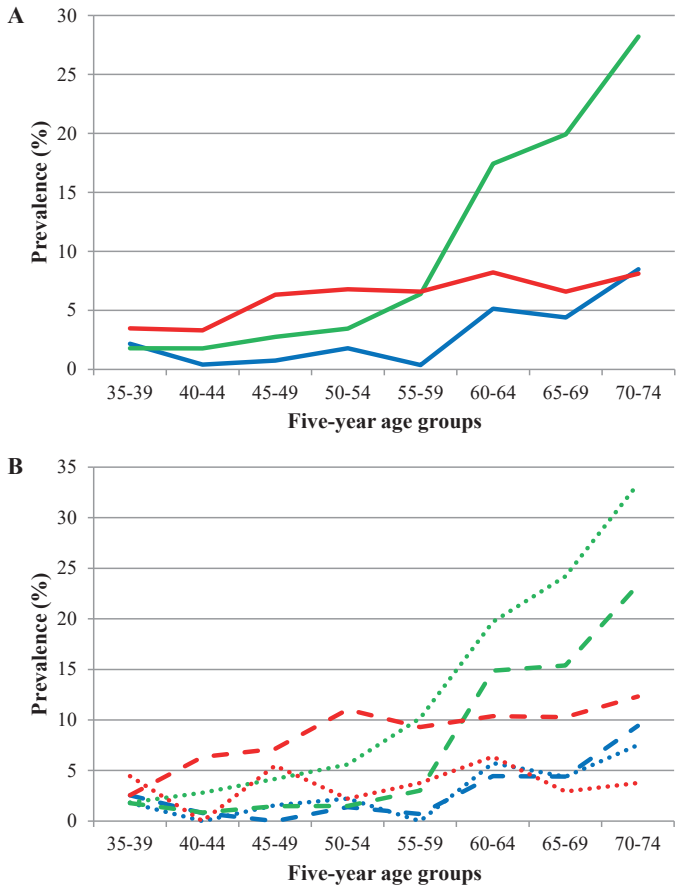

Figure 4.2. Prevalence in five-year age groups per frailty domain for the total population (A) and stratified by sex (B). Blue line: physical frailty; green line: cognitive frailty; red line: psychological frailty; straight line: total population; striped line: women; dotted line: men.

Candidate (bio)markers for different frailty domains: results from univariate analyses

All three frail groups reported to have worse health compared to the non–frail group (**Table 4.2**). In *physically* frail people, significantly lower levels of α–carotene, selenium (total and sub–fraction), β–cryptoxanthin, and β–carotene were observed compared to people without physical frailty, while higher monocyte levels were observed. In *cognitively* frail people, levels of β–cryptoxanthin, zeaxanthin, cholesterol, α–tocopherol, and guanidinoacetate were significantly lower compared to people without cognitive frailty, while cytomegalovirus (CMV) antibody levels were significantly higher. In *psychologically* frail people, levels of α–carotene were significantly lower compared to people without psychological frailty, and also level of education was statistically significantly lower (**Table 4.2**). Age stratified boxplots of the biomarkers listed in **Table 4.2**, visualise the spread in expression of each biomarker and clarify the level of overlap in expression when comparing the frail and non–frail groups (**Supplementary Figures 4.2 A–S**).

Table 4.2. Candidate (bio)markers for physical, cognitive, and psychological frailty: Results from univariate analyses.

Domain	Biomarker or marker	Adjusted p–value	Median non–frail group[a]	Median frail group[a]	Difference between medians[b]	Relative difference (%)[c]
Physical	Self–reported health*[d]	<.0001				
	Excellent/ Very good/ Good		90%	48%		
	Poor/ Fair		10%	52%		
	α–Carotene (µmol/l)	0.0078	0.142	0.096	–0.046	–32.4
	Selenium (ppb)	0.0098	109.333	99.495	–9.838	–9.0
	β–Cryptoxanthin (µmol/l)	0.0130	0.211	0.152	–0.059	–28.0
	SeAlbSeP (ppb)[e]	0.0206	86.978	78.183	–8.795	–10.1
	Monocytes (10^6/l)	0.0229	452.600	526.300	73.700	16.3
	β–Carotene (µmol/l)	0.0242	0.565	0.394	–0.171	–30.3
Cognitive	Self–reported health*[d]	<.0001				
	Excellent/ Very good/ Good		92%	73%		
	Poor/ Fair		8%	27%		
	β–Cryptoxanthin (µmol/l)	<.0001	0.215	0.148	–0.067	–31.2
	Zeaxanthin (µmol/l)	0.0002	0.044	0.036	–0.008	–18.2
	CMV (U/l)	0.0017	19.300	36.540	17.240	89.3
	Cholesterol (mmol/l)	0.0086	5.510	5.340	–0.170	–3.1
	α–Tocopherol (µmol/l)	0.0148	27.790	26.570	–1.220	–4.4
	Guanidinoacetate 3.97s (area (a.u.))[f]	0.0480	8.466	7.616	–0.85	–10.0

(Table 4.2 continues)

Domain	Biomarker or marker	Adjusted p-value	Median non-frail group[a]	Median frail group[a]	Difference between medians[b]	Relative difference (%)[c]
Psychological	Self-reported health*[d]	<.0001				
	Excellent/ Very good/ Good		91%	55%		
	Poor/ Fair		9%	45%		
	Level of education*[g]	0.0002				
	Low		10%	25%		
	Middle-low		33%	39%		
	Middle-high		39%	28%		
	High		18%	8%		
	α-Carotene (µmol/l)	0.0224	0.142	0.119	-0.023	-16.2

Rank-ANOVA with adjustment for age and sex and correction for multiple testing.

Abbreviation: CMV, cytomegalovirus antibodies.

*Note: Self-reported health and level of education are binary and categorical variables, respectively. Therefore, the percentages per category of these two variables are presented and not the median as presented for the (continuous) biomarker variables.

[a] Medians are of untransformed biomarker levels and are not adjusted for age and sex. Participants in the non-frail group of a certain domain could be frail on another domain.

[b] Difference of median biomarker levels between individuals with and without frailty.

[c] Relative difference of median biomarker levels between individuals with and without frailty expressed in percentages.

[d] Self-reported health was defined as: 1= 'fair','poor'; 0='excellent','very good','good'.

[e] SeAlbSeP (ppb): Plasma Selenium eluting with retention time of Albumin or Selenoprotein P (absolute value in ppb).

[f] Relative Guanidinoacetate 3.97s concentration.

[g] Level of education was divided into four categories:

Low = 'Never went to school' or 'Did not finish elementary school (*i.e.* up to age 10)' or 'Finished elementary school'

Middle-low = 'First stage of secondary level education' or 'Second stage of secondary level education'

Middle-high = 'Recognised third level education: a third level education other than university degree' or 'Recognised third level education:

an initial university degree or recognised equivalent'

High = 'Recognised third level education: a higher university degree or post graduate'.

Sensitivity analyses: results from univariate analyses with stratification for sex
In *physically* frail women, levels of α–carotene and β–carotene were significantly lower compared to women without physical frailty. In p*sychologically* frail women, level of education was significantly lower compared to women without psychological frailty (**Table 4.3a**).

In *cognitively* frail men, levels of β–cryptoxanthin and guanidinoacetate were significantly lower, while levels of CMV antibodies were significantly higher compared to men without cognitive frailty. In *psychologically* frail men, levels of copper not bound to ceruloplasmin were significantly lower compared to men without psychological frailty (**Table 4.3b**).

Both men and women in all three frail groups reported to have worse health compared to the non–frail groups (**Tables 4.3a** and **4.3b**). Age and sex stratified boxplots of the biomarkers listed in **Tables 3a** and **3b**, visualise the spread in expression of each biomarker and clarify the level of overlap in expression when comparing the frail and non–frail groups (**Supplementary Figures 4.2 A–S**).

Table 4.3a. Candidate (bio)markers for physical, cognitive, and psychological frailty in women: Results from univariate analyses.

Domain	**Women**					
	Biomarker or marker	Adjusted p–value	Median non–frail group[a]	Median frail group[a]	Difference between medians[b]	Relative difference (%)[c]
Physical	Self–reported health*[d]	<.0001				
	Excellent/ Very good/ Good		89%	47%		
	Poor/ Fair		11%	53%		
	α–Carotene (µmol/l)	0.0216	0.172	0.103	–0.069	–40.1
	β–Carotene (µmol/l)	0.0315	0.656	0.413	–0.243	–37.0
Cognitive	Self–reported health*[d]	<.0001				
	Excellent/ Very good/ Good		91%	68%		
	Poor/ Fair		9%	32%		
Psychological	Self–reported health*[d]	<.0001				
	Excellent/ Very good/ Good		91%	59%		
	Poor/ Fair		9%	41%		
	Level of education*[e]	<.0001				
	Low		10%	28%		
	Middle–low		32%	40%		
	Middle–high		41%	27%		
	High		17%	4%		

Table 4.3b. Candidate (bio)markers for physical, cognitive, and psychological frailty in men: Results from univariate analyses.

Domain	**Men**					
	Biomarker or marker	Adjusted p-value	Median non-frail group[a]	Median frail group[a]	Difference between medians[b]	Relative difference (%)[c]
Physical	Self-reported health*[d]	<.0001				
	Excellent/ Very good/ Good		91%	50%		
	Poor/ Fair		9%	50%		
Cognitive	Self-reported health*[d]	<.0001				
	Excellent/ Very good/ Good		93%	76%		
	Poor/ Fair		7%	24%		
	β-Cryptoxanthin (µmol/l)	0.0002	0.176	0.122	-0.054	-30.7
	CMV (U/l)	0.0004	14.645	37.180	22.535	153.9
	Guanidinoacetate 3.97s (area (a.u.))[f]	0.0382	8.180	7.136	-1.044	-12.8
Psychological	Self-reported health*[d]	<.0001				
	Excellent/ Very good/ Good		92%	46%		
	Poor/ Fair		8%	54%		
	CunotCp (ppb)[g]	0.0214	165.848	144.792	-21.060	-12.7

Rank-ANOVA with adjustment for age, stratification for sex, and correction for multiple testing.
Abbreviation: CMV, cytomegalovirus antibodies.
*Note: Self-reported health and level of education are binary and categorical variables, respectively. Therefore, the percentages per category of these two variables are presented and not the median as presented for the (continuous) biomarker variables.
[a] Medians are of untransformed biomarker levels and are not adjusted for age. Participants in the non-frail group of a certain domain could be frail on another domain.
[b] Difference of median biomarker levels between individuals with and without frailty.
[c] Relative difference of median biomarker levels between individuals with and without frailty expressed in percentages.
[d] Self-reported health was defined as: 1= 'fair','poor'; 0='excellent','very good','good'.
[e] Level of education was divided into four categories:
Low = 'Never went to school' or 'Did not finish elementary school (*i.e.* up to age 10)' or 'Finished elementary school'.
Middle-low = 'First stage of secondary level education' or 'Second stage of secondary level education'.
Middle-high = 'Recognised third level education: a third level education other than university degree' or ' Recognised third level education:
an initial university degree or recognised equivalent'.
High = 'Recognised third level education: a higher university degree or post graduate'.
[f] Relative Guanidinoacetate 3.97s concentration.
[g] CunotCp (ppb): Plasma Copper not bound to Ceruloplasmin (absolute value in ppb).

Candidate (bio)markers for different frailty domains: results from multivariate analyses

The multivariate approach did not lead to a selection of (bio)markers that were predictive of *physical* and *psychological* frailty, based on an average selection probability >0.5 (**Table 4.4**). However, for *cognitive* frailty an average selection probability >0.5 was obtained for β–cryptoxanthin and zeaxanthin (**Table 4.4**). People with high levels (90th percentile) of β–cryptoxanthin had a 9.3% lower risk of being cognitively frail compared to people with low levels (10th percentile) of β–cryptoxanthin. People with high levels of zeaxanthin had 6.3% lower risk of being cognitively frail compared to people with low levels of zeaxanthin (**Table 4.5**).

Table 4.4. Candidate (bio)markers for physical, cognitive, and psychological frailty: Results from multivariate analyses.

Physical		**Cognitive**		**Psychological**	
Biomarker or marker	Selection probability	Biomarker or marker	Selection probability	Biomarker or marker	Selection probability
α-Carotene (µmol/l)	0.47	β-Cryptoxanthin (µmol/l)	**0.75**	Haemoglobin (g/l)	0.45
Ascorbic acid (mg/l)	0.41	Zeaxanthin (µmol/l)	**0.53**	Haematocrit (%)	0.30
Monocytes (10^6/l)	0.37	CMV (U/l)	0.41	ApoJ/Clu (µg/ml)	0.28
β-Cryptoxanthin (µmol/l)	0.32	LDL1 Cholesterol (mg/dl)	0.36	Tetanus IgG antibodies (IU/ml)	0.25
β-Carotene (µmol/l)	0.31	PSA (ng/ml)	0.36	25-Hydroxy-Vitamin-D (nmol/l)	0.25
Lutein (µmol/l)	0.29	HDL cholesterol (mmol/l)	0.33	Urinary 8-isoprostane (mM/l/mM creatinine)	0.24
Insulin (µIU/ml)	0.21	β-Carotene (µmol/l)	0.30	Systolic blood pressure	0.24
Research center no2[a]	0.18	Lutein (µmol/l)	0.30	Haemoglobin (g/l)[b]	0.23
γ-Tocopherol (µmol/l)	0.18	Neutrophils (10^9/l)	0.28	Hb-MCHC[c]	0.18
BMI (kg/m^2)	0.17	Monocytes (10^6/l)	0.24	FHL2 CpG6[d]	0.17

In this table, the ten biomarkers with the highest selection probability for each frailty domain are presented. Biomarkers with an average selection probability of >0.5 (in bold) were used for further analyses.

Abbreviations: BMI, body mass index; CMV, cytomegalovirus antibodies; LDL1 Cholesterol, serum concentration of cholesterol in large low-density lipoprotein particles; PSA, Prostate specific antigen; HDL cholesterol, High-density lipoproteins cholesterol; ApoJ/Clu, Apolipoprotein J/Clusterin serum levels.

[a] Research center number 2 is BioTeSys (Germany).

[b] Hemoglobin of frozen blood.

[c] Hb-MCHC: Mean corpuscular hemoglobin concentration.

[d] FHL2 CpG6: DNA methylation level of a specific CpG in FHL2 gene.

Table 4.5. Delta for candidate biomarkers with a selection probability >0.5 from the multivariate analyses.

Domain	Biomarker	Delta (90th vs 10th percentile)
Physical	–	–
Cognitive	β-Cryptoxanthin	-9.3%
	Zeaxanthin	-6.3%
Psychological	–	–

Note: The delta, the risk of being frail, was calculated by subtracting the average value of the 10th percentile for a certain biomarker from the 90th percentile from that biomarker.

Associations between candidate biomarkers and cognitive frailty adjusted for confounders

Based on both the univariate and multivariate analyses, we identified β-cryptoxanthin and zeaxanthin as candidate biomarkers for *cognitive* frailty. With binomial logistic regression, we studied the association between these log-transformed carotenoids and cognitive frailty. In model 1, we adjusted for sex and age. In model 2, we adjusted for sex, age, level of education, BMI, smoking status, depressive symptoms and season of blood collection. In model 1, log β-cryptoxanthin (odds ratio 0.642; 95% CI, 0.538-0.765) p<0.0001 and log zeaxanthin (odds ratio 0.626; 95% CI, 0.509-0.771) p<0.0001 were associated with a lower risk of being cognitively frail. Also in model 2, log β-cryptoxanthin (odds ratio 0.742; 95% CI, 0.604-0.911) p=0.0043 and log zeaxanthin (odds ratio 0.752; 95% CI, 0.588-0.960) p=0.0225 were associated with a lower risk of being cognitively frail (**Table 4.6**).

Table 4.6. Logistic regression analyses on biomarkers resulted from both the univariate and multivariate analyses.

Domain	Biomarker	Model 1 OR (95% CI)[a]	Model 2 OR (95% CI)[b]
Cognitive	Log β-Cryptoxanthin	0.642 (0.538-0.765)	0.742 (0.604-0.911)
	Log zeaxanthin	0.626 (0.509-0.771)	0.752 (0.588-0.960)

Abbreviations: OR, odds ratio; 95% CI, 95% Confidence Interval.

Note: Odds ratios and 95% confidence intervals are presented in this table for biomarkers with an average selection probability of >0.5.

[a] Model 1 is a model adjusted for sex and age.

[b] Model 2 is a model adjusted for sex, age, level of education, BMI, smoking status, depressive symptoms and season of blood collection.

Discussion

We showed that women are more frequently *psychologically* frail, while men are more frequently *cognitively* frail. The prevalence of both *physical* and *cognitive* frailty increased with age, whereas the prevalence of *psychological* frailty hardly did (**Figure 4.2**). Hence, the *physical* and *cognitive* frailty domains seem to be age-related, while *psychological* frailty does not seem to be age-related. These findings are in line with our previous study in a different cohort, *i.e.* the Doetinchem Cohort Study (16). In addition, both independent cohorts, the MARK-AGE Study (**Figure 4.1**) and the Doetinchem Cohort Study (16), showed limited overlap between the frailty domains. Therefore, we identified candidate biomarkers for each frailty domain independently in the MARK-AGE Study. Two complementary approaches, *i.e.* univariate and multivariate analyses, were applied from which three types of antioxidants (*i.e.* carotenoids, metals, tocopherol) emerged for one or more frailty domains.

Carotenoids were identified for all three domains. Univariately, we found lower levels of carotenoids in *physically* frail people compared to people without physical frailty when correcting for sex. When stratifying for sex we found lower levels of carotenoids in physically frail women. Other cross-sectional studies also found lower carotenoid levels in physically frail men and women (36) and in physically frail women (35) compared to people without physical frailty. Both univariately and multivariately, we found lower levels of two carotenoids, *i.e.* β-cryptoxanthin and zeaxanthin, in *cognitively* frail people. This is in line with Dominguez and Barbagallo (37), they suggested the involvement of antioxidants in cognitive frailty. Moreover, studies do report associations between carotenoids, such as zeaxanthin, and cognitive functioning (38, 39). When stratifying for sex in the univariate analyses, we found lower levels of β-cryptoxanthin for cognitively frail men, but not for women. When studying the association between these carotenoids and cognitive frailty adjusted for important confounders, we showed that having higher levels of carotenoids was significantly associated with a lower risk of being cognitively frail. Univariately, we found lower levels of α-carotene in *psychologically* frail people. Black *et al.* (40) found that depressive symptoms were cross-sectionally associated with decreased carotenoid levels including α-carotene. The percentage of people with and without frailty in our study population with low fruit, vegetable, or vitamin supplement consumption does not seem to differ considerably (**Table 4.1**). However, previously we showed

that several of these carotenoids were associated with age of which a subset remained significant after adjusting for important confounders including dietary habits (41). Hence, the difference in carotenoid levels between frail and non–frail people might be caused by differences in nutrient intake but also by differences in nutrient absorption in the digestive system or in micronutrient metabolism with variation between carotenoids.

Similar to carotenoids, metals may also serve as pro–oxidants (42, 43). We found lower levels of selenium in *physically* frail people, which has been reported before (36). We also found lower levels of copper (not eluting in the ceruloplasmin region) in *psychologically* frail men. It has been suggested that a mineral deficiency might trigger low–grade chronic inflammation (44).

We identified one type of tocopherol, *i.e.* α–tocopherol (a form of vitamin E), as a biomarker for *cognitive* frailty. This is in line with the suggested involvement of antioxidants in cognitive frailty (37).

Antioxidants are, to a greater or lesser extent, associated with all frailty domains. Antioxidants could minimize the damaging effect of pro–inflammatory compounds such as inflammatory cytokines, like IL–6 and TNF–α. The production of cytokines is upregulated via the activation of redox–sensitive transcription factors like nuclear factor kappa B (NF–ƙB) in response to a high level of reactive oxygen species (ROS) which causes oxidative stress (45). Indeed, it has previously been hypothesised that both inflammation and oxidative stress are associated with frailty (6, 46). Some of the biomarkers we identified are related to inflammatory processes such as monocytes, which we identified as a biomarker for physical frailty, and CMV, which we identified as a biomarker for cognitive frailty.

Further, we identified self–reported health as a marker for all frailty domains in the univariate analyses when adjusting for sex, and when stratifying for sex. Self–reported health, perhaps in combination with certain biomarkers, could help detect those at increased risk of frailty. Longitudinal studies are instrumental to study this further.

In the univariate analyses adjusting for sex, we found multiple biomarkers for physical and cognitive frailty, while for psychological frailty we found only one biomarker, *i.e.* α–carotene. Possibly, a larger number of biological processes

may be involved in physical and cognitive frailty while different factors play a role in psychological frailty. Given the overlap in types of biomarkers identified for physical and cognitive frailty, the underlying mechanisms for these domains may be related.

Frailty is recognised as a multidimensional syndrome and therefore we believe it is important to take the cognitive frailty domain into account. We deviated from the current definition for cognitive frailty (47), because this definition also includes physical components. Since it is possible to be cognitively frail without being physically frail (**Figure 4.1**), we adjusted our operationalisation accordingly.

We used two approaches, univariate and multivariate analyses, to identify (bio) markers for different frailty domains both providing relevant information (28). The results from the univariate analyses showed significant differences in the levels of certain (bio)markers between the frail and the non–frail groups. The advantage of this analysis is that the results are easy to interpret. The results from the multivariate analyses showed which biomarkers are associated with a certain frailty domain taking inter–variable relations into account. In our approach, we had to deal with many missing values limiting the number of (bio)markers used for the multivariate versus the univariate analyses.

The RASIG population of the MARK–AGE Study spans the 'middle age range' in industrialized countries and is a relatively young population for studying (bio)markers of frailty. The low prevalence of frailty in this population made it more challenging to identify candidate (bio)markers in a multivariate approach. Furthermore, the participants for the RASIG population were recruited via newspapers, which may have introduced a selection bias. This may be reflected by the relatively high educational level of the population. Also, the biological material was obtained from participants in several European countries. This may have influenced the sample collection, storage, and measurements of the biological material. However, due to the standardised, elaborate protocol these potential differences are assumed to be kept to a minimum. In addition, the category 'recruitment center' was included as a variable in both the univariate and multivariate analyses and did not seem to have an effect.

The potential (bio)markers for frailty we identified need to be replicated in other studies and causality could be examined in a longitudinal cohort. In particular,

the latter will indicate whether antioxidants and self-reported health could serve as (bio)markers to detect people at risk of becoming frail. For preventive purposes, causality needs to be established with respect to antioxidant levels and frailty. Underlying questions are: Is the difference caused by nutritional intake, gastrointestinal absorption, or micronutrient metabolism and does genetics play a role in (one) of these processes? Collectively, the identified biomarkers may indicate the involvement of inflammation in frailty, especially for physical and cognitive frailty.

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Supplementary Methods

Frailty criteria

Physical frailty

Participants were considered physically frail if they fulfilled ≥2 of 4 frailty criteria described by Fried (1): unintentional weight loss, exhaustion, low physical activity and reduced handgrip strength. Unintentional weight loss was defined as weight loss >5% in 12 months time and the participant answering 'most of the time' or 'a good part of the time' to the question: 'I eat as much as I used to'. The presence of exhaustion was assessed using the following statement: 'Did you feel worn out?' (referring to the past two weeks). Participants answering 'all of the time', 'most of the time', or 'a good bit of the time' were considered to be exhausted. Low physical activity was defined using the question: 'Do you do any kind of light housework or exercise (e.g. vacuum–cleaning, sweeping, mopping floors, ironing, gardening, gymnastics or short walks)?'. If the answer was 'yes' participants answered the following question: 'How often?'. If the answer was 'several times a week' or less, the participant was considered insufficiently physical active. Handgrip strength was measured using a dynamometer (SMEDLYS' dynamometer, Scandidact, Kvistgaard, Denmark). Cut–off points for handgrip strength in kilogram (kg) were stratified by sex and body mass index (BMI), as described by Fried (1). Cut–off points for men were ≤29.0 kg for BMI ≤24.0, ≤30.0 kg for BMI 24.1–26.0, ≤30 kg for BMI 26.1–28.0, and ≤32.0 kg for BMI >28.0. Cut–off points for women were ≤17.0 kg for BMI ≤23.0, ≤17.3 kg for BMI 23.1–26.0, ≤18.0 kg for BMI 26.1–29.0, and ≤21.0 kg for BMI >29.0. Because gait speed was not available, we used 4 instead of 5 criteria for physical frailty and therefore we adapted the cut–off point for physical frailty accordingly.

Supplementary references

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Statistical analyses

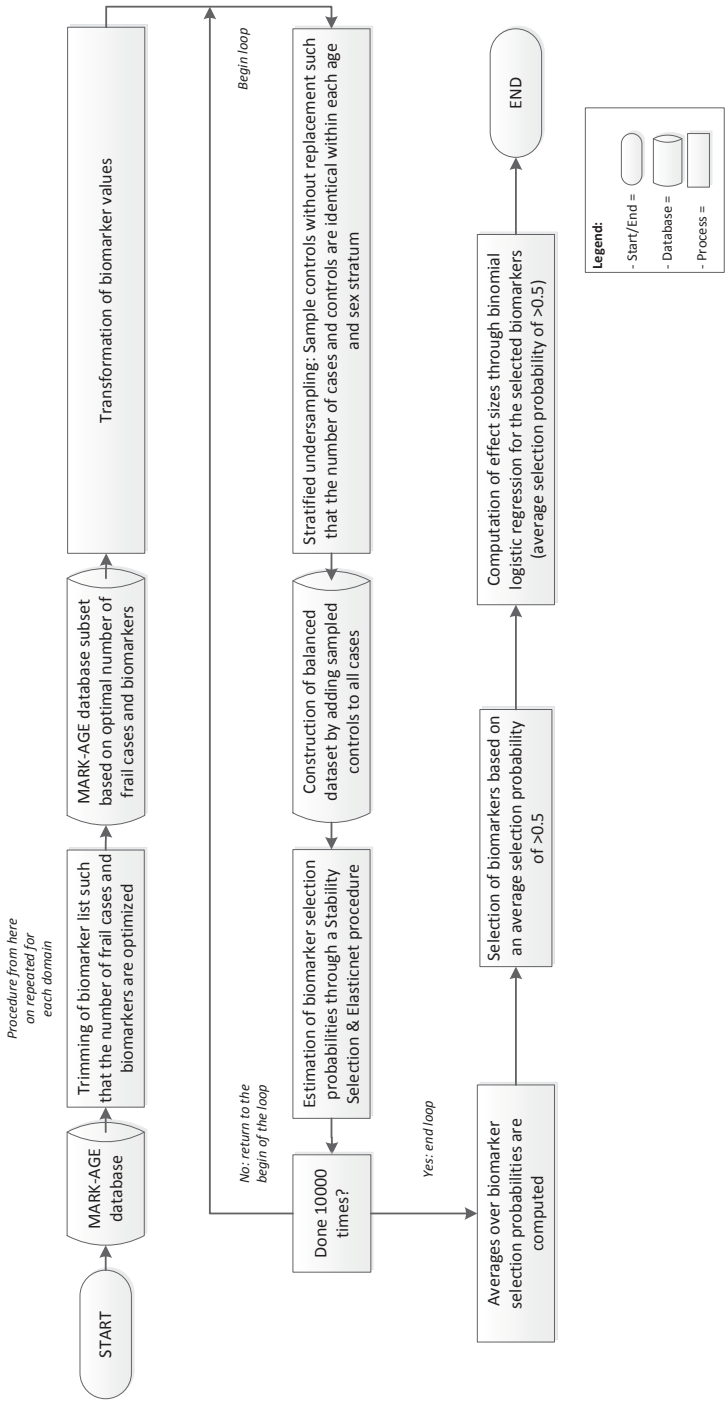
Following Saccenti *et al.* (1), we performed univariate and multivariate analyses to identify candidate (bio)markers for three frailty domains. While multivariate approaches seem to be preferred because they would result in more 'pure' estimates of associations, they argue that it is ambitious to find the most relevant covariates out of a large pool of candidates, when the majority of the candidates most likely add little but noise. They also showed that multivariate approaches could come with a loss of power compared to univariate approaches. However, in univariate approaches, the inter–variable relationships are not taken into account, which is important as certain (bio)markers can affect the level of others. Thus, for each frailty domain we used both approaches, and considered them complementary to each other.

The multivariate approach can only be applied on observations that have no missing values. And since most (bio)markers were not measured for all individuals, we trimmed the candidate (bio)marker–list such that the sum of the fraction of covariates and fraction of frail individuals included in the sample was maximized. This resulted in 55 cases and 222 (bio)markers for physical frailty, 141 cases and 275 (bio)markers for cognitive frailty, and 117 and 219 (bio)markers for psychological frailty. Imputation strategies were not pursued, as the fraction of missings was high in many biomarkers and thus potentially troublesome for imputation procedures such as Multivariate Imputation by Chained Equations (MICE) (2).

We used a variable selection procedure based on Stability Selection (3) in combination with elastic net (4) to identify candidate (bio)markers. Elastic net is a regression method that finds models that yield good fitting, that are not overly complex (*i.e.*, have too many variables), avoid overfitting, and are therefore (more) generalizable. Since it is suggested that elastic net can still lead to many false positives (3), we used a Stability Selection procedure which involves repeatedly splitting the dataset into halves, fitting elastic net for each half, and then determining the proportion of all fitted models in which a variable is selected (3, 5). This yielded a ranking list of variable selection probabilities, where a higher selection probability implied an association between the variable and the outcome that is more 'robust' to sampling variability. Variables with an average selection probability >0.5 were used for further analyses.

We made further adjustments in the variable selection procedure to deal with the particularities of our dataset. First, (bio)markers with a skewed probability distribution were transformed using the Yeo–Johnson power transformation (6). Second, the low number of frail cases compared to the high number of controls (*i.e.*, imbalanced classes) led to the elastic net model being more inclined to properly identify controls rather than cases. Third, the number of cases increased strongly with age. Given that age is the strongest predictor of frailty, we had to adjust for this accordingly. Fourth, several biomarker mechanisms are known to differ by sex, consequently this also had to be accounted for. To deal with points two to four, we used a form of undersampling prior to applying the Stability Selection procedure. Undersampling involves forming a balanced dataset through sampling as many controls as there are cases, and using this newly formed dataset as input for the variable selection procedure (7, 8). We used stratified undersampling, where we sampled controls such that within each age and sex stratum (age operationalized as five-year categories) the number of cases and controls were identical. As undersampling leads to extra sampling variability, we repeatedly applied our procedure (10,000 iterations), where in each iteration stratified undersampling was used to create a balanced dataset, the variable selection procedure was applied, and a list of variable selection probabilities was obtained. A final ranking of variable selection probabilities was obtained by averaging the selection probabilities over all iterations. This approach can be seen as an ensemble for feature selection (9, 10). The analyses were carried out in R 3.3.2.

After the selection of (bio)markers based on the univariate and multivariate approaches, we used logistic regression with additional confounders to estimate effect sizes for these (bio)markers. We considered confounders based on literature (11) being sex, age, level of education, body mass index (BMI), smoking status, and season of blood collection. For cognitive frailty, we adjusted for depressive symptoms in addition. Age, BMI, and depressive symptoms (measured with the ZUNG depression scale) were used as continuous variables. Level of education was categorized into low (never went to school, not finished elementary school, finished elementary school), middle–low (first stage of secondary level education, second stage of secondary level education), middle–high (third level education other than university degree, university degree or recognised equivalent), and high (higher university degree or post graduate). Smoking status was categorized into current smoker and non-smoker (including ex-smokers). Season was categorized in winter (December– February), spring (March– May), summer (June– August), and fall (September– November) with summer as reference category.



Supplementary Figure 4.1. Schematic representation of the machine learning procedure.

Supplementary references

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Supplementary Table 4.1. Biomarker and marker list.

	Physical frailty		Cognitive frailty		Psychological frailty	
	n=64		n=199		n=134	
	missings(#)	missings(%)	missings(#)	missings(%)	missings(#)	missings(%)
consume_dairy_products	0	0,0	0	0,0	0	0,0
consume_fruit	0	0,0	0	0,0	0	0,0
consume_meat	0	0,0	0	0,0	0	0,0
consume_vegetables	0	0,0	0	0,0	0	0,0
falls_last_twelve_month	0	0,0	1	0,5	0	0,0
hospitalized_last_twelve_month	0	0,0	0	0,0	0	0,0
marital_status	0	0,0	0	0,0	0	0,0
past_diseases_hip_fracture	0	0,0	0	0,0	0	0,0
past_diseases_infarction	0	0,0	0	0,0	0	0,0
past_diseases_pneumonia	0	0,0	0	0,0	0	0,0
past_diseases_stroke	0	0,0	0	0,0	0	0,0
past_diseases_tumor	0	0,0	0	0,0	1	0,7
problem_angina_pectoris	0	0,0	0	0,0	0	0,0
problem_arthritis	0	0,0	0	0,0	0	0,0
problem_autoimmune	0	0,0	0	0,0	0	0,0
problem_backpain	0	0,0	0	0,0	0	0,0
problem_blood_pressure	0	0,0	0	0,0	0	0,0
problem_cholesterol	0	0,0	0	0,0	0	0,0
problem_diabetes	0	0,0	0	0,0	0	0,0
problem_hearing	0	0,0	0	0,0	1	0,7
problem_heart_beat	0	0,0	0	0,0	0	0,0
problem_heart_failure	0	0,0	0	0,0	0	0,0
problem_hyperthyroidism	0	0,0	0	0,0	0	0,0
problem_hyperthyroidism	0	0,0	0	0,0	0	0,0
problem_memory	0	0,0	0	0,0	0	0,0
problem_mental	0	0,0	1	0,5	1	0,7
problem_neurological	0	0,0	0	0,0	0	0,0
problem_osteoporosis	0	0,0	0	0,0	0	0,0
problem_pain_leg	0	0,0	0	0,0	0	0,0
problem_renal_failure	0	0,0	0	0,0	0	0,0
problem_respiratory_diseases	0	0,0	0	0,0	0	0,0
problem_venous	0	0,0	0	0,0	0	0,0
problem_vison	0	0,0	0	0,0	0	0,0
vaccinated_hepatitis_b	5	7,8	4	2,0	4	3,0
vaccinated_tetanus	1	1,6	1	0,5	5	3,7
females_hormonal_use	25	39,1	122	61,3	38	28,4
medicine_antibiotics	0	0,0	0	0,0	0	0,0
prescribed_medicine_use	0	0,0	0	0,0	0	0,0
smoke_current	0	0,0	0	0,0	0	0,0
smoke_previous	0	0,0	0	0,0	0	0,0
AGE_350_norm	2	3,1	9	4,5	2	1,5
AGE_405_350	5	7,8	14	7,0	7	5,2
AGE_405_norm	5	7,8	14	7,0	7	5,2

(**Supplementary Table 4.1** continues)

	Physical frailty		**Cognitive frailty**		**Psychological frailty**	
	n=64		n=199		n=134	
	missings(#)	missings(%)	missings(#)	missings(%)	missings(#)	missings(%)
AGE_440_350	2	3,1	9	4,5	2	1,5
AGE_440_norm	2	3,1	9	4,5	2	1,5
AGE_535_350	2	3,1	9	4,5	2	1,5
Alanine	9	14,1	31	15,6	21	15,7
Alexa_active	34	53,1	114	57,3	60	44,8
Alexa_basal	34	53,1	114	57,3	60	44,8
alpha2_macroglobulin	5	7,8	10	5,0	8	6,0
Anti_nuclear_auto_antibodies	8	12,5	11	5,5	6	4,5
basic_FGF	57	89,1	169	84,9	109	81,3
beta_Hydroxyisovalerate	9	14,1	31	15,6	21	15,7
Carotine	8	12,5	27	13,6	19	14,2
CD16_CD56__CD45__	16	25,0	58	29,1	51	38,1
CD178_FasL	61	95,3	184	92,5	121	90,3
CD19__CD45_	16	25,0	58	29,1	51	38,1
CD3__CD45_	16	25,0	58	29,1	51	38,1
CD3_CD4__CD45_	16	25,0	58	29,1	51	38,1
CD3_CD4__CD8_	16	25,0	58	29,1	51	38,1
CD3_CD4_CD8__CD45_	16	25,0	58	29,1	51	38,1
CD3_CD8__CD45_	16	25,0	58	29,1	51	38,1
CD3_lymph_diff	16	25,0	58	29,1	51	38,1
CD4_Eno__	57	89,1	178	89,4	122	91,0
CD4_Eno_MdX	57	89,1	178	89,4	122	91,0
CD4_MdX_Trx	57	89,1	178	89,4	122	91,0
CD4_Trx__	57	89,1	178	89,4	122	91,0
cell_counts	19	29,7	59	29,6	42	31,3
Ceruloplasmin	6	9,4	10	5,0	8	6,0
Cholesterol	4	6,3	10	5,0	7	5,2
CML_norm	2	3,1	9	4,5	2	1,5
Creatinine_urine	9	14,1	31	15,6	21	15,7
Cytomegalovirus_antibodies	8	12,5	11	5,5	6	4,5
Dnmt1_relative_expression	13	20,3	58	29,1	30	22,4
Dnmt3b_relative_expression	13	20,3	58	29,1	30	22,4
ELOVL_2_CpG_10	8	12,5	19	9,5	9	6,7
ELOVL_2_CpG_11_12_13_14	5	7,8	11	5,5	7	5,2
ELOVL_2_CpG_15_16_17	2	3,1	10	5,0	4	3,0
ELOVL_2_CpG_18_19_20_21	3	4,7	14	7,0	8	6,0
ELOVL_2_CpG_2_3	5	7,8	21	10,6	10	7,5
ELOVL_2_CpG_22_23_24	2	3,1	9	4,5	5	3,7
ELOVL_2_CpG_27	6	9,4	29	14,6	15	11,2
ELOVL_2_CpG_28_29	2	3,1	9	4,5	4	3,0
ELOVL_2_CpG_30_31_32	2	3,1	10	5,0	4	3,0
ELOVL_2_CpG_33	2	3,1	9	4,5	5	3,7
ELOVL_2_CpG_34_35_36	2	3,1	9	4,5	5	3,7
ELOVL_2_CpG_5	4	6,3	16	8,0	9	6,7

(Supplementary Table 4.1 continues)

	Physical frailty		Cognitive frailty		Psychological frailty	
	n=64		n=199		n=134	
	missings(#)	missings(%)	missings(#)	missings(%)	missings(#)	missings(%)
ELOVL_2_CpG_6_7	2	3,1	9	4,5	4	3,0
ELOVL_2_CpG_8	2	3,1	9	4,5	4	3,0
ELOVL_2_CpG_9	2	3,1	9	4,5	4	3,0
Fe_saturation_of_transferrin	6	9,4	11	5,5	8	6,0
Ferritin	4	6,3	10	5,0	7	5,2
FHL2_CpG_1	2	3,1	9	4,5	3	2,2
FHL2_CpG_11_12	2	3,1	9	4,5	3	2,2
FHL2_CpG_13_14_15	2	3,1	9	4,5	3	2,2
FHL2_CpG_16_17	6	9,4	13	6,5	8	6,0
FHL2_CpG_18	20	31,3	54	27,1	38	28,4
FHL2_CpG_19_20	2	3,1	9	4,5	3	2,2
FHL2_CpG_2	2	3,1	9	4,5	3	2,2
FHL2_CpG_21	2	3,1	9	4,5	3	2,2
FHL2_CpG_5	2	3,1	9	4,5	3	2,2
FHL2_CpG_6	2	3,1	9	4,5	3	2,2
FHL2_CpG_7_8	2	3,1	9	4,5	3	2,2
FHL2_CpG_9_10	2	3,1	9	4,5	3	2,2
Free_fatty_acids	4	6,3	10	5,0	7	5,2
Galactose_residue_b	26	40,6	101	50,8	55	41,0
Gamma_glutamyl_ transferase	4	6,3	11	5,5	7	5,2
Glutathione_DNA	4	6,3	10	5,0	9	6,7
Glycine	9	14,1	31	15,6	21	15,7
GM_CSF	56	87,5	169	84,9	104	77,6
Guanidinoacetate_3_81_s	9	14,1	31	15,6	21	15,7
Guanidinoacetate_3_97_s	9	14,1	31	15,6	21	15,7
HDL_Cholesterol	6	9,4	12	6,0	10	7,5
HDL_Triglycerides	6	9,4	12	6,0	10	7,5
HDL1_Cholesterol	6	9,4	12	6,0	10	7,5
HDL1_Triglycerides	6	9,4	12	6,0	10	7,5
HDL2_Cholesterol	6	9,4	12	6,0	10	7,5
HDL2_Triglycerides	6	9,4	12	6,0	10	7,5
High_density_lipoproteins_ choles	4	6,3	10	5,0	7	5,2
Hippurate	9	14,1	31	15,6	21	15,7
IFN_g	56	87,5	169	84,9	104	77,6
IL_10	56	87,5	169	84,9	104	77,6
IL_12p70	56	87,5	169	84,9	104	77,6
IL_13	56	87,5	169	84,9	104	77,6
IL_1b	56	87,5	169	84,9	104	77,6
IL_2	56	87,5	169	84,9	104	77,6
IL_6	56	87,5	169	84,9	104	77,6
IL_8	56	87,5	169	84,9	104	77,6
Immunoglobulin_A	5	7,8	10	5,0	8	6,0
Immunoglobulin_E	8	12,5	10	5,0	8	6,0

(**Supplementary Table 4.1** continues)

	Physical frailty		Cognitive frailty		Psychological frailty	
	n=64		n=199		n=134	
	missings(#)	missings(%)	missings(#)	missings(%)	missings(#)	missings(%)
Immunoglobulin_G	4	6,3	10	5,0	8	6,0
Immunoglobulin_M	4	6,3	10	5,0	8	6,0
Influenza_A_IgG_antibodies	25	39,1	66	33,2	41	30,6
Influenza_B_IgG_antibodies	25	39,1	66	33,2	41	30,6
LDL_Cholesterol	6	9,4	12	6,0	10	7,5
LDL_Triglycerides	6	9,4	12	6,0	10	7,5
LDL1_Cholesterol	6	9,4	12	6,0	10	7,5
LDL1_Triglycerides	6	9,4	12	6,0	10	7,5
LDL2_Cholesterol	6	9,4	12	6,0	10	7,5
LDL2_Triglycerides	6	9,4	12	6,0	10	7,5
LDLox_protein	7	10,9	14	7,0	10	7,5
LDLox_serum	7	10,9	14	7,0	10	7,5
Low_density_lipoproteins_cholest	4	6,3	10	5,0	7	5,2
Lymphocytes	2	3,1	4	2,0	3	2,2
Lymphocytes__MESF_	20	31,3	48	24,1	33	24,6
Lymphocytes__Metallo_Blank__MESF	20	31,3	48	24,1	33	24,6
Lymphocytes__Metallo_Blank__MFI	20	31,3	48	24,1	32	23,9
Lymphocytes_Blank__MESF_	20	31,3	48	24,1	33	24,6
Lymphocytes_Blank__MFI_	20	31,3	48	24,1	32	23,9
Lymphocytes_Fluorescence__MFI_	20	31,3	48	24,1	32	23,9
Lymphocytes_Metallothionein__MES	20	31,3	48	24,1	33	24,6
Lymphocytes_Metallothioneins__MF	20	31,3	48	24,1	32	23,9
Lymphosum	16	25,0	58	29,1	51	38,1
MCHC	0	0,0	0	0,0	0	0,0
MCP_1	56	87,5	169	84,9	104	77,6
MDA_NOX	7	10,9	16	8,0	10	7,5
mean_21q	2	3,1	11	5,5	4	3,0
mean_21q_nosn1	2	3,1	11	5,5	4	3,0
mean_5p_bis	2	3,1	11	5,5	3	2,2
Methionine_sulfoxide_reductase_a	56	87,5	171	85,9	118	88,1
Monocytes__MESF_	20	31,3	48	24,1	32	23,9
Monocytes__Metallo_Blank__MESF	20	31,3	48	24,1	33	24,6
Monocytes__Metallo_Blank__MFI	20	31,3	48	24,1	32	23,9
Monocytes_Blank__MESF_	20	31,3	48	24,1	33	24,6
Monocytes_Blank__MFI_	20	31,3	48	24,1	32	23,9
Monocytes_Fluorescence__MFI_	20	31,3	48	24,1	32	23,9
Monocytes_Metallothionein__MESF_	20	31,3	48	24,1	32	23,9

(**Supplementary Table 4.1** continues)

	Physical frailty		**Cognitive frailty**		**Psychological frailty**	
	n=64		n=199		n=134	
	missings(#)	missings(%)	missings(#)	missings(%)	missings(#)	missings(%)
Monocytes_ Metallothionenines__MF	20	31,3	48	24,1	32	23,9
p_telom_cnt	6	9,4	30	15,1	30	22,4
p_telom_len_se	17	26,6	77	38,7	54	40,3
p_telom_short_pc	6	9,4	30	15,1	30	22,4
PARP_activity	34	53,1	114	57,3	60	44,8
Parp1_relative_expression	13	20,3	58	29,1	30	22,4
Parp2_relative_expression	13	20,3	58	29,1	30	22,4
PBMC__MESF_	20	31,3	48	24,1	32	23,9
PBMC__Metallo_Blank__ MESF	20	31,3	48	24,1	32	23,9
PBMC__Metallo_Blank__MFI	20	31,3	48	24,1	32	23,9
PBMC_Blank__MESF_	20	31,3	48	24,1	32	23,9
PBMC_Blank__MFI_	20	31,3	48	24,1	31	23,1
PBMC_Eno__	57	89,1	178	89,4	122	91,0
PBMC_Eno_MdX	57	89,1	178	89,4	122	91,0
PBMC_Fluorescence__MFI_	20	31,3	48	24,1	31	23,1
PBMC_Metallothinein__ MESF_	20	31,3	48	24,1	32	23,9
PBMC_Metallothioneins__ MFI_	20	31,3	48	24,1	32	23,9
PBMC_Trx__	57	89,1	178	89,4	122	91,0
PBMC_Trx_MdX	57	89,1	178	89,4	122	91,0
PCS	9	14,1	31	15,6	21	15,7
Phenylacetylglutamine	9	14,1	31	15,6	21	15,7
plasma_Protein_ concentration__UP	16	25,0	54	27,1	34	25,4
Prostate_specific_antigen	9	14,1	10	5,0	8	6,0
Proteasome_peptidase_ activity	16	25,0	54	27,1	34	25,4
Proteasome_peptidase_ activity_no	16	25,0	54	27,1	34	25,4
S_log_p1_p6	1	1,6	7	3,5	3	2,2
S_p1_n_glycan	1	1,6	7	3,5	3	2,2
S_p2_n_glycan	1	1,6	7	3,5	3	2,2
S_p3_n_glycan	1	1,6	7	3,5	3	2,2
S_p4_n_glycan	1	1,6	7	3,5	3	2,2
S_p5_n_glycan	1	1,6	7	3,5	3	2,2
S_p6_n_glycan	1	1,6	7	3,5	3	2,2
S_p7_n_glycan	1	1,6	7	3,5	3	2,2
S_p8_n_glycan	1	1,6	7	3,5	3	2,2
S_p9_n_glycan	1	1,6	7	3,5	3	2,2
s_vimentin_conc	58	90,6	192	96,5	123	91,8
Serotonin	9	14,1	11	5,5	8	6,0

(**Supplementary Table 4.1** continues)

	Physical frailty		**Cognitive frailty**		**Psychological frailty**	
	n=64		n=199		n=134	
	missings(#)	missings(%)	missings(#)	missings(%)	missings(#)	missings(%)
Serum_Fe	5	7,8	11	5,5	8	6,0
Serum_glucose	4	6,3	10	5,0	7	5,2
serum_hemolysis_index	4	6,3	9	4,5	7	5,2
serum_icterus_index	4	6,3	9	4,5	7	5,2
serum_lipemia_index	4	6,3	9	4,5	7	5,2
Serum_transferrin	6	9,4	10	5,0	8	6,0
Testosterone	4	6,3	10	5,0	7	5,2
Threonine_Lactate	9	14,1	31	15,6	21	15,7
TNF	56	87,5	169	84,9	104	77,6
Total_Cells__ml	19	29,7	59	29,6	42	31,3
Total_Cholesterol	6	9,4	12	6,0	10	7,5
Total_serum_protein__ RIVM_	4	6,3	10	5,0	7	5,2
Total_Triglycerides	6	9,4	12	6,0	10	7,5
Triglycerides	4	6,3	10	5,0	7	5,2
Trigonelline	9	14,1	31	15,6	21	15,7
Trimethylaminoxid	9	14,1	31	15,6	21	15,7
vital_stat_weight	0	0,0	0	0,0	0	0,0
Vitamin_C	4	6,3	10	5,0	9	6,7
Vitamin_E	4	6,3	10	5,0	9	6,7
VLDL_Cholesterol	6	9,4	12	6,0	10	7,5
VLDL_Triglycerides	6	9,4	12	6,0	10	7,5
VLDL1_Cholesterol	6	9,4	12	6,0	10	7,5
VLDL1_Triglycerides	6	9,4	12	6,0	10	7,5
VLDL2_Cholesterol	6	9,4	12	6,0	10	7,5
VLDL2_Triglycerides	6	9,4	12	6,0	10	7,5
__Nitrotyrosine	2	3,1	6	3,0	2	1,5
_1q_cpg_1_2_sn1	3	4,7	11	5,5	5	3,7
_1q_cpg_10_11_12_13_14	2	3,1	11	5,5	4	3,0
_1q_cpg_15	2	3,1	11	5,5	4	3,0
_1q_cpg_16_17	2	3,1	11	5,5	4	3,0
_1q_cpg_18_19	2	3,1	11	5,5	4	3,0
_1q_cpg_20_21	2	3,1	11	5,5	6	4,5
_1q_cpg_3	2	3,1	11	5,5	4	3,0
_1q_cpg_4_sn1	2	3,1	11	5,5	4	3,0
_1q_cpg_5_sn1	2	3,1	11	5,5	4	3,0
_1q_cpg_8	2	3,1	11	5,5	4	3,0
_5_Hydroxy_Vitamin_D	1	1,6	5	2,5	2	1,5
_bab_norm	2	3,1	9	4,5	2	1,5
_p_bis_cpg_1	2	3,1	11	5,5	3	2,2
_p_bis_cpg_2	7	10,9	15	7,5	6	4,5
_p_bis_cpg_3_sn1	2	3,1	11	5,5	3	2,2
_p_bis_cpg_5	2	3,1	11	5,5	3	2,2

(Supplementary Table 4.1 continues)

	Physical frailty n=64		Cognitive frailty n=199		Psychological frailty n=134	
	missings(#)	missings(%)	missings(#)	missings(%)	missings(#)	missings(%)
APOE	1	1,6	6	3,0	3	2,2
gender_sex	0	0,0	0	0,0	0	0,0
plasma_hemolysis_index	1	1,6	5	2,5	2	1,5
plasma_icterus_index	1	1,6	5	2,5	2	1,5
research_center_no	0	0,0	0	0,0	0	0,0
selfrep_health	0	0,0	0	0,0	0	0,0
ses4	1	1,6	0	0,0	0	0,0
Adiponectin	1	1,6	5	2,5	2	1,5
AGE_350	2	3,1	8	4,0	2	1,5
AGE_405	2	3,1	8	4,0	2	1,5
AGE_440	2	3,1	8	4,0	2	1,5
AGE_535	1	1,6	5	2,5	2	1,5
AGE_535_norm	1	1,6	6	3,0	2	1,5
Age_years	0	0,0	0	0,0	0	0,0
Alanine_aminotransferase	1	1,6	6	3,0	2	1,5
Albumin	1	1,6	6	3,0	2	1,5
Alpha_carotene	1	1,6	5	2,5	2	1,5
ApoJ_Clu	1	1,6	4	2,0	2	1,5
Arg_Pyrimidine	2	3,1	8	4,0	2	1,5
Ascorbic_acid	1	1,6	5	2,5	2	1,5
Basophils	3	4,7	3	1,5	1	0,7
Beta_carotene	1	1,6	5	2,5	2	1,5
Beta_cryptoxanthin	1	1,6	5	2,5	2	1,5
blood_pressure_diastolic	0	0,0	0	0,0	0	0,0
blood_pressure_systolic	0	0,0	0	0,0	0	0,0
BMI	0	0,0	0	0,0	0	0,0
C_reactive_protein	0	0,0	1	0,5	1	0,7
Carboxylmethyllysine	2	3,1	8	4,0	2	1,5
Creatinine	1	1,6	6	3,0	2	1,5
Cu__ppb_	1	1,6	4	2,0	2	1,5
Cu_Zn	1	1,6	4	2,0	2	1,5
CuCp____	1	1,6	9	4,5	3	2,2
CuCp__ppb_	1	1,6	9	4,5	3	2,2
CunotCp__ppb_	1	1,6	9	4,5	3	2,2
dehydroepiandrosteron_sulfate	1	1,6	6	3,0	2	1,5
Eosinophils	2	3,1	3	1,5	0	0,0
Fe__ppb_	1	1,6	4	2,0	2	1,5
FeAlb__ppb_	1	1,6	6	3,0	2	1,5
FeTransf____	1	1,6	6	3,0	2	1,5
FeTransf__ppb_	1	1,6	6	3,0	2	1,5
Fibrinogen	1	1,6	6	3,0	2	1,5
Gamma_tocopherol	1	1,6	5	2,5	2	1,5
Glutathione	1	1,6	5	2,5	2	1,5

(**Supplementary Table 4.1** continues)

	Physical frailty		Cognitive frailty		Psychological frailty	
	n=64		n=199		n=134	
	missings(#)	missings(%)	missings(#)	missings(%)	missings(#)	missings(%)
Glycosylated_haemoglobin_A1C	1	1,6	5	2,5	3	2,2
Haemoglobin_of_frozen_blood	1	1,6	5	2,5	3	2,2
HCT	1	1,6	2	1,0	0	0,0
heart_rate	0	0,0	0	0,0	0	0,0
Hepatitis_B_core_antibodies	0	0,0	0	0,0	1	0,7
Hepatitis_C_virus_antibodies	0	0,0	0	0,0	1	0,7
Heteroplasmy	1	1,6	6	3,0	2	1,5
HGB	0	0,0	0	0,0	0	0,0
Homocysteine	1	1,6	6	3,0	2	1,5
Insulin	1	1,6	6	3,0	2	1,5
Lutein	1	1,6	5	2,5	2	1,5
Malondialdehyde	1	1,6	5	2,5	2	1,5
MCH	1	1,6	2	1,0	0	0,0
MCV	1	1,6	2	1,0	0	0,0
Measles_IgG_antibodies	1	1,6	7	3,5	3	2,2
Monocytes	2	3,1	3	1,5	0	0,0
Neutrophils	2	3,1	3	1,5	0	0,0
NOx_plasma	1	1,6	7	3,5	2	1,5
NOx_plasma_replicate_1	1	1,6	7	3,5	2	1,5
pe_6bab_md	2	3,1	8	4,0	2	1,5
pe_age350_md	2	3,1	8	4,0	2	1,5
pe_age405_md	2	3,1	8	4,0	2	1,5
pe_age440_md	2	3,1	8	4,0	2	1,5
pe_age535_md	1	1,6	5	2,5	2	1,5
pe_cml_md	2	3,1	8	4,0	2	1,5
Plasma_8_isoprostane	1	1,6	4	2,0	2	1,5
Plasma_Alpha_tocopherol	1	1,6	5	2,5	2	1,5
Plasma_Cysteine	1	1,6	5	2,5	2	1,5
plasma_lipemia_index	1	1,6	5	2,5	2	1,5
Plasma_Lycopene	1	1,6	5	2,5	2	1,5
Platelets	2	3,1	1	0,5	0	0,0
Protein	1	1,6	6	3,0	2	1,5
Protein_carbonyls	1	1,6	5	2,5	2	1,5
RBC	0	0,0	1	0,5	0	0,0
Se__ppb_	1	1,6	4	2,0	2	1,5
SeAlbSeP____	1	1,6	6	3,0	2	1,5
SeAlbSeP__ppb_	1	1,6	6	3,0	2	1,5
SeGPX__ppb_	1	1,6	6	3,0	2	1,5
Serum_Retinol	1	1,6	5	2,5	2	1,5
Tetanus_IgG_antibodies	1	1,6	7	3,5	3	2,2
Thyreoglobulin_auto_antibodies	1	1,6	6	3,0	2	1,5

(Supplementary Table 4.1 continues)

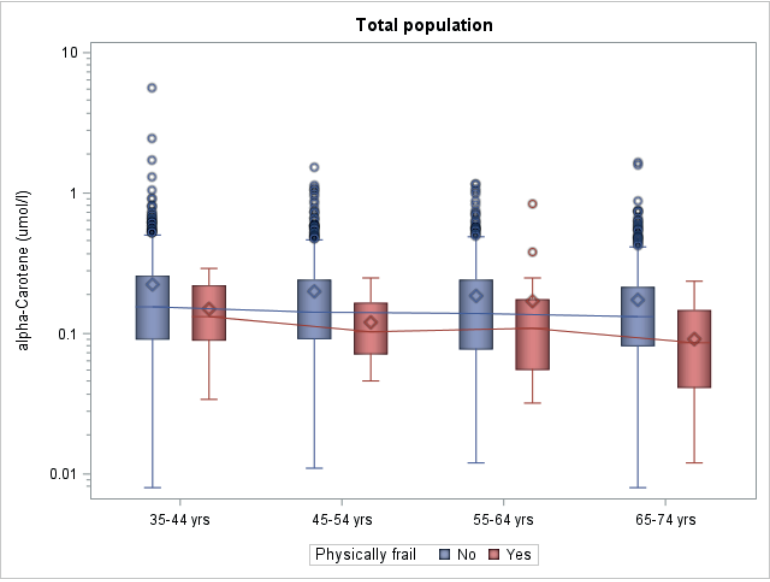
	Physical frailty		Cognitive frailty		Psychological frailty	
	n=64		n=199		n=134	
	missings(#)	missings(%)	missings(#)	missings(%)	missings(#)	missings(%)
U_p1_n_glycan	1	1,6	7	3,5	3	2,2
U_p2_n_glycan	1	1,6	7	3,5	3	2,2
U_p3_n_glycan	1	1,6	7	3,5	3	2,2
U_p4_n_glycan	1	1,6	7	3,5	3	2,2
U_p5_n_glycan	1	1,6	7	3,5	3	2,2
U_p6_n_glycan	1	1,6	7	3,5	3	2,2
U_p7_n_glycan	1	1,6	7	3,5	3	2,2
U_p8_n_glycan	1	1,6	7	3,5	3	2,2
U_p9_n_glycan	1	1,6	7	3,5	3	2,2
Urea	1	1,6	6	3,0	2	1,5
Uric_acid	1	1,6	5	2,5	2	1,5
Uric_acid_molar	1	1,6	6	3,0	2	1,5
Urinary_8_isoprostane	1	1,6	6	3,0	2	1,5
Urinary_8_isoprostane__direct_	1	1,6	6	3,0	2	1,5
Urinary_creatinine	1	1,6	6	3,0	2	1,5
Urinary_isoprostane_metabolite	1	1,6	6	3,0	2	1,5
vital_stat_waist	0	0,0	0	0,0	0	0,0
WBC	1	1,6	1	0,5	0	0,0
Zeaxanthin	1	1,6	5	2,5	2	1,5
Zn__ppb_	1	1,6	4	2,0	2	1,5
Influenza_A_antigen	54	84,4	170	85,4	117	87,3
Influenza_B_antigen	54	84,4	170	85,4	117	87,3
Measles_antigen	54	84,4	171	85,9	117	87,3
p_sjtrec	44	68,8	156	78,4	106	79,1
past_diseases_hip_fracture_age_f	62	96,9	195	98,0	131	97,8
past_diseases_infarction_age_fir	61	95,3	188	94,5	130	97,0
problem_other_age_onset	63	98,4	189	95,0	127	94,8
Tetanus_antigen	54	84,4	170	85,4	117	87,3
u_cmv	44	68,8	149	74,9	102	76,1
u_hhv6a	44	68,8	149	74,9	102	76,1
u_hhv6b	44	68,8	149	74,9	102	76,1
u_hhv7	44	68,8	148	74,4	101	75,4
Cell_Debris__ml	19	29,7	59	29,6	42	31,3
Cell_free_DNA	25	39,1	60	30,2	39	29,1
DNA_integrity_after_3_8Gy	9	14,1	35	17,6	32	23,9
initial_DNA_integrity	9	14,1	35	17,6	32	23,9
Reference_value__BioTeSys_	3	4,7	4	2,0	2	1,5
Viable_Cells__ml__UKON_	19	29,7	59	29,6	42	31,3
Vitality_of_cells____INRCA_	21	32,8	47	23,6	31	23,1
__cell_viability	19	29,7	59	29,6	42	31,3
__DNA_repair	9	14,1	35	17,6	32	23,9

Supplementary Figures 4.2 A–S

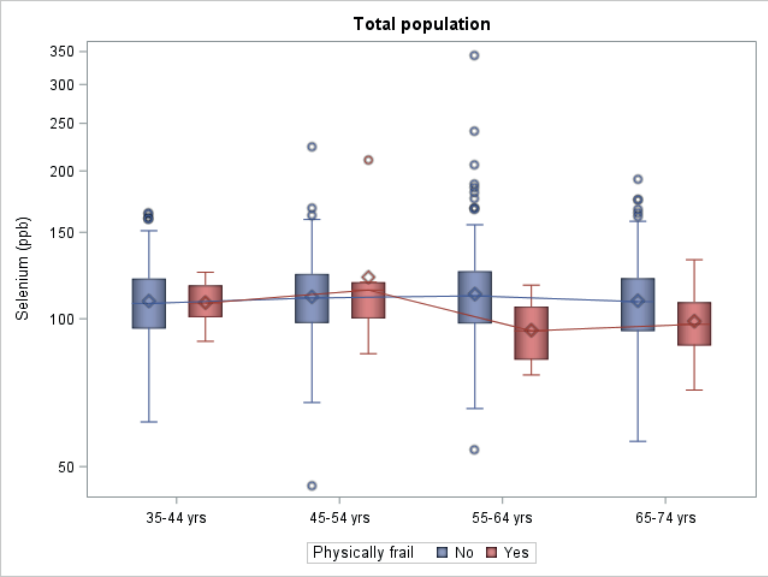
The boxes represent the interquartile range and a solid line connects the medians of the ten-year age groups for the frail and non-frail. The mean is represented as a diamond. The whiskers represent the minimum and maximum values. The circles outside the whiskers represent outliers. A logarithmic scale (log_{10}) is applied to the y-axis to preserve the dynamic range and plot all outlier values.

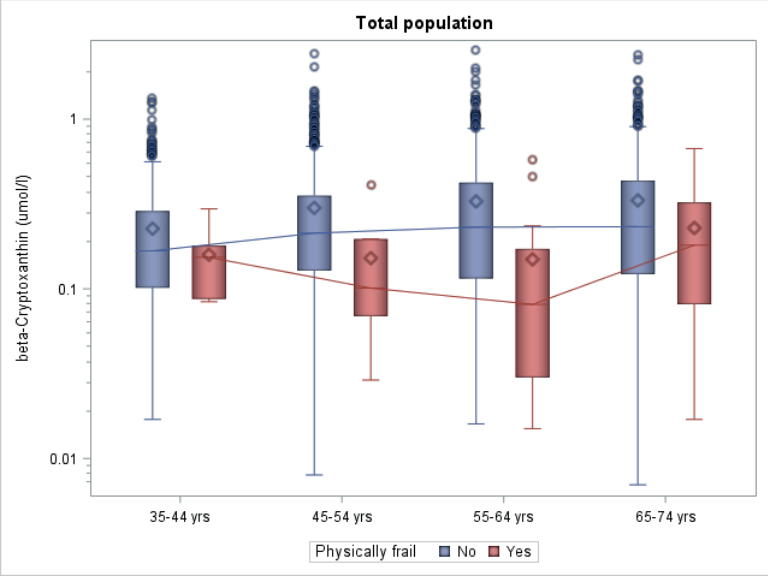
Age stratified boxplots of the biomarkers listed in **Table 4.2** of the manuscript.

Physical frailty:



(Supplementary Figures 4.1 A–S continue)





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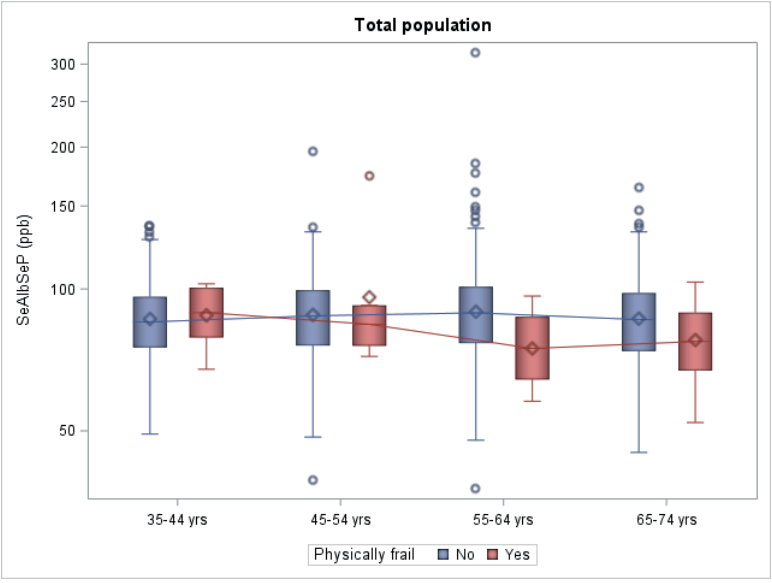

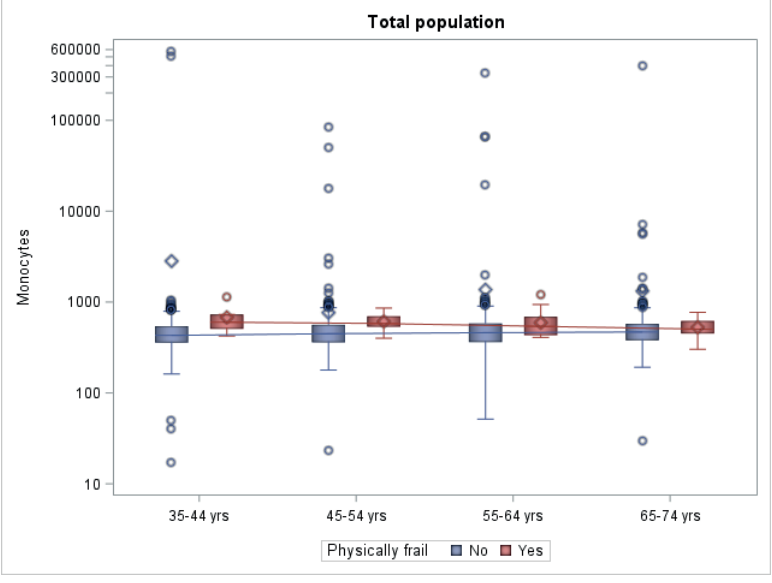

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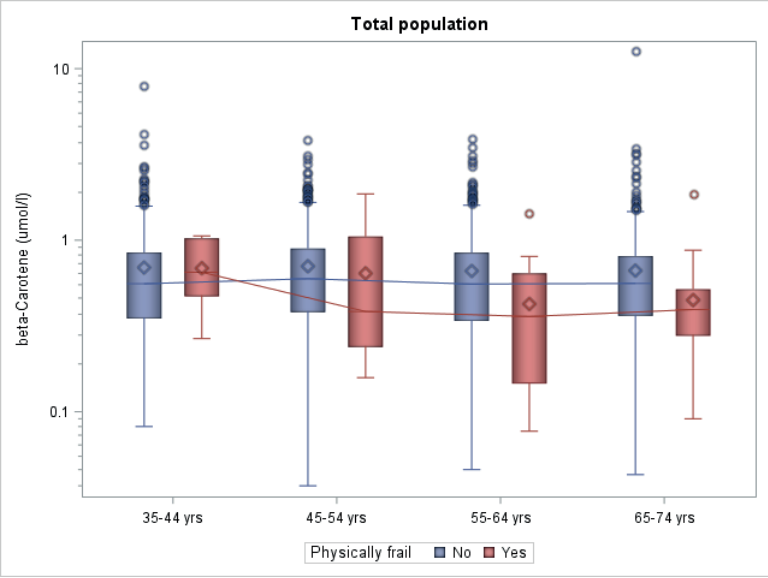
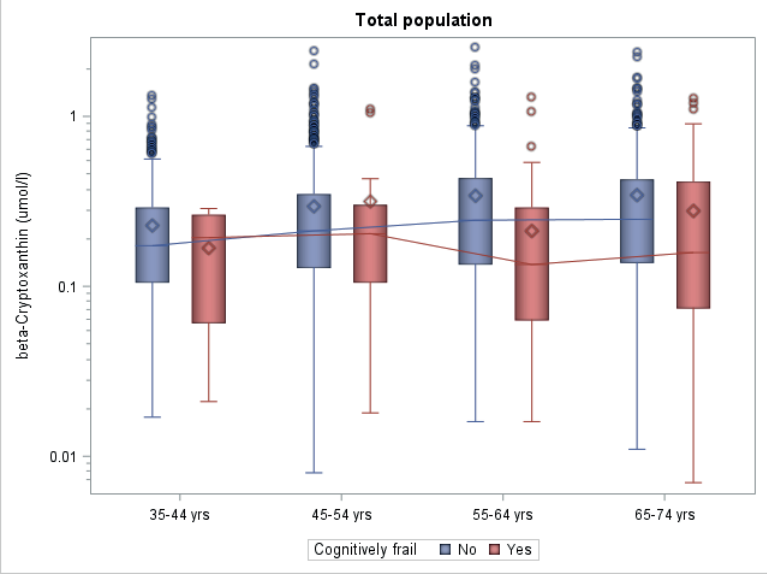
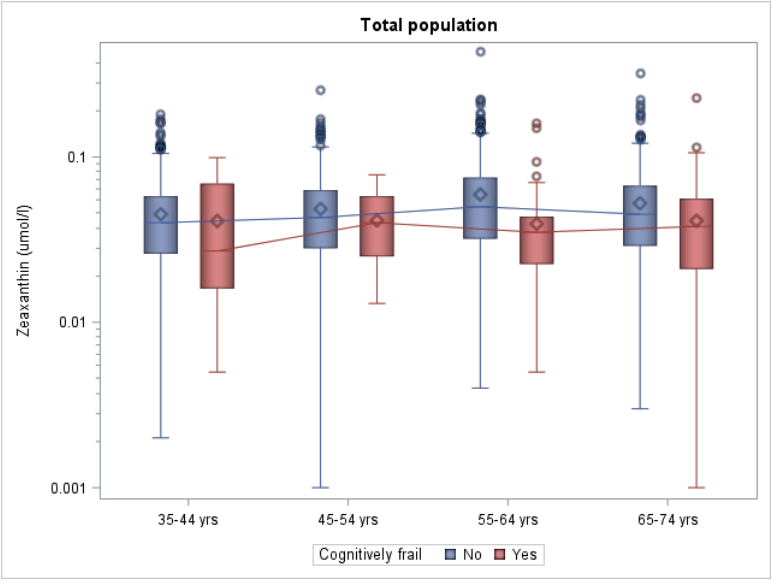
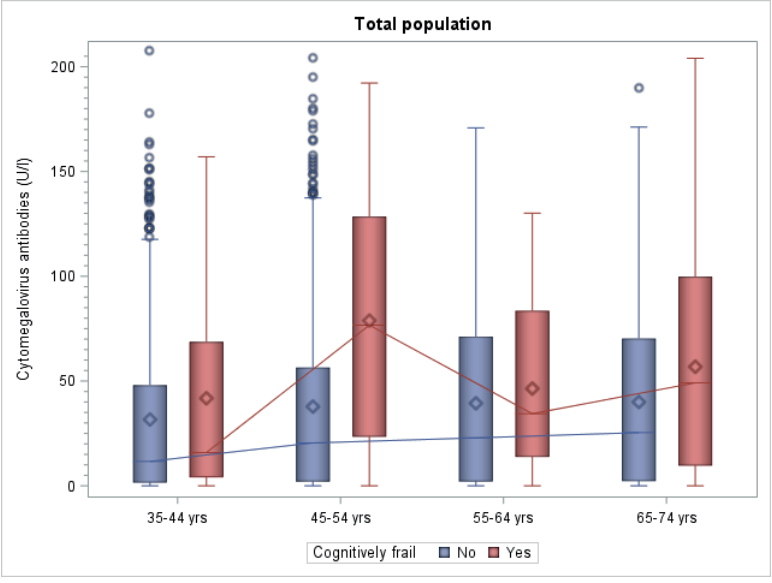


Cognitive frailty:

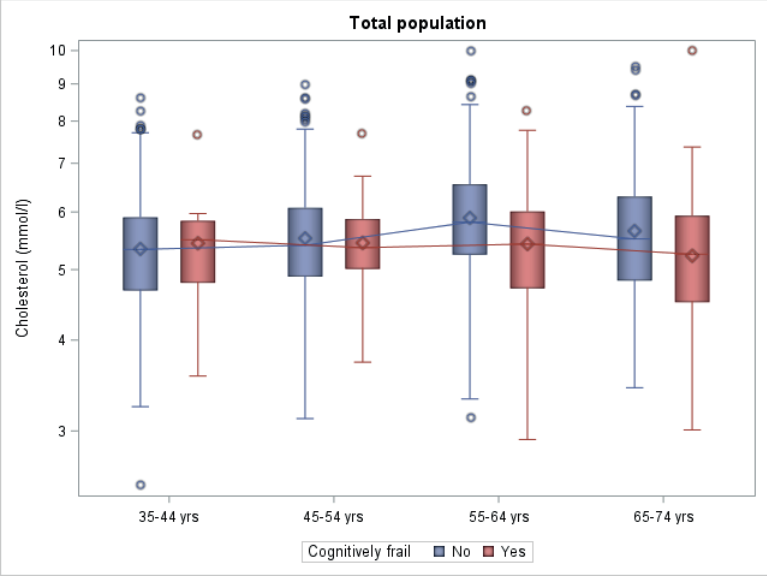


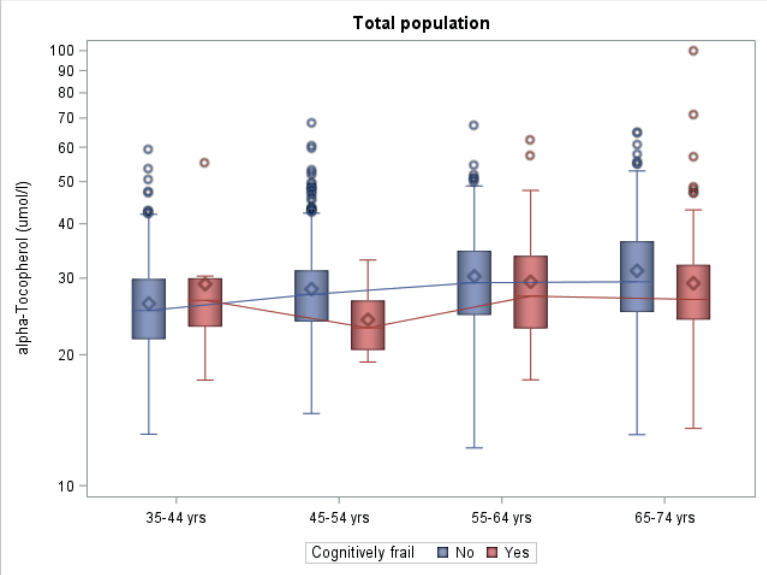
(Supplementary Figures 4.1 A–S continue)





(Supplementary Figures 4.1 A–S continue)





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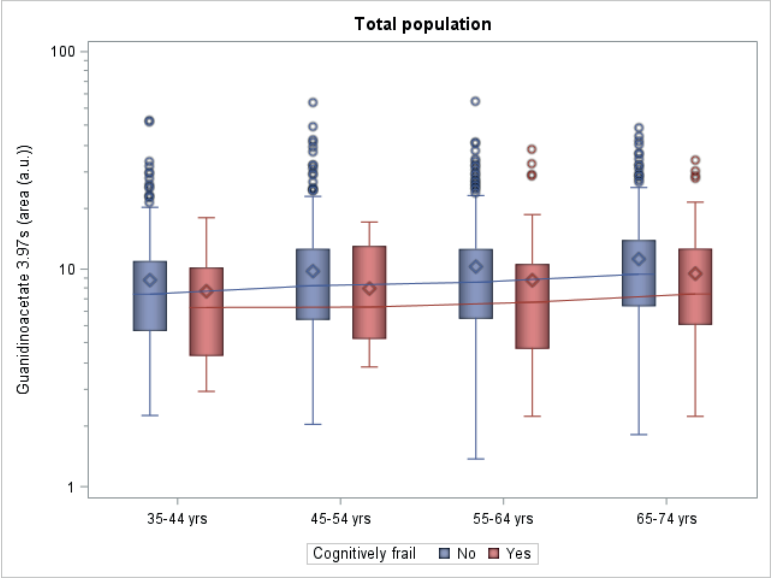
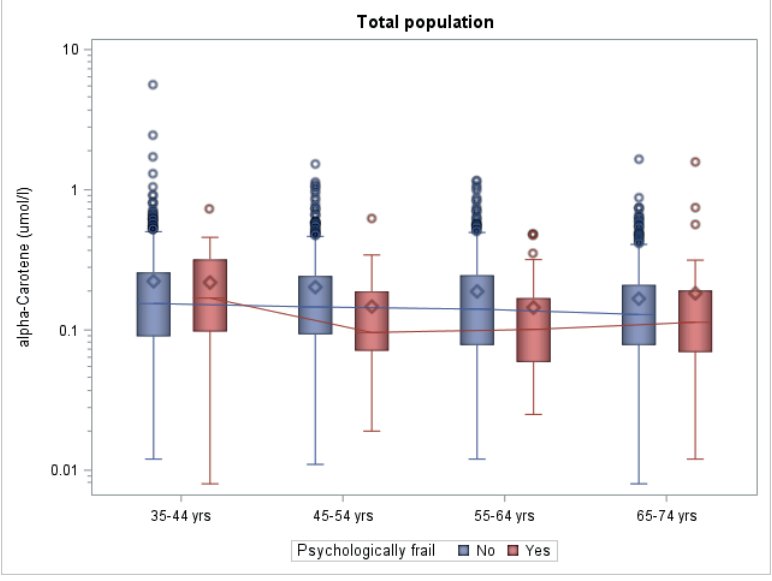


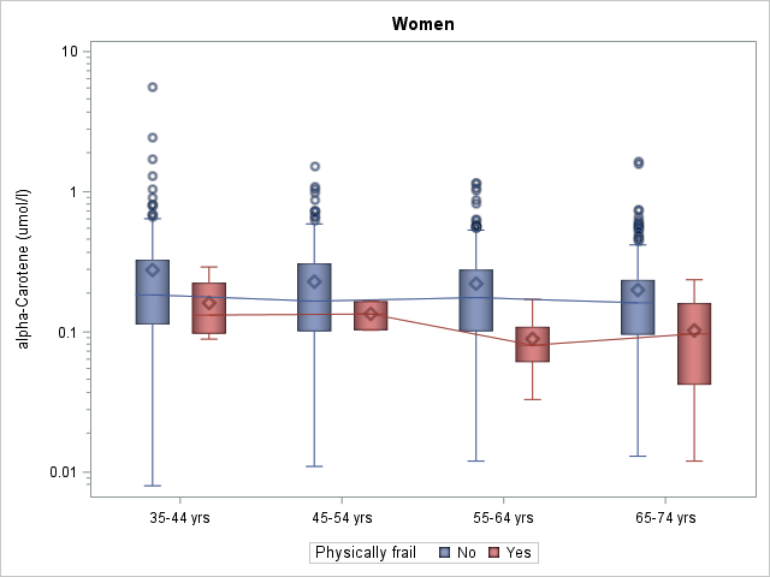
Psychological frailty:

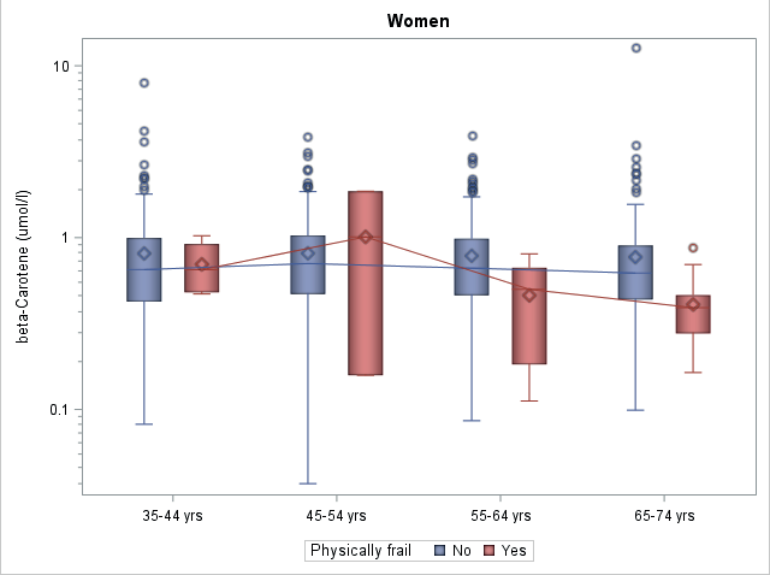


(Supplementary Figures 4.1 A–S continue)

Age and sex stratified boxplots of the biomarkers listed in **Table 3a** of the manuscript.

Physical frailty:

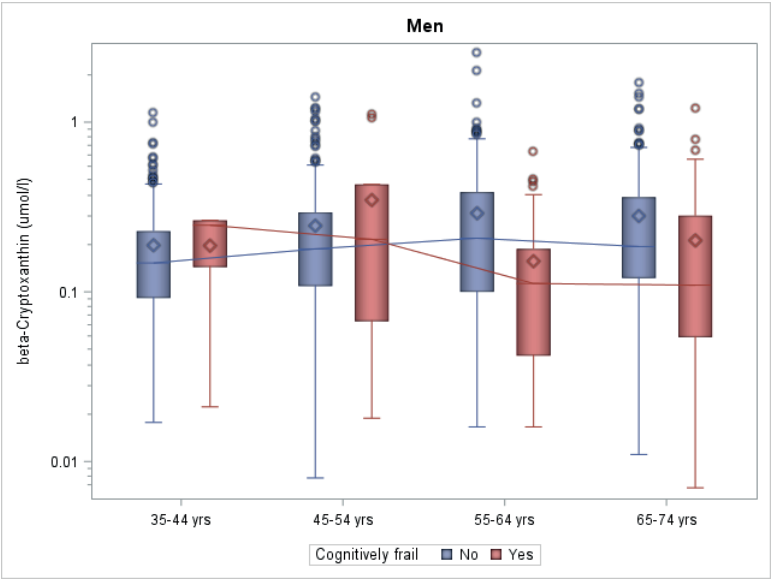


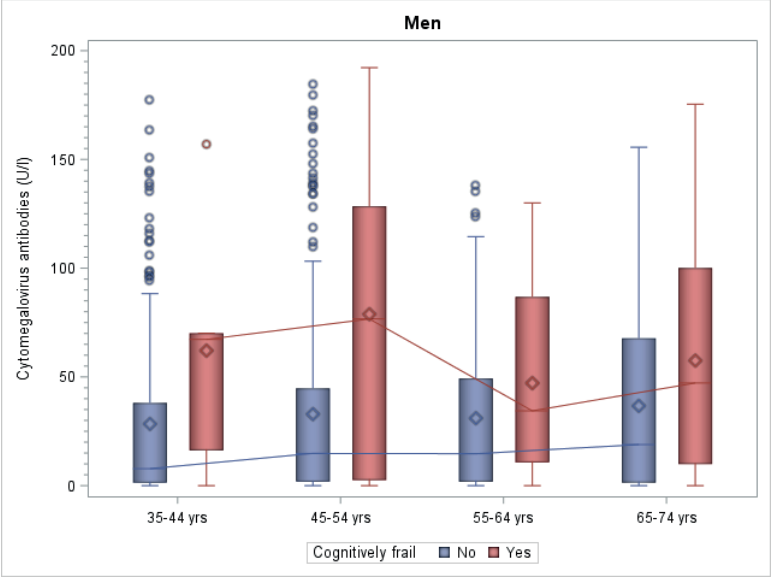


(Supplementary Figures 4.1 A–S continue)

Age and sex stratified boxplots of the biomarkers listed in **Table 3b** of the manuscript.

Cognitive frailty:





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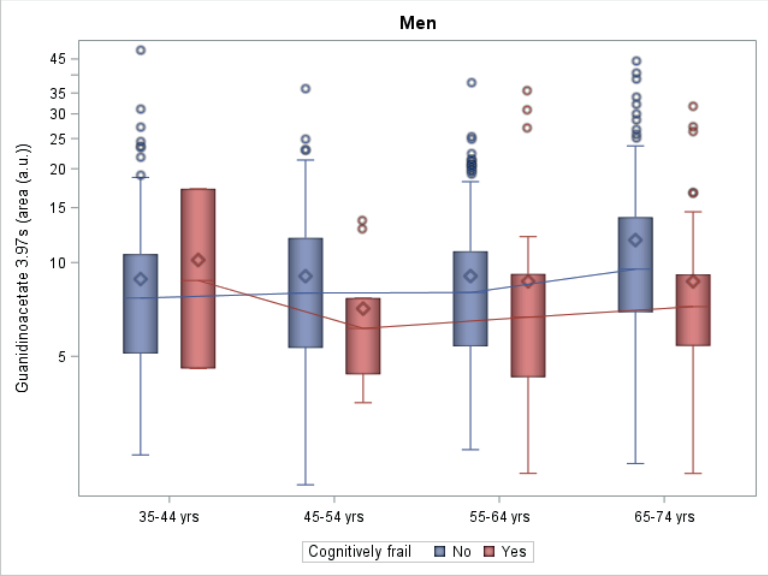
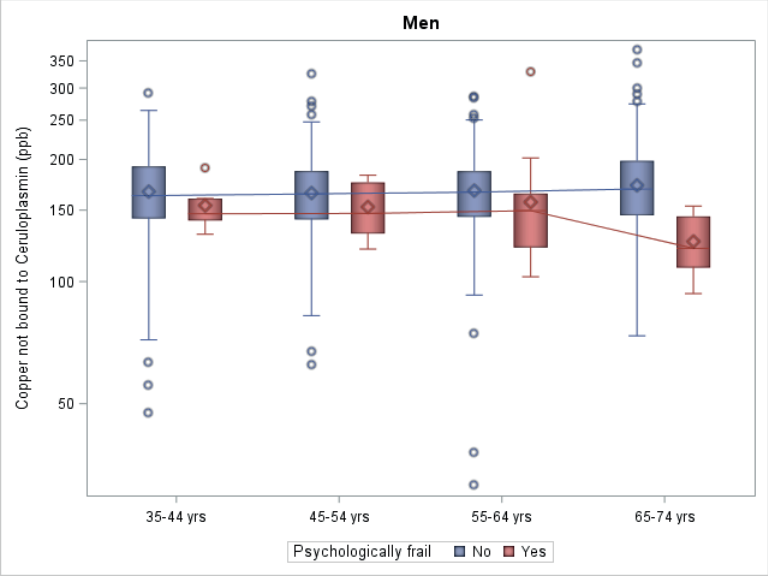


Psychological frailty:



Part II

Exploring (bio)markers of cognitive frailty, general cognitive function and cognitive decline using longitudinal data

5

Trajectories of (bio)markers during the development of cognitive frailty in the Doetinchem Cohort Study

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Frontiers in Neurology, section Neuroepidemiology, 2019

Abstract

Background

Long-term changes in (bio)markers for cognitive frailty are not well characterized. Therefore, our aim is to explore (bio)marker trajectories in adults who became cognitively frail compared to age- and sex-matched controls who did not become cognitively frail over a 15 year follow-up. We hypothesise that those who become cognitively frail have more unfavourable trajectories of (bio)markers compared to controls.

Methods

The Doetinchem Cohort Study is a longitudinal population-based study that started in 1987-1991 in men and women aged 20-59 years, with follow-up examinations every 5 years. For the current analyses, we used data of 17 potentially relevant (bio)markers (*e.g.* body mass index (BMI), urea) from rounds 2-5 (1993-2012). A global cognitive functioning score (based on memory, speed and flexibility) was calculated for each round and transformed into education and examination round-adjusted z-scores. The z-score that corresponded to the 10th percentile in round 5 (z-score=-0.77) was applied as cut-off point for incident cognitive frailty in rounds 2-5. In total, 455 incident cognitively frail cases were identified retrospectively and were compared with 910 age- and sex-matched controls. Trajectories up to 15 years before and 10 years after incident cognitive frailty were analysed using generalized estimating equations with stratification for sex and adjustment for age and, if appropriate, medication use. Results were further adjusted for level of education, depressive symptoms, BMI, and lifestyle factors.

Results

In men, (bio)marker trajectories did not differ as they ran parallel and the difference in levels was not statistically significant between those who became cognitively frail compared to controls. In women, total cholesterol trajectories first increased and thereafter decreased in cognitively frail women and steadily increased in controls, gamma-glutamyltransferase trajectories were more or less stable in cognitively frail women and increased in controls, and urea trajectories increased in cognitively frail women and remained more or less stable in controls. Results were similar after additional adjustment for potential confounders.

Conclusions

Out of the 17 (bio)markers included in this explorative study, differential trajectories for three biomarkers were observed in women. We do not yet consider any of the studied (bio)markers as promising biomarkers for cognitive frailty.

Introduction

Frailty is a state of increased vulnerability to adverse health outcomes when exposed to stressors caused by the cumulative decline in one or more domains of functioning, including the cognitive domain (1–4). Moderate cognitive decline is part of the normal ageing process (5). Some elderly are confronted with accelerated cognitive decline, which could eventually lead to (mild) cognitive impairment or dementia. A (reversible) state of cognitive vulnerability within mild cognitive impairment has been termed 'Cognitive frailty' (6). Although the existence of cognitive frailty and its definition are still under debate (7), there seems to be broad agreement that cognitively frail people experience accelerated cognitive decline (*i.e.* cognitive dysfunction) without having a form of dementia (8). In this study, people were considered to be cognitively frail when their global cognitive functioning was poor, given their level of education.

It is not yet fully understood how cognitive frailty develops and whether it can be detected at an early stage. However, there are indications that processes of inflammation and oxidative stress are involved and that C–reactive protein (CRP) could potentially serve as a biomarker (9). In addition, in previous studies we observed associations between body mass index (BMI), self–reported health, several biomarkers (*e.g.* β–cryptoxanthin and zeaxanthin) and cognitive frailty (10, 11). Unfavourable changes in these and other (bio)marker levels may precede cognitive frailty. Studying these changes can provide insight into the molecular pathways involved and could point out promising biomarkers for cognitive frailty.

In the Doetinchem Cohort Study (DCS), various markers (*e.g.* self–reported health, BMI) and biomarkers (*e.g.* CRP, urea), have been measured over a time span of at least 15 years (12). Out of these (bio)markers, we identified 17 possibly relevant (bio)markers for cognitive frailty. These are mainly cardiometabolic, inflammatory, and oxidative stress markers. These types of markers have been linked to cognitive decline (13) and could therefore possibly serve as biomarkers for cognitive frailty. Since cognitive frailty arises gradually, it is meaningful to study how (bio)markers 'behave' in the course of developing cognitive frailty. These insights can be helpful for the development of treatment and prevention. Therefore, our aim is to explore the trajectories of several (bio)markers during the development of cognitive frailty in adults and compare these to the trajectories of age– and sex–matched controls. We hypothesise that those who become cognitively frail have more unfavourable trajectories of (bio)markers compared to controls.

Methods

Cohort

The DCS is a longitudinal population–based cohort study starting in 1987–1991 (round 1) examining 7769 men and women aged 20–59 years living in Doetinchem, a town in the Netherlands. Adults who participated in the first round were invited for follow–up examinations in 1993–1997 (round 2, n=6117, mean age: 46 years), 1998–2002 (round 3, n=4918, mean age: 51 years), 2003–2007 (round 4, n=4520, mean age: 56 years), and 2008–2012 (round 5, n=4018, mean age: 60 years). Response rates were 75% or higher in all rounds. Verschuren *et al.* (14) and Picavet *et al.* (12) have described the study design in more detail. All participants gave written informed consent in each round and the study was approved by the Medical Ethics Committee of the University Medical Center Utrecht.

Incident cognitive frailty

Cognitive functioning was assessed among participants aged ≥45 years. Trained personnel carried out the cognitive tests according to a standardised protocol. In rounds 2–5, global cognitive functioning was measured with a neuropsychological test battery assessing three domains: memory function, information processing speed and cognitive flexibility. These were tested using the 15 Words Verbal Learning Test (VLT) (immediate and delayed recall) (15), the Stroop Color–Word Test (16), the Word Fluency Test (17) and the Letter Digit Substitution Test (18). Nooyens *et al.* (19) have described the cognitive tests in more detail. From the separate test scores, one global cognitive functioning score was calculated for each round. Next, the global cognitive functioning scores were transformed into z–scores, based on the mean and standard deviation in round 5, and were adjusted for level of education and for the number of tests performed during follow–up to take a possible learning effect into account. The z–score that corresponded to the 10th percentile in round 5 (z–score=–0.77) was applied as cut–off point for incident cognitive frailty in rounds 2, 3, 4, and 5. This is consistent with the definition used in one of our previous studies, where we also defined people as being cognitively frail when their global cognitive functioning was poor, given their level of education (11). Since the prevalence of frailty naturally increases with age, this was not included in the definition of cognitive frailty. Participants with a score below the cut–off point were considered incident cognitively frail and participants with a score above this value were considered not cognitively frail. As cognitive tests were only performed among participants aged ≥45 years, we

defined participants <45 years as not being cognitively frail. Participants aged ≥45 years without data on cognitive functioning were excluded.

Measurements

Markers

Weight and height (to calculate the BMI), waist circumference, and diastolic and systolic blood pressure were measured according to standard protocols (14). Standardised questionnaires were used to obtain data on self-reported health, depressive symptoms (assessed with the Mental Health Inventory-5 and the Vitality dimension of the *36-Item Short-Form Health Survey)* (20, 21), level of education, smoking status, alcohol consumption, physical activity (categorised using the Cambridge Physical Activity Index) (22), use of anti-hypertensive medication, cholesterol-lowering medication, and glucose-lowering medication.

Biomarkers

Total and high-density lipoprotein (HDL) cholesterol were measured with standardised enzymatic methods. In 2013-2014, standardised enzymatic methods were used to retrospectively determine triglycerides, alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), high sensitivity CRP, uric acid, cystatin C, and creatinine of rounds 2-5 using blood plasma that had been stored in freezers. Participants with only one measurement of the (bio) markers were excluded and participants who were pregnant in a particular round were excluded for that round only. Details of all measurements are described in the **Supplementary Methods**.

Statistical analyses

Matching

The time of incident cognitive frailty was the first examination round in which participants scored below the cut-off point of -0.77. From this point in time, we were able to investigate trajectories with a maximum of 15 years before and 10 years after incident cognitive frailty. There were not enough observations to study the trajectories up to 15 years after incident cognitive frailty. Both cognitive frailty and biomarker levels vary strongly by sex and age. Therefore, we used a matching design in which each incident cognitively frail person was matched to two controls based on sex, age (with 5-year age categories), and examination round. We excluded two incident cognitively frail cases, as no suitable controls could be identified.

(Bio)marker trajectories

Trajectories of (bio)markers for incident cognitively frail cases and controls were analysed using generalized estimating equations (GEE) with an unstructured correlation structure. The GEE analysis was performed for each (bio)marker (dependent variable) separately and with cognitive frailty ('yes' versus 'no') as the main determinant. This resulted in separate estimates (*i.e.* adjusted marginal means) at each point in time for cases and controls. With these estimates, trajectories for the cognitively frail and the controls were constructed. This approach is consistent with the method used by Hulsegge *et al.* (23).

Analyses were stratified for sex and the model included age (linear and quadratic), examination round (categorical variable with round 5 as reference category), and time (categorical variable). Time consisted of six categories ranging from T_{-15} to T_{+10} with T_0 as the moment of incident cognitive frailty. Age was centred at 60 years because this was approximately the mean age at round 5, which resulted in (bio)marker levels of someone who was hypothetically 60 years old. Trajectories of systolic and diastolic blood pressure were adjusted for self-reported anti-hypertensive medication, trajectories of total cholesterol, HDL cholesterol and triglycerides were adjusted for self-reported cholesterol-lowering medication, and trajectories of glucose were adjusted for the self-reported use of glucose-lowering medication.

Triglycerides, ALT, GGT, and CRP had a skewed distribution. Therefore, we log-transformed these biomarkers and reported back-transformed geometric means. Differences between trajectories of cognitively frail people and their controls were tested using an overall interaction term between cognitive frailty and time, and a p-value lower than 0.1 was considered statistically significant. This was obtained via the joint tests for GEE, where differences in slopes were calculated based on five interaction terms (frailty*T_{-15}, frailty*$T_{-10,}$ frailty*$T_{-5,}$ frailty*$T_{+5,}$ frailty*T_{+10}) with T_0 (*i.e.* moment of incident cognitive frailty) as reference category.

To summarise, trajectories up to 15 years before and 10 years after incident cognitive frailty were analysed and compared to controls using GEE with stratification for sex and adjustment for age and, if appropriate, medication use (model 1). We verified whether the results changed after additional adjustment for level of education, depressive symptoms (model 2), BMI, smoking, alcohol intake, and physical activity (model 3). Trajectories of BMI and waist circumference were

not adjusted for BMI.

We performed a sensitivity analysis to study the potential impact of loss to follow–up due to mortality on our results. To this end, we excluded participants who died during the follow–up period (1993–2012) along with their matched case and/or control(s) and compared the results to those obtained in the total population. All analyses were performed using SAS 9.4 software (SAS Institute, Cary, North Caroline, USA).

Results

Population characteristics

After excluding participants ≥45 years without data on cognitive functioning and applying the additional exclusion criteria, 5139 participants remained for further analyses. Over the course of the study, 6 participants were defined as incident cognitively frail in round 2, 116 participants in round 3, 134 participants in round 4, and 202 participants in round 5. In total, 455 participants became cognitively frail of which 303 (67%) were men and 152 (33%) were women. At incident cognitive frailty (T_0), men had an average age of 65.5 (SD 7.6) and women of 66.9 (SD 7.8). Cognitively frail people more often had a low level of education and a slightly higher BMI, waist circumference, and systolic blood pressure than controls (**Table 5.1**). In addition, cognitively frail men and women more often reported to have poor or fair health and their medication use was higher compared to controls.

Table 5.1. Population characteristics for incident cognitively frail men and women and their controls at T_0.

	Men		Women	
	Controls N=606	Cognitively frail N=303	Controls N=304	Cognitively frail N=152
Socio-demographic factors				
Age (years), mean (SD)	63.0 (7.8)	65.5 (7.6)	64.6 (8.0)	66.9 (7.8)
Low level of education, %	42	49	62	70
Lifestyle factors				
Current smoker, %	15	24	17	14
Alcohol consumption (1 or more glasses/week), %	77	76	60	39
Medication use				
Anti-hypertensive, %	24	27	29	34
Cholesterol-lowering, %	17	22	16	20
Glucose-lowering, %	6	10	6	7
Health				
Poor or fair self-reported health, %	16	23	22	34
Mental health (range 0-100), mean (SD)	81.3 (14.3)	78.2 (15.9)	75.6 (15.4)	70.2 (17.3)
Vitality (range 0-100), mean (SD)	71.0 (17.4)	68.9 (18.0)	65.8 (17.4)	59.9 (19.8)
Anthropometric data				
BMI (kg/m^2), mean (SD)	26.9 (3.5)	27.4 (3.7)	27.1 (4.7)	28.3 (5.5)
Waist circumference (cm), mean (SD)	100.7 (10.0)	102.7 (10.5)	93.5 (12.0)	97.4 (13.3)
Systolic blood pressure (mmHg), mean (SD)	134.7 (17.2)	138.0 (18.6)	133.8 (18.5)	137.8 (19.4)
Diastolic blood pressure (mmHg), mean (SD)	81.9 (9.8)	81.4 (9.9)	79.9 (9.7)	80.4 (10.1)
Biomarkers				
Total cholesterol (mmol/L), median (IQR)	5.5 (4.8 to 6.1)	5.3 (4.4 to 6.3)	5.9 (5.1 to 6.7)	5.9 (5.4 to 6.5)
HDL cholesterol (mmol/L), median (IQR)	1.21 (1.00 to 1.44)	1.17 (0.98 to 1.42)	1.51 (1.23 to 1.81)	1.39 (1.17 to 1.67)
Glucose (mmol/L), median (IQR)	5.3 (4.8 to 6.1)	5.4 (4.9 to 6.1)	5.2 (4.8 to 5.8)	5.3 (4.9 to 5.9)
Triglycerides (mmol/L), median (IQR)	1.45 (1.06 to 2.07)	1.55 (1.10 to 2.08)	1.40 (1.05 to 1.90)	1.54 (1.11 to 2.10)
ALT (U/L), median (IQR)	18 (14 to 24)	18 (14 to 24)	16 (12 to 20)	14 (11 to 18)
GGT (U/L), median (IQR)	28 (19 to 40)	27 (20 to 41)	19 (15 to 29)	19 (14 to 26)
CRP (mg/L), median (IQR)	1.17 (0.62 to 2.40)	1.61 (0.81 to 3.07)	1.38 (0.68 to 2.89)	1.50 (0.70 to 2.48)
Albumin (g/L), median (IQR)	45 (43 to 46)	45 (43 to 46)	45 (43 to 47)	45 (43 to 46)
Cystatin C (mg/L), median (IQR)	0.86 (0.78 to 0.97)	0.91 (0.81 to 1.02)	0.81 (0.72 to 0.94)	0.89 (0.79 to 0.99)
Creatinine (umol/L), median (IQR)	84 (76 to 93)	83 (76 to 91)	67 (60 to 74)	69 (62 to 77)
Uric acid (mmol/L), median (IQR)	0.34 (0.29 to 0.38)	0.33 (0.29 to 0.38)	0.27 (0.23 to 0.30)	0.27 (0.22 to 0.32)
Urea (mmol/L), median (IQR)	6.2 (5.4 to 7.1)	6.2 (5.3 to 7.4)	5.8 (4.9 to 6.6)	6.1 (5.1 to 7.2)

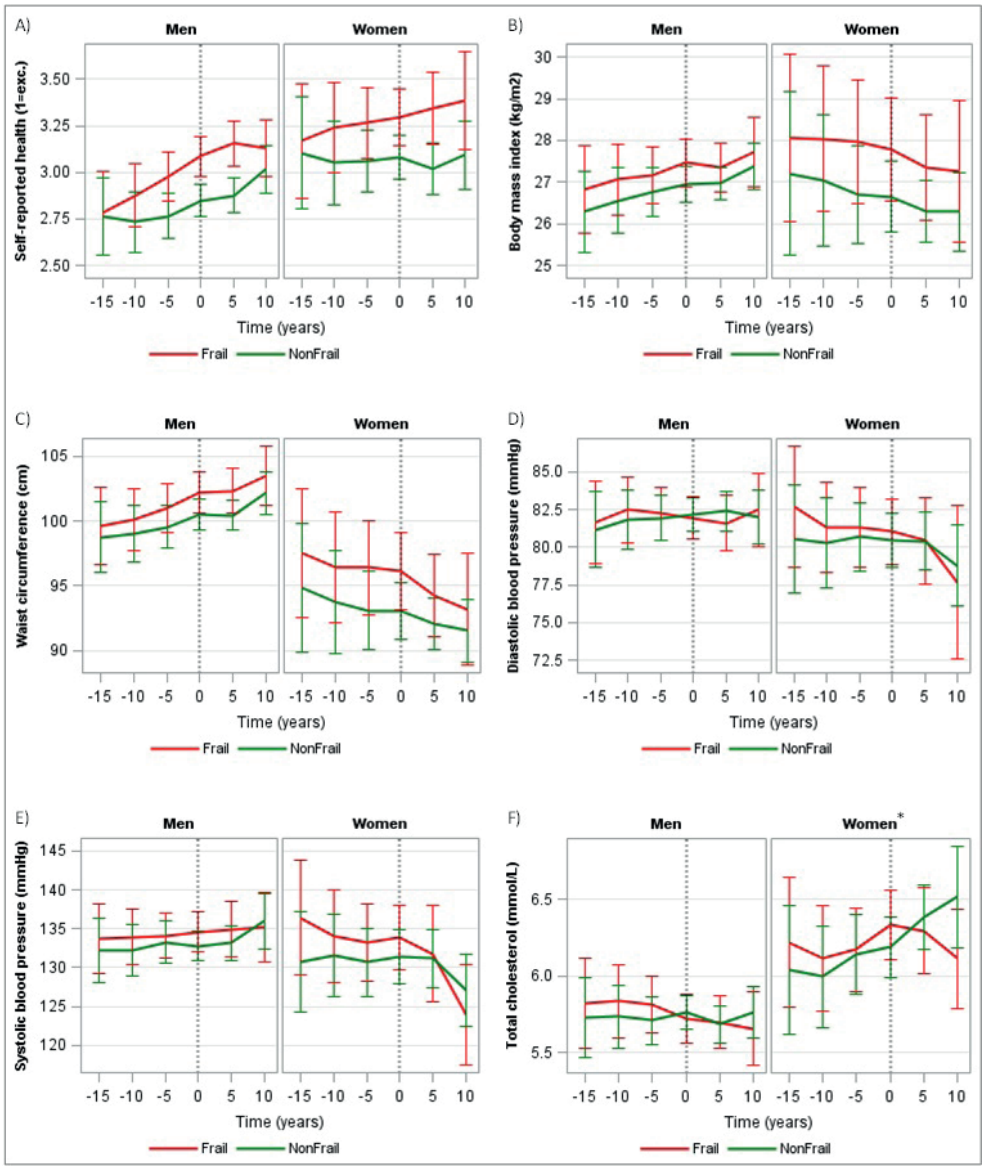
Abbreviations: BMI, body mass index; HDL cholesterol, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; CRP, C-reactive protein.

(Bio)marker trajectories

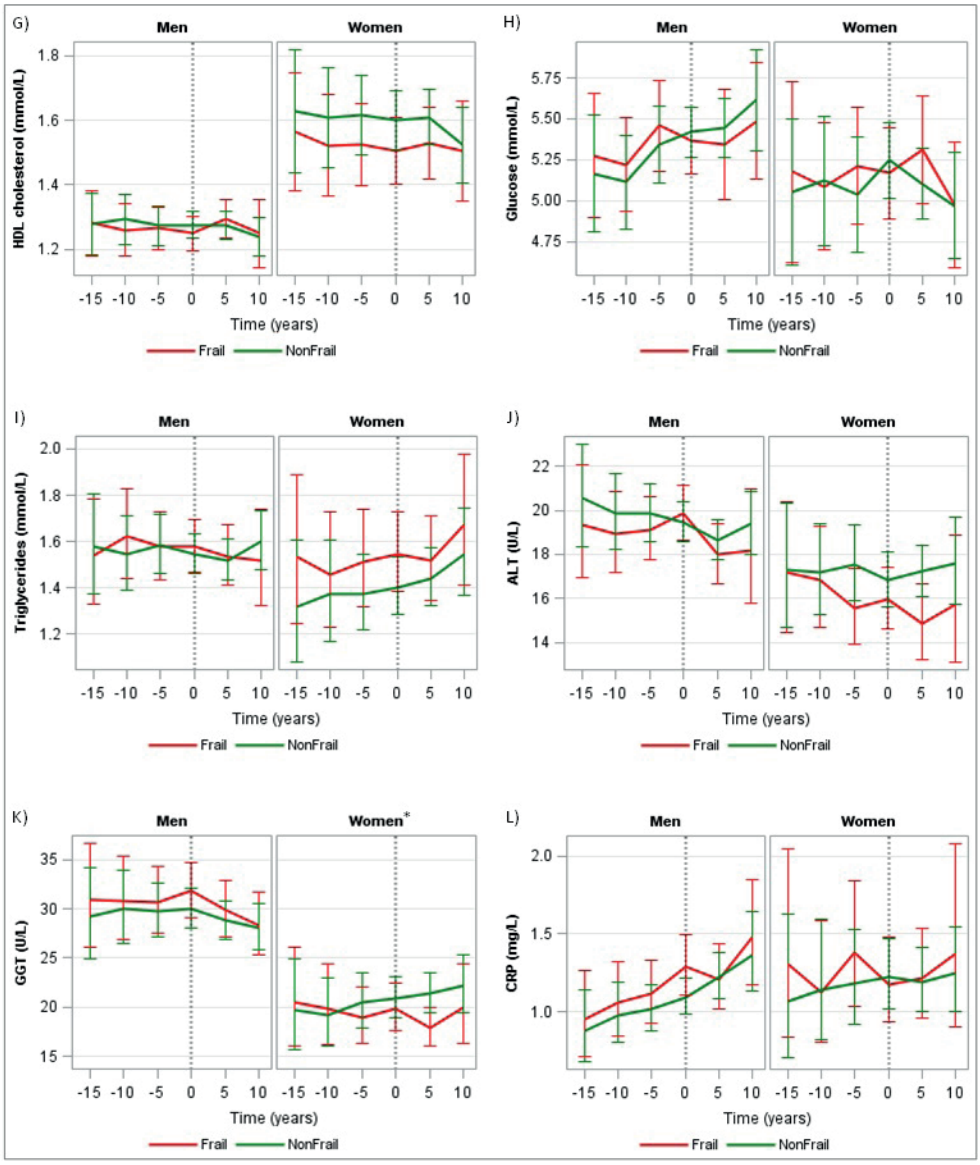
In our main model (*i.e.* model 1), we observed no differences in (bio)marker trajectories between incident cognitively frail men and controls as the trajectories ran parallel and the difference in levels was not statistically significant. In women, we observed differences in the shape of the trajectories of total cholesterol (p=0.067), GGT (p=0.008), and urea (p=0.002) between incident cognitively frail women and controls (**Figure 5.1** and **Supplementary Table 5.1**). Total cholesterol increased before women became cognitively frail and decreased after incident cognitive frailty (T_0), while in controls, total cholesterol levels steadily increased over time. GGT was more or less stable in incident cognitively frail women, while in controls, GGT levels slowly increased from T_{-10} onwards. Urea increased over time in incident cognitively frail women, while in controls, urea levels remained more or less stable.

After further adjustment for level of education and depressive symptoms (model 2), we found no differences in (bio)marker trajectories for men. In women, we found differences in the same biomarker trajectories as in model 1 (*i.e.* total cholesterol, GGT, urea) and additionally observed a difference in trajectories for ALT in women (p=0.046) (**Supplementary Table 5.1**). When further adjusting for BMI and lifestyle factors (model 3), we still observed no differences in (bio)marker trajectories for men. In women, differences in the trajectories for GGT, urea, and ALT between incident cognitively frail women and controls remained, but the difference in total cholesterol trajectory (p=0.109) was just above our threshold (p–value for interaction<0.1).

To explore the potential impact of loss to follow–up due to mortality on our results, we performed a sensitivity analysis using model 1 in which we excluded participants who died (n=130) during the follow–up period (1993–2012). In men, 61 cognitively frail and 40 controls died. In women, 16 cognitively frail and 13 controls died. Excluding these participants, along with their matched case and/or control(s), resulted in the exclusion of 333 participants in total. After exclusion, in men, the trajectories of total cholesterol (p=0.061) and BMI (p=0.082) differed between cognitively frail men and controls. In women, consistent with the observed differences in the total population, the trajectories of total cholesterol (p=0.066), GGT (p=0.018), and urea (p=0.001) differed between cognitively frail women and controls. In addition, the trajectories of ALT (p=0.057), and albumin (p=0.057) also became different between cognitively frail women and controls (**Supplementary Figure 5.1**).



(Figure 5.1 continues)



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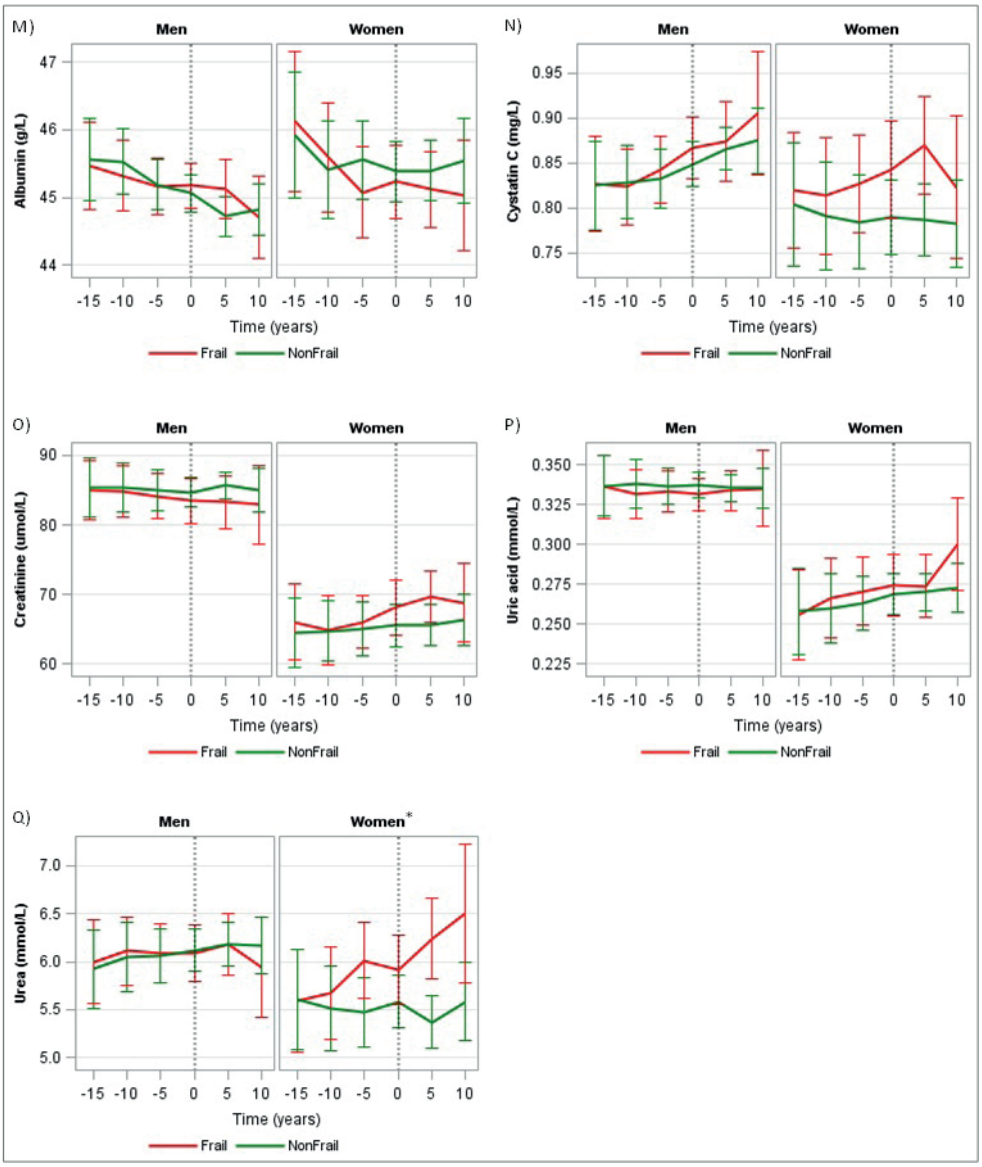

Figure 5.1. Trajectories of (bio)markers for incident cognitively frail men and women and their controls. Abbreviations: HDL cholesterol, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; CRP, C-reactive protein. Geometric means are shown for triglycerides, ALT, GGT and CRP.

Trajectories of self-reported health (A), body mass index (B), waist circumference (C), diastolic blood pressure (D), systolic blood pressure (E), total cholesterol (F), HDL cholesterol (G), glucose (H),

triglycerides (I), ALT (J), GGT (K), CRP (L), albumin (M), cystatin C (N), creatinine (O), uric acid (P), and urea (Q) of incident cognitively frail people (red lines) and controls (green lines) with 95% confidence intervals stratified by sex and corrected for age and, if appropriate, medication use (model 1), where men and women were hypothetically 60 years old at the time of incident cognitive frailty. A difference (p-value for interaction<0.1) in (bio)marker trajectory between those with and without incident cognitive frailty are indicated by an asterisk.

Discussion

Our aim was to examine whether (bio)marker trajectories differ for those who become cognitively frail compared to those who do not over a follow–up of 15 years. In addition, we hypothesised more unfavourable trajectories for those who became cognitively frail compared to controls. In our main model (model 1), we observed no differences in (bio)marker trajectories between incident cognitively frail men and controls as the trajectories ran parallel and the difference in levels was not statistically significant. In women, we observed differences in the shape of the trajectories of total cholesterol, GGT, and urea between incident cognitively frail women and controls.

Against our expectations, most of the 17 (bio)markers included in this study did not show deviating trajectories between those who became cognitively frail and those who did not. This was, for example, surprising for the inflammation marker CRP, since this biomarker has, longitudinally, been linked to physical frailty (24), cognitive decline (25), and risk of dementia (26, 27), and therefore could serve as a biomarker for cognitive frailty. However, also other epidemiological studies report inconsistent findings regarding longitudinal measures of inflammation markers and cognitive decline (28, 29). In addition, Soysal *et al.* (30) showed that higher CRP and interleukin–6 (IL–6) levels are cross–sectionally associated to physical frailty, but not longitudinally. Although this study focused on physical frailty, it seems consistent with our null findings regarding cognitive frailty.

We did find a difference in the trajectory for total cholesterol in women. Total cholesterol increased before women became cognitively frail and decreased after incident cognitive frailty (T_0), while in controls, total cholesterol levels steadily increased over time. In a previous cross–sectional study in MARK–AGE, we also found lower cholesterol levels among people who were already cognitively frail compared to people without frailty (10). Solomon *et al.* (31) found that non–demented people with high total cholesterol levels around age 50 had poorer cognition 20 years later. In addition, their total cholesterol levels decreased after age 50. The pattern Solomon *et al.* describe seems comparable to the trajectory we found in incident cognitively frail women. The decline we observed in total cholesterol levels after becoming cognitively frail could be caused by various factors, one of which is medication use. The analyses were adjusted for self–reported use of cholesterol–lowering medication, but information about medication type, dose

and therapy compliance were not collected and could therefore not be included in these analyses. Hence, it is unclear whether the trajectories differ due to the occurrence of cognitive frailty, or whether other effects, like a treatment effect, is underlying this difference.

We also observed different urea and GGT trajectories in incident cognitively frail women compared to controls. Higher urea levels can be caused by disrupted blood flow through the kidneys for example through heart failure (32). In contrast, we found lower GGT levels in incident cognitively frail women, while heart failure would also cause increased, and not decreased, GGT levels (33). Lower GGT levels could be caused, for example, by the use of clofibrate, a lipid–lowering agent controlling high cholesterol and triglyceride levels. When adjusting for the use of cholesterol–lowering medication for GGT, results remained similar. It is suggested that serum GGT within the normal range is an early marker for oxidative stress (34). Oxidative stress has been suggested to be associated with frailty (35) and increased GGT levels in later life (80 years and older) were associated with cognitive decline (36). However, we unexpectedly observed lower instead of higher GGT levels in cognitively frail women compared to controls, indicating that cognitive frail women might have less oxidative stress. Overall, there does not seem to be a reasonable explanation for the course of these trajectories. We cannot exclude the possibility that the trajectories of GGT and urea are chance findings.

Differences in (bio)marker trajectories were observed among women, but not among men. We found that more men had poor cognitive functioning compared to women (*i.e.* more men were identified as incident cognitively frail than women). On the other hand, differences in cognitive functioning between the incident cognitively frail men and their controls were smaller compared to the differences observed in women. Possibly, the women who we identified as incident cognitively frail were relatively worse off than the incident cognitively frail men, having relatively poorer cognitive function and potentially poorer overall health and therefore we only found differences between trajectories for women.

We studied the potential impact of loss to follow–up due to mortality on our results. This was appropriate since some of the trajectories for women indicated a (rapidly) deteriorating health. For example, decreasing cholesterol levels could be caused by malnutrition and increasing urea levels could be caused by heart failure. As expected, mortality rate was higher among those who became cognitively

frail compared to those who did not. However, excluding these participants did not materially change the results. In fact, the differences in trajectories for total cholesterol, GGT, and urea in women remained the same and seemed therefore rather robust.

We considered people as being cognitively frail when their global cognitive functioning was poor, given their level of education. Since the prevalence of frailty naturally increases with age, this was not included in the definition of cognitive frailty. The most important difference between our operationalisation of cognitive frailty and the classic operationalisation for mild cognitive impairment (MCI) (37) is that our definition of cognitive frailty did not include subjective memory complaints. Also, we did not include self–reported activities of daily living which is part of the MCI definition. Recently, a definition for cognitive frailty was proposed combining physical frailty with MCI (38). Since we have previously observed that it is possible to be cognitively frail without being physically frail (10), we defined cognitive frailty only based on cognitive functioning. In our manuscript, the term 'cognitive frailty' represents cognitive dysfunction, independent of other (physical) limitations and is only based on poor cognitive functioning given the level of education. We explicitly adjusted for level of education, since highly educated people can also experience cognitive dysfunction and this would otherwise be masked by their cognitive reserves.

One of the strengths of this study is that both cognitive functioning, using a comprehensive neuropsychological test battery, and multiple (bio)markers were objectively measured in four rounds over a follow–up of 15 years, making this a unique cohort for studying the relation between (bio)markers and cognitive functioning over time. In addition, all biomarkers of rounds 2–5 were measured in a single run, limiting inter–assay variation.

This study has some limitations. We tried to include all relevant confounders in the analyses (model 3) but residual confounding may still be present. However, adjustment for confounders had a marginal effect in the results. Further, cognitive functioning was not assessed in participants younger than 45 years. We defined these participants as not being cognitively frail under the assumption that people become cognitively frail with advancing age, mostly from 60 years onwards. In addition, since this population was still relatively young, it could have been too young to find (bio)markers for cognitive frailty. Moreover, since cognitive frailty

can be described as a complex syndrome, multiple factors can influence the development of this syndrome. This makes it challenging to identify biomarkers for cognitive frailty. Further, we were unable to exclude participants with dementia. However, given the age–distribution of the cohort, the prevalence and incidence of dementia would be quite low and is therefore unlikely to have influenced our results. Finally, due to the age– and sex–matched study design, the power was limited to analyse differences at any time point. However, more power would probably not lead to other differences in trajectories, because most trajectories run almost parallel and that aspect is unlikely to change with more power.

In conclusion, out of the 17 (bio)markers included in this explorative study, different trajectories between incident cognitively frail women and their controls were found for three biomarkers. However, the relation between these biomarkers and the development of cognitive frailty is unclear. Future studies are needed to confirm these findings. Given the results of this study, we do not yet consider any of the studied (bio)markers as promising (bio)markers for cognitive frailty.

Acknowledgement
We would like to thank the epidemiologists and fieldworkers of the Municipal Health Service in Doetinchem for their contribution to the data collection for this study.

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Supplementary Methods

Biomarkers

Total cholesterol and high-density lipoprotein (HDL) cholesterol were measured until 1998 in non-fasting EDTA-plasma and from 1998 onwards in serum at the Lipid Reference Laboratory of the University Hospital Dijkzigt in Rotterdam, using standardized enzymatic methods. Random plasma glucose was measured with the hexokinase method. In 2013-2014, standardized enzymatic methods (Roche/Hitachi Modular P analyzer, Mannheim, Germany) were used to retrospectively determine biochemical markers from waves 2-5 in non-fasting plasma samples that had been stored at -20 degree Celsius until June 1995 and at -80 degree Celsius from July 1995 onwards. Triglycerides (GPO-PAP assay), alanine aminotransferase (ALT) (kinetic UV assay), gamma-glutamyltransferase (GGT), albumin, and uric acid were measured with a colorimetric method. ALT measurements until June 1995 were recoded as missing (n=2495) because during those years blood plasma was stored at -20 degree Celsius, a temperature at which ALT has poor stability (1). ALT and GGT values greater than three times the upper normal reference were not taken into consideration for the estimation of the trajectories for this particular round since this may indicate liver problems (2). High sensitivity CRP was measured with the principle of particle-enhanced immunological agglutination (Tina-quant CRP). CRP values above 10 mg/L were excluded for the estimation of the trajectories for this particular round because it may indicate an acute-phase response to infection (3). Cystatin C measurement was based on a particle enhanced-turbidimetric immunoassay using reagents from Gentian (Gentian, Moss, Norway) and creatinine was measured with a Creatinine Plus assay (IDMS traceable).

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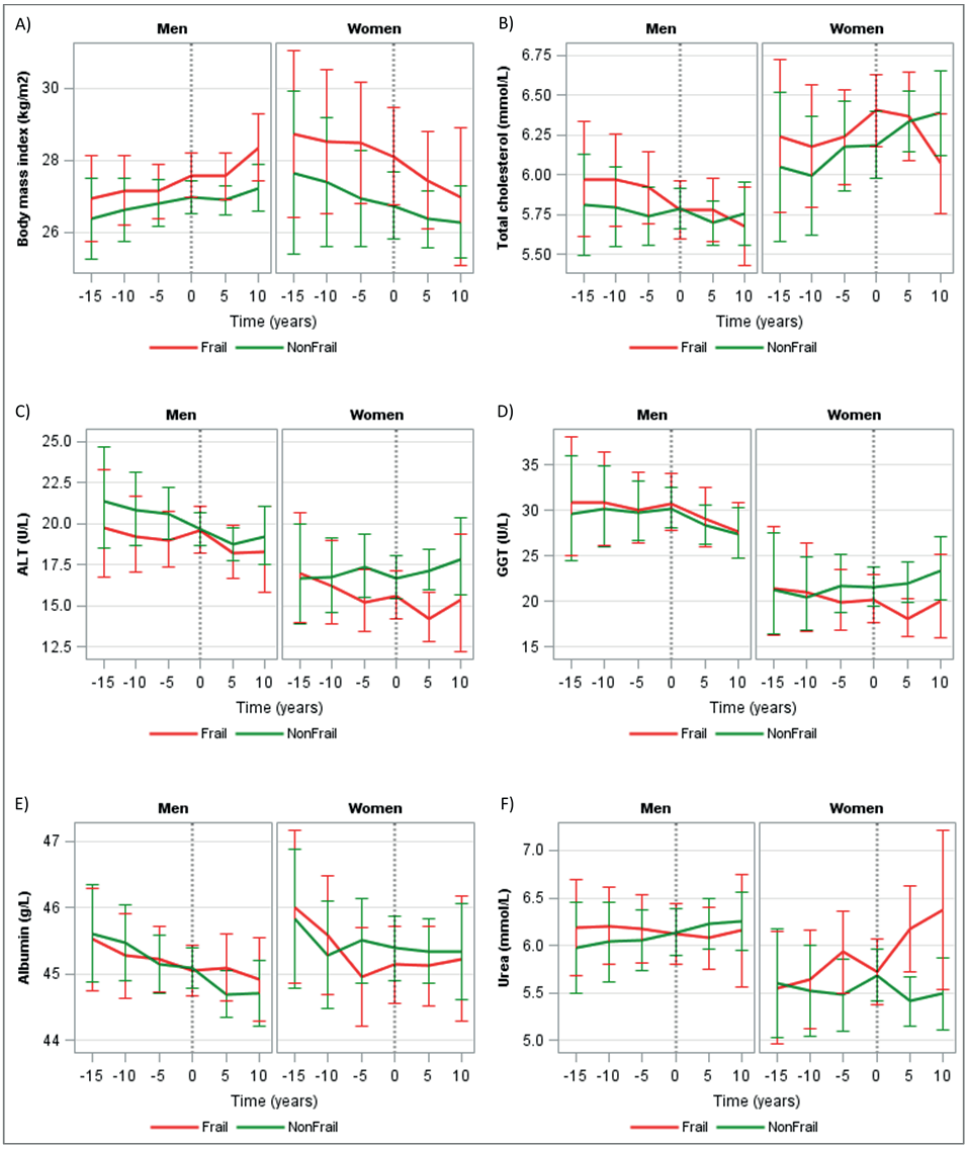
Supplementary Table 5.1. Differences in (bio)marker trajectories between those with and without incident cognitive frailty, stratified by sex.

Markers and biomarkers	Men						Women					
	Model 1		Model 2		Model 3		Model 1		Model 2		Model 3	
	χ2	Pr > ChiSq	χ2	Pr > ChiSq	χ2	Pr > ChiSq	χ2	Pr > ChiSq	χ2	Pr > ChiSq	χ2	Pr > ChiSq
Self-reported health	9.22	0.101	1.93	0.858	1.39	0.926	3.55	0.616	2.91	0.714	2.78	0.734
BMI	2.28	0.809	3.21	0.668	4.36	0.499	2.22	0.818	0.71	0.983	0.51	0.992
Waist circumference	2.79	0.733	2.79	0.733	3.17	0.673	2.12	0.833	2.42	0.788	3.58	0.612
Systolic blood pressure	1.43	0.921	0.95	0.966	0.93	0.968	4.04	0.544	3.11	0.683	2.45	0.785
Diastolic blood pressure	1.97	0.854	2.93	0.712	3.60	0.609	1.38	0.927	0.56	0.990	0.63	0.987
Total cholesterol	8.17	0.147	7.22	0.205	7.37	0.195	**10.32**	**0.067**	**10.59**	**0.060**	9.01	0.109
HDL cholesterol	8.83	0.116	5.92	0.314	5.15	0.398	1.64	0.897	1.98	0.853	2.45	0.784
Glucose	2.66	0.752	3.46	0.629	3.63	0.604	4.02	0.547	3.93	0.559	4.27	0.511
Triglycerides	5.07	0.408	5.36	0.373	4.66	0.459	3.56	0.614	2.36	0.797	2.32	0.803
ALT	6.64	0.249	5.77	0.330	5.87	0.319	8.17	0.147	**11.29**	**0.046**	**12.96**	**0.024**
GGT	2.31	0.804	2.23	0.817	2.34	0.800	**15.71**	**0.008**	**17.48**	**0.004**	**20.05**	**0.001**
CRP	5.02	0.413	5.91	0.315	4.62	0.465	6.96	0.223	7.31	0.199	8.36	0.138
Albumin	7.34	0.196	6.27	0.281	6.54	0.257	8.18	0.147	6.70	0.244	6.10	0.297
Cystatin C	3.35	0.646	2.35	0.799	1.96	0.855	6.63	0.250	5.91	0.315	4.47	0.484
Creatinine	0.96	0.966	2.47	0.781	2.41	0.790	4.24	0.516	5.00	0.416	5.42	0.367
Uric Acid	2.45	0.784	1.90	0.863	1.88	0.866	4.86	0.433	6.42	0.267	5.74	0.333
Urea	1.18	0.946	2.69	0.747	2.87	0.720	**19.08**	**0.002**	**17.14**	**0.004**	**14.60**	**0.012**

Model 1: age (linear and quadratic), examination round, and time
Model 2: age (linear and quadratic), examination round, time, level of education, and depressive symptoms
Model 3: age (linear and quadratic), examination round, time, level of education, depressive symptoms, BMI, smoking, alcohol intake, and physical activity.
Note: The trajectories of BMI and waist circumference were not adjusted for BMI.
Additional adjustment in all three models: Trajectories of systolic and diastolic blood pressure were adjusted for anti-hypertensive medication, trajectories of total cholesterol, HDL cholesterol and triglycerides were adjusted for cholesterol-lowering medication, and trajectories of glucose were adjusted for the use of glucose-lowering medication;
A difference (p-value for interaction<0.1) in (bio)marker trajectory between those with and without cognitive frailty is indicated in bold;
Abbreviations: BMI, body mass index; HDL cholesterol, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; CRP, C-reactive protein.



Supplementary Figure 5.1. Sensitivity analysis for loss to follow-up due to mortality.
Abbreviations: ALT, alanine aminotransferase; GGT, gamma glutamyltransferase. Geometric means are shown for ALT and GGT.
Participants who died during the follow-up period (1993–2012) along with their matched case and/or control(s) were excluded in these analyses (n=333). Trajectories are shown of body mass index (A), total cholesterol (B), ALT (C), GGT (D), albumin (E), and urea (F) of incident cognitively frail people (red lines) and controls (green lines) with 95% confidence intervals stratified by sex and corrected for age and, if appropriate, medication use (model 1), where men and women were hypothetically 60 years old at the time of incident cognitive frailty. A difference (p-value for interaction<0.1) in (bio)marker trajectory between those with and without incident cognitive frailty are indicated by an asterisk.

6

The APOE locus is linked to decline in general cognitive function: 20-years follow-up in the Doetinchem Cohort Study

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Submitted for publication

Abstract

Background

Cognitive decline is part of the normal ageing process. However, some people experience a more rapid decline than others due to environmental and genetic factors. Numerous single nucleotide polymorphisms (SNPs) have been linked to cognitive function, but only a few to cognitive decline. To understand whether cognitive function and cognitive decline are driven by the same mechanisms, we investigated whether 433 SNPs previously linked to cognitive function and 2 SNPs previously linked to cognitive decline are associated with both general cognitive functioning at baseline and general cognitive decline up to 20-years follow-up in the Doetinchem Cohort Study (DCS).

Methods

The DCS is a longitudinal population-based study that enrolled men and women aged 20-59 years between 1987-1991, with follow-up examinations every 5 years. We used data of rounds 2-6 (1993-2017, n=2559). General cognitive function was assessed using four cognition tests measuring memory, speed, fluency and flexibility. With these test scores, standardised residuals (adjusted for sex, age and examination round) were calculated for each cognition test at each round and subsequently combined into one general cognitive function measure using principal component analyses.

Results

None of 435 previously identified variants were associated with baseline general cognitive function in the DCS. Only rs429358-C, a coding apolipoprotein E (APOE) SNP and one of the variants previously associated with cognitive decline, was associated with general cognitive decline in our study as well (p-value=$1x10^{-5}$, Beta=-0.013).

Conclusion

Our study suggests that general cognitive function is possibly regulated by different mechanisms than general cognitive decline.

Introduction

For healthy ageing it is essential to maintain optimal cognitive function throughout the course of life. Preserving good cognitive function is important to remain self-reliant and to prevent or postpone cognitive impairment and dementia (1). Decline in cognitive function is part of the normal ageing process (2), but there is large inter-individual heterogeneity in the rate of decline. Moreover, accelerated cognitive decline is a predictor of dementia and mortality (3–5). Multiple risk factors that negatively affect cognitive function and cognitive decline are known, such as lifestyle factors (physical inactivity, smoking, unhealthy diet), metabolic factors (hypertension, obesity), diabetes mellitus, and a lower educational level (6–9). In addition to these (partly) modifiable factors, genetic factors play an important role. For example, it has been shown that apolipoprotein E (APOE) ε4 carriers have accelerated cognitive decline, while APOE ε2 carriers have decelerated cognitive decline compared to ε3 carriers (10, 11).

Studying the role of genetic factors, through for example genome-wide associations studies (GWASs), could disclose underlying biological mechanisms affecting cognitive health. In addition, it may be of even greater value to identify single nucleotide polymorphisms (SNPs) associated with a decline in cognitive function, rather than with cognitive functioning at a single point in time. This may reveal specific mechanisms behind cognitive decline, preceding cognitive impairment and dementia. In addition, genetic markers can also help identify people who are at risk of (accelerated) cognitive decline and possibly postpone or reduce cognitive decline, for example by increasing the cognitive reserves (12).

Numerous SNPs have been linked to cognitive function. In a recent GWAS by Davies and Lam *et al.*, including over 300,000 participants, 434 independent SNPs (*i.e.* SNPs with a p-value of $\leq 5\times10^{-8}$ and $r^2<0.6$) in 148 genomic loci were associated with general cognitive function cross-sectionally (13). Only a few SNPs have been linked to cognitive decline in GWASs, possibly due to the limited number of cohort studies in which cognitive functioning is repeatedly measured. These studies showed that APOE is associated with cognitive decline in people with different genomic backgrounds (14–16). Surprisingly, the APOE locus was not amongst the associated loci in the GWAS by Davies and Lam *et al.* on cross-sectional cognitive function (13). This raises the question whether different genetically determined pathways influence the level of cognitive function and the

rate of cognitive decline. Therefore, we investigated whether the recently identified independent SNPs by Davies and Lam *et al.* (13) along with two APOE SNPs, were associated with general cognitive functioning at baseline and general cognitive decline in the Doetinchem Cohort Study (DCS) over an extended period of time (up to 20–years follow–up) and with up to five repeated cognition measurements in older adults (n=2559).

Methods

Cohort

The DCS is a longitudinal population-based cohort study including 7769 men and women aged 20-59 years living in Doetinchem between in 1987-1991 (round 1). Adults who participated in the first round were invited for follow-up examinations in 1993-1997 (round 2, n=6117, mean age: 46 years), 1998-2002 (round 3, n=4918, mean age: 51 years), 2003-2007 (round 4, n=4520, mean age: 56 years), 2008-2012 (round 5, n=4018, mean age: 60 years), and 2013-2017 (round 6, n=3438, mean age: 64 years). Response rates were 75% or higher in all rounds. The design of this study has previously been described in more detail (17, 18). All participants gave written informed consent in each round. The study was approved by the Medical Ethics Committee of the Netherlands Organization of Applied Scientific Research and the Medical Ethics Committee of the University of Utrecht according to the guidelines of the Helsinki Declaration.

Measurements

Weight (kg), height (cm), waist circumference (cm), and systolic and diastolic blood pressure (mmHg) were measured according to standard protocols (18). BMI was calculated as weight divided by height squared (kg/m^2). Obesity was defined as having a BMI ≥30 kg/m^2. Standardised questionnaires were used to obtain data on education level (low, intermediate, high), smoking status (never smoker (including former smokers), current smoker), alcohol consumption (never, stopped consuming, <1 glass/week, 1 or more glasses/week), physical activity (categorised using the Cambridge Physical Activity Index; inactive, moderately inactive, moderately active, active) (19), and self-reported health (poor, fair, good, very good, excellent). Education level was measured as the highest level reached during follow-up and categorised into low (intermediate secondary education or less), intermediate (intermediate vocational and higher secondary education) and high (higher vocational education or university). Participants who were physically inactive or moderately inactive were defined as being physically inactive.

Cognitive function was assessed in rounds 2-6 using a neuropsychological test battery among participants aged 45 years and older by trained personnel following a standardised protocol. General cognitive functioning was measured using four tests assessing four domains: memory function, information processing speed, verbal fluency and cognitive flexibility. These four tests were the 15 Words Verbal

Learning Test (VLT) (number of correct words on the delayed recall) (20), the Letter Digit Substitution Test (total of correct answers) (21), the Word Fluency Test (number of correct animals) (22), and the Stroop Color–Word Test (card III, *i.e.* total time needed for the interference test) (23). The cognitive tests have previously been described in more detail (24).

The STROOP test was log-transformed. The other tests had a normal distribution. The first cognition measurement of a study participant was considered the baseline measurement, *i.e.* timepoint zero (T0). Since cognition measurements started when participants had reached the age of 45 and was introduced half-way in round 2 of the DCS, T0 was not confined to a particular round. Most participants had their T0 measurement in round 3, but there were also participants who had their T0 measurement in round 2, 4, 5 or 6 (**Figure 6.1**). Timepoints range from T0–T20 with 5-year intervals.

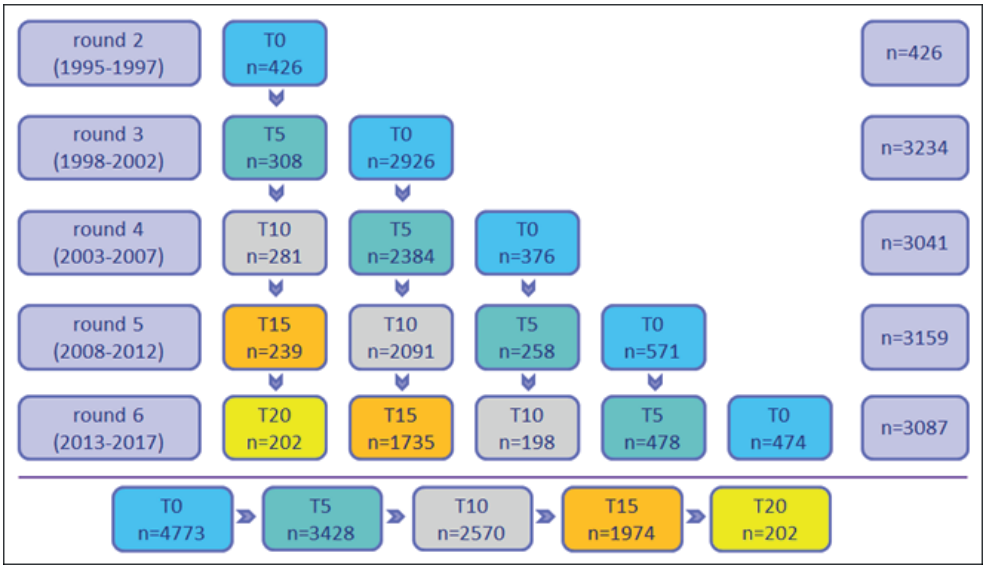

Figure 6.1. Overview of cognition measurements (T0–T20) through rounds 2–6 in the Doetinchem Cohort Study. This figure shows the number of participants with one or more cognition measurements (from T0 up to T20 (time in years)) through rounds 2–6 of the Doetinchem Cohort Study (DCS) during the 20-year follow-up.
Note: This figure includes all participants with cognition measurements in the DCS. In the present study, part of these participants were excluded (see **Figure 6.2**).
Legend: T0= blue, T5=green, T10=grey, T15=orange, T20=yellow.

Genotyping, quality control and imputation

Genomic DNA was isolated from venous blood samples of 5088 individuals at the Dutch National Institute for Public Health and the Environment, and genotyped in the HUman GEnomics Facility (HUGE-F) Rotterdam using the Illumina Infinium Global Screening Array-24 Kit (GSA) (Illumina Inc., San Diego, California, United States of America) (25). The R package GenABEL 1.8-0 (26), was used to perform the quality control for both participants and genetic variants.

Participants were excluded if (**Figure 6.2**): 1) there was a sex mismatch (n=45), 2) samples were duplicates (n=18) or monozygotic twins (n=1) (one individual per pair), 3) heterozygosity rate was high (false discovery rate (FDR) <1%) (n=37), 4) the sample call rate was <95% (n=20), and 5) participants were widely diverged (*i.e.* being genetically distant based on visual inspection of a genomic principal component (gPC) plot) regarding their genetic background based on the first two gPCs that were constructed using a kinship matrix, in two steps (n=114). First, the more distant participants compared to the group as a whole were excluded. Next, as a single iterative step new gPCs were generated in the remaining sample population and additional participants were removed.

Genetic variants were excluded when: 1) minor allele frequencies (MAF) were < 1/(2*5088) (n=5088, this is the population before quality control), *i.e.* the chance of finding the allele once in the study population, thus representing monomorphic variants (n=109129), 2) genotype call rates were <95% (n=8005), 3) variants were not in Hardy-Weinberg equilibrium (FDR<0.2) (n=0), and 4) X-linked markers were likely to be autosomal (n=421). Subsequently, the HRC-1000G-check-bim.pl script from Rayner (27) was used for quality control and to convert the Plink genotype data (28) to separate VCF files per chromosome. This pre-imputation step of quality control filtered additional SNPs based on genotype call rate < 98% (n=15013) and Hardy-Weinberg $p<10^{-6}$ (n=0). Finally, genotypes were imputed to the Haplotype Reference Consortium (HRC) panel (version r1.1 2016) (29) with the Michigan Imputation Server (30) using NCBI Genome Reference Consortium Human Build 37. Pre-phasing was performed on the imputation server with Eagle v2.3 (29) and imputation with Minimac3 (31). After quality control and imputation of the GSA-data, a total of 4853 participants were left for further analyses (**Figure 6.2**).

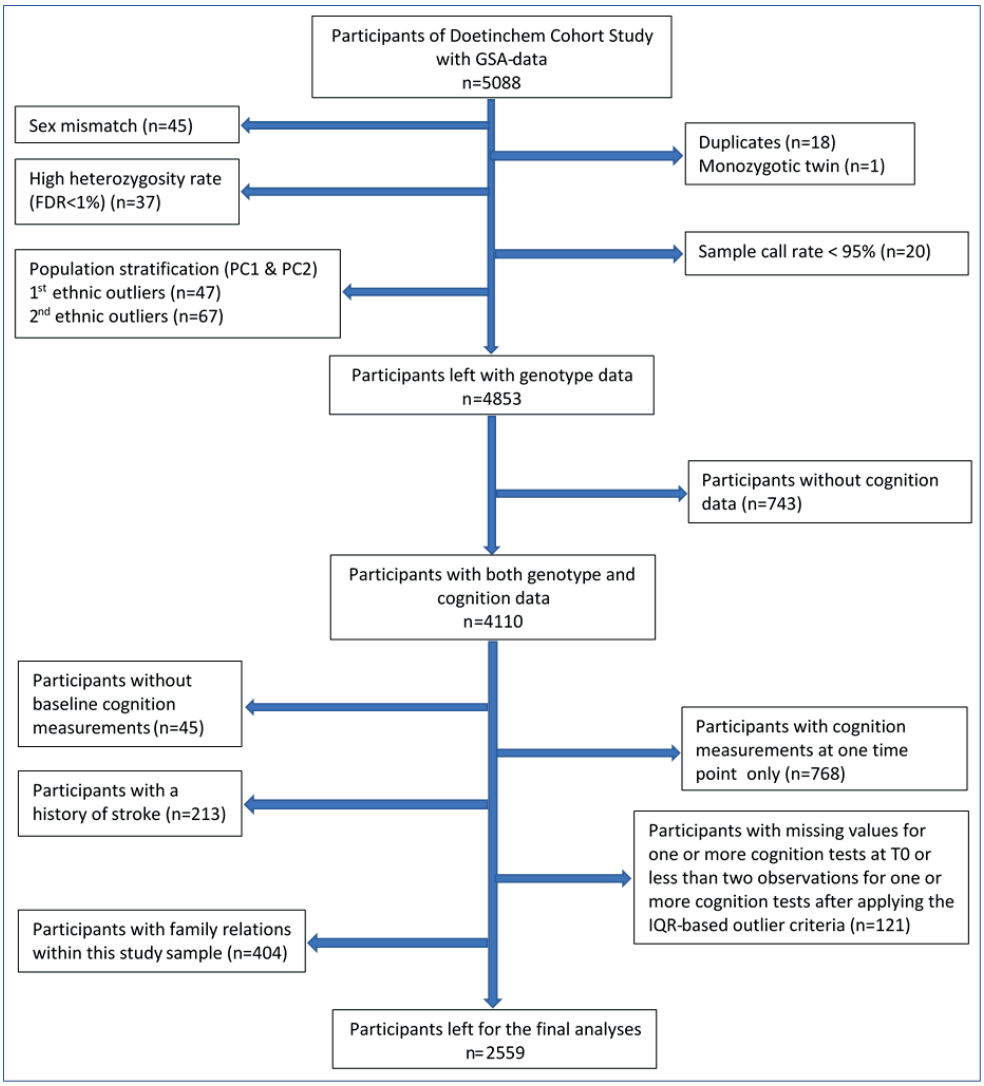

Figure 6.2. Flowchart of quality control steps and exclusion criteria.
Abbreviations: GSA–data, Illumina Infinium Global Screening Array–24 Kit–data; FDR, false discovery rate; PC, principal component; IQR, interquartile range.
This figure shows the number of participants excluded in each step. The quality control steps were performed in order to obtain high quality genotype data of each participant. The exclusion criteria were performed in order to obtain high quality cognition data of each participant.

General cognitive function at baseline and during follow-up

For 4110 participants both genotype and cognition data were available (**Figure 6.2**). Before constructing the general cognitive function measure, participants without measurements on all four cognition tests were made missing for that particular round. Participants were excluded when: the previous step resulted in missing values for the cognition tests at T0 (n=45), had cognition measurements at only one time point (n=768), or had a history of stroke (diagnosed or self-reported (n=213)) at any measurement.

Using the four tests, we constructed a measure of general cognitive function as described by Davies and Lam *et al.* (13) and Trampush *et al.* (32). In brief, sex, age and examination round-adjusted standardised residuals were calculated for each cognition test at each round. Next, these four adjusted test scores were combined into one general cognitive function measure using a principal component analysis. In the Supplementary material, we describe each of these steps in more detail. After all the steps had been taken, also shown in **Figure 6.2**, 2559 participants were left to study the association between SNPs and general cognitive function and decline.

SNP selection

Davies and Lam *et al.*, identified 11.600 SNPs that were statistically significantly (p-value of $\leq 5\times10^{-8}$) associated with general cognitive function cross-sectionally. Of these SNPs, they identified 434 'independent' SNPs (at an $r2$ cut-off < 0.6 (13)). They used NCBI build 37 as reference, which is the same build we used to impute our data. Since in previous GWASs it was shown that APOE gene variants are associated with age related cognitive decline (14, 15), this locus was also part of our interest. Hence, we added rs429358 (chromosome (chr):base pair (bp) 19:45411941) and rs7412 (chr:bp 19:45412079) to our SNP-dataset. Thus, we selected the 434 'independent' SNPs and the two aforementioned exonic APOE SNPs for our study resulting in a total of 436 SNPs. Genetic variants with an imputation quality (R2) below 0.4 or a minor allele frequency (MAF) below 0.01 were not considered for analysis in the present study. Since one of the 434 'independent' SNPs had a MAF<0.01 (rs541507329, chr:bp 1:22428398), the final SNP selection consisted of 435 SNPs based on 433 'independent' SNPs and 2 APOE SNPs.

Statistical analyses

Population characteristics

Descriptive analyses were carried out in RStudio version 1.1.456 (33). Trajectories of general cognitive function up to 20–years follow–up were visualised using ggplot2 version 3.0.0 (34).

Association between SNPs and general cognitive function at baseline

We studied the association between the 435 SNPs (independent variables) and general cognitive function at baseline (T0) (dependent variable). A linear regression model was fitted per SNP and in each model we adjusted for sex, age, and population stratification using the first two gPCs using RVTESTS version 20190205 (35). We corrected for multiple testing based on the Bonferroni adjustment (*i.e.* p–value is $0.05/435=1x10^{-4}$). Hence, a p–value < $1x10^{-4}$ was considered statistically significant.

Since it is known that education level strongly influences the level of general cognitive function, but most likely not that of cognitive decline (36, 37) (see also **Figure 6.4**), we performed a sensitivity analysis in which we studied the effect of education level in the association between SNPs and general cognitive function, with the linear regression model (for baseline cognitive function) as described above to which we added education level as a covariate.

Association between SNPs and general cognitive decline

To study the association between the 435 SNPs (independent variables) and decline in general cognitive function (T0–T20) (dependent variable) we used LME4 version 1.1–17 (38). This package can handle missing values, as long as each participant has at least two observations. A linear mixed model was fitted for each SNP and in each model we adjusted for sex, age at baseline, and population stratification using the first two gPCs. In addition, we included time (0–20 years with 5–year intervals, *i.e.* 5 time points) into the model, and the interaction terms SNP*time and age at baseline*time. For this model we used a correlated random intercept and slope, since participants with higher cognitive function at baseline (intercept) may have a steeper decline (slope), and vice versa. A p–value of 0.1, instead of 0.05, was considered statistically significant since we are now interested in an interaction term instead of a main effect. We corrected for multiple testing based on the Bonferroni adjustment (*i.e.* $0.1/435=2x10^{-4}$) for the interaction term SNP*time. Hence, a p–value $<2x10^{-4}$ was considered statistically significant.

Results

Population characteristics

The study sample consisted of 2559 participants at T0, 2434 at T5, 1832 at T10, 1423 at T15, and 130 at T20. There were 707 participants with two cognition measurements, 556 with three, 1184 with four, and 112 with five cognition measurements, resulting in 8378 observations. Participants did not always have consecutive measurements, meaning that participants could for example have cognition measurements at T0, T10, and T20. The population characteristics at baseline (T0) are summarised in **Table 6.1**.

Figure 6.3 shows the trajectories of all 2559 participants for general cognitive function. We also visualised the trajectories of the sex, age and examination round–adjusted standardised residuals of the four individual cognition tests (**Supplementary Figures 6.1–6.4**).

Table 6.1. Population characteristics at baseline (T_0).

	Characteristics at T_0 N=2559
Socio–demographic factors	
Sex (men)	47 %
Age (years), mean (SD)	54.5 (6.3)
Low education level	41 %
Lifestyle factors	
Physically inactive	23 %
Current smoker	22 %
Alcohol consumption (1 or more glasses/week)	73 %
Anthropometric data	
BMI (kg/m^2), mean (SD)	26.3 (3.9)
Obesity (≥ 30 kg/m^2)	14 %
Waist circumference (cm), mean (SD)	93.9 (11.3)
Systolic blood pressure (mmHg), mean (SD)	129.2 (17.4)
Diastolic blood pressure (mmHg), mean (SD)	81.6 (10.5)
Health	
Poor or fair self–reported health	12 %

Abbreviations: SD, standard deviation; BMI, body mass index.

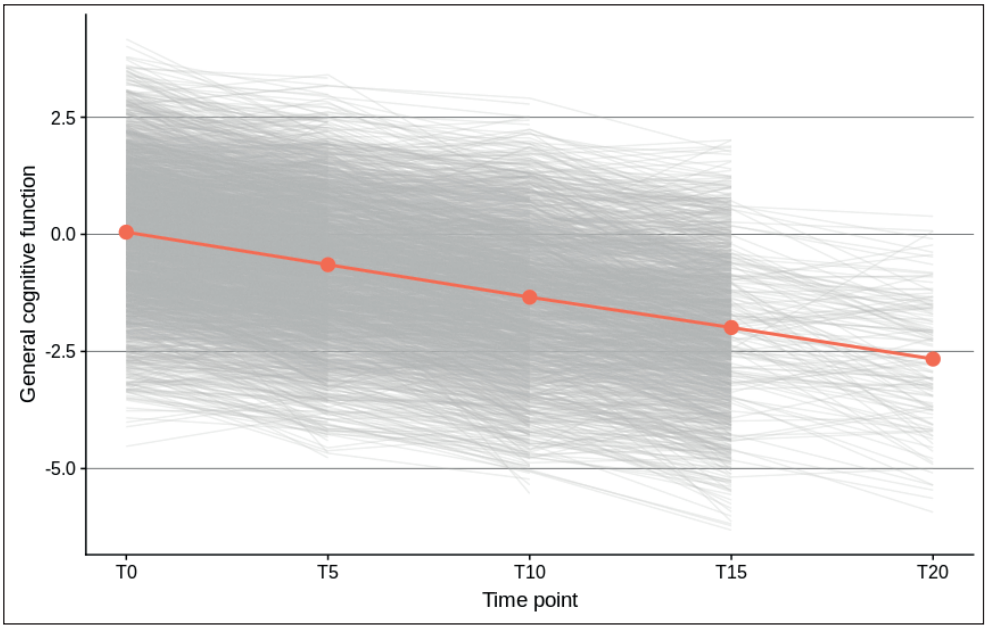

Figure 6.3. Trajectories of general cognitive function. This figure shows the trajectories of general cognitive function up to 20-years follow-up (n=2559). The general cognitive function measure is based on sex, age and examination round-adjusted standardised residuals. T0-T20 represents time in years. The red dots represent mean general cognitive function at each time point.

Association between SNPs and general cognitive function at baseline

After adjustment for multiple testing, none of the 435 SNPs were significantly associated with general cognitive function at baseline (**Supplementary Table 6.1**). The SNP with the lowest p-value (p-value=2x10^{-4}) was rs2782653 located at chr:bp 1:43950265. The alternative allele G was inversely associated with general cognitive function at baseline (Beta=-0.16) compared to the reference allele C.

Additional adjustment for level of education did not change the results (**Supplementary Table 6.1**). **Figure 6.4** shows trajectories of general cognitive function stratified by education level up to 20-years follow-up.

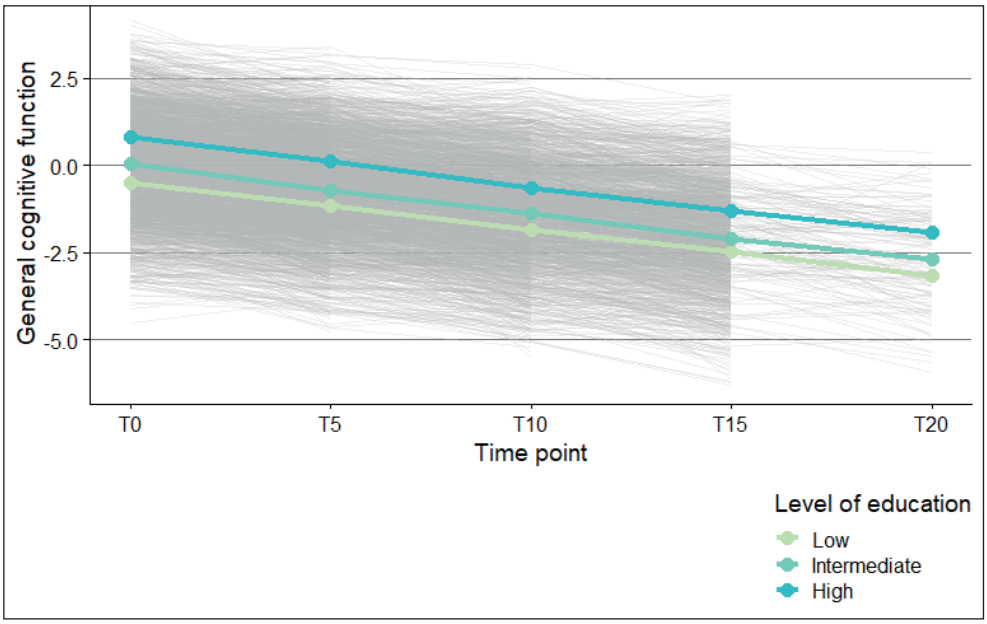

Figure 6.4. Trajectories of general cognitive function stratified by education level. This figure shows the trajectories of general cognitive function stratified by education level up to 20-years follow-up (n=2559). Education level was categorised into low (intermediate secondary education or less), intermediate (intermediate vocational and higher secondary education) and high (higher vocational education or university). T0-T20 represents time in years.

Association between SNPs and general cognitive decline

After adjusting for multiple testing, rs429358, one of the two APOE SNPs, was statistically significantly associated with decline in general cognitive function (p-value=1x10^{-5}, Beta=-0.013) with T as reference allele and C as alternative allele (**Supplementary Table 6.2**). **Figure 6.5** shows the trajectories stratified by rs429358 genotype up to 20-years follow-up. None of the other SNPs were significantly associated with a decline in general cognitive function.

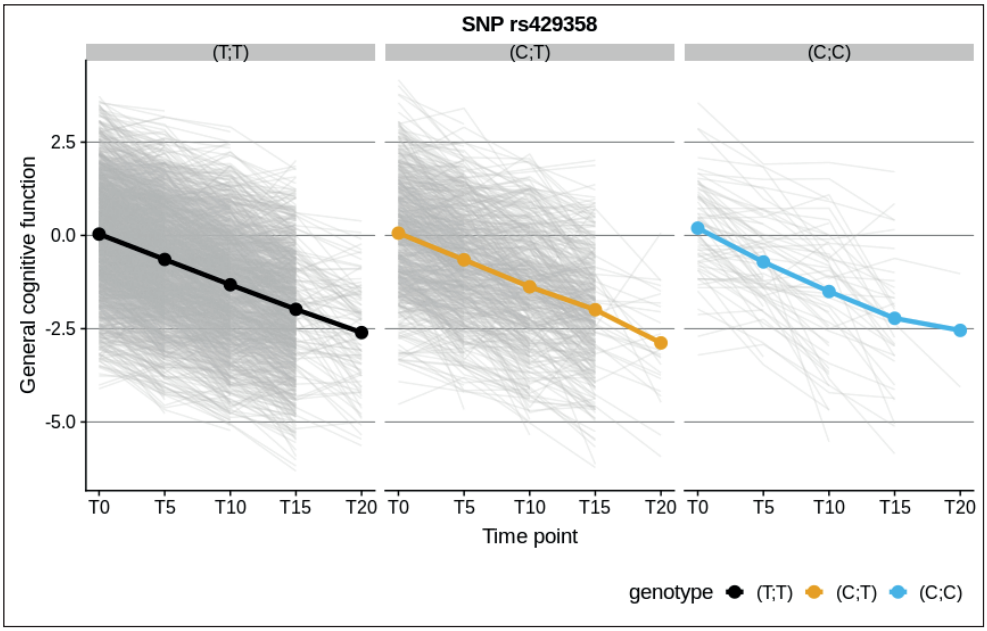

Figure 6.5. Trajectories of general cognitive function stratified by rs429358 genotype. This figure shows the trajectories of general cognitive function stratified by rs429358 genotype up to 20-years follow-up (n=2559). T0–T20 represents time in years.

Discussion

To understand whether cognitive function and cognitive decline are driven by the same mechanisms, we investigated whether 433 SNPs previously linked to cognitive function and 2 SNPs previously linked to cognitive decline were associated with both general cognitive functioning at baseline and general cognitive decline in the DCS. We found that rs429358-C, one of the APOE SNPs, was associated with long-term general cognitive decline, but not with general cognitive function at baseline. None of the other previously identified SNPs for cognitive function or decline were significantly associated with general cognitive function at baseline, nor with cognitive decline.

One of the strengths of the DCS is that cognitive functioning was repeatedly (up to five measurements) and objectively measured with a standardised, comprehensive and validated neuropsychological test battery in adults over an extended period of time (up to 20-years follow-up), making this a unique cohort to study cognitive ageing. In addition, for this study we used data from a single cohort, *i.e.* the DCS, and used an identical neuropsychological test battery at all examinations resulting in a more homogenous outcome than in a meta-analysis where the included cohorts often use different neuropsychological test batteries. A limitation to this study was the number of included participants. After applying all exclusion criteria there were 2559 participants left for the association analyses. The number of participants may have limited our power since general cognitive function is a complex trait for which a higher number of participants is likely to be needed to gain sufficient power (39).

We were unable to replicate the findings of Davies and Lam *et al.* (13), *i.e.* none of their 433 'independent' SNPs were associated with general cognitive function at baseline in our study. There are some explanations for these different results that need to be discussed. First, the age range in the DCS was 45-74 years at baseline, while the age range in the study of Davies and Lam was 16-102 years. Cognitive function changes over the course of life and has an inverted U-shape in which the brain and cognitive functions of adolescents still develop (40), while older adults may experience cognitive decline due to ageing of the brain (41). In both the DCS study as well as in the study of Davies and Lam *et al.*, adjustment for age was performed. However, since age has such a strong impact on the level of cognitive function, it could still have affected the obtained results differentially. Second,

the heterogeneous phenotype could also have influenced our results regarding baseline cognitive function. General cognitive function is a heterogenous outcome in two respects. 1) participants can score differently on the individual cognitive functioning tests while they can have the same score on the overall measure. For example, one participant can have a low memory test score, while another participant can have a low executive functioning test score. This can lead to the same overall score, *i.e.* general cognitive function, while they score differently on the underlying tests. Between studies, therefore, the average total score may not reflect similar underlying functioning of the participants. 2) frequently, different tests are used to measure cognitive function in different cohort studies. In the DCS, the same neuropsychological test battery was used through all rounds for all participants. In the meta-analyses of Davies and Lam *et al.*, data of multiple cohorts were used in which cognitive function was tested using different test batteries. As a result, phenotypic heterogeneity may be larger in the Davies and Lam study. Possibly, the phenotype of Davies and Lam *et al.* represents different aspects of general cognitive function compared to our phenotype. Finally, we cannot exclude the possibility that we had limited power to detect the associations found in the Davies and Lam study. Our study sample was considerably smaller compared to the number of participants included in the Davies and Lam study. Hence, our phenotype was probably more homogeneous than the phenotype of Davies and Lam *et al.*, but this may not have outweighed the smaller number of observations.

The SNP with the lowest p-value (but not statistically significant after adjustment for multiple testing) associated with general cognitive function at baseline was rs2782653 (p-value=$2x10^{-4}$, beta=-0.16) located at chr:bp 1:43950265. The C allele was associated with a lower general cognitive function at baseline, which was similar to the effect found in the Davies and Lam study. Rs2782653 was previously found to be associated with lower attained education level in the UK Biobank (42, 43). A sensitivity analysis, in which we adjusted for education level, did not change our results.

Our result for the association between rs429358 in the APOE coding region and cognitive decline is in line with the three GWASs on cognitive decline (14–16). De Jager *et al.* (15) identified rs4420638, another SNP at the APOE locus which is in strong LD (r^2=0.7) with rs429358 (44). Davies *et al.* (14) also found rs429358 to be significantly associated with cognitive decline.

Our findings suggest that it might very well be possible that cognitive decline is driven by different mechanisms than the level of cognitive function at a certain time point. This hypothesis is supported by our finding that the APOE locus was significantly associated with cognitive decline, but not to cognitive function at baseline. This was also found in two studies of Davies, in which the APOE locus was not associated with cognitive function (13), but was associated with cognitive decline (14). In addition, this was confirmed in a recent study that showed that APOE ε4 status was not statistically significant association with cognition level, but was associated with cognitive decline (45). Another recent study showed that APOE ε4 carriers have accelerated breakdown of the blood–brain barrier (BBB) in the hippocampus and medial temporal lobe contributing to cognitive decline independent of Alzheimer's disease pathology (46). It could be hypothesised that APOE ε4 affects cognitive decline, but not the level of cognitive function, via breakdown of the BBB.

In conclusion, we confirm that rs429358, and thereby the APOE locus, is significantly associated to general cognitive decline, but not to general cognitive function at baseline. Baseline general cognitive function could be regulated by different mechanisms than general cognitive decline.

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Supplementary Methods

General cognitive function at baseline and during follow-up

Using the four tests, we constructed a measure of general cognitive function as described by Davies and Lam *et al.* (1) and Trampush *et al.* (2). In brief, sex, age and examination round–adjusted standardised residuals were calculated for each cognition test at each round. Next, these four adjusted test scores were combined into one general cognitive function measure using a principal component analysis. In de section below, we describe each of these steps in more detail.

Age was centred at 55 years because this was the mean age at T0 (*i.e.* baseline cognition measurement). Next, every individual cognitive test score was adjusted for sex, centred baseline age, (centred baseline age)², sex*centred baseline age, and sex*(centred baseline age)² at T0 using linear regression analyses (model 1). In model 2, each individual cognitive test score was, on top of the variables in model 1, also adjusted for the number of cognition measurements and time at T0–T20 using linear mixed models with correlated random slope and intercept. This was done to account for a potential learning effect because the neuropsychological test battery was identical in every examination round. Next, the residuals were calculated, for each participant on each test and each time point, by extracting the value based on sex and age at T0 (model 1) from the value adjusted for number of cognition measurements at T0–T20 (model 2). Subsequently, the residuals were standardised (*i.e.* creating z-scores) by using the mean and SD of T0 for all time points (*i.e.* T0–T20). The standardised residuals were checked per cognition test and per time point. Standardised residuals with a distance from the nearest quartile that was greater than 1.5 times the interquartile range (IQR) in either direction were made missing, which is in line with Trampush *et al.* (3). When this resulted in missing values for one or more cognition tests at T0 or in cognition measurements at only one time point, participants were excluded (n=121). Next, the Cronbach's Alpha was calculated for each time point resulting in Alpha's between 0.65 and 0.69. The Cronbach's Alpha is a measure for internal consistency and is used in this study to test whether the different cognitive tests measure the same construct (*i.e.* general cognitive function). Dropping one of the four cognition tests did not improve the Alpha at any time point (*i.e.* T0–T20), therefore all four cognition tests were used for cognition principal component analyses (cPCAs) to construct the general cognitive function measure. The individual cPCA scores were calculated at T0. The percentage of explained variance of the first unrotated

cPCA was 49.6%. Next, a linear regression model was fitted with the first unrotated cPCA as dependent variable and the standardised residuals of the four cognition tests as independent variables to obtain the estimates of these four tests at T0. Subsequently, by using the estimates of the linear regression analyses at T0 in a prediction model, the cPCA scores at T5–T20 could be calculated. Just as for calculating the z-scores, T0 was used as basis for calculating the cPCA scores. Finally, to overcome relatedness regarding first and second degree kinship (*i.e.* an identity by descent (IBD) >0.185 between pairs of individuals) participants with an IBD above the cut-off point and having the lowest call-rate were excluded (n=404) (4). After all the steps had been taken, also shown in **Figure 6.2** of the manuscript, 2559 participants were left to study the association between SNPs and general cognitive function and decline.

All previous steps were performed in R with RStudio interface version 1.2.1335 (5) using the following packages: haven version 2.0.0, dplyr version 0.7.8, tidyr version 0.8.2, lme4 version 1.1-20, ggplot2 version 3.1.0, xlsx version 0.6.1, psych version 1.8.12, factoextra version 1.0.5 , FactoMineR version 1.41, and tibble version. 2.0.1.

Supplementary references

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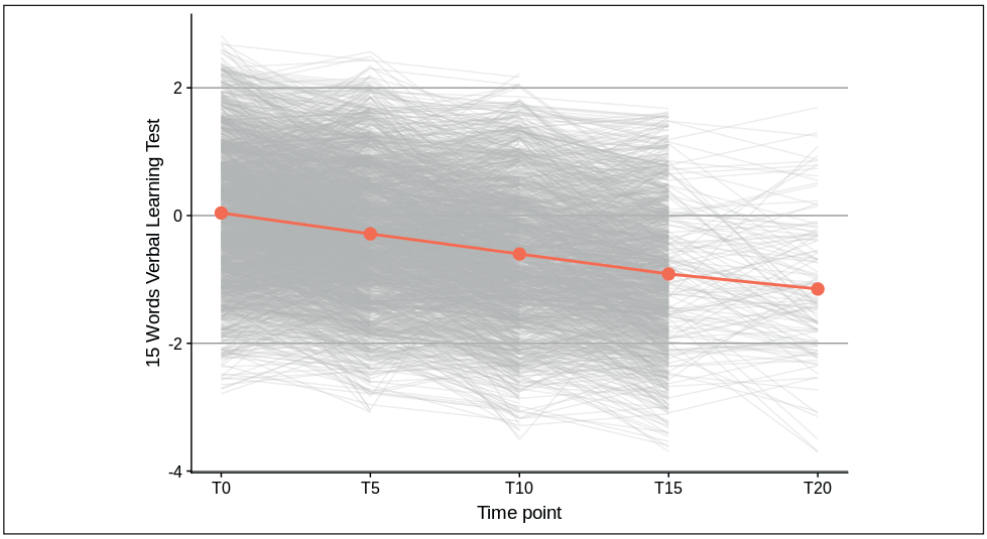
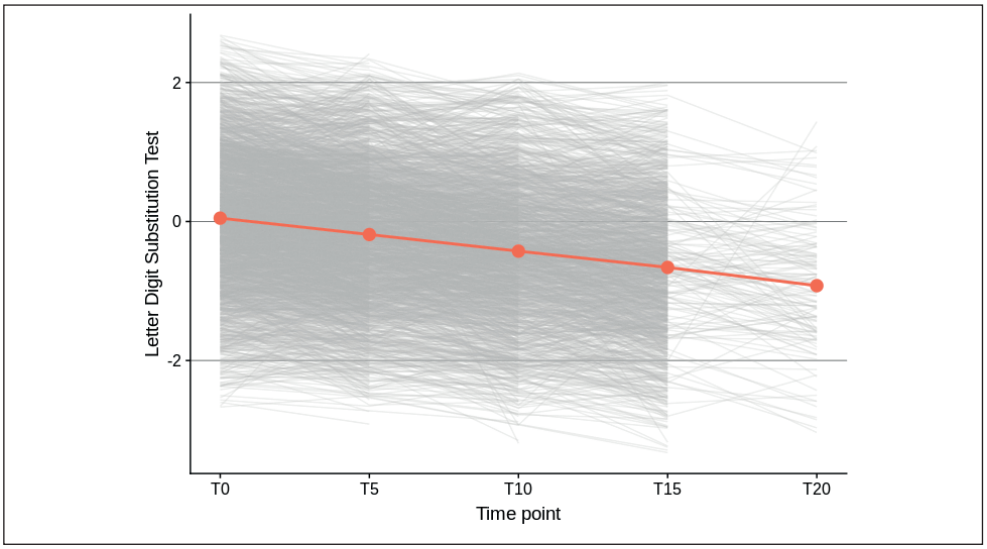
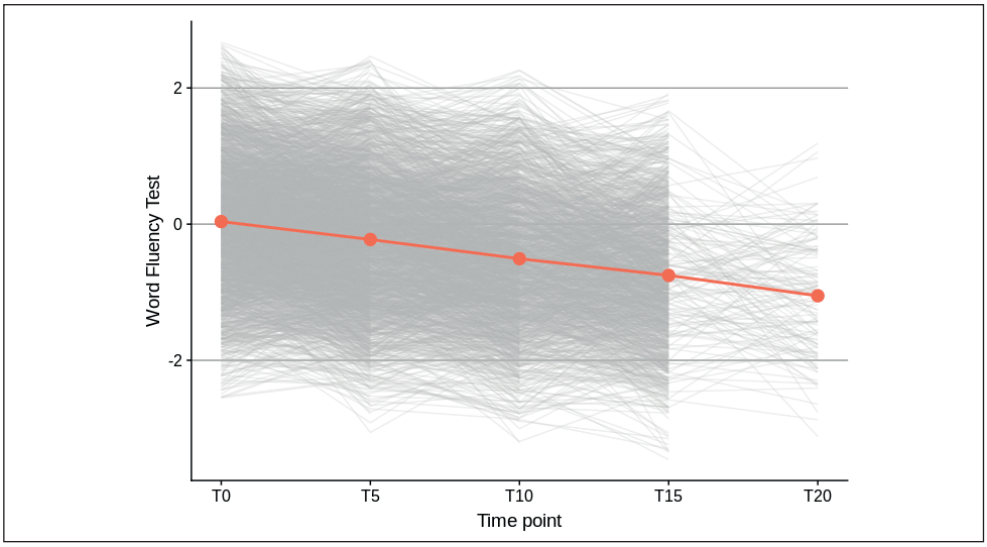
Supplementary Figures 6.1–6.4



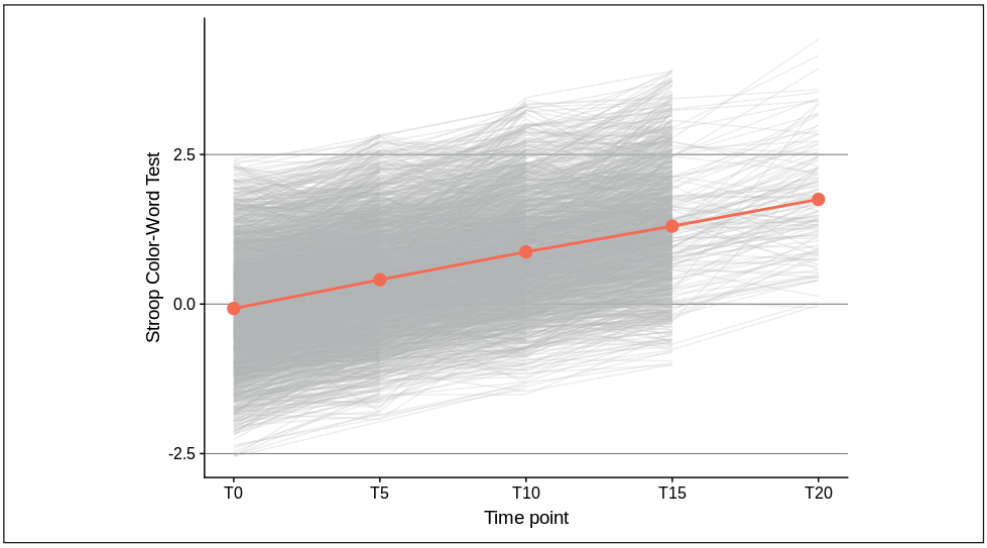
Supplementary Figure 6.1. Trajectories of the 15 Words Verbal Learning Test. Sex, age and examination round–adjusted standardized residuals of the 15 Words Verbal Learning Test up to 20–years follow–up. T0–T20 represents time in years.



Supplementary Figure 6.2. Trajectories of the Letter Digit Substitution Test. Sex, age and examination round–adjusted standardized residuals of the Letter Digit Substitution Test up to 20–years follow–up. T0–T20 represents time in years.



Supplementary Figure 6.3. Trajectories of the Word Fluency Test. Sex, age and examination round-adjusted standardized residuals of the Word Fluency Test up to 20-years follow-up. T0-T20 represents time in years.



Supplementary Figure 6.4. Trajectories of the Stroop Color-Word Test. Sex, age and examination round-adjusted standardized residuals of the Stroop Color-Word Test up to 20-years follow-up. T0-T20 represents time in years.

Supplementary Table 6.1 Association between 435 SNPs and general cognitive function.

Association between 435 SNPs and general cognitive function														
							Results for general cognitive function				Results for general cognitive function adjusted for education level			
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value
rs2782653	1	43950265	C	G	2559	0,7111	–	-0,1622	0,0443	0,0002	–	-0,1438	0,0407	0,0004
rs2819336	1	44015809	T	C	2559	0,6297	–	-0,1323	0,0404	0,0010	–	-0,1072	0,0372	0,0039
rs11717383	3	52287468	G	T	2559	0,5301	+	0,1056	0,0395	0,0075	+	0,0859	0,0364	0,0183
rs187084	3	52261031	A	G	2559	0,4369	+	0,1061	0,0398	0,0076	+	0,1079	0,0366	0,0032
rs3095340	6	30726939	A	C	2559	0,1456	+	0,2380	0,0900	0,0082	+	0,2541	0,0828	0,0022
rs13105581	4	103228830	C	T	2559	0,0567	–	-0,2216	0,0849	0,0090	–	-0,2093	0,0781	0,0074
rs6819372	4	67970101	A	G	2559	0,5042	+	0,1011	0,0400	0,0116	+	0,0678	0,0369	0,0660
rs1428472	5	66500842	A	C	2559	0,3147	+	0,1072	0,0430	0,0127	+	0,1042	0,0396	0,0084
rs67474621	1	72577950	A	T	2559	0,4890	–	-0,1027	0,0414	0,0132	–	-0,0914	0,0381	0,0166
rs4852252	2	71539301	T	C	2559	0,5876	+	0,0964	0,0404	0,0171	+	0,0864	0,0372	0,0202
rs9384679	6	108864419	C	T	2559	0,3729	–	-0,0962	0,0407	0,0181	–	-0,0865	0,0374	0,0208
rs10914462	1	32125943	A	G	2559	0,4138	–	-0,0942	0,0400	0,0186	–	-0,0674	0,0368	0,0673
rs12489732	3	52566820	C	A	2559	0,3446	+	0,0930	0,0411	0,0235	+	0,0800	0,0378	0,0344
rs4587178	6	98421991	T	C	2559	0,4020	–	-0,0901	0,0402	0,0250	–	-0,0522	0,0370	0,1586
rs4852259	2	71682950	C	G	2559	0,4098	+	0,0898	0,0401	0,0251	+	0,0782	0,0369	0,0340
rs3129977	6	30745800	A	C	2559	0,1224	+	0,2620	0,1172	0,0253	+	0,2427	0,1078	0,0243
rs11759522	6	3450814	C	G	2559	0,4246	–	-0,0854	0,0394	0,0300	–	-0,0740	0,0362	0,0412
rs11970030	6	20930113	G	C	2559	0,1021	–	-0,1395	0,0661	0,0349	–	-0,1755	0,0609	0,0039
rs36004779	5	59603880	A	G	2559	0,1659	+	0,1111	0,0537	0,0385	+	0,1000	0,0494	0,0429
rs17199964	4	102707791	G	A	2559	0,0615	–	-0,1740	0,0841	0,0386	–	-0,1680	0,0774	0,0299
rs4976976	8	143311653	G	A	2559	0,4021	+	0,0833	0,0404	0,0393	+	0,0662	0,0372	0,0753
rs79046494	16	28527326	G	A	2559	0,3534	–	-0,0827	0,0410	0,0436	–	-0,0737	0,0377	0,0509
rs34811474	4	25408838	G	A	2559	0,2222	+	0,1027	0,0510	0,0441	+	0,1159	0,0469	0,0135
rs9467777	6	26534616	C	A	2559	0,4384	–	-0,0829	0,0412	0,0441	–	-0,0640	0,0379	0,0914
rs145104523	21	40644110	C	T	2559	0,1409	+	0,1176	0,0588	0,0453	+	0,1332	0,0541	0,0138
rs11191270	10	104124605	C	T	2559	0,1910	–	-0,1012	0,0510	0,0473	–	-0,0741	0,0470	0,1147
rs4731992	7	133702097	A	G	2559	0,7823	+	0,0966	0,0489	0,0479	+	0,0729	0,0450	0,1050
rs2726032	16	28338043	T	C	2559	0,4277	–	-0,0795	0,0402	0,0480	–	-0,0748	0,0370	0,0432
rs9398173	6	109000316	T	C	2559	0,7192	+	0,0872	0,0442	0,0485	+	0,0951	0,0407	0,0194
rs6568547	6	108856378	C	T	2559	0,2071	–	-0,0954	0,0485	0,0493	–	-0,0581	0,0447	0,1932
rs2925635	16	28622993	A	G	2559	0,6097	+	0,0823	0,0424	0,0521	+	0,0884	0,0390	0,0234
rs13262595	8	143316970	A	G	2559	0,5508	+	0,0762	0,0396	0,0541	+	0,0578	0,0364	0,1127

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function														
							Results for general cognitive function				Results for general cognitive function adjusted for education level			
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value
rs7365380	1	173625710	T	C	2559	0,5033	–	-0,0757	0,0395	0,0553	–	-0,0660	0,0363	0,0694
rs4690029	4	2722815	T	C	2559	0,4603	–	-0,0771	0,0403	0,0554	–	-0,0544	0,0371	0,1424
rs7487854	12	50150266	G	T	2559	0,0683	+	0,1544	0,0820	0,0597	+	0,1511	0,0754	0,0452
rs2517592	6	30093137	C	T	2559	0,2181	–	-0,1154	0,0616	0,0612	–	-0,0793	0,0567	0,1622
rs74370218	17	57095212	C	T	2559	0,3646	–	-0,0769	0,0414	0,0629	–	-0,0749	0,0381	0,0491
rs4687662	3	52870324	T	C	2559	0,2189	–	-0,0889	0,0479	0,0637	–	-0,1063	0,0441	0,0159
rs11577684	1	44254280	C	G	2559	0,3547	+	0,0729	0,0405	0,0718	+	0,0538	0,0373	0,1486
rs4812279	20	59777491	C	A	2559	0,6140	+	0,0737	0,0410	0,0722	+	0,0517	0,0378	0,1710
rs1158660	17	43945288	G	A	2559	0,2960	+	0,0783	0,0438	0,0738	+	0,0403	0,0403	0,3173
rs1787003	18	13104251	A	G	2559	0,5840	–	-0,0710	0,0399	0,0748	–	-0,0687	0,0367	0,0610
rs2526376	17	56427142	A	C	2559	0,6122	+	0,0718	0,0406	0,0769	+	0,0753	0,0373	0,0437
rs230489	4	103388441	T	C	2559	0,0892	–	-0,1216	0,0690	0,0778	–	-0,1129	0,0635	0,0752
rs9379870	6	26374410	A	G	2559	0,2860	+	0,0807	0,0459	0,0790	+	0,0729	0,0423	0,0845
rs62194185	2	161908278	C	T	2559	0,3352	–	-0,0733	0,0418	0,0797	–	-0,0296	0,0385	0,4430
rs2844790	6	30094113	C	G	2559	0,1303	+	0,1715	0,0985	0,0815	+	0,1877	0,0906	0,0382
rs749694	10	103519784	A	G	2559	0,5620	–	-0,0700	0,0402	0,0819	–	-0,0604	0,0370	0,1030
rs2284871	2	162888055	C	T	2559	0,3279	+	0,0719	0,0420	0,0872	+	0,0576	0,0387	0,1367
rs1702877	12	56427808	C	T	2559	0,3390	+	0,0703	0,0413	0,0887	+	0,0811	0,0380	0,0329
rs9879090	3	52648265	T	C	2559	0,5652	+	0,0682	0,0402	0,0896	+	0,0673	0,0370	0,0689
rs34592089	4	102926923	G	A	2559	0,0296	–	-0,2021	0,1201	0,0923	–	-0,2170	0,1105	0,0495
rs3755799	3	52809193	G	A	2559	0,3471	+	0,0685	0,0408	0,0934	+	0,0624	0,0376	0,0966
rs12918191	16	50945156	A	G	2559	0,2297	–	-0,0761	0,0461	0,0990	–	-0,0569	0,0424	0,1799
rs11209943	1	72750500	A	G	2559	0,5762	–	-0,0651	0,0395	0,0993	–	-0,0778	0,0364	0,0323
rs62422686	6	98661637	G	C	2559	0,2891	+	0,0729	0,0443	0,0997	+	0,0314	0,0408	0,4408
rs11126396	2	73565143	C	T	2559	0,2933	–	-0,0705	0,0432	0,1031	–	-0,0523	0,0398	0,1885
rs28657585	3	48736216	A	T	2559	0,1352	+	0,0930	0,0573	0,1046	+	0,0784	0,0527	0,1369
rs58859557	1	44010456	C	T	2559	0,0655	+	0,1328	0,0823	0,1066	+	0,1106	0,0758	0,1444
rs1895471	19	31942961	A	G	2559	0,3352	–	-0,0663	0,0415	0,1100	–	-0,0684	0,0382	0,0732
rs1363119	19	18444809	A	G	2559	0,5363	+	0,0653	0,0409	0,1105	+	0,0472	0,0376	0,2096
rs2428507	6	30229306	A	G	2559	0,3863	–	-0,0740	0,0464	0,1106	–	-0,0539	0,0427	0,2064
rs71413877	2	100924822	G	A	2559	0,0347	+	0,1730	0,1105	0,1175	+	0,1165	0,1017	0,2521
rs12713315	2	56458509	T	C	2559	0,2941	–	-0,0668	0,0427	0,1182	–	-0,0712	0,0393	0,0700
rs6772177	3	52497778	C	T	2559	0,1473	–	-0,0883	0,0567	0,1192	–	-0,1068	0,0522	0,0406

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function														
							Results for general cognitive function				Results for general cognitive function adjusted for education level			
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value
rs9949444	18	50695904	A	C	2559	0,3626	+	0,0627	0,0403	0,1197	+	0,0417	0,0371	0,2605
rs62037363	16	28865042	T	C	2559	0,4170	–	-0,0609	0,0396	0,1245	–	-0,0543	0,0365	0,1364
rs6503863	17	56517034	G	A	2559	0,4449	+	0,0593	0,0388	0,1269	+	0,0542	0,0357	0,1292
rs3799378	6	26404374	A	G	2559	0,2338	+	0,0764	0,0504	0,1296	+	0,0611	0,0464	0,1879
rs9477970	6	19082663	T	A	2559	0,1814	+	0,0779	0,0515	0,1305	+	0,0907	0,0474	0,0556
rs4244612	8	145741702	C	G	2559	0,4154	+	0,0641	0,0427	0,1331	+	0,0761	0,0393	0,0530
rs2056923	6	27689939	T	C	2559	0,1566	–	-0,0933	0,0625	0,1352	–	-0,0744	0,0575	0,1958
rs2591125	2	155730202	A	G	2559	0,7376	+	0,0654	0,0440	0,1371	+	0,0589	0,0405	0,1458
rs58874647	22	42386269	C	T	2559	0,2939	+	0,0645	0,0435	0,1381	+	0,0801	0,0400	0,0452
rs31770	5	165496438	C	G	2559	0,6775	–	-0,0625	0,0422	0,1385	–	-0,0717	0,0388	0,0644
rs3843738	17	43739194	A	G	2559	0,4675	+	0,0577	0,0390	0,1388	+	0,0288	0,0359	0,4221
rs12485060	22	34265796	T	C	2559	0,4020	+	0,0604	0,0409	0,1394	+	0,0576	0,0376	0,1259
rs6778735	3	52565100	T	C	2559	0,5631	+	0,0588	0,0399	0,1405	+	0,0473	0,0367	0,1972
rs2388334	6	98591622	A	G	2559	0,4448	+	0,0575	0,0392	0,1429	+	0,0411	0,0361	0,2554
rs36051354	3	52986012	T	C	2559	0,3020	–	-0,0629	0,0432	0,1452	–	-0,0820	0,0397	0,0390
rs74796574	2	155654998	T	C	2559	0,0471	+	0,1379	0,0953	0,1481	+	0,1230	0,0877	0,1608
rs4949460	1	32143138	G	A	2559	0,5514	+	0,0576	0,0399	0,1486	+	0,0341	0,0367	0,3525
rs242934	17	43936664	G	C	2559	0,4451	+	0,0573	0,0400	0,1517	+	0,0225	0,0368	0,5419
rs6855246	4	103112470	A	G	2559	0,0495	–	-0,1349	0,0947	0,1542	–	-0,1778	0,0871	0,0413
rs9257802	6	29343355	C	T	2559	0,1815	+	0,0856	0,0602	0,1551	+	0,0656	0,0554	0,2366
rs10914457	1	32094261	C	A	2559	0,4214	+	0,0564	0,0405	0,1640	+	0,0303	0,0373	0,4174
rs7412	19	45412079	C	T	2559	0,0868	–	-0,0961	0,0695	0,1668	–	-0,0812	0,0640	0,2042
rs34506349	2	100598726	G	A	2559	0,0335	+	0,1571	0,1140	0,1682	+	0,0734	0,1050	0,4844
rs35792732	7	132929804	C	T	2559	0,2128	–	-0,0676	0,0491	0,1684	–	-0,0319	0,0452	0,4807
rs13023088	2	100576304	C	T	2559	0,1493	+	0,0780	0,0570	0,1715	+	0,0256	0,0526	0,6266
rs7758926	6	98785796	A	G	2559	0,1434	–	-0,0780	0,0572	0,1724	–	-0,0382	0,0526	0,4678
rs10798879	1	32096815	T	C	2559	0,6332	+	0,0559	0,0411	0,1741	+	0,0458	0,0379	0,2264
rs9864170	3	85610908	A	G	2559	0,8818	+	0,0830	0,0612	0,1754	+	0,0624	0,0563	0,2681
rs2523933	6	29932292	G	T	2559	0,3220	+	0,0717	0,0529	0,1754	+	0,0537	0,0487	0,2705
rs935116	15	87973743	G	A	2559	0,6269	–	-0,0551	0,0407	0,1755	–	-0,0502	0,0375	0,1803
rs6741949	2	162910223	G	C	2559	0,4572	–	-0,0550	0,0406	0,1760	–	-0,0545	0,0374	0,1444
rs6882046	5	87968864	A	G	2559	0,2613	+	0,0605	0,0451	0,1799	+	0,0506	0,0415	0,2227

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function															
							Results for general cognitive function				Results for general cognitive function adjusted for education level				
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value	
rs246776	1	44274699	C	T	2559	0,4573	+	0,0518	0,0393	0,1881	+	0,0274	0,0362	0,4488	
rs35116560	17	43804186	T	C	2559	0,4076	+	0,0528	0,0402	0,1892	+	0,0458	0,0370	0,2163	
rs1724425	17	43781747	C	T	2559	0,4289	–	–0,0520	0,0397	0,1903	–	–0,0402	0,0366	0,2712	
rs7518151	1	32061851	T	C	2559	0,7126	+	0,0566	0,0436	0,1943	+	0,0514	0,0402	0,2002	
rs1343775	1	41757993	A	G	2559	0,2122	–	–0,0617	0,0480	0,1993	–	–0,0499	0,0442	0,2594	
rs3130127	6	30391975	A	T	2559	0,2543	+	0,0741	0,0584	0,2048	+	0,0536	0,0538	0,3188	
rs55985762	14	89715495	A	T	2559	0,7753	+	0,0616	0,0487	0,2054	+	0,0642	0,0448	0,1516	
rs8070135	17	56459063	G	A	2559	0,3436	–	–0,0519	0,0416	0,2124	–	–0,0687	0,0383	0,0730	
rs117793008	10	104198528	A	G	2559	0,0620	–	–0,1057	0,0851	0,2143	–	–0,0143	0,0785	0,8554	
rs7699872	4	166195670	T	A	2559	0,4046	–	–0,0513	0,0415	0,2156	–	–0,0533	0,0381	0,1623	
rs111599092	3	49502779	G	A	2559	0,1169	–	–0,0753	0,0610	0,2170	–	–0,0800	0,0561	0,1541	
rs3893463	6	29935405	C	T	2559	0,4275	+	0,0553	0,0452	0,2208	+	0,0381	0,0416	0,3597	
rs61874768	10	103880118	G	T	2559	0,1808	–	–0,0626	0,0511	0,2209	–	–0,0805	0,0470	0,0869	
rs11884495	2	162059969	A	T	2559	0,4901	–	–0,0469	0,0387	0,2256	–	–0,0115	0,0357	0,7473	
rs17420654	4	16414810	C	T	2559	0,3134	–	–0,0515	0,0427	0,2280	–	–0,0213	0,0393	0,5874	
rs2346449	7	133733917	G	T	2559	0,6395	+	0,0508	0,0422	0,2286	+	0,0670	0,0388	0,0846	
rs7590388	2	101042122	T	G	2559	0,5452	+	0,0483	0,0402	0,2292	–	–0,0044	0,0370	0,9045	
rs62253086	3	85393815	T	G	2559	0,0828	–	–0,0861	0,0718	0,2305	–	–0,0727	0,0661	0,2710	
rs62047280	16	61582586	G	A	2559	0,1861	+	0,0601	0,0507	0,2365	–	–0,0115	0,0468	0,8066	
rs56151722	3	71482840	A	G	2559	0,7606	–	–0,0545	0,0462	0,2380	–	–0,0314	0,0425	0,4595	
rs56016333	7	104596248	T	C	2559	0,3705	+	0,0484	0,0411	0,2390	+	0,0253	0,0378	0,5044	
rs8065165	17	43970317	C	T	2559	0,1679	+	0,0634	0,0541	0,2414	+	0,0273	0,0498	0,5836	
rs180020	6	16296824	A	G	2559	0,1883	–	–0,0591	0,0505	0,2416	–	–0,0832	0,0465	0,0736	
rs9921412	16	71988106	C	T	2559	0,7155	+	0,0510	0,0435	0,2418	+	0,0315	0,0401	0,4319	
rs151411	4	103138628	A	C	2559	0,2039	+	0,0566	0,0485	0,2432	+	0,0449	0,0446	0,3145	
rs2765042	14	103990745	G	A	2559	0,3384	+	0,0479	0,0412	0,2442	+	0,0617	0,0379	0,1032	
rs2457192	16	12197441	C	A	2559	0,6731	–	–0,0503	0,0433	0,2451	–	–0,0374	0,0398	0,3472	
rs77944304	1	72582779	C	T	2559	0,3013	+	0,0492	0,0428	0,2508	+	0,0464	0,0394	0,2386	
rs11896887	2	163830427	A	G	2559	0,5433	+	0,0454	0,0398	0,2536	+	0,0029	0,0367	0,9359	
rs3113262	7	69993342	G	C	2559	0,4992	+	0,0453	0,0397	0,2538	+	0,0413	0,0365	0,2575	
rs74944275	5	102726073	C	T	2559	0,0497	+	0,1032	0,0908	0,2560	+	0,0682	0,0836	0,4142	
rs11720236	3	127161689	T	G	2559	0,1585	+	0,0613	0,0543	0,2591	+	0,0717	0,0499	0,1511	

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function														
							Results for general cognitive function				Results for general cognitive function adjusted for education level			
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value
rs1541102	21	40628765	C	T	2559	0,3524	+	0,0463	0,0412	0,2611	+	0,0406	0,0379	0,2842
rs10806984	6	24181831	A	C	2559	0,3497	–	–0,0462	0,0412	0,2627	–	–0,0265	0,0379	0,4855
rs17411061	18	50795113	C	T	2559	0,4471	–	–0,0438	0,0392	0,2643	–	–0,0520	0,0361	0,1495
rs2064853	20	47675176	T	A	2559	0,5883	–	–0,0449	0,0402	0,2648	–	–0,0582	0,0370	0,1160
rs13101632	4	103034391	G	A	2559	0,0737	–	–0,0887	0,0806	0,2713	–	–0,0970	0,0742	0,1908
rs7873907	9	92225483	T	C	2559	0,5124	+	0,0446	0,0406	0,2725	+	0,0312	0,0374	0,4045
rs28892332	2	118536571	C	T	2559	0,0419	+	0,1085	0,0989	0,2726	+	0,0590	0,0910	0,5168
rs421211	5	139581773	G	A	2559	0,5538	+	0,0432	0,0394	0,2731	+	0,0340	0,0363	0,3480
rs2685200	2	82205627	A	G	2559	0,3153	–	–0,0458	0,0423	0,2789	–	–0,0551	0,0389	0,1569
rs2024449	16	53494617	T	C	2559	0,4379	–	–0,0422	0,0393	0,2835	–	–0,0298	0,0362	0,4101
rs55658584	7	104994721	G	A	2559	0,3155	+	0,0472	0,0442	0,2856	+	0,0432	0,0406	0,2880
rs112223196	16	28298418	A	C	2559	0,2522	–	–0,0493	0,0464	0,2880	–	–0,0259	0,0427	0,5442
rs2420551	1	69438777	T	A	2559	0,9218	+	0,0786	0,0740	0,2881	+	0,0666	0,0681	0,3280
rs640177	5	88002084	T	A	2559	0,5877	–	–0,0426	0,0402	0,2895	–	–0,0186	0,0370	0,6157
rs10786662	10	103989812	G	C	2559	0,5608	–	–0,0411	0,0392	0,2939	–	–0,0350	0,0360	0,3310
rs9379850	6	26343057	C	G	2559	0,1766	+	0,0601	0,0573	0,2945	+	0,0511	0,0528	0,3325
rs497523	16	28577931	T	C	2559	0,3682	+	0,0447	0,0432	0,3007	+	0,0437	0,0397	0,2716
rs6066861	20	47422577	G	A	2559	0,3226	–	–0,0453	0,0440	0,3030	–	–0,0211	0,0405	0,6018
rs35741362	6	27007687	T	C	2559	0,1295	–	–0,0710	0,0690	0,3038	–	–0,0749	0,0635	0,2383
rs2624839	3	50202231	T	C	2559	0,3997	–	–0,0411	0,0402	0,3065	–	–0,0279	0,0370	0,4518
rs1391438	4	106151843	T	C	2559	0,6642	–	–0,0417	0,0411	0,3107	–	–0,0435	0,0378	0,2505
rs13201341	6	25822661	C	T	2559	0,0906	+	0,0811	0,0805	0,3142	+	0,0070	0,0742	0,9245
rs2074404	17	44865439	T	G	2559	0,2321	–	–0,0471	0,0469	0,3151	–	–0,0230	0,0432	0,5948
rs72793807	16	28651732	C	T	2559	0,2866	–	–0,0448	0,0449	0,3180	–	–0,0306	0,0413	0,4587
rs77418166	6	98204498	C	T	2559	0,0845	+	0,0755	0,0757	0,3187	+	0,0182	0,0697	0,7945
rs8016504	14	33308021	C	A	2559	0,5223	–	–0,0385	0,0389	0,3226	–	–0,0363	0,0358	0,3107
rs13107325	4	103188709	C	T	2559	0,0381	–	–0,1010	0,1026	0,3250	–	–0,1304	0,0945	0,1675
rs17032400	4	103190229	T	C	2559	0,4526	+	0,0396	0,0402	0,3251	+	0,0246	0,0370	0,5067
rs2647249	4	106185663	C	T	2559	0,7967	–	–0,0478	0,0486	0,3255	–	–0,0470	0,0447	0,2935
rs11648192	16	28618708	C	T	2559	0,4255	+	0,0416	0,0424	0,3257	+	0,0329	0,0390	0,3992
rs79245415	6	98323549	C	G	2559	0,1193	+	0,0626	0,0638	0,3268	+	0,0306	0,0587	0,6024
rs2471738	17	44076063	C	T	2559	0,2087	+	0,0467	0,0481	0,3318	+	0,0652	0,0443	0,1408

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function														
							Results for general cognitive function				Results for general cognitive function adjusted for education level			
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value
rs73843290	3	85145836	T	C	2559	0,0292	-	-0,1140	0,1176	0,3321	-	-0,0977	0,1082	0,3664
rs9836970	3	23949839	G	A	2559	0,2022	-	-0,0466	0,0489	0,3399	-	-0,0482	0,0450	0,2841
rs39302	7	21467190	T	C	2559	0,7850	-	-0,0459	0,0482	0,3411	-	-0,0327	0,0444	0,4608
rs830386	5	165479749	A	C	2559	0,7487	-	-0,0438	0,0462	0,3431	-	-0,0447	0,0425	0,2931
rs4772729	13	106647578	C	G	2559	0,8417	-	-0,0522	0,0550	0,3434	-	-0,0091	0,0507	0,8569
rs3823572	7	133680601	G	A	2559	0,4480	+	0,0383	0,0407	0,3463	+	0,0578	0,0374	0,1227
rs62358087	5	107170148	A	G	2559	0,1849	+	0,0473	0,0504	0,3486	+	0,0392	0,0464	0,3985
rs4716325	6	19025741	G	C	2559	0,2917	+	0,0406	0,0434	0,3493	+	0,0324	0,0399	0,4169
rs1150735	6	30045199	G	A	2559	0,4056	+	0,0441	0,0474	0,3524	+	0,0468	0,0436	0,2836
rs233811	4	103211254	A	C	2559	0,3161	+	0,0391	0,0422	0,3539	+	0,0412	0,0388	0,2883
rs34180096	7	104608592	T	C	2559	0,4782	+	0,0364	0,0394	0,3556	+	0,0188	0,0362	0,6033
rs28433345	16	28883327	T	C	2559	0,6332	-	-0,0381	0,0416	0,3604	-	-0,0366	0,0383	0,3397
rs13428598	2	144250487	C	T	2559	0,3938	-	-0,0363	0,0397	0,3606	-	-0,0577	0,0365	0,1142
rs2007481	7	69996371	A	G	2559	0,3398	+	0,0378	0,0414	0,3617	+	0,0334	0,0381	0,3805
rs1732664	12	79747487	T	C	2559	0,6504	+	0,0420	0,0472	0,3739	+	0,0534	0,0434	0,2191
rs885916	6	30202571	C	T	2559	0,1444	+	0,0805	0,0907	0,3750	+	0,0713	0,0835	0,3931
rs10858096	1	109967104	C	A	2559	0,5285	+	0,0354	0,0404	0,3806	+	0,0319	0,0372	0,3917
rs1046953	3	50197097	C	T	2559	0,4504	+	0,0349	0,0398	0,3806	+	0,0111	0,0366	0,7607
rs11168951	12	49661715	C	T	2559	0,0847	+	0,0632	0,0726	0,3841	+	0,0651	0,0668	0,3300
rs17589603	1	72632885	A	G	2559	0,1233	+	0,0533	0,0615	0,3859	+	0,0408	0,0566	0,4713
rs71324996	3	49834767	A	C	2559	0,2806	+	0,0378	0,0437	0,3876	+	0,0516	0,0402	0,1994
rs4652802	1	183426758	A	G	2559	0,4459	+	0,0344	0,0399	0,3885	+	0,0119	0,0367	0,7463
rs3131064	6	30763893	T	C	2559	0,1465	+	0,0756	0,0886	0,3939	+	0,0645	0,0815	0,4291
rs2336670	3	52998504	T	G	2559	0,6666	-	-0,0362	0,0426	0,3946	-	-0,0328	0,0392	0,4023
rs926300	6	27059443	A	T	2559	0,1904	-	-0,0470	0,0552	0,3950	-	-0,0427	0,0508	0,4003
rs143699161	17	43748712	A	G	2559	0,2541	+	0,0393	0,0463	0,3954	+	0,0116	0,0426	0,7848
rs770463	5	88194950	C	T	2559	0,5272	+	0,0327	0,0387	0,3986	+	0,0128	0,0356	0,7191
rs10911301	1	183282575	A	G	2559	0,4816	-	-0,0332	0,0396	0,4016	-	-0,0123	0,0365	0,7351
rs1144	7	104756355	T	C	2559	0,3644	-	-0,0344	0,0410	0,4021	-	-0,0241	0,0377	0,5230
rs424029	3	85010039	G	A	2559	0,0327	-	-0,0949	0,1134	0,4027	-	-0,1204	0,1044	0,2487
rs62170823	2	155664656	A	G	2559	0,3432	+	0,0348	0,0417	0,4031	+	0,0412	0,0383	0,2829
rs1620977	1	72729142	A	G	2559	0,7439	+	0,0380	0,0456	0,4047	+	0,0699	0,0420	0,0962

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function															
							Results for general cognitive function				Results for general cognitive function adjusted for education level				
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value	
rs61754230	12	72179446	C	T	2559	0,0135	+	0,1397	0,1690	0,4083	+	0,1110	0,1555	0,4754	
rs429358	19	45411941	T	C	2559	0,1592	+	0,0441	0,0535	0,4096	+	0,0462	0,0492	0,3477	
rs11716948	3	49431903	C	T	2559	0,2655	+	0,0371	0,0451	0,4101	+	0,0529	0,0415	0,2024	
rs12938031	17	43854502	A	G	2559	0,3843	–	–0,0323	0,0397	0,4163	–	–0,0254	0,0365	0,4877	
rs2280405	3	49895052	G	C	2559	0,4779	–	–0,0330	0,0409	0,4188	–	–0,0338	0,0376	0,3687	
rs1977200	6	26466392	G	A	2559	0,7654	+	0,0411	0,0511	0,4213	+	0,0535	0,0470	0,2558	
rs1044258	10	103605714	T	C	2559	0,3734	–	–0,0333	0,0414	0,4215	–	–0,0222	0,0381	0,5605	
rs4778988	15	82540337	T	C	2559	0,3689	–	–0,0325	0,0408	0,4260	–	–0,0205	0,0376	0,5853	
rs2273558	6	26466035	A	C	2559	0,3201	+	0,0349	0,0443	0,4314	+	0,0322	0,0408	0,4297	
rs6760964	2	73387386	C	G	2559	0,3752	+	0,0316	0,0405	0,4341	+	0,0262	0,0372	0,4811	
rs4294650	13	21356540	C	T	2559	0,3066	+	0,0330	0,0423	0,4351	+	0,0356	0,0389	0,3605	
rs77179555	2	100309124	C	G	2559	0,0228	+	0,1058	0,1359	0,4361	+	0,0262	0,1251	0,8340	
rs6914699	6	29934022	T	C	2559	0,3949	–	–0,0403	0,0518	0,4363	–	–0,0378	0,0477	0,4280	
rs9916613	17	28682453	T	A	2559	0,3656	–	–0,0318	0,0409	0,4364	–	–0,0522	0,0376	0,1651	
rs79470265	14	98649129	A	C	2559	0,1947	+	0,0391	0,0504	0,4374	+	0,0274	0,0464	0,5549	
rs2426132	20	47723127	G	C	2559	0,4573	–	–0,0309	0,0400	0,4398	–	–0,0464	0,0368	0,2071	
rs9480861	6	108858460	C	T	2559	0,4092	–	–0,0303	0,0393	0,4403	–	–0,0291	0,0362	0,4202	
rs61572747	17	43460891	A	G	2559	0,2574	–	–0,0352	0,0465	0,4484	–	–0,0105	0,0428	0,8052	
rs10761765	10	65188973	A	G	2559	0,8161	+	0,0378	0,0499	0,4492	+	0,0337	0,0460	0,4632	
rs28645341	14	30174078	T	C	2559	0,3122	–	–0,0327	0,0432	0,4497	–	–0,0480	0,0398	0,2276	
rs7613360	3	49916710	C	T	2559	0,3722	–	–0,0301	0,0398	0,4505	–	–0,0098	0,0367	0,7894	
rs12441495	15	41222487	G	C	2559	0,1237	–	–0,0461	0,0616	0,4542	–	–0,0163	0,0567	0,7740	
rs7978070	12	14511806	T	C	2559	0,4728	+	0,0293	0,0399	0,4624	–	–0,0061	0,0368	0,8687	
rs2571400	6	29927722	C	G	2559	0,4990	–	–0,0325	0,0453	0,4729	–	–0,0273	0,0417	0,5129	
rs4720476	7	44773310	C	T	2559	0,5660	–	–0,0295	0,0415	0,4782	–	–0,0186	0,0382	0,6265	
rs7618519	3	49772708	A	T	2559	0,5261	–	–0,0272	0,0387	0,4820	–	–0,0357	0,0356	0,3156	
rs702223	9	23813840	C	A	2559	0,3614	+	0,0291	0,0415	0,4827	+	0,0146	0,0382	0,7011	
rs6074798	20	14761710	G	A	2559	0,4990	+	0,0279	0,0397	0,4828	+	0,0033	0,0366	0,9284	
rs10846167	12	15515945	C	T	2559	0,2975	–	–0,0300	0,0428	0,4838	+	0,0151	0,0395	0,7014	
rs11596211	10	101840119	T	A	2559	0,3363	–	–0,0294	0,0421	0,4852	–	–0,0221	0,0387	0,5679	
rs151231	16	28582849	A	G	2559	0,5429	–	–0,0282	0,0408	0,4894	–	–0,0297	0,0375	0,4277	
rs11678106	2	82444107	C	T	2559	0,5207	–	–0,0275	0,0398	0,4903	–	–0,0179	0,0367	0,6244	

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function														
							Results for general cognitive function				Results for general cognitive function adjusted for education level			
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value
rs77482156	6	25199853	T	C	2559	0,0571	+	0,0614	0,0892	0,4912	–	-0,0130	0,0821	0,8740
rs62236533	22	41992169	G	A	2559	0,0998	+	0,0473	0,0687	0,4918	+	0,0625	0,0633	0,3229
rs4500960	2	162818621	C	T	2559	0,4768	–	-0,0270	0,0394	0,4935	–	-0,0228	0,0363	0,5304
rs7802313	7	132927792	T	C	2559	0,5619	–	-0,0270	0,0398	0,4976	–	-0,0167	0,0366	0,6489
rs36162392	6	26569135	T	C	2559	0,1067	+	0,0538	0,0802	0,5025	+	0,0169	0,0739	0,8192
rs1796518	6	26388672	C	T	2559	0,3624	+	0,0283	0,0425	0,5048	+	0,0024	0,0391	0,9502
rs79582714	1	20883608	C	T	2559	0,1093	+	0,0417	0,0626	0,5053	+	0,0660	0,0576	0,2516
rs75477016	6	98781196	C	T	2559	0,1277	+	0,0392	0,0615	0,5237	+	0,0318	0,0566	0,5741
rs10133551	14	33282232	T	C	2559	0,4125	–	-0,0254	0,0401	0,5257	–	-0,0263	0,0369	0,4765
rs7646366	3	49470668	G	A	2559	0,3013	–	-0,0267	0,0424	0,5287	–	-0,0270	0,0390	0,4891
rs2189373	6	30223428	C	T	2559	0,1831	+	0,0437	0,0695	0,5290	+	0,0364	0,0639	0,5687
rs429479	6	29372323	A	G	2559	0,1080	+	0,0650	0,1033	0,5295	+	0,0059	0,0951	0,9505
rs6906737	6	98345853	T	A	2559	0,4500	+	0,0246	0,0392	0,5308	–	-0,0036	0,0361	0,9213
rs527825	1	183340238	A	G	2559	0,5845	–	-0,0250	0,0400	0,5324	–	-0,0041	0,0368	0,9116
rs13425585	2	157487273	C	G	2559	0,4697	–	-0,0249	0,0399	0,5326	–	-0,0158	0,0367	0,6678
rs1233578	6	28712247	A	G	2559	0,1409	+	0,0445	0,0715	0,5337	+	0,0440	0,0658	0,5035
rs28374519	16	28489342	G	A	2559	0,4058	+	0,0257	0,0419	0,5397	+	0,0219	0,0386	0,5708
rs750155	16	28620572	C	T	2559	0,4916	–	-0,0252	0,0412	0,5400	–	-0,0293	0,0379	0,4393
rs13253386	8	14002020	T	G	2559	0,5034	+	0,0242	0,0395	0,5403	+	0,0275	0,0363	0,4495
rs9467714	6	26340785	G	A	2559	0,1232	+	0,0434	0,0714	0,5432	–	-0,0068	0,0657	0,9178
rs5758605	22	42541349	G	A	2559	0,4639	–	-0,0238	0,0392	0,5440	–	-0,0460	0,0361	0,2026
rs61104616	5	88163771	G	A	2559	0,5124	–	-0,0235	0,0389	0,5454	–	-0,0028	0,0358	0,9367
rs6964771	7	21410035	C	G	2559	0,4383	+	0,0236	0,0391	0,5466	–	-0,0024	0,0360	0,9466
rs11210871	1	44029353	C	G	2559	0,7047	+	0,0262	0,0435	0,5467	+	0,0267	0,0400	0,5050
rs34734335	9	23911098	G	A	2559	0,5680	–	-0,0241	0,0403	0,5488	–	-0,0446	0,0371	0,2286
rs12709186	16	7249472	G	A	2559	0,3227	+	0,0248	0,0416	0,5519	+	0,0219	0,0383	0,5672
rs11865256	16	7265070	C	T	2559	0,4169	+	0,0238	0,0402	0,5535	+	0,0242	0,0370	0,5132
rs179994	6	16320987	A	C	2559	0,4029	–	-0,0237	0,0401	0,5543	–	-0,0280	0,0369	0,4479
rs2309812	2	100869702	C	T	2559	0,3806	–	-0,0238	0,0403	0,5547	–	-0,0507	0,0371	0,1723
rs10031823	4	103125031	T	C	2559	0,6145	+	0,0237	0,0403	0,5561	+	0,0211	0,0371	0,5697
rs1699462	9	23857083	G	T	2559	0,4172	–	-0,0234	0,0400	0,5594	–	-0,0304	0,0368	0,4094
rs3823427	6	27256346	G	C	2559	0,2334	–	-0,0288	0,0494	0,5595	–	-0,0272	0,0454	0,5495
rs9856289	3	123898877	A	C	2559	0,2859	+	0,0252	0,0436	0,5634	+	0,0047	0,0401	0,9071
rs11130226	3	49993191	T	G	2559	0,4891	–	-0,0223	0,0389	0,5657	–	-0,0028	0,0358	0,9379

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function														
							Results for general cognitive function				Results for general cognitive function adjusted for education level			
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value
rs7146202	14	33303517	A	G	2559	0,5437	–	-0,0223	0,0389	0,5659	–	-0,0090	0,0358	0,8004
rs6801153	3	24113697	T	C	2559	0,7671	–	-0,0269	0,0472	0,5696	–	-0,0635	0,0435	0,1447
rs9262120	6	30565853	G	A	2559	0,1323	–	-0,0549	0,0980	0,5755	–	-0,0406	0,0902	0,6524
rs1951167	14	37008149	G	A	2559	0,5546	+	0,0219	0,0393	0,5765	+	0,0217	0,0362	0,5491
rs9295486	6	20896553	T	C	2559	0,4683	+	0,0216	0,0389	0,5788	+	0,0447	0,0358	0,2117
rs11640533	16	53412862	C	T	2559	0,4227	+	0,0222	0,0400	0,5790	+	0,0184	0,0368	0,6168
rs3817334	11	47650993	C	T	2559	0,3878	+	0,0218	0,0400	0,5866	+	0,0578	0,0369	0,1168
rs7599488	2	60718347	C	T	2559	0,4292	–	-0,0215	0,0396	0,5873	–	-0,0100	0,0365	0,7844
rs62484026	7	127792094	G	A	2559	0,2494	–	-0,0260	0,0483	0,5906	–	-0,0060	0,0445	0,8936
rs10875914	12	49422094	A	G	2559	0,3918	+	0,0218	0,0406	0,5907	+	0,0227	0,0373	0,5431
rs11130194	3	49475689	G	A	2559	0,4118	+	0,0217	0,0404	0,5923	+	0,0156	0,0372	0,6750
rs6095417	20	47678201	G	A	2559	0,3349	–	-0,0219	0,0416	0,5985	–	-0,0314	0,0383	0,4114
rs7998050	13	58473866	A	G	2559	0,6898	+	0,0226	0,0435	0,6031	+	0,0410	0,0400	0,3053
rs7609050	2	156021862	A	C	2559	0,5229	+	0,0201	0,0389	0,6045	+	0,0317	0,0358	0,3754
rs6890684	5	60615886	A	G	2559	0,5084	–	-0,0207	0,0400	0,6049	–	-0,0165	0,0368	0,6535
rs11764631	7	127179493	G	A	2559	0,4083	–	-0,0203	0,0395	0,6069	–	-0,0327	0,0364	0,3690
rs1408579	10	101912194	C	T	2559	0,4860	–	-0,0205	0,0399	0,6072	–	-0,0035	0,0367	0,9241
rs200991	6	27815494	C	A	2559	0,1499	–	-0,0332	0,0652	0,6109	–	-0,0466	0,0600	0,4371
rs11793831	9	23362311	G	T	2559	0,4190	–	-0,0202	0,0401	0,6145	–	-0,0639	0,0369	0,0835
rs4851300	2	100958804	C	A	2559	0,3622	+	0,0208	0,0415	0,6170	–	-0,0270	0,0383	0,4799
rs1362104	6	30101656	G	A	2559	0,3847	–	-0,0237	0,0478	0,6205	–	-0,0145	0,0440	0,7421
rs6124077	20	59815020	A	G	2559	0,5080	+	0,0196	0,0401	0,6256	–	-0,0025	0,0369	0,9450
rs516902	1	96700020	C	T	2559	0,6161	+	0,0200	0,0411	0,6274	+	0,0144	0,0378	0,7043
rs62439690	7	21417556	G	A	2559	0,2509	–	-0,0222	0,0458	0,6276	–	-0,0392	0,0422	0,3528
rs12170228	22	42247695	C	T	2559	0,4541	–	-0,0193	0,0398	0,6281	–	-0,0429	0,0367	0,2418
rs80170948	5	64020316	T	G	2559	0,0432	+	0,0504	0,1044	0,6292	+	0,0287	0,0961	0,7653
rs10853627	18	50706073	T	C	2559	0,4462	+	0,0189	0,0394	0,6309	+	0,0138	0,0362	0,7027
rs9366673	6	26893329	C	G	2559	0,2223	+	0,0251	0,0529	0,6351	+	0,0270	0,0487	0,5790
rs112780312	1	153797015	G	A	2559	0,2733	–	-0,0210	0,0445	0,6368	–	-0,0323	0,0410	0,4305
rs10218712	1	41832297	T	G	2559	0,2940	–	-0,0204	0,0436	0,6395	–	-0,0209	0,0401	0,6016
rs10996430	10	67213589	A	G	2559	0,2599	–	-0,0215	0,0458	0,6397	–	-0,0358	0,0422	0,3958
rs7186834	16	7673255	T	C	2559	0,3947	–	-0,0185	0,0400	0,6432	+	0,0104	0,0368	0,7784

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function															
							Results for general cognitive function				Results for general cognitive function adjusted for education level				
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value	
rs7746199	6	27261324	C	T	2559	0,1594	+	0,0276	0,0596	0,6440	+	0,0199	0,0549	0,7163	
rs407102	1	109846278	C	T	2559	0,7060	+	0,0195	0,0431	0,6504	+	0,0207	0,0397	0,6023	
rs9558651	13	106617233	C	A	2559	0,2022	–	–0,0226	0,0501	0,6528	–	–0,0495	0,0461	0,2829	
rs2517602	6	30190040	C	T	2559	0,3328	+	0,0222	0,0494	0,6535	+	0,0275	0,0455	0,5455	
rs78066960	7	127716892	G	A	2559	0,2972	–	–0,0189	0,0437	0,6648	–	–0,0131	0,0402	0,7439	
rs754593	17	44054696	G	A	2559	0,5951	+	0,0175	0,0404	0,6652	–	–0,0004	0,0372	0,9907	
rs28549862	8	64662318	G	A	2559	0,2756	+	0,0185	0,0431	0,6668	–	–0,0055	0,0396	0,8903	
rs7534501	1	96221514	G	A	2559	0,5075	–	–0,0174	0,0405	0,6682	–	–0,0215	0,0373	0,5638	
rs66886492	6	28089731	C	T	2559	0,0961	+	0,0395	0,0940	0,6745	–	–0,0037	0,0865	0,9658	
rs7773141	6	98537134	G	A	2559	0,4938	–	–0,0161	0,0390	0,6796	–	–0,0072	0,0359	0,8416	
rs7583067	2	100796850	C	T	2559	0,2286	–	–0,0192	0,0471	0,6826	–	–0,0499	0,0433	0,2492	
rs7193413	16	28644663	A	T	2559	0,6853	–	–0,0182	0,0446	0,6836	–	–0,0028	0,0410	0,9455	
rs2906455	1	44341895	T	C	2559	0,9148	+	0,0279	0,0685	0,6842	+	0,0513	0,0631	0,4163	
rs1892423	1	41849340	A	C	2559	0,5744	+	0,0157	0,0392	0,6894	+	0,0164	0,0361	0,6488	
rs4331524	2	100876111	C	T	2559	0,5041	+	0,0153	0,0391	0,6949	–	–0,0095	0,0360	0,7919	
rs7010173	8	13969304	C	T	2559	0,6182	+	0,0159	0,0412	0,7001	+	0,0215	0,0379	0,5700	
rs10872224	6	98435125	G	T	2559	0,6245	–	–0,0152	0,0402	0,7051	–	–0,0029	0,0370	0,9378	
rs11662271	18	50907365	T	C	2559	0,5457	–	–0,0148	0,0391	0,7055	–	–0,0143	0,0360	0,6910	
rs12646225	4	696848	C	T	2559	0,1018	+	0,0246	0,0654	0,7069	+	0,0161	0,0602	0,7890	
rs2451729	6	26705859	C	T	2559	0,6306	+	0,0159	0,0424	0,7084	+	0,0090	0,0391	0,8180	
rs7038525	9	23772277	C	T	2559	0,1914	–	–0,0188	0,0511	0,7133	+	0,0084	0,0471	0,8581	
rs12655172	5	88010829	C	T	2559	0,1036	+	0,0241	0,0661	0,7154	+	0,0095	0,0608	0,8764	
rs28646281	17	43977846	T	G	2559	0,3408	+	0,0151	0,0415	0,7157	+	0,0308	0,0382	0,4208	
rs34305371	1	72733610	G	A	2559	0,0855	+	0,0264	0,0730	0,7172	+	0,0080	0,0672	0,9048	
rs62056490	16	71852833	C	A	2559	0,7899	+	0,0176	0,0486	0,7173	+	0,0223	0,0448	0,6183	
rs2074613	5	139714564	C	T	2559	0,5442	–	–0,0143	0,0400	0,7209	–	–0,0097	0,0368	0,7916	
rs117431198	20	58219763	C	T	2559	0,0286	+	0,0420	0,1192	0,7248	+	0,0246	0,1097	0,8223	
rs34627	5	60528806	G	A	2559	0,4815	+	0,0137	0,0389	0,7249	+	0,0056	0,0358	0,8757	
rs7683416	4	106152984	T	C	2559	0,5290	–	–0,0139	0,0396	0,7251	–	–0,0314	0,0364	0,3880	
rs7638524	3	52874861	G	A	2559	0,2288	+	0,0164	0,0471	0,7271	+	0,0140	0,0433	0,7473	
rs56116382	3	49606188	A	C	2559	0,1861	+	0,0174	0,0499	0,7278	+	0,0214	0,0459	0,6416	
rs79738691	6	25148560	A	G	2559	0,0463	+	0,0335	0,0970	0,7296	–	–0,0298	0,0893	0,7388	
rs3850610	15	83221392	C	A	2559	0,2801	–	–0,0152	0,0445	0,7326	–	–0,0342	0,0410	0,4037	

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function															
							Results for general cognitive function				Results for general cognitive function adjusted for education level				
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value	
rs942353	13	106631574	C	T	2559	0,3845	–	-0,0135	0,0401	0,7358	–	-0,0463	0,0370	0,2100	
rs7775835	6	28678357	T	C	2559	0,8808	–	-0,0288	0,0852	0,7358	–	-0,0239	0,0784	0,7602	
rs7814022	8	118902809	C	A	2559	0,5631	+	0,0132	0,0400	0,7403	+	0,0336	0,0368	0,3609	
rs9489945	6	98311875	G	C	2559	0,4010	+	0,0134	0,0404	0,7404	+	0,0207	0,0372	0,5774	
rs17251689	12	93067870	G	C	2559	0,2008	–	-0,0162	0,0490	0,7416	+	0,0006	0,0451	0,9898	
rs4781499	16	13630944	A	G	2559	0,1351	+	0,0183	0,0569	0,7483	+	0,0002	0,0523	0,9977	
rs13001130	2	100668699	G	A	2559	0,1899	+	0,0162	0,0510	0,7516	–	-0,0239	0,0470	0,6113	
rs62570303	9	23910400	G	T	2559	0,2626	–	-0,0142	0,0451	0,7524	–	-0,0148	0,0415	0,7207	
rs6120993	20	34185161	G	T	2559	0,1607	+	0,0166	0,0532	0,7543	–	-0,0049	0,0490	0,9207	
rs7963801	12	79685226	T	C	2559	0,5527	+	0,0134	0,0429	0,7548	+	0,0212	0,0394	0,5910	
rs3094127	6	30697447	A	G	2559	0,1807	–	-0,0217	0,0698	0,7556	–	-0,0037	0,0642	0,9544	
rs3092566	20	47499170	T	C	2559	0,3932	+	0,0128	0,0417	0,7593	+	0,0320	0,0384	0,4046	
rs6950324	7	133054712	T	C	2559	0,6105	–	-0,0129	0,0422	0,7599	–	-0,0076	0,0388	0,8455	
rs4925114	17	17711270	A	G	2559	0,6598	–	-0,0124	0,0408	0,7615	–	-0,0041	0,0376	0,9128	
rs4636654	11	28642653	G	A	2559	0,4023	+	0,0122	0,0405	0,7633	+	0,0025	0,0372	0,9454	
rs810681	9	23799239	T	A	2559	0,2419	–	-0,0141	0,0469	0,7636	–	-0,0025	0,0431	0,9540	
rs10273635	7	127158357	T	G	2559	0,6284	+	0,0122	0,0406	0,7641	+	0,0069	0,0373	0,8526	
rs56150095	7	71759069	C	A	2559	0,5411	–	-0,0116	0,0394	0,7691	–	-0,0051	0,0363	0,8878	
rs743134	20	14804964	G	A	2559	0,3868	+	0,0118	0,0404	0,7696	+	0,0210	0,0372	0,5729	
rs3858954	15	82558034	G	C	2559	0,6768	–	-0,0119	0,0417	0,7757	+	0,0053	0,0384	0,8904	
rs6122773	20	47866656	G	A	2559	0,5410	–	-0,0108	0,0393	0,7830	+	0,0140	0,0362	0,6981	
rs12707116	7	133424909	T	C	2559	0,4776	+	0,0109	0,0399	0,7849	+	0,0014	0,0368	0,9690	
rs61544985	7	127102681	G	A	2559	0,4073	–	-0,0106	0,0399	0,7909	–	-0,0305	0,0368	0,4066	
rs13146981	4	152720703	T	C	2559	0,5404	+	0,0103	0,0391	0,7924	+	0,0093	0,0359	0,7967	
rs3129817	6	30342753	G	A	2559	0,1180	+	0,0299	0,1146	0,7944	+	0,0293	0,1055	0,7811	
rs830237	5	165405678	A	C	2559	0,2774	+	0,0116	0,0447	0,7956	+	0,0417	0,0412	0,3118	
rs2303907	2	73470497	T	C	2559	0,4731	–	-0,0101	0,0393	0,7963	–	-0,0216	0,0362	0,5498	
rs13214027	6	26573631	C	G	2559	0,1321	+	0,0179	0,0693	0,7964	–	-0,0046	0,0638	0,9426	
rs9467707	6	26327905	G	C	2559	0,1057	+	0,0196	0,0771	0,7992	–	-0,0528	0,0710	0,4574	
rs1343700	3	123571754	G	A	2559	0,6667	–	-0,0104	0,0413	0,8013	–	-0,0024	0,0380	0,9495	
rs8067056	17	44083948	T	C	2559	0,3851	+	0,0103	0,0410	0,8016	+	0,0387	0,0377	0,3054	
rs17814604	6	98585502	A	G	2559	0,2659	+	0,0111	0,0444	0,8035	–	-0,0067	0,0409	0,8700	

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function														
							Results for general cognitive function				Results for general cognitive function adjusted for education level			
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value
rs4810883	20	47495794	C	T	2559	0,3177	+	0,0106	0,0430	0,8062	–	-0,0299	0,0396	0,4505
rs2425161	20	34427192	A	G	2559	0,2005	–	-0,0120	0,0492	0,8067	–	-0,0083	0,0452	0,8539
rs11628208	14	36994709	C	A	2559	0,4090	+	0,0098	0,0403	0,8073	+	0,0179	0,0371	0,6288
rs1526123	17	43783340	T	C	2559	0,4377	–	-0,0096	0,0395	0,8078	+	0,0052	0,0364	0,8868
rs6759626	2	163886350	C	A	2559	0,2360	–	-0,0114	0,0468	0,8079	–	-0,0380	0,0431	0,3787
rs10733389	9	23378220	A	G	2559	0,6471	–	-0,0099	0,0414	0,8108	–	-0,0590	0,0382	0,1223
rs2091329	1	110042079	A	G	2559	0,3179	–	-0,0100	0,0416	0,8110	–	-0,0057	0,0383	0,8821
rs13062354	3	35528166	G	A	2559	0,4890	–	-0,0093	0,0397	0,8153	–	-0,0307	0,0365	0,4000
rs111342015	6	43227141	G	A	2559	0,1133	+	0,0146	0,0626	0,8158	+	0,0298	0,0576	0,6048
rs3132681	6	30060041	C	A	2559	0,4080	+	0,0104	0,0449	0,8164	+	0,0011	0,0413	0,9787
rs9541479	13	69221551	C	T	2559	0,4819	+	0,0092	0,0404	0,8204	+	0,0126	0,0371	0,7340
rs6928583	6	98398142	A	G	2559	0,1743	+	0,0116	0,0528	0,8255	+	0,0102	0,0486	0,8335
rs1953522	1	216837241	A	C	2559	0,6599	–	-0,0087	0,0411	0,8318	+	0,0034	0,0379	0,9274
rs9451649	6	92049630	T	C	2559	0,3724	–	-0,0084	0,0398	0,8335	–	-0,0089	0,0366	0,8079
rs7809066	7	104568249	C	A	2559	0,4586	+	0,0081	0,0392	0,8358	–	-0,0027	0,0360	0,9411
rs10281827	7	133081810	A	G	2559	0,3281	–	-0,0088	0,0428	0,8366	+	0,0063	0,0394	0,8732
rs2239647	14	33292743	A	C	2559	0,5403	–	-0,0079	0,0389	0,8387	–	-0,0117	0,0358	0,7436
rs6931604	6	98578215	C	T	2559	0,5864	+	0,0080	0,0393	0,8389	+	0,0189	0,0362	0,6007
rs12438326	15	51037974	A	G	2559	0,3326	–	-0,0080	0,0413	0,8461	+	0,0120	0,0380	0,7521
rs9520384	13	89016992	G	A	2559	0,3046	+	0,0083	0,0433	0,8482	+	0,0141	0,0399	0,7233
rs3101338	1	72750353	C	A	2559	0,7491	–	-0,0085	0,0455	0,8520	–	-0,0282	0,0419	0,5012
rs381262	20	47933479	G	A	2559	0,2359	+	0,0085	0,0468	0,8550	–	-0,0152	0,0430	0,7248
rs745086	20	47433515	G	A	2559	0,4443	–	-0,0076	0,0415	0,8551	+	0,0267	0,0382	0,4851
rs10514301	5	87939654	C	T	2559	0,1037	+	0,0120	0,0656	0,8553	–	-0,0022	0,0603	0,9708
rs1473634	8	20915316	A	G	2559	0,3202	+	0,0077	0,0425	0,8559	–	-0,0119	0,0392	0,7612
rs11716820	3	50228316	C	T	2559	0,1001	+	0,0123	0,0682	0,8572	–	-0,0076	0,0627	0,9041
rs73189617	3	184069126	C	G	2559	0,3070	+	0,0077	0,0434	0,8591	+	0,0008	0,0400	0,9840
rs2517664	6	30073259	C	T	2559	0,1755	+	0,0124	0,0710	0,8612	+	0,0039	0,0653	0,9530
rs13278931	8	30879207	G	T	2559	0,4549	+	0,0068	0,0396	0,8630	+	0,0113	0,0364	0,7566
rs2636699	4	106312813	T	C	2559	0,3478	+	0,0065	0,0414	0,8746	+	0,0069	0,0381	0,8558
rs12025777	1	41748897	G	T	2559	0,0729	–	-0,0119	0,0755	0,8752	–	-0,0203	0,0695	0,7704
rs1775118	9	23842340	A	T	2559	0,5467	+	0,0060	0,0402	0,8808	–	-0,0050	0,0370	0,8915
rs17321073	4	106258989	A	G	2559	0,3295	+	0,0061	0,0422	0,8841	+	0,0171	0,0388	0,6601

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function														
							Results for general cognitive function				Results for general cognitive function adjusted for education level			
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value
rs909674	22	39859169	C	A	2559	0,7358	+	0,0061	0,0437	0,8892	–	-0,0293	0,0402	0,4666
rs7749823	6	26158079	A	C	2559	0,1319	–	-0,0089	0,0653	0,8916	–	-0,0210	0,0600	0,7261
rs114030538	1	72588658	T	C	2559	0,0623	+	0,0115	0,0852	0,8927	+	0,0164	0,0784	0,8338
rs9966005	18	50440366	A	G	2559	0,4698	–	-0,0053	0,0391	0,8930	+	0,0212	0,0360	0,5566
rs2077610	3	70911271	T	A	2559	0,5621	+	0,0051	0,0403	0,8994	–	-0,0016	0,0371	0,9653
rs6890611	5	60847272	T	G	2559	0,5834	–	-0,0047	0,0418	0,9099	–	-0,0007	0,0384	0,9848
rs1318878	12	15543479	C	A	2559	0,1952	–	-0,0056	0,0495	0,9100	+	0,0089	0,0455	0,8448
rs28612284	14	69712437	T	G	2559	0,0118	–	-0,0207	0,1861	0,9116	–	-0,0040	0,1712	0,9814
rs3092073	20	44595649	G	A	2559	0,4574	+	0,0041	0,0392	0,9174	+	0,0179	0,0360	0,6195
rs7763264	6	21960065	C	T	2559	0,3804	–	-0,0043	0,0417	0,9183	–	-0,0169	0,0384	0,6596
rs153286	5	165409177	G	A	2559	0,4292	+	0,0040	0,0395	0,9189	+	0,0407	0,0364	0,2636
rs11153822	6	98214814	C	T	2559	0,4895	–	-0,0039	0,0405	0,9225	–	-0,0123	0,0372	0,7408
rs17663165	3	71677981	C	A	2559	0,4083	–	-0,0038	0,0396	0,9228	–	-0,0119	0,0365	0,7447
rs2903385	4	106094427	G	A	2559	0,4885	+	0,0038	0,0397	0,9241	+	0,0094	0,0365	0,7974
rs750472	8	145701453	A	C	2559	0,4962	+	0,0036	0,0400	0,9290	–	-0,0264	0,0368	0,4740
rs3914453	9	120677877	A	T	2559	0,5759	–	-0,0035	0,0404	0,9303	+	0,0072	0,0372	0,8457
rs12073420	1	20871181	G	T	2559	0,1681	–	-0,0044	0,0524	0,9334	+	0,0017	0,0482	0,9718
rs113714691	8	85804582	C	T	2559	0,1338	–	-0,0049	0,0588	0,9335	–	-0,0006	0,0541	0,9912
rs1933720	6	98297832	T	C	2559	0,3384	+	0,0034	0,0420	0,9362	–	-0,0020	0,0386	0,9579
rs7633520	3	23966425	G	T	2559	0,2691	–	-0,0032	0,0439	0,9419	+	0,0072	0,0404	0,8592
rs11720523	3	71545170	C	A	2559	0,4209	+	0,0029	0,0400	0,9424	+	0,0004	0,0368	0,9907
rs12885221	14	33279010	G	C	2559	0,3157	+	0,0030	0,0428	0,9438	+	0,0062	0,0394	0,8742
rs1502233	18	50990435	T	C	2559	0,6172	+	0,0029	0,0411	0,9441	–	-0,0001	0,0378	0,9972
rs73234901	7	127398248	T	A	2559	0,3815	+	0,0027	0,0404	0,9466	+	3,0200	0,0372	0,9994
rs9597254	13	56325337	A	T	2559	0,3244	–	-0,0028	0,0424	0,9473	+	0,0012	0,0391	0,9760
rs2726513	4	106217358	G	T	2559	0,3982	–	-0,0025	0,0407	0,9506	–	-0,0101	0,0374	0,7866
rs10916805	1	20818659	G	C	2559	0,1743	–	-0,0032	0,0524	0,9512	+	0,0175	0,0482	0,7170
rs889956	2	57404834	G	A	2559	0,3989	+	0,0024	0,0398	0,9513	–	-0,0159	0,0366	0,6631
rs9934041	16	7229579	C	T	2559	0,3376	–	-0,0023	0,0412	0,9551	+	0,0101	0,0379	0,7894
rs242559	17	44025888	C	A	2559	0,7802	–	-0,0026	0,0476	0,9566	–	-0,0226	0,0438	0,6063
rs59663815	2	68473755	T	G	2559	0,2860	–	-0,0023	0,0435	0,9586	–	-0,0103	0,0401	0,7975
rs78565420	8	85703065	C	T	2559	0,0591	–	-0,0039	0,0874	0,9640	+	0,0348	0,0804	0,6648
rs7002959	8	145685396	T	C	2559	0,4227	+	0,0017	0,0407	0,9666	–	-0,0128	0,0375	0,7319

(Supplementary Table 6.1 continues)

Association between 435 SNPs and general cognitive function														
							Results for general cognitive function				Results for general cognitive function adjusted for education level			
rs-number	chr	position	reference allele	alternative allele	N	EAF	direction	effect	SE	raw p-value	direction	effect	SE	raw p-value
rs2333604	14	30071738	C	A	2559	0,3825	–	-0,0013	0,0400	0,9748	–	-0,0113	0,0368	0,7591
rs1445653	2	155661432	A	G	2559	0,5992	–	-0,0012	0,0404	0,9768	+	0,0177	0,0371	0,6342
rs72801843	16	53508802	T	A	2559	0,3128	–	-0,0012	0,0424	0,9768	+	0,0086	0,0390	0,8259
rs10145335	14	98547748	G	A	2559	0,2475	–	-0,0012	0,0457	0,9788	+	0,0113	0,0421	0,7889
rs704444	3	64880377	G	A	2559	0,5446	–	-0,0010	0,0399	0,9808	–	-0,0104	0,0367	0,7763
rs34172651	16	24766841	T	C	2559	0,2831	–	-0,0009	0,0435	0,9827	–	-0,0063	0,0400	0,8750
rs35891966	11	20129311	G	A	2559	0,0654	+	0,0017	0,0795	0,9830	–	-0,0513	0,0732	0,4833
rs2267442	22	42280361	G	A	2559	0,6162	–	-0,0007	0,0402	0,9857	–	-0,0140	0,0370	0,7061
rs2478295	13	106635505	C	G	2559	0,7486	–	-0,0007	0,0458	0,9886	+	0,0397	0,0422	0,3463
rs11977339	7	132970674	C	T	2559	0,3575	+	0,0001	0,0422	0,9972	+	0,0023	0,0388	0,9532
rs954734	3	24055654	A	C	2559	0,3146	+	8,0200	0,0414	0,9985	+	0,0197	0,0381	0,6048
rs713522	17	44065263	T	C	2559	0,4393	+	5,4700	0,0398	0,9989	+	0,0181	0,0366	0,6213
rs35076622	17	43856458	G	A	2559	0,3717	–	-0,6280	0,0409	0,9999	+	0,0081	0,0377	0,8289

Notes:

- chr: chromosome
- N: number of participants
- EAF: estimate of allele frequency (best linear unbiased estimator (BLUE) for alternative allele frequencies in related samples by McPeek et al. (2004))
- direction: direction of the effect estimate
- SE: standard error
- raw p-value: the reported p-value is unadjusted for multiple testing, a p-value < 1x10-4 (based on Bonferroni adjustment (0.05/435=1x10-4)) is considered statistically significant

Supplementary Table 6.2 Association between 435 SNPs and general cognitive decline.

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs429358	19	45411941	C	G	1861,891098	-0,012546	0,002831	-4,432278	0,000010
rs1318878	12	15543479	T	C	1818,743978	0,008102	0,002631	3,079328	0,002106
rs1977200	6	26466392	G	T	1753,091154	-0,007411	0,002430	-3,050245	0,002321
rs74370218	17	57095212	A	G	1782,206680	0,005838	0,002184	2,673632	0,007572
rs1428472	5	66500842	A	C	1771,269307	-0,005620	0,002250	-2,498171	0,012574
rs9451649	6	92049630	C	T	1826,053756	0,005119	0,002097	2,440729	0,014752
rs13262595	8	143316970	A	G	1758,402972	-0,005057	0,002084	-2,426878	0,015329
rs3858954	15	82558034	A	C	1791,773164	0,005135	0,002218	2,315115	0,020719
rs2647249	4	106185663	A	T	1768,289144	0,005815	0,002518	2,309907	0,021008
rs72801843	16	53508802	T	C	1836,515075	-0,005075	0,002253	-2,252584	0,024403
rs9864170	3	85610908	C	T	1766,636895	0,007061	0,003194	2,210935	0,027168
rs7038525	9	23772277	A	G	1779,698402	-0,005302	0,002679	-1,979248	0,047942
rs2526376	17	56427142	C	A	1817,737151	-0,004228	0,002169	-1,949910	0,051340
rs28612284	14	69712437	T	C	1857,825206	-0,019157	0,009859	-1,942961	0,052172
rs2425161	20	34427192	C	G	1686,355167	0,004820	0,002535	1,901410	0,057419
rs56116382	3	49606188	A	C	1817,086647	0,004976	0,002651	1,877479	0,060613
rs5758605	22	42541349	C	G	1754,080109	0,003865	0,002063	1,873585	0,061154
rs112223196	16	28298418	G	C	1780,270705	0,004563	0,002448	1,864273	0,062448
rs13278931	8	30879207	A	G	1768,883412	-0,003867	0,002087	-1,852878	0,064066
rs4778988	15	82540337	G	A	1762,905991	0,003935	0,002143	1,835880	0,066544
rs11716820	3	50228316	G	A	1826,894357	0,006615	0,003608	1,833601	0,066876
rs113714691	8	85804582	G	A	1705,970793	-0,005631	0,003076	-1,830962	0,067281
rs77179555	2	100309124	G	A	2000,936867	0,013232	0,007419	1,783455	0,074664
rs1620977	1	72729142	C	A	1781,516428	-0,004300	0,002413	-1,782419	0,074851
rs8070135	17	56459063	C	T	1770,792284	0,003928	0,002208	1,778524	0,075489
rs4720476	7	44773310	C	T	1785,833433	-0,003910	0,002203	-1,774751	0,076109

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs35076622	17	43856458	A	G	1777,586231	-0,003820	0,002185	-1,748474	0,080555
rs1445653	2	155661432	T	C	1786,481958	0,003653	0,002115	1,726884	0,084361
rs1796518	6	26388672	T	C	1754,349513	0,003679	0,002137	1,721269	0,085378
rs180020	6	16296824	C	T	1739,992138	0,004554	0,002661	1,711407	0,087184
rs12441495	15	41222487	A	G	1825,240655	-0,005658	0,003316	-1,706423	0,088100
rs7609050	2	156021862	A	G	1785,801849	0,003392	0,002044	1,659855	0,097119
rs6964771	7	21410035	T	C	1732,651584	0,003329	0,002045	1,627887	0,103731
rs34506349	2	100598726	T	C	1879,475295	0,009914	0,006100	1,625305	0,104265
rs1343775	1	41757993	G	T	1835,080123	0,004093	0,002535	1,614869	0,106511
rs35741362	6	27007687	C	T	1806,525445	0,004886	0,003050	1,602033	0,109323
rs78565420	8	85703065	C	T	1801,779677	-0,007584	0,004750	-1,596654	0,110518
rs9379850	6	26343057	T	C	1765,821688	-0,004306	0,002720	-1,582903	0,113623
rs111342015	6	43227141	C	G	1769,369297	0,005176	0,003274	1,580866	0,114087
rs2388334	6	98591622	C	A	1787,338723	-0,003256	0,002072	-1,571391	0,116269
rs56151722	3	71482840	G	A	1823,044482	0,003869	0,002466	1,569328	0,116745
rs2074613	5	139714564	A	G	1777,356664	0,003308	0,002112	1,566298	0,117457
rs230489	4	103388441	A	C	1762,528596	0,005702	0,003644	1,564581	0,117861
rs17663165	3	71677981	T	C	1787,540032	0,003254	0,002097	1,551649	0,120923
rs55985762	14	89715495	A	G	1724,795343	-0,003851	0,002555	-1,507185	0,131946
rs3914453	9	120677877	C	T	1781,491946	0,003203	0,002129	1,504690	0,132581
rs1391438	4	106151843	C	G	1749,682425	0,003223	0,002147	1,501260	0,133469
rs11640533	16	53412862	A	G	1819,813411	-0,003193	0,002132	-1,497471	0,134444
rs421211	5	139581773	C	T	1798,448984	0,003127	0,002089	1,497004	0,134568
rs179994	6	16320987	C	T	1754,391947	-0,003137	0,002106	-1,489594	0,136511
rs11209943	1	72750500	T	C	1756,809489	0,003043	0,002076	1,466099	0,142800
rs4716325	6	19025741	G	A	1809,274473	-0,003364	0,002302	-1,461276	0,144113

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs62170823	2	155664656	G	A	1707,554624	0,003092	0,002156	1,433982	0,151760
rs9520384	13	89016992	A	G	1811,451190	0,003214	0,002305	1,394228	0,163420
rs7518151	1	32061851	A	G	1736,544661	-0,003193	0,002291	-1,393509	0,163644
rs17032400	4	103190229	G	C	1787,937868	0,002936	0,002125	1,381847	0,167191
rs6095417	20	47678201	C	T	1820,963029	0,003059	0,002214	1,381300	0,167356
rs67474621	1	72577950	A	T	1789,870277	0,003035	0,002199	1,380311	0,167663
rs2685200	2	82205627	C	T	1766,368447	0,003039	0,002232	1,361276	0,173600
rs62194185	2	161908278	A	G	1784,411618	-0,002992	0,002200	-1,359988	0,174005
rs10514301	5	87939654	A	G	1762,648192	0,004620	0,003439	1,343223	0,179373
rs2074404	17	44865439	A	G	1798,352867	-0,003266	0,002496	-1,308102	0,191006
rs12170228	22	42247695	G	A	1761,287446	0,002689	0,002079	1,293520	0,196001
rs7590388	2	101042122	T	C	1737,224798	-0,002729	0,002111	-1,292973	0,196192
rs6120993	20	34185161	C	T	1676,389308	0,003529	0,002737	1,289188	0,197511
rs6931604	6	98578215	A	C	1772,669440	-0,002668	0,002071	-1,288278	0,197817
rs8067056	17	44083948	T	C	1792,020250	-0,002795	0,002176	-1,284463	0,199146
rs61874768	10	103880118	G	A	1794,075965	0,003425	0,002683	1,276562	0,201922
rs6772177	3	52497778	A	G	1823,419917	0,003766	0,003024	1,245567	0,213084
rs11191270	10	104124605	T	A	1855,347855	0,003383	0,002729	1,239584	0,215286
rs754593	17	44054696	C	G	1778,803121	0,002648	0,002148	1,232766	0,217826
rs79245415	6	98323549	T	C	1769,734683	-0,004213	0,003422	-1,230910	0,218520
rs10996430	10	67213589	A	G	1751,084085	0,002961	0,002406	1,230763	0,218577
rs3113262	7	69993342	C	T	1826,330943	-0,002600	0,002121	-1,225637	0,220493
rs10733389	9	23378220	C	G	1807,528600	0,002671	0,002197	1,216151	0,224086
rs2420551	1	69438777	A	G	1707,736965	0,004629	0,003845	1,203756	0,228851
rs1343700	3	123571754	T	C	1781,134954	0,002606	0,002166	1,203148	0,229079
rs7365380	1	173625710	T	C	1759,193855	-0,002512	0,002090	-1,202109	0,229483

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs17589603	1	72632885	A	G	1760,544399	-0,003868	0,003225	-1,199451	0,230514
rs111599092	3	49502779	T	C	1754,508074	-0,003874	0,003250	-1,192087	0,233388
rs7010173	8	13969304	T	C	1798,587662	0,002598	0,002188	1,187445	0,235209
rs58874647	22	42386269	G	A	1728,771305	-0,002693	0,002286	-1,178001	0,238958
rs2591125	2	155730202	G	C	1770,069765	0,002721	0,002313	1,176056	0,239731
rs9257802	6	29343355	A	G	1801,608165	0,003125	0,002666	1,172171	0,241283
rs3850610	15	83221392	C	T	1787,832300	0,002721	0,002337	1,164073	0,244550
rs9379870	6	26374410	C	A	1765,795230	-0,002655	0,002284	-1,162560	0,245165
rs1699462	9	23857083	C	T	1827,734489	0,002477	0,002143	1,155737	0,247940
rs61572747	17	43460891	G	A	1767,202865	-0,002832	0,002460	-1,151474	0,249693
rs11793831	9	23362311	C	T	1798,996006	0,002415	0,002108	1,145273	0,252248
rs34734335	9	23911098	C	T	1799,244748	0,002444	0,002153	1,134947	0,256549
rs745086	20	47433515	A	G	1801,646388	-0,002480	0,002199	-1,127814	0,259548
rs6906737	6	98345853	T	C	1765,338278	-0,002331	0,002071	-1,125865	0,260376
rs6855246	4	103112470	A	G	1725,786131	0,005639	0,005034	1,120077	0,262837
rs36004779	5	59603880	G	T	1763,133850	-0,003142	0,002811	-1,117983	0,263726
rs7746199	6	27261324	G	A	1807,370659	0,003102	0,002789	1,112209	0,266196
rs13146981	4	152720703	G	C	1806,622129	0,002303	0,002074	1,110306	0,267015
rs4652802	1	183426758	A	G	1795,580084	0,002330	0,002112	1,103294	0,270048
rs35891966	11	20129311	C	T	1947,842724	-0,004677	0,004271	-1,095149	0,273587
rs2451729	6	26705859	T	C	1808,022661	-0,002336	0,002151	-1,086302	0,277490
rs11720523	3	71545170	C	T	1794,019849	0,002273	0,002108	1,078102	0,281133
rs13107325	4	103188709	T	C	1834,709956	0,005865	0,005447	1,076767	0,281726
rs2782653	1	43950265	A	G	1860,732685	0,002537	0,002358	1,075773	0,282168
rs74796574	2	155654998	A	T	1805,289398	-0,005394	0,005071	-1,063748	0,287585
rs6074798	20	14761710	A	T	1765,169006	-0,002191	0,002087	-1,049866	0,293923

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs749694	10	103519784	G	A	1779,775582	0,002223	0,002124	1,046413	0,295512
rs4810883	20	47495794	A	G	1815,566654	0,002377	0,002275	1,045048	0,296140
rs1150735	6	30045199	T	A	1768,617710	-0,002164	0,002073	-1,043982	0,296636
rs7773141	6	98537134	G	A	1799,056858	-0,002151	0,002077	-1,035662	0,300499
rs10273635	7	127158357	C	T	1746,059686	-0,002194	0,002142	-1,023983	0,305985
rs3843738	17	43739194	G	T	1762,457836	0,002108	0,002062	1,022472	0,306698
rs6066861	20	47422577	A	T	1781,115349	0,002363	0,002338	1,011062	0,312124
rs702223	9	23813840	C	T	1791,155653	-0,002224	0,002201	-1,010128	0,312570
rs10806984	6	24181831	G	T	1757,161409	0,002184	0,002166	1,008680	0,313267
rs28433345	16	28883327	T	G	1797,274622	-0,002205	0,002208	-0,998685	0,318082
rs4949460	1	32143138	T	G	1792,425494	-0,002103	0,002110	-0,996938	0,318929
rs9921412	16	71988106	G	A	1781,157869	-0,002297	0,002311	-0,993621	0,320543
rs7699872	4	166195670	A	G	1734,150212	0,002170	0,002196	0,987980	0,323300
rs942353	13	106631574	T	C	1758,836390	-0,002076	0,002120	-0,979600	0,327418
rs4636654	11	28642653	C	T	1823,994152	0,002088	0,002143	0,974566	0,329905
rs7683416	4	106152984	A	G	1763,925830	0,002021	0,002075	0,974130	0,330126
rs6819372	4	67970101	C	T	1788,153776	-0,002040	0,002114	-0,964928	0,334711
rs12938031	17	43854502	A	C	1805,827662	0,002021	0,002099	0,962506	0,335924
rs2903385	4	106094427	G	A	1835,211103	-0,002018	0,002097	-0,962059	0,336147
rs9934041	16	7229579	C	A	1802,125728	-0,002077	0,002173	-0,955887	0,339257
rs3094127	6	30697447	C	T	1766,068897	-0,002546	0,002665	-0,955162	0,339626
rs7873907	9	92225483	A	G	1794,689328	-0,002071	0,002171	-0,953854	0,340286
rs62570303	9	23910400	G	C	1767,598086	0,002258	0,002381	0,948390	0,343061
rs34811474	4	25408838	C	T	1782,016142	-0,002552	0,002706	-0,943151	0,345731
rs1775118	9	23842340	T	G	1786,072206	0,001999	0,002137	0,935121	0,349852
rs11717383	3	52287468	C	T	1743,255365	-0,001959	0,002096	-0,934710	0,350067

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs12885221	14	33279010	A	C	1810,264302	0,002097	0,002267	0,924714	0,355238
rs11865256	16	7265070	C	T	1815,049662	-0,001964	0,002130	-0,922253	0,356519
rs7802313	7	132927792	T	A	1736,161951	-0,001937	0,002101	-0,922181	0,356562
rs3095340	6	30726939	G	A	1756,109956	-0,002760	0,003003	-0,918969	0,358238
rs10914457	1	32094261	T	C	1728,222395	0,001945	0,002126	0,914997	0,360321
rs2457192	16	12197441	C	T	1829,152808	-0,002094	0,002291	-0,914171	0,360747
rs6741949	2	162910223	G	A	1778,933828	-0,001943	0,002134	-0,910361	0,362755
rs3823572	7	133680601	A	G	1772,912400	-0,001964	0,002160	-0,909041	0,363452
rs11126396	2	73565143	T	C	1766,169520	0,002075	0,002288	0,907024	0,364518
rs10872224	6	98435125	G	A	1771,658444	0,001907	0,002124	0,897843	0,369391
rs2024449	16	53494617	A	C	1811,785077	-0,001864	0,002080	-0,896061	0,370339
rs6760964	2	73387386	T	A	1814,724520	0,001909	0,002133	0,894844	0,370989
rs9398173	6	109000316	T	A	1784,789342	-0,002055	0,002329	-0,882297	0,377735
rs34592089	4	102926923	G	C	1688,003341	0,005522	0,006272	0,880351	0,378795
rs3101338	1	72750353	C	G	1810,953628	0,002125	0,002419	0,878592	0,379739
rs4294650	13	21356540	T	C	1770,651819	0,001942	0,002222	0,873992	0,382241
rs4500960	2	162818621	G	A	1799,104292	-0,001781	0,002077	-0,857480	0,391294
rs2267442	22	42280361	T	C	1772,553455	0,001828	0,002132	0,857407	0,391336
rs62439690	7	21417556	T	C	1743,731691	0,002038	0,002391	0,852281	0,394175
rs9366673	6	26893329	T	C	1742,504136	-0,002135	0,002508	-0,851435	0,394645
rs2077610	3	70911271	C	T	1761,593086	-0,001797	0,002133	-0,842338	0,399713
rs2056923	6	27689939	T	G	1786,100783	0,002359	0,002814	0,838372	0,401934
rs7963801	12	79685226	C	T	1792,904091	-0,001903	0,002272	-0,837614	0,402359
rs9597254	13	56325337	C	T	1769,305748	0,001875	0,002241	0,836512	0,402980
rs7534501	1	96221514	C	A	1771,902298	0,001780	0,002132	0,834724	0,403986
rs7646366	3	49470668	C	T	1783,827725	0,001877	0,002249	0,834633	0,404036
rs4244612	8	145741702	T	C	1765,248586	-0,001878	0,002253	-0,833851	0,404478

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs2273558	6	26466035	C	T	1766,351982	-0,001808	0,002215	-0,816288	0,414446
rs3823427	6	27256346	C	T	1791,544369	0,001994	0,002444	0,816037	0,414587
rs6778735	3	52565100	C	G	1703,241854	-0,001683	0,002081	-0,808576	0,418872
rs810681	9	23799239	C	T	1765,756034	-0,001990	0,002472	-0,804822	0,421031
rs4851300	2	100958804	T	C	1813,269828	-0,001769	0,002202	-0,803327	0,421891
rs7599488	2	60718347	G	A	1786,348057	-0,001676	0,002089	-0,802116	0,422593
rs2303907	2	73470497	T	C	1817,328280	0,001653	0,002088	0,791655	0,428665
rs9384679	6	108864419	A	C	1828,667697	0,001675	0,002166	0,773060	0,439587
rs527825	1	183340238	C	G	1804,034199	-0,001633	0,002115	-0,772286	0,440046
rs1044258	10	103605714	G	A	1806,929155	0,001688	0,002186	0,772198	0,440098
rs6503863	17	56517034	A	G	1782,230060	-0,001546	0,002044	-0,756590	0,449396
rs13105581	4	103228830	G	C	1829,283738	0,003423	0,004549	0,752523	0,451833
rs10145335	14	98547748	G	A	1826,422023	0,001828	0,002438	0,749870	0,453429
rs4690029	4	2722815	A	C	1773,279415	-0,001577	0,002105	-0,749099	0,453897
rs2007481	7	69996371	T	C	1818,976600	-0,001652	0,002208	-0,748200	0,454436
rs61754230	12	72179446	T	C	1654,975009	0,006753	0,009065	0,744926	0,456422
rs2523933	6	29932292	C	T	1803,322344	-0,001623	0,002184	-0,742989	0,457585
rs12438326	15	51037974	A	G	1759,467565	-0,001614	0,002175	-0,741830	0,458289
rs10031823	4	103125031	T	C	1803,464081	0,001576	0,002134	0,738685	0,460195
rs4587178	6	98421991	C	T	1748,347460	0,001559	0,002119	0,736015	0,461820
rs4976976	8	143311653	C	A	1750,580735	-0,001567	0,002133	-0,734537	0,462720
rs7412	19	45412079	C	T	1735,703959	0,002649	0,003625	0,730856	0,464966
rs2064853	20	47675176	C	T	1805,266020	-0,001545	0,002134	-0,724080	0,469110
rs7193413	16	28644663	A	G	1786,346726	-0,001695	0,002355	-0,719949	0,471651
rs114030538	1	72588658	A	C	1736,768221	0,003169	0,004420	0,716879	0,473545
rs9558651	13	106617233	A	G	1781,229553	-0,001875	0,002631	-0,712421	0,476298
rs12713315	2	56458509	T	C	1749,538255	0,001595	0,002241	0,711902	0,476620

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs13062354	3	35528166	T	G	1775,815402	0,001460	0,002089	0,698731	0,484812
rs2428507	6	30229306	A	T	1733,917339	0,001473	0,002109	0,698278	0,485097
rs1895471	19	31942961	A	G	1756,224448	0,001520	0,002177	0,698147	0,485178
rs11678106	2	82444107	C	T	1794,276996	0,001460	0,002102	0,694370	0,487540
rs640177	5	88002084	A	G	1811,055710	-0,001445	0,002121	-0,681395	0,495709
rs12709186	16	7249472	T	C	1809,254435	-0,001490	0,002192	-0,679768	0,496738
rs3817334	11	47650993	G	A	1781,167975	0,001426	0,002115	0,674414	0,500136
rs17321073	4	106258989	A	G	1778,380172	-0,001489	0,002216	-0,672080	0,501620
rs2284871	2	162888055	A	G	1765,773297	0,001478	0,002219	0,665889	0,505569
rs2765042	14	103990745	C	T	1724,299421	0,001429	0,002150	0,664403	0,506521
rs28657585	3	48736216	T	C	1833,599254	0,002009	0,003042	0,660280	0,509157
rs2726032	16	28338043	C	T	1788,558018	0,001401	0,002131	0,657289	0,511079
rs2478295	13	106635505	A	G	1758,190632	0,001575	0,002403	0,655352	0,512327
rs242559	17	44025888	G	C	1748,556614	0,001624	0,002502	0,649213	0,516286
rs750472	8	145701453	G	A	1749,426802	0,001345	0,002097	0,641504	0,521279
rs13001130	2	100668699	T	C	1898,411893	0,001750	0,002730	0,641131	0,521515
rs8065165	17	43970317	T	C	1771,760753	-0,001821	0,002876	-0,633029	0,526796
rs62056490	16	71852833	A	C	1819,251048	-0,001627	0,002587	-0,629091	0,529368
rs10911301	1	183282575	C	G	1795,682045	-0,001316	0,002094	-0,628174	0,529970
rs73189617	3	184069126	C	T	1760,313947	-0,001435	0,002289	-0,626874	0,530823
rs66886492	6	28089731	C	G	1784,876848	0,002159	0,003478	0,620612	0,534934
rs17420654	4	16414810	T	C	1765,798243	-0,001379	0,002238	-0,615947	0,538009
rs3092566	20	47499170	T	A	1790,567219	0,001357	0,002219	0,611720	0,540801
rs3755799	3	52809193	A	C	1802,933647	0,001317	0,002159	0,610162	0,541831
rs6950324	7	133054712	G	C	1791,406915	0,001358	0,002228	0,609789	0,542079
rs4852252	2	71539301	C	T	1793,290113	-0,001305	0,002151	-0,606752	0,544093

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs35116560	17	43804186	A	G	1807,633047	0,001299	0,002143	0,605953	0,544622
rs713522	17	44065263	A	G	1741,395640	-0,001242	0,002089	-0,594540	0,552228
rs2471738	17	44076063	T	C	1737,243643	-0,001510	0,002539	-0,594483	0,552266
rs7749823	6	26158079	C	T	1747,984159	0,001791	0,003015	0,593937	0,552631
rs73843290	3	85145836	G	C	1740,740115	0,003619	0,006130	0,590319	0,555053
rs4772729	13	106647578	T	C	1773,104211	0,001673	0,002885	0,579754	0,562154
rs7758926	6	98785796	C	G	1719,047458	0,001727	0,003006	0,574479	0,565719
rs1233578	6	28712247	C	T	1806,236255	0,001691	0,002948	0,573539	0,566351
rs9467777	6	26534616	A	T	1825,107319	-0,001210	0,002132	-0,567482	0,570456
rs62236533	22	41992169	C	A	1815,434167	0,002103	0,003711	0,566679	0,571002
rs71413877	2	100924822	G	A	1916,169587	0,003346	0,005940	0,563208	0,573359
rs13253386	8	14002020	C	T	1802,447765	0,001182	0,002101	0,562714	0,573700
rs11130194	3	49475689	T	A	1763,198498	-0,001204	0,002149	-0,560389	0,575285
rs11210871	1	44029353	A	G	1815,929968	0,001295	0,002312	0,560307	0,575339
rs79582714	1	20883608	C	T	1838,083206	-0,001833	0,003290	-0,557320	0,577376
rs2906455	1	44341895	T	C	1857,353958	0,002068	0,003728	0,554713	0,579157
rs7775835	6	28678357	G	A	1785,350171	-0,001746	0,003160	-0,552413	0,580734
rs80170948	5	64020316	C	T	1751,666507	0,002942	0,005390	0,545718	0,585329
rs10846167	12	15515945	T	C	1817,772489	0,001233	0,002276	0,541678	0,588107
rs59663815	2	68473755	T	C	1766,887666	0,001239	0,002304	0,537682	0,590864
rs1702877	12	56427808	C	T	1813,587175	-0,001179	0,002196	-0,536936	0,591378
rs2517664	6	30073259	C	T	1831,817337	0,001451	0,002737	0,530085	0,596117
rs1046953	3	50197097	C	T	1760,275627	0,001110	0,002097	0,529584	0,596467
rs1526123	17	43783340	T	C	1783,372511	-0,001094	0,002100	-0,520975	0,602449
rs11884495	2	162059969	G	A	1800,831040	0,001058	0,002047	0,516867	0,605312
rs62037363	16	28865042	C	T	1779,092087	-0,001074	0,002092	-0,513361	0,607763

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs1951167	14	37008149	A	G	1762,051005	-0,001045	0,002062	-0,506700	0,612428
rs4687662	3	52870324	T	A	1785,813760	0,001282	0,002558	0,501122	0,616347
rs7618519	3	49772708	A	G	1746,683127	0,001019	0,002040	0,499469	0,617512
rs77418166	6	98204498	C	G	1806,544006	0,002003	0,004017	0,498578	0,618138
rs6568547	6	108856378	A	G	1856,389647	0,001286	0,002585	0,497474	0,618914
rs9541479	13	69221551	G	A	1744,365129	-0,001052	0,002124	-0,495096	0,620595
rs151411	4	103138628	C	T	1803,404574	-0,001271	0,002576	-0,493379	0,621805
rs7186834	16	7673255	T	G	1849,451309	0,001045	0,002121	0,492671	0,622303
rs1892423	1	41849340	G	A	1818,214998	0,001027	0,002086	0,492148	0,622674
rs61104616	5	88163771	G	A	1821,250719	0,001005	0,002073	0,484718	0,627935
rs39302	7	21467190	G	A	1779,372917	0,001218	0,002521	0,483264	0,628968
rs1787003	18	13104251	C	G	1760,088913	0,001017	0,002112	0,481671	0,630099
rs1363119	19	18444809	C	G	1774,434369	-0,001044	0,002169	-0,481389	0,630299
rs12489732	3	52566820	G	A	1791,563220	0,001037	0,002164	0,479392	0,631718
rs9262120	6	30565853	G	A	1804,025287	-0,001468	0,003073	-0,477789	0,632858
rs7763264	6	21960065	C	T	1787,817701	0,001053	0,002212	0,476112	0,634053
rs7809066	7	104568249	A	C	1788,144543	0,000986	0,002073	0,475396	0,634562
rs7002959	8	145685396	C	T	1717,781087	-0,000998	0,002127	-0,468993	0,639134
rs1144	7	104756355	T	C	1760,480886	0,001014	0,002162	0,468901	0,639198
rs3129817	6	30342753	G	T	1814,457429	-0,001522	0,003249	-0,468523	0,639467
rs11577684	1	44254280	G	C	1798,951732	-0,000998	0,002164	-0,461035	0,644829
rs9489945	6	98311875	A	C	1812,468187	-0,000982	0,002148	-0,457459	0,647396
rs17411061	18	50795113	T	G	1777,552681	-0,000944	0,002068	-0,456620	0,648000
rs9949444	18	50695904	A	G	1772,586253	0,000940	0,002138	0,439735	0,660183
rs13201341	6	25822661	T	C	1811,800946	-0,001578	0,003601	-0,438242	0,661263
rs6914699	6	29934022	G	A	1736,027374	-0,000914	0,002109	-0,433524	0,664688

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs6122773	20	47866656	G	A	1861,058361	0,000915	0,002113	0,433242	0,664889
rs55658584	7	104994721	T	C	1777,375986	-0,001004	0,002338	-0,429227	0,667810
rs233811	4	103211254	C	T	1825,459668	-0,000951	0,002228	-0,426625	0,669703
rs935116	15	87973743	C	T	1845,853569	0,000920	0,002172	0,423553	0,671941
rs830237	5	165405678	C	T	1823,844135	-0,000993	0,002349	-0,422593	0,672642
rs78066960	7	127716892	G	A	1760,840484	0,000970	0,002298	0,422123	0,672987
rs79470265	14	98649129	A	G	1854,467470	0,001127	0,002689	0,419237	0,675092
rs6890611	5	60847272	G	A	1752,560072	0,000923	0,002205	0,418735	0,675461
rs11168951	12	49661715	G	A	1851,474231	-0,001581	0,003791	-0,417129	0,676633
rs12918191	16	50945156	A	G	1803,703591	0,001006	0,002456	0,409575	0,682166
rs2517592	6	30093137	A	C	1768,072689	0,001016	0,002506	0,405256	0,685338
rs3132681	6	30060041	A	G	1740,627972	-0,000839	0,002086	-0,402479	0,687381
rs12655172	5	88010829	G	A	1818,252485	0,001386	0,003497	0,396309	0,691924
rs11896887	2	163830427	C	T	1788,966092	0,000814	0,002088	0,389885	0,696668
rs10914462	1	32125943	C	A	1759,573014	0,000812	0,002111	0,384553	0,700615
rs6928583	6	98398142	G	T	1850,617172	-0,001091	0,002865	-0,380749	0,703433
rs7638524	3	52874861	C	A	1801,427700	0,000938	0,002489	0,376736	0,706414
rs31770	5	165496438	G	A	1795,778927	-0,000823	0,002212	-0,372178	0,709804
rs497523	16	28577931	A	G	1820,951694	-0,000851	0,002294	-0,370792	0,710835
rs830386	5	165479749	C	T	1817,246231	-0,000897	0,002430	-0,369343	0,711915
rs56150095	7	71759069	G	A	1791,701401	0,000768	0,002081	0,368924	0,712228
rs3092073	20	44595649	C	T	1807,135247	-0,000767	0,002085	-0,367744	0,713107
rs62422686	6	98661637	T	G	1806,913060	-0,000868	0,002363	-0,367410	0,713356
rs10875914	12	49422094	T	C	1772,634748	0,000785	0,002147	0,365649	0,714671
rs6890684	5	60615886	C	G	1763,288151	-0,000770	0,002113	-0,364419	0,715589
rs62358087	5	107170148	G	A	1816,349458	-0,000964	0,002655	-0,363235	0,716472

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs9856289	3	123898877	T	G	1750,363081	-0,000827	0,002282	-0,362568	0,716971
rs10916805	1	20818659	A	G	1811,183022	0,000999	0,002774	0,360067	0,718839
rs112780312	1	153797015	T	C	1765,366027	-0,000843	0,002349	-0,358911	0,719705
rs62253086	3	85393815	C	T	1771,009680	-0,001320	0,003731	-0,353843	0,723499
rs9836970	3	23949839	C	T	1772,955586	0,000904	0,002555	0,353630	0,723658
rs885916	6	30202571	C	A	1716,684708	0,001050	0,002971	0,353426	0,723812
rs17814604	6	98585502	C	T	1817,631616	-0,000838	0,002380	-0,351915	0,724943
rs242934	17	43936664	G	A	1747,718066	0,000734	0,002097	0,350135	0,726279
rs56016333	7	104596248	G	A	1794,225339	-0,000759	0,002183	-0,347945	0,727923
rs11662271	18	50907365	G	A	1802,069352	0,000720	0,002082	0,345620	0,729669
rs6801153	3	24113697	G	A	1748,172376	-0,000845	0,002448	-0,345177	0,730003
rs1724425	17	43781747	C	T	1766,553219	-0,000723	0,002109	-0,342696	0,731868
rs17251689	12	93067870	G	A	1790,029929	0,000889	0,002600	0,341701	0,732616
rs4925114	17	17711270	C	T	1786,203332	-0,000732	0,002148	-0,340809	0,733288
rs9916613	17	28682453	A	T	1778,395865	0,000731	0,002160	0,338397	0,735104
rs3893463	6	29935405	T	C	1764,487768	0,000681	0,002021	0,337003	0,736155
rs11716948	3	49431903	A	C	1741,076537	-0,000778	0,002374	-0,327620	0,743239
rs6882046	5	87968864	C	T	1796,572672	-0,000779	0,002385	-0,326750	0,743895
rs4852259	2	71682950	C	T	1812,331730	0,000698	0,002139	0,326447	0,744124
rs12485060	22	34265796	G	T	1802,562566	-0,000697	0,002169	-0,321623	0,747776
rs10798879	1	32096815	T	C	1822,683342	-0,000701	0,002189	-0,320361	0,748732
rs62047280	16	61582586	C	T	1775,733885	0,000845	0,002645	0,319375	0,749480
rs2624839	3	50202231	C	T	1788,195831	0,000676	0,002117	0,319184	0,749624
rs73234901	7	127398248	C	T	1758,699544	0,000671	0,002123	0,316035	0,752013
rs117793008	10	104198528	C	T	1751,337875	0,001433	0,004538	0,315830	0,752169
rs9879090	3	52648265	T	G	1766,653850	0,000669	0,002127	0,314712	0,753017

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs2571400	6	29927722	G	A	1764,055457	0,000642	0,002041	0,314686	0,753038
rs1933720	6	98297832	C	A	1748,295869	-0,000697	0,002221	-0,313546	0,753904
rs28374519	16	28489342	C	T	1854,557695	-0,000699	0,002251	-0,310315	0,756356
rs3131064	6	30763893	C	T	1779,512643	0,000901	0,002913	0,309405	0,757050
rs9477970	6	19082663	G	A	1830,084286	-0,000839	0,002742	-0,306140	0,759533
rs12646225	4	696848	T	C	1771,843492	-0,001051	0,003454	-0,304295	0,760939
rs424029	3	85010039	G	A	1803,716619	0,001803	0,005947	0,303242	0,761740
rs34305371	1	72733610	A	C	1796,566333	0,001161	0,003882	0,299019	0,764960
rs2517602	6	30190040	A	G	1774,171878	0,000656	0,002194	0,298884	0,765064
rs1158660	17	43945288	C	A	1750,985417	-0,000683	0,002303	-0,296385	0,766971
rs117431198	20	58219763	C	T	1623,326532	-0,001831	0,006193	-0,295694	0,767502
rs34627	5	60528806	T	C	1822,389289	-0,000609	0,002067	-0,294814	0,768170
rs9467714	6	26340785	C	A	1797,731209	0,000929	0,003166	0,293400	0,769250
rs3799378	6	26404374	G	C	1730,634919	-0,000703	0,002420	-0,290563	0,771420
rs75477016	6	98781196	G	C	1818,816627	-0,000948	0,003285	-0,288651	0,772881
rs13101632	4	103034391	A	G	1766,734926	-0,001240	0,004309	-0,287759	0,773565
rs770463	5	88194950	G	A	1788,386669	-0,000587	0,002048	-0,286408	0,774599
rs36162392	6	26569135	G	T	1776,148392	-0,000939	0,003329	-0,282112	0,777891
rs77482156	6	25199853	G	T	1836,697572	0,001224	0,004411	0,277524	0,781409
rs4731992	7	133702097	T	C	1863,724216	0,000699	0,002625	0,266457	0,789917
rs10786662	10	103989812	A	G	1777,601849	-0,000545	0,002060	-0,264402	0,791501
rs2333604	14	30071738	T	C	1783,372037	-0,000532	0,002111	-0,251818	0,801211
rs7583067	2	100796850	T	C	1865,535265	0,000625	0,002520	0,247977	0,804180
rs72793807	16	28651732	A	G	1757,613154	0,000579	0,002370	0,244308	0,807021
rs1473634	8	20915316	G	A	1718,235143	-0,000544	0,002227	-0,244243	0,807072
rs13214027	6	26573631	T	A	1742,698169	0,000727	0,003004	0,242005	0,808805

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs4331524	2	100876111	T	G	1795,240297	-0,000502	0,002080	-0,241405	0,809269
rs1732664	12	79747487	C	A	1769,965359	-0,000600	0,002501	-0,239925	0,810416
rs28892332	2	118536571	G	A	1852,487980	0,001254	0,005235	0,239494	0,810749
rs2925635	16	28622993	G	C	1762,432224	-0,000523	0,002226	-0,235173	0,814102
rs3129977	6	30745800	G	A	1756,453662	-0,000747	0,003195	-0,233616	0,815310
rs62484026	7	127792094	T	C	1794,446456	0,000579	0,002556	0,226668	0,820707
rs36051354	3	52986012	G	A	1787,362432	-0,000515	0,002283	-0,225776	0,821402
rs12707116	7	133424909	T	C	1779,089180	0,000478	0,002118	0,225721	0,821445
rs909674	22	39859169	G	A	1753,720811	0,000519	0,002300	0,225600	0,821539
rs407102	1	109846278	A	C	1741,320054	0,000491	0,002284	0,214832	0,829923
rs429479	6	29372323	T	C	1762,904785	-0,000712	0,003331	-0,213739	0,830776
rs13023088	2	100576304	C	G	1832,398622	0,000632	0,002998	0,210656	0,833179
rs6124077	20	59815020	G	C	1763,322728	-0,000440	0,002120	-0,207607	0,835560
rs743134	20	14804964	G	A	1800,344723	0,000442	0,002137	0,206978	0,836050
rs926300	6	27059443	T	C	1782,632482	0,000552	0,002679	0,205870	0,836916
rs2726513	4	106217358	A	G	1793,563333	0,000439	0,002157	0,203624	0,838671
rs74944275	5	102726073	C	T	1729,262594	-0,000945	0,004756	-0,198803	0,842440
rs2309812	2	100869702	A	G	1792,126365	0,000424	0,002134	0,198641	0,842566
rs2346449	7	133733917	C	A	1802,572902	-0,000447	0,002250	-0,198497	0,842679
rs61544985	7	127102681	T	C	1806,298283	0,000405	0,002113	0,191594	0,848082
rs516902	1	96700020	C	A	1768,415575	-0,000414	0,002175	-0,190393	0,849023
rs17199964	4	102707791	A	G	1730,751382	0,000828	0,004374	0,189367	0,849827
rs11759522	6	3450814	A	G	1767,916590	-0,000393	0,002082	-0,188516	0,850494
rs34172651	16	24766841	G	A	1848,421003	-0,000436	0,002322	-0,187576	0,851229
rs13425585	2	157487273	G	A	1804,903751	-0,000393	0,002106	-0,186407	0,852147
rs79738691	6	25148560	C	A	1867,103045	0,000892	0,004976	0,179272	0,857744

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs2091329	1	110042079	C	T	1749,623933	0,000392	0,002203	0,177710	0,858971
rs11764631	7	127179493	A	G	1803,383603	-0,000365	0,002091	-0,174366	0,861597
rs1953522	1	216837241	A	C	1762,660108	0,000367	0,002172	0,169076	0,865756
rs10761765	10	65188973	T	C	1847,990235	-0,000442	0,002654	-0,166718	0,867610
rs12025777	1	41748897	C	A	1979,882234	0,000679	0,004128	0,164392	0,869439
rs7613360	3	49916710	A	G	1741,543381	-0,000341	0,002080	-0,163902	0,869827
rs34180096	7	104608592	A	C	1774,235940	-0,000341	0,002084	-0,163726	0,869966
rs954734	3	24055654	C	T	1738,687721	0,000346	0,002154	0,160608	0,872421
rs6759626	2	163886350	A	G	1773,317798	0,000396	0,002472	0,160352	0,872622
rs750155	16	28620572	G	A	1805,334805	0,000349	0,002175	0,160342	0,872629
rs11130226	3	49993191	C	A	1764,390919	-0,000324	0,002049	-0,158279	0,874255
rs3130127	6	30391975	G	A	1802,816890	-0,000377	0,002401	-0,157170	0,875128
rs1408579	10	101912194	G	A	1762,466511	-0,000322	0,002095	-0,153729	0,877841
rs11970030	6	20930113	C	T	1776,530594	0,000533	0,003486	0,152776	0,878592
rs200991	6	27815494	A	G	1757,913156	0,000426	0,002854	0,149409	0,881248
rs7487854	12	50150266	C	T	1793,679102	-0,000637	0,004321	-0,147354	0,882869
rs10853627	18	50706073	C	G	1774,790497	-0,000306	0,002084	-0,147065	0,883097
rs10133551	14	33282232	C	T	1787,837987	-0,000297	0,002114	-0,140263	0,888468
rs28646281	17	43977846	G	T	1767,668308	-0,000286	0,002190	-0,130842	0,895915
rs10858096	1	109967104	T	C	1773,477532	-0,000278	0,002130	-0,130662	0,896058
rs2636699	4	106312813	G	T	1769,083841	-0,000285	0,002189	-0,130146	0,896466
rs143699161	17	43748712	A	T	1748,057493	0,000318	0,002466	0,129091	0,897301
rs9966005	18	50440366	A	G	1813,619625	0,000259	0,002073	0,125025	0,900517
rs1502233	18	50990435	C	A	1769,545095	0,000270	0,002171	0,124175	0,901191
rs7998050	13	58473866	A	C	1763,320893	0,000277	0,002262	0,122638	0,902408
rs4812279	20	59777491	T	C	1807,234587	-0,000265	0,002165	-0,122273	0,902697
rs153286	5	165409177	A	G	1795,756584	0,000253	0,002078	0,121940	0,902961

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs10281827	7	133081810	T	A	1751,782809	-0,000260	0,002252	-0,115635	0,907955
rs11628208	14	36994709	T	G	1789,480863	-0,000239	0,002114	-0,112854	0,910159
rs889956	2	57404834	C	A	1737,750964	-0,000234	0,002074	-0,112734	0,910254
rs2336670	3	52998504	T	G	1755,972366	-0,000249	0,002249	-0,110614	0,911935
rs11720236	3	127161689	G	A	1823,225168	0,000310	0,002883	0,107443	0,914449
rs246776	1	44274699	C	T	1782,876407	-0,000220	0,002093	-0,104955	0,916423
rs58859557	1	44010456	G	A	1744,677014	-0,000455	0,004337	-0,104858	0,916500
rs2189373	6	30223428	C	T	1734,244627	0,000283	0,002707	0,104359	0,916896
rs2426132	20	47723127	C	A	1810,101820	-0,000222	0,002126	-0,104257	0,916977
rs151231	16	28582849	G	A	1803,742542	-0,000219	0,002159	-0,101311	0,919315
rs28549862	8	64662318	A	C	1769,529337	0,000229	0,002274	0,100588	0,919889
rs28645341	14	30174078	A	T	1745,676669	0,000225	0,002253	0,099800	0,920515
rs187084	3	52261031	G	T	1793,832066	0,000210	0,002111	0,099581	0,920688
rs11977339	7	132970674	C	T	1741,680132	0,000218	0,002212	0,098619	0,921452
rs1362104	6	30101656	T	C	1745,684187	-0,000202	0,002116	-0,095357	0,924042
rs71324996	3	49834767	G	T	1712,597179	0,000214	0,002301	0,093009	0,925908
rs2239647	14	33292743	C	A	1780,956542	-0,000189	0,002046	-0,092479	0,926328
rs11648192	16	28618708	G	C	1782,493120	0,000192	0,002234	0,086158	0,931350
rs9467707	6	26327905	T	C	1760,255060	0,000276	0,003341	0,082626	0,934158
rs79046494	16	28527326	T	A	1807,732934	-0,000165	0,002160	-0,076489	0,939038
rs381262	20	47933479	A	T	1825,875214	0,000191	0,002501	0,076267	0,939215
rs7814022	8	118902809	G	T	1780,200705	0,000159	0,002113	0,075439	0,939874
rs8016504	14	33308021	G	C	1768,875003	-0,000143	0,002037	-0,070453	0,943841
rs1541102	21	40628765	G	A	1766,672944	0,000153	0,002183	0,070147	0,944084
rs2819336	1	44015809	C	T	1845,084299	0,000145	0,002156	0,067126	0,946489
rs7633520	3	23966425	C	A	1746,949880	-0,000152	0,002274	-0,066809	0,946742
rs4781499	16	13630944	T	G	1746,379823	0,000196	0,003003	0,065433	0,947837

(Supplementary Table 6.2 continues)

Association between 435 SNPs and general cognitive decline									
					Results of interaction effect between each SNP and Time (i.e. SNP*Time)				
rs-number	chromosome	position	reference allele	alternative allele	df	estimate	std. error	t-value	raw p-value
rs145104523	21	40644110	C	T	1673,347045	0,000185	0,003058	0,060368	0,951869
rs2280405	3	49895052	T	C	1791,501990	-0,000130	0,002156	-0,060300	0,951923
rs12073420	1	20871181	C	A	1783,121098	0,000161	0,002749	0,058550	0,953317
rs704444	3	64880377	A	G	1743,491694	0,000118	0,002091	0,056581	0,954885
rs9295486	6	20896553	T	A	1771,002158	-0,000109	0,002047	-0,053443	0,957385
rs9480861	6	108858460	G	A	1791,936627	0,000091	0,002091	0,043492	0,965314
rs11153822	6	98214814	G	A	1742,120581	-0,000083	0,002134	-0,038961	0,968926
rs11596211	10	101840119	T	C	1752,858544	-0,000080	0,002219	-0,035944	0,971331
rs7146202	14	33303517	G	A	1804,085158	-0,000068	0,002051	-0,033010	0,973670
rs35792732	7	132929804	G	A	1760,304841	-0,000081	0,002567	-0,031533	0,974848
rs7978070	12	14511806	C	G	1759,422408	0,000051	0,002107	0,024025	0,980836
rs13428598	2	144250487	C	T	1796,643574	0,000045	0,002091	0,021539	0,982818
rs77944304	1	72582779	A	C	1788,975171	-0,000038	0,002264	-0,016775	0,986618
rs10218712	1	41832297	T	C	1832,801405	-0,000027	0,002320	-0,011568	0,990771
rs2844790	6	30094113	G	A	1820,006070	0,000014	0,003119	0,004557	0,996364

Notes:

- df: degrees of freedom
- std. error: standard error
- raw p-value: the reported p-value is unadjusted for multiple testing, a p-value < 2x10-4 (based on Bonferroni adjustment (0.1/435=2x10-4)) is considered statistically significant

7

General discussion

Frailty is an age-associated syndrome and can be described as a state of increased vulnerability to adverse health outcomes when exposed to stressors, caused by the cumulative decline in one or more domains of functioning. An important characteristic of frailty is that it is reversible, thus frail older people can return to a non-frail state when frailty is identified in an early stage (1–5). Hence, early identification of frailty is important to prevent progression and stimulate reversal. Further, evidence is accumulating that chronic low-grade inflammation, and thereby an ageing immune system, may be involved in the development of frailty (6–10). In this thesis, a multi-domain approach was used to study frailty in adults and to identify (bio)markers for the physical, cognitive, psychological, and social frailty domains, which could be indicative of the underlying processes that cause frailty and could possibly help to detect people at increased risk.

To address these aims, data of the Doetinchem Cohort Study (DCS) and the European study to establish biomarkers of human Ageing (MARK-AGE) were used comprising a wide variety of (bio)markers, such as questionnaire data, anthropometric markers, biochemical markers, and genetic data. Moreover, different study designs and analysis techniques were used for the exploratory studies described in this thesis. In **Chapters 2**, **3**, and **4**, (bio)markers for the four domains of frailty were studied cross-sectionally (**Part I** of this thesis). In **Chapters 5** and **6**, (bio)markers for cognitive frailty and general cognitive function were studied longitudinally (**Part II** of this thesis). The major focus in frailty research worldwide has been on the physical domain. Although the cognitive domain has been studied less frequently, this domain is very important too, amongst others for remaining self-reliant. Therefore, we studied the cognitive domain in depth using longitudinal data. In this general discussion, the main findings are summarised and reviewed. Further, conceptual and methodological aspects are discussed, as well as the implications of these findings for public health and future research.

Main findings of Part I: Exploring (bio)markers for the four frailty domains cross-sectionally

Limited overlap between the four frailty domains

We found that the physical, cognitive, psychological, and social frailty domains entail distinct groups of frail people, but that these groups have shared characteristics (**Chapters 2**, **3** and **4**). Below, the results are discussed per frailty domain. For the multivariate analyses only the statistically significant results are discussed.

Physical frailty

Descriptive analyses showed that the prevalence of physical frailty was higher at higher ages, was higher among women than men, and physically frail people were lower educated compared to non-frail people. Physically frail people were less physically active, were more often smokers, had a higher BMI, had more often multimorbidity and had worse self-reported health compared to non-frail people (**Chapters 2, 3** and **4**). In addition, total cholesterol levels were lower in physically frail people compared to non-frail people (**Chapter 4**) (**Table 7.1**).

Multivariate regression analyses showed that advanced age (*i.e.* belonging to the highest age group (70–81 years) compared to younger age groups), current smoking, being underweight or obese, having multimorbidity, and a short sleep duration were associated with a higher risk of being physically frail (**Chapters 2, 3 and 4**). In addition, an intermediate educational level, having a paid job, sufficient physical activity, a healthy diet, and consuming alcohol on a weekly basis were associated with a lower risk of being physically frail (**Chapter 2**) (**Table 7.2**).

Psychological frailty

Descriptive analyses showed that the prevalence of psychological frailty was not age related, was more common among women than men, and psychologically frail people were lower educated compared to non-frail people. Psychologically frail people were less physically active, were more often smokers, had a higher BMI, had more often multimorbidity and had worse self-reported health compared to non-frail people (**Chapters 2**, **3** and **4**) (**Table 7.1**).

Multivariate regression analyses showed that female sex, a low educational level, current smoking, having multimorbidity, and both short and long sleep duration were associated with a higher risk of being psychologically frail (**Chapter 2**). In addition, being married and physically active were associated with a lower risk of being psychologically frail (**Chapter 2**) (**Table 7.2**).

Social frailty

Descriptive analyses showed that the prevalence of social frailty was slightly higher at higher ages, and was not different between men and women. Socially frail people were lower educated compared to non-frail people. Socially frail people were less physically active, were more often smokers, had a higher BMI, had more often multimorbidity and had worse self-reported health compared to non-frail people (**Chapters 2** and **3**) (**Table 7.1**).

Multivariate regression analyses showed that a low educational level and short sleep duration were associated with a higher risk of being socially frail (**Chapter 2**). In addition, being a woman, married, and physically active were associated with a lower risk of being socially frail (**Chapter 2**) (**Table 7.2**).

Cognitive frailty

Descriptive analyses showed that the prevalence of cognitive frailty was higher at higher ages, was more frequent among men than women, and cognitively frail people were lower educated compared to non–frail people. Cognitively frail people were less physically active, had a higher BMI, had more often multimorbidity and had worse self–reported health compared to non–frail people (**Chapters 2**, **3** and **4**). In addition, total cholesterol levels were lower in cognitively frail people compared to non–frail people (**Chapter 4**) (**Table 7.1**).

Multivariate regression analyses showed that advanced age (*i.e.* belonging to the highest age group (70–81 years) compared to younger age groups) and being obese were associated with a higher risk of being cognitively frail (**Chapters 2** and **3**). In addition, female sex, having a paid job, adhering to a healthy diet, being physically active, higher plasma β–cryptoxanthin levels, and higher plasma zeaxanthin levels were associated with a lower risk of being cognitively frail (**Chapters 2** and **4**) (**Table 7.2**).

Table 7.1. Results of the descriptive cross-sectional analyses of Chapters 2, 3, and 4, for people who are physically, psychologically, socially and/or cognitively frail compared to the total or non-frail study population.

	Physically frail	Psychologically frail	Socially frail	Cognitively frail
Socio-demographic				
age	↑	≈	≈/↑[a]	↑
sex (women)	↑	↑	≈	↓
educational level	↓	↓	↓	↓
married	↓	↓	↓	↓
paid job	↓	↓	↓	↓
Lifestyle				
physically active[b]	↓	↓	↓	↓
healthy diet[c]	↓	≈	≈	≈/↓[a]
current smoker	↑	↑	↑	≈
weekly alcohol consumption[d]	↓	↓	↓	≈
Body-composition				
BMI, kg/m^2	↑	↑	↑	↑
underweight[e]	↑	↑	↑	–
obesity[f]	↑	↑	↑	↑
Biomarkers				
total cholesterol serum levels, mmol/l	↓	≈	–	↓
β-cryptoxanthin plasma levels, µmol/l	↓	↓	–	↓
zeaxanthin plasma levels, µmol/l	↓	↓	–	↓
Health and disease				
self-reported health	↓	↓	↓	↓
multimorbidity[g]	↑	↑	↑	↑
sleep duration				
≤5 hours	↑	↑	↑	↑
<6 hours	↑	↑	↑	↓
7 or 8 hours	↓	↓	↓	↓
>9 hours	↑	↑	↑	↑

Abbreviation: BMI = body mass index.

[a] The results between chapters differ; both results are summarised.

[b] Physically active: adherence to the Dutch physical activity guideline, which recommends ≥ 30 min of moderate to vigorous physical activity on at least 5 days per week.

[c] Healthy diet: the World Health Organisation's dietary recommendations for the prevention of chronic disease were applied. Score on the healthy diet indicator ranged from 0 to 9 and was based on the sum of the number of nutrients (out of a group of seven nutrients) and the number of products from two food groups for which intake was within the recommended range.

[d] Weekly alcohol consumption: ≥1 glass/week.

[e] Underweight: <20 kg/m^2.

[f] Obesity: ≥30 kg/m^2.

[g] Multimorbidity: having two or more conditions out of diabetes, cancer, myocardial infarction, cerebrovascular accident, and chronic respiratory symptoms.

Legend: ↑ = more often/higher in the frail population compared to the total or non-frail study population; ↓ = less often/lower in de frail population compared to the total or non-frail study population; ≈ = similar for the frail and the total or non-frail study population; – = could not be studied.

Table 7.2. Results of the multivariate cross-sectional regression analyses of Chapters 2, 3, and 4 for people who are physically, psychologically, socially and/or cognitively frail compared to the total or non-frail study population.

	Physically frail[a]	Psychologically frail[a]	Socially frail[a]	Cognitively frail[a]
Socio-demographic				
advanced age (70–81 yrs.)	↑	ns	ns	↑
sex (women)	ns	↑	↓	↓
educational level				
low	ns	↑	↑	ns
intermediate	↓	ns	ns	ns
high	reference	reference	reference	reference
married	ns	↓	↓	ns
paid job	↓	ns	ns	↓
Lifestyle				
physically active[b]	↓	↓	↓	↓
healthy diet[c]	↓	ns	ns	↓
current smoker	↑	↑	ns	ns
weekly alcohol consumption[d]	↓	ns	ns	ns
Body-composition				
underweight[e]	↑	ns	ns	–
obesity[f]	↑	ns	ns	↑
Biomarkers				
β-cryptoxanthin plasma levels, µmol/l	ns	ns	–	↓
zeaxanthin plasma levels, µmol/l	ns	ns	–	↓
Health and disease				
multimorbidity[g]	↑	↑	*ne*	*ne*
sleep duration				
≤5 hours	↑	↑	↑	ns
<6 hours	ns	↑	ns	ns
7 or 8 hours	reference	reference	reference	reference
>9 hours	ns	↑	ns	ns

[a] Each result summarised in this table is based on one chapter only.

[b] Physically active: adherence to the Dutch physical activity guideline, which recommends ≥ 30 min of moderate to vigorous physical activity on at least 5 days per week.

[c] Healthy diet: the World Health Organisation's dietary recommendations for the prevention of chronic disease were applied. Score on the healthy diet indicator ranged from 0 to 9 and was based on the sum of the number of nutrients (out of a group of seven nutrients) and the number of products from two food groups for which intake was within the recommended range.

[d] Weekly alcohol consumption: ≥1 glass/week.

[e] Underweight: <20 kg/m^2.

[f] Obesity: ≥30 kg/m^2.

[g] Multimorbidity: having two or more conditions out of diabetes, cancer, myocardial infarction, cerebrovascular accident, and chronic respiratory symptoms.

Legend: ↑ = a higher risk of being frail; ↓ = a lower risk of being frail; ns = no significant difference in risk; – = could not be studied.

Interpretation of Part I

The importance of distinguishing different frailty domains

The concept of frailty originates from studies of the physical domain. In more recent years, a holistic approach of frailty has been advocated, focussing not only on the physical domain, but also on other domains of functioning, such as the psychological and social domains. In response, many frailty instruments have been developed (11). Three frailty instruments that are often used are: 1) the Frailty Phenotype (FP) focusing on physical frailty (12), 2) the Frailty Index (FI) that sums the accumulation of deficits (13), 3) the Tilburg Frailty Indicator (TFI) using a multi-domain approach to identify frail older people (14). In this thesis, a multi-domain approach was used to study frailty. In multi-domain frailty instruments, the cognitive frailty domain is sometimes combined with the psychological frailty domain. Since cognitive and psychological functioning are two distinct domains of functioning, we studied the cognitive and psychological frailty domains separately, along with the physical and social frailty domains. This approach revealed that the different frail populations (physical, cognitive, psychological, and social) entail distinct groups of frail people. These four frail populations have overlapping, but also partly distinct characteristics in terms of socio-demographic factors, lifestyle, and multimorbidity. This is in line with previous research that observed differences in population characteristics between frailty domains (15, 16). This suggests that effective prevention and intervention strategies need to be tailored to each frailty domain.

In the following sections, several notable findings for the different frailty domains are discussed.

Physical frailty

Different frailty instruments lead to different results: the TFI versus the FP

In **Chapter 2**, physical frailty was operationalised based on the TFI, while in **Chapter 3** this domain was operationalised based on the FP (**Table 7.3**). Both studies were performed using data of the DCS. Comparing physically frail people identified with the TFI (**Table 2.2**) versus the FP (**Table 3.2**), indicates that the TFI identifies physically frail people who are older (mean age 69 years with TFI and 64 years with FP), more often low educated (69% with TFI and 51% with FP) and less healthy (higher BMI (30kg/m^2 with TFI and 28kg/m^2 with FP), more multimorbidity (39% with TFI and 21% with FP)) compared to the physically frail people identified

using the FP. The differences between the physically frail population identified by the TFI versus the FP might also be reflected in the observed associations. For example, we observed that being underweight or obese was not associated with physical frailty in **Chapter 2** (where the TFI was used), while in **Chapter 3** (where the FP was used), both underweight and obesity were associated with physical frailty. The sensitivity analysis in **Chapter 3** showed that there was also a U–shaped association between BMI and physical frailty based on the TFI. However, this association was less strong compared to the U–shaped association between BMI and physical frailty based on the FP. These differences were also observed in multiple studies in which a U–shaped association between BMI and physical frailty was found. For example, Strawbridge *et al.* (17) used a multi–domain approach to measure overall frailty based on four domains of functioning (*i.e.* physical, cognitive, sensory, and nutritive) and did not find an association between obesity and overall frailty. In contrast, studies that used the FP or FI did find an association between obesity and (physical) frailty (18–21). In these studies, the different frailty instruments used to measure physical frailty may also explain the differences in associations between BMI and physical frailty.

This thesis provides some insight into the physical frailty domain defined according to the TFI versus the FP. The choice of frailty instrument affects the composition of the frail population that is identified, as shown for physical frailty based on the TFI versus the FP (**Chapter 3**). This, in turn, affects which associations are observed and the strength of these associations. Hence, in deciding which frailty instrument to use and in interpreting the results, it is important to be aware of the specifications (and possible advantages and disadvantages) of the chosen instrument. In addition, when systematically reviewing the literature, the different frailty instruments and their proxies should be taken into account.

Table 7.3. Overview of the criteria of the original frailty instrument used to operationalise physical, cognitive, psychological, and social frailty in the Doetinchem Cohort Study and in MARK-AGE.

Domains	Criteria	Cut-off	Based on
FP (*i.e.* physical frailty) (12)	– unintentional weight loss	≥ 3 criteria	– unintentional weight loss: ○ In the last year, have you lost more than 10 pounds unintentionally (i.e., not due to dieting or exercise)? ○ At follow-up: > 5% weight loss between in one year.
	– exhaustion		– exhaustion: 2 statements of the Center for Epidemiologic Studies Depression scale (22)
	– low physical activity		– low physical activity: Based on the short version of the Minnesota Leisure Time Activity questionnaire. This variable is stratified by sex. ○ Men: Those with Kcals of physical activity per week <383 are frail. ○ Women: Those with Kcals per week <270 are frail.
	– reduced handgrip strength		– handgrip strength: dynamometer
	– slow walk speed		– slow walk speed: cut-off for time to walk 15 feet ○ Men with height ≤ 173 cm: cut-off time is ≥ 7 seconds ○ Men with height >173 cm: cut-off time is ≥ 6 seconds ○ Women with height ≤159 cm: cut-off time is ≥ 7 seconds ○ Women with height >159 cm: cut-off time is ≥ 6 seconds
Operationalisation of physical frailty based on FP in the DCS	– unintentional weight loss	≥ 2 criteria	– unintentional weight loss: > 5% weight loss between round 4 and 5 and not being on a diet
	– exhaustion		– exhaustion: 2 statements of the Center for Epidemiologic Studies Depression scale (22)
	– low physical activity		– low physical activity: meeting all of the following three criteria: 1) < 10th percentile of a physical activity score 2) <25th percentile of walking hours per week during the last 12 months 3) failing to meet the Dutch physical activity guideline
	– reduced handgrip strength		– handgrip strength: dynamometer

(Table 7.3 continues)

Domains	Criteria	Cut-off	Based on
Operationalisation of physical frailty based on FP in MARK-AGE	– unintentional weight loss	≥ 2 criteria	– weight loss >5% in 12 months' time and the participant answering 'most of the time' or 'a good part of the time' to the question: 'I eat as much as I used to'.
	– exhaustion		– participants were considered exhausted when answering 'all of the time', 'most of the time', or 'a good bit of the time' to the statement 'Did you feel worn out?'
	– low physical activity		– low physical activity was defined using the question: 'Do you do any kind of light housework or exercise (e.g. vacuum-cleaning, sweeping, mopping floors, ironing, gardening, gymnastics or short walks)?'. If the answer was 'yes' participants answered the following question: 'How often?'. If the answer was 'several times a week' or less, the participant was considered insufficiently physical active.
	– reduced handgrip strength		– handgrip strength: dynamometer
Physical domain of TFI (14)	– unintentional weight loss	*	– unintentional weight loss: Have you lost a lot of weight recently without wishing to do so? ('a lot' is: 6 kg or more during the last six months, or 3 kg or more during the last month)
	– perceived health		– perceived health: Do you feel physically healthy?
	– limited in walking		– Other criteria: Do you experience problems in your daily life due to: ○ Difficulty in walking?
	– disturbed balance		○ Difficulty maintaining your balance?
	– hearing impairment		○ Poor hearing?
	– vision impairment		○ Poor vision?
	– reduced handgrip strength		○ Lack of strength in your hands?
	– exhaustion		○ Physical tiredness?
Operationalisation of physical frailty based on TFI in the DCS	– unintentional weight loss	≥ 4 criteria	– unintentional weight loss (see FP)
	– perceived health		– perceived health: one question of 36-Item Short-Form Health Survey (23, 24)
	– limited in walking		– 1 question on 100 m walking
	– disturbed balance		– Tandem Stand Balance Test
	– hearing impairment		– 3 questions regarding hearing
	– vision impairment		– 3 questions regarding vision
	– reduced handgrip strength		– handgrip strength (see FP)
	– exhaustion		– exhaustion (see FP)

(Table 7.3 continues)

Domains	Criteria	Cut-off	Based on
Cognitive domain of TFI (14)	– memory problems	*	– Do you have problems with your memory?
Operationalisation of cognitive frailty based on TFI in the DCS	– reduced global cognitive functioning	<10th percentile	– global cognitive functioning score based on tests for memory, speed and flexibility: ○ 15 Words Verbal Learning Test (25) ○ Stroop Colour–Word Test (26) ○ Word Fluency Test (27) ○ Letter Digit Substitution Test (28)
Operationalisation of cognitive frailty based on TFI in the MARK-AGE	– reduced global cognitive functioning	< 10th percentile	– global cognitive functioning score based on tests for memory, speed and flexibility: ○ 15-Picture Word Learning Test ○ Stroop Colour-Word Test ○ Digit Symbol Substitution Test
Psychological domain of TFI (14)	– depressive symptoms and mental health – coping style	*	– Have you felt down during the last month? – Have you felt nervous or anxious during the last month? – Are you able to cope with problems well?
Operationalisation of psychological frailty based on TFI in the DCS	– depressive symptoms – mental health	= 2 criteria	– Centre for Epidemiologic Studies Depression scale – Mental Health Inventory 5 (24)
Operationalisation of psychological frailty based on TFI in the MARK-AGE	– depressive symptoms – mental health	= 2 criteria	– Zung self-rating depression scale (29) – Mental Health Inventory 5 (30)
Social domain of TFI (14)	– loneliness – little social support – low social participation	*	– Do you live alone? – Do you receive enough support from other people? – Do you sometimes miss having people around you?
Operationalisation of social frailty based on TFI in the DCS	– loneliness – little social support – low social participation	≥ 2 criteria	– Loneliness Scale (31) – Social Support List-12 (32) – Questionnaire Dutch Elderly Monitor (33)

Abbreviations: FP, Frailty Phenotype; TFI, Tilburg Frailty Indicator.

Notes: Physical frailty based on the TFI and social frailty were not measured in MARK-AGE; For each frailty domain, first the criteria of the original frailty instrument are summarised and thereafter the operationalisation in the DCS is summarised; The TFI uses a cut-off point for overall frailty and not for the separate frailty domains. An individual is considered frail if the total TFI-score is five or higher.

Psychological frailty

Psychologically frail people have poor health already at a relatively young age

When comparing the frail populations for the different frailty domains, the psychologically frail population is relatively young (mean age is 59 years in **Chapters 2** and **3** and 58 years in **Chapter 4**). Yet, the prevalence of poor self–reported health and objectively measured markers in this group is similar to that in the older physically, cognitively, and socially frail populations. At least forty–five percent of the psychologically frail population reported to have poor health (**Chapters 2** and **3**). In addition, the psychologically frail group had the highest percentage of falls, of over 19% (**Chapter 4**), which is remarkable because falls are generally more common among people of older age (34). The relatively young age and already poor self–reported health of the psychologically frail population underlines the urgency of developing effective prevention and intervention strategies for this specific group.

The prevalence of psychological frailty was not age–related. Risk factors for developing psychological problems or disorders include environmental factors, such as life events and chronic stressors (35), personality traits *e.g.* coping style (36), and genetic factors (37). In **Chapter 2**, we did not observe an association between adverse life events and psychological frailty, but others did find evidence for this association (38–40). Personality traits may be important risk factors for the development of psychological frailty, and these might explain why psychological frailty is already common in middle–aged adults. The association between personality traits and psychological frailty could not be studied in the DCS nor in MARK–AGE since there was no data available regarding this topic.

Social frailty

The positive effect of being married and physical activity on social frailty

In a review study on social frailty, it was found that a lack of social resources, social behaviours, social activities, and self–management abilities are components of the concept of social frailty (41). Therefore, the authors suggested that interventions should focus on all these aspects of social frailty. Our multivariate analyses showed that being married and being physically active was associated with a lower risk of being socially frail (**Chapter 2**). In a review study it was shown that social support, especially from family members, stimulates older people to be physically active (42). Another study showed that an unsatisfactory social network

predicts a reduction in physical activity in older people (43). These studies showed that having social resources encourages older people to be physically active. The reverse may also be true, *i.e.* older people who are physically active acquire more social resources through these activities. Thus, being married and being physically active might both be associated with a lower risk of social frailty, both directly and through their positive effect on physical activity and social resources, respectively.

Cognitive frailty

The role of a healthy diet in the development of cognitively frailty

Multivariate regression analyses showed that a healthy diet, higher plasma β–cryptoxanthin levels, and higher plasma zeaxanthin levels were associated with a lower risk of being cognitively frail (**Chapters 2** and **4**) (**Table 7.2**). These findings suggest that, especially in the cognitive frailty domain, nutrition could play an important role. The importance of a healthy diet for cognitive function has been demonstrated by others and was also observed previously in the DCS (44). Antioxidants, for example, can reduce oxidative stress, which is important since the brain is highly susceptible to oxidative damage (44), and higher levels of antioxidants are associated with less cognitive decline (45, 46). Although it is interesting to understand the role of individual nutritional components, an overall healthy diet is probably most important (47). One diet that has been studied often and also seems to have a positive effect on cognitive outcomes is the Mediterranean diet (44). This diet consists of fruits, vegetables, whole grains, legumes, nuts, fish, monounsaturated fats from olive oil and moderate alcohol consumption and reduces the risk of developing cardiovascular disease, obesity, type 2 diabetes mellitus, depression, cognitive decline and other negative health related outcomes (48–50). This diet contains a wide variety of nutrients such as antioxidants and could possibly be a part of strategies for the prevention of cognitive frailty, or for early intervention.

Main findings of Part II: Exploring (bio)markers of cognitive frailty, general cognitive function and cognitive decline using longitudinal data

Cognitive frailty

In **Chapter 5**, we explored differences between (bio)marker trajectories of people who became cognitively frail compared to controls who did not become cognitively

frail, based on the hypothesis that those who would become cognitively frail would have more unfavourable trajectories of (bio)markers compared to controls. Over a follow-up period of 15 years, although not statistically significantly different, we observed that self-reported health was consistently worse, and BMI and waist circumference were consistently higher among cognitively frail people compared to non-frail people. Out of the 17 (bio)markers included in this explorative study, we only found statistically significant differences in the shape of the trajectories of total cholesterol, gamma-glutamyltransferase, and urea between incident cognitively frail women and controls. The trajectory of total cholesterol showed a decline around the onset of cognitive frailty among women, which was not observed in non-frail women. In cognitively frail men, the total cholesterol trajectory declined earlier, 5 years before the onset of cognitive frailty, and this decline was less steep compared to the decline in total cholesterol trajectories in cognitively frail women.

General cognitive function and decline

In **Chapter 6**, we studied the association between a subset of genetic biomarkers (*i.e.* single nucleotide polymorphisms (SNPs)) and cognitive function and cognitive decline up to 20 years follow-up in the DCS to understand whether cognitive function and cognitive decline are driven by the same mechanisms. We found that out of the 435 SNPs, rs429358-C, a SNP linked to an Alzheimer's disease related variant of apolipoprotein E (APOE) (*i.e.* APOE ε4), was associated with cognitive decline, but not with general cognitive function at baseline.

Interpretation of Part II

Cognitive frailty

A decline in total cholesterol levels accompanies cognitive frailty

In this thesis, it was shown that in cognitively frail women, a decline in the total cholesterol trajectory was observed at the onset of cognitive frailty. In cognitively frail men, the total cholesterol trajectory declined earlier, 5 years before the onset of cognitive frailty, and to a lesser extent than in cognitively frail women (**Chapter 5**). It has been shown that total cholesterol levels increase over the life course and then tend to decrease in old age (51). Further, high total cholesterol levels at midlife, but not at advanced age, are associated with cognitive impairment later in life (52). Moreover, persistent high or decreasing cholesterol levels are associated with a higher mortality risk (in participants over 40 years of age) (53), while high

total cholesterol levels in the oldest old (>85 years) are associated with lower mortality risk (54). Possibly, a decline in total cholesterol indicates a decline in cognitive functioning and/or overall health in middle–aged and older adults.

General cognitive function and decline

Genetic and modifiable risk factors contributing to cognitive decline

In **Chapter 6**, we found that, out of the 435 studied SNPs, the APOE genotype was associated to cognitive decline, but not to baseline cognitive function, indicating that a decline of general cognitive function is influenced by other mechanisms than those that are involved in the regulation of general cognitive function.

None of the other 433 independent SNPs (*i.e.* SNPs with a p–value of $\leq 5\times10^{-8}$ and $r^2<0.6$) were, after adjustment for multiple testing using the Bonferroni correction, associated with general cognitive function. However, before adjustment for multiple testing, 30 SNPs were associated with general cognitive function (based on p–value <0.05 for main effect). The direction of effect of the association between these SNPs and general cognitive function was similar to that observed by Davies and Lam *et al.* (55). The fact that after adjustment for multiple testing no significant associations remained, is likely explained by limited power due to a relatively small sample size.

Except for the APOE genotype, we did not find genotypes of other genes that were associated with cognitive decline. Apart from genotype, which cannot be altered, it is important to consider the contribution of modifiable risk factors to cognitive decline. In this context, it should be noted that there might also be a genetic component to modifiable risk factors such as educational level, lifestyle and metabolic factors. Hence, these are in fact partly modifiable risk factors. It has been suggested that these (partly) modifiable factors have a stronger influence on cognitive decline than genetic factors (56). In particular educational level seems to be important. In the 2020 report on dementia by the Lancet Commission, twelve modifiable risk factors for the development of dementia were identified, namely: less education, smoking, excessive alcohol consumption, physical inactivity, obesity, hypertension, diabetes, hearing impairment, traumatic brain injury, depression, low social contact and air pollution (57). Given that cognitive decline precedes dementia, some or all of these (partly) modifiable risk factors might be related to cognitive decline too. We found that physical activity and obesity were associated with cognitive frailty (**Table 7.2**). The APOE ε4 genotype possibly

amplifies the effects of both positive and negative (modifiable) risk factors (such as healthy nutrition, physical inactivity, and smoking) in dementia (47, 58). This highlights the important contribution of (partly) modifiable risk factors to cognitive decline, development of dementia and cognitive frailty. Ideally, the general population should be made aware of the contribution of modifiable risk factors to cognitive function over the life course. Since multiple lifestyle factors play an important role in the development of cognitive decline and dementia, prevention strategies targeting cognitive health should at least include supporting a healthy lifestyle.

Interpretation of Part I and II

Overall frailty

Similarities between all four frail groups: unhealthy lifestyle and higher BMI and multimorbidity rates

The descriptive analyses of all four frail groups show that they more often had a low educational level, were less often married, less often had a paid job, were more often physically inactive, had a higher BMI, were more often obese, had more often multimorbidity, a long or short sleep duration, and had worse self–reported health compared to the total or non–frail study population. The multivariate regression analyses showed that being physically active was consistently associated with a lower risk of being frail on all four domains **(Chapter 2)**.

Frail people have an unhealthy lifestyle and higher multimorbidity rates (4, 12). These findings were confirmed in this thesis for all four frailty domains (**Chapters 2**, **3**, and **4**). In addition, we observed that the four frail populations have a higher BMI than the non–frail population. When focusing on BMI trajectories over the life course, **Chapter 5** also showed that BMI trajectories in cognitively frail men and women are persistently higher than BMI trajectories of non–frail people. Overall, this implicates that prevention strategies aimed at improving someone's lifestyle, for example with more physical activity and a healthier diet, might be effective for all four frailty domains if, indeed, the observed associations are causal.

Biological mechanisms underlying frailty

One of the aims of this thesis was to identify (bio)markers, which would be indicative of the underlying processes causing frailty. A small number of biomarkers

(plasma levels of β-cryptoxanthin and zeaxanthin, total cholesterol and the APOE genotype) were identified in this thesis, and we confirmed the importance of other markers such as obesity, sufficient physical activity, and a healthy diet. In this section, biological mechanisms for the physical and cognitive frailty domains will be discussed, since these domains have a strong biological component.

It has been hypothesised that frailty is caused by the age-related loss of functional homeostatic reserves in multiple physiological systems (59, 60). Proposedly, the main systems involved are the immune system, endocrine system, nervous system, and musculoskeletal system (59, 61–63). The results presented in this thesis might, indirectly, indicate that there could be a prominent role for the immune system in the development of physical and cognitive frailty.

The ageing immune system

Ageing of the immune system can manifest itself in a decline in stem cells, changes in T-lymphocyte production, dampening of the B-cell dependent antibody response, and reduced neutrophil, macrophage and natural killer cell activity (62, 64, 65). The aged immune system is also characterised by chronic low-grade inflammation, *i.e.* 'inflammaging' (66). Inflammation is an immune response to traumatic, infectious, post-ischaemic, toxic or autoimmune injury (67). There is considerable evidence that chronic low-grade inflammation is linked to frailty, cognitive decline, and a high BMI (7, 68–75). In this thesis, it was shown that physically, cognitively, psychologically, and socially frail groups had on average a higher BMI compared to the non-frail population (**Chapters 2**, **3** and **4**). Only in physically and cognitively frail people we observed a higher prevalence of obesity **(Chapter 3)**. Since these people are frail ánd have a higher BMI, it is likely that they also have higher levels of inflammatory markers. In **Chapters 4** and **5**, the commonly used inflammatory marker C-reactive protein (CRP) was studied. In these multivariate analyses, we did not observe an association between this inflammatory marker and any of the four frailty domains. However, the descriptive analyses of **Chapter 5** showed that CRP levels are higher in cognitively frail people than in non-frail people.

The descriptive analyses in **Chapter 4** also showed that the physically, cognitively and psychologically frail populations have lower carotenoid levels compared to the non-frail population. The multivariate regression analyses showed that people with higher carotenoid levels have a lower risk of being cognitively frail (**Chapter**

4). Another study on frailty performed in a subcohort of the DCS, measuring frailty with the FI, also showed that overall frail people have higher CRP levels compared to non–frail people (76). Antioxidants (like carotenoids) are part of the immune system since they help to preserve adequate functioning of immune cells against homeostatic disturbances caused by oxidative stress (77). The 'Free radical theory of aging' hypothesises that part of the ageing process and age–related diseases is driven by a relative overproduction of reactive oxygen species (ROS) causing tissue damage when defence mechanisms fail to act (78). Older people are possibly more vulnerable to oxidative stress since they have less efficient endogenous antioxidant systems (79). The imbalance between ROS and antioxidants can cause oxidative stress (80). It has been suggested that oxidative stress possibly underlies physical and cognitive frailty (81, 82). We observed that older people with higher levels of antioxidants have a lower risk of being cognitively frail. However, we do not know whether the older people with cognitive frailty also had increased ROS and in turn oxidative stress. This deserves further investigation.

The descriptive analyses of **Chapter 4** also showed higher monocyte counts and cytomegalovirus (CMV) antibody levels among the physically, cognitively and psychologically frail populations compared to the non–frail population. These biomarkers are also linked to the immune system. Another study based on a subcohort of the DCS where frailty was measured with the FI, also found higher monocyte counts in frail people (76, 83).

The results shown in this thesis indicate that the ageing immune system plays a role in the development of frailty, which has also been suggested by others (76, 84). However, a recent review concluded that results regarding inflammatory biomarkers are still contradictory and other (bio)markers (such as nutritional, endocrine, haematological and genetic markers) are not yet studied thoroughly (85). More research is needed to understand the exact biological mechanisms underlying the development of frailty.

Conceptual and methodological considerations

Operationalisation of the four frailty domains

As mentioned before, we used the TFI (14) as basis for the definition of frailty. Further, in **Chapter 2**, the operationalisation of physical frailty was based on the

physical domain as defined in the TFI, while in **Chapters 3** and **4**, physical frailty was based on the FP (12). This approach allowed us to compare the physical frailty domain of the TFI with that of the FP, which is the most widely used instrument to measure physical frailty.

Not all criteria of the TFI and FP could be assessed from the available data of the DCS and MARK–AGE. Within that limitation, we approximated the operationalisation of the TFI and FP as closely as possible. Yet, this could have affected the prevalence of the four frailty domains. For example, originally the FP is based on five instead of four criteria (**Table 7.3**). Since in the DCS and MARK–AGE, no data was available on walking speed, we were not able to include this variable. Further, the TFI is solely based on self–report, but we were able to combine self–reported and objectively measured variables to define frailty. For example, for cognitive frailty memory problems were measured objectively with neuropsychological tests instead of obtaining this information through self–report only. Hence, in this thesis, some frailty criteria were measured more precise, while other criteria were measured less precise relative to the criteria used in the original frailty instruments. Comparing the prevalence of the four frailty domains obtained in this thesis with other studies is difficult because of these adjustments in the operationalisation of the frailty domains (**Table 7.3**), and because of the differences in age range (>65 years in many frailty studies versus >40 years in the DCS and >35 years in MARK–AGE). Despite these differences the associations we found are consistent with other studies, such as the U–shaped association between BMI and physical frailty.

Research limitations

Limited power

In this thesis, multiple (bio)markers of frailty were studied, which resulted in several consistent observations, but also in numerous null findings. For example, in **Chapter 4**, we found an association between two carotenoids and cognitive frailty, while we did not observe an association for any of the other 331 (bio)markers and the two other frailty domains. In MARK–AGE, the prevalence of physical (3.0%), cognitive (10.2%), and psychological (6.3%) frailty was relatively low. Due to the low number of participants with physical and/or psychological frailty, statistical power was low which most likely explains our null findings for these domains. Further, in **Chapter 6**, none of the 435 SNPs were associated with general cognitive function at baseline, while another study did find these associations (55). The difference

was that the study by Davies and Lam *et al.* had far more participants (over 300.000) than our study with over 2500 participants. In both **Chapters 4** and **6**, there were few to none significant associations left after adjustment for multiple testing, *i.e.* reducing the alfa. It is therefore very likely that some of our null findings can be explained by limited power caused by the relatively small sample sizes in these studies.

Generalisability of cohort studies

Two study populations were used in this thesis. The DCS, which is a Dutch longitudinal population–based cohort study, and MARK–AGE which is a European cross–sectional cohort study. These cohorts are not necessarily a representative sample of the current Dutch and European populations. In general, cohort studies are prone to selection bias, because healthier (and higher educated) people are more likely to participate and remain in the study than less healthy people. Due to the selection bias, the frailty prevalence reported in this thesis, is most likely an underestimation of the actual prevalence in the general population and this could have influenced the measured (bio)markers levels, since healthier people usually have more favourable (bio)markers levels. This selection bias could limit generalisability of the estimated frailty prevalence to the general population. However, the selection bias most likely did not affect the studied (bio)marker–frailty associations, since biological mechanisms are the same in cohort studies and in the general population. Hence, the associations observed in our studies could, when confirmed in other studies, be generalised to the general population.

Research strengths

Incorporating the cognitive frailty domain

The importance of the cognitive frailty domain is recognised more and more and has been, over time, included in different frailty instruments. Some multi–domain approaches, such as the Comprehensive Frailty Assessment Instrument (CFAI) (86), include cognitive frailty as a separate domain, but some combine it with the psychological domain (14). Broadening the focus from studying physical and overall frailty to also cognitive frailty can result in new insights. For example, in this thesis we observed that cognitive frailty is more common among men, while overall and physical frailty is more common among women. And we also observed that both underweight and obesity are associated with physical and overall frailty, while only obesity is associated with cognitive frailty. These two findings reinforce our view that the cognitive frailty domain should be included as a separate entity

in frailty instruments and frailty research.

The importance of using multiple cohort studies, designs, and analysis techniques
Biomarker research in the epidemiological setting is particularly sensitive to the composition of the particular study population (87). Further, repeated measurements are an advantage in biomarker research as they allow to track the change in biomarker levels over time. We had the opportunity to conduct biomarker research using multiple cohort studies, study designs and analysis techniques that provided relevant insights and supported the aim of this thesis, *i.e.* to identify (bio)markers for different domains of frailty (*i.e.* physical, cognitive, psychological, social).

Using multiple cohorts
In this thesis, data of two cohort studies were used, *i.e.* the DCS and MARK-AGE. The results of the descriptive analyses were similar for the various frailty domains in both cohorts, implying that these characteristics were not cohort-specific. For example, in both cohort studies we found that cognitive frailty was more common among men, and that total cholesterol levels were lower in cognitively frail people compared to non-frail people. Hence, given that similar results were obtained in different cohorts, these findings seem robust.

Using multiple study designs
In this thesis, we studied (bio)marker levels using a cross-sectional design, and (bio)marker trajectories using a longitudinal design. This provided important information. For example, cross-sectionally we found that total cholesterol levels were lower in cognitively frail compared to non-frail people (**Chapter 4**). By comparing total cholesterol trajectories of cognitively frail people with those of non-frail people (**Chapter 5**), we also showed that before people became cognitively frail, their total cholesterol levels were actually higher than in non-frail people. However, once people had become cognitively frail, their total cholesterol levels were lower compared to non-frail people due to a decline in their total cholesterol levels. The association between certain biomarkers and frailty has been reported previously by others using a cross-sectional design but not how the associations change over time, which requires a longitudinal design (88). The study design may therefore play an important role in the results obtained, with a longitudinal design providing a better understanding of the development of a certain biomarker over time in relation to frailty.

Using multiple analysis techniques

We also used multiple analysis techniques, *i.e.* univariate Rank–ANOVA, multivariate regression and machine learning. All these techniques have their advantages and disadvantages. Univariate Rank–ANOVA is a straightforward and intuitive method to compare biomarker levels between two populations. This gives an idea of the differences in biomarker levels between the two groups, if any. The down–side of Rank–ANOVA is that there is very limited room to adjust for confounders. Given that biomarker levels are likely to be affected by confounders, the use of regression methods is an advantage. The disadvantage of regression models is that they can only handle a limited number of variables, depending on the number of cases versus non–cases. Using machine learning, hundreds of biomarkers can be studied at the same time along with adjustment for all variables included. This is an advantage, since usually it is not known which of the co–variables are confounders. Thus, in regression analyses, confounders must be specifically selected by the researcher since only a limited number of covariates can be included in a regression model, whereas in machine learning, much more variables can be included and a selection does not need to be made. However, results from machine learning techniques are more difficult to interpret than results from regression analyses, as the outcomes do not translate to meaningful clinical values directly, which is important especially in public health research (89). A possible solution is to use machine learning for selecting the most important determinants for the outcome and in addition to perform a regression analysis to study the relation between the selected determinants and the outcome in more detail. Another difference between regression methods and machine learning is that regression methods are hypothesis–driven, while machine learning is data–driven, which is therefore very suitable for conducting explorative research.

In conclusion, in (bio)marker research, it is important to use multiple cohorts, study designs, and analysis techniques to verify one's findings. This is particularly important in biomarker research because it matters whether the study is conducted in a sample of the general population or in a population within a clinical setting, which can greatly affect biomarker levels. Further, many biomarker levels increase or decrease over the life course. Therefore, the age distribution of the cohort used can also affect the obtained results. In addition, biomarker levels studied in a cross–sectional design, and biomarkers trajectories studied in a longitudinal design, can tell a different story. Further, the biomarkers of interest may pose a challenge, since many biomarkers have a highly skewed distribution, are

correlated, and there are many confounders that can influence biomarker levels, some of which are known, but many of which are not. Given these challenges, choosing the appropriate analysis technique, or a combination of techniques, in biomarker research is therefore essential.

Public health

Implications for practice

Monitoring for frailty among older adults

Life expectancy rises and, as a result, populations are ageing in many countries worldwide (90). The proportion of older people in the Netherlands will rise sharply in the coming 20 years from over 800.000 in 2020 up to 1.6 million in 2040 (91). As life expectancy rises, frailty will become a public health challenge that deserves attention as the number of older adults who become frail increases rapidly. In the Netherlands, there is an Adult and Elderly Health Monitor. Via a survey, every four years data concerning the health of a representative sample of the Dutch population is collected (92). In the surveys of 2012 and 2016 (part of), the TFI was included in a limited number of participating municipalities. It would be desirable to include a frailty instrument, such as the TFI, as a core element of the survey of the Adult and Elderly Health Monitor in Dutch municipalities. Monitoring the prevalence of frailty among the general population is important since frail people have a higher risk of negative health outcomes, such as falls, disability, hospitalisation, and mortality (60, 93–95). Since people can start becoming frail at an early age, early detection and prevention strategies can reduce the future burden of disease and mitigate public health problems when today's adults reach advanced age.

The importance of monitoring ageing populations is also recognised by the World Health Organization (WHO) (96). The WHO does not focus on frailty, but on healthy ageing. Currently, the WHO and their Collaborating Centres in Aging are developing a toolkit with the aim to measure and monitor healthy ageing among populations worldwide.

Preventing frailty: support a healthy lifestyle that includes physical activity

Frailty prevention is important for enabling older people to stay self–reliant. An important characteristic of frailty is that it is reversible, thus frail older people can

return to a non-frail state when frailty is identified in an early stage. This thesis shows that the four frail groups are distinct groups, but have overlapping and group-specific characteristics. For prevention strategies, it may be efficient to first focus on factors relevant to all four frailty domains. In this thesis we have shown that being physically active was associated with a lower risk of being physically, cognitively, psychologically, and socially frail (**Chapter 2**). Therefore, prevention strategies should at least focus on promotion of physical activity, tailored to (pre-) frail individuals. Only a limited number of randomised control trials (RCTs) focussing on effective prevention strategies for frailty have been conducted. Two RCTs showed that exercise can enhance muscle strength, improve balance, reduce the incidence of falls, and reduce frailty prevalence (97, 98). In a meta-analysis of RCTs it was shown that physical activity was the most effective intervention for frailty (99).

A healthy diet and having higher carotenoid levels were also associated with a lower risk of being physically and cognitively frail (**Chapter 2 and 3**). Given that malnutrition is associated with a higher risk of developing frailty (100), appropriate nutrition should be part of an effective prevention strategy for frailty. Adherence to a Mediterranean diet is associated with a lower risk of developing physical frailty and cognitive decline (101). Another study showed that a higher protein intake, but not energy intake, was associated with a lower physical frailty prevalence (102). A review study found that the type and amount of nutrition seemed important in the development of overall frailty, but the exact role of nutrition for prevention or reversal of overall frailty was unclear (103). The results of multiple studies indicate that supporting a healthy lifestyle can be an effective strategy for preventing and possibly reversing frailty, amongst others by becoming and/or staying physically active and maintaining a proper nutritional state into older age.

Future research

In this thesis, a broad range of (bio)markers for the four frailty domains was studied. However, we only identified a limited number of (bio)markers for the different frailty domains, among which were socio-demographic characteristics, lifestyle factors, and biological markers and we did not define a set of biomarkers that can be used to identify high risk groups. First, more research is needed to be able to identify people at risk of becoming frail at an early stage, in order to prevent, delay or even reverse frailty. Second, more research is needed to understand the

mechanisms involved in development of frailty. This research should provide us with knowledge for developing tailored prevention and intervention strategies for the different high risk groups.

Biological factors can be useful for early identification. This would allow people at high risk of frailty to be identified long before the first phenotypic symptoms become apparent. With new assays and laboratory techniques there are new horizons in biomarker research to be explored, with the beckoning prospect that frail or other risk groups can be identified on the basis of a number of (bio)markers. Metabolomics–data seems promising and, for example, it has been shown that a group of 12 metabolites can be used to a distinguish between frail and non–frail groups (104). In addition to metabolomics, other –omics data are available such as transcriptomics and proteomics data. And where previously only single–omics datasets were available, based on one type of assay where, for example, only metabolomics had been measured, multi–omics datasets are now becoming accessible based on multiple assays measuring different types of omics in the same samples. Currently, these assays are still quite expensive, which may limit the use of one or more of these assays in larger cohorts. In the DCS, metabolomics data of rounds 2 and 6 have become available recently. A possible and exciting next step could be to study the metabolomics data of the physically, cognitively, psychologically and socially frail groups with the aim to distinguish them from non–frail people.

Second, more research is needed to understand the underlying biological mechanisms in the development of frailty. Understanding underlying mechanisms is important since it could generate new targets for the prevention and treatment of frailty. To this aim, more life–course research using data of longitudinal studies is needed for linking (bio)markers or other factors at middle–age (or earlier) to frailty later in life. Studying the role of the immune system and its biomarkers in the development of frailty would be an appropriate starting point. Also here the –omics data can contribute to identify pathways of interest, since this type of data provides a detailed snapshot of the underlying biology of the biological system of interest. An interesting step based on the DCS metabolomics data would be to study the metabolomic change in the four frail groups, which would provide insight into the pathways and mechanisms involved in the development of frailty.

The above mentioned research steps should provide insight into the elements

that should comprise tailored interventions for the various frail groups. Different RCTs focusing on frailty prevention and intervention strategies via lifestyle factors have been conducted (97–99, 101–103). These studies indicate that physical activity and possibly also nutrition can be interesting targets to focus on when starting an intervention.

Overall conclusion

In this thesis, it was shown that the physical, cognitive, psychological and social frailty domains encompass different groups of frail people, but that these groups have overlapping characteristics. This illustrates the importance of adopting a broad definition of frailty and of studying the frailty domains separately. Based on the results discussed in this thesis, we adapted the conceptual model of the TFI, in which the cognitive frailty domain is incorporated as a separate frailty domain.

Especially physical and cognitive frailty are age-related, while psychological frailty is not. This suggests that, besides the accumulation of deficits which needs time to develop, another more constant risk factor is involved in the development of psychological frailty, such as someone's personality traits. Given that people can already become frail before being 60 years of age, it is important to ensure frailty research and frailty monitoring and prevention strategies also include middle-aged individuals.

The aim of this thesis was to identify (bio)markers of frailty. At this point we do not yet have defined a set of biomarkers that can be used to identify high risk groups, and that can be used to better target preventive measures. However, based on the current studies in this thesis we do have clues about the underlying biological mechanisms involved, such as inflammation and the possible involvement of the immune system in the development of physical and cognitive frailty. Further, we have found clear evidence of the importance of adopting a healthy lifestyle for various frailty domains with sufficient physical activity and a healthy diet.

All frail groups were found to be less healthy with higher BMI levels and multimorbidity rates in all four frail groups compared to non-frail people. Further, all frail groups had an unhealthier lifestyle compared to non-frail people. Prevention strategies can focus on many lifestyle factors, but in this thesis it was shown that being physically active was associated with a lower risk of being frailty in all four

frailty domains. Therefore, prevention strategies targeting (pre–)frail should at least focus on promoting physical activity to prevent and potentially reverse frailty. A healthy lifestyle, including physical activity and a healthy diet, over the life course positively affects many age–related diseases, including the four domains of frailty studied in this thesis.

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8

Summary

Frailty is an age-associated syndrome and can be described as a state of increased vulnerability to adverse health outcomes when exposed to stressors (*e.g.* an infection), caused by the cumulative decline in one or more domains of functioning. To prevent frailty, we need to better understand the underlying mechanisms and identify at an early stage people at risk of becoming frail. The aim of this thesis is therefore to identify (bio)markers (based on questionnaire data, anthropometric measurements, biochemical markers, and genetic data) of frailty, which could be indicative of the underlying processes that cause frailty and/or could help to detect people at risk. In this thesis, frailty is defined using a multi-domain approach based on the Tilburg Frailty Indicator (TFI) which allowed us to study (bio)markers for the physical, cognitive, psychological, and social frailty domains. For this research we used data of two cohort studies, the Doetinchem Cohort Study (DCS) and the European study to establish biomarkers of human Ageing (MARK-AGE). In addition, we used multiple research designs and various analysis techniques. **Chapter 1** provides the background and objectives of this thesis.

The remaining chapters in this thesis are divided into two parts. In **Part I** (**Chapters 2-4**), (bio)markers for four domains of frailty, *i.e.* the physical, cognitive, psychological, and social domains, were studied cross-sectionally. The major focus in frailty research worldwide has been on the physical domain. The cognitive domain is also essential for healthy ageing and for remaining self-reliant. Therefore, the focus in **Part II** (**Chapters 5** and **6**) of this thesis was on identifying (bio)markers for cognitive frailty and general cognitive function using longitudinal data.

Part I: Exploring (bio)markers for the four frailty domains cross-sectionally
Chapter 2 is based on data of the DCS and shows that sociodemographic factors, lifestyle and multimorbidity contributed differently to the four frailty domains. Physical activity was the only factor associated with all four frailty domains. People who were physically active had a lower risk of being physically, cognitively, psychologically, or socially frail. In view of the effectiveness of prevention strategies, it may be efficient to focus on factors relevant to all four frailty domains. Therefore, prevention strategies should at least include physical activity.

Chapter 3 is also based on data of the DCS and shows that there is limited overlap between the different frailty domains, indicating that the domains entailed distinct groups of frail people. Therefore, prevention strategies should target multiple frailty domains (*e.g.* physical, cognitive, psychological, and social frailty) rather than one domain only. This study also shows that both underweight and obesity were associated with physical frailty and thus confirm the U-shaped association between BMI and physical frailty, which has been observed by others. Obesity was also associated with cognitive frailty. Although no causal inferences from this study can be drawn, these findings strengthen the importance of maintaining a healthy body weight throughout the life course. This study further shows that the prevalence of physical, cognitive and social frailty increased with age, whereas the prevalence of psychological frailty did not. Finally, this study shows that frailty is not limited to older people, but can also be present at younger ages (<65 years of age).

Chapter 4 is based on data of MARK-AGE and also shows that there is limited overlap between the physical, cognitive and psychological frailty domains. In addition, in line with the results from the DCS it shows that the prevalences of physical and cognitive frailty increased with age, while the prevalence of psychological frailty did not. This chapter further shows that from over 300 (bio) markers analysed, a limited set was associated with cognitive frailty, particularly in the class of antioxidants. Plasma levels of the carotenoids β-cryptoxanthin and zeaxanthin were inversely associated with the risk of being cognitively frail. The biomarkers identified may indicate the involvement of inflammation in frailty, in particular for cognitive frailty.

Part II: Exploring (bio)markers of cognitive frailty, general cognitive function and cognitive decline using longitudinal data

Chapter 5 describes the study analysing 17 (bio)marker trajectories for the cognitive frailty domain. In men, (bio)marker trajectories did not differ. That is, they ran parallel and the difference in levels was not statistically significant between those who became cognitively frail and controls who did not. In women, differential trajectories for three biomarkers were observed: total cholesterol trajectories first inclined and thereafter declined in cognitively frail women, while steadily inclining in controls. Gamma-glutamyltransferase trajectories were more or less stable in cognitively frail women and inclined in controls, and urea trajectories inclined in cognitively frail women and remained more or less stable in controls. Yet, these

findings do not support a role for any of the studied (bio)markers in the early identification of cognitive frailty.

Chapter 6 focuses on general cognitive function instead of cognitive frailty. Cognitive decline is part of the normal aging process. However, some people experience a more rapid decline than others due to environmental and genetic factors. Numerous single nucleotide polymorphisms (SNPs) have been linked to cognitive function, but only a few to cognitive decline. To understand whether cognitive function and cognitive decline are driven by the same mechanisms, we investigated whether 433 SNPs previously linked to cognitive function and 2 SNPs previously linked to cognitive decline were associated with both general cognitive functioning at baseline and general cognitive decline for up to 20–years of follow–up in the DCS. None of the 435 previously identified variants were associated with baseline general cognitive function in the DCS. But rs429358–C, one of the well–known APOE variants associated with cognitive decline, was associated with general cognitive decline in our study as well. These findings suggest that general cognitive decline is influenced by other mechanisms than those that are involved in the regulation of general cognitive function.

General discussion

Finally, in **Chapter 7** the main findings are summarised and reviewed. Further, conceptual and methodological aspects are discussed, as well as the implications of these findings for public health and future research.

Lessons regarding the conceptual model of frailty

The choice of frailty instrument affects the composition of the frail population that is identified. This, in turn, affects which associations are observed and the strength of these associations. Hence, in deciding which frailty instrument to use and in interpreting the results, it is important to be aware of the specifications (and possible advantages and disadvantages) of the chosen instrument.

Broadening the scope from studying physical and overall frailty to also cognitive, psychological and social frailty can result in new insights. For example, we observed that cognitive frailty was more common among men, while overall and physical frailty was more common among women and that both underweight and obesity were associated with physical and overall frailty, while only obesity was associated with cognitive frailty. These two findings, and the limited overlap we

observed between the four frail populations, reinforce our view that the cognitive frailty domain should be included as a separate entity in frailty instruments and frailty research.

The ageing immune system
We showed that the physically, cognitively and psychologically frail populations have lower carotenoid levels compared to the non-frail population and that people with higher carotenoid levels have a lower risk of being cognitively frail, other factors also taken into account. In addition, we observed higher monocyte counts and cytomegalovirus (CMV) antibody levels among the physically, cognitively and psychologically frail populations compared to the non-frail population. These biomarkers are all linked to the immune system and may indicate that the ageing immune system plays a role in the development of frailty.

Frailty prevention should at least focus on supporting a healthy lifestyle
The four frail groups are distinct groups, but have overlapping and group-specific characteristics. For prevention strategies, it may be efficient to first focus on factors relevant to all four frailty domains. In this thesis we have shown that being physically active was associated with a lower risk of being physically, cognitively, psychologically, and socially frail. In addition, a healthy diet and having higher carotenoid levels were also associated with a lower risk of being physically and cognitively frail. These results indicate that supporting a healthy lifestyle can be an effective strategy for preventing and possibly reversing frailty, amongst others by becoming and/or staying physically active and maintaining a proper nutritional state into older age.

In conclusion, we showed that the physical, cognitive, psychological and social frailty domains entail different groups of frail people, but that these groups have overlapping characteristics. Therefore, prevention strategies should target multiple frailty domains (*e.g.* physical, cognitive, psychological, and social frailty) rather than one domain only. At this point we have not yet defined a set of biomarkers that can be used to identify high risk groups and that can be used to better target preventive measures. With the development of new assays and laboratory techniques in the field of omics (metabolomics, transcriptomics and proteomics) one might be able to identify frail groups at an early stage and gain more insight in the near future in the underlying processes that cause frailty. However, based on the current studies in this thesis we do have clues about the underlying biological

mechanisms involved, such as inflammation and the possible involvement of the immune system in the development of physical and cognitive frailty. Further, we have found clear evidence of the importance of adopting a healthy lifestyle for various frailty domains with sufficient physical activity and a healthy diet. The findings of this thesis underline the importance of a healthy lifestyle for healthy ageing.

Samenvatting

Kwetsbaarheid (*frailty* in het Engels) kan worden omschreven als het hebben van beperkingen in één of meer domeinen van functioneren en neemt toe met de leeftijd. Kwetsbare mensen hebben een verhoogd risico voor ongunstige gezondheidsuitkomsten wanneer zij worden blootgesteld aan stressoren, zoals een infectie. Om kwetsbaarheid te voorkomen, is het nodig om de onderliggende mechanismen beter te begrijpen en in een vroeg stadium mensen te identificeren die het risico lopen kwetsbaar te worden. Het doel van dit proefschrift is daarom om (bio)merkers (gebaseerd op vragenlijstgegevens, antropometrische metingen, biochemische merkers, en genetische gegevens) van kwetsbaarheid te identificeren, die indicatief kunnen zijn voor de onderliggende oorzaken van kwetsbaarheid en/of mensen met een verhoogd risico op kwetsbaarheid kunnen identificeren. In dit proefschrift wordt een brede definitie van kwetsbaarheid gehanteerd die is gebaseerd op de *Tilburg Frailty Indicator* (TFI), waarin verschillende domeinen van kwetsbaarheid worden onderscheiden. Dit instrument stelde ons in staat (bio)merkers te bestuderen voor de fysieke, cognitieve, psychische, en sociale domeinen van kwetsbaarheid. Voor dit onderzoek gebruikten we gegevens van twee cohortstudies, de Doetinchem Cohort Studie (DCS) en de European study to establish biomarkers of human Ageing (MARK-AGE). Daarnaast hebben we gebruik gemaakt van meerdere onderzoeksdesigns en verschillende analysetechnieken. **Hoofdstuk 1** geeft de achtergrond en doelstellingen van dit proefschrift.

De overige hoofdstukken in dit proefschrift zijn verdeeld in twee delen. In **deel I** (**hoofdstukken 2-4**) zijn (bio)merkers voor vier domeinen van kwetsbaarheid, namelijk het fysieke, cognitieve, psychische en sociale domein, cross-sectioneel onderzocht. Internationaal wordt vooral veel onderzoek gedaan naar het fysieke domein van kwetsbaarheid. Het cognitieve domein is ook essentieel voor gezond ouder worden en zelfredzaam blijven. Daarom lag de focus in **deel II** (**hoofdstukken 5** en **6**) van dit proefschrift op het identificeren van (bio)merkers voor cognitieve kwetsbaarheid en algemeen cognitief functioneren met behulp van longitudinale data.

Deel I: Cross-sectionele verkenning van (bio)merkers voor de vier domeinen van kwetsbaarheid

Hoofdstuk 2 is gebaseerd op gegevens van de DCS en laat zien dat

sociodemografische factoren, leefstijl en multimorbiditeit verschillend bijdragen aan de vier domeinen van kwetsbaarheid. Fysieke activiteit was de enige factor die geassocieerd was met alle vier de domeinen. Mensen die fysiek actief waren hadden een lager risico om fysiek, cognitief, psychisch of sociaal kwetsbaar te zijn. Met het oog op de effectiviteit van preventiestrategieën, kan het aantrekkelijk zijn om zich te richten op factoren die relevant zijn voor alle vier de domeinen kwetsbaarheid. Daarom zouden preventiestrategieën in ieder geval fysieke activiteit moeten omvatten.

Hoofdstuk 3 is ook gebaseerd op gegevens van de DCS en laat zien dat er beperkte overlap is tussen de verschillende domeinen van kwetsbaarheid. Dit geeft aan dat de domeinen verschillende groepen kwetsbare mensen betreffen. Om alle groepen kwetsbare mensen te bereiken moeten preventiestrategieën daarom gericht zijn op meerdere domeinen van kwetsbaarheid (bijvoorbeeld fysieke, cognitieve, psychische en sociale kwetsbaarheid) in plaats van op slechts één domein. Deze studie laat ook zien dat zowel ondergewicht als obesitas waren geassocieerd met fysieke kwetsbaarheid. Deze resultaten bevestigen daarmee de U–vormige associatie tussen BMI en fysieke kwetsbaarheid, die ook door anderen is aangetoond. Obesitas was ook geassocieerd met cognitieve kwetsbaarheid. Hoewel uit deze studie geen oorzakelijke conclusies kunnen worden getrokken, bevestigen deze bevindingen het belang van het behouden van een gezond lichaamsgewicht gedurende het hele leven. Deze studie laat verder zien dat de prevalentie van fysieke, cognitieve en sociale kwetsbaarheid toeneemt met de leeftijd, terwijl de prevalentie van psychische kwetsbaarheid niet leeftijdsafhankelijk is. Tenslotte laat dit onderzoek zien dat kwetsbaarheid niet beperkt is tot ouderen, maar ook op jongere leeftijd (<65 jaar) kan voorkomen.

Hoofdstuk 4 is gebaseerd op gegevens van MARK–AGE en laat eveneens zien dat er een beperkte overlap is tussen de fysieke, cognitieve en psychische domeinen van kwetsbaarheid. Bovendien bleek, in lijn met de resultaten van de DCS, dat de prevalenties van fysieke en cognitieve kwetsbaarheid toenamen met de leeftijd, terwijl de prevalentie van psychische kwetsbaarheid gelijk was over de verschillende leeftijdsgroepen. Dit hoofdstuk laat verder zien dat van de meer dan 300 geanalyseerde (bio)merkers, een beperkte set geassocieerd was met cognitieve kwetsbaarheid, met name antioxidanten. Plasma niveaus van de carotenoïden β–cryptoxanthine en zeaxanthine waren invers geassocieerd met het risico op cognitieve kwetsbaarheid. De geïdentificeerde biomerkers kunnen

wijzen op de betrokkenheid van inflammatie bij kwetsbaarheid, vooral voor cognitieve kwetsbaarheid.

Deel II: Onderzoek naar (bio)merkers van cognitieve kwetsbaarheid, algemeen cognitief functioneren en cognitieve achteruitgang met behulp van longitudinale gegevens

Hoofdstuk 5 beschrijft de studie waarin 17 (bio)merker trajecten voor cognitieve kwetsbaarheid werden geanalyseerd over een periode van 15 jaar. Bij mannen verschilden de (bio)merker trajecten niet: de trajecten liepen parallel en het verschil in niveau was niet statistisch significant tussen degenen die cognitief kwetsbaar werden en controles die dat niet werden. Bij vrouwen werden voor drie biomerkers verschillende trajecten waargenomen: het totaal cholesterolgehalte steeg eerst en daalde daarna bij vrouwen met cognitieve kwetsbaarheid, terwijl het bij de controles gestaag steeg. Gamma–glutamyltransferase trajecten waren min of meer stabiel bij cognitief kwetsbare vrouwen en stegen bij controles, en ureum trajecten stegen bij cognitief kwetsbare vrouwen en bleven min of meer stabiel bij controles. Op basis van deze bevindingen is het echter nog niet mogelijk vroegtijdig cognitieve kwetsbaarheid te identificeren.

Hoofdstuk 6 richt zich op algemeen cognitief functioneren in plaats van op cognitieve kwetsbaarheid. Cognitieve achteruitgang is onderdeel van het normale verouderingsproces. Bij sommige mensen gaat de cognitieve achteruitgang echter sneller dan bij anderen als gevolg van omgevings- en genetische factoren. Talrijke single nucleotide polymorfismen (SNPs) zijn in verband gebracht met cognitieve functie, maar slechts een paar met cognitieve achteruitgang. Om te begrijpen of cognitieve functie en cognitieve achteruitgang gedreven worden door dezelfde mechanismen, onderzochten wij of 433 SNPs die eerder gelinkt waren aan cognitieve functie en 2 SNPs die eerder gelinkt waren aan cognitieve achteruitgang geassocieerd waren met zowel algemeen cognitief functioneren op baseline als algemene cognitieve achteruitgang gedurende 20 jaar in de DCS. Geen van de 435 eerder geïdentificeerde varianten waren geassocieerd met algemene cognitieve functie op baseline in de DCS. Maar rs429358-C, één van de bekende APOE varianten geassocieerd met cognitieve achteruitgang, was in onze studie ook geassocieerd met algemene cognitieve achteruitgang. Deze bevindingen suggereren dat cognitieve achteruitgang beïnvloed wordt door andere mechanismen dan de mechanismen die betrokken zijn bij de regulatie van het niveau van cognitieve functie.

Algemene discussie

In **hoofdstuk 7** ten slotte worden de belangrijkste bevindingen samengevat en bediscussieerd. Verder worden conceptuele en methodologische aspecten besproken, evenals de implicaties van deze bevindingen voor de volksgezondheid en toekomstig onderzoek.

Lessen met betrekking tot het conceptuele model van kwetsbaarheid

De keuze van het instrument om kwetsbaarheid te meten beïnvloedt de samenstelling van de populatie die als kwetsbaar wordt geïdentificeerd. Dit beïnvloedt op zijn beurt welke associaties worden waargenomen en de sterkte van deze associaties. Bij de keuze van het instrument om kwetsbaarheid te meten en de interpretatie van de resultaten is het dan ook van belang de specificaties (en eventuele voor- en nadelen) van het gekozen instrument te kennen en mee te wegen.

Het verbreden van de studie van fysieke en algemene kwetsbaarheid naar cognitieve, psychische en sociale kwetsbaarheid kan tot nieuwe inzichten leiden. Wij stelden bijvoorbeeld vast dat cognitieve kwetsbaarheid vaker voorkwam bij mannen, terwijl algemene en fysieke kwetsbaarheid vaker bij vrouwen voorkwam, en dat zowel ondergewicht als obesitas geassocieerd waren met fysieke en algemene kwetsbaarheid, terwijl alleen obesitas geassocieerd was met cognitieve kwetsbaarheid. Deze twee bevindingen, en de beperkte overlap die we zagen tussen de vier kwetsbare populaties, versterken onze visie dat het cognitieve domein van kwetsbaarheid als een aparte entiteit moet worden opgenomen in instrumenten om kwetsbaarheid te meten en in toekomstig onderzoek naar kwetsbaarheid.

Het verouderende immuunsysteem

Wij lieten zien dat de fysiek, cognitief en psychisch kwetsbare populaties lagere carotenoïdenniveaus hebben dan de niet-kwetsbare populatie en dat mensen met hogere carotenoïdenniveaus een lager risico hebben om cognitief kwetsbaar te zijn, ook als rekening gehouden wordt met andere relevante factoren. Bovendien zagen wij hogere aantallen monocyten en cytomegalovirus (CMV) antilichaam niveaus bij de fysiek, cognitief en psychisch kwetsbare populaties in vergelijking met de niet-kwetsbare populatie. Deze biomerkers hebben allemaal te maken met het immuunsysteem en kunnen erop wijzen dat het verouderende immuunsysteem een rol speelt bij de ontwikkeling van kwetsbaarheid.

Preventie van kwetsbaarheid moet in ieder geval gericht zijn op ondersteuning van een gezonde leefstijl
De vier domeinen van kwetsbaarheid bestaan uit afzonderlijke groepen die deels dezelfde kenmerken hebben maar ook groepsspecifieke kenmerken hebben. Voor preventiestrategieën kan het effectief zijn om zich eerst te richten op factoren die relevant zijn voor alle vier de domeinen van kwetsbaarheid. In dit proefschrift hebben we laten zien dat fysiek actief zijn geassocieerd was met een lager risico op fysieke, cognitieve, psychische en sociale kwetsbaarheid. Daarnaast waren gezonde voeding en hogere carotenoïde niveaus ook geassocieerd met een lager risico op fysieke en cognitieve kwetsbaarheid. Deze resultaten wijzen erop dat het ondersteunen van een gezonde leefstijl een effectieve strategie kan zijn om kwetsbaarheid te voorkomen en mogelijk om te keren, onder andere door fysiek actief te worden en/of te blijven en een goede voedingstoestand te behouden tot op hogere leeftijd.

Concluderend hebben wij laten zien dat de vier domeinen van kwetsbaarheid bestaan uit verschillende groepen kwetsbare mensen met deels vergelijkbare kenmerken. Daarom wordt aanbevolen om preventiestrategieën te richten op meerdere domeinen van kwetsbaarheid (bijvoorbeeld fysieke, cognitieve, psychische en sociale kwetsbaarheid) in plaats van op slechts één domein. Op basis van onze resultaten is het (nog) niet mogelijk een set van biomerkers te selecteren waarmee hoog risicogroepen kunnen worden geïdentificeerd en waarmee preventieve maatregelen beter gericht kunnen worden ingezet. Met nieuwe assays en laboratoriumtechnieken op het gebied van omics (metabolomics, transcriptomics en proteomics) kunnen we in de toekomst wellicht beter kwetsbare groepen in een vroeg stadium identificeren en meer inzicht krijgen in de onderliggende processen die kwetsbaarheid veroorzaken. Op basis van de studies in dit proefschrift hebben we wel aanwijzingen over de onderliggende biologische mechanismen die een rol spelen, zoals inflammatie en de mogelijke betrokkenheid van het immuunsysteem bij de ontwikkeling van fysieke en cognitieve kwetsbaarheid. Verder hebben we duidelijk bewijs gevonden voor het belang van een gezonde leefstijl, met name voldoende fysieke activiteit en een gezonde voeding, voor verschillende domeinen van kwetsbaarheid. De bevindingen uit dit proefschrift onderstrepen het belang van een gezonde leefstijl voor gezonde veroudering.

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Liset

About the author

Liset Rietman was born on February 11, 1986 in Almelo (The Netherlands). After completing her Atheneum at O.S.G. Erasmus in 2004, she moved to Groningen to study Psychology. During her Bachelor, she followed several courses in Madrid (Spain) as an Erasmus exchange student. She received her Bachelor's degree in Psychology at the University of Groningen in 2008. In 2009, she completed her Master's degree in Psychology within the track Brain and Behaviour at the University of Groningen. Given her great interest in the functioning of the brain, both at the cognitive and molecular level, Liset moved in 2009 to Utrecht to start the Master Neuroscience and Cognition, a two-year Biomedical Research Master at Utrecht University. During this Master, she performed research and wrote her Master's thesis at the Smurfit Institute of Genetics at Trinity College Dublin (Ireland). She graduated in 2011.

In 2012, Liset became manager of a newly established induced Pluripotent Stem Cell (iPSC) Facility at the Rudolf Magnus Brain Centre at the Utrecht University Medical Centre (UMC). Her primary task was to set up this new facility. Thereafter, she was also involved in conducting fundamental iPSC research.

Given Liset's passion for building bridges via translational and multidisciplinary research, she was motivated to work in a field where she could unite all her background knowledge.

In 2014 she was given this opportunity when she started her PhD project at the Dutch National Institute for Public Health and the Environment (RIVM) that led to this thesis, in collaboration with the Julius Centre for Health Sciences and Primary Care of the University Medical Centre Utrecht. During her PhD project, Liset graduated from the Master Epidemiology Postgraduate in 2018 within the track Medical Statistics at Utrecht University. From 2017–2019, Liset also represented the Netherlands in the Joint Action ADVANTAGE, a European Joint Action with the aim of preventing frailty in older people in Europe. After completing her PhD project in 2021, Liset continues to work in the ageing research field as postdoctoral researcher for the World Health Organization (WHO) Collaborating Centre for Life Course and Ageing (in formation) at RIVM, and she is member of the project team of the Doetinchem Cohort Study.

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