Role of cardiac biomarkers in cognitive impairment and functional decline

Mahin Rad, S.

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ROLE OF CARDIAC BIOMARKERS IN COGNITIVE IMPAIRMENT AND FUNCTIONAL DECLINE

Simin Mahin Rad

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Role of Cardiac Biomarkers in Cognitive Impairment and Functional Decline

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	Prof. dr. M.J.A.P. Daemen	Academic Medical Center, Amsterdam, the Netherlands
	Prof. dr. F.A. Sorond	Northwestsern University, the Feinberg School of Medicine, Chicago, IL, USA

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Burden of Cognitive Impairment and Functional Decline in Old Age

The recent increase in life expectancy and decreased birth rate have resulted in a rapid rise of older individuals worldwide[1]. In particular, 8.3% of the Europes' population are aged above 75 years, and this proportion is projected to increase up to 10.7% by 2030[2]. Such population ageing has a profound impact on the burden of age-related disorders such as dementia and functional disability[3]. In fact, currently 47 million people are living with dementia worldwide, while these numbers are expected to rise to 75 million by 2030[2]. Despite these alarming public health projections, current treatment strategies have largely been unsuccessful in delaying the progression of cognitive impairment and functional decline[4]. Consequently, such ageing-related disorders create an important challenge for medicine, public health services and society[1].

Mounting evidence suggest that cognitive impairment and functional decline have a long preclinical phase[5]. In fact, several older subjects develop mild cognitive impairment years before progression to dementia[6]. As such, effective identification of risk factors in an early stage may provide an opportunity for the timely start of disease modifying interventions[5]. This premise, however, necessitates elucidating the link between the pathological processes and the onset of clinical symptoms[5].[7]

Cardiac Function and Cognitive Impairment: Pathophysiological Perspectives

Over the past couple of decades, a large number of preclinical and clinical studies have focused on identifying the main causes of dementia[8]. Despite huge investments, strategies targeting the neurodegenerative changes in the brain of demented subjects have proven to be unsuccessful[9]. While Alzheimer's disease is the most common type of dementia, vascular dementia is largely recognized as an independent contributor to cognitive impairment[10]. Interestingly, it has been shown that neuro-vascular damage is a common feature in Alzheimer's disease[4]. Meanwhile, vascular dementia and Alzheimer's disease have also been shown to be highly prevalent in heart failure patients[11, 12]. Furthermore, vascular damage such as atherosclerosis and peripheral arterial disease are shown to be predictors of functional decline[13, 14]. Together, these findings suggest a potential contribution of cardiac and vascular disturbances to cognitive impairment and functional decline in old age[15].

Cardiac and vascular contributions to cognitive brain ageing have been captured in the heart-brain connection hypothesis[16]. This hypothesis states that cardiac and vascular pathologies affect the hemodynamic status of the brain resulting in cerebral hypo-perfusion, neuronal crisis and dysregulation of the neurovascular unit[17]. Ultimately, this might lead to neuronal injury and impairment of the structural and functional integrity of the brain[18]. In fact, animal studies showed a negative influence of cerebral hypo-perfusion on structure and function of the brain[19]. In human subjects, the detrimental effects of cerebral hypo-perfusion have been frequently studied in heart failure patients[20]. It was shown that heart failure patients are at increased risk of dementia[21], and restoration of cardiac function improved cerebral blood flow and cognitive function[22, 23]. On the other hand, recent findings suggest that the link between cardiac dysfunction and cognitive impairment might not be limited to overt cardiac damage[17]. Instead, it has been suggested that individuals with suboptimal cardiac function might also be at increased risk of dementia[24]. This is supported by studies demonstrating a link between graded decrease in cardiac function and features of brain ageing[25, 26]. However, there has been lack of studies on the link between subclinical cardiac dysfunction and cognitive impairment, and the mechanisms underlying this association remain largely unknown.

The physiological connection between the heart and the brain is rather complex and not limited to vascular and hemodynamic factors[17]. Particularly, neuronal and hormonal mediators act in concrete to maintain homeostasis of the cerebro- and cardiovascular systems[27, 28]. It is known that the autonomic nervous system regulates the adoptive response of the cardiovascular system to the rapidly changing external stimuli[28]. Furthermore, cardiac hormones and hormones released from the hypothalamus-pituitary-adrenal glands act in a tangible feedback loop to regulate body fluid homeostasis, blood pressure, stress responses and inflammation[29]. Notably, increased levels of plasma cardiac hormones have been shown to associate with dementia and cognitive impairment, even in the absence of overt cardiac damage[24, 30]. However, the pathological mechanism linking cardiac hormones with cognitive impairment remains largely unknown.

Building on this background, the aim of this thesis was to explore the link between cardiac biomarkers, cognitive impairment and functional decline in older subjects with a focus on non-invasive markers that are routinely available in clinical practice.

Cardiac Biomarkers Derived from Electrocardiogram

Heart Rate and Heart Rate Variability

The autonomic nervous system is a branch of the peripheral nervous system that plays an important role in regulation of cardiovascular homeostasis[28]. Particularly, the sympathetic and parasympathetic branches of the autonomic nervous system innervate the sinoatrial node and regulate heart rate[31]. By modulating the heart rate, the autonomic nervous system keeps blood pressure within an optimal range and ensures adequate perfusion to vital organs[32]. This cardiac autonomic control is frequently assessed by means of heart rate variability[7]. Heart rate variability is characterized as the beat to beat variations in consecutive heart beat intervals that represents the synchronized action of the autonomic nervous system on the sinoatrial node[33] (Figure1). Importantly, increased heart rate and reduced heart rate variability indicate a disturbed cardiac autonomic control and decreased resilience to stressors[34].

Heart rate variability is usually measured from a 12-lead electrocardiogram (ECG) recording by means of “time domain” or “frequency domain” methods. The “time domain” methods are among the most commonly used measures of heart rate variability[7]. Their measurement depends on detection of the QRS complexes and normal-to-normal (NN) RR intervals. The NN interval refers to the interval between the neighboring QRS complexes that are generated from the sinoatrial node. The most simple "time domain" measures include the mean NN interval, the mean heart rate, and the difference between the longest and shortest NN interval. More complex "time-domain" measures include the standard deviation of NN intervals (SDNN), heart rate variability triangular index, standard deviation of the average NN intervals (SDANN) and the square root of the mean squared differences of successive NN intervals (RMSSD)[7].

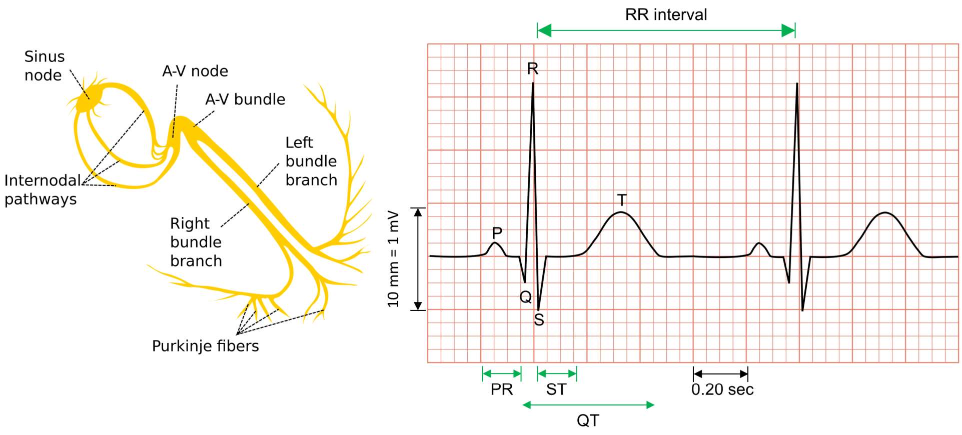

Figure 1. Cardiac conduction system and electrocardiogram waveforms. The figure shows a schematic view of the components of the electrical conduction system of the heart and an ECG-recording. The electrical impulses generated from the sinus node travel to the A-V node. After a delay, the electrical impulses spread through the cardiac ventricles via bundle branches and Purkinje fibers. The ECG captures these electrical impulses in forms of ECG-waveforms. The P wave represents depolarization of atrium; the QRS complex represents depolarization of ventricles and the T wave captures repolarization of ventricles. Sources: Wikipedia and the cardiovascular physiology website (http://www.cvphysiology.com).

Left Ventricular Hypertrophy

The cardiac wall tissue consists of an elastic network of myocyte cells encompassed in a collagen matrix that connects the myocytes and supports the coronary vasculature[35]. An increase in the cardiac workload results in a range of changes in the cardiac tissue architecture that manifests as thickening of the cardiac wall structure[36]. For example, a chronic increase in cardiac workload such as that in hypertension leads to thickening of the cardiac left ventricle walls. This cardiac structural remodeling known as left ventricular hypertrophy may in turn hamper diastolic function and decrease cardiac output[36]. Particularly, left ventricular hypertrophy is considered a precursor of clinical heart failure in the form of diastolic heart failure[37]. As such, a long lasting hemodynamic stress manifested in cardiac structural remodeling can be detected by means of assessment of left ventricular hypertrophy[8].

There are different ECG-markers for measurements of left ventricular hypertrophy that are frequently based on the amplitude of R and S waves. Since the duration of the QRS complex is frequently increased in left ventricular hypertrophy[38], the QRS complex is incorporated in the calculation of a number of ECG markers[39]. The Sokolow-Lyon product is among such markers that is commonly used in evaluation of left ventricular hypertrophy patients from ECG[39, 40]. In practice, the Sokolow-Lyon product is defined as the sum of S wave in the V1 lead plus the R wave in V5 or V6 leads, multiplied by the duration of the QRS complex[40]. Of note, most ECG markers for left ventricular hypertrophy have a high specify ranging from 85% to 90%[39].

Spatial QRS-T angle

The cardiac action potential consists of waves of depolarization and repolarization. During a cardiac cycle, the loss of electrical charges in cardiac cells result in depolarization (excitation), that is followed by a period of electrical inactivity and repolarization (relaxation), i.e. restoration of electrical charge in the cardiac cells[41]. The depolarization and repolarization waves are generated through a cascade of ionic movements between the intra- and extracellular spaces that result in cardiac muscle contraction/relaxation[42]. An important feature of the cardiac action potential is the ventricular gradient: heterogeneous restoration of electrical charges in the ventricles[43]. This phenomenon is a result of delayed repolarization in the endocardium (inner cardiac tissue layer) compared to the epicedium (outer cardiac tissue layer)[43]. In fact, the depolarization waves start in the endocardium and travel toward the epicardium, while the repolarization begins in the epicardium rather than the endocardium. As a result, the electrical changes generated by depolarization (QRS axis) and the electrical changes generated by repolarization (T-wave axis) are in opposing directions, forming an angular shape. This angle is known as the QRS-T angle. The QRS-T angle has been originally measured by means of vectorcardiogram in three orthonormal leads of X,Y and Z and referred to as the spatial QRS-T angle[43, 44](Figure 2). Using modern computerized methods, the spatial QRS-T angle can be reconstructed using information derived from a 12-lead ECG[45].

The spatial QRS-T angle provides novel additional information about the function of cardiac ventricles that is not captured with a routine 12-lead ECG. Importantly, cardiac pathophysiological changes such as ischemia, fibrosis, ionic channel disturbances or infarction result in an abnormally wide spatial QRS-T angle indicating localized non-physiological changes[43]. Hence, a wide spatial QRS-T angle might indicate early hemodynamic stresses to the cardiac ventricles.

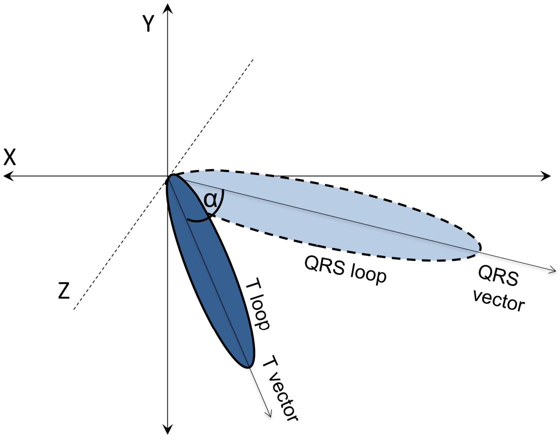

Figure 2. Spatial QRS-T angle in a three dimensional space. Figure shows orthonormal leads of X, Y and Z in a 3-dimentional space. Using T loop and QRS loop, the main T and QRS vectors (arrows) are calculated. The T vector shows the mean direction of ventricular repolarization and the QRS vector shows the mean direction of ventricular depolarization. The angle between the T and QRS vectors (α) represents the spatial QRS-T angle. Adopted from *Vahedi, Farzad, et al. Journal of Applied Physiology 113.3 (2012): 368-376.*

Cardiac Biomarkers Derived from Plasma and Brain Tissue

Natriuretic Peptides

Natriuretic peptides (NP), often referred to as cardiac hormones, are cardiac biomarkers that are used in clinical practice for diagnosis of heart failure and cardiac wall stress[46]. They consist of a family of structurally related peptides that are released into the circulation in response to cardiac wall stretch and volume overload[47]. The opposing action of NP on the renin–angiotensin–aldosterone system and their vasodilatory effect contribute to natriuresis, diuresis and lowering the blood pressure[24] (Figure 3).

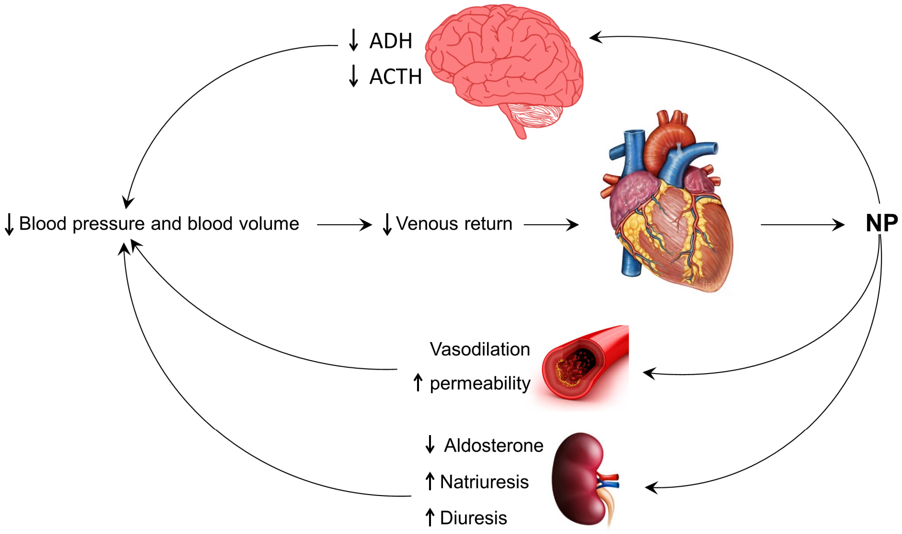

Figure 3. The main physiological functions of the natriuretic peptides in the systemic circulation. Natriuretic peptides are released into the circulation in response to volume overload. Upon release, they stimulate the natriuretic peptide receptors that are located on the kidney, vessel walls and the brain. Consequently, this results in activation and/or deactivation of several biological pathways in the systemic circulation as well as in the brain. NPs: natriuretic peptides, ADH: antidiuretic hormone, ACTH: Adrenocorticotropic hormone. Adopted from: *Moro, Cedric, and Max Lafontan. American Journal of Physiology-Heart and Circulatory Physiology 304.3 (2013): H358-H368.*

Furthermore, a gradual increase in the plasma levels of NP indicates a graded decrease in cardiac function and severity of heart failure[24, 48]. Several studies have shown that higher plasma levels of NP associate with cognitive impairment, even in subjects free of heart failure[30, 49, 50]. Interestingly, growing evidence suggests that the role of natriuretic peptides is not limited to the cardiovascular system, but that they might as well be essential in regulation of brain homeostasis[51]. Differential evidence from animal and human models suggests the presence and expression of natriuretic peptides in brain tissue and cerebrospinal fluid[52]. In fact, natriuretic peptides might be involved in regulation of several brain functions such as neurovascular integrity, synaptic transmutation and neruo-inflammation[51]. However, the role of centrally acting natriuretic peptides in the brain of human subjects remains unknown.

Outline of this thesis

Chapter 2 tests the association of resting heart rate, heart rate variability and functional decline in older subjects. **Chapter 3** explores the independent link between heart rate variability and cognitive function in older subjects. **Chapter 4** is devoted to studying the relation between left ventricular hypertrophy and cognitive function; and **Chapter 5** assesses the relation between spatial QRS-T angle and cognitive function in older subjects. **Chapter 2, 3, 4 and 5** are performed using participants of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) cohort who are at increased risk of cardiovascular disease[53]. The physiology of natriuretic peptides and the role of centrally acting natriuretic peptides in relation to cognitive impairment is described in **Chapter 6**. **Chapter 7** explores the distribution of natriuretic peptides in the brain of post-mortem human subjects and their relation to Alzheimer’s disease. **Chapter 8** summarizes the main findings of this thesis and discusses the future perspectives.

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CHAPTER 2

RESTING HEART RATE, HEART RATE VARIABILITY AND FUNCTIONAL DECLINE

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Abstract

Background: Heart rate and heart rate variability, markers of cardiac autonomic function, have been linked with cardiovascular disease. We investigated whether heart rate and heart rate variability are associated with functional status in older adults, independent of cardiovascular disease. **Methods:** We obtained data from the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). A total of 5042 participants were included in the present study, and mean follow-up was 3.2 years. Heart rate and heart rate variability were derived from baseline 10-second electrocardiograms. Heart rate variability was defined as the standard deviation of normal-to-normal RR intervals (SDNN). Functional status in basic (ADL) and instrumental (IADL) activities of daily living was measured using Barthel and Lawton scales, at baseline and during follow-up. **Results:** The mean age of the study population was 75.3 years. At baseline, higher heart rate was associated with worse ADL and IADL, and lower SDNN was related to worse IADL (all p values < 0.05). Participants in the highest tertile of heart rate (range 71–117 beats/min) had a 1.79-fold (95% confidence interval [CI] 1.45–2.22) and 1.35-fold (95% CI 1.12–1.63) higher risk of decline in ADL and IADL, respectively (p for trend < 0.001 and 0.001, respectively). Participants in the lowest tertile of SDNN (range 1.70–13.30 ms) had 1.21-fold (95% CI 1.00–1.46) and 1.25-fold (95% CI 1.05–1.48) higher risk of decline in ADL and IADL, respectively (both p for trends < 0.05). All associations were independent of sex, medications, cardiovascular risk factors and comorbidities. **Interpretation:** Higher resting heart rate and lower heart rate variability were associated with worse functional status and with higher risk of future functional decline in older adults, independent of cardiovascular disease. This study provides insight into the role of cardiac autonomic function in the development of functional decline.

Introduction

Elevated heart rate and reduced heart rate variability — the beat-to-beat variation in heart rate intervals — both reflect an altered balance of the autonomic nervous system tone characterized by increased sympathetic and/or decreased parasympathetic activity[1-3]. Sympathetic overactivity has been linked to a procoagulant state and also to risk factors for atherosclerosis, including metabolic syndrome, obesity and subclinical inflammation[2-4]. Moreover, increased heart rate is related to atherosclerosis, not only as an epiphenomenon of sympathetic overactivity, but also through hemodynamic mechanisms, such as high pulsatile shear stress, which leads to endothelial dysfunction[5].

Atherosclerosis has been linked to increased risk of functional decline in older people via cardiovascular events[6]. As the world population is aging, the burden of functional disability is expected to increase[6]. It has been hypothesized that heart rate and heart rate variability are markers of frailty, an increased vulnerability to stressors and functional decline[7]. However, the direct link between these 2 parameters and risk of functional decline has not been fully established, and it is uncertain whether this association is independent of cardiovascular comorbidities.

In this study, we examined whether heart rate and heart rate variability were cross-sectionally and longitudinally associated with functional status in older adults at high risk of cardiovascular disease, independent of cardiovascular risk factors and comorbidities.

Methods

Study design and participants

The data in this study were obtained from the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER), a randomized controlled trial on the effect of pravastatin in a cohort of older men and women (70–82 yr) with pre-existing vascular disease or risk factors thereof. A total of 5804 individuals were recruited from 3 collaborating centres in Ireland, Scotland and the Netherlands. Details of study design, population recruitment and characteristics have been previously reported[8, 9]. Exclusion criteria included physical or mental inability to attend clinic visits, poor cognitive function at baseline (Mini Mental State Examination score < 24), advanced heart failure (New York Heart Association functional class III or IV), electrocardiographic (ECG) evidence of atrial fibrillation or other major arrhythmias and implanted cardiac pacemakers. Participants were followed up for a mean of 3.2 years.

From the original population, we excluded 150 participants with missing heart rate and/or heart rate variability measurements at baseline, 489 participants with cardiac rhythm not generated by sinoatrial node and 123 participants with missing data on functional status at baseline or during follow-up. We included participants from both the pravastatin and placebo arms because the PROSPER study group had previously shown that pravastatin did not affect functional status during follow-up[9]. Hence, 5042 participants were included in the present study. The PROSPER study complied with the Declaration of Helsinki and was approved by the medical ethics committees of the 3 centres. All participants provided written informed consent.

Measurement of heart rate and heart rate variability

We measured resting heart rate and heart rate variability from a 10-second, 12-lead ECG, recorded in the morning of the first enrolment visit to limit circadian variability. All ECGs were transmitted electronically for storage at the University of Glasgow ECG Core Laboratory based at Glasgow Royal Infirmary, Scotland, and interpreted using the same software[10]. We computed the standard deviation of normal-to-normal RR intervals (SDNN), one of the most frequently used and easily calculated indices of heart rate variability, by deriving it from normal-to-normal RR intervals[11]. Normal-to-normal RR intervals were defined as the time between 2 successive normally conducted QRS complexes.

Functional status

Functional status was assessed using 2 questionnaires: the Barthel Index[12] and the Lawton Instrumental Activities of Daily Living Scale (IADL)[13].The Barthel Index measures performance in basic activities of daily living (ADL) and consists of 10 items: fecal continence, urinary continence, grooming, toilet use, feeding, transfers (e.g., from chair to bed), walking, dressing, climbing stairs and bathing. The Lawton IADL evaluates more complex instrumental activities and includes 7 items: doing housework, taking medication as prescribed, managing money, shopping, using a phone or other forms of communication, using technology and taking transportation within the community. Scores for ADLs and IADLs range from 0 to 20 and from 0 to 14, respectively, with higher scores indicating higher independence and better functional status. Functional status using the 2 questionnaires was measured at baseline; after 9, 18 and 30 months; and at the end of the study, which varied between 36 and 42 months. Based on changes in functional status scores during follow-up, participants were classified as either declining or not declining in ADL and IADL.

Statistical analysis

We used SPSS version 20 for all the analyses. We reported baseline characteristics of participants as number of participants (percentage) for categorical variables and as mean (standard deviation) for continuous variables. We tested differences in baseline characteristics first across heart rate tertiles and then across SDNN tertiles, using analysis of variance for continuous variables and $\chi 2$ test for categorical variables. Linear regression analyses tested the cross-sectional associations of heart rate and SDNN with functional status. Dependent variables were the scores on each of the 2 functional status tests. We computed p values for trend using tertiles of heart rate and SDNN. We performed binary logistic regression analyses to investigate longitudinal associations of heart rate and SDNN with risk of decline in functional status. Independent variables were heart rate and SDNN. The outcome variable was the risk of declining in each of the functional status tests. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) in tertiles of heart rate and

SDNN, respectively. The reference categories were the lowest tertile of heart rate and the highest tertile of SDNN. We calculated *p* values for trend using tertiles of heart rate and SDNN. We performed all cross-sectional and longitudinal analyses in 2 steps. In the first step, analyses were adjusted for age, sex, country of enrolment and education (minimally adjusted model). In the second step, we further adjusted for cardiovascular risk factors (smoking status, body mass index [BMI], history of hypertension, history of diabetes mellitus), cardiovascular morbidities (history of myocardial infarction, history of stroke or transient ischemic attack, history of claudication), use of medications and statin treatment group. In the longitudinal analyses we also adjusted for baseline functional status (fully adjusted model).

To test whether the association of heart rate and SDNN with functional status is independent of β-blocker use, we repeated the longitudinal analyses after exclusion of participants taking β-blockers. Furthermore, we repeated the longitudinal analyses after stratifying the participants by sex, history of hypertension, history of vascular diseases, use of β-blockers, calcium channel blockers or statin treatment to explore the potential modifying effect of these covariates. We computed interaction terms by multiplying heart rate and SDNN, as continuous variables, per these covariates. To explore the influence of vascular events on the longitudinal associations, we performed sensitivity analyses from which we excluded the following: 1) participants with incident stroke, 2) participants with incident coronary events and 3) participants who were admitted to hospital for heart failure during follow-up. Furthermore, to check whether the longitudinal associations are affected by baseline functional status or by duration of follow-up, we performed sensitivity analyses including only 1) participants with maximum functional status at baseline and 2) participants who completed 36 months of follow-up. To check whether the association between SDNN and functional status is independent of heart rate, we repeated the analyses after standardizing SDNN for heart rate (dividing SDNN by heart rate)[14]. Finally, we repeated the longitudinal analyses by dividing the participants in the lowest tertile of heart rate into 2 groups of participants with a heart rate of less than 50 beats/min and participants with a heart rate of 50–60 beats/min.

Results

The mean age of the study population was 75.3 years. A total of 2619 (51.9%) participants were female (Table 1). The median resting heart rate and SDNN were 65 beats/min and 18.6 ms, respectively. Participants with a higher resting heart rate were older, were more likely to be female and current smokers, and had a higher BMI and a higher prevalence of diabetes mellitus. In contrast, participants with a lower resting heart rate used β-blockers more frequently and had a higher prevalence of myocardial infarction (all *p* values < 0.05) (Appendix 1). Participants with lower heart rate variability as measured by SDNN had a higher BMI, a higher prevalence of diabetes mellitus and less frequently used β-blockers (all *p* values < 0.05) (Appendix 2).

Table 1. Characteristics of study population at baseline

Characteristics	Values
Socio-demographics	
Age, years, mean (SD)	75.3 (3.3)
Female, n (%)	2619 (51.9)
Age left school, years, mean (SD)	15.1 (2.1)
Cardiovascular risk factors	
History of hypertension, n (%)	3127 (62.0)
History of stroke or TIA, n (%)	552 (10.9)
History of MI, n (%)	662 (13.1)
History of claudication, n (%)	336 (6.7)
History of diabetes mellitus, n (%)	517 (10.3)
Current smoking, n (%)	1334 (26.5)
BMI, kg/m ^{2} , mean (SD)	26.8 (4.2)
Medications	
ß-blockers, n (%)	1320 (26.2)
Calcium channel blockers, n(%)	1275 (25.3)
Statins, n (%)	2504 (49.7)

Abbreviations: HR: heart rate; BMI: body mass index; MI: myocardial infarction and TIA: transient ischemic attack.

Table 2. Baseline functional status in tertiles of resting heart rate and SDNN

	Tertiles of HR/SDNN			
	Low	Middle	High	*P* for trend
Heart Rate	n=1649	n=1742	n=1651	
HR, beats/min, range	34-60	61-70	71-117	
ADL score				
Model 1	19.79 (0.02)	19.78 (0.02)	19.71 (0.02)	0.004
Model 2	19.27 (0.25)	19.26 (0.25)	19.21 (0.25)	0.02
IADL score				
Model 1	13.67 (0.03)	13.62 (0.02)	13.52 (0.03)	<0.001
Model 2	12.94 (0.34)	12.89 (0.34)	12.80 (0.33)	<0.001
SDNN	n=1689	n=1670	n=1683	
SDNN range, msec	1.70-13.30	13.40-26.50	26.60-422.60	
ADL score				
Model 1	19.73 (0.02)	19.75 (0.02)	19.80 (0.02)	0.01
Model 2	19.23 (0.25)	19.24 (0.25)	19.27 (0.25)	0.11
IADL score				
Model 1	13.55 (0.03)	13.62 (0.03)	13.65 (0.02)	0.004
Model 2	12.84 (0.33)	12.90 (0.34)	12.91 (0.34)	0.03

ADL and IADL scores are presented as means (standard errors). Model 1: adjusted for country, age, sex, education. Model 2: adjusted for country, age, sex, education, smoking, body mass index, history of hypertension, history of diabetes mellitus, history of claudication, history of myocardial infarction, history of stroke/transient ischemic attack, statin treatment, diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers, calcium-channel blockers, nitrates, aspirin and anticoagulants. Abbreviations: HR: heart rate; SDNN: standard deviation of the normal to normal R-R intervals; ADL: basic activities of daily living; IADL: instrumental activities of daily living.

Table 2 shows the associations of resting heart rate and SDNN with functional status at baseline. In the minimally adjusted model, participants with a higher resting heart rate had a worse performance in both functional status scales (*p* for trend < 0.05, for both). These associations remained significant in the fully adjusted model (*p* for trend < 0.05, for both). Likewise, participants with lower SDNN had a worse performance in both functional status scales in the minimally adjusted model (*p* for trend < 0.05, for both). After full adjustment, the same association persisted between SDNN and IADL (*p* for trend = 0.03). The same trend was observed between SDNN and ADL, although it did not reach significance (*p* for trend = 0.11).

During a mean follow-up of 3.2 years, 779 participants (15.5%) declined in ADL score and 1128 participants (22.4%) declined in IADL score. Among the participants who declined in ADL score, 406 (52.1%) declined 1 point, 141 (18.1%) 2 points and 232 (29.8%) 3 or more points. Among the participants who declined in IADL score, 402 (35.6%) declined 1 point, 224 (19.9%) 2 points and 502 (44.5%) 3 or more points.

Figure 1 shows the longitudinal associations of resting heart rate and SDNN with risk of decline in functional status after full adjustment. Participants with a resting heart rate in the highest tertile had a 1.79-fold (95% CI 1.45–2.22) and a 1.35-fold (95% CI 1.12–1.63) higher risk of decline in ADL and IADL scores, respectively (*p* for trend < 0.001 and 0.001, respectively). Participants with SDNN in the lowest tertile had 1.21-fold (95% CI 1.00–1.46) and 1.25-fold (95% CI 1.05–1.48) higher risk of decline in ADL and IADL scores, respectively (*p* for trend < 0.05, for both groups). These associations were similar in the minimally adjusted model (*p* for trend < 0.05, for all groups) (Appendix 3).

Table 3 shows the sensitivity analyses after exclusion of the 1320 participants receiving treatment with β-blockers. Higher resting heart rate and lower SDNN remained significantly related to a higher risk of decline for both ADL and IADL in the fully adjusted model (*p* for trend < 0.05, for all groups). To clarify whether cardiovascular events during follow-up might affect the longitudinal associations between resting heart rate/SDNN and risk of decline in functional status, we performed a series of sensitivity analyses after exclusion of 1) participants with incident stroke during follow-up (*n* = 220); 2) participants with incident coronary events during follow-up (*n* = 541); and 3) participants who were admitted to hospital for heart failure during follow-up (*n* = 196). Results did not materially change (Appendices 4, 5 and 6).

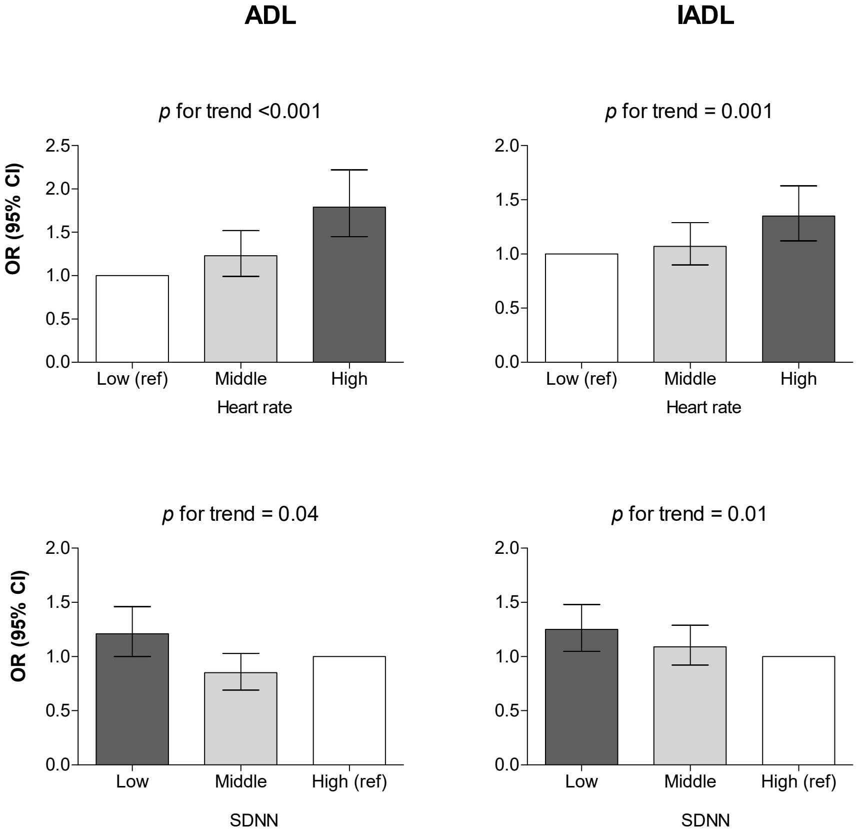

Figure 1: Risk of decline in functional status in tertiles of resting heart rate and heart rate variability. All analyses are adjusted for country, age, sex, education, basic activities of daily living (ADL) and instrumental activities of daily living (IADL) scores at baseline, smoking, body mass index, history of hypertension, history of diabetes mellitus, history of claudication, history of myocardial infarction, history of stroke/transient ischemic attack, statin treatment, diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, ß-blockers, calcium channel blockers, nitrates, acetylsalicylic acid and anticoagulants. Abbreviations: CI = confidence interval, OR = odds ratio.

To explore whether poor functional status at baseline might affect the longitudinal relation between resting heart rate/SDNN and risk of decline in functional status, we performed further sensitivity analyses including only participants with maximum functional status scores at baseline (*n* = 4343 participants with maximum ADL score, *n* = 4129 participants with maximum IADL score). Results did not materially change (Appendix 7). To test whether short duration of follow-up might affect the results, we repeated the longitudinal analyses including only participants who completed 36 months of follow-up (*n* = 4552). The longitudinal associations between resting heart rate/SDNN and risk of decline in functional status remained significant (Appendix 8).

Table 3. Risk of decline in functional status in tertiles of resting heart rate and SDNN after exclusion of participants using beta-blockers

	Tertiles of HR/SDNN			
	Low	Middle	High	P for trend
Heart Rate	n=863	n=1379	n=1480	
ADL, OR (95% CI)				
Model 1	1 (ref)	1.27 [0.97;1.67]	1.95 [1.50;2.53]	<0.001
Model 2	1 (ref)	1.25 [0.95;1.65]	1.86 [1.43;2.42]	<0.001
IADL, OR (95% CI)				
Model 1	1 (ref)	1.09 [0.87;1.37]	1.46 [1.17;1.81]	<0.001
Model 2	1 (ref)	1.07 [0.85;1.35]	1.39 [1.11;1.74]	0.002
SDNN	n=1312	n=1192	n=1218	
ADL, OR (95% CI)				
Model 1	1.31 [1.06;1.63]	0.85 [0.67;1.08]	1 (ref)	0.009
Model 2	1.25 [1.00;1.55]	0.82 [0.65;1.04]	1 (ref)	0.03
IADL, OR (95% CI)				
Model 1	1.30 [1.07;1.58]	1.09 [0.89;1.34]	1 (ref)	0.008
Model 2	1.26 [1.03;1.53]	1.07 [0.87;1.31]	1 (ref)	0.02

Model 1: adjusted for country, age, sex, education. Model 2: adjusted for country, age, sex, education, ADL/IADL at baseline, smoking, body mass index, history of hypertension, history of diabetes mellitus, history of claudication, history of myocardial infarction, history of stroke/transient ischemic attack, statin treatment, diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium-channel blockers, nitrates, aspirin and anticoagulants. Abbreviations: HR: heart rate; SDNN: standard deviation of the normal to normal R-R intervals; ADL: basic activities of daily living; IADL: instrumental activities of daily living; OR: odds ratio; CI: confidence interval.

The associations of resting heart rate and SDNN with functional decline were not modified by sex, history of hypertension or vascular diseases, use of β-blockers, calcium channel blockers or statin treatment (*p* for interaction > 0.05, for all groups) (Appendix 9 for heart rate; data not shown for SDNN). In an extra analysis, we tested whether the observed associations were independent of baseline cognitive function as assessed by the Mini Mental State Examination. The associations did not materially change after adjustment for baseline cognitive function (data not shown). Likewise, these associations remained unchanged when we standardized SDNN for heart rate (Appendices 10 and 11). Furthermore, we observed no difference in risk of functional decline between participants with a heart rate of less than 50 beats/min (*n* = 284) and those with a heart rate of 50–60 beats/ min (*n* = 1365). Participants in the highest tertile had a higher risk of functional decline compared with the participants in the group with a heart rate of 50–60 beats/min (Appendix 12).

Interpretation

In our study, higher resting heart rate and lower heart rate variability were associated with worse functional performance at baseline and with higher risk of future functional decline in older adults at high cardiovascular risk. These associations were independent of cardiovascular risk factors, cardiovascular morbidities and use of medications.

The results of our study are in line with the results of the Prevention Regimen for Effectively Avoiding Second Stroke (PRoFESS) trial, which showed that higher heart rate was related to worse functional outcomes in patients with a recurrent stroke[15]. Our results are also consistent with findings from the Women's Health and Aging Study-I (WHAS-I), which showed a cross-sectional association between lower heart rate variability and frailty in disabled older women living in the community[16]. Our study extends the findings of WHAS-I to older adults at risk for cardiovascular disease with preserved functional status. Furthermore, we showed that the association of heart rate variability with functional decline was independent of sex.

Different pathophysiological mechanisms may underlie these associations. First, higher heart rate and lower heart rate variability have been consistently associated with incident cardiovascular events in previous studies[1-3]. In this study, the strength of the associations between heart rate/ heart rate variability and functional decline did not materially change after exclusion of participants with incident cardiovascular events. This might suggest that mechanisms other than macrovascular damage play roles in the association between heart rate/heart rate variability and functional decline. Second, lower heart rate is associated with better cardiovascular fitness, which is a protective factor for brain aging and functional decline[17]. In particular, lower heart rate is related to less myocardial oxygen consumption and more prolonged time available for diastolic heart chamber filling and coronary perfusion[18]. Furthermore, higher heart rate has been suggested to increase pulsatile shear stress, which leads to endothelial dysfunction and accelerated atherosclerosis[5, 19]. In this setting, use of ivabradine, a pure heart rate lowering agent, in relation to cardiovascular outcomes has been tested with conflicting results[20-22]. Third, heart rate and heart rate variability reflect the autonomic nervous system's control over cardiac function. Cardiac autonomic control regulates the interaction between circulation and respiration. Higher heart rate variability in synchrony with respiration improves the efficiency of gas exchange at the level of the lung via efficient ventilation and perfusion matching[23]. Furthermore, cardiac autonomic control keeps blood pressure constant within a certain range to maintain adequate perfusion to vital organs, including the brain. A preserved cardiac autonomic control buffers variations in blood pressure in response to stressors. Indeed, participants with lower heart rate variability present higher blood pressure variability in response to psychological challenge or tilt test[24, 25]. Higher blood pressure variability is associated with atherosclerosis[26] and silent brain damage[27]. Finally, the autonomic nervous system is connected to regions of the central nervous system[28, 29], which are involved in mood regulation. Lower heart rate variability has been associated with depression[30, 31], which is a cause of disability[6].

Strengths and limitations

A strength of our study was the longitudinal design, which allowed us to show that high heart rate and low heart rate variability preceded the decline in functional status. We also showed that this association was independent of potential confounders such as vascular diseases and use of antihypertensive and cardioprotective medications. However, causality cannot be inferred given the observational nature of this study. Further strengths are the large study population of older adults and the multicentre design. A limitation of our study was that all participants were older adults at high cardiovascular risk, which may limit the generalizability of our findings. Nevertheless, a considerable number of older adults carry high loads of cardiovascular pathologies and comorbidities. Moreover, we categorized our participants into the clinically distinguishable groups of those who declined and those who did not decline, although this categorization may result in loss of information. Another possible limitation is the use of a 10-second ECG; nonetheless, we were able to show a significant association of resting heart rate and heart rate variability with functional status even by using a short ECG recording, which is more feasible in clinical practice than longer recordings. Heart rate variability measured from standard 10-second ECG recordings correlates with heart rate variability measured from longer ECG recordings[11].

Conclusion

We found that higher resting heart rate and lower heart rate variability were associated with worse functional status in older adults, independent of cardiovascular risk factors and comorbidities. This study provides insight into the role of cardiac autonomic function in the development of functional decline. Because functional disability has a long preclinical phase, it is crucial to identify potential interventions to delay it. Further research is needed to establish whether heart rate and heart rate variability are risk markers and/or potentially modifiable risk factors for functional decline. Pharmacologic and nonpharmacologic interventions (e.g., drugs with antiadrenergic properties, physical exercise, nervus vagus stimulation) aimed at modulating cardiac autonomic function may be beneficial in preserving functional status. It is well established that physical activity is a key contributor in autonomic regulation and is linked with preservation of functional status[32, 33]. However, future studies are needed to test the influence of physical activity on functionality through autonomic regulation in older adults.

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CHAPTER 3

HEART RATE VARIABILITY AND COGNITIVE IMPAIRMENT

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Abstract

Objective: To investigate the cross-sectional and longitudinal associations of 10-second heart rate variability (HRV) with various domains of cognitive function in older participants at risk of cardiovascular disease. **Methods:** We studied 3,583 participants, mean age of 75.0 years, who were enrolled in the Prospective Study of Pravastatin in the Elderly at Risk. From baseline 10-second ECGs, standard deviation of normal-to-normal intervals was calculated as the index of HRV. Four cognitive domains were assessed at baseline and repeated during a mean follow-up period of 3.2 years. **Results:** Lower HRV at baseline was associated with worse performance in reaction time (mean difference between low third vs high third of HRV 5 1.96 seconds, 95% confidence interval [CI] 0.20 to 3.71) and processing speed (20.57 digits coded, 95% CI 21.09 to 20.05). During follow-up, participants with lower HRV had a steeper decline in processing speed (mean annual change between low third vs high third of HRV 5 20.16 digits coded, 95% CI 20.28 to 20.04). There was no difference in annual changes of reaction time or immediate and delayed memory among HRV thirds during follow-up. All these associations remained unchanged after adjustment for medications, cardiovascular risk factors, and comorbidities. **Conclusions:** Participants with lower 10-second HRV have worse performance in reaction time and processing speed and experience steeper decline in their processing speed, independent of medications, cardiovascular risk factors, and comorbidities

Introduction

Heart rate variability (HRV), the variation in consecutive heart beat intervals, results from the constant interaction between the sympathetic and parasympathetic arms of the autonomic nervous system[1]. Reduced HRV is shown to be a strong predictor of cardiovascular morbidity and mortality[2, 3]; and has been linked to several vascular risk factors such as hypertension, diabetes mellitus and subclinical inflammation[4].

Current evidence indicates that vascular risk factors are independently associated with cognitive impairment in older participants. Cardiovascular risk factors and morbidities contribute to the development of cognitive impairment possibly by affecting the neurovascular integrity of the brain[5]. Neurovascular integrity of the brain is dependent on adequate and constant cerebral blood flow and regulation of cerebral blood flow requires intact function of autonomic nervous system[5, 6]. Hence, participants with lower HRV, as a reflection of autonomic dysfunction, might be at increased risk of cognitive decline.

HRV is typically measured using long or short-term electrocardiogram (ECG) recordings. Long-term measurements provide detailed information during physiological conditions such as activity and rest. Despite merits of long-term measurements, they are time-consuming and involve patients' discomfort which might limit their application in routine clinical practice. On the other hand, measuring HRV from a 10-second ECG recording is more practical and easier to apply in daily practice. It has been suggested that 10-second HRV may predict 5-minute cardiac vagal tones accurately[7]; and has a comparable predictive value for cardiac mortality in older participants[8]. However, to date there is no study evaluating the association of HRV with cognitive function using 10-second HRV measurements. In this study, we assessed the cross-sectional and longitudinal association of HRV, using 10-second ECG recordings, with various domains of cognitive function in older participants at high risk of cardiovascular disease.

Methods

Study Design and participants

The data for this study were drawn from PROSPER (PROspective Study of Pravastatin in the Elderly at Risk), a large prospective study of 5804 men and women aged 70 to 82 years. PROSPER was a randomized controlled trial designed to examine the effect of pravastatin in older participants with pre-existing or at high risk of cardiovascular diseases. The mean follow-up time was 3.2 years. The PROSPER study design, inclusion and exclusion criteria have been described elsewhere[9, 10]. We received approval from the institutional ethics review boards of the three centers on human experimentation and the PROSPER study complied with the Declaration of Helsinki. All participants in the study provided written informed consent[10].

In this study, we excluded all participants with cardiac arrhythmias and/or cardiac rhythms not generated by the sino-atrial node including premature ventricular and/or atrial contractions (n=414), ectopic atrial rhythm (n=161), supraventricular arrhythmia (n=139), atrial fibrillation (n=89), atrial flutter (n=13) and other arrhythmias (n=85) from the original PROSPER cohort. Individuals with sinus arrhythmia were also excluded (n=314). Furthermore, participants with missing HRV measurements at baseline (n=148) and with missing cognitive measurements at baseline or during follow-up (n=858) were excluded. Accordingly, 3583 participants were included in this study. Included participants were slightly younger and had lower degrees of cardiovascular co-morbidities (table e-1). We included participants from both pravastatin and placebo groups as it has been shown that treatment with pravastatin does not affect cognitive function[11]. Moreover, we adjusted our analyses for pravastatin treatment groups.

HRV measurements

Standard 10-second ECG recordings were obtained in resting, supine position using a Burdick Eclipse 850i electrocardiograph in the morning of the first enrolment visit before initiation of statin treatment. These digital data were subsequently transferred to the University of Glasgow ECG Core Lab based at Glasgow Royal Infirmary, Scotland, for storage[12]. HRV was measured using the University of Glasgow resting ECG program – a fully automated method – to ensure the reproducibility of the measurements and interpreted using the same software[13]. We used one of the most frequently used and easily calculated time domain measurements of HRV defined as the standard deviation of normal-to-normal R-R intervals (SDNN) in the 10-second ECG recording period. For each ECG, the onset of every QRS complex was recorded and then the dominant or normal-to-normal R-R intervals were calculated. Dominant R-R intervals are defined as the time between two normally conducted QRS complexes. The standard deviation of dominant R-R intervals was calculated thereafter.

Cognitive function measurements

The mini-mental state examination (MMSE) was used to measure global cognitive function at baseline. The cutoff point of 24 or more was applied as the inclusion criterion and participants with poor cognitive function (MMSE < 24) were excluded from enrolment in PROSPER. In this study, we used four neuropsychological performance tests to assess different domains of cognitive function. The Stroop test was used to assess selective attention and reaction time. The outcome variable was the time (number of seconds) taken to complete the test, with higher scores indicating worse performance. The Letter-Digit Coding test was used to measure the general cognitive processing speed. The outcome variable was the total number of correct digits entered in 60 seconds; a higher score indicates better performance. Memory was assessed using the Picture-Word Learning Test, which tests the immediate and delayed memory. The outcome variable was the accumulated number of correctly recalled pictures over 3 trials and the number of pictures recalled during delayed recall; a higher score indicates better performance. The test/re-test correlation of Stroop and Letter-Digit Coding tests were shown to be high (r = 0.80 and 0.88, respectively). The reliability of immediate and delayed Picture-Word Learning tests were shown to be acceptable (r = 0.66 and 0.63, respectively). In addition, the test/re-test correlations were independent of age and education[14]. Cognitive function was measured at baseline, after 9, 18 and 30 months, and at the end of the study. The time point at the end of the study varied among participants and ranged from 36 to 48 months.

Statistical analyses

Baseline characteristics of participants are reported as mean (SD) for continuous variables and as number of participants (%) for categorical variables across thirds of HRV. To test the cross-sectional and longitudinal association of HRV and cognitive domains, we used linear regression models. In longitudinal analyses, regression coefficient of the change in each cognitive test score per year was calculated for each participant, which indicates the annual changes in cognitive domains during follow-up time. This allowed us to test the longitudinal associations more accurately by using repeated measurements of cognitive tests. In both cross-sectional and longitudinal analyses, probability values were calculated using continuous log-transformed values of baseline SDNN as the determinant, since it was not normally distributed. Using analysis of covariance, we calculated the adjusted mean values of baseline and annual changes of cognitive scores in thirds of HRV.

All cross-sectional and longitudinal analyses were performed in 2 steps. In the first step (minimally adjusted model), the analyses were adjusted for age, sex, education (age at which the participants left school), country of enrolment and version of cognitive tests where appropriate. In the second step (fully adjusted model), the analyses were further adjusted for cardiovascular risk factors and morbidities and use of antihypertensive medications. In the longitudinal analyses, both models were additionally adjusted for baseline cognitive domain scores, and the fully adjusted model was additionally adjusted for statin treatment.

To explore the effect of cardiovascular events on the longitudinal associations, we performed a series of additional analyses in which we stratified for participants who did and did not develop incident stroke, incident heart failure hospitalization and incident coronary events. To test whether the difference between participants who did and did not develop cardiovascular events is significant, *p* value for interaction was calculated using linear regression models. To test whether the association of HRV with cognitive domains is independent of ß-blockers and medications with antiarrhythmic or anticholinergic properties, the longitudinal analyses were repeated after exclusion of participants who used those medications. Finally, to check whether the relation between HRV and cognitive domains is independent of heart rate, the cross-sectional and longitudinal analyses were repeated after standardizing HRV for heart rate (SDNN was divided by heart rate)[15]. A *p* value of < 0.05 was considered as statistically significant.

Results

The mean age of the study population was 75.0 years and 1675 (46.7%) participants were male. Median HRV as measured by SDNN was 17.00 milliseconds. Table 1 shows the baseline characteristics of participants in thirds of HRV. Participants in the lowest third of HRV were older, had higher resting heart rate, higher body mass index and used beta-blockers less frequently (all *p* values < 0.05).

Table 2 shows the cross-sectional association of HRV with cognitive domains in the minimally adjusted model. At baseline, participants with lower HRV had worse performance on the Stroop test (mean score of 64.71 seconds in the lowest third, 64.46 seconds in the middle third, and 62.75 seconds in the highest third, *p* = 0.008) and the Letter-Digit Coding test (mean score of 23.62 digits coded in the lowest third, 23.67 digits coded in the middle third, and 24.18 digits coded in the highest third, *p* = 0.008). Lower HRV was not associated with worse performance in the immediate and delayed Picture-Word Learning tests. Figure 1_shows the cross-sectional association of HRV with cognitive domains after full adjustment for medications, cardiovascular risk factors, and comorbidities. Full adjustments did not change the cross-sectional results, meaning that lower HRV remained associated with worse performance in the Stroop and Letter-Digit Coding tests.

Table 1. Baseline characteristics of participants in thirds of heart rate variability

	Thirds of SDNN, ms			
	Low n = 1197	Middle n = 1193	High n = 1193	*p* Value
Socio-demographics				
Age, y , mean (SD)	75.24 (3.38)	74.98 (3.25)	74.92 (3.28)	0.047
Male, n (%)	553 (46.2)	563 (47.2)	559 (46.9)	0.885
Age left school, y, mean (SD)	15.23 (2.09)	15.24 (2.14)	15.15 (2.11)	0.507
Cardiovascular risk factors				
HR, beats/min, mean (SD)	70.01 (11.71)	64.80 (10.40)	61.71 (9.80)	<0.001
History of stroke or TIA, n (%)	136 (11.4)	131 (11.0)	106 (8.9)	0.103
History of MI, n (%)	146 (12.2)	140 (11.7)	153 (12.8)	0.718
History of DM, n (%)	143 (11.9)	127 (10.6)	108 (9.1)	0.070
SBP, mm Hg, mean (SD)	155.87 (21.4)	154.52 (22.8)	153.17 (21.3)	0.010
DBP, mm Hg, mean (SD)	84.6 (11.0)	84.0 (11.6)	82.6 (10.8)	<0.001
BMI, kg/m ^{2} ,mean (SD)	27.21 (4.2)	26.83 (4.1)	26.74 (4.0)	0.015
Current smoking, n (%)	286 (23.9)	325 (27.2)	323 (27.1)	0.110
Antihypertensive medications				
ß-blockers, n (%)	283 (23.6)	337 (28.2)	366 (30.7)	<0.001
Calcium channel blocker, n (%)	303 (25.3)	303 (25.4)	301 (25.2)	0.996

Abbreviations: BMI: body mass index; DBP: diastolic blood pressure; DM: diabetes mellitus; HR: heart rate; MI: myocardial infarction; SBP: systolic blood pressure; SDNN: standard deviation of normal-to-normal R-R intervals. The differences in characteristics across thirds of SDNN were examined using analysis of variance test for continuous variables and x^2 test for categorical variables.

Table 3 shows the association of baseline HRV and changes in cognitive domains during a mean follow-up of 3.2 years. In the minimally adjusted model, participants with lower HRV had a steeper decline in the Stroop test performance (mean annual change of 1.63 seconds in the lowest third, 0.96 seconds in the middle third and 1.11 seconds in the highest third), although this association was marginal ($p = 0.073$). Similarly, participants with lower HRV had a steeper decline in the Letter-Digit Coding test score (mean annual change of −0.50 digits coded in the lowest third, −0.49 digits coded in the middle third and −0.35 digits coded in the highest third, $p = 0.016$). In contrast, low HRV was not associated with accelerated decline in the immediate and delayed Picture-Word learning test scores during follow-up. After full adjustment for cardiovascular risk factors, co-morbidities and use of medications, the estimates of the difference in cognitive domains between the HRV groups did not change essentially. Lower HRV remained associated with a steeper decline in the Letter-Digit coding test score (*p* = 0.038), whereas for the Stroop test and the immediate and delayed Picture-Word learning tests, the associations did not reach statistical significance (*p* value = 0.084, 0.337 and 0.738, respectively).

Table 2. Baseline cognitive domains in relation to heart rate variability

	Thirds of SDNN, ms			
	Low (1.70-12.60) n = 1197	Middle (12.70-22.90) n = 1193	High (23.00- 128.40) n = 1193	*p* *V*alue[a]
Stroop, s	64.71 (0.63)	64.46 (0.63)	62.75 (0.63)	0.008
LDCT, digits coded	23.62 (0.19)	23.67 (0.19)	24.18 (0.19)	0.008
PLTi, pictures remembered	9.44 (0.05)	9.38 (0.05)	9.47 (0.05)	0.353
PLTd, pictures remembered	10.28 (0.07)	10.26 (0.07)	10.41 (0.07)	0.130

Abbreviations: LDCT: Letter-Digit Coding Test; PLTd: Picture-Word Learning Test delayed; PLTi: Picture-Word Learning Test immediate; SDNN: standard deviation of normal-to-normal R-R intervals. Data represent mean score (standard error) of each cognitive test. Adjusted for country, age, sex, education, and version of LDCT and PLT tests. [a] The *p* values were calculated using the continuous values of log-transformed SDNN.

In additional analyses, we combined the lowest and the middle thirds of HRV and repeated the cross-sectional and longitudinal analyses. Results show that compared to the high HRV group, the combined middle and low HRV group was associated with worse performance in the Stroop and the Letter-Digit Coding test (table e-2). The longitudinal results indicate that the combined middle and low HRV thirds is associated with a steeper decline on the Letter-Digit Coding test and the immediate Picture-Word Learning test (table e-3).

Table 3. Annual changes of cognitive domains in relation to heart rate variability

	Thirds of SDNN, ms			
	Low n = 1197	Middle n = 1193	High n = 1193	*p Value*[a]
Stroop, s				
Minimally adjusted model	1.63 (0.30)	0.96 (0.30)	1.11 (0.30)	0.073
Fully adjusted model	1.62 (0.30)	0.94 (0.30)	1.13 (0.30)	0.084
LDCT, digits coded				
Minimally adjusted model	−0.50 (0.04)	−0.49 (0.04)	−0.35 (0.04)	0.016
Fully adjusted model	−0.50 (0.04)	−0.49 (0.04)	−0.35 (0.04)	0.038
PLTi, pictures remembered				
Minimally adjusted model	−0.06 (0.02)	−0.05 (0.02)	−0.01 (0.02)	0.257
Fully adjusted model	−0.06 (0.02)	−0.05 (0.02)	−0.01 (0.02)	0.337
PLTd, pictures remembered				
Minimally adjusted model	−0.11 (0.03)	−0.10 (0.03)	−0.09 (0.03)	0.698
Fully adjusted model	−0.11 (0.03)	−0.10 (0.03)	−0.10 (0.03)	0.738

Abbreviations: LDCT: Letter-Digit Coding Test; PLTd: Picture-Word Learning Test delayed; PLTi: Picture-Word Learning Test immediate; SDNN: standard deviation of normal-to-normal R-R intervals. Data represent mean annual change (standard error) in each cognitive test. Minimally adjusted model: adjusted for country, age, sex, education, cognitive scores at baseline, and version of LDCT and PLT tests. Fully adjusted model: adjusted for country, age, sex, education, baseline cognitive scores, version of LDCT and PLT tests, body mass index, smoking, systolic blood pressure, diastolic blood pressure, history of stroke/TIA, history of myocardial infarction, history of diabetes mellitus, statin treatment, and antihypertensive medications (diuretics, ß-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers).[a]The p values were calculated using the continuous values of log-transformed SDNN.

Figure 2 shows the association of HRV with cognitive decline, stratified by cardiovascular events during follow-up including stroke or TIA (n=199), heart failure hospitalization (n=78) and coronary events (n=269). There was no difference in change of cognitive domains during follow-up between participants who did and did not develop cardiovascular events (all *p* for interaction >0.05). Likewise, stratification of participants based on the presence of history of stroke or TIA at baseline did not change the longitudinal results (data not shown).

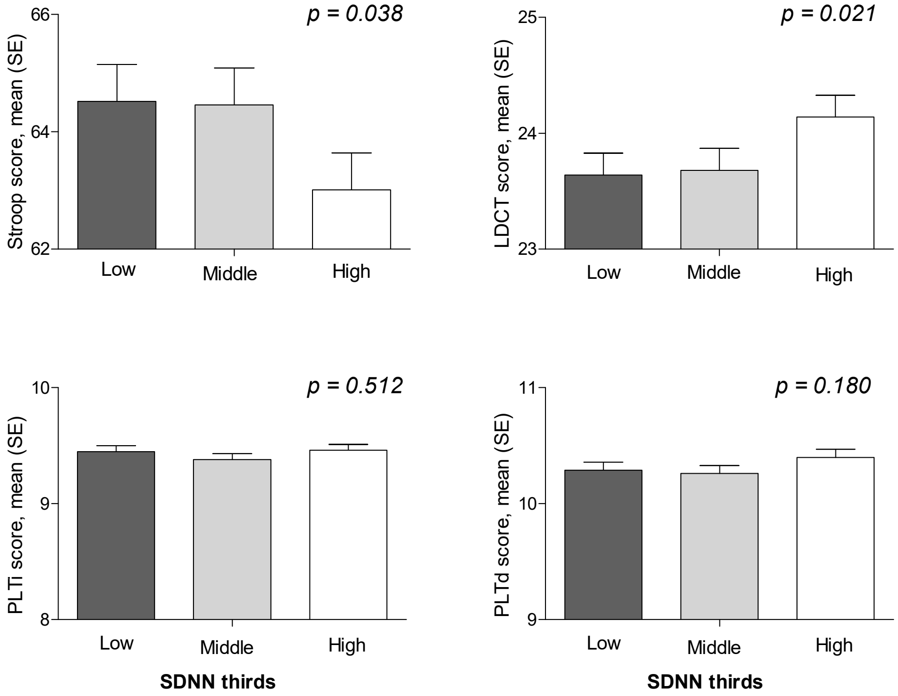

Figure 1. Baseline cognitive domains in relation to heart rate variability in the fully adjusted model. All analyses were performed in the fully adjusted model. PLTd: Picture-Word Learning Test delayed; PLTi: Picture-Word Learning Test immediate; SDNN: standard deviation of normal-to-normal R-R intervals.

Furthermore, the sensitivity analyses after exclusion of participants who used β-blockers (n = 986) and medications with antiarrhythmic (n = 75) or anticholinergic (n = 98) properties did not change the associations between HRV and cognitive decline (table e-4). After exclusion of participants who used β-blockers (n = 986, 27.5% of the population), the association between HRV and Letter-Digit Coding test scores remained essentially the same, with marginal *p* values (table e-4). Finally, standardization of SDNN for heart rate did not change the cross-sectional and longitudinal results (table e-5 and 6).

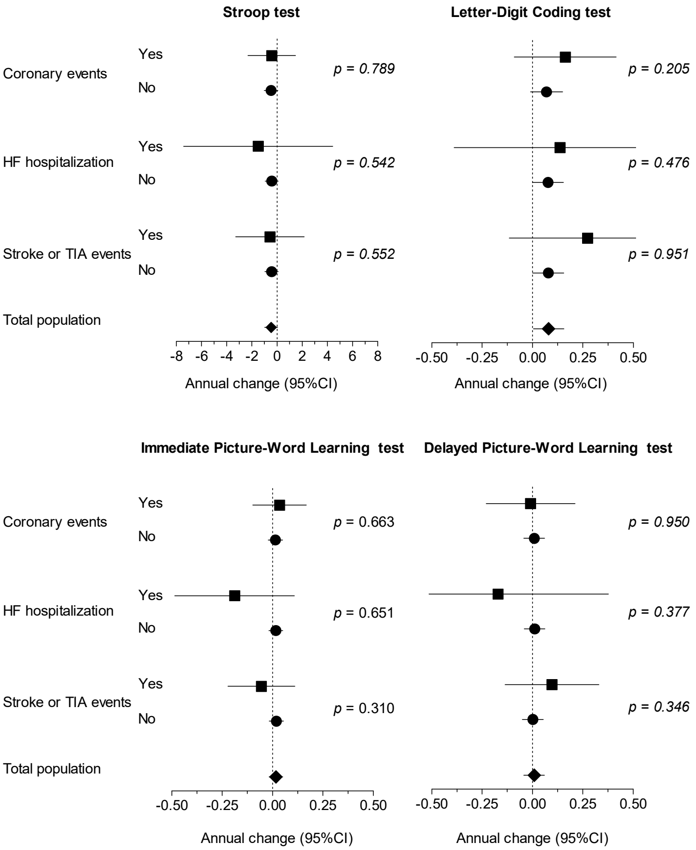

Figure 2. Annual changes of cognitive domains in relation to heart rate variability, stratified for cardiovascular events during follow-up. Data represent annual change (95% CI) per 1 millisecond increase in log-transformed SDNN for each cognitive test, stratified by cardiovascular events during follow-up. Adjusted for country, age, sex, education, version of cognitive tests, BMI, smoking, systolic blood pressure, diastolic blood pressure, history of stroke/TIA, history of myocardial infarction, history of diabetes mellitus, statin treatment, and antihypertensive medications. The p values show p for interaction. HF: heart failure.

Discussion

In this study, we show that older participants at risk of cardiovascular disease with lower 10-second HRV have worse performance in reaction time and processing speed and experience steeper decline in their processing speed during a mean period of 3.2 years. These associations were independent of cardiovascular risk factors and morbidities.

Our findings are in line with some studies on the association between HRV and cognitive function. For example, cross-sectional results from 869 Mexican Americans with a mean age of 75 years have shown that reduced 5-minute HRV was associated with worse performance on the MMSE test, but not with verbal memory[16]. Results from the Vietnam Era Twin Registry on healthy middle aged men showed that reduced 24-hour HRV was associated with poor verbal, but not visual and memory performance[17]. The cross-sectional results from the Irish longitudinal study on ageing (TILDA) showed that reduced 5-minure HRV was most strongly associated with worse performance in memory recall and language[18]. To date, the only prospective study on the longitudinal association between HRV and cognitive function is the UK Whitehall II study, which showed no cross-sectional and longitudinal associations[19]. However, in that study the cognitive battery used was not able to assess the executive function in details. Furthermore, their population consisted of middle-aged adults who were much younger than the PROSPER participants.

There are several explanations that might describe the observed associations between HRV and cognitive function. First, cardiovascular risk factors such as hypertension, subclinical inflammation and diabetes mellitus have been linked to both reduced HRV and cognitive impairment[4, 20, 21]. This might suggest that cardiovascular risk factors play role as extraneous factors on the association between HRV and cognitive function. Nevertheless, we observed that adjustment of our analyses for several well-established cardiovascular risk factors did not change the associations. Second, reduced HRV is associated with future cardiovascular events[3, 22], which in turn might result in neurocognitive deficits and cognitive decline. We observed that the association between HRV and cognitive function was not different in participants without cardiovascular events during follow-up time. In this setting, reduced HRV might serve as an early manifestation of brain damage mirrored in disturbed autonomic nervous system. Third, low HRV as a reflection of autonomic dysfunction might directly link to cognitive impairment by causing dysregulations in cerebral perfusion[23].

Furthermore, it is possible that lower HRV might reflect established cerebral lesions and neurodegenerative processes in the brain[18]. Finally, given that low HRV have been associated with higher blood pressure variability[24] and that higher blood pressure variability has been shown to be associated with cognitive decline and structural brain changes[25, 26], it is likely that altered HRV is associated with cognitive decline by increasing blood pressure variability.

In this study, we show that reduced HRV is related to worse performance and future decline of executive function. Executive function is mainly controlled by the prefrontal cortex of the brain. It has been shown that reduced HRV is associated with hypo-activity of the prefrontal cortex, which might in turn disturb executive function[27, 28]. In a meta-analyses, Thayer and colleagues have shown that HRV is closely related to neuronal activities in the ventromedial prefrontal cortex[29]. Furthermore, it has been shown that the frontal cortex is able to adjust HRV via subcortical structures such as the amygdala. This cortico-subcortical inhibitory circuit is the structural connection between neuropsychological processes such as cognitive function and physiological processes such as HRV. Abnormalities in the cortico-subcortical circuit can be reflected in HRV[28]. In this setting, future brain imaging studies might bring new insights in the biology of observed associations.

The selective association of lower HRV with cognitive domains involving speed needs further exploration. Previously, it has been shown that the detrimental effects of cardiovascular risk factors are more evident in such cognitive domains [30], however it is also possible that HRV is basically related to the pace of performing a certain task and not necessarily to the cognitive ability of the participants. In addition, we observed that the largest changes in cognitive scores were between the “high” HRV group and the remaining two-third of the population. It is important to mention that there is no well-established clinical cut-off value for categorization of HRV indices which might hamper grouping of participants and therefore the comparisons should be performed cautiously.

This study has certain strength and limitations. The major strengths are the large sample size and the prospective design which allowed us to examine the temporality of associations. Furthermore, we used an extensive set of neuropsychological tests consisting of four cognitive tests to assess different domains of cognitive function. We could also show that the results are independent of cardiovascular risk factors and co-morbidities. As limitations, the participants in this study were at high risk of cardiovascular disease which makes it difficult to generalize our findings to a healthy elderly population. Nevertheless, a considerable proportion of older adults have a number of cardiovascular pathologies and our results were independent of cardiovascular risk factors, co-morbidities and use of medications. Using a 10-second HRV might serve as a possible limitation as it does not allow capturing the circadian changes. However, we were able to show that reduced HRV associates with cognitive impairment even by using 10-second HRV which is widely used in clinical practice and is more feasible for assessment. Another limitation could be the relatively small changes in the absolute scores of cognitive domains. This might be due to the PROSPER inclusion criteria (MMSE ≥ 24 points) resulting in participants with a relatively preserved cognitive function at baseline. Of note, although the magnitude of associations were modest, the effect estimates were comparable with the effect estimates of apolipoprotein E4 in the same population[31].

In our cohort of older participants at high risk of cardiovascular disease, participants with lower 10-second HRV have worse performance in reaction time and processing speed and experience steeper decline in their processing speed, independent of cardiovascular risk factors and co-morbidities.

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Appendices (e-tables) are available online:

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CHAPTER 4

LEFT VENTRICULAR HYPERTROPHY AND COGNITIVE IMPAIRMENT

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*Equal contribution

Abstract

Background: Patients with advanced heart failure run a greater risk of dementia. Whether early cardiac structural changes also associate with cognitive decline is yet to be determined. **Objective:** We tested whether left ventricular hypertrophy (LVH) derived from electrocardiogram associates with cognitive decline in older subjects at risk of cardiovascular disease. **Methods:** We included 4233 participants (mean age 75.2 years, 47.8% male) from PROSPER (PROspective Study of Pravastatin in the Elderly at Risk). LVH was assessed from baseline electrocardiograms by measuring the Sokolow-Lyon index. Higher levels of Sokolow-Lyon index indicate higher degrees of LVH. Cognitive domains involving selective attention, processing speed, immediate and delayed memory were measured at baseline and repeated during a mean follow-up of 3.2 years. **Results:** At baseline, LVH was not associated with worse cognitive function. During follow-up, participants with higher levels of LVH had a steeper decline in cognitive function including in selective attention ($p = 0.009$), processing speed ($p = 0.010$), immediate memory ($p < 0.001$) and delayed memory ($p = 0.002$). These associations were independent of cardiovascular risk factors, co-morbidities and medications. **Conclusion:** LVH assessed by electrocardiogram associates with steeper decline in cognitive function of older subjects independent of cardiovascular risk factors and co-morbidities. This study provides further evidence on the link between subclinical cardiac structural changes and cognitive decline in older subjects.

Introduction

Patients with advanced heart failure have an increased risk of dementia and Alzheimer’s disease[1, 2]. Recent research suggests that subclinical cardiac dysfunction increases the risk of dementia and stroke in older subjects free of cardiac disease[3]. In line with this, a graded decrease in cardiac function has been also associated with structural and functional alterations in the brain of older subjects[4]. Together, these findings might suggest that not only overt cardiac dysfunction, but also suboptimal cardiac function contributes to development of cognitive impairment.

Left ventricular hypertrophy (LVH) is one of the early structural changes in the heart that occur in response to cardiovascular risk factors, particularly hypertension[5]. The anatomical remodeling of the heart during LVH results in diastolic dysfunction which might hamper cardiac output[6]. Since the heart is the driving force for cerebral perfusion, a chronic decrease in cardiac output might jeopardize the integrity of the brain[4]. In line with this premise, LVH has been associated with major cerebrovascular events such as stroke[7], as well as with asymptomatic brain lesions such as lacunar infarcts[8]. Hence, as LVH might be a potential marker of suboptimal cardiac function, we hypothesize that older patients with LVH are at increased risk of developing cognitive impairment.

In this study, we sought to investigate the cross-sectional and longitudinal association of LVH with various domains of cognitive function in older subjects at risk of cardiovascular disease. Furthermore, we tested whether the associations are independent of cardiovascular risk factors, cardiovascular co-morbidities and use of medications.

Methods

Study design

The data for this study were drawn from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). PROSPER was a randomized controlled trial designed to assess the effect of pravastatin in prevention of vascular events in the elderly with pre-existing cardiovascular diseases or risk factors. A total of 5804 men and women aged 70 to 82 years were included from three collaborating centers in Ireland, Scotland and the Netherlands. About 50% of the participants had a history of vascular disease defined as stable angina, intermittent claudication, stroke, transient ischemic attack, myocardial infarction and vascular surgery. The rest of the participants had at least one major cardiovascular risk factor defined as smoking, hypertension and diabetes mellitus. The mean follow-up time was 3.2 years. A detailed description of PROSPER study design, inclusion and exclusion criteria has been published previously[9]. In brief, exclusion criteria included physical or mental inability to attend the clinic for study visits, poor cognitive function at baseline, congestive heart failure (New York Heart Association functional class III or IV), previous atrial fibrillation, significant arrhythmias and implanted cardiac pacemakers. This study was approved by the institutional ethics committees of the three collaborating centers and all participants gave written informed consent.

Study participants

From the original PROSPER cohort, we excluded 148 participants with missing ECG measurements at baseline, 475 participants with ECG data indicative of cardiac arrhythmias and/or rhythms not generated by the sino-atrial node (including atrial fibrillation, atrial flutter, ectopic atrial rhythms, supraventricular arrhythmias, junctional rhythms, intermittent conduction defect and other arrhythmias); and 948 participants with missing cognitive measurements at baseline or during follow-up. Participants with at least the baseline and one follow-up cognitive measurement were included. Therefore, we included a total of 4233 participants in the present study. Compared to the excluded participants, those included were younger and had lower prevalence of cardiovascular risk factors and co-morbidities (Supplementary Table 1a, b).

ECG measurements

On the morning of the first enrolment visit, standard 12-lead ECGs were recorded in the resting supine position using a Burdick Eclipse 850i electrocardiograph before initiation of statin treatment. These digital data were transferred to the University of Glasgow ECG Core Lab based at Glasgow Royal Infirmary in Scotland and interpreted using the University of Glasgow (Uni-G) ECG analysis program. We used a fully automated method to ensure reproducibility of the measurements[10]. In addition, all the automated ECG recordings were reviewed by two experts (P.W.M and E.N.C) and any incorrect recordings were replaced by the correct interpretation[11]. The software provided numerous measurements including QRS durations, R-wave amplitudes in leads V5 and V6 and S-wave amplitude in lead V1, from which we determined the Sokolow-Lyon voltage index for LVH (sum of S wave amplitude in lead V1 and R wave amplitude in lead V5 or V6, whichever is the higher)[12]. Previous studies have shown that the product of QRS duration and voltage index significantly improves the accuracy of LVH detection from the ECG[13, 14]. Therefore, we computed the product of Sokolow-Lyon voltage by multiplying it with QRS duration: Sokolow-Lyon product=Sokolow-Lyon voltage×QRS duration. Higher Sokolow-Lyon product values indicate higher degrees of LVH.

Cognitive function measurements

In PROSPER, the global cognitive function was assessed using the mini-mental state examination test (MMSE) at baseline and participants with a MMSE score of < 24 were excluded. In this study, we used four tests to assess different domains of cognitive function. The Stroop interference test was used to test selective attention. The participants were asked to read the name of a color which was typed with ink of color different from the color being named. The outcome was the total number of seconds to complete the test; a higher score indicates worse performance. The Letter-Digit Coding Test was used to assess general cognitive speed. The subjects had to match digits with letters according to a key provided. The outcome parameter was the total number of correct digits entered in 60 seconds; higher scores indicate better performance. Immediate and delayed memory were tested by the Picture-Word Learning Test. 15 pictures were shown to the participants and they were asked to recall as many pictures as possible in three trials. To measure their delayed recall, participants were asked to repeat the test after 20 minutes. The outcome variable was the accumulated number of correctly recalled pictures; and a higher score indicates better performance. The test/re-test correlations of the four cognitive tests were shown to be high and acceptable, indicating the reliability of cognitive tests (r = 0.80, 0.88, 0.66 and 0.63 for Stroop interference test, Letter-Digit Coding test, immediate and delayed Picture-Word Learning tests, respectively). To avoid learning effect, we used different versions of cognitive tests at each visit[15]. Cognitive function was measured at five time points: at baseline, after 9, 18 and 30 months and at the end of the study, which varied among participants ranging from 36 to 48 months. The baseline cognitive function was assessed before initiation of PROSPER clinical trial. A more detailed description of the cognitive tests has been published elsewhere[15].

Statistical analysis

We used linear regression models to assess the cross-sectional association of LVH with cognitive function. The dependent variable was the score of each cognitive test at baseline. Linear mixed models were used to test the longitudinal association between LVH and cognitive decline over time. The models incorporated Sokolow-Lyon product levels, time (in years) and the interaction between time and Sokolow-Lyon product. Subjects were defined as random factors and all other variables were defined as fixed factors. Probability values were calculated using the interaction term between time and Sokolow-Lyon product. The Stroop interference test scores were log-transformed because they were not normally distributed. All analyses were performed in two steps. In the first step (Model 1), analyses were adjusted for age, sex, education (age at which the participant left school), country of enrolment and version of cognitive tests where applicable. In the second step (Model 2), analyses were further adjusted for cardiovascular risk factors and co-morbidities (history of diabetes, history of vascular disease, body mass index, smoking status, systolic blood pressure, diastolic blood pressure, total cholesterol and HDL-cholesterol levels), antihypertensive medications (diuretics, beta-blockers, calcium channel blockers, angiotensin converting enzyme inhibitors [ACEI] and angiotensin receptors blockers [ARB]) and anticoagulant medications. In the longitudinal analyses, adjustments for statin treatment groups were added. In an additional analysis, we categorized the participants in two groups based on the clinical cut-off value of Sokolow-Lyon voltage, since there is no well-established clinical cut-off value for Sokolow-Lyon product[16]. Participants were categorized as follow: 1) participants with Sokolow-Lyon voltage < 3500 µV; 2) participants with Sokolow-Lyon voltage ≥ 3500 µV. We then repeated the cross-sectional and longitudinal analyses in these two groups. Probability values were calculated using the interaction term between time and continuous values of Sokolow-Lyon voltage.

Furthermore, to explore the effect of cardiovascular events on longitudinal associations, we stratified for participants who did and did not develop coronary events, stroke or transient ischemic attack events, incident atrial fibrillation and heart failure hospitalization during follow-up. The interaction terms were tested in linear mixed models for composite executive function and memory function scores. The composite executive function was calculated by averaging the Z scores of the Stroop interference test and Letter-Digit Coding tests. The composite memory function was calculated by averaging the Z scores of the immediate and delayed Picture-Word Learning tests. To test the effect of ACEI and ARB medications, participants were stratified in two groups of ACEIs users and ARBs users and tested for interaction. Similarly, to test the effect of sex on longitudinal associations participants were stratified in two groups of males and females. Finally, to test the effect of cardiovascular risk factors and co-morbidities on longitudinal associations, a series of additional analyses were performed in which we excluded participants with a history of hypertension, history of diabetes mellitus, history of myocardial infarction, history of stroke or transient ischemic attack, high cholesterol levels (>6.21 mmol/L) and current smokers and repeated the longitudinal analyses.

Results

The mean age of the participants was 75.2 years and a total of 2025 (47.8%) participants were male. The mean Sokolow-Lyon product and Sokolow-Lyon voltage were 224.3 µVs and 2285.1 µV, respectively (Table 1). Participants with higher Sokolow-Lyon product were older, more likely to be male, had lower body mass index, had lower prevalence of diabetes mellitus, had higher systolic and diastolic blood pressure, and used calcium channel blockers and beta-blockers more frequently (all p values < 0.05) (Supplementary Table 2).

Table 2 shows the cross-sectional association of Sokolow-Lyon product and cognitive function at baseline. In Model 1, higher Sokolow-Lyon product was not associated with worse performance of participants in the Stroop interference test, Letter-Digit Coding test and delayed Picture-Word Learning tests. Participants with higher Sokolow-Lyon product had a non-significant trend of better performance in the immediate Picture-Word Learning test. Consistently, full adjustment for cardiovascular risk factors, co-morbidities and use of medications in Model 2 did not change the observed associations (estimates and 95% confidence intervals are presented in Supplementary Table 3).

Table 3 shows the longitudinal association of Sokolow-Lyon product and cognitive function during follow-up. In Model 1, participants with higher Sokolow-Lyon product had a steeper decline in Stroop interference test performance ($p = 0.009$), Letter-Digit Coding Test score ($p = 0.010$), immediate Picture-Word Learning Test score ($p < 0.001$) and delayed Picture-Word Learning Test score ($p = 0.002$). These results were similar after adjustment for cardiovascular risk factors, co-morbidities and use of medication (Model 2) (estimates and 95% confidence intervals are presented in Supplementary Table 3). Exclusion of outliers (>3 SD, < 2% of the data) did not change the results.

Table 1. Baseline characteristics of participants

Characteristics	Value
Socio-demographics	
Age, y, mean (SD)	75.2 (3.3)
Male, n (%)	2025 (47.8)
Age left school, y, mean (SD)	15.2 (2.1)
Vascular risk factors	
History of vascular disease, n (%)	1850 (43.7)
History of MI, n (%)	546 (12.9)
History of stroke or TIA, n (%)	438 (10.3)
History of diabetes mellitus, n (%)	435 (10.3)
SBP, mmHg, mean (SD)	154.6 (21.9)
DBP, mmHg, mean (SD)	83.7 (11.3)
BMI, kg/m^2, mean (SD)	26.9 (4.1)
Current smoking, n (%)	1099 (26.0)
Cognitive function	
MMSE, score, median (IQR)	28.0 (27.0-29.0)
Stroop interference, seconds, median (IQR)	58.2 (48.0-73.5)
LDCT, mean (SD)	23.7 (7.8)
PLTi, mean (SD)	9.4 (1.8)
PLTd, mean (SD)	10.3 (2.6)
Medications	
Diuretics, n (%)	1689 (39.9)
Beta-blockers, n (%)	1130 (26.7)
Calcium channel blockers, n (%)	1070 (25.3)
ACE inhibitors, n (%)	675 (15.9)
ARBs, n (%)	90 (2.1)
Anti-diabetic medication, n (%)	281 (6.6)
ECG indices for LVH	
Sokolow-Lyon voltage, µV, mean (SD)	2285.1 (799.4)
Sokolow-Lyon product, µVs, mean (SD)	224.3 (90.5)

Abbreviations: MI: myocardial infarction; TIA: transient ischemic attack; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; MMSE: Mini-Mental State Examination test; LDCT: Letter Digit Coding Test; PLTi: Picture-Word Learning Test immediate; PLTd: Picture-Word Learning Test delayed; ACE: angiotensin converting enzyme inhibitors; ARBs: angiotensin receptor blockers.

At baseline, participants in the high Sokolow-Lyon voltage group did not have worse performance in any of the cognitive tests (Supplementary Table 4). Figure 1 shows the longitudinal association of Sokolow-Lyon voltage categories and cognitive function after full adjustment for measured cardiovascular risk factors, co-morbidities and use of medications. Participants in the high Sokolow-Lyon voltage group had a steeper decline in Letter-Digit Coding test score ($p = 0.007$), immediate Picture-Word Learning test score ($p < 0.001$), and delayed Picture-Word Learning test score ($p = 0.024$). The relation between high Sokolow-Lyon voltage and decline in Stroop interference test performance was marginally significant ($p = 0.072$). All these associations were similar in Model 1 (data not shown).

Table 2. The association between LVH and cognitive function at baseline

	Sokolow-Lyon product (µVs)			
	Low 17.11 – 177.16 n = 1411	Medium 177.20 – 245.55 n = 1411	High 245.70 – 902.53 n = 1411	*p* Value*
Stroop interference, seconds, mean (SE)				
Model 1	63.60 (0.61)	63.79 (0.60)	63.80 (0.61)	0.978
Model 2	65.69 (1.98)	66.17 (1.96)	66.35 (1.96)	0.712
LDCT, digits coded, mean (SE)				
Model 1	24.07 (0.18)	24.26 (0.18)	23.74 (0.18)	0.351
Model 2	23.25 (0.57)	23.33 (0.57)	22.72 (0.57)	0.119
PLTi, pictures remembered, mean (SE)				
Model 1	9.41 (0.05)	9.53 (0.05)	9.56 (0.05)	0.090
Model 2	9.29 (0.15)	9.41 (0.15)	9.46 (0.15)	0.065
PLTd, pictures remembered, mean (SE)				
Model 1	10.35 (0.07)	10.34 (0.07)	10.45 (0.07)	0.240
Model 2	10.14 (0.22)	10.12 (0.21)	10.23 (0.21)	0.238

**p* values were calculated using continuous values of Sokolow-Lyon product. Model 1: adjusted for age, sex, country, education and version of cognitive tests where applicable. Model 2: adjusted for age, sex, country, education, version of cognitive tests where applicable, body mass index, smoking status, systolic blood pressure, diastolic blood pressure, history of diabetes, history of vascular disease, total cholesterol level, HDL-cholesterol level, antihypertensive medications (diuretics, beta-blockers, calcium channel blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blockers) and anticoagulant medications. SE: standard error; LDCT: letter digit coding test; PLTi: picture-word learning test immediate; PLTd: picture-word learning test delayed.

Figure 2 shows the longitudinal association of Sokolow-Lyon product and cognitive function, stratified by incident cardiovascular events. During follow-up, 432 participants developed coronary events, 281 participants developed stroke or transient ischemic attack events, 357 participants developed incident atrial fibrillation and 151 participants were hospitalized for heart failure. There was no significant difference in annual changes of cognitive function between participants who did and those who did not develop cardiovascular events, except that participants with atrial fibrillation had a less prominent decline in executive function (marginal p for interaction = 0.055). There was no significant difference in annual changes of cognitive function between participants taking ACEI medications vs participants taking ARB medications (Supplementary Table 5). Similarly, we did not observe sex dependent differences in annual changes of cognitive function during follow-up (data not shown).

Table 3. The association between LVH and cognitive function during follow-up

	Sokolow-Lyon product (µVs)			
	Low 17.11 – 177.16 n = 1411	Medium 177.20 – 245.55 n = 1411	High 245.70 – 902.53 n = 1411	*p* Value*
*Stroop interference, seconds, mean** (SE)*				
Model 1	0.523 (0.13)	0.507 (0.13)	0.841 (0.13)	0.009
Model 2	0.525 (0.13)	0.510 (0.13)	0.845 (0.13)	0.009
LDCT, digits coded, mean (SE)				
Model 1	-0.329 (0.03)	-0.389 (0.03)	-0.437 (0.03)	0.010
Model 2	-0.329 (0.03)	-0.390 (0.03)	-0.438 (0.03)	0.010
PLTi, pictures remembered, mean (SE)				
Model 1	0.016 (0.01)	-0.009 (0.01)	-0.043 (0.01)	<0.001
Model 2	0.015 (0.01)	-0.010 (0.01)	-0.043 (0.01)	<0.001
PLTd, pictures remembered, mean (SE)				
Model 1	-0.054 (0.02)	-0.060 (0.02)	-0.094 (0.02)	0.002
Model 2	-0.054 (0.02)	-0.060 (0.02)	-0.095 (0.02)	0.002

**p* values were calculated using the interaction term between continuous levels of Sokolow-Lyon product and time. ** represents mean annual decline in cognitive scores during follow-up. Model 1: adjusted for age, sex, country, education and version of cognitive test where applicable. Model 2: Model 1 + body mass index, smoking, systolic blood pressure, diastolic blood pressure, history of diabetes, history of vascular disease, antihypertensive medications (diuretics, beta-blockers, calcium channel blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blockers), statin treatment groups, HDL, total cholesterol and anticoagulants. SE: standard error; LDCT: letter digit coding test; PLTi: picture-word learning test immediate; PLTd: picture-word learning test delayed.

After exclusion of participants with a history of hypertension (n=2634), diabetes mellitus (n=435), myocardial infarction (n=546), stroke or transient ischemic attack (n=438), high cholesterol levels (n=1174) and current smokers (n=1099) at baseline, the longitudinal associations did not materially change (Supplementary Table 6)

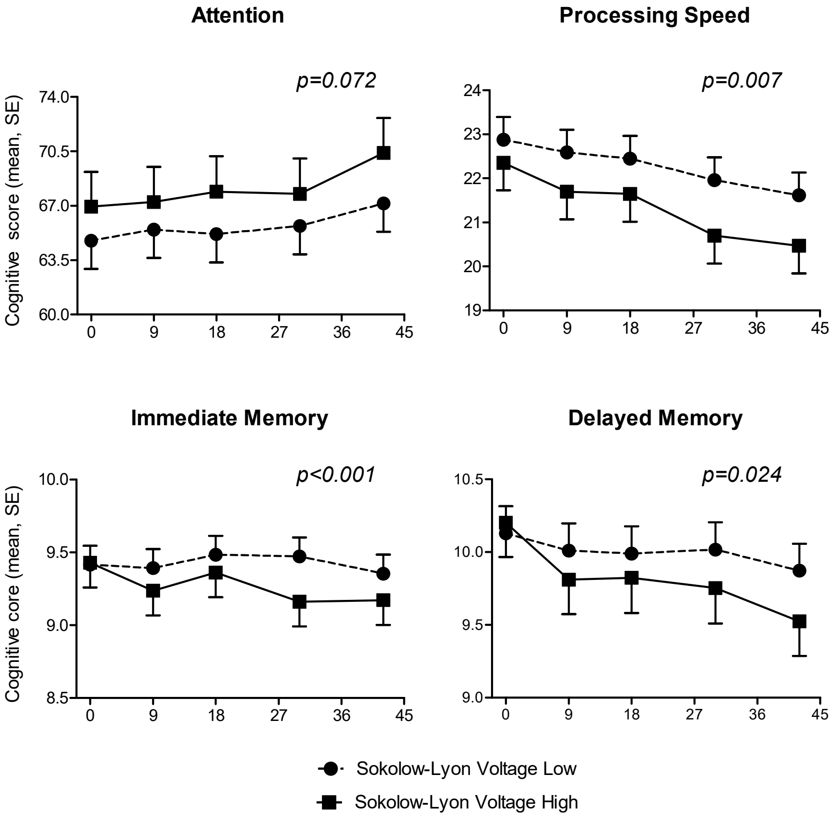

Figure 1. The association between clinical categories of left ventricular hypertrophy and annual changes of cognitive function during follow-up. The time point at the end of study is the mean of the end time points (36 to 48). Data shows fully adjusted models. Abbreviations: LDCT: letter digit coding test; PLTi: picture-word learning test immediate; PLTd: picture-word learning test delayed.

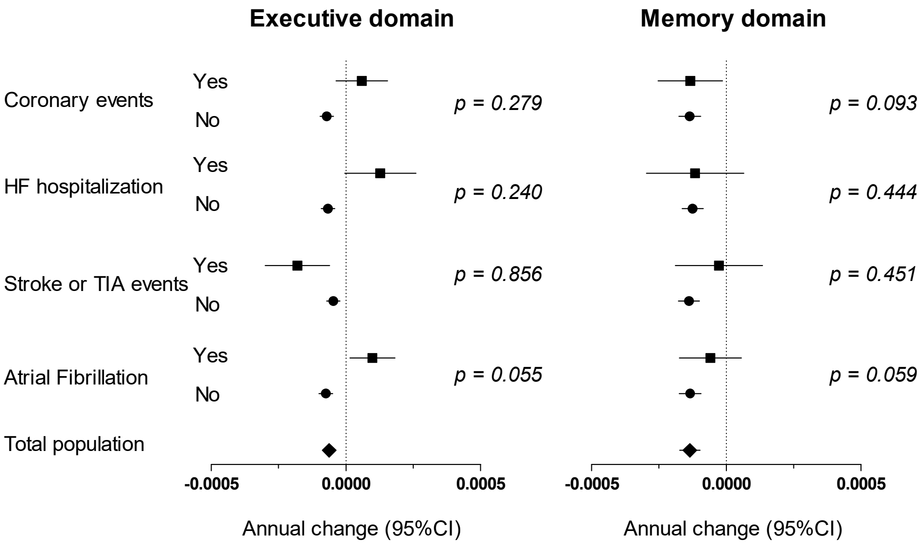

Figure 2. The association between left ventricular hypertrophy and annual changes of cognitive function, stratified for cardiovascular events during follow-up. Data represent annual change (95%CI) per each unit (µVs) increase in Sokolow-Lyon product for each cognitive test, stratified by cardiovascular events during follow-up. P-values were show p for interaction. Adjusted for age, sex, country, education, version of cognitive tests where applicable, history of diabetes, history of vascular disease, body mass index, smoking status, systolic blood pressure, diastolic blood pressure, total cholesterol level, HDL-cholesterol level, antihypertensive medications, anticoagulant medications and statin treatment groups.

Discussion

In this study we found that higher degrees of LVH as assessed by ECG associate with a steeper decline in cognitive function of older subjects independent of measured cardiovascular risk factors, cardiovascular co-morbidities and use of medications.

Previous cross-sectional studies have reported an association between echocardiographic measured LVH and cognitive function. For example, Scuteri et al. showed that increased left ventricular mass associated with worse MMSE score in 400 participants (mean age 79 ± 6 years) independent of blood pressure and large artery stiffness[17]. The

Framingham Offspring Study on 1673 participants (mean age 57 ± 9 years) showed similar results. However, this association was attenuated after adjustment for cardiovascular risk factors and morbidities[18]. Echocardiographic measured LVH was also associated with a 5-year decline in MMSE score in a small group of older adults in the Helsinki Ageing study[19]. Nevertheless, very few studies have tested the link between LVH assessed by ECG and cognitive function. Of note, ECG detected LVH has been shown to predict development of cognitive impairment in stroke free individuals in The Reasons for Geographic and Racial Differences in Stroke (REGARDS) study[20]. In that study, cognitive function was assessed using a single-item measure of global cognitive function (The Six-item Screener) while the population consisted of middle aged adults (mean age 64 years).

The observed link between LVH and cognitive function can be explained through a number of pathophysiological mechanisms. First, LVH is recognized as cardiovascular target organ damage[6] and the role of vascular risk factors in development of cognitive impairment is well-established[21]. This suggests that cardiovascular risk factors and morbidities might play role as confounders in the association between LVH and cognitive function. Nevertheless, adjustment of our analyses for several well-established cardiovascular risk factors and co-morbidities, and exclusion of participants with cardiovascular co-morbidities at baseline, did not alter the observed associations. Second, LVH has been associated with future cardiovascular events[6], which might in turn contribute to neurovascular damage and cognitive impairment. Finally, it can be hypothesized that a subclinical decrease in cardiac output predisposes subjects to chronic cerebral hypoperfusion, which might adversely affect structural and functional integrity of the brain[1]. Consistent with this hypothesis, Jefferson et al. have shown that a lower cardiac index (cardiac output divided by body surface area), even in subjects without prevalent cardiovascular disease, associates with abnormal brain aging[2]. Recently, we have shown that a graded decrease in cardiac function is linked to lower brain parenchymal and grey matter volume, as well as worse cognitive function [4].

We did not observe a cross-sectional association between LVH and cognitive function at baseline. It should be noted that the participants in this study had preserved cognitive function at baseline (due to PROSPER inclusion criteria: MMSE ≥ 24 points), which might have underestimated the relation between LVH and cognitive function. Furthermore, the presence of longitudinal association between LVH and cognitive function strengthens the temporality of the observed associations. We used the ECG to detect LVH in our patients. Although the ECG has a low sensitivity for LVH compared to echocardiography, both ECG and echocardiography measured LVH have been shown to associate with silent cerebral lesions such as punctate lesions, lacunas and cerebral artery territorial lesions[8]. This is in line with our results, suggesting that the ECG might be able to detect the presence of previously established vascular brain damage. Moreover, the specificity of ECG detected LVH compared to various diagnostic standards (e.g. echocardiography[14, 22], cardiac magnetic resonance imaging[23] and left ventricular mass at autopsy[13]), is shown to be quite high ranging from 85% to 90%[16]. As a result, the rate of false-positive detection is low and participants with higher values of ECG indices for LVH are very likely to actually have LVH.

This study has certain strengths and limitations. The major strengths are the large sample size, the prospective design, and the usage of an extensive set of neuropsychological tests to assess different domains of cognitive function. Furthermore, we show that the results are independent of several cardiovascular risk factors and co-morbidities. As limitations, the participants in this study were at high risk of cardiovascular disease which makes it difficult to generalize our findings to a healthy elderly population. Nevertheless, older adults frequently have a number of cardiovascular pathologies and our results were independent of measured cardiovascular risk factors, cardiovascular co-morbidities and use of medications. The observational nature of this study makes it difficult to infer any causality. Finally, we cannot exclude the possibility of selection bias given that some participants had missing cognitive measurements.

In conclusion, a higher degree of LVH assessed from ECG associates with a steeper decline in cognitive function independent of cardiovascular risk factors, cardiovascular co-morbidities and use of medications. Older adults with LVH may need to be recognized as a potentially high risk group for cognitive impairment. To establish a potential causal link, future research should determine whether interventions that halt or slow down the pace of LVH have favorable effects on cognitive function of older subjects at risk of cardiovascular disease.

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CHAPTER 5

SPATIAL QRS-T ANGLE AND COGNITIVE IMPAIRMENT

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Abstract

An abnormally wide spatial QRS-T angle on an ECG is a marker of heterogeneity in electrical activity of cardiac ventricles and is linked with cardiovascular events. Growing evidence suggests that cardiac dysfunction might signal future risk of cognitive impairment. In this study, we investigated whether spatial QRS-T angle associates with future cognitive decline in older subjects at high cardiovascular risk. We included 4172 men and women (mean age 75.2 ± 3.3 years) free of cardiac arrhythmias from PROSPER cohort. Spatial QRS-T angle was calculated from baseline 12-lead ECGs using a matrix transformation method. Different domains of cognitive function including reaction time, processing speed, immediate memory and delayed memory were measured at baseline and repeatedly during a mean follow-up time of 3.2 years. Using linear mixed models, we calculated annual changes of cognitive scores in sex-specific thirds of spatial QRS-T angle. Our results showed that participants with wider spatial QRS-T angle had a steeper decline in reaction time ($\beta = 0.0004$, $p = 0.055$), processing speed ($\beta = -0.0106$, $p = 0.004$), immediate memory ($\beta = -0.0049$, $p = 0.001$) and delayed memory ($\beta = -0.0055$, $p = 0.013$). All associations were independent of arrhythmias, cardiovascular risk factors, comorbidities, medication use, cardiovascular events and other ECG abnormalities including QRS duration, QTc interval, T wave abnormalities and left ventricular hypertrophy. In conclusion, abnormal cardiac electrical activity characterized by wide spatial QRS-T angle associates with accelerated cognitive decline independent of conventional cardiovascular factors. These findings suggest a link between a non-traditional ECG measure of pre-clinical cardiac pathology and future cognitive decline.

Introduction

The electrical activity of the heart is generated by waves of myocardial depolarization and repolarization leading to a harmonized cardiac muscle contraction and relaxation[1]. Alterations in the sequence of ventricular depolarization and repolarization are not only associated with cardiovascular events[2, 3] but may also precede cerebrovascular events and increase risk of stroke[4]. Apart from overt cerebrovascular accidents, ventricular depolarization inhomogeneity has shown to be closely related to a higher load of cerebral small vessel disease[5]. In the last couple of years, mounting evidence has supported a major contribution of small and large vessel disease in development and progression of cognitive impairment[6, 7]. Indeed cardiac and cerebrovascular dysfunction is a common phenomenon in patients with cognitive deficit[6]. Hence, it has been suggested that early changes in cardiac function might signal future risk of cognitive impairment and such markers can assist in identification of high-risk populations[8].

The spatial QRS-T angle is a non-invasive subclinical marker of electrical activity of cardiac ventricles[9]. It quantifies the deviation in the direction of cardiac ventricular depolarization and repolarization in a three-dimensional space[10]. A wide spatial QRS-T angle reflects the pathophysiological changes in the ionic channels affecting the repolarization profiles, or structural abnormalities affecting the sequence of depolarization[11]. A wider spatial QRS-T angle has been associated with incident coronary heart disease[2], all-cause and cardiovascular mortality[12] and ischemic stroke[13]. Since a wide spatial QRS-T angle is a marker of greater inhomogeneity of electrical activity of cardiac ventricles, we aimed to study the independent link between spatial QRS-T angle and cognitive decline in older subjects at high risk of cardiovascular disease.

Methods

Study design and participants

The study population consists of the participants from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). PROSPER was a multicenter randomized clinical trial which aimed to assess the effect of pravastatin in prevention of vascular events in older subjects with pre-existing cardiovascular risk factors or diseases[14]. In brief, a total of 5804 men and women at high cardiovascular risk (defined as being due to smoking, hypertension or diabetes mellitus) or with a history of vascular disease (defined as stable angina, intermittent claudication, stroke, transient ischemic attack, myocardial infarction [MI] and vascular surgery), aged 70 to 82 years, were enrolled from three collaborating centers in Ireland, Scotland and the Netherlands. The mean follow-up time was 3.2 years. The study was approved by the institutional ethics committees of the three collaborating centers and all participants gave written informed consent. The original PROSPER study was approved by the Medical Ethics Committees of the three collaborating centres and complied with the Declaration of Helsinki. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

From the baseline electrocardiogram (ECG) recordings, we excluded participants with missing spatial QRS-T angle measurements and those with evidence of atrial fibrillation, atrial flutter, ectopic atrial rhythms, supraventricular arrhythmias, junctional rhythms, intermittent conduction defect and other arrhythmias (n= 520). Furthermore, we included only participants with at least two cognitive measurements: the baseline and one follow-up measurement. Hence, a total of 4172 participants were included for analysis. Compared to the excluded participants, the included participants were younger and had a lower prevalence of cardiovascular risk factors and co-morbidities (Supplementary Table 1). We included participants from both treatment groups since it has been shown that treatment with pravastatin does not affect cognitive function[15].

Spatial QRS-T angle measurement

Standard 12-lead ECGs were recorded using a Burdick Eclipse 850i electrocardiograph in the resting supine position. All the ECGs were recorded in the morning of the first enrolment visit (at baseline) before initiation of statin treatment. A fully computerized automated method was used to ensure reproducibility of the measurements. The digital ECG recordings were transferred to the University of Glasgow ECG Core Lab in Scotland and interpreted using the University of Glasgow (Uni-G) ECG analysis program software[16]. The software provided an interpretation of the ECG recordings, Minnesota Codes, and numerous measurements including the QRS complex and T waves[16-18]. To calculate the spatial QRS-T angle, we first reconstructed the Frank XYZ vectocardiographic leads from the 12-lead ECG recordings using the inverse Dower method[19]. We then measured the spatial QRS-T angle as the angle between the maximum QRS vector and the maximum T vector. The maximum QRS and T vectors were defined as the point of maximum magnitude of the spatial QRS vector and the spatial T vector within the 3-dimensional QRS loop and T loop, respectively[9]. Since the cut-point for spatial QRS-T angle has varied among previous studies[9], we first classified the spatial QRS-angle based on the sex-specific tertiles in three groups of low (0°-38° for women and 0°-45° for men), middle (39°-75° for women and 46°- 84° for men) and high (76°-180° for women and 85°-180° for men). Secondly, based on a cut-point used in previous studies for older subjects[12, 20], we classified the spatial QRS-T angle levels in three groups of normal (0° to 104°), borderline (105° to 134°), and abnormal (135° to 180°). The QT interval was calculated from the onset of QRS complex to the T offset and was corrected for heart rate (QTc interval) using the Hodges formula[21]. T wave abnormalities were defined as Minnesota Codes 5-1, 5-2 and 5-3[17]. Left ventricular hypertrophy (LVH) was defined by using the Sokolow-Lyon voltage criteria[22].

Cognitive function assessment

In PROSPER, the mini-mental state examination test (MMSE) was used at baseline for screening purposes and participants with poor cognitive function (MMSE score <24) were excluded from enrolment in the study[23]. Four cognitive tests were used to assess different domains of cognitive function. The cognitive tests included the Stroop test, the letter-digit coding test (LDCT), immediate picture-word learning test (PLTi) and delayed picture-word learning test (PLTd). A detailed description of the cognitive assessments has been published elsewhere[23]. In brief, the Stroop test measures the selective attention and reaction time. The outcome variable is the total number of seconds to complete the test; a higher score indicates worse performance. The LDCT measures the processing speed and the outcome variable is the total number of correct digits entered in 60 seconds; a higher score indicates better performance. The PLTi and PLTd tests measure immediate and delayed memory, respectively. The outcome variables are the accumulated number of correctly recalled pictures for the immediate and delayed trials; a higher score indicates better performance. Cognitive function was assessed at baseline, after 9, 18, 30 months and at the end of the study which varied between 36 and 48 months. During each visit, different versions of cognitive tests were used to avoid learning effects[23].

Statistical analysis

Differences in baseline characteristics of participants in the three groups of spatial QRS-T angle were compared using the ANOVA test for continuous variables and Chi-squared test for categorical variables. Linear regression models were used to test the cross-sectional association between spatial QRS-T angle and cognitive function at baseline. The dependent variable was the score of each cognitive test at baseline. Using analysis of covariance, we calculated the adjusted mean score of cognitive tests in sex-specific thirds of spatial QRS-T angle. The Stroop test scores were log-transformed because they were not normally distributed. Linear mixed models (random regression model with random intercepts and slopes) were used to test the longitudinal association between spatial QRS-T angle and changes in cognitive scores over time. The models incorporated spatial QRS-T angle, time (in years) and the interaction term between time and spatial QRS-T angle. Subjects were defined as random factors and all other variables were defined as fixed factors. To assess the mean annual change of cognitive scores in thirds of spatial QRS-T angle, we computed the estimates of interaction between time and sex-specific thirds of spatial QRS-T angle using linear mixed models. To calculate the estimate of change in cognitive scores per 10 degree increase in spatial QRS-T angle, we divided the spatial QRS-T angle levels by ten. All probability values were calculated using continuous values of spatial QRS-T angle. Both cross-sectional and longitudinal analyses were first adjusted for age, sex, education (age at which the participant left school), country of enrolment and version of cognitive tests where appropriate (model 1). In the next step, adjustment for cardiovascular diseases, cardiovascular risk factors and medication use (diuretics, beta-blockers, calcium channel blockers, angiotensin converting enzyme inhibitors [ACEI] and angiotensin receptors blockers [ARB]) were added (model 2). In longitudinal analysis, further adjustment for statin treatment groups was added in model 2. To test the effect of other ECG parameters on the longitudinal associations, adjustments for T wave abnormalities, QTc interval, QRS duration and LVH were added in model 3.

To test the effect of cardiovascular events on the longitudinal associations, a series of sensitivity analyses were performed in which we stratified for the participants according to who did and who did not develop incident atrial fibrillation, heart failure hospitalization, coronary events, fatal or non-fatal stroke or transient ischemic attack (TIA) and non-fatal MI during follow-up. To test whether the difference between participants who did and who did not develop cardiovascular events is significant, p for interactions were calculated using linear mixed models. Finally, in a series of additional analyses, we tested the effect of baseline cardiovascular co-morbidities and use of anti-arrhythmic medications on longitudinal associations by excluding participants who had history of hypertension, history of diabetes mellitus, history of MI, history of stroke or TIA, and participants using anti-arrhythmic medications.

Results

Of the 4172 participants in this study, the mean age was 75.2 ± 3.3 years and 1998 (47.9%) participants were male. A total of 2593 (62.2%) had history of hypertension, 427 (10.2%) had history of diabetes mellitus, 541 (13.0%) had history of MI and 430 (10.3%) had history of stroke or TIA. The mean systolic blood pressure was 154.5 ± 21.8 mmHG, mean diastolic blood pressure was 83.7 ± 11.3 mmHG and mean body mass index was 26.9 ± 4.1 kg/m^2. Subjects with higher spatial QRS-T angle were slightly older, had higher prevalence of cardiovascular diseases and risk factors, were more likely to have T-wave abnormalities, had higher QTc interval and QRS duration, used ACEI more frequently and used beta-blockers less frequently (Table 1). Moreover, participants with higher spatial QRS-T angle were more likely to develop incident atrial fibrillation, heart failure hospitalization, coronary events, and non-fatal MI during follow-up period (Table 1).

Table 2 shows the cross-sectional association between spatial QRS-T and cognitive function at baseline. In model 1, higher spatial QRS-T angle was marginally associated with worse Stroop performance ($\beta = 0.002$; $p = 0.049$) and LDCT score ($\beta = -0.042$; $p = 0.064$). After full adjustments for cardiovascular risk factors, cardiovascular co-morbidities and medication use, higher spatial QRS-T angle was not associated with any of the cognitive tests scores at baseline.

Table 3 and Figure 1 show the longitudinal association between spatial QRS-T angle and cognitive decline over a period of 3.2 years. In model 1, higher spatial QRS-T angle was marginally associated with a steeper decline in the Stroop performance ($\beta = 0.0004$; $p = 0.055$). Higher spatial QRS-T angle was significantly associated with a steeper decline in the LDCT ($\beta = -0.0106$; $p = 0.004$), PLTi ($\beta = -0.0049$; $p = 0.001$) and PLTd ($\beta = -0.0055$; $p = 0.013$) scores during follow-up. After full adjustment for cardiovascular risk factors, cardiovascular co-morbidities and medication use, the mean annual change of cognitive scores in thirds of spatial QRS-T angle remained essentially the same (Table 3, model 2). Adjustment for baseline level of cognitive scores did not essentially change these results (data not shown).

Table 1. Baseline characteristics of participants in thirds of Spatial QRS-T angle

Characteristics	Spatial QRS-T angle thirds Low n=1383	 Middle n=1393	 High n=1396	*p* *V*alue
Demographics				
Age, y, mean (SD)	75.04 (3.29)	75.09 (3.30)	75.33 (3.37)	0.049
Age left school, y, mean (SD)	15.22 (2.15)	15.25 (2.11)	15.12 (2.08)	0.253
Cardiovascular diseases and risk factors				
History of vascular disease, n (%)	543 (39.3)	568 (40.8)	713 (51.1)	<0.001
History of stroke or TIA, n (%)	126 (9.1)	140 (10.1)	164 (11.7)	0.068
History of MI, n (%)	116 (8.4)	156 (11.2)	269 (19.3)	<0.001
History of DM, n (%)	123 (8.9)	134 (9.6)	170 (12.2)	0.011
History of hypertension, n (%)	841 (60.8)	883 (63.4)	869 (62.2)	0.373
LVH, n (%)*	41 (3.0)	112 (8.0)	157 (11.3)	<0.001
BMI, kg/m^2, mean (SD)	26.87 (4.17)	26.69 (3.97)	27.19 (4.25)	0.006
SBP, mmHG, mean (SD)	152.17 (21.48)	154.78 (21.51)	156.58 (22.33)	<0.001
DBP, mmHG, mean (SD)	83.09 (11.40)	83.67 (10.74)	84.35 (11.61)	0.012
Current smoking, n (%)	373 (27.0)	362 (26.0)	351 (25.1)	0.547
ECG parameters				
Heart rate, beats/min, mean (SD)	65.70 (11.06)	65.48 (11.47)	66.67 (11.62)	0.014
T wave abnormality, n (%)†	184 (13.3)	353 (25.3)	560 (40.1)	<0.001
QTc interval, sec, mean (SD)	0.424 (0.02)	0.426 (0.03)	0.433 (0.03)	<0.001
QRS duration, sec, mean (SD)	0.095 (0.02)	0.094 (0.01)	0.105 (0.02)	<0.001
Medications				
Beta-blocker, n (%)	413 (29.9)	379 (27.2)	325 (23.3)	<0.001
Diuretic, n (%)	538 (38.9)	561 (40.3)	557 (39.9)	0.747
ACE inhibitor, n (%)	188 (13.6)	208 (14.9)	268 (19.2)	<0.001
ARB, n (%)	26 (1.9)	24 (1.7)	39 (2.8)	0.107
Calcium channel blocker, n (%)	324 (23.4)	350 (25.1)	376 (26.9)	0.103
Incident cardiovascular events during follow-up				
Atrial fibrillation, n (%)	105 (7.6)	105 (7.5)	141 (10.1)	0.021
Heart failure hospitalization, n (%)	34 (2.5)	37 (2.7)	75 (5.4)	<0.001
Coronary events, n (%)	110 (8.0)	123 (8.8)	191 (13.7)	<0.001
Non-fatal MI, n (%)	99 (7.2)	99 (7.1)	137 (9.8)	0.011
Fatal/non-fatal stroke or TIA, n (%)	89 (6.4)	91 (6.5)	99 (7.1)	0.756

Abbreviations: TIA: Transient Ischemic Attack; MI: Myocardial Infarction; DM: Diabetes Mellitus; LVH: Left Ventricular Hypertrophy; BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; ACE inhibitors: Angiotensin Converting Enzyme inhibitors; ARBs: Angiotensin Receptor Blockers.

To further explore the effect of other ECG parameters affecting the sequence of ventricular repolarization/depolarization on the longitudinal associations, adjustment for T wave abnormalities, QTc interval, QRS duration and LVH were added in model 3. Further adjustment for these parameters did not essentially change the results, meaning that higher spatial QRS-T angle remained significantly associated with a steeper decline in LDCT, PLTi and PLTd test scores (all $p < 0.05$) and marginally associated with a steeper decline in the Stroop test performance ($p = 0.052$) (Table 3). Furthermore, we tested whether an interaction exists between spatial QRS-T angle and these ECG parameters in relation to cognitive function and found no significant interaction (all p for interactions > 0.05). The estimate (β) and 95% confidence intervals that were assessed using continuous levels of spatial QRS-T angle are presented in Supplementary Table 2. When we categorized the spatial QRS-T angle according to a cut-point that is reported in literature (Normal: 0° to 104°, Borderline: 105° to 134° and Abnormal: 135° to 180°), the abnormal group was associated with a steeper decline in the Stroop, LDCT, PLTi and PLTd tests performances (Supplementary Table 3).

Table 2. Cognitive function in relation to spatial QRS-T angle at baseline

	Spatial QRS-T angle thirds			
	Low n=1383	Middle n=1393	High n=1396	*p V*alue*
Stroop, seconds, mean (SE)				
Model 1	63.45 (0.61)	62.90 (0.61)	64.64 (0.61)	0.049
Model 2	66.04 (1.57)	65.46 (1.57)	66.84 (1.52)	0.166
LDCT, digits coded, mean (SE)				
Model 1	24.21 (0.18)	24.06 (0.18)	23.87 (0.18)	0.064
Model 2	23.21 (0.45)	23.05 (0.45)	22.99 (0.44)	0.171
PLTi, pictures remembered, mean (SE)				
Model 1	9.50 (0.05)	9.55 (0.05)	9.46 (0.05)	0.299
Model 2	9.40 (0.12)	9.46 (0.12)	9.39 (0.12)	0.436
PLTd, pictures remembered, mean (SE)				
Model 1	10.38 (0.07)	10.47 (0.07)	10.35 (0.07)	0.530
Model 2	10.25 (0.17)	10.35 (0.17)	10.28 (0.17)	0.876

**p* values were calculated using continuous values of spatial QRS-T angle. Model 1: adjusted for age, sex, country, education and version of cognitive tests where applicable; Model 2: adjusted for all the variables in model 1 + history of vascular disease, history of diabetes, systolic and diastolic blood pressure, body mass index, smoking status and antihypertensive medications. Abbreviations: LDCT: letter digit coding test; PLTi: picture-word learning test immediate; PLTd: picture-word learning test delayed.

Table 3. Cognitive function in relation to spatial QRS-T angle during follow-up

	Spatial QRS-T angle thirds			
	Low n=1383	Middle n=1393	High n=1396	*p* Value*
Stroop, seconds, mean annual change (SE)				
Model 1	0.488 (0.13)	0.622 (0.13)	0.775 (0.13)	0.055
Model 2	0.491 (0.13)	0.625 (0.13)	0.781 (0.13)	0.054
Model 3	0.492 (0.13)	0.622 (0.13)	0.781 (0.13)	0.052
LDCT, digits coded, mean annual change (SE)				
Model 1	-0.350 (0.03)	-0.345 (0.03)	-0.461 (0.03)	0.004
Model 2	-0.350 (0.03)	-0.346 (0.03)	-0.462 (0.03)	0.004
Model 3	-0.350 (0.03)	-0.346 (0.03)	-0.463 (0.03)	0.004
PLTi, pictures remembered, mean annual change (SE)				
Model 1	0.007 (0.01)	-0.009 (0.01)	-0.034 (0.01)	0.001
Model 2	0.007 (0.01)	-0.009 (0.01)	-0.034 (0.01)	0.001
Model 3	0.007 (0.01)	-0.010 (0.01)	-0.035 (0.01)	0.001
PLTd, pictures remembered, mean annual change (SE)				
Model 1	-0.060 (0.02)	-0.054 (0.02)	-0.094 (0.02)	0.013
Model 2	-0.060 (0.02)	-0.054 (0.02)	-0.095 (0.02)	0.013
Model 3	-0.060 (0.02)	-0.054 (0.02)	-0.095 (0.02)	0.012

**p* values were calculated using the interaction term between continuous values of spatial QRS-T angle and time. Means represent the mean annual decline in the score of each cognitive test. Model 1: adjusted for age, sex, country, education and version of cognitive tests where applicable; Model 2: adjusted for all the variables in model 1+ history of vascular disease, history of diabetes, systolic and diastolic blood pressure, body mass index, smoking status, antihypertensive medications and statin treatment groups. Model 3: adjusted for all the variables in model 2 + LVH, T wave abnormalities, QTc interval and QRS duration. Abbreviations: LDCT: letter digit coding test; PLTi: picture-word learning test immediate; PLTd: picture-word learning test delayed.

Over a period of 3.2 years, a total of 351 participants developed incident atrial fibrillation, 146 participants were hospitalized for heart failure, 424 participants developed coronary events, 279 participants developed stroke or TIA and 335 participants developed non-fatal MI. A total of 3235 subjects were free of these cardiovascular events during follow-up. Figure 2 shows the longitudinal association of spatial QRS-T angle and cognitive function stratified by incident cardiovascular events during follow-up. We did not observe a significant difference in annual changes of cognitive function between participants who did and those who did not develop incident atrial fibrillation, coronary events, stroke or TIA, heart failure hospitalization, non-fatal MI during follow-up (all p for interactions > 0.05). Finally, when we excluded participants with additional cardiovascular co-morbidities at baseline and participants taking anti-arrhythmic medications, the longitudinal results did not change except that after exclusion of participants with history of hypertension (n=1579, >37% of the population) higher spatial QRS-T angle was not associated with steeper decline in Stroop and LDCT tests performances (Supplementary Table 4).

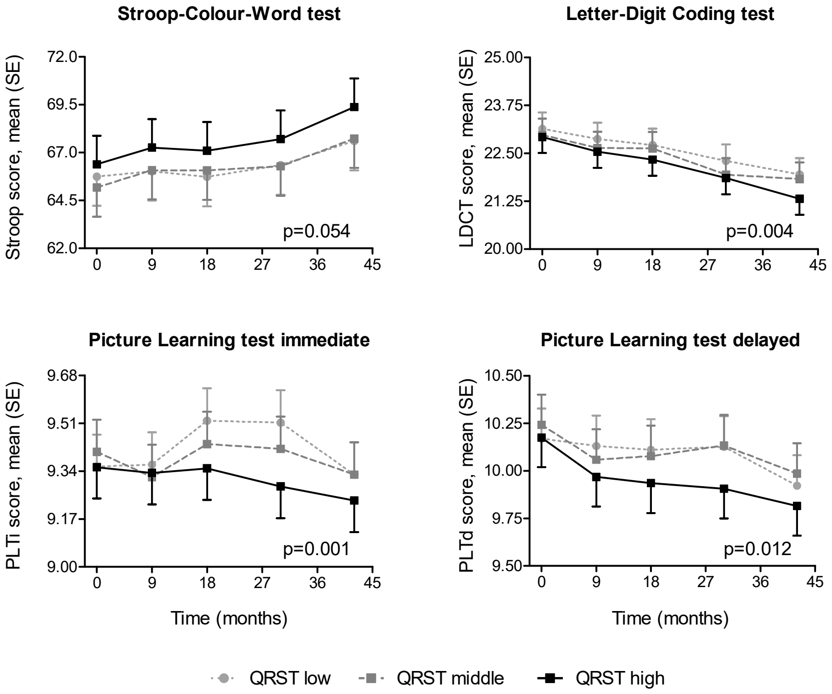

Figure 1. Cognitive function in relation to spatial QRS-T angle during follow-up. Data represent mean (standard error) decline in the score of each cognitive test in three groups of spatial QRS-T angle. The time point at the end of the study is the mean of end time points (36 to 48 months). Analyses were adjusted for age, country, education, version of cognitive tests where applicable, history of diabetes, history of vascular disease, body mass index, smoking status, systolic blood pressure, diastolic blood pressure, antihypertensive medications and statin treatment groups.

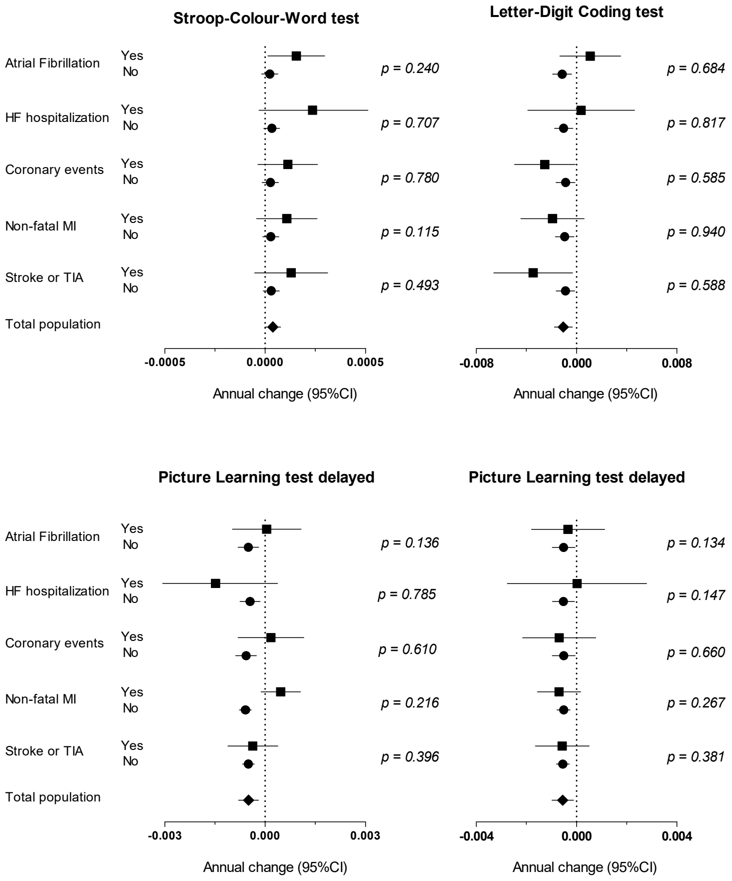

Figure 2. Association of spatial QRS-T angle and annual changes of cognitive function stratified for cardiovascular events during follow-up. Data represent annual change (95% confidence interval) in cognitive function per each unit (degree) increase in spatial QRS-T angle (the X axis), stratified for subjects who did and those who did not develop cardiovascular events during follow-up. Dark circles and squares correspond to the annual change (estimate) and the lines correspond to the 95% confidence intervals. P-values show the p for interaction. Analyses were performed in the fully adjusted model. Abbreviation: MI: myocardial infarction; HF: heart failure hospitalization and TIA: transient ischemic attack

Discussion

Our findings suggest that a wide spatial QRS-T angle, as a reflection of greater heterogeneity in electrical activity of cardiac ventricles, associates with accelerated decline in cognitive function of older subjects at high cardiovascular risk. These associations were independent of arrhythmias, cardiovascular risk factor and co-morbidities, medication use, incident cardiovascular events and other ECG parameters.

Previous research has shown that different ECG-parameters measuring either the excitation or relaxation phase of the cardiac ventricles are related to cerebrovascular accidents and cognitive impairment. Pathologic Q waves in men suggesting unrecognized MI have a two-fold increased risk of dementia and carry a higher burden of cerebral small vessel disease[5]. Similarly, patients with Alzheimer’s disease and mild cognitive impairment have higher values of QT dispersion and QT corrected dispersion[24]. Our current study suggests that an abnormal spatial QRS-T angle might precede the decline in cognitive function in a large cohort of older adults at high cardiovascular risk. Furthermore, the persistence of longitudinal associations in subjects without cardiac arrhythmias suggests that even subtle alterations in the ventricular electrical activity are linked to cognitive decline.

The observed association between spatial QRS-T angle and cognitive decline can be explained through a number of mechanisms. First, the spatial QRS-T angle and cognitive decline may both reflect cardiovascular damage and hence share a common cause. Previous research has shown that spatial QRS-T angle is a strong non-invasive predictor of cardiovascular morbidity and mortality2, 25, 26 and a wide angle is linked with higher electric instability of the cardiac ventricles that might increase the risk of cardiac arrhythmias and ischemia10. The role of cardiovascular risk factors and diseases in development of cognitive impairment is well established8. In line with this, our results show that subjects with higher spatial QRS-T angle have higher prevalence of cardiovascular co-morbidities and are more likely to develop incident cardiovascular events during follow-up. Furthermore, exclusion of subjects with a history of hypertension at baseline attenuated the longitudinal relation of spatial QRS-T angle and executive function. However, the relation between spatial QRS-T angle and memory function was independent of measured cardiovascular co-morbidities and incident cardiovascular events during follow-up. This might suggest that the association of spatial QRS-T angle and cognitive function is not limited to vascular pathologies. An abnormal spatial QRS-T angle might reflect cardiac changes that results in hemodynamic stress and altered brain perfusion, which might in turn lead to global cognitive decline. In line with this, a wide QRS-T angle have been associated with cardiac abnormalities such as ventricular remodeling, diastolic dysfunction, impaired ionic channel and calcium homeostasis in the heart27. Such alterations may cause subtle hemodynamic instability, which may over time, lead to cerebral small vessel disease and ultimately to parenchymal damage manifesting as cognitive impairment8. In fact, recently we found that even early alterations in cardiac hemodynamic stability are linked with features of accelerated brain ageing28. Nevertheless, we cannot rule out the effect of residual confounding and unmeasured cardiovascular factors in our study, which might affect both cognitive function and spatial QRS-T angle 29. Finally, it is also possible that a wide QRS-T angle mirrors the already established cerebrovascular damage in the brain which may in turn contribute to cognitive impairment13.

It is important to mention that the spatial QRS-T angle is superior to other traditional ECG parameters measuring cardiac electrical activity since it quantifies the spatial aspects of ventricular action potential that are not measured by other ECG-parameters20. Furthermore, the spatial QRS-T angle is a more robust parameter since it is less susceptible to measurement errors and noises due to determination of the T wave end on the surface of ECG10, 30. Other ECG measures of ventricular electrical activity such as QT dispersion and QTc interval are highly dependent on the determination of ECG wave points31. Moreover, the spatial QRS-T angle is distinct from other well-known markers of cardiac dysfunction such as troponin and B-type natriuretic peptide. Such plasma markers of cardiac dysfunction such as B-type natriuretic peptide (BNP) or troponin are released from cardiac muscles in response to cardiac wall stretch and/or cardiac muscle damage32. However, the spatial QRS-T angle is increased as a result of cardiac ionic channel disturbances and inhomogeneity of cardiac electrical activity10. As such, it might be considered as a non-invasive marker for detection of subtle cardiac abnormalities in an early stage.

In our analysis, we did not find a cross-sectional association between spatial QRS-T angle and cognitive function at baseline. This might be due to the characteristics of participants in the original PROSPER cohort in which subjects with poor cognitive function were excluded14. Since our population consists of older subjects with a fairly preserved cognitive function at baseline, the observed association between QRS-T angle and cognitive function might be underestimated. Nevertheless, a wide spatial QRS-angle was associated with a significant decline in all four cognitive tests during follow-up, strengthening the temporal order of the associations. Furthermore, the threshold for abnormal spatial QRS-T angle varied among studies20, 26, 33 and there is no established cut-off point. When we repeated our analysis using a cut-off point from previous literature12, 20, the results did not essentially change. Future research should determine the optimal age and gender specific threshold for abnormal spatial QRS-T angle.

The strengths of this study include the large sample size (more than 4000 participants), prospective multicentre design and the use of different cognitive tests assessing various domains of cognitive function. Furthermore, the non-invasive nature of ECG makes the QRS-T angle a suitable tool for use in routine clinical practice and supports its use for cardiovascular prevention strategies as well as efforts to prevent cognitive decline. Although measurement of spatial QRS-T angle requires a computer program, the implementation of this program using modern electrocardiographs is quite easy20. One limitation is that the participants had pre-existing cardiovascular risk factors or disease which might limit generalizability of our findings to a healthy older population. Another limitation is that PROSPER had a relatively short duration of follow-up which results in small magnitude of associations and jeopardizes the clinical significance of our findings. Moreover, only 10% of the population developed incident events during follow-up which might have underpowered our analysis in finding moderating effects of cardiovascular events on the reported associations. Finally, this was a secondary analysis of a randomized-controlled trial and was not designed to investigate a causal association between ECG abnormalities and cognitive decline.

In conclusion, we found that wider spatial QRS-T angle as a reflection of electrical instability in cardiac ventricles associates with accelerated cognitive decline independent of several conventional cardiovascular risk factors and comorbidities. These findings provide insight into the link between early electrical abnormalities of the heart and development of cognitive decline in future. Further work should investigate the prognostic accuracy of a widened QRS-T angle and future cognitive decline, with the aim of early intervention into subtle cardiovascular abnormality in order to prevent cerebrovascular alterations and cognitive impairment.

Supplementary Material

Supplementary Table 1. Baseline characteristics of included and excluded participants

Characteristics	Included n=4172	Excluded n=1632	*p* Value
Socio-demographics			
Age, y, mean (SD)	75.2 (3.3)	75.8 (3.4)	<0.001
Male, n (%)	1998 (47.9)	806 (49.4)	0.305
Age left school, y, mean (SD)	15.2 (2.1)	15.0 (1.8)	<0.001
Cardiovascular risk factors			
History of vascular disease, n (%)	1824 (43.7)	741 (45.4)	0.245
History of stroke or TIA, n (%)	430 (10.0)	219 (13.4)	0.001
History of MI, n (%)	541 (13.0)	235 (14.4)	0.097
History of DM, n (%)	427 (10.2)	196 (12.0)	0.050
SBP, mmHg, mean (SD)	154.5 (21.8)	155.0 (21.8)	0.431
DBP, mmHg, mean (SD)	83.7 (11.3)	83.9 (11.9)	0.480
BMI, kg/m^2,mean (SD)	26.9 (4.1)	26.6 (4.3)	0.015
Current smoking, n (%)	1086 (26.0)	472 (28.9)	0.025
Antihypertensive medications			
Beta-blockers, n (%)	1117 (26.8)	385 (23.6)	0.013
Diuretics, n (%)	1656 (39.7)	702 (43.0)	0.021
ACE inhibitors, n (%)	664 (15.9)	287 (17.6)	0.122
ARBs, n (%)	89 (2.1)	27 (1.7)	0.241
Calcium channel blockers, n (%)	1050 (25.2)	408 (25.0)	0.895

Abbreviations: TIA: transient ischemic attack; MI: myocardial infarction; DM: diabetes mellitus; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; ACE inhibitors: angiotensin converting enzyme inhibitors; ARBs: angiotensin receptor blockers.

Supplementary Table 2. The estimates (β) and 95% confidence intervals for cross-sectional and longitudinal analyses

	Cross-sectional Estimate (95% CI)	Change over time Estimate (95% CI)	Additional annual change Estimate (95% CI)
Stroop test, seconds			
Model 1*	0.002 (0.00001, 0.004)	0.004 (0.0006, 0.007)	0.0004 (-0.000007, 0.0008)
Model 2*	0.001 (-0.001, 0.003)	0.004 (0.0007, 0.007)	0.0004 (-0.000006, 0.0008)
LDCT, digits coded			
Model 1	-0.042 (-0.086, 0.002)	-0.312 (-0.37, -0.25)	-0.0106 (-0.018, -0.003)
Model 2	-0.031 (-0.076, 0.013)	-0.312 (-0.37, -0.25)	-0.0106 (-0.018, -0.003)
PLTi, pictures remembered			
Model 1	-0.006 (-0.018, 0.006)	0.022 (-0.002, 0.047)	-0.0049 (-0.008, -0.002)
Model 2	-0.005 (-0.017, 0.007)	0.022 (-0.002, 0.047)	-0.0049 (-0.008, -0.002)
PLTd, pictures remembered			
Model 1	-0.005 (-0.022, 0.011)	-0.031 (-0.067, 0.005)	-0.0055 (-0.098, -0.001)
Model 2	-0.001 (-0.018, 0.016)	-0.031 (-0.067, 0.005)	-0.0055 (-0.098, -0.001)

*estimates and 95% CIs for the natural log-transformed values of Stroop test. The term *cross-sectional* represents the cross-sectional association between spatial QRS-T angle and cognitive function at baseline. The term *changes over time* represents the annual changes of cognitive scores during follow-up. The term *additional annual change* represents the extra yearly change in cognitive scores per 10 degree increase in the spatial QRS-T angle values. Model 1 is adjusted for age, sex, country, education and version of cognitive tests where appropriate; Model 2 is additionally adjusted for history of vascular disease, history of diabetes, systolic and diastolic blood pressure, body mass index, smoking status, antihypertensive medications and statin treatment groups. Abbreviations: LDCT: Letter Digit Coding test; PLTi: Picture-Word Learning test immediate; PLTd: Picture-Word Learning test delayed.

Supplementary Table 3. Cognitive function in relation to spatial QRS-T angle during follow-up, using cut-offs from previous studies

	Spatial QRS-T angle thirds			
	Normal (0°-104°) 3223	Borderline (105°-134°) 370	Abnormal (135°-180°) 579	*p* Value*
Stroop, seconds, mean annual decline (SE)				
Model 1	0.536 (0.08)	0.933 (0.25)	0.954 (0.20)	0.055
Model 2	0.540 (0.08)	0.941 (0.25)	0.956 (0.20)	0.054
LDCT, digits coded, mean annual decline (SE)				
Model 1	-0.362 (0.02)	-0.463 (0.05)	-0.467 (0.04)	0.004
Model 2	-0.363 (0.02)	-0.465 (0.05)	-0.467 (0.04)	0.004
PLTi, pictures remembered, mean annual decline (SE)				
Model 1	-0.002 (0.008)	-0.028 (0.02)	-0.059 (0.02)	0.001
Model 2	-0.002 (0.008)	-0.028 (0.02)	-0.059 (0.02)	0.001
PLTd, pictures remembered, mean annual decline (SE)				
Model 1	-0.054 (0.01)	-0.100 (0.03)	-0.132 (0.03)	0.013
Model 2	-0.055 (0.01)	-0.101 (0.03)	-0.133 (0.03)	0.013

*p-values were calculated using the interaction term between continuous values of spatial QRST angle and time.
Means represent the mean annual decline in the score of each cognitive test. Model 1: adjusted for age, sex, country, education and version of cognitive tests where applicable; Model 2: adjusted for all the variables in model 1+ history of vascular disease, history of diabetes, systolic and diastolic blood pressure, body mass index, smoking status, antihypertensive medications and statin treatment groups. Abbreviations: SE: Standard error; LDCT: Letter Digit Coding test; PLTi: Picture-Word Learning test immediate; PLTd: Picture-Word Learning test delayed.

Supplementary Table 4. Cognitive function in relation to spatial QRS-T angle during follow-up, after exclusion of participants with cardiovascular co-morbidities at baseline

	Spatial QRS-T angle thirds			*p* Value*
	Low	Medium	High	
Stroop, seconds, mean (SE)				
No history of hypertension (n=1579)	0.785 (0.20)	0.707 (0.21)	1.008 (0.20)	0.152
No history of DM (n=3745)	0.422 (0.13)	0.624 (0.13)	0.725 (0.13)	0.035
No history of MI (n=3631)	0.487 (0.14)	0.582 (0.14)	0.836 (0.14)	0.029
No history of stroke or TIA (n=3742)	0.415 (0.14)	0.593 (0.14)	0.724 (0.14)	0.045
No anti-arrhythmic use (n= 4079)	0.524 (0.13)	0.637 (0.13)	0.737 (0.13)	0.117
LDCT, digits coded, mean (SE)				
No history of hypertension (n=1579)	-0.431 (0.04)	-0.355 (0.04)	-0.418 (0.04)	0.943
No history of DM (n=3745)	-0.326 (0.03)	-0.323 (0.03)	-0.459 (0.03)	0.002
No history of MI (n=3631)	-0.359 (0.03)	-0.338 (0.03)	-0.446 (0.03)	0.029
No history of stroke or TIA (n=3742)	-0.345 (0.03)	-0.347 (0.03)	-0.472 (0.03)	0.002
No anti-arrhythmic use (n= 4079)	-0.355 (0.03)	-0.347 (0.03)	-0.461 (0.03)	0.009
PLTi, pictures remembered, mean (SE)				
No history of hypertension (n=1579)	0.010 (0.02)	-0.035 (0.02)	-0.037 (0.02)	0.017
No history of DM (n=3745)	0.007 (0.01)	0.001 (0.01)	-0.028 (0.01)	0.008
No history of MI (n=3631)	0.004 (0.01)	-0.007 (0.01)	-0.037 (0.01)	0.001
No history of stroke or TIA (n=3742)	0.007 (0.01)	-0.001 (0.01)	-0.034 (0.01)	0.002
No anti-arrhythmic use (n= 4079)	0.006 (0.01)	-0.008 (0.01)	-0.034 (0.01)	0.002
PLTd, pictures remembered, mean (SE):				
No history of hypertension (n=1579)	-0.010 (0.03)	-0.111 (0.03)	-0.074 (0.03)	0.030
No history of DM (n=3745)	-0.061 (0.02)	-0.042 (0.02)	-0.088 (0.02)	0.040
No history of MI (n=3631)	-0.066 (0.02)	-0.058 (0.02)	-0.094 (0.02)	0.025
No history of stroke or TIA (n=3742)	-0.053 (0.02)	-0.047 (0.02)	-0.096 (0.02)	0.009
No anti-arrhythmic use (n= 4079)	-0.059 (0.02)	-0.052 (0.02)	-0.095 (0.02)	0.014

**p* values were calculated using continuous values of spatial QRS-T angle. All analyses were performed in the fully adjusted models (model 2). Abbreviations: SE: standard error; LDCT: Letter Digit Coding test; PLTi: Picture-Word Learning test immediate; PLTd: Picture-Word Learning test delayed; DM: diabetes mellitus; MI: myocardial infarction; TIA: transient ischemic attack.

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CHAPTER 6

NATRIURETIC PEPTIDES IN THE BRAIN: NOVEL TARGETS FOR COGNITIVE IMPAIRMENT

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Abstract

Natriuretic peptides (NPs) are traditionally known as cardiac hormones with diuretic, natriuretic and blood pressure lowering properties. Evidence indicates that NPs and their receptors are abundant in the central nervous system, suggesting their involvement in regulation of various brain functions. It has been shown that NPs are involved in the regulation of neurovascular and blood-brain barrier integrity, neuro-inflammation, neuroprotection, synaptic transmission and brain fluid homeostasis. In addition, NPs might contribute to the brain's inhibitory control over the hypothalamic-pituitary-adrenal axis. Studies have also shown that high systemic levels of NPs are associated with cognitive impairment independent of cardiovascular risk factors. In this review we discuss the potential roles of NPs in regulating structural and functional integrity of the brain. Based on the available neurobiological and clinical evidence, we propose that NPs might represent as potential novel diagnostic and therapeutic targets for cognitive impairment.

Introduction

Natriuretic peptides (NPs) are commonly recognized as cardiac hormones regulating several cardiovascular functions. This family comprises three members, namely atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). These peptides are involved in the control of body fluid homeostasis and have natriuretic, diuretic and vasodilatory properties[1].

Increasing evidence shows that elevated plasma levels of NPs are associated with accelerated cognitive decline. While higher circulating levels of NPs are correlated with cardiovascular risk factors and impaired cardiac function[2], we and others have shown that the association between circulating NPs and cognitive impairment is independent of cardiac and cardiovascular factors[3-5]. This independent association might suggest that NPs directly influence the brain and its vasculature. Indeed, all three NPs and their receptors are abundantly present in the central nervous system (CNS) and regulate various CNS functions. It has been shown that NPs are involved in the regulation of neurovascular and blood-brain barrier integrity, neuro-inflammation, neuroprotection, synaptic transmission, the stress responsive hypothalamus-pituitary-adrenal (HPA) axis, and brain fluid homeostasis[6]. Since disturbances in these functions have been implicated as potential mechanisms for cognitive impairment and dementia, any disruptions in the activity of NPs in the CNS might affect the integrity of the brain and lead to cognitive impairment.

In this review, we provide a brief overview of the roles of NPs in various aspects of CNS physiology and discuss their relevance to cognitive impairment and dementia in the context of amyloid beta (Aβ) and tau protein depositions, neurovascular dysfunction, neuro-inflammation, neuronal damage, synaptic failure, anxiety and disturbed fluid homeostasis in the CNS. Given the available neurobiological and clinical evidence, we propose that NPs might serve as potential novel diagnostic and therapeutic targets for cognitive impairment.

Molecular structure and localization of NPs and their receptors in the CNS

All NPs share a ring structure of 17-amino-acides mediated by a disulfide bridge between two cysteine residues[7]. The amino acid sequence of human ANP and BNP consist of 28 and 32 amino-acids, respectively. In the human CNS, the molecular weight of BNP was suggested to be larger than that of BNP-32 and the molecular structure of ANP was found to be different than that of ANP in the heart[8]. It was shown that immunoreactive CNP contains 22 or 53 amino-acids, with the CNP-53 being the predominant form in human brain[9].

NPs bind to specific types of membrane receptors to exert their biological functions. Three receptors for NPs include natriuretic peptide receptor A (NPR-A), natriuretic peptide receptor B (NPR-B) and natriuretic peptide receptor C (NPR-C). It has been shown that all three NPs have similar affinity for NPR-C, while ANP and BNP bind primarily to NPR-A and CNP binds to NPR-B with high specificity[10]. NPR-A and NPR-B share four structural features: an extracellular domain, a transmembrane region, a kinase-like domain and a guanylate cyclase catalytic domain. Activation of these receptors increases the intracellular concentration of cyclic guanosine monophosphate (cGMP), which has been shown to regulate most of the biological effects of NPs[11]. NPR-C lacks the guanylate cyclase catalytic domain and functions mostly as a clearance receptor[12], although there is evidence for a signaling function of this receptor as well[13].

Shortly after discovery of NPs in the rat myocardia[14], all NPs have been found to have extensive distribution in rodent and human CNS. The distributions of CNP and BNP in the CNS were shown to be higher than that of ANP. It was shown that in human brain the level of CNP-like immunoreactivity is 10 times higher than that of ANP or BNP, suggesting that it mainly functions in the CNS[15]. Evidence from animal studies showed highest concentration of NPs in the hypothalamus, and abundant concentrations in the telencephalon, cerebellar cortex, spinal cord and retina[16]. Human studies showed the presence of CNP and BNP in the cerebral cortex, thalamus, hypothalamus, pons and cerebellum, with CNP having the highest concentrations in the hypothalamus[8, 17]. In human brain autopsy samples, the highest concentration of ANP was found in the preoptic, supraoptic and paraventricular nuclei of the hypothalamus, choroid plexus and ventricular ependymal cells[18]. ANP was also detected in the brain stem, cerebral cortex, basal nuclei and thalamus of human CNS[18]. Furthermore, all NPs have been detected in human cerebrospinal fluid (CSF) with CNP being the most abundant type[19].

The binding sites for ANP have been detected in various brain regions of animal and human species[20]. In mammalian species, the highest concentration of all NPRs were found in the hypothalamus. In summary, the expression patterns of NPRs in the brain seem to complement each other, with NPR-C having the highest concentrations in the CNS. As discussed in detail by Cao and Yang, most studies on the localization of NPRs in the CNS have focused on animal models[16] and there is need for mapping of NPRs in the human CNS.

The expression of NPs in astroglial cells has been repeatedly reported. ANP-immunoreactive astrocytes have been found in canine brain and in human cerebral and cerebellar cortices. In human cerebellum, the most abundant form of ANP-immunoreactive astrocytes were found to be Bergman glia[21]. The expression of all NPs and their receptors were also detected in astrocytes and vascular structures of human retina[22]. It was shown that NPs are stored in vesicles of astrocytes and that their release occurs by calcium dependent exocytosis[23]. Moreover, existing evidence suggests that NPRs are mainly localized in astroglia cells of various CNS regions. For example, NPs stimulated cGMP accumulation in cultured rat astrocytes with CNP being the strongest one; and astrocytes in diencephalon accumulated more cGMP than cortical astrocytes suggesting a region specific expression of NPRs[24]. These findings suggests the potential roles of astroglia cells in physiological activities of NPs in the brain.

In summary, NPs and their receptors are present not only in the systemic circulation but also in the CNS. Interestingly, research suggests that the central and systemic NPs might function in a feedback loop, such that increased plasma NPs during volume expansion and cardiac wall stretch might down-regulate the NPRs in the brain. This notion is supported by animal studies showing that rats with myocardial infarction have significantly decreased levels of central ANP in several brain regions, including the circumventricular organs[25]. Since the circumventricular organs are outside the blood-brain barrier and have high density of NPRs, the concentration of NPs in this region is proportional to their receptor density and indicates the possibility of down-regulation of NPRs[25]. Besides, the presence of NPRs in endothelial cells of cerebral micro-vessels have been previously reported[26] which might be another possible pathway of the communication between central and systemic NP systems. Alternatively, it has been also postulated that the central NPs might influence their systemic production/function by showing that lesion in the AV3V region (anterior ventral region of the third ventricle) of rats inhibits volume-induced increase in systemic ANP[27]. Obviously, there is a need for future research to elucidate the cross-talk between central and systemic NPs.

Relevance of NPs to cognitive impairment

Cognitive impairment and its ultimate presentation; dementia is a complex medical condition that affects a considerable proportion of the elderly population[28]. Increasing evidence suggests that multiple pathological pathways are responsible for development of cognitive impairment. Among them, Aβ deposition, tau protein abnormalities, neurovascular dysfunction, oxidative and inflammatory damage, synaptic failure and glia cell activation are commonly believed as the key features of cognitive impairment and dementia[29, 30]. Nevertheless, to date there is no effective treatment for these devastating disorders. Given the abundance of NPs in the brain, numerous studies have attempted to delineate their roles in the physiology of the CNS. It was shown that NPs play key roles in the regulation of neurotransmitters release and re-uptake, synaptic transmission and plasticity, microglial cell activation, neuro-inflammation, neuroprotection and blood-brain barrier integrity. Since brain NPs regulate several functions that are disturbed during the course of cognitive impairment, their functional disturbances might be responsible for development of cognitive impairment. Below we discuss the relevance of NPs functions in the brain to cognitive impairment in a random order.

NPs, Aβ and Tau protein depositions in the CNS

Alzheimer disease (AD) is the most common type of dementia[29]. The dominant hypothesis for AD pathogenesis is the Aβ hypothesis, stating that the deposition of Aβ in senile plaques of the extracellular brain tissues is the main cause of AD. The Aβ peptide is produced during β-amyloid precursor protein (APP) processing. The Aβ hypothesis was supported by the observations that the gene for the APP is localized on chromosome 21, and patients with Down syndrome (trisomy 21) commonly develop AD by the age of 40. The imbalance between production and clearance of Aβ peptides from the brain is believed to play essential roles in the pathology of AD. The accumulation of neurotoxic Aβ substances disturbs synaptic activity and neurotransmitters' function, which then might lead to memory loss and other clinical symptoms of cognitive impairment and AD[31]. Another leading hypothesis is the tau protein hypothesis, proposing tau protein abnormalities as the main initiators of AD. Hyperphosphorylation of tau protein leads to formation of neurofibrillary tangles, which disrupts the organization of microtubules and the cytoskeleton of the cells. This results in impairment of synaptic transport and thus impaired neuronal function[32].

Recently, the results of the "Alzheimer's Disease Neuroimaging Initiative" have shown that higher circulating levels of BNP are associated with lower CSF Aβ42 levels and higher t-tau/Aβ42 ratios, with effect estimates comparable to the number of *APOE4* alleles[33]. Earlier, in an attempt to identify CSF biomarkers for early identification of mild cognitive impairment and AD, it was reported that the CSF levels of N-terminal pro BNP (NTproBNP) are elevated in subjects with mild cognitive impairment in comparison to non-demented subjects. It was also shown that CSF levels of NTproBNP are positively correlated with tau, p-tau 181 and tau/Aβ42 ratio[34]. This might be explained by the findings that no BNP mRNA have been detected in the CNS, proposing the possible peripheral source of BNP in the CSF of subjects with cognitive impairment[35]. Another study has suggested that ANP and Aβ share a common clearance pathway from the brain; and that the insulin-degrading enzyme is the possible mediator in this pathway. It was postulated that since ANP has a higher affinity for insulin-degrading enzyme than Aβ, higher ANP levels in the brain might influence the clearance of Aβ protein from the CNS[36]. This evidence, although still in their infancy, opens novel areas of research,that aim to identify the roles of NPs in formation of neurofibrillary tangles, senile plaques, and clearance of Aβ from the CNS of AD patients. The observed correlations need to be further studied to validate the prognostic value of NPs, in plasma and in CSF, in patients with mild cognitive impairment and AD. Although studies have mostly measured BNP or ANP, measuring CNP in the CNS and/or CSF of cognitively impaired subjects is of critical importance considering the widespread concentrations of this peptide in the CNS.

NPs and neurovascular dysfunction

Recently, it has become more appreciated that neurovascular dysfunction plays an important role in the pathophysiology of cognitive impairment which might occur years before its clinical onset[37]. Amongst others, disruption in blood-brain barrier (BBB) function may result in impaired clearance of Aβ and other toxic substances from the brain, and consequently contribute to development and aggravation of AD[38]. Accumulating evidence suggests the involvement of NPs in regulation of BBB and cerebral blood flow. Animal studies have detected specific binding sites for ANP and BNP in cerebral vasculature including brain capillary endothelial cells[26, 39]. It has been shown that ANP increases cGMP in rabbit and pig cerebral microvessels; and that CNP increases cGMP in primary cultured cerebral microvessels in a dose dependent manner suggesting the involvement of these peptides in modulation of the BBB[40]. Furthermore, roles of NPs in regulation of water and brain electrolyte balance have been suggested in rats by showing that ANP decreased brain water and sodium in areas of brain edema using MRI techniques[41]; and intracerebroventricular administration of ANP decreased brain edema after hemorrhagic brain injury[42]. The existence of tight junctions between endothelial cells of CNS contributes to the functions of the BBB. The cytoplasmic proteins zonula occludens (ZO-1) is involved in forming the tight junction of BBB. Recently, it has been shown that CNP increases the permeability of BBB by altering the expression of ZO-1 *in vivo* and *in vitro* and this was proposed to have a cGMP dependent mechanism. As such, CNP might play a critical role in permeability of the BBB, which might be beneficial in improving the transport and delivery of drugs to the CNS[43].

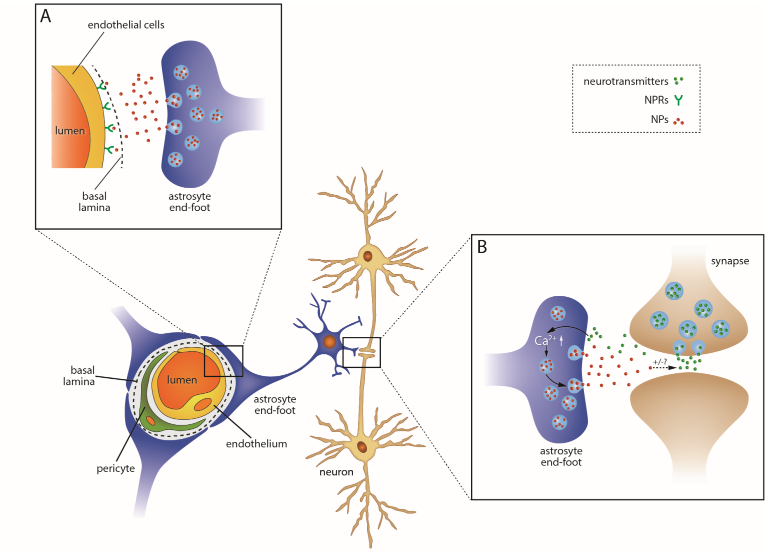

Figure 1. The interaction between astrocytes and NPs in regulation of brain physiology. The end-feets of astrocytes are in contact with several neuronal networks and at the same time with the cerebral blood vessels, hence regulating the function of both structures. **A**: The NPRs are located in endothelial cells of brain vasculature in various regions including choroid plexus. The release of NPs from astrocytes in response to neuronal function might lead to activation of NPRs. This in turn might increase the space between tight junctions of endothelium and facilitate transport of chemicals across the blood-brain barrier. NPs might also exert vasodilating functions on cerebral microvessels, in particular CNP. **B**: NPs are stored in vesicles in the astrocytes. Neuronal activity and neurotransmitters release increase the calcium concentrations in the astrocytes, which in turn stimulates the exocytosis release of NPs from astrocytes in the extracellular space. NPs then might regulate the action of several neurotransmitters by inhibiting or stimulating their release and re-uptake. In this way, neurotransmitters and NPs might function in a feed-back loop.

NPs might exert modulatory functions on brain vasculature and BBB via glia cells, in particular astrocytes. The end-feet of astrocytes is in contact with the synaptic membrane of neurons and blood vessels at the same time. Neuronal activity might increase calcium concentrations in astrocytes and hence increase exocytosis of NPs[44], which in turn might affect permeability of BBB and/or vascular tones through stimulation of NPRs on endothelial cells of brain vessels (Figure 1). On the other hand, the BBB itself might also play role in clearance of NPs from the CNS and regulate their brain concentrations. Ito and colleagues have identified the expression of NPR-A and NPR-C in rat brain capillaries and demonstrated that NPR-C mediates the efflux transport of ANP from brain to blood, suggesting that BBB efflux transport is involved in elimination of NPs from the brain[45].

NPs and neuro-inflammation

Animal and clinical studies strongly suggest the involvement of neuro-inflammation in the pathophysiology of dementia and AD[30]. Several studies have shown that the concentration of inflammatory markers such as the inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor α (TNF-α) is increased in the brain of AD patients[32]. TNF-α in the CNS exerts its action by inhibiting the transport of Aβ from brain to periphery. Consequently, increased TNF-α concentrations might increase the levels of Aβ in brain. Similarly, IL-6 may result in increased Aβ42 production by affecting APP. Furthermore, activation of microglia cells during the course of AD has been repeatedly reported. Microglia cells in the CNS are activated during brain damage. Increased activity of activated microglia cells may result in enhanced production of cytokines, reactive oxygen and nitrogen species that may have unfavorable effects in the CNS. Aβ can also stimulate microglial cells to produce neurotoxic and inflammatory factors[32]. It is proposed that the inflammatory response of microglia cells is mediated via the cGMP pathways. In line with this, intraperitoneal injection of a cGMP-phosphodiesterase inhibitor – zaprinast – decreased the activation of microglia/macrophage cells in mouse models with cortical cryoinjury and lowered oxidative stress and decelerated neurodegeneration[46].

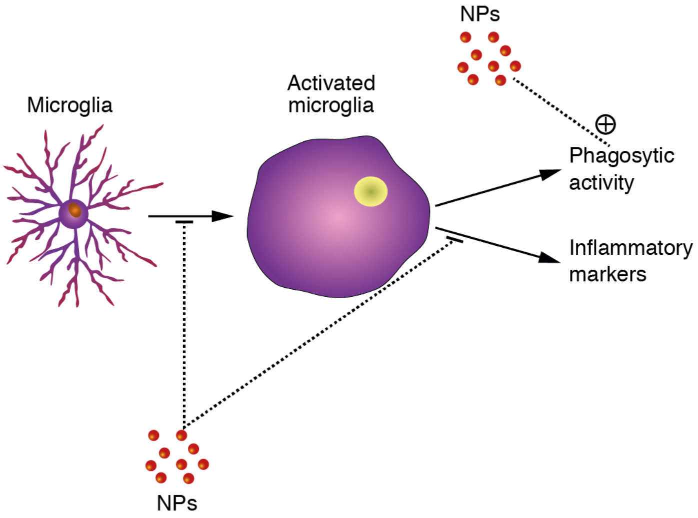

Figure 2. Anti-inflammatory actions of natriuretic peptides (NPs) in the brain. NPs might act on several pathways to reduce inflammation in the brain, in particular through their effects on microglia cells. They might inhibit the transformation of microglia cells to activated microglia cells. NPs might also directly inhibit the production of inflammatory markers or increase the phagocytic activity of microglia cells in the central nervous system

Existing evidence supports the involvement of NPs in regulation of inflammatory processes in the CNS, in particular of ANP. It was shown that in lipopolysaccharide (LPS) activated macrophages, ANP binds to NPR-A and inhibits production of nitrite and IL-1β through inhibition of the pro-inflammatory transcription factors of nuclear factor κB and activator protein-1, suggesting the involvement of ANP-NPR-A system in the regulation of neuro-inflammation[47]. Intravenous administration of human recombinant BNP (nesiritide) to mouse models with traumatic brain injury and intracerebral hemorrhage reduced microglia cell activation and inflammatory markers TNF-α and IL-6[48]. Boran and colleagues have shown that ANP decreases the LPS-induced expression of inflammatory genes, namely nitric oxide synthase Type 2 and TNF-α in rat microglia cells. ANP could also increase the phagocytic activity of microglia cells in primary and hippocampal organotypic cultures of rat. ANP might exert favorable effects on damaged brain tissue and suppresses the expression of anti-inflammatory genes[49]. These data suggest that the regulatory actions of NPs on inflammatory processes in the CNS is at the level of microglia cells. NPs may exert their anti-inflammatory actions by reducing the activation of microglia cells, inhibiting the secretion of pro-inflammatory markers and stimulating phagocyte activity of microglia cells (Figure 2). Together, evidence points toward the importance of NPs in regulation of inflammatory processes in the CNS and their potential value in management of cognitive impairment and dementia as anti-inflammatory substances.

NPs and neuroprotection

Several studies have provided evidence on neuroprotective effects of NPs. Cortical spreading depression (CSD) – a spreading wave of electrical hyperactivity in the brain which is followed by subsequent inhibition of electrical activities – is known to be protective against cerebral ischemia. It was shown that cortical expression of ANP mRNA is increased in rats preconditioned with a CSD episode and that this is accompanied by increasing cGMP levels, suggesting the involvement of an ANP-cGMP pathway in neuroprotective effects of NPs[50]. ANP and BNP could suppress the apoptotic fragmentation of DNA in cultured PC12 cells and improve their survival by prolonging the elevation of cGMP levels[51]. The same group has shown that pretreatment with ANP protects NG108-15 cells, a cholinergic-neuron-like cell line, against nitric oxide induced apoptosis by increasing cGMP levels. The authors hypothesized that the neuroprotective effect of NPs via elevation of cGMP might be protective against neurodegenerative disorders in which nitric oxide is responsible for neuronal apoptosis[52]. Furthermore, Kuribayashi and colleagues showed neuroprotective effect of ANP in rat retinal neurons. They have demonstrated that ANP-NPR-A pathway is protective against N-methyl-D-aspartate-induced neurotoxicity possibly by activation of dopamine D1 receptors[53]. CNP was also shown to protect rat retinal ganglion cells against apoptotic damage both *in vitro* and *in vivo*[54].

Using immunohistochemistry methods, it has been shown that the number of ANP-immunoreactive glial cells is increased in the white matter around brain infarcts[55].

Intravenous administration of BNP increased cerebral blood flow and reduced inflammatory markers (TNF-a and IL-6) and neuronal degeneration in mouse models[48]. In line with this, ANP decreased sodium and water accumulation and reduced brain edema after cerebral ischemia and intracerebral hemorrhage in rats[42]. These findings suggest that the neuroprotective effects of NPs might be mediated via regulation of cerebral blood flow and NPs might possess protective effect on brain neurovascular structures, although further studies are warranted to explore their precise mechanisms.

NPs and synaptic regulation

Synaptic alterations and synaptic failure have been proposed as possible pathophysiological mechanisms behind AD and other types of dementia[56]. In AD, Aβ accumulation leads to synaptic disassociation, which in turn may disturb neurotransmitters and synaptic functions and hence the presence of clinical symptoms such as memory impairment. It is suggested that the disturbances in synaptic function begin in the hippocampal areas prior to neurodegeneration[57]. In line with this, evidence suggests that NPs might control the action of neurotransmitters including noradrenalin, dopamine and glycine. It has been shown that ANP negatively regulates the release of noradrenalin in slices of rat hypothalamus[58] and increases the uptake of noradrenalin in rat hypothalamus and medulla oblongata[59]. Furthermore, intracerebroventricular injection of CNP inhibits cocaine-induced release of dopamine in caudate putamen, suggesting a regulatory role on dopaminergic neurons[60]. Recently, Maeda and colleagues showed that ANP directly acts on the glycinergic presynaptic nerve terminals and inhibits the release of glycine in spinal cord sensory circuits of rats[61].

NPs may also be involved in the regulation of synaptic plasticity and processing of information. For example, application of CNP to hippocampal slices of rat decreased population spike amplitude after high frequency stimulation and affected long-term potentiation[62]. CNP could decrease hippocampal network oscillations that are related to short- and long-term memory and the *in vitro* results from the same group demonstrated that CNP is involved in the regulation of bidirectional plasticity in hippocampal area CA1[63].

Consistently, other studies reported that CNP influences anxiety, arousal, learning and memory processes in rats. It was also shown that treatment with receptor blockers of dopamine, acetylcholine and nitric oxide inhibited learning effects of CNP in rats[64].

Apart from the effects of NPs on neurotransmitter release and uptake, neurotransmitters themselves might also regulate the release of NPs from astrocyte cells. Although astrocytes have long been known for their supporting actions on neighboring neuronal cells, growing evidence indicates that they also release several chemicals known as glio-transmitters in response to various physiological and/or pathological stimuli. Interestingly, it has been shown that NPs are among glio-transmitters secreted from astrocytes, and this release was shown to be calcium dependent. Calcium fluctuations in astrocyte in response to neurotransmitters might stimulate exocytosis release of NPs. In this way, NPs might play important roles in processing of information through neuro-glia interactions[23] (Figure 1).

NPs and anxiety

Anxiety has been implicated in cognitive impairment. It has been shown that older subjects with cognitive impairment have elevated anxiety levels, and increased anxiety has been related to poor cognitive function as well as accelerated future cognitive decline. Different pathological mechanisms have been proposed to play a role in the relation between anxiety and cognitive impairment, including alterations in neurotransmitter functions and availability, brain structures and HPA-axis activity[65]. Amongst others, it has been suggested that chronic activation of the HPA-axis might reduce the hippocampal volume and contribute to cognitive decline. Furthermore, accumulation of lifetime stress has been hypothesized to reduce the feedback inhibition of the HPA-axis and therefore lead to hypercortisolism, which might in turn accelerate the ageing process[66]. Interestingly, it has been shown that increased anxiety and Aβ levels are correlated with decreased memory function in healthy older adults. In this study, subjects with increased Aβ and anxiety symptoms had accelerated decline in cognitive domains that are controlled by temporal and prefrontal cortex of the brain[67].

It is suggested that NPs have modulatory effects on anxiety disorders by affecting the HPA axis. It has been shown that ANP inhibits the release of corticotropin releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) and directly inhibits cortisol release[68]. Intracerebroventricular administration of ANP and CNP decreased anxiety related behaviors in rats[69, 70], and the inhibitory effect of CNP on anxiety was show to be dose dependent[71]. In an attempt to test the inhibitory role of brain NPs on the HPA-axis in humans, ANP was administrated intranasally to reach the brain directly in 18 male subjects. ANP was able to strongly inhibit hypoglycemia induced release of ACTH and cortisol, suggesting inhibition of the HPA-axis in hypothalamus[66]. Together these data suggest potential beneficial effect of central NPs on anxiety related symptoms that might provide more feasible strategies to manage cognitive impairment in older adults.

NPs and memory

CNP have been shown to participate in regulation of learning and memory. Passive avoidance memory is a type of conditioning during which the subject learns to avoid certain behaviors in order to prevent the occurrence of aversive stimuli. Telegdy and colleagues have shown that intracerebroventricular injection of CNP improved learning when injected 30 minutes before learning trial, and consolidation of passive avoidance memory when injected 30 minutes after learning trial. They have also shown that administration of ANP and BNP to the lateral brain ventricle improved passive and active avoidance learning behaviors[64, 72]. Moreover, the relation between CNP and neuroplasticity has been studied using electrophysiological methods. As discussed previously, CNP was able to regulate hippocampal network oscillations which are responsible for storage of information and consolidation of memory; regulate the magnitude of long-term potentiation and long-term-depression; and was shown to be involved in the regulation of bidirectional plasticity in hippocampal slices[62, 63]. Because the hippocampus is linked to memory processes and CNP and its receptors are abundantly located in hippocampal areas, it can be hypothesized that CNP contributes to the regulation of memory and learning behaviors, although further studies are needed to assess the direct relation of CNP with memory functions.

NPs and fluid homeostasis in the CNS

One of the pathological features of AD is that the volume of CSF is increased due to atrophic loss of brain mass. However, the ability of CSF to renew itself - CSF turnover - is reduced which jeopardizes the clearance of harmful metabolites such as Aβ from the brain. It has been shown that during ageing and AD the turnover rate of CSF decreases by 3 to 4 times. This altered fluid homeostasis in the CNS during neurodegenerative disorders might negatively affect neuronal processes and cognitive function[73].

In the periphery, NPs are secreted from the heart in response to volume overload, which results in increased natriuresis, diuresis and decreased blood pressure. Evidence suggests that these peptides might have similar functions in the CNS that is regulation of CNS fluid homeostasis. NPs and their receptors are found in the choroid plexus and circumventricular organs, which are the sites of CSF production. As a result, it is possible that they regulate fluid homeostasis in the CNS by adjusting the production of CSF in the choroid plexus. Indeed, it has been shown that ANP regulates intracranial pressure by reducing CSF formation[74]. Furthermore, Yamasaki and colleagues showed that intracranial hypertension was positively related to increased CSF levels of ANP in neurological patients, while this was not accompanied by raised circulating levels of ANP[75]. Schouten and colleagues found that CSF N-terminal pro CNP (NT-proCNP) and CNP are inversely related to plasma concentration of CNP, which suggests that CSF levels of CNP might be regulated differently than their systemic concentrations[76]. Interestingly, Mori and colleagues have shown that extraluminal administration of CNP produces a dose dependent vasodilatory effect on cerebral arteriole of rats[77]. The vasodilatory action of CNP was proposed in patients with subarachnoid hemorrhage (SAH) as well. It was shown that CSF levels of CNP are increased one day after SAH, whereas plasma levels of CNP were not changed[78]. Recently, it has been shown that elevated systemic concentrations of CNP in pregnant sheep did not increase CNP levels in the CSF[79]. Together, these evidence supports the hypothesis that NPs in the brain contribute to the production and flow of CSF within the CNS, which is independent of their systemic concentrations. In line with this, one study examined the effect of elevated systemic levels of NPs on their central concentrations. They found that in ovine, pacing-induced heart failure was associated with lower central levels of BNP, in particular in pituitary region[80]. Furthermore, the inverse relation between CSF and circulating levels of NPs makes the central NPs as potential novel targets for diagnosis of CNS disorders. In this setting, it has been shown that CSF levels of CNP are decreased in patients with Parkinson's disease and lower CSF levels of CNP at baseline were related to worse functional outcomes[1]. However, more studies are needed to clarify the specific role of CSF NPs in neurodegenerative disorders.

Plasma NPs and brain structural integrity

Despite the wealth of evidence from animal studies, limited human data is available on the roles of brain NPs in the structural and functional integrity of the brain. Several studies reported that higher circulating levels of BNP and its precursor NTproBNP are associated with impaired cognition. Elevated plasma levels of BNP have been associated with increased risk of cerebrovascular events, structural brain changes and subclinical brain damages such as with matter hyper-intensities[4]. The Framingham offspring study of 3127 stroke-free individuals showed that high plasma BNP levels increased the risk of stroke or transient ischemic attack and improved the risk prediction of the Framingham Stroke Risk Profile, independent of blood pressure, cardiac and renal diseases[81]. The results of the Atherosclerosis Risk in Communities study showed that higher NTproBNP plasma levels were associated with silent brain infarcts and white matter lesions independent of cardiovascular risk factors[82]. Furthermore, recently we have shown that higher serum NTproBNP levels are associated with lower brain volume, cognitive impairment and depression independent of cardiovascular risk factors and cardiac output[4]. Shibazaki and colleagues have shown that patients with intracerebral hemorrhage have higher plasma BNP levels; and plasma BNP was related with intraventricular extension and resulting hydrocephalus[83]. The available evidence implies that low CNS and high peripheral concentration of NPs are linked with structural and functional alterations in the brain. Higher NPs in CNS are crucial for maintenance of homeostasis in the brain while elevated systemic concentration of NPs might suppress central NPs and their receptors[80].

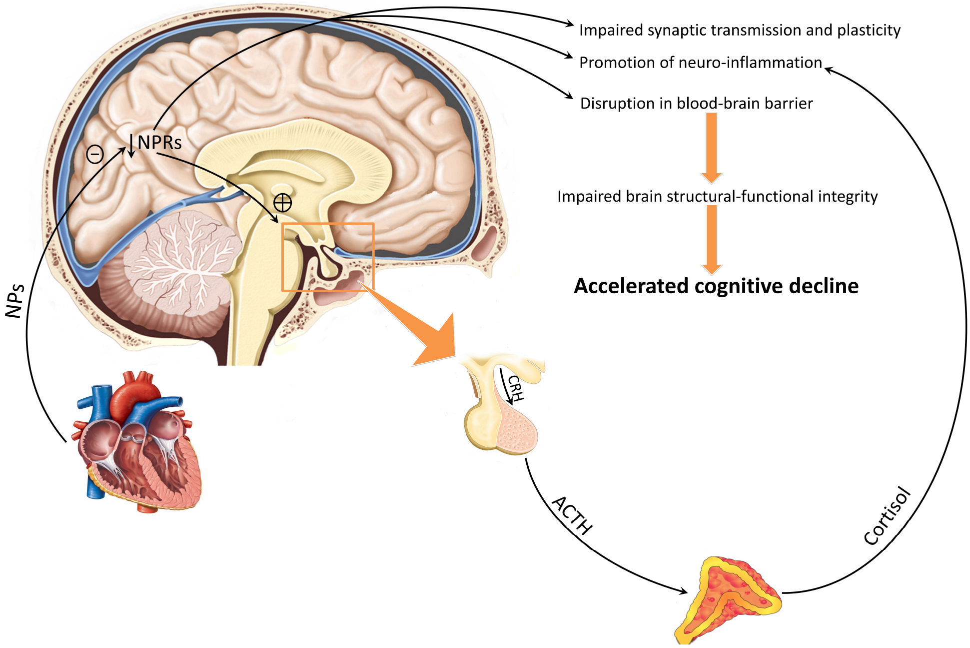

Figure 3. The potential role of natriuretic peptides (NPs) and their receptors (NPRs) in cognitive impairment. High systemic levels of NPs may lead to down-regulation of NPRs in the brain which in turn would result in dysregulation of synaptic transmission and plasticity, promotion of neuro-inflammation and disruption of the blood brain barrier. These brain deficiencies are responsible for impaired structural and functional integrity of the brain that ultimately accelerates cognitive decline. Abbreviations: CRH: corticotropin releasing hormone, ACTH: adrenocorticotropic hormone

Summary and conclusion

Finding effective disease modifying strategies for cognitive impairment still represents an unmet goal. There is an increasing body of evidence showing that disturbances in various pathways act in concert to initiate and promote neuronal injuries, cell death and functional impairments in the neuronal networks in the brain of cognitively impaired patients. This extraordinary level of complexity in the pathophysiology of cognitive impairment has been marked as a possible reason for failure of the recent trials targeting specific pathologies. Hence, further attention needs to be paid to strategies that can identify subjects at risk in early stages and also modify the course of cognitive decline by acting on various key pathologic pathways. Although various mechanisms for cognitive impairment have been proposed, neurovascular dysfunction, glial cell activation, oxidative and inflammatory damage and synaptic failure are among the key features of cognitive impairment. Therefore, disease modifying treatments that will be effective on multiple pathways can potentially decelerate the pace of cognitive decline. In line with this, we suggest that NPs play essential roles in multiple pathways including regulation of neuro-inflammation, synaptic transmission, CNS fluid homeostasis and modulation of systemic and CNS stress response. Animal studies have shown that activation of the brain NPRs improves memory and learning behavior. Furthermore, elevated plasma NPs might down-regulate the expression of NPRs in the CNS. Hence, we hypothesize that reduced central action of NPs in the brain, possibly as a result of their elevated systemic levels, might lead to impairment in several key physiological functions of the brain and disturb the integrity of the CNS, which might ultimately put subjects at higher risk of accelerated cognitive decline. Furthermore, reduced central action of NPs in the brain might increase the production of stress hormones of the HPA axis including CRH, ACTH and cortisol. This might alter the stress response of brain to various stimuli and lead to emotional impairments including anxiety. Elevated circulating cortisol levels, on the other hand, might accelerate neuro-inflammation and exaggerate the functional impairments in the CNS (Figure 3). Yet, future large-sample studies are needed to investigate (i) the function of NPs in the CNS in subjects with and without neurological disorders, (ii) the interaction between systemic NPs and their CNS levels to identify if the two pathways in the periphery and the CNS function separately or in a feed-back loop fashion, and (iii) the relation between CSF levels of NPs in patients with and without AD and their influence on future clinical outcomes. Among three members of NPs family, CNP seems to be the most promising target given the abundance of this peptide and its receptors in the CNS and the specific role of CNP in the CNS needs to be further explored. Available evidence suggests that NPs are important regulators of overall integrity of the brain, and might represent novel targets in management of subjects with cognitive impairments.

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CHAPTER 7

NATRIURETIC PEPTIDES IN POST-MORTEM BRAIN TISSUE AND CEREBROSPINAL FLUID OF NON-DEMENTED HUMANS AND ALZHEIMER'S PATIENTS

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Mahinrad, S., Bulk, M., van der Velpen, I., Mahfouz, A., van Roon-Mom, W., Fedarko, N., Yasar, S., Sabayan, B., van Heemst, D. & van der Weerd, L. Natriuretic peptides in post-mortem brain tissue and cerebrospinal fluid of non-demented humans and Alzheimer's disease patients.

Abstract

Animal studies suggest the involvement of natriuretic peptides (NP) in several brain functions that are known to be disturbed during Alzheimer's disease (AD). However, it remains unclear whether such findings extend to humans. In this study, we aimed to: 1) map the gene expression and localization of NP and their receptors (NPR) in human post-mortem brain tissue; 2) compare the relative amounts of NPR between the brain tissue of AD patients and non-demented controls and 3) compare the relative amounts of NP between the cerebrospinal fluid (CSF) of AD patients and non-demented controls. Using the publicly available Allen Human Brain Atlas dataset, we mapped the gene expression of NP and NPR in healthy humans. Using immunohistochemistry, we visualized the localization of NP and NPR in the frontal cortex of AD patients (n=10, mean age 85.8 ± 6.2 years) and non-demented controls (mean age = 80.2 ± 9.1 years). Using Western blotting and ELISA, we quantified the relative amounts of NP and NPR in the brain tissue and CSF of these AD patients and non-demented controls. Our results showed that NP and NPR genes were ubiquitously expressed throughout the brain in healthy humans. NP and NPR were present in various cellular structures including in neurons, astrocyte-like structures, and cerebral vessels in both AD patients and non-demented controls. Furthermore, we found higher amounts of NPR type-A in the brain of AD patients (p=0.045) and lower amounts of NP type-B in the CSF of AD patients (p=0.029). In conclusion, this study show the abundance of NP and NPR in the brain of humans suggesting involvement of NP in various brain functions. In addition, our findings suggest alterations of NP levels in the brain of AD patients. The role of NP in the development and progression of AD remains to be elucidated.

Introduction

Natriuretic peptides (NP) refer to a group of peptides that are mostly known for their actions within the cardiovascular system[1]. Three members of this family are atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP)[2]. The biological activities of NP are mediated through activation of three transmembrane receptors including natriuretic peptide receptor A (NPR-A), natriuretic peptide receptor B (NPR-B) and natriuretic peptide receptor C (NPR-C)[3] (Figure 1). While NP have been initially discovered in cardiac myocytes[4], extensive distribution of NP and their receptors in the brain of animal species has been repeatedly reported [5]. It was shown that NP and their receptors are present not only in the neuronal structures, but are also abundant in the glial cells and cerebral vessels[5, 6]. Notably, findings from animal studies suggest that NP are involved in regulation of neuroplasticity[7], blood-brain barrier integrity[8], neuro-inflammation[9, 10] and memory function[11, 12].

The biological roles of NP in the central nervous system (CNS) have been mainly described in rodent and mammalian species[13]. Existing evidence from human subjects indicates that higher plasma levels of NP associate with dementia and accelerated cognitive decline[14]. Although this link was mainly attributed to cardiovascular pathologies[15], recent findings suggest that plasma NP associate with cognitive decline independent of cardiovascular disease[16, 17]. Moreover, higher circulating levels of BNP were linked with lower cerebrospinal fluid (CSF) amyloid beta42 (Aβ42) and total tau/Aβ42 ratios[18]. Interestingly, some studies have reported the presence of NP and their receptors in the brain tissue and CSF of human subjects[19, 20]. Hence, based on the available biological and clinical evidence, we have recently hypothesized that NP may be a potential diagnostic and/or therapeutic markers for Alzheimer's disease (AD)[13].

To substantiate this hypothesis, in this study we aimed to: 1) map the gene expression and localization of NP and their receptors (NPR) in human post-mortem brain tissue, 2) compare the relative amounts of NPR between the brain tissue of AD patients and non-demented controls and 3) compare the relative amounts of NP between the cerebrospinal fluid (CSF) of AD patients and controls.

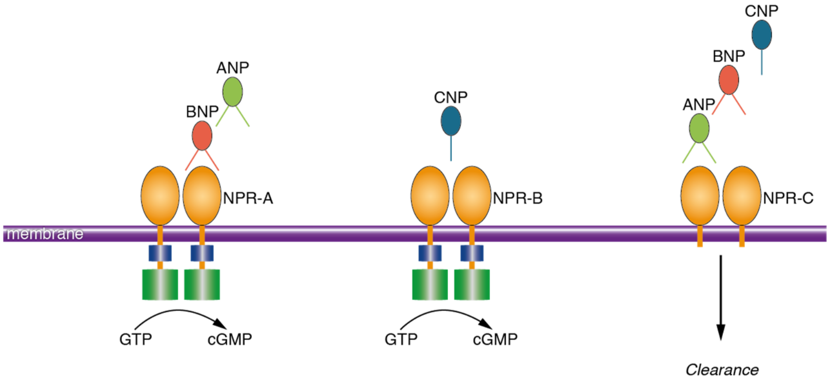


Figure 1. Schematic presentation of the ligand preference of natriuretic peptide receptors. Natriuretic peptides bind to three transmembrane receptors, namely NPR-A, NPR-B and NPR-C. ANP and BNP bind to NPR-A; CNP binds to NPR-B and all the NP (ANP, BNP and CNP) bind to NPR-C. Activation of the NPR increases the intracellular concentration of cyclic guanosine monophosphate (cGMP), being the main mediator of the biological activity of the NP. NPR-C is a clearance receptor.

Methods and Materials

Experimental design

We had access to biomaterials of 17 non-demented controls (mean age = 80 ± 8.5 years, 59% female) and 10 patients with a definitive pathological diagnosis of AD (mean age 85.8 ± 6.2 years, 60% female) (Table 1). The definitive diagnosis of the donors was provided by expert neuropathologists that considered both histopathological results and the clinical diagnosis of the donors. To investigate the localization of NP and NPR in the brain, we performed immunohistochemistry using post-mortem brain tissue of a subset of 13 non-demented controls and 10 AD patients (Table 1). For quantitative assessment of NPR in the brain, we performed Western blotting using a sub-set of donors from whom frozen brain tissue was available (10 controls and 8 AD patients, Table 1). NP in the CSF were detected by enzyme-linked immunosorbent assay (ELISA) in another subset of the donors that had a frozen CSF sample available (10 controls and 10 AD patients, Table 1). Age and gender were not significantly different between controls and AD patients in any of the subsets (all $p>0.05$).

Brain tissue samples

Post-mortem brain tissue from the frontal lobe – middle frontal gyrus – of 10 AD patients and 8 controls were obtained from the Netherlands Brain Bank (NBB, Netherlands Institute for Neuroscience Amsterdam); and for 6 controls tissues were obtained from the Normal Ageing Brain Collection Amsterdam (NABCA). All CSF samples were provided by the NBB. The CSF was collected from the lateral ventricles during autopsy and stored at -80° C for later analysis. All donors gave written informed consent for brain autopsy and for the use of their specimens and medical records for research purposes. According to national ethical guidelines, all samples were coded to maintain the anonymity of donors.

Gene expressions

To investigate the gene expression of NP and NPR in the brain, we used BrainScope[21] (http://brainscope.nl/brainscope). BrainScope is a web-portal for visual analysis of gene expression in the brain of healthy human subjects, based on data from the Allen Human Brain Atlas[22, 23] (http://human.brain-map.org/static/download). The Allen Human Brain Atlas provides the mRNA expression of about 20,000 genes using six healthy adult donors (age range from 24 to 57 years). Gene expression was measured with a customized micro-array chip using ~3,700 samples taken from anatomically annotated regions of the brain. From this data, BrainScope uses 105 expression values per gene corresponding to anatomically annotated brain regions that were sampled for all the six donors. The expression values were averaged to provide a single expression value for each sample across the subjects[21]. Accordingly, we used BrainScope to explore the expression of the following genes: natriuretic peptide A (*NPPA*, encodes ANP protein, Gene ID 4878), natriuretic peptide B (*NPPB*, encodes BNP protein, Gene ID 4879), natriuretic peptide C (*NPPC*, encodes CNP protein, Gene ID 4880), natriuretic peptide receptor 1 (*NPR1*, encodes NPR-A protein, Gene

ID 4881), natriuretic peptide receptor 2 (*NPR2*, encodes NPR-B protein, Gene ID 4882) and natriuretic peptide receptor 3 (*NPR3*, encodes NPR-C protein, Gene ID 4883).

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues were serially cut into 5-µm-thick sections and mounted on coated glass slides (SuperFrost® Plus, VWR). The sections were deparaffinized in xylene and rehydrated through graded ethanol concentrations (100%, 90% and 70%). To block endogenous peroxidase activity, the sections were incubated for 20 minutes in methanol with 0.3% hydrogen peroxide (H_2O_2). This was followed by antigen retrieval by cooking the sections for 20 minutes at 0.76 bar steam pressure in an acidic pH 6 solution (H-3300, Vector labs). The antigen retrieval step was performed depending on the primary antibody (Supplementary Table 1). Next, the sections were washed in phosphate-buffered saline (PBS) and incubated with the primary antibody overnight at room temperature in the blocking buffer (1% bovine serum albumin [BSA] in PBS). The sections were then incubated for 1 hour with a biotin-labelled secondary anti- rabbit or anti- mouse antibody followed by 30 minutes incubation with avidin-biotin complex (ABC, Vector Labs, CA, USA). For the anti-NPR-C primary antibody, the secondary antibody was conjugated to horseradish peroxidase (HRP) instead of biotin. Staining was visualized using 3, 3’-Diaminobenzidine (DAB) which was activated by H_2O_2. Finally, the sections were counterstained with Harris Haematoxylin and coverslipped with Entellan. The sections were scanned using an automatic bright field slide scanner (Philips IntelliSite Ultra-Fast Scanner, Digital pathology slide scanner, Netherlands) for microscopic evaluation.

All sections were evaluated for the presence/absence of NP or NPR staining in the grey matter (GM), white matter (WM) and cerebral vessels. The assessments were performed by two trained observers (SM and IV) who were blinded to the clinical diagnosis of the subjects. Disagreements (n=151 cases, 9.8% of the sections) between the two reviewers were resolved by discussion with the third reviewer (MB).

Table 1. Characteristics of study subjects

Diagnosis	Source	Age	Gender	PMD (h)	IHC	WB	CSF
Control	NBB	84	F	5:36	✓	✓	✓
Control	NBB	70	F	7:35	✓	NA	✓
Control	NBB	64	F	5:40	✓	NA	NA
Control	NBB	83	M	5:15	✓	NA	✓
Control	NBB	91	F	3:47	✓	✓	✓
Control	NBB	73	M	8:00	✓	✓	✓
Control	NBB	72	F	6:50	✓	✓	✓
Control	NBB	89	F	6:30	✓	✓	✓
Control	NBB	88	M	11:10	NA	NA	✓
Control	NBB	82	M	5:20	NA	NA	✓
Control	NBB	73	F	5:30	NA	NA	✓
Control	NABCA	82	M	5:30	✓	NA	NA
Control	NABCA	87	F	8:30	✓	✓	NA
Control	NABCA	72	F	7:15	✓	✓	NA
Control	NABCA	93	M	8:30	✓	✓	NA
Control	NABCA	82	M	7:30	✓	✓	NA
Control	NABCA	73	F	6:30	NA	✓	NA
AD	NBB	88	M	5:30	✓	✓	✓
AD	NBB	85	F	4:05	✓	✓	✓
AD	NBB	86	M	5:10	✓	NA	✓
AD	NBB	81	M	7:50	✓	✓	✓
AD	NBB	88	F	4:40	✓	✓	✓
AD	NBB	73	M	4:45	✓	✓	✓
AD	NBB	96	F	7:55	✓	✓	✓
AD	NBB	82	F	4:35	✓	NA	✓
AD	NBB	90	F	3:55	✓	✓	✓
AD	NBB	89	F	4:30	✓	✓	✓

Abbreviations: AD: Alzheimer's disease; NBB: Netherlands Brain Bank; NABCA: Netherlands Ageing Brain Collection Amsterdam; PMD: post-mortem delay (in hours); IHC: immunohistochemistry; WB: Western blot; NA: not available and CSF: cerebrospinal fluid

Protein isolation and Western blotting

The frozen brain tissues were chopped in GM and WM pieces on dry ice. We were not able to separate the WM of four AD patients due to severe atrophy of the brain tissue. Next, the GM and WM samples were suspended in lysis buffer (50 mM tris pH 7.5 and 1% triton in 10 ml Milli-Q) supplemented with cOmplete Mini Protease Inhibitor Cocktail Tablets (Roche). This was followed by homogenizing the samples in a bullet blender electric homogenizer (Next Advance) for 3 minutes using 0.5 mm stainless steel beads. The homogenized samples were centrifuged for 30 minutes at full speed before collection of the supernatants. Protein concentration was calculated using the bicinchoninic acid kit (Thermo Fisher Scientific, Waltham, USA) with bovine serum albumin as a standard. The supernatant was further diluted in sample buffer and denatured by boiling for 10 minutes in 95°.

We used 50 µg of the GM and WM protein lysates per sample. Proteins were separated by SDS-PAGE by running through a 4-20% sodium dodecyl sulphate polyacrylamide gel (Bio-Rad) alongside a proteins size marker (PageRuler^{TM}, Thermo Fisher Scientific). Proteins were transferred to a nitrocellulose membrane using the Trans-blot Turbo Transfer system (Bio-Rad) for 30 minutes at 1.0 A. The transfer of proteins was checked with Ponceau red followed by washing the membrane in Tris-Buffered Saline (TBS). The membrane was blocked in 5% low fat milk in TBS-Tween (mTBST) for 1 hour and incubated with primary antibody (diluted in 5% mTBST) at room temperature for 90 minutes, 2 hours or overnight at -4° (Supplementary table 1). After washing in TBS buffer, the membrane was incubated with secondary antibodies: anti-Rabbit or anti-Mouse IRDye800CW (LI-COR, Lincoln, USA) at 1:5000 for 1.5 hours. For β-actin loading control, the membrane was incubated with mouse β-actin at 1:5000 followed by a secondary anti Mouse IRDye680CW antibody (LI-COR, Lincoln, USA). Target bands were visualized using an Odyssey infrared imaging system (LI-COR). Target bands were visualized using an Odyssey infrared imaging system (LI-COR). The relative densities of the bands were measured using Image Studio Lite software (version 5.0).

CSF analysis using ELISA

The frozen CSF samples were shipped to Johns Hopkins University, Baltimore, USA, for ELISA experiments. ANP, BNP and CNP were measured using commercial ELISAs following the manufacturer's protocol (ALPCO, Salem, NH, USA), except that CSF was concentrated prior to analysis by centifuguation/lyophilisation. For ANP and BNP, samples were reconstituted or diluted in diluent 35 (Mesoscale Diagnostics, Rockville, MD, USA) and for a subset of donors CSF was spiked with a mid-range NP standard and recovery was assessed. A sandwich pro-ANP ELISA (1-98) was used to quantify ANP where CSF samples taken to dryness were resuspended in a volume to generate a 5-fold concentration. The sensitivity of the assay was 0.05 nmol/L and the dynamic range was 0.63 to 10 nmol/L. The amino terminal BNP fragment (nt-pro-BNP 8-29) was measured by competitive ELISA where CSF samples were also concentrated 5-fold. Under the conditions used in the laboratory, the sensitivity was 34 pmol/L and the dynamic range was between 40 and 1600 pmol/L. Determination of levels of amino-terminal pro-CNP was performed using a sandwich ELISA where CSF was diluted 1:30 with diluent 35 prior to analysis. The sensitivity was 0.7 pmol/L and the dynamic range was 0.1 to 128 pmol/L. When 9 CSF samples were spiked with 1.25 nmol/L pro-ANP, the recovery was 87 ± 9%. Nine samples spiked with 400 pmol/L pro-BNP yielded a recovery of 103 ± 5%, while for 9 samples spiked with 20 pmol/L pro-CNP the recovery was 93 ± 5%.

Statistical analysis

All data represent mean and standard deviations. Depending on the distribution of the outcome, unpaired two-tailed t-test or Mann-Whitney U test were used to compare between AD patients and controls. Statistical analyses were performed using SPSS software and a p-value <0.05 was considered as statistically significant.

Results

Gene expression of NP and NPR in the brain of healthy subjects

Figure 2 shows the mean expression of genes coding for NP and NPR across the brains of six healthy human donors from the Allen Human Brain Atlas. The NP and NPR genes were expressed throughout the CNS, although the different NP had distinctly diverse regional expression levels. In particular, the gene coding for ANP (*NPPA*) had the highest expression in cortical regions, followed by a lower expression in the telencephalic white matter regions (TEWM) and the lowest expression in the subcortical structures. In contrast, the gene coding for BNP (*NPPB*) had the highest expression in subcortical structures (basal ganglia), followed by a lower expression in the TEWM and very low expression throughout the cortical regions. The gene coding for CNP (*NPPC*) had the highest expression in subcortical structures (thalamic nuclei), followed by a lower expression in the cortical regions and the lowest expression in TEWM. The gene coding for NPR-A (*NPR1*) had the highest expression in subcortical structures (basal ganglia and thalamic nuclei), followed by a lower expression in cortical regions and the lowest expression in the TEWM. The genes coding for NPR-B (*NPR2*) and NPR-C (*NPR3*) had the highest expression in subcortical structures followed with a very low expression in cortical and TEWM regions

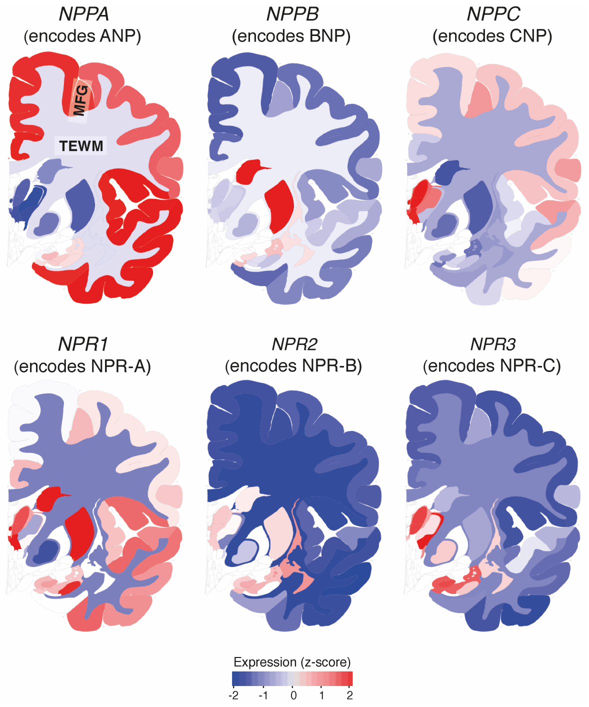


Figure 2. Expression of natriuretic peptides and natriuretic peptide receptors genes in the brain of healthy subjects. The mean mRNA expression of genes coding for natriuretic peptides and natriuretic peptide receptors is shown in a coronal view of the left hemisphere. The Expression of each gene is z-score normalized to the average expression of the gene in the whole brain. Data from the Allen Human Brain Atlas (http://human.brain-map.org/) is visualised using BrainScope (http://w ww.brainscope.nl/brainscope). MFG: middle frontal gyrus; TEWM: telencephalic white matter.

Localization of NP and NPR in the frontal lobe

In the cortex of control subjects, the immunohistochemistry study showed positive staining of all NP and NPR proteins in the neuronal structures. ANP, BNP and CNP positive stainings were observed in the cytoplasmic body and neuronal processes of pyramidal neurons (layers II-VI) (Figure 3A, 3B and 3C, respectively). BNP-positive staining was mainly localized to Nissl bodies that were characterized as granular bodies within the cytoplasm of neurons (Figure 3B). In addition, the CNP-positive staining was observed in networks of short and long fibers throughout the cortex (Supplementary Figure 4A). NPR-A, NPR-B and NPR-C positive stainings were also localized to the cytoplasmic body and neuronal processes of pyramidal neurons (layers II-VI) (Figure 5A, 5B and 5C, respectively). Furthermore, a strong NPR-A positive staining was observed in the Nissl body of neurons throughout the cortex. These Nissl bodies were characterized as large granular bodies in the cytoplasm with a stronger staining pattern compared to the cytosol of neurons (Supplementary Figure 4B). In addition to the cytoplasm, the NPR-B-positive neurons also showed a prominent staining in the nucleolus of neurons (Supplementary Figure 4C). We observed no staining in the negative control sections using only the secondary antibody (Supplementary Figure 1).

In the WM, we observed ANP-positive astrocytes-like structures that consisted of short packed processes (Figure 3D). These astrocytes-like structures were spread throughout the WM, occasionally associated with blood vessels and their cell bodies were not always distinct. Some ANP-positive astrocytes-like structures were also observed throughout the cortical layers. Similarly, we observed NPR-positive astrocyte-like staining in the WM of some control subjects (Figure 5D, 5E and 5F). We did not observe BNP or CNP-positive astrocyte-like structures in the WM or GM (Figure 3E and 3F). Other glial cells were not stained for NP or NPR.

In the cerebral vessels, ANP-positive staining was observed in the leptomeningeal and parenchymal vessels, and was localized to the endothelium and smooth muscle layers (Figure 3G). BNP-positive staining was only weakly present in the endothelium of some leptomeningeal vessels (Figure 3H), while CNP staining was not detected in the cerebral vessels at all (Figure 3I). NPR-A and NPR-B stainings were positive in the endothelium and smooth muscle layers of leptomeningeal and parenchymal vessels (Figure 5G and 5H), while NPR-C staining was less prominent in the cerebral vessels (Figure 5I). Supplementary Figure 2 and 3 show the localization of the NP and NPR in the frontal lobe of AD patients. We observed a similar pattern of NP and NPR staining in the frontal lobe of AD patients and control subjects.

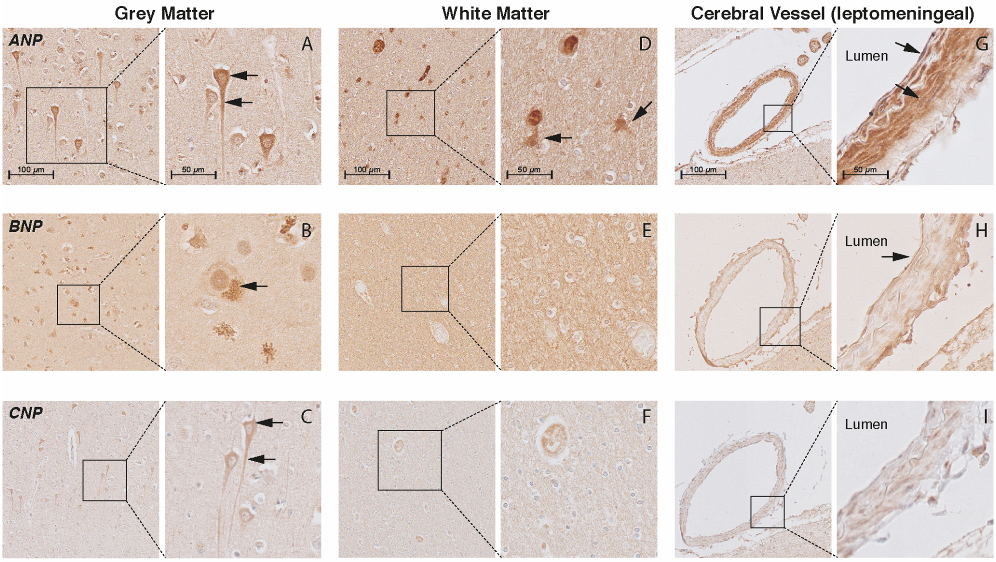


Figure 3. Localization of ANP, BNP and CNP in the frontal lobe of non-demented subject. NP immunohistochemistry in the frontal lobe (middle frontal gyrus) of non-demented control subjects. A, B and C) ANP, BNP and CNP-positive neurons. Arrows point to cytoplasm (ANP and CNP), neuronal processes (ANP and CNP) and Nissl bodies (BNP); D) ANP-positive astrocyte-like cells in the white matter; E and F) negative BNP and CNP staining in the white matter; G) ANP-positive endothelium and smooth muscles; H) BNP-positive endothelium and I) negative CNP staining in the leptomeningeal vessels.

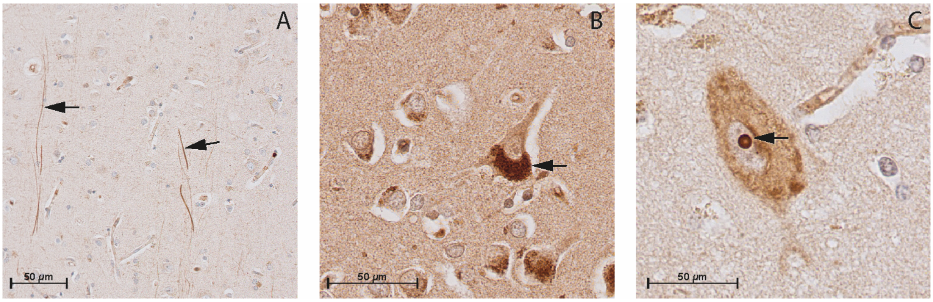


Figure 4. Immunohistochemistry of CNP, NPR-B and NPR-A in the grey matter. A) Immunohistochemistry of CNP in the grey matter. Arrows point to CNP-positive fibres in the grey matter; B) Immunohistochemistry of NPR-A in the grey matter. Arrow point to the granular bodies resembling Nissl bodies in the cytoplasm of neuron and C) Immunohistochemistry of NPR-B in the grey matter. Arrow point to the nucleolus of the neuron.

Quantitative comparison of NP and NPR between AD and control subjects

For quantitative comparisons in brain tissue, we performed Western blots using GM and WM samples isolated from each donor. Using an antibody against NPR-A, we observed specific bands of ~150 kDa in both AD patients and controls. The NPR-A specific bands were highly pronounced in the GM while no or very weak bands were present in the WM (Supplementary Figure 4). Bands reacting with anti-NPR-C antibody were observed at ~60 kDa in both AD patients and controls; and in GM and WM samples (Supplementary Figure 4). We were not able to detect any NPR-B specific signals despite the use of different antibodies. Figure 6 shows quantification of Western blot bands in the GM of AD patients and controls. We found significantly higher amounts of NPR-A in the GM of AD patients compared to controls (t(16)= -2.18, p=0.045, independent sample t-test). NPR-C signals appeared to be higher in the GM of controls, although the differences between AD patients and controls were not statistically different (Mann-Whitney U=18.0, p=0.051 two-tailed). In the WM, none of the Western blot bands was significantly different between AD patients and controls (data not shown).

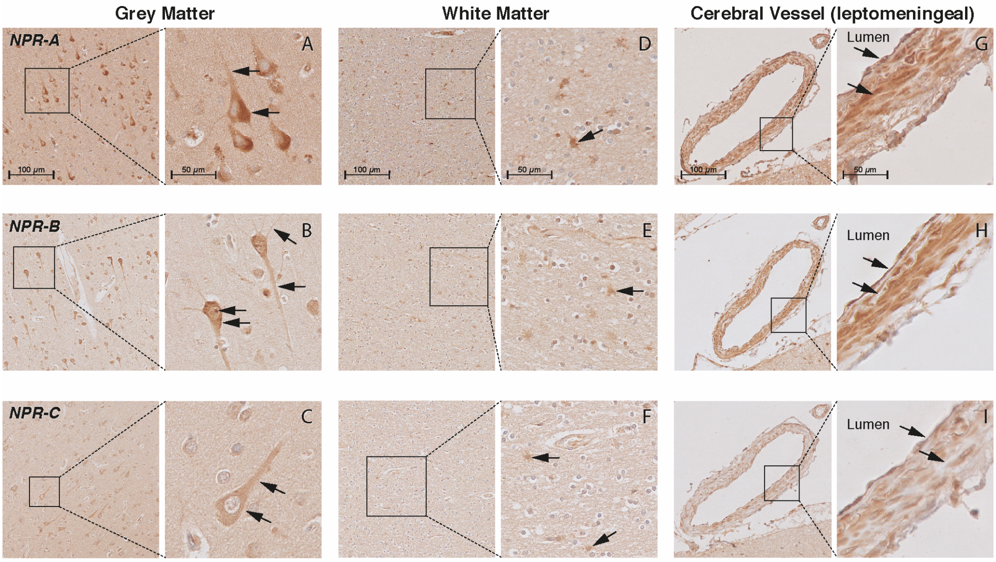


Figure 5. Localization of NPR-A, NPR-B and NPR-C in the frontal lobe of non-demented subject. NPR immunohistochemistry in the frontal lobe (middle frontal gyrus) of non-demented control subjects. A, B and C) NPR-A, NPR-B and NPR-C-positive neurons. Arrows point to cytoplasm (all NPR), neuronal processes (all NPR) and Nissl bodies (NPR-A); D, E and F) NPR-A, NPR-B and NPR-C-positive astrocyte-like cells in the white matter; G, H and I) NPR-A, NPR-B and NPR-C-positive endothelium and smooth muscles in leptomeningeal vessels.

When comparing between GM and WM, NPR-A-specific signals were significantly higher in GM compared to WM ($p < 0.001$), while NPR-C-specific signals were not different between GM and WM. Comparison of the gene expression data (Figure 2) with our Western blot findings (Figure 6) showed similar patterns: the gene coding for NPR-A was highly expressed in the cortex (expression z-score > 0) compared to the TEWM (expression z-score <-1). On the other hand, the gene coding for NPR-C had low mRNA expression in both GM and WM (expression z-score <0 in GM and WM).

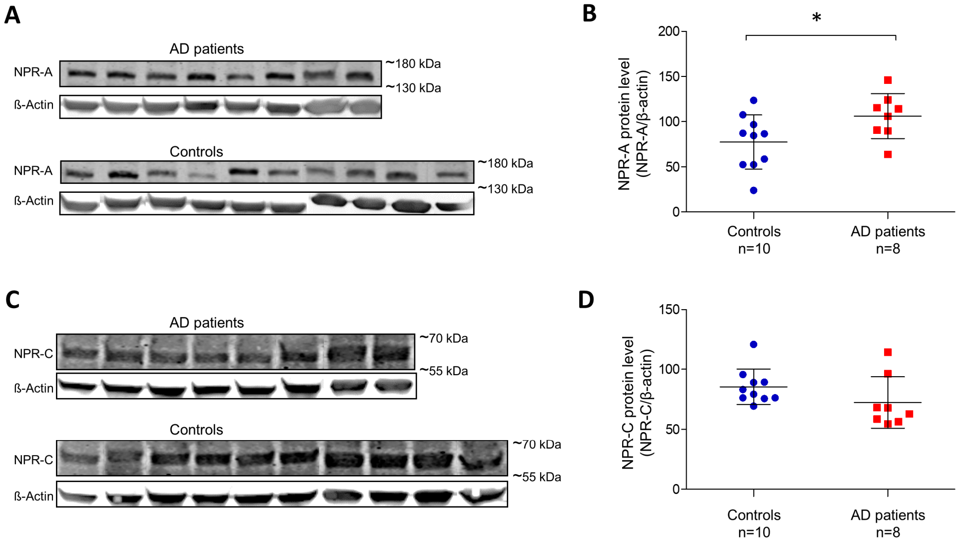


Figure 6. Relative amounts of NPR-A and NPR-C in the grey matter of non-demented controls and Alzheimer's disease patients. A) NPR-A and β-Actin Western blot bands in AD patients and non-demented controls; B) quantification of NPR-A Western blots between AD patients and controls; C) NPR-C and β-Actin Western blot bands in AD patients and non-demented controls; D) quantification of NPR-C Western blots between AD patients and controls. Bars show the mean (standard deviation) of the normalized signals. Analyses were performed using t-test for NPR-A and Mann Whitney U test for NPR-C. *show p-value <0.05. Abbreviations: NPR-A: natriuretic peptide receptor A; NPR-C: natriuretic peptide receptor C and AD: Alzheimer's disease.

Quantitative comparison of NP in the CSF between AD patients and controls

ANP, BNP and CNP levels in the CSF ranged from 56.48 to 192.36 pmol/L, 44.12 to 127.39 pmol/L and 125.25 to 1180.13 pmol/L, respectively. Figure 7 shows the comparison of ANP, BNP and CNP levels between AD patients and controls in post-mortem CSF. We observed significantly lower amounts of BNP in the CSF of AD patients compared to controls (Mann-Whitney U = 21.0, p=0.029 two-tailed). Similarly, ANP and CNP levels appeared to be lower in the CSF of AD patients although these differences were not statistically significant (Figure 7).

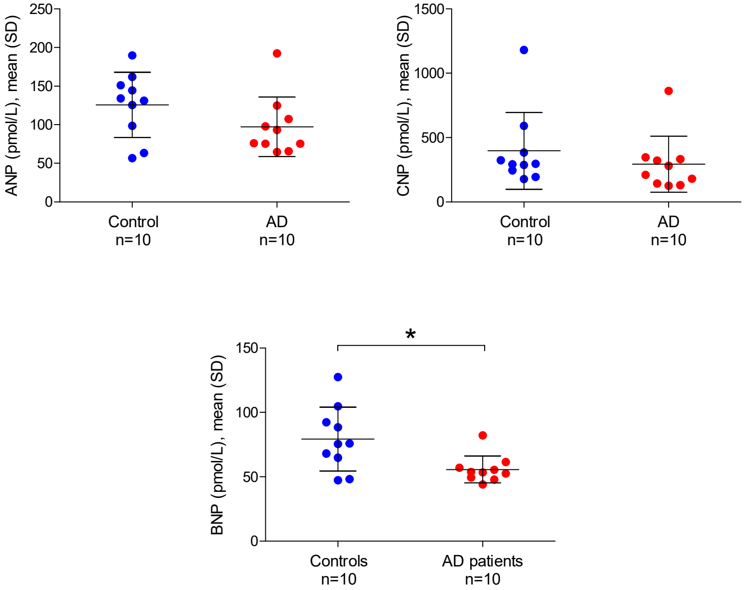


Figure 7. ANP, BNP and CNP in the post-mortem cerebrospinal fluid of non-demented controls and Alzheimer's disease patients. Bars represent the mean (standard deviation) of each NP in the cerebrospinal fluid of AD patients and controls. Analyses were performed using Mann Witney U test. *shows p-value <0.05. Abbreviations: CSF: cerebrospinal fluid; AD: Alzheimer's disease; ANP: atrial natriuretic peptide; BNP: brain natriuretic peptide; CNP: C-type natriuretic peptide and SD: standard deviation.

Discussion

In this study, we first showed that the genes coding for NP and NPR are ubiquitously expressed across the brain of healthy humans. We detected NP and NPR proteins in neuronal structures, cerebral vessels, and in structures resembling astrocytes in the frontal lobe of non-demented individuals and patients with AD. When comparing between AD patients and non-demented controls, NPR-A levels were higher in the brain tissue of AD patients while BNP levels were lower in the CSF of AD patients.

Previous animal studies have detected the expression and localization of NP and NPR in numerous structures of the CNS[13]. For example, ANP was detected in neuronal structures of the hypothalamus, telencephalon and cerebellum [5], and in the astrocytes of human and canine cortex[24, 25]. Similarly, BNP and CNP were found to be widely distributed in the neuronal structures of the cerebral cortex, hypothalamus, spinal cord and retina[5, 13]. Furthermore, the receptors for ANP and BNP were detected on the luminal membrane of cerebral vessels *in vitro* and *in vivo*[26, 27]. Despite the wealth of evidence from animal studies, limited data on the distribution and expression of NP and NPR in the brain of human subjects is available. In this study, we extended the evidence from animal species to human subjects by providing a detailed mapping of NP and NPR in the frontal lobe of human subjects. As in animal species, in humans the expression of NP and NPR genes seems to be complementary in various brain regions[28]. This suggest that the NP are not only produced in cardiac myocytes, but are also locally produced in the CNS of humans[5]. Furthermore, we found that NP and NPR are present in various cellular structures of the frontal lobe, suggesting a wide range of NP functions in the human brain. In particular, the presence of NP and NPR in both neuronal and cerebral vessels might indicate a potential role of NP in neurovascular functioning[29]. In line with this, recent *in vitro* studies have shown that administration of ANP and BNP resulted in a significant dose-dependent relaxation of the middle cerebral artery and basilar artery[30]. On the other hand, NP might also be involved in synaptic transmission and processing of information. In support of this, animal and *in vivo* studies have demonstrated that NP regulate the release and re-uptake of neurotransmitters such as noradrenalin, dopamine and glycine[13]. Furthermore, we could also detect ANP and NPR in structures resembling astrocytes in the WM. This is consistent with previous studies showing the presence of ANP and NPR in astrocyte of the cortex[24], suggesting a potential role of astrocytes in physiological functions of NP in the brain[13]. Collectively, our qualitative and gene expression data are consistent with previous animal studies indicating NP as neuropeptides that might regulate several functions in the brain[31]. Nevertheless, future research is needed to further disentangle the putative role(s) of centrally acting NP in the brain of human subjects.

Given the involvement of NP in several of the pathological pathways that are also disturbed during the course of AD, we have recently hypothesized NP as potential markers for diagnosis and/or treatment of AD[13]. We postulated that decreased action of NP in the brain might impair the structural and/or functional integrity of the brain and predispose subjects to a higher risk of cognitive decline[13]. In line with this hypothesis, we found lower levels of BNP in the CSF of AD patients, coupled with higher amounts of NPR-A in the brain tissue of AD patients. This may suggest impaired function of NP in the brain of AD patients, which could in turn accelerate neuro-inflammation, oxidative stress and neurodegeneration. One explanation for reduced levels of BNP in the CSF could be attributed to their elevated levels in the systemic circulation. In fact, systemic and central NP might act in a feedback loop such that increased NP in the plasma inhibits production and/or biological activity of NP in the brain[13, 32]. In line with this, previous research has shown that higher plasma BNP in ovine sheep associated with decreased BNP levels in the hypothalamus[32]. Interestingly, recent findings in humans suggest that higher plasma BNP associates with lower CSF Aβ42 and higher t-tau/Aβ42 ratios[18]. Collectively, our pilot results point towards a potential role of NP in the pathology of AD. Nevertheless, larger scale studies are needed to replicate our findings and explore the potential association of centrally acting NP with markers of neurodegeneration.

We used frontal lobe to detect NP and NPR in the brain tissue of AD patients and non-demented controls. Frontal lobe involvement is a well-described feature of AD pathology [33-35]. Furthermore, atrophy of the middle frontal gyrus has been demonstrated in patients with mild cognitive impairment[36]. It is worth mentioning that previous research have detected NP and NPR in other brain regions such as thalamus and hypothalamus[28]. In animal models, the highest concentration of NP and NPR were reported in various nuclei of hypothalamus[28]. Consistent with this, we observed high expression of NP and NPR in the subcortical structures and hypothalamus of healthy humans. In this study, future studies focusing on other brain regions are needed to further disentangle the localization and function of NP in different brain regions of humans.

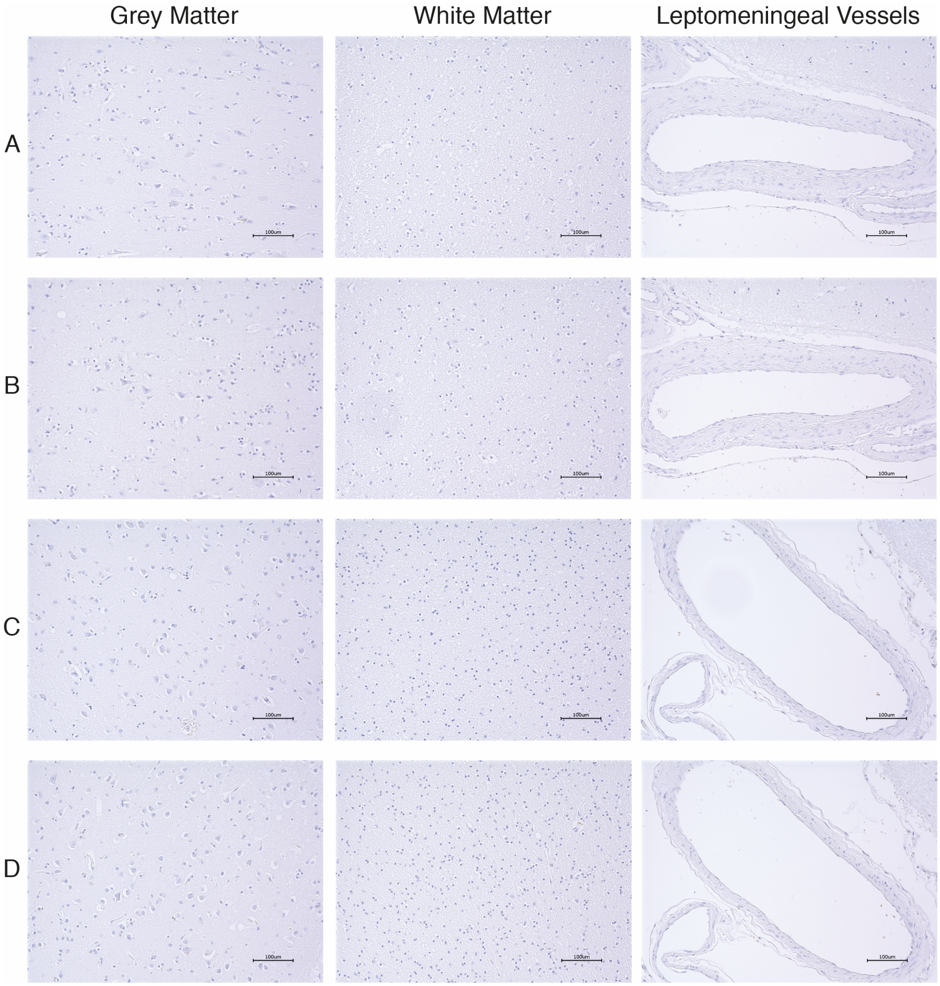
In summary, we showed the widespread presence of all NP and their receptors in the brain of humans. In line with our hypothesis, we observed higher amounts of NPR-A in the brain tissue and lower levels of BNP in the CSF of AD patients. These findings further highlight that NP may be potential markers for AD. Given the widespread distribution of NP in different structures of the human brain, future research should determine the specific function of NP in the brain of humans, in health and disease.

Supplementary Material

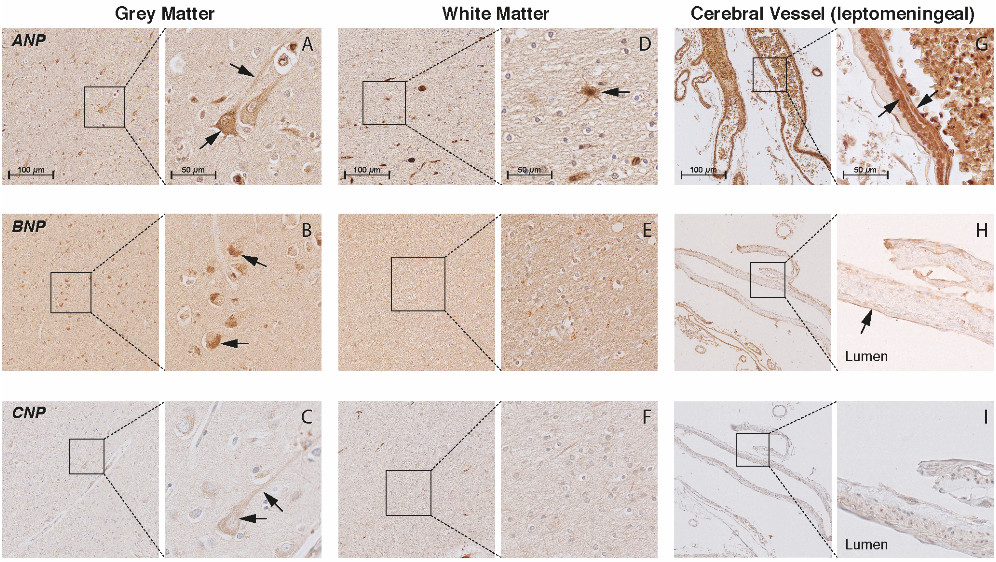
Supplementary Table 1. List of antibodies in this study

ID	Company	Description	Host	Antigen recognized	AR
ab14356	Abcam	Anti-NPRA	Rb (pc)	aa 294-308 of h-NPRA	+
ab55724	Abcam	Anti-NPRB	Ms (mc)	aa 131-231 of h-NPRB	+
ab37617	Abcam	Anti-NPRC	Rb (pc)	aa 67-97 (N terminal) h-NPRC	+
ab19646	Abcam	Anti-BNP	Rb (pc)	h-BNP	+
ab91250	Abcam	Anti-ANP	Rb (pc)	aa 30-56 of h-ANP	–
HPA035362	Sigma	Anti-NPPC	Rb (pc)	aa 29-126 of h-CNP	+

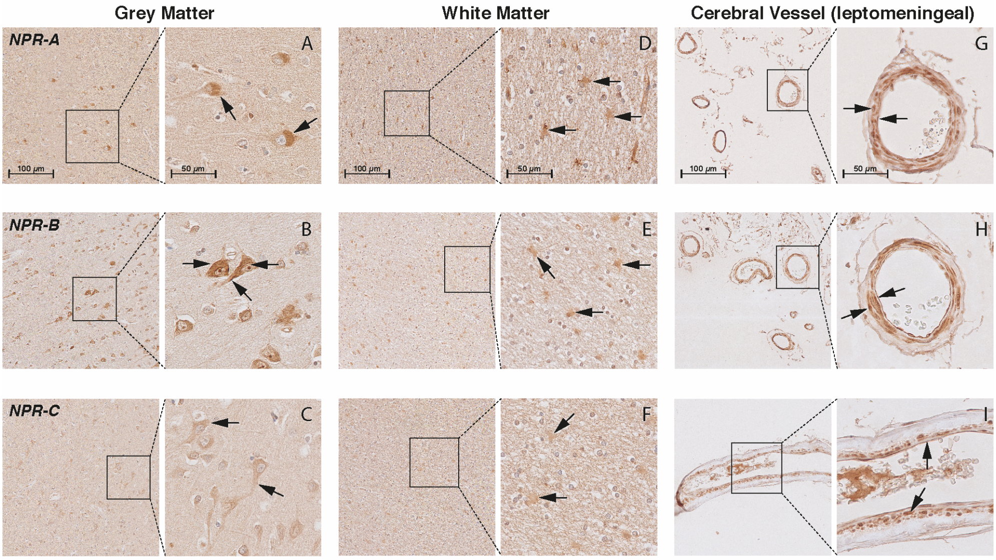
Abbreviations: Rb: rabbit; Ms: mouse; pc: polyclonal; mc: monoclonal; AR: antigen retrieval; h: human and aa: amino acid.



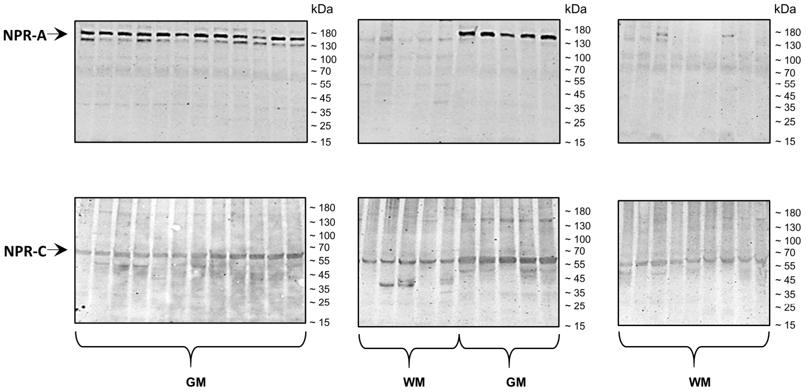
Supplementary Figure 2. Negative controls treated only with secondary antibodies. A) Frontal cortex of a control subject incubated only with secondary goat anti rabbit antibody and B) secondary rabbit anti mouse antibody. C) Frontal cortex of an AD patient incubated only with secondary goat anti rabbit antibody and D) secondary rabbit anti mouse antibody



Supplementary Figure 2. Localization of ANP, BNP and CNP in the frontal lobe of AD subjects. NP immunohistochemistry in the frontal lobe (middle frontal gyrus) of Alzheimer's disease patients. A, B and C) ANP, BNP and CNP-positive neurons. Arrows point to cytoplasm (ANP and CNP), neuronal processes (ANP, and CNP) and Nissle bodies (BNP); D) ANP-positive astrocyte-like cells in the white matter; E and F) negative BNP and CNP staining in the white matter; G) ANP-positive endothelium and smooth muscles; H) BNP-positive endothelium and I) negative CNP staining in the leptomeningeal vessels.



Supplementary Figure 3. Localization of NPR-A, NPR-B and NPR-C in the frontal lobe of AD subjects. NPR immunohistochemistry in the frontal lobe (middle frontal gyrus) of Alzheimer's disease patients. A, B and C) NPR-A, NPR-B and NPR-C-positive neurons. Arrows point to cytoplasm (all NPR), neuronal processes (all NPR) and Nissl bodies (NPR-A); D, E and F) NPR-A, NPR-B and NPR-C-positive astrocyte-like cells in the white matter; G, H and I) NPR-A, NPR-B and NPR-C-positive endothelium and smooth muscles in leptomeningeal vessels



Supplementary Figure 4. Western blots of natriuretic peptide receptors. Each subject has one band corresponding to grey matter and one band corresponding to white matter. All images were scanned using the Image Studio software with the same resolution and image quality. Abbreviations: NPR-A: natriuretic peptide receptor A; NPR-C: natriuretic peptide receptor C; GM: grey matter and WM: white matter.

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CHAPTER 8

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Discussion

In this thesis, the current knowledge on the heart-brain connection was extended by providing further evidence for potential mechanisms linking suboptimal cardiac function with cognitive impairment and functional decline. Another important aspect of this thesis is the preliminary results on the role of cardiac hormones in cognitive impairment, indicating a complex interplay between the heart and the brain that might extend beyond vascular factors. This chapter reviews the key findings of this thesis, discusses them in the context of current knowledge and provides perspectives for future research.

ECG-derived Markers of Cardiac Dysfunction, Cognitive Impairment and Functional Decline

Cardiac autonomic dysfunction as reflected in heart rate and heart rate variability

Elevated heart rate and reduced heart rate variability are markers of cardiac autonomic dysfunction[1]. These markers reflect failure of the autonomic nervous system in regulation of cardiovascular homeostasis[2]. The prognostic importance of high heart rate and low heart rate variability have been demonstrated for a wide range of pathogenic states and for mortality. For example, low heart rate variability has been linked to inflammation[3], insulin resistance[4] and cardiovascular factors[2, 5]; and high heart rate has been related to atherosclerosis and endothelial dysfunction[6]. Interestingly, it has been hypothesized that high heart rate and low heart rate variability are potential markers of frailty and functional decline[7]. One potential mechanism linking these markers with functional decline might be through the detrimental effect of cardiovascular factors on both cardiac autonomic control and functional status[5, 8]. In **Chapter 2**, we show that higher heart rate and lower heart rate variability are directly linked with higher risk of functional decline independent of cardiovascular risk factors and comorbidities. These findings highlight the link between cardiac autonomic imbalance and development of functional decline in future, even in subjects with preserved functional status at baseline. In this setting, future research should determine whether interventions aimed at preservation of cardiac autonomic control are beneficial for functional status. In **Chapter 3**, we tested whether cardiac autonomic dysfunction – as measured by heart rate variability – also relates to cognitive decline in older subjects. **Chapter 3** shows that lower heart rate variability associates with worse performance and steeper decline in executive function of older subjects at high cardiovascular risk. Our findings indicate a direct link between low heart rate variability and accelerated cognitive decline, independent of several cardiovascular risk factors, co-morbidities and medication use. The observed direct link between low heart rate variability and cognitive decline might be attributed to a chronic failure of the autonomic nervous system in sustaining adequate cerebral perfusion[9]. A long lasting cerebral hypo-perfusion may in turn hamper the neurovascular integrity of the brain and contribute to brain functional decline[10]. On the other hand, given that low heart rate variability was mainly associated with decline in executive function, the potential mediating effect of unmeasured vascular factors needs to be further addressed in future research. Nevertheless, our findings points toward a potential role of cardiac autonomic disturbance in accelerated cognitive brain ageing and functional decline in older subjects.

Cardiac structural changes as reflected in left ventricular hypertrophy

Cerebral blood flow provides the brain with an adequate and constant supply of oxygen and nutrients[11]. Long-term cerebral hypo-perfusion has been shown to negatively affect the structural and functional integrity of the brain in animals[12]. In humans, the effect of cerebral hypo-perfusion on the brain has been frequently studied in congestive heart failure patients[13]. Patients with advanced heart failure have around 30% lower cerebral blood flow compared to normal healthy subjects[14]. Furthermore, cognitive impairment, memory problems and confusion are common in heart failure patients[15]. Recent studies suggest that not only heart failure, but also a graded decrease in cardiac functioning associates with features of brain ageing[16]. It was shown that each unit lower cardiac output associates with 3.9 mL lower total brain volume and grey matter volume[17]. In addition, cardiac index (cardiac output/body mass surface) was shown to positively correlate with total brain volume and information processing speed, while it was negatively correlated with lateral ventricular volume[18]. Whether subjects with a suboptimal cardiac function free of heart failure are at increased risk of dementia is yet to be determined. In **Chapter 4**, we show that left ventricular hypertrophy measured from 12-lead ECG recordings associates with accelerated cognitive decline, even in subjects free of heart failure. While the effect of cardiovascular factors and cognitive impairment has been mainly attributed to a sequence of pathologic events[19], we provided evidence on the direct link between cardiac dysfunction and cognitive impairment independent of cardiovascular risk factors and co-morbidities. These findings shed light on the potential role of early structural changes in the heart – as reflected in left ventricular hypertrophy – in development of cognitive impairment. The complex mechanism(s) underlying these associations, however, needs to be further studied to allow for the development of targeted treatment strategies in future.

Cardiac electrical abnormality as reflected in spatial QRS-T angle

Growing evidence indicates that cardiac electrical abnormalities are linked with future risk of cerebrovascular accidents[20]. In fact, ECG markers such as pathologic Q waves and QRS/QT duration have emerged as potential predictors of stroke[20-22]. In addition, ventricular depolarization abnormality – as reflected in abnormal Q-waves – has been related to a higher load of cerebral small vessel disease[23]. A well-established tool for characterization of cardiac electrical changes is the spatial QRS-T angle[24]. The power of spatial QRS-T lies in its ability to assess ventricular repolarization abnormalities secondary to depolarization changes[25]. Particularly, a widened spatial QRS-T angle reflects greater heterogeneity of cardiac electrical activity due to either distortion of cardiac ionic channels or micro-myocardial damage that distorts the spread of electrical forces through the myocardium[26]. The prognostic utility of wide spatial QRS-T angle has been shown in a variety of pathologic states such as ventricular arrhythmias, sudden cardiac death and cardiovascular mortality[24]. On the other hand, an abnormally wide QRS-T angle >90° has been associated with ischemic stroke[27]. In **Chapter 5** of this thesis, a wider spatial QRS-T angle was found to associate with accelerated decline in executive and memory function of older subjects at high cardiovascular risk. The observed association was independent of cardiovascular diseases, occurrence of cardiovascular events during follow-up, medication use and other ECG markers of cardiac electrical abnormality. Therefore, we postulated that localized pathologic changes in the heart as reflected in wide spatial QRS-T angle may result in subtle hemodynamic disturbances[24]. These hemodynamic disturbances may in turn contribute to cerebral small vessel disease and ultimately parenchymal brain damage and cognitive impairment[28, 29]. These findings extend the current knowledge on the heart-brain connection by showing a link between subtle alterations in the electrical activity of the heart and accelerated cognitive decline in old age. In this setting, effective characterization of cardiac electrical abnormalities may provide a deeper understanding of the pathologic pathways connecting cardiac dysfunction with accelerated cognitive decline.

Neuro-hormonal Aspects of the Heart-Brain Connection: Beyond Vascular Factors

The physiological connection between the heart and the brain comprises a complex interaction of vascular, neuronal and hormonal factors[16, 30]. Nevertheless, studies of the heart-brain connection have mostly focused on vascular factors and there is limited research on the effect of hormonal mediators. Natriuretic peptides have long been known as cardiac hormones that are released into the systemic circulation in response to cardiac wall stretch and systemic overload[31]. Growing evidence, however, indicates that natriuretic peptides are not only present in the systemic circulation, but are also abundant in the central nervous system[32]. In fact, animal studies showed that natriuretic peptides are locally expressed in the central nervous system and their receptors are located in numerous regions of the brain[33]. These remarkable findings have opened a new area of research focusing on the biological roles of natriuretic peptides in the central nervous system of animals and humans[34]. In **Chapter 6** of this thesis, we provided a comprehensive overview of the existing literature on the role of natriuretic peptides in the central nervous system of animal species and human subjects. The available biological and clinical evidence points toward the essential involvement of natriuretic peptides in multiple key functions of the brain including inflammation and oxidative stress, synaptic transmission, brain fluid homeostasis and the stress response of the central nervous system through the hypothalamus-pituitary-adrenal axis[32-34]. Furthermore, we postulated a cross-talk between central and systemic levels of natriuretic peptides such that increased levels of natriuretic peptides in the systemic circulation might result in reduced action of central natriuretic peptides[35]. Finally, given the involvement of natriuretic peptides in several key pathways that are also disturbed during the course of cognitive impairment[32, 36], we hypothesized that natriuretic peptides could be novel markers for diagnosis and/or treatment of cognitive impairment. To substantiate this hypothesis, in **Chapter 7** we performed pilot experiments using the post-mortem human tissue and cerebrospinal fluid of non-demented controls and Alzheimer's disease human subjects. Given the limited evidence on the gene expression and localization of natriuretic peptides in the brain of human subjects, we first provided detailed cellular mapping of natriuretic peptides and their receptors in the brain of non-demented controls, as well as of their gene expression in the brain of healthy humans. Our results indicate the presence of natriuretic peptides and their receptors in various neuronal structures such as neuronal cytoplasm, neuronal processes and Nissl bodies. Furthermore, we showed that natriuretic peptides and their receptors are abundantly present in cerebral leptomeningeal and cerebral parenchymal vessels. We also demonstrated the gene expression of natriuretic peptides and their receptors in the central nervous system of healthy human subjects indicating the local production of natriuretic peptides in the brain. Taken together, these findings provide further evidence on the biological importance of natriuretic peptides in the central nervous system of human subjects. In particular, our findings of presence of natriuretic peptides in both neuronal and cerebral vessels suggest a potential role of natriuretic peptides in regulation of the blood-brain barrier integrity and/or neurovascular coupling. When comparing between controls and Alzheimer's disease patients, we showed that the level of natriuretic peptide receptor type A (NPR-A) is higher in the brain of Alzheimer's disease patients, while its ligand

– natriuretic peptide type B – is decreased in the cerebrospinal fluid of Alzheimer's disease patients. This is consistent with our earlier provided hypothesis indicating up-regulation of NPR-A which might occur in response to decreased natriuretic peptides activity in the brain[35]. An increased understanding of the complex role of natriuretic peptides in the brain of human subjects is needed to further disentangle the potential involvement of natriuretic peptides in cognitive impairment.

Conclusions and Future perspectives

The demographic shift in the life expectancy of the populations worldwide has resulted in a rapid rise of seniors with multiple age-related medical conditions[37]. Cognitive impairment and functional disability are prevalent disorders of old age[38]. Nevertheless, current strategies in reducing the progression of cognitive impairment and functional decline have been largely unsuccessful[39]. Therefore, there is an urgent need for identification of effective risk factors in an early stage[40]. The findings from **Chapter 2, 3, 4** and **5** of this thesis points toward a direct link of suboptimal cardiac dysfunction – as detected by ECG – with cognitive impairment and functional decline in old age. In particular, we show that cardiac autonomic dysfunction, cardiac structural changes and subtle cardiac electrical abnormalities may contribute to accelerated cognitive decline. Moreover, we show that cardiac autonomic dysfunction also relates to a higher risk of functional decline in older subjects. The findings from this thesis are timely given the growing research and awareness on the role of cardiovascular pathologies in relation to cognitive brain ageing and functional decline[41]. As such, detection of cardiac changes in an early stage prior to manifestation of overt cardiovascular diseases may provide effective strategies in risk stratification of subjects at increased risk of dementia[28]. In line with this, several ECG-markers of cardiac dysfunction such as left ventricular hypertrophy and atrial fibrillation have already been implemented in risk stratification of stroke patients[20, 42]. Whether such markers provide accurate prognostic information for subjects at increased risk of cognitive impairment and functional decline is a matter of future research. Furthermore, we performed our analyses using a well-established cohort of older subjects at high risk for cardiovascular diseases[43]. Although cardiovascular diseases are common in old age, the generalizability of our findings to healthy elderly subjects should be determined in future research. On the other hand, the observational nature of our studies does not imply causality. Therefore, future intervention strategies are needed to determine the effect of improved cardiac hemodynamic in decelerating the peace of cognitive and functional decline.

Another important aspect of this thesis is the hypothesis proposed along with preliminary findings about the potential role of natriuretic peptides in cognitive impairment as presented in **Chapters 6 and 7**. We proposed natriuretic peptides as novel markers for cognitive impairment and provided indications about their local production in the brain of human subjects. Importantly, our results extend the findings from animal studies[32, 34] to human subjects and provides evidence on the abundant distribution and localization of the natriuretic peptide family in the brain of human subjects. On the basis of our findings, we also postulate a potential up-regulation of natriuretic peptide receptors in the brain of Alzheimer’s disease patients. It is important to mention that the biological role of natriuretic peptides in the brain of human subjects is largely unexplored and therefore remains mostly unknown[32]. Therefore, it is critical to carry out more research in unrevealing the biological pathways that are critical for functioning of natriuretic peptides in the human brain. On the other hand, while our findings are based on post-mortem experiments, future research should focus on exploring the implication of our findings *in vivo.* Given our findings about the abundant distribution of natriuretic peptides in the vascular structures, research aiming at unravelling the potential contribution of natriuretic peptides in regulation of blood-brain barrier integrity and/or neurovascular coupling is needed. Finally, we should keep in mind that the brain is a complex organ and multiple pathways act together to maintain the structural and functional integrity of the brain[37, 44]. This extraordinary level of complexity calls for more sophisticated approaches for a deeper understanding of the mechanisms underlying neurodegenerative disorders in the brain.

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CHAPTER 9

SUMMARY/SAMENVATTING

Summary in English

This thesis aimed to investigate the link between cardiac biomarkers, cognitive impairment and functional decline in older subjects with a focus on non-invasive markers that are routinely available in clinical practice. The findings of this thesis show that markers of subclinical cardiac dysfunction are linked with accelerated cognitive decline and a higher risk of functional decline. In addition, we provide preliminary findings on the potential role of centrally acting natriuretic peptides in relation to cognitive impairment. Collectively, these findings highlight a complex pathophysiological heart-brain coupling that might extend beyond vascular and hemodynamic factors. Future studies are needed to investigate whether such link warrants mutual screening of the heart and the brain functions, and whether such practice could improve early diagnosis of dementia.

Following is a summary of the chapters in this thesis:

Chapter 1 provides a background on the burden and demographics of cognitive impairment and functional decline in old age. Furthermore, a background on the potential pathophysiological pathways connecting cardiac dysfunction with cognitive impairment is provided. Finally, a brief description of non-invasive cardiac biomarkers used in this thesis to characterize cardiac dysfunction are highlighted.

Chapter 2 shows that higher heart rate and lower heart rate variability are associated with worse functional status and with higher risk of functional decline in older subjects at high cardiovascular risk. We showed that the found link between heart rate, heart rate variability and functional decline was independent of cardiovascular factors, cardiovascular events and medication use. We also discuss the potential pathophysiological mechanisms linking markers of cardiac autonomic dysfunction to functional decline.

Chapter 3 shows that lower heart rate variability, a marker of cardiac autonomic dysfunction, associates with worse executive function and with accelerated decline in executive function of older subjects at high cardiovascular risk. We showed that the found link between heart

rate variability and impairment in executive function was independent of several measured cardiovascular factors, comorbidities and medication use.

Chapter 4 highlights the role of subclinical cardiac remodeling in development of cognitive decline in future. We show that left ventricular hypertrophy measured from ECG, a maker of cardiac structural abnormality, associates with accelerated decline in several domains of cognitive function in older subjects at high cardiovascular risk. The found link between left ventricular hypertrophy and cognitive decline was independent of cardiovascular risk factors and co-morbidities, occurrence of cardiovascular events during follow-up and medication use.

Chapter 5 shows a link between cardiac electrical abnormality and accelerated cognitive decline. We showed that a wider spatial QRS-T angle derived from ECG– a marker of greater inhomogeneity in cardiac electrical activity – associates with accelerated decline in several domains of cognitive function in older subjects at high cardiovascular risk. In addition, we showed that the link between spatial QRS-T angle and cognitive decline was independent of other ECG abnormalities, cardiac arrhythmias, measured cardiovascular factor, cardiovascular events and medication use.

Chapter 6 provides a detailed review on the role of natriuretic peptides in the central nervous system of human subjects. Importantly, **Chapter 6** discusses the relevance of natriuretic peptides to cognitive impairment in the context of amyloid beta and tau protein depositions, neurovascular dysfunction, neuro-inflammation, neuronal damage, synaptic failure, anxiety and disturbed fluid homeostasis in the brain. Based on the available evidence, we hypothesized that natriuretic peptides in the central nervous system might be potential novel markers for diagnosis and/or treatment of cognitive impairment.

In **Chapter 7**, we first showed that the genes for natriuretic peptides and their receptors are widely expressed throughout the central nervous system of healthy human subjects. Second, we showed that natriuretic peptides are present in various cellular structures in the human brain including in neurons, astrocytes-like structures and cerebral vessels. Finally, our pilot experiments showed higher levels of “natriuretic peptide receptor type A” in the brain tissue, and lower levels of “natriuretic peptide type B” in the cerebrospinal fluid of Alzheimer’s patients compared to controls.

Chapter 8 summarizes the key findings of this thesis, discusses them in the context of current knowledge and provides perspectives for future research.

Nederlandse samenvatting

Dit proefschrift had als doel om de relatie tussen cardiale markers, cognitieve stoornissen en functionele achteruitgang in ouderen te onderzoeken, waarbij de focus lag op niet-invasieve markers die routinematig gemeten worden in de kliniek. De bevindingen uit dit proefschrift tonen aan dat markers van subklinische cardiale dysfunctie associëren met versnelde cognitieve achteruitgang en een hoger risico op functionele achteruitgang. Daarnast, geven we inzicht in de mogelijke rol die op het centrale zenuwstelsel inwerkende natriuretische peptiden hebben met betrekking tot cognitieve stoornissen. Tezamen belichten deze resultaten de complexe pathofysiologische verbinding tussen het hart en de hersenen, welke mogelijk verder gaat dan alleen vasculaire en hemodynamische factoren. Nieuwe studies zijn nodig om te onderzoeken of een dergelijke link gezamenlijke screening van zowel cardiale als cognitieve functies rechtvaardigt, en of deze screening voor vroegtijdige diagnose van dementie van meerwaarde kan zijn.

Hieronder een samenvatting van de hoofdstukken van dit proefschrift:

Hoofdstuk 1 geeft achtergrondinformatie over de demografie van cognitieve stoornissen op oudere leeftijd, over potentiële pathofysiologische reactiepaden die hartdysfunctie met cognitieve stoornissen verbinden, en een beschrijving van de niet-invasieve cardiale markers die in dit proefschrift worden gebruikt om hartdysfunctie te karakteriseren, waaronder het ECG en de natriuretische peptiden.

Hoofdstuk 2 laat zien dat een hogere hartslag en een lagere hartslagvariabiliteit geassocieerd zijn met een slechtere functionele status en met een hoger risico op functionele achteruitgang bij oudere personen met een hoog cardiovasculair risico. We hebben aangetoond dat dat de gevonden link tussen hartslag, hartslagvariatie en functionele achteruitgang onafhankelijk is van cardiovasculaire factoren, cardiovasculaire events en medicatiegebruik. We bespreken ook de mogelijke pathofysiologische mechanismen die cardiale autonome dysfunctie verbinden met functionele achteruitgang.

Hoofdstuk 3 laat zien dat een lagere hartslagvariabiliteit - een marker voor cardiale autonome dysfunctie - associeert met stoornissen in de executieve functie, zowel cross-sectioneel als longitudinaal bij oudere personen met een verhoogd cardiovasculair risico. We hebben aangetoond dat de gevonden link tussen 10-seconden hartslagvariabiliteit en cognitieve dysfunctie onafhankelijk is van verschillende cardiovasculaire factoren, cardiovasculaire events en medicatiegebruik.

Hoofdstuk 4 benadrukt de rol van subklinische remodeling van het hart in de ontwikkeling van cognitieve achteruitgang. We laten zien dat op ECG-gemeten linkerventrikelhypertrofie - een marker van structurele afwijkingen van het hart - associeert met een versnelde afname in verschillende domeinen van cognitieve functies bij oudere personen met een verhoogd cardiovasculair risico. De gevonden link tussen linkerventrikelhypertrofie en cognitieve achteruitgang was onafhankelijk van cardiovasculaire risicofactoren en co-morbiditeiten, het optreden van cardiovasculaire events tijdens de follow-up periode en het medicatiegebruik.

Hoofdstuk 5 laat zien dat een op ECG-gemeten bredere spatiële QRS-T hoek - een marker van grotere Heterogeniteit in elektrische activiteit van het hart - associeert met een versnelde achteruitgang in verschillende cognitieve domeinen bij oudere personen met een verhoogd cardiovasculair risico. Bovendien toonden we aan dat het verband tussen de QRS-T-hoek en cognitieve achteruitgang onafhankelijk was van overige ECG-afwijkingen, hartritmestoornissen, gemeten cardiovasculaire factoren, cardiovasculaire events en medicatiegebruik.

Hoofdstuk 6 geeft een gedetailleerd overzicht van de rol van natriuretische peptiden in het centrale zenuwstelsel van menselijke proefpersonen. In het bijzonder beschrijft **Hoofdstuk 6** de relevantie van natriuretische peptiden voor cognitieve stoornissen in de context van amyloïde bèta- en tau-eiwitafzettingen, neurovasculaire disfunctie, neuro-inflammatie, neuronale schade, synaptisch falen, angst en verstoorde vochthomeostase in de hersenen. Op basis van het beschikbare bewijs formuleerden wij de hypothese dat natriuretische peptiden in het centrale zenuwstelsel mogelijke nieuwe markers voor de diagnose en/of behandeling van cognitieve stoornissen kunnen zijn.

In **Hoofdstuk 7** hebben we eerst aangetoond dat de genen voor natriuretische peptiden en hun receptoren op grote schaal tot expressie worden gebracht in het centrale zenuwstelsel van gezonde menselijke proefpersonen. Ten tweede hebben we aangetoond dat natriuretische peptiden aanwezig zijn in verschillende cellulaire structuren in het menselijk brein, inclusief in neuronen, astrocyt-achtige structuren en cerebrale vaten. Ten slotte toonden onze pilot-experimenten een toename in het niveau van "natriuretisch peptide-receptor type A" in de hersenen, en afname in het niveau van “natriuretisch peptide type B” in het hersenvocht van Alzheimer-proefpersonen.

Hoofdstuk 8 vat de belangrijkste bevindingen van dit proefschrift samen, bespreekt deze in de context van de huidige kennis en biedt perspectieven voor toekomstig onderzoe

List of Publications

Peer-reviewed Journal papers:

1. **Mahinrad S***, Vriend AE*, Jukema JW, van Heemst D, Sattar N, Blauw GJ, Macfarlane PW, Clark EN, de Craen AJ, Sabayan B. Left ventricular hypertrophy and cognitive decline in old age. *Journal of Alzheimer's Disease*. 2017 Jan 1;58(1):275-83. (*Joint first-author)

2. **Mahinrad S**, de Craen AJ, Yasar S, van Heemst D, Sabayan B. Natriuretic peptides in the central nervous system: Novel targets for cognitive impairment. *Neuroscience & Biobehavioral Reviews*. 2016 Sep 1;68:148-56.

3. **Mahinrad S**, Jukema JW, van Heemst D, Macfarlane PW, Clark EN, de Craen AJ, Sabayan B. 10-Second heart rate variability and cognitive function in old age. *Neurology*. 2016 Mar 22;86(12):1120-7.

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6. **Mahinrad S**, Ferguson I, Macfarlane PW, Clark EN, Stott DJ, Ford I, Mooijaart SP, Trompet S, van Heemst D, Jukema JW, Sabayan B. Spatial QRS-T angle and cognitive function in old age. *Submitted*

7. **Mahinrad, S.**, Bulk, M., van der Velpen, I., Mahfouz, A., van Roon-Mom, Fedarko, N., Yasar, S., W., Sabayan, B., van Heemst, D. & van der Weerd, L. Natriuretic peptides in post-mortem brain tissue and cerebrospinal fluid of non-demented humans and Alzheimer's disease patients.

Conference abstracts:

8. **Mahinrad S,** Vriend AE, Jukema JW, Macfarlane PW, van Buchem MA, van der Grond J, Sabayan B, On behalf of PROSPER study group. QT Interval Prolongation and Cognitive Impairment in Older Subjects. *VasCog 2016, Amsterdam, the Netherlands – Oral presentation*

9. **Mahinrad S**, Van Heemst D, Macfarlane PW, Stott DJ, Jukema JW, de Craen, AJM, Sabayan B, On behalf of PROSPER study group. Short-term heart rate variability and cognitive function in older subjects at risk of cardiovascular disease. *25th European Conference on Hypertension and Cardiovascular Protection. Milan, Italy, June2015– Oral presentation*

10. **Mahinrad S**, Bulk M, Van der Velpen I, Mahfouz A, van Roon-Mom W, Sabayan B, van Heemst D, van der Weerd L. Mapping of natriuretic peptides and their receptors in the brains of non-demented human subjects and patients with Alzheimer's disease. *AAIC 2018, Chicago, US – Poster presentation*

About the Author

Simin Mahin Rad was born on March, 1986 in Tehran, Iran. After completing her high school education in Tehran, she moved to Minsk, Belarus with her family. She received her medical degree from Belarusian State Medical University, Minsk, Belarus in 2011. In the same year, she obtained a scholarship from Leiden University Medical Centre (LUMC), the Netherlands, to study the Master of Science program on Vitality and Ageing. After receiving her MSc degree in 2012, she joined the department of Gerontology and Geriatrics at LUMC as a PhD student and scientific researcher. During her PhD, she has collaborated with the department of Gerontology and Geriatrics, Human Genetics and Radiology at LUMC, and the John Hopkin's Medicine to investigate the contribution of cardiac biomarkers in cognitive brain ageing. Currently, she is a postdoctoral fellow at the department of Neurology, the cerebrovascular laboratory of prof. Sorond at Northwestern University in Chicago, USA. Her current research focusses on exploring early markers of cerebrovascular damage in midlife and their relation to clinical and brain MRI outcomes.

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