Supplementary Material

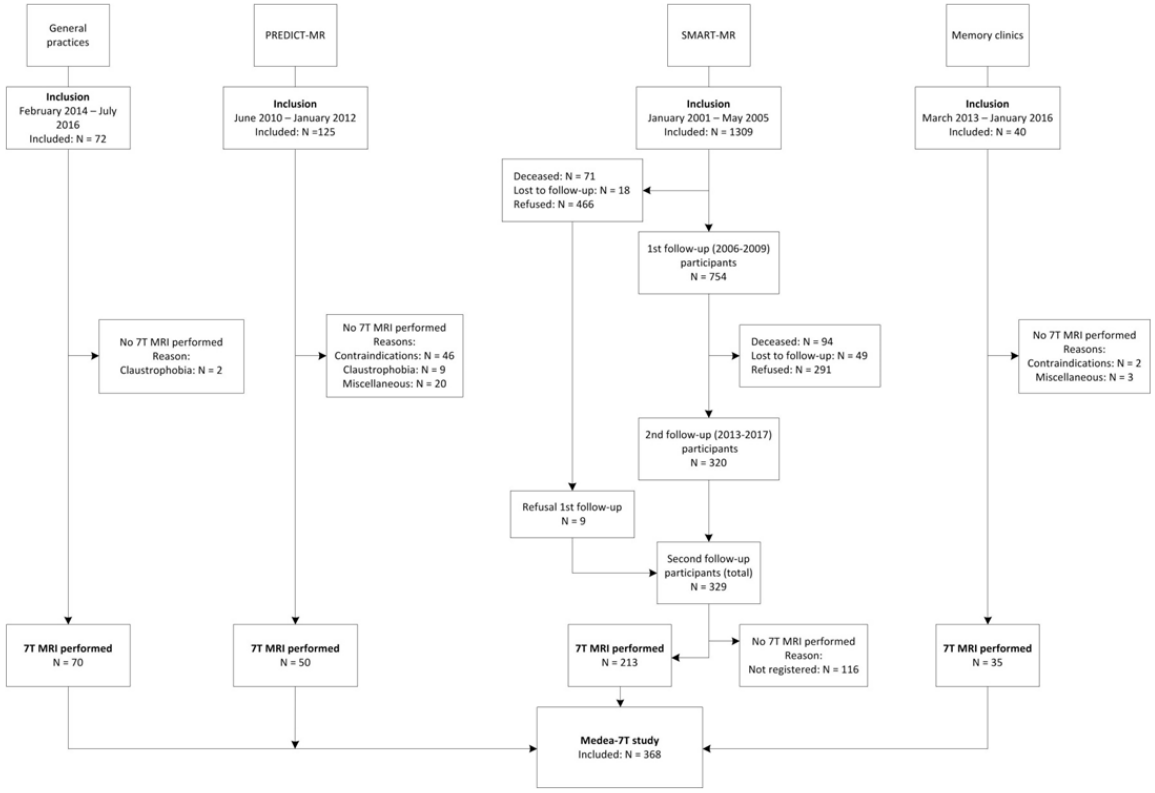
Vascular Risk Factors of Hippocampal Subfield Volumes in Persons without Dementia: The Medea 7T Study

Supplementary Methods

Cohort description Medea-7T study

The Memory Depression and Aging (Medea)-7T study is a cohort study at the University Medical Center (UMC) Utrecht, with the objective to investigate risk factors and outcomes of brain changes defined on 7T MRI. A total of 368 participants were included between January 2010 and October 2017, 70 from the general practice, 50 from the PREDICT-MR study, 213 from the SMART-MR study, and 35 from the memory clinics (Supplementary Figure 1).

Supplemental Figure 1. Flowchart of the Medea-7T study



General practices

Participants with normal cognition were recruited through one general practice in the city of Utrecht, the Netherlands. The general practitioners were asked to select from their records all persons who were 60 years or older and had no clinical diagnosis of mild cognitive impairment (MCI) or dementia or other neurological conditions that affect cognition; had no terminal illness; no previous medical evaluations for cognitive complaints; and a Clinical Dementia Rating Scale (CDR) 0. Eligible persons were then invited via a letter by the research team in which the main purpose of the study was explained. If the person was willing to participate, exclusion criteria were verified by telephone by a member of the research team. If eligible, an appointment was made for a single visit to the University Medical Center Utrecht. Participants who visited the hospital received a standardized work-up from the memory clinic, including general physical, neurological and cognitive assessments, vascular risk factor assessment, blood sampling, questionnaires, a 3T brain MRI, and a 7T brain MRI. The work-up was identical to that of the MCI and Alzheimer's disease (AD) patients, with the exception that the older persons with normal cognition had an additional questionnaire to assess psychosocial vulnerability and stress factors.

In total, 701 persons were invited to participate. Of these, 90 responded positive to the invitation and 72 participants consented to participate in the study. Inclusion took place between February 2014 and July 2016. In total, 70 finished the protocol including a 3T and 7T brain MRI.

PREDICT-MR study

The PREDICT-MR study [1] is an ancillary study to the PREDICT-NL study [2] with the objective to examine determinants and consequences of brain changes on MRI in adults. The PREDICT-MR study originates from a multicenter prospective cohort study (PredictD study

[3]) with the aim to predict major depressive disorder in primary care patients aged 18 years or older in six European countries, including the Netherlands, and Chile [4]. For PredictD, primary care patients were recruited in waiting rooms of general practices, irrespective of the reason for consulting the general practitioner. Participants were followed-up after 6 and 12 months. In the Netherlands, an additional follow-up was conducted after 39 months [2]. Between June 2010 and January 2012 (84 months after the baseline measurements) 125 participants were examined at the University Medical Center of Utrecht as part of the PREDICT-MR study [1]. Examinations included questionnaires and clinical assessment for risk factors, medical history, functioning, a diagnostic depression interview, neuropsychological testing, blood sampling, and a 1.5T brain MRI. Of the 125 included participants, 50 were eligible and willing to undergo an additional 7T brain MRI [1]. The PredictD, PREDICT-NL, and PREDICT-MR studies were approved by the ethical committee of our institution, according to the guidelines of the Declaration of Helsinki of 1975, and all participants gave written informed consent.

SMART-MR study and SMART-Medea study

The Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study is a prospective cohort study at the University Medical Center Utrecht, the Netherlands, with the aim to investigate risk factors and consequences of brain changes on MRI in patients with symptomatic atherosclerotic disease [5]. In brief, from 2001 through 2005, 1309 middle-aged and older adult persons newly referred to the University Medical Center Utrecht for treatment of symptomatic atherosclerotic disease (coronary artery disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm) and without MRI contraindications were enrolled in the SMART-MR study. On a one day visit to our medical center the participants received a 1.5T brain MRI, neuropsychological tests, a physical

examination, ankle-brachial index assessment, ultrasonography of the carotid arteries, blood and urine sampling, and questionnaires to assess risk factors, medical history, and daily functioning. Between January 2006 and May 2009, after an average of 4 years of follow-up, 754 patients had follow-up measurements for the SMART-MR cohort, and measurements were then added as part of the Second Manifestations of ARTerial disease-Memory, depression and aging (SMART-Medea) study [6], including depression assessment, psychosocial risk factor questionnaires, saliva sampling for stress hormones, and a 3-dimensional T1-weighted MR image to assess hippocampal volumes [7]. The SMART-Medea study has the objective to investigate psychosocial vulnerability and stress factors and vascular risk factors of brain changes on MRI, course of depressive symptoms, and cognitive decline [8]. Questionnaires were used for assessing demographics, risk factors and medical history, medication use, functioning, psychosocial vulnerability and stress factors, and depressive symptoms. Between November 2013 and October 2017, after an average of 12 years of follow-up since baseline, 329 surviving participants of the SMART-MR and SMART-Medea cohort had follow-up measurements, including neuropsychological and depression assessment, and a 1.5T brain MRI scan. In addition, 213 participants had a 7T brain MRI. The SMART-MR study and the SMART-Medea study were approved by the medical ethics committee of our institution according to the guidelines of the Declaration of Helsinki of 1975 and written informed consent was obtained from all participants.

Memory clinics

Patients with MCI or early AD were recruited from the outpatient memory clinics of the departments of Geriatrics and Neurology at the University Medical Center Utrecht and the memory clinic of the Diakonessenhuis Zeist. Patients with moderate or severe AD were not included in the study because we are interested in the early stages of AD and we wanted to

ensure that patients were mentally competent to give informed consent and were able to undergo the procedures including 7T brain MRI. Inclusion criteria were age ≥60 years, a diagnosis of possible or probable AD, according to the NINCDS-ADRDA workgroup criteria [9], or MCI according to Petersen criteria [10]; a CDR 0.5 or 1; and a Mini-Mental State Examination score of ≥20. Exclusion criteria were contra- indications for MR imaging and not being able to understand the Dutch language. Patients who visited the memory clinics received a standardized work-up, including general physical, neurological and cognitive assessment, vascular risk factor assessment, questionnaires and a 3T brain MRI as part of regular clinical care. A multidisciplinary team of neurologist and/or geriatrician, nurse and neuropsychologist decided on the diagnosis. For research purposes, a 7T brain MRI was added as well as blood sampling. The study was approved by the medical ethics committee of our institution, according to the guidelines of the Declaration of Helsinki of 1975, and all participants signed informed consent. In total, 40 patients were included between March 2013 and January 2016. Of these, 5 patients did not have a 7T brain MRI, leaving 35 patients of whom 13 had early AD; 20 had aMCI; and 2 had subjective cognitive decline but not fulfilling aMCI criteria.

SUPPLEMENTARY REFERENCES

[1] Wisse LEM, Biessels GJ, Stegenga BT, Kooistra M, Van Der Veen PH, Zwanenburg JJM, Van Der Graaf Y, Geerlings MI (2015) Major depressive episodes over the course of 7 years and hippocampal subfield volumes at 7 tesla MRI: The PREDICT-MR study. *J Affect Disord* **175**, 1–7.

[2] Stegenga BT, Kamphuis MH, King M, Nazareth I, Geerlings MI (2012) The natural course and outcome of major depressive disorder in primary care: The PREDICT-NL study. *Soc Psychiatry Psychiatr Epidemiol* **47**, 87–95.

[3] King M, Walker C, Levy G, Bottomley C, Royston P, Weich S, Bellón-Saameño JÁ, Moreno B, Švab I, Rotar D, Rifel J, Maaroos HI, Aluoja A, Kalda R, Neeleman J, Geerlings MI, Xavier M, Carraça I, Gonçalves-Pereira M, Vicente B, Saldivia S, Melipillan R, Torres-Gonzalez F, Nazareth I (2008) Development and validation of an international risk prediction algorithm for episodes of major depression in general practice attendees: The predictD study. *Arch Gen Psychiatry* **65**, 1368–1376.

[4] King M, Weich S, Torres-González F, Švab I, Maaroos HI, Neeleman J, Xavier M, Morris R, Walker C, Bellón-Saameño JA, Moreno-Küstner B, Rotar D, Rifel J, Aluoja A, Kalda R, Geerlings MI, Carraça I, De Almeida MC, Vicente B, Saldivia S, Rioseco P, Nazareth I (2006) Prediction of depression in European general practice attendees: the PREDICT study. *BMC Public Health* **6**, 6.

[5] Geerlings MI, Appelman AP, Vincken KL, Algra A, Witkamp TD, Mali WP, van der Graaf Y, Group SS (2010) Brain volumes and cerebrovascular lesions on MRI in patients with atherosclerotic disease. The SMART-MR study. *Atherosclerosis* **210**, 130–136.

[6] Grool AM, Gerritsen L, Zuithoff NP, Mali WP, van der Graaf Y, Geerlings MI (2013) Lacunar infarcts in deep white matter are associated with higher and more fluctuating

depressive symptoms during three years follow-up. *Biol Psychiatry* **73**, 169–176.

[7] Gerritsen L, Comijs H, Van Der Graaf Y, Knoops A, Penninx B, Geerlings MI (2011) Depression, hypothalamic pituitary adrenal axis, and hippocampal and entorhinal cortex volumes-the SMART Medea study. *Biol Psychiatry* **70**, 373–380.

[8] Kooistra M, van der Graaf Y, Grool AM, Zuithoff NP, Biessels G, Geerlings M, Group S-MS (2016) The natural course of elevated levels of depressive symptoms in patients with vascular disease over eight years of follow-up. The SMART-Medea study. *J Affect Disord* **202**, 95–101.

[9] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34**, 939–944.

[10] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E (1999) Mild cognitive impairment: Clinical characterization and outcome. *Arch Neurol* **56**, 303–308.