

ORIGINAL ARTICLE

Genetic, psychosocial and clinical factors associated with hippocampal volume in the general population

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The hippocampus—crucial for memory formation, recall and mood regulation—is involved in the pathophysiology of dementia and depressive disorders. Recent genome-wide association studies (GWAS) have identified five genetic loci associated with hippocampal volume (HV). Previous studies have described psychosocial and clinical factors (for example, smoking, type 2 diabetes and hypertension) to have an impact on HV. However, the interplay between genetic, psychosocial and clinical factors on the HV remains unclear. Still, it is likely that genetic variants and clinical or psychosocial factors jointly act in modifying HV; it might be possible they even interact. Knowledge of these factors might help to quantify ones individual risk of or rather resilience against HV loss. We investigated subjects ($N = 2463$; 55.7% women; mean age 53 years) from the Study of Health in Pomerania (SHIP-2; SHIP-TREND-0) who underwent whole-body magnetic resonance imaging (MRI) and genotyping. HVs were estimated with FreeSurfer. For optimal nonlinear model fitting, we used regression analyses with restricted cubic splines. Genetic variants and associated psychosocial or clinical factors were jointly assessed for potential two-way interactions. We observed associations between HV and gender ($P < 0.0001$), age ($P < 0.0001$), body height ($P < 0.0001$), education ($P = 0.0053$), smoking ($P = 0.0058$), diastolic blood pressure ($P = 0.0211$), rs7294919 ($P = 0.0065$), rs17178006 ($P = 0.0002$), rs6581612 ($P = 0.0036$), rs6741949 ($P = 0.0112$) and rs7852872 ($P = 0.0451$). In addition, we found three significant interactions: between rs7294919 and smoking ($P = 0.0473$), rs7294919 and diastolic blood pressure ($P = 0.0447$) and between rs7852872 and rs6581612 ($P = 0.0114$). We suggest that these factors might have a role in the individual susceptibility to hippocampus-associated disorders.

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INTRODUCTION

The hippocampus—a core region in the limbic system—represents an important brain structure for memory¹ and stress regulation.² Decreased hippocampal volume (HV) is associated with executive dysfunction.^{3,4} In addition, HV and hippocampus-dependent memory measures (such as short-delay retention, long-delay retention and discriminability) are positively correlated.⁵ Patients with hippocampal degradation seem to have negative clinical outcome in depression.^{3,6} Cumulative factors lead to differences in the HV with increasing age. Therefore, it seems to be important to investigate factors influencing HV changes.

A broad range of clinical and psychosocial determinants influencing the HV and its function have been identified. Depression,^{7,8} alcohol consumption^{9–11} and smoking have been associated with decreased HVs.¹² In addition, cardiometabolic factors have been associated with decreased HV: abdominal obesity,^{13,14} hypertension,^{15,16} type 2 diabetes^{17–19} and elevated total cholesterol levels.¹² In contrast, exercise intervention increased the HV and subsequently improved memory

function.^{20,21} High education buffered the age-related decrease in the HV.²²

In twin studies, the heritability of HV has been estimated at 40–64%.^{23,24} Two genome-wide association studies (GWAS) have been performed to search for genetic markers related to HV.^{25,26} We have contributed with our samples (SHIP-2, SHIP-TREND-0) to these analyses, leading to the identification of the intergenic variant rs7294919,²⁶ replicated by Bis *et al.*²⁵ Rs7294919 is located on 12q24.22, a chromosomal region comprising the genes *TESC*, *HRK* and *FBXW8*. The biological mechanism how rs7294919 influences on HV is currently unknown. However, all three neighboring genes could be involved. *TESC* has an important role in cell proliferation and differentiation for HV and brain development.^{26,27} Harakiri (*HRK*) is a regulator of apoptosis, which exhibits death-inducing activity in cells.^{28,29} *FBXW8* encodes a member of the F-box protein family, which modifies neuronal dendrite growth and elaboration.³⁰ Furthermore, Bis *et al.*²⁵ identified promising loci at 12q14 (*WIF1* and *MSRB3*), 2q24 (within *DPP4*, dipeptidylpeptidase) and 9p33 (within *ASTN2*).

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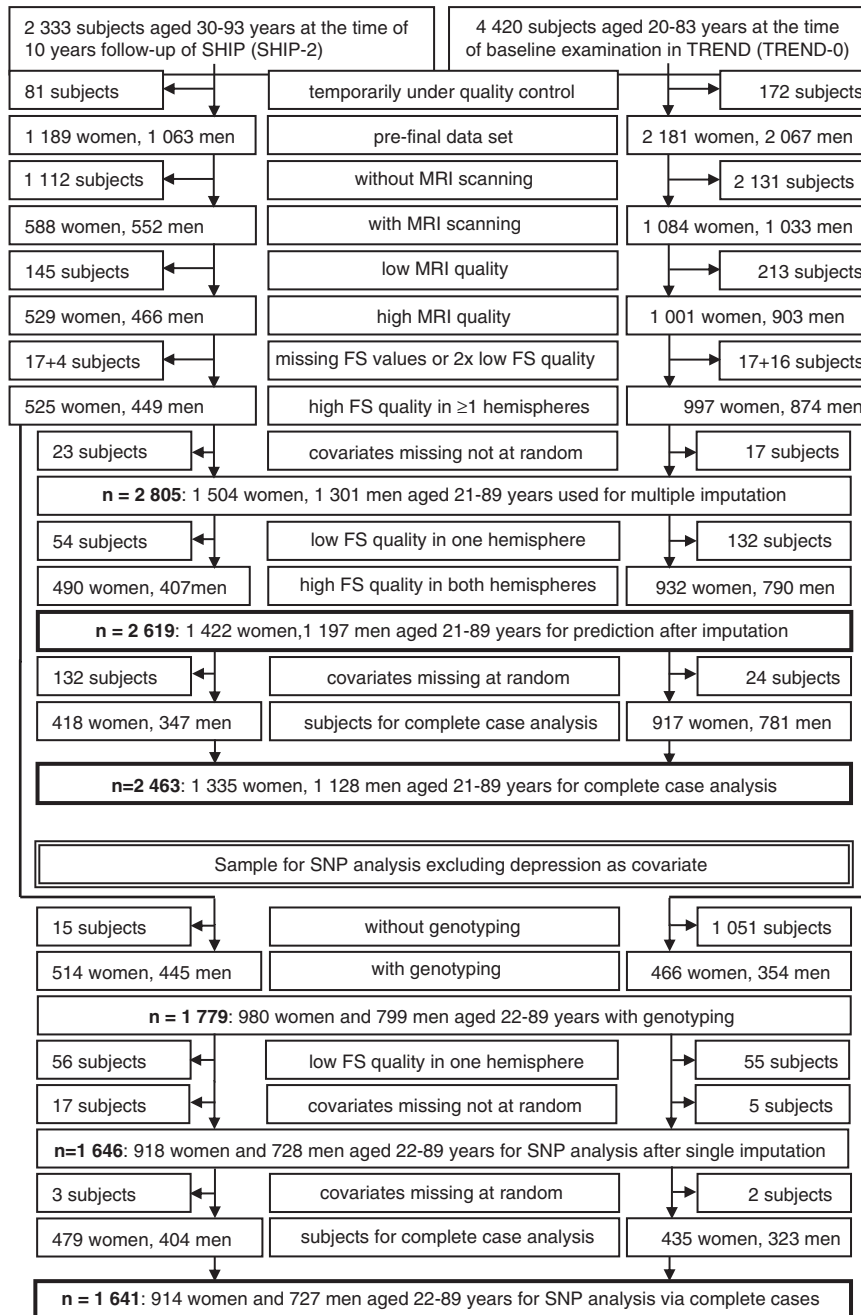


Figure 1. Flow chart: inclusion and exclusion criteria for participants from the Study of Health in Pomerania (SHIP-2 and SHIP-TREND-0).

Those markers may be of relevance to the physiology of the hippocampus: Wingless-related integration site (WNT) inhibitory factor 1 is a protein that inhibits WNT proteins, which have an important role in the embryonic development, in the regulation of morphogenesis in the central nervous system and which can affect neuronal functions in adulthood.^{31,32} *MSRB3* encodes proteins that catalyze the reduction of methionine sulfoxide to methionine, thus preserving the biological activity of proteins after oxidative damage.^{33,34} *DPP4* is a protein encoded by the *DPP4* gene. DPP4 inhibitors, as a new antidiabetic treatment approach, inhibit the degradation of Glucagon-like Peptide 1. The DPP-4 inhibitor moderated the impaired neuronal insulin receptor function and brain mitochondrial function caused by high-fat diet and showed improved cognitive behavior in an animal model.³⁵ *Astrotactin2 (ASTN2)* is expressed in the brain among others in the

dendate gyrus of the hippocampus and, together with *ASTN1*, involved in neuronal migration.³⁶

However, the interplay between genetic, psychosocial and clinical factors on the HV remains poorly understood. These factors might jointly act or interact in modifying HV. Knowledge of these factors and their interactions might help to quantify ones individual risk of or rather resilience against hippocampus-related diseases (for example, dementia) with HV loss and hippocampal loss function. It might improve our understanding of structural changes from this vulnerable brain tissue. We included the above mentioned, well-described and highly epidemiological relevant clinical and psychosocial factors to affect HV in our study.

The aim of our study was to explore direct associations of genetic candidate markers and potential psychosocial and clinical factors with HV in the general adult population. Moreover, we

investigated putative gene–gene and gene–environment interactions in relation to HVs.

Our aims were threefold:

- (1) to explore clinical and psychosocial determinants of HV in large adult population-based samples (SHIP-2 and SHIP-TREND-0);
- (2) to test associations between single-nucleotide polymorphisms (SNPs; known from GWAS^{25,26}) and HV;
- (3) to examine two-way interactions between those SNPs and psychosocial and clinical factors and interactions between these SNPs.

MATERIALS AND METHODS

Participants

Data from the Study of Health in Pomerania (SHIP-2 and SHIP-TREND-0) were used.^{37–39} SHIP-0 comprised adult German residents in northeastern Germany living in three cities and 29 communities, with a total population of 212 157. A two-stage stratified cluster sample (aged 20–79 years at baseline) was randomly drawn from local registries. In all, 4308 Caucasian subjects participated at baseline SHIP-0 (1997–2001). The first follow-up was conducted 5 years later (SHIP-1; $N=3300$ subjects). The second follow-up examination (SHIP-2; $N=2333$) was carried out about 10 years after baseline. In 2007, the LEGEND study was started (evaluation of lifetime diagnosis of depression in the SHIP-2 sample).³⁷ Concurrent with SHIP-2, a new independent sample in the same area was drawn in 2008 and similar examinations were undertaken (SHIP-TREND-0; $N=4420$).

For inclusion and exclusion criteria see flow chart (Figure 1).

Magnetic resonance imaging (MRI) scanning and segmentation of the hippocampus

All images were obtained from the same scanner (1.5 Tesla Magnetom Avanto; Siemens Medical Solutions, Erlangen, Germany). We used the multiplanar reconstruction T1-weighted axial MRI sequence with the following parameters: 1900 ms repetition time, 3.4 ms echo time, flip angle = 15° and a voxel size of 1.0 × 1.0 × 1.0 mm. After file format conversion from DICOM to NIfTI, the whole preprocessing of the T1-weighted images and segmentation of the hippocampus was carried out with FreeSurfer v5.1.0 (Cambridge, MA, USA).

Interview (psychosocial factors) and clinical examination

Sociodemographic factors and medical history were assessed by a computer-assisted face-to-face interview. Smoking was defined as current smoking (occasional; 1–14 cigarette (s) per day; ≥ 15 cigarettes per day), former smoking (occasional; 1–14 cigarette (s) per day; ≥ 15 cigarettes per day) and never smoking. The highest level of education was assessed by interview and was divided into three groups of school education: 8 years, 10 years and 12 years. Leisure time physical activity (weekly sportive activity or exercise, for example, jogging, biking and swimming) was assessed by interview in three categories: no activity (neither in summer nor in winter), high activity (> 2 h per week as well in summer and winter) and moderate activity (residual category). Alcohol consumption was calculated from the interview in g per day as a continuous variable. Having completed the interview, participants underwent medical examinations: including the measurement of height and weight to calculate the body mass index (continuous variable). Waist circumference was measured in cm (continuous variable). After a 5-min resting period, blood pressure was measured three times on the right arm of seated subjects using a digital blood pressure monitor (HEM-705CP, Omron, Tokyo, Japan), with each reading being followed by a further resting period of 3 min. Cuffs were applied according to the circumference of the participant's arm. The mean of the second and third measurements (mm Hg) was used for the analyses (continuous variables). All subjects were informed to bring in their packing containers of all medication they had taken during the last 7 days, as well as their drug prescription sheets. Every compound was recorded and categorized according to the Anatomical Therapeutic Chemical classification.⁴⁰ We focused on antidepressants (ATC-code N06A*), antihypertensives (C02*, C03*, C07*, C08* and C09*), antidiabetic drugs (A10*) and lipid-lowering drugs (C10*).

Assessment of depression

The lifetime diagnosis of depressive disorders was assessed with the Munich-Composite International Diagnostic Interview (M-CIDI⁴¹) in SHIP-LEGEND³⁷ and in SHIP-TREND-0. The M-CIDI is a standardized, fully structured instrument for assessing psychiatric disorders over the lifespan according to DSM-IV criteria.

Genotyping

Serum, EDTA, citrate plasma and DNA are stored at –80 °C in a biobank. In SHIP-0, 4081 individuals were successfully genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA). The overall genotyping efficiency of the GWA was 98.6%. Imputation of genotypes in the SHIP cohort was performed with the software IMPUTE v0.5.0 (Oxford, UK) against the HapMap II (CEU v22, Build 36; International HapMap Consortium) reference panel using 869 224 genotyped SNPs. The total number of SNPs after imputation and quality control was 2 748 910.

Genotyping of the SHIP-TREND-0 subjects ($N=986$) was performed using the Illumina HumanOmni2.5-Quad (San Diego, CA, USA). DNA from whole blood was prepared using the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Subsequent sample processing and array hybridization were performed as described by the manufacturer (Illumina) at the Helmholtz Zentrum München. The final sample call rate was 99.51%. Imputation of genotypes in the SHIP-TREND-0 cohort was performed with the software IMPUTE v2.1.2.3 against the HapMap II (CEU v22, Build 36) reference panel. The total number of SNPs after imputation and quality control was 3 437 411. Allele frequencies were all in Hardy–Weinberg equilibrium $P>0.2$ and imputation quality was $P>0.90$, except for rs17178006 (Supplementary eTable 1).

Purity and concentration of DNA was determined using a NanoDrop ND-1000 UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA). The integrity of all DNA preparations was validated with electrophoresis using 0.8% agarose-1x TBE gels stained with ethidium bromide. Genotypes in SHIP-0 were determined using the Birdseed2 clustering algorithm. Genotypes in SHIP-TREND-0 were called with the GenCall algorithm of GenomeStudio Genotyping Module v1.0 (Illumina). Arrays with a call rate below 94%, duplicate samples as identified by estimated IBD as well as individuals with reported and genotyped gender mismatch were excluded. The genetic data analysis workflow was created using the Software InforSense (London, UK). Genetic data were stored using the database Caché (InterSystems, Cambridge, MA, USA). Hardy–Weinberg equilibrium P -values are: rs7294919: $P=0.913$; rs17178006: $P=0.844$; rs6581612: $P=0.658$; rs6741949: $P=0.215$ and rs7852872: $P=0.4233$.

Laboratory work

Glycated hemoglobin (HbA1c, measured in %) concentrations were determined with high-performance liquid chromatography (Bio-Rad Diamat, Munich, Germany). Skilled technical personnel performed all assays according to the manufacturer's recommendations. In addition, the laboratory participates in official quarterly German external proficiency testing programs. HbA1c, glucose, triglycerides, total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured as dimensional scores. Triglyceride and HDL cholesterol concentrations were measured photometrically (Hitachi 704, Roche, Mannheim, Germany), whereas follow-up HDL concentrations were quantified with lipid electrophoresis (HELENA SAS-3 system, Helena 7 BioSciences Europe, Tyne & Wear, UK). Triglyceride and glucose concentrations were determined enzymatically using reagents from Roche Diagnostics (Hitachi 717, Roche Diagnostics).

To ensure comparability in the longitudinal HDL analyses, we used baseline HDL concentrations as the reference and calculated corrected follow-up HDL concentrations based on a previously published conversion formula ($HDL_{fu_corr} = -80 + (1.158 * HDL_{fu})$).⁴² Doing so, we found that the average HDL concentrations produced by the two methods were virtually identical, suggesting that the differences in HDL will be small within the range of practical relevance. Serum LDL was measured by applying a precipitation procedure using dextran sulphate (Immuno, Heidelberg, Germany) on an Epos 5060 (Eppendorf, Hamburg, Germany).

Statistical methods

As recommended,⁴³ we applied advanced statistical methods implemented in the 'rms: Regression Modeling Strategies' R package⁴⁴ (including redundancy analysis with the 'redun' procedure, cluster analysis and

Table 1. Clinical prediction model

Factor	Complete case analysis (n = 2463)				Imputation (n = 2619)				
	Effects estimated				Penalized estimation				
	Low (median = 50th percentile)	High (90th percentile)	Difference or reference		Conventional estimation	Penalized estimation	Conventional estimation		
				Effect	Robust s.e.	P (factor)	Effect	Robust s.e.	P (factor)
Women			Men	-199.7	25.3	< 0.0001	-170.0	20.2	< 0.0001
Age, years	52.1	69.9	17.8	-290.7	25.0	< 0.0001	-291.4	18.1	< 0.0001
Body height, cm	170	182	12	129.8	25.6	< 0.0001	140.0	17.0	< 0.0001
Education						0.0053			0.0120
Education, 10 years			8 Years	21.3	23.7	0.3684	14.7	20.3	0.4705
Education, 12 years			8 Years	65.6	24.5	0.0076	53.2	21.3	0.0125
Cigarette smoking						0.0058			0.0329
Ex-smoker, occasional			Never	-49.3	20.3	0.0151	-41.7	18.5	0.0241
Ex-smoker, 1-14 cigarette per day			Never	-67.4	24.6	0.0063	-53.3	22.0	0.0160
Ex-smoker, ≥ 15 cigarette per day			Never	-41.8	23.4	0.0748	-34.2	20.1	0.0896
Current smoker, occasional			Never	-16.9	41.7	0.6853	-24.6	27.5	0.3711
Current smoker, 1-14 cigarette per day			Never	-81.5	23.9	0.0007	-65.4	21.1	0.0019
Current smoker, ≥ 15 cigarette per day			Never	-37.5	28.1	0.1825	-31.9	22.0	0.1481
Alcohol consumption, g per day	4.0	22.3	18.3	-9.7	24.1	0.6885	-5.3	12.9	0.9456
Major depression, lifetime			No	3.1	19.0	0.8692	-0.5	17.0	0.9765
Physical activity						0.1468			0.2256
> 0-2 h Per week			0 h Per week	36.3	19.2	0.0588	29.7	17.4	0.0872
> 2 h Per week			0 h Per week	19.7	22.6	0.3843	18.4	19.9	0.3557
Waist circumference, cm	88.6	106.0	17.4	-5.6	17.4	0.2654	-2.6	16.4	0.3717
HbA1c, %	5.2	6.0	0.8	-24.7	14.6	0.1374	-13.7	8.6	0.2695
HDL cholesterol, mmol l ⁻¹	1.42	1.98	0.56	30.0	14.9	0.1166	20.3	14.9	0.3957
LDL cholesterol, mmol l ⁻¹	3.37	4.61	1.24	4.5	15.3	0.5089	5.4	13.4	0.5249
Diastolic blood pressure, mm Hg	77.5	91.0	13.5	-38.2	13.7	0.0211	-31.4	13.6	0.0654
Antidepressants			No	-60.5	30.9	0.0500	-42.8	25.4	0.0926
Antidiabetics			No	-37.7	36.2	0.2971	-22.8	30.4	0.4522
Lipid-lowering drugs			No	-16.3	27.1	0.5473	-16.3	22.3	0.4647
Antihypertensive medication			No	-26.2	17.4	0.1336	-27.8	16.3	0.0886
SHIP-2			TREND-0	1.1	15.7	0.9449	-1.0	17.0	0.9480

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein. P for joint test including major depression, antidepressants: robust/penalized/imputed: 0.1404/0.2308/0.2109, P for joint test including HbA1c, antidiabetics: robust/penalized/imputed: 0.0408/0.1859/0.0116. P for joint test including HDL, LDL, lipid-lowering drugs: robust/penalized/imputed: 0.3037/0.5607/0.1501. P for joint test including diastolic blood pressure, antihypertensive medication: robust/penalized/imputed: 0.0232/0.0429/0.0091.

Spearman correlations). Intracranial volume (ICV) was not considered to be a confounding factor because two of the three criteria for a confounding factor do not meet.⁴⁵ First, the ICV includes the HV and is a consequence rather than a source of HV. Second, if an exposure affects the ICV, then the ICV is an intermediate step in the causal path between the exposure and the HV and, therefore, to be excluded from analysis.

Because of redundancy, systolic blood pressure, total cholesterol, triglycerides, glucose and body mass index were removed from further analysis. To avoid power loss and unjustified linearity assumptions, continuous variables were modeled as restricted cubic splines.⁴³

To test interactions between genetic and psychosocial or clinical factors, we chose an approach in two steps. In the first step we identified statistically significant psychosocial or clinical factors in the sample with 2463 subjects. A statistically significant psychosocial or clinical factor had to meet two conditions: (1) significance ($P \leq 0.05$) and (2) for factors that had to be controlled for medication, the related group of clinical predictors including medication had to be significant in the corresponding joint test; a categorical variable was tested by joint test across categories ($P \leq 0.05$). (Specification of the related groups for joint test: included major depression and antidepressants; HbA1c and antidiabetics; HDL, LDL and lipid-lowering drugs; and diastolic blood pressure and antihypertensive medication.)

In the second step, interactions between genetic variants and clinical or psychosocial factors (significant in the previous step) were modeled and analyzed in 1641 subjects with complete genetic and clinical data (data not shown).

We restricted interactions to be linear in continuous predictors. All two-way interactions that met the conditions were modeled in a single model. All factors from the first step were left in the interaction model.⁴³ Only depression was excluded from this step because the proportion of missing values was higher than 5% ($N = 116$), and results in the first step were not significant.

Analyses and graphics were performed using the R software⁴⁶ and the STATA/MP software, release 12.1 (Stata, College Station, TX, USA).

Sensitivity analysis

Effects of current and ex-smoking showed no statistical significance (contrast between current and ex-smoking in occasional smokers: $32.4 \pm 43.5 \text{ mm}^3$, $P = 0.4575$; contrast between current and ex-smoking across three categories each 7.5 ± 21.4 , $P = 0.7254$). As a result, these six categories were collapsed for further analyses.

Imputation

In addition to the complete case analysis ($N = 2463$), we conducted an extra analysis for the clinical prediction model with imputed data ($N = 2619$) to validate the consistency of the associations with larger sample sizes (Table 1).

Imputation of missing data is recommended in general,⁴⁷ therefore, two imputations were planned, one for the model without genetic data and one for the gene interaction model.

Additional 156 subjects revealed data 'missing at random' (for example, blood pressure, blood lipids; for details see Table 2). The overall proportion (SHIP-2/TREND combined) of missing data was 6% (SHIP-2 sample separate: 15%), which is acceptable for single imputation.⁴³ SHIP-2 and TREND-0 data were separately imputed using chained equations. One hundred sixteen subjects did not participate in the psychiatric diagnostic interview (highest number of missing data in the diagnosis of lifetime depression); however, most of them took part in the CID screener from SHIP-0 and SHIP-1. Two items of the CID screener are also part of the depression interview and were used for recursive partitioning and imputation of depression. Observations with missing data of HV in one hemisphere were multiply-imputed (all variables were used); however, observations with missing outcome data dropped.⁴⁷ Data not missing at random (for example, questions of alcohol consumption) were not imputed.

Given the insignificant P -value for depression in the second step of analysis, we decided to remove depression from the model, thereby avoiding a need for imputation in gene interaction analysis. Using imputed values sample data ($N = 2619$), however, HbA1c and HDL cholesterol additionally achieved significant associations with HV (Table 1).

Table 2. Variables in clinical and genotype models

Characteristic	Clinical prediction model		Analysis of gene interaction ^a (n = 1641)
	Included (n = 263)	Excluded (n = 156)	
Mean hippocampal volume, mean (s.d.), mm ³	3947.8 (423.7)	3892.5 (415.9)	3935.0 (430.4)
Women, N (%)	1335 (54.2)	87 (55.8)	914 (55.7)
Age, mean (s.d.), years	52.1 (13.6)	55.8 (12.7) ^b	53.0 (13.1)
Body height, mean (s.d.), cm	170 (9.3)	169 (9.3)	170 (9.2)
<i>Education, mean (s.d.)</i>			
< 10 Years	372 (15.1)	37 (23.9) ^c	253 (15.4)
10 Years	1382 (56.1)	83 (53.6)	924 (56.3)
> 10 Years	709 (28.8)	35 (22.6)	464 (28.3)
<i>Cigarette smoking, N (%)</i>			
Non-smoker	991 (40.2)	56 (38.6)	677 (41.3)
Former smoker, occasional	389 (15.8)	13 (9.0)	Former:
Former smoker, 1–14 cigarettes per day	206 (8.4)	18 (12.4)	624 (38.0)
Former smoker, ≥ 15 cigarettes per day	310 (12.6)	24 (16.6)	
Current smoker, occasional	79 (3.2)	2 (1.4)	Current:
Current smoker, 1–14 cigarettes per day	253 (10.3)	17 (11.7)	340 (20.7)
Current smoker, ≥ 15 cigarettes per day	235 (9.5)	15 (10.3)	
<i>Alcohol consumption, N (%)</i>			
0 g Per day	314 (12.8)	18 (11.5)	171 (10.4)
> 0–5 g Per day	1035 (42.0)	57 (36.5)	693 (42.2)
> 5–10 g Per day	427 (17.3)	37 (23.7)	303 (18.5)
> 10–20 g Per day	393 (16.0)	18 (11.5)	257 (15.7)
> 20–30 g Per day	137 (5.6)	14 (9.0)	97 (5.9)
> 30 g Per day	157 (6.4)	12 (7.7)	120 (7.3)
Major depression, lifetime ^d , N (%)	446 (18.1)	3 (15.0) ^e	254 (16.8)
<i>Physical activity, N (%)</i>			
No	429 (17.4)	37 (23.9)	269 (16.4)
> 0–2 h Per week	1459 (59.2)	87 (56.1)	971 (59.2)
> 2 h Per week	575 (23.4)	31 (20.0)	401 (24.4)
Waist circumference (cm), N (%)	88.9 (12.8)	90.5 (13.1) ^f	88.5 (12.6)
Body mass index (kg m ⁻²), N (%)	27.4 (4.4)	27.7 (4.3)	27.3 (4.3)
HbA1c (%) mean (s.d.)	5.3 (0.7)	5.4 (0.8)	5.3 (0.7)
Glucose (mmol l ⁻¹)	5.6 (1.3)	5.6 (1.5)	5.5 (1.1)
Triglycerides (mmol l ⁻¹), mean (s.d.)	1.6 (1.1)	1.8 (1.4) ^{c,g}	1.6 (1.2)
Total cholesterol (mmol l ⁻¹), mean (s.d.)	5.5 (1.1)	5.5 (1.1) ^g	5.5 (1.1)
HDL-cholesterol (mmol l ⁻¹), mean (s.d.)	1.5 (0.4)	1.5 (0.4) ^g	1.5 (0.4)
LDL cholesterol (mmol l ⁻¹), mean (s.d.)	3.4 (0.9)	3.3 (1.0) ^g	3.4 (0.9)
Diastolic blood pressure (mm Hg), mean (s.d.)	78.0 (9.9)	80.2 (10.4) ^{c,h}	78.6 (10.0)
Systolic blood pressure (mm Hg), mean (s.d.)	127.2 (17.3)	131.8 (19.9) ^{b,h}	127.7 (17.7)
<i>Medication</i>			
Antidepressants, N (%)	138 (5.6)	11 (7.0)	90 (5.5)
Antidiabetics, N (%)	95 (3.9)	13 (8.3) ^c	43 (2.6)
Lipid-lowering drugs, N (%)	257 (10.4)	18 (11.5)	181 (11.0)

Table 2. (Continued)

Characteristic	Clinical prediction model		Analysis of gene interaction ^a (n = 1641)
	Included (n = 263)	Excluded (n = 156)	
Antihypertensive medication, N (%)	772 (31.3)	51 (32.7)	514 (31.3)
rs7294919 (TESC, HRK, FBXW8), N (%)			
CC			18 (1.1)
CT			326 (19.9)
TT			1297 (79.0)
rs17178006 (MSRB3), N (%)			
GG			21 (1.3)
GT			284 (17.3)
TT			1336 (81.4)
rs6581612 (WIF1), N (%)			
AA			908 (55.3)
AC			613 (37.4)
CC			120 (7.3)
rs6741949 (DPP4), N (%)			
CC			265 (16.2)
CG			773 (47.1)
GG			603 (36.8)
rs7852872 (ASTN2), N (%)			
CC			636 (38.8)
CG			763 (46.5)
GG			242 (14.8)

^aExcluded major depression, lifetime diagnosis. ^b $P < 0.01$. ^c $P < 0.05$. ^d $N = 1\ 510$. ^e $N = 20$. ^f $N = 156$. ^g $N = 153$. ^h $N = 152$.

Penalized regression

Results of penalized regression based on the 'pentrace' procedure were presented because 'A shrinkage approach will often result in regression coefficient estimates that while biased are lower in the mean squared error and hence are more likely to be close to the true unknown parameter values'.⁴³

Mediator and pathway analyses

For two genes—rs17178006 and rs6581612—a mediator analysis (data not shown) was conducted. For the gene–gene interaction—rs7852872 and rs6581612—a pathway analyses was conducted⁴⁸ (see supplement and discussion).

RESULTS

Baseline characteristics

The description of the sample is given in Table 2.

Associations of psychosocial and clinical factors with HV ($N = 2463$) Besides gender, age and body height, education, smoking and diastolic blood pressure were associated with HV (Table 1). Participants with high education had larger HV than participants with low education ($P = 0.0076$; Table 1). Current and former smokers revealed smaller HV than non-smokers ($P = 0.0058$; Table 1). Diastolic blood pressure was negatively associated with HV in participants having more than 80 mm Hg and slightly positively associated in participants having less than 75 mm Hg ($P = 0.0211$; Table 1). Although antidepressant medication was associated with smaller HV, the corresponding joint test (including depression) did not indicate an association.

We did not find significant associations for the following psychosocial and clinical factors: waist circumference, alcohol consumption, physical activity, lifetime major depression diagnosis, HDL cholesterol, LDL cholesterol and HbA1c (Table 1).

Associations of genetic variants with HV ($N = 1641$)

We confirmed significant associations for genetic variants with HV (Table 3).

Interactions between clinical or psychosocial factors and genetic markers ($N = 1641$)

We identified two interactions between genetic markers and clinical factors. Rs7294919 modified both: the effects of smoking and diastolic blood pressure. In non-smokers, carriers of the C allele had larger HVs than non-smokers with TT genotype. In smokers, C allele carriers and non-carriers did not differ (Figure 2a; P for interaction = 0.0473). Moreover, participants with low diastolic blood pressure carrying the C allele had larger HVs than TT carriers (Figure 2b; P indication of interaction = 0.0447).

Interactions between genetic markers ($N = 1641$)

We found one gene–gene interaction: CG carriers of rs7852872 combined with CC genotype of rs6581612 had larger HVs than CG carriers combined with the A allele of rs6581612 (Figure 2c; P for interaction = 0.0114).

DISCUSSION

In this adult population-based study, we confirmed genetic, psychosocial and clinical factors that are associated with HV: gender, age and body height, as well as education, smoking and diastolic blood pressure. As genetic factors, we found rs7294919 to modify effects of smoking and diastolic blood pressure. Moreover, we identified an interaction between genetic markers known as candidates of neural function.

The novelty of this study consists of the joint analysis of potential predictors within one model, allowing for nonlinear relationship between continuous predictors and HV in a large population-based sample.

Table 3. Genetic markers associated with hippocampal volume (comparison with Bis et al.²²)

SNP	SHIP/TREND $N = 1641$				Bis et al. ²²	SHIP/TREND ($N = 1641$) versus Bis et al. ²²
	Allele coded	Effect adjusted for sex and age	Robust s.e.	P-value		
rs7294919 (TESC, HRK, FBXW8)	T	−52.7	19.4	0.0065	−107.8	0.0045
rs17178006 (MSRB3)	G	−72.9	19.7	0.0002	−123.8	0.0100
rs6581612 (WIF1)	C	−41.2	14.1	0.0036	−63.3	0.1194
rs6741949 (DPP4)	G	−31.7	12.5	0.0112	−52.8	0.0916
rs7852872 (ASTN2)	C	−26.6	13.3	0.0451	−47.7	0.1126

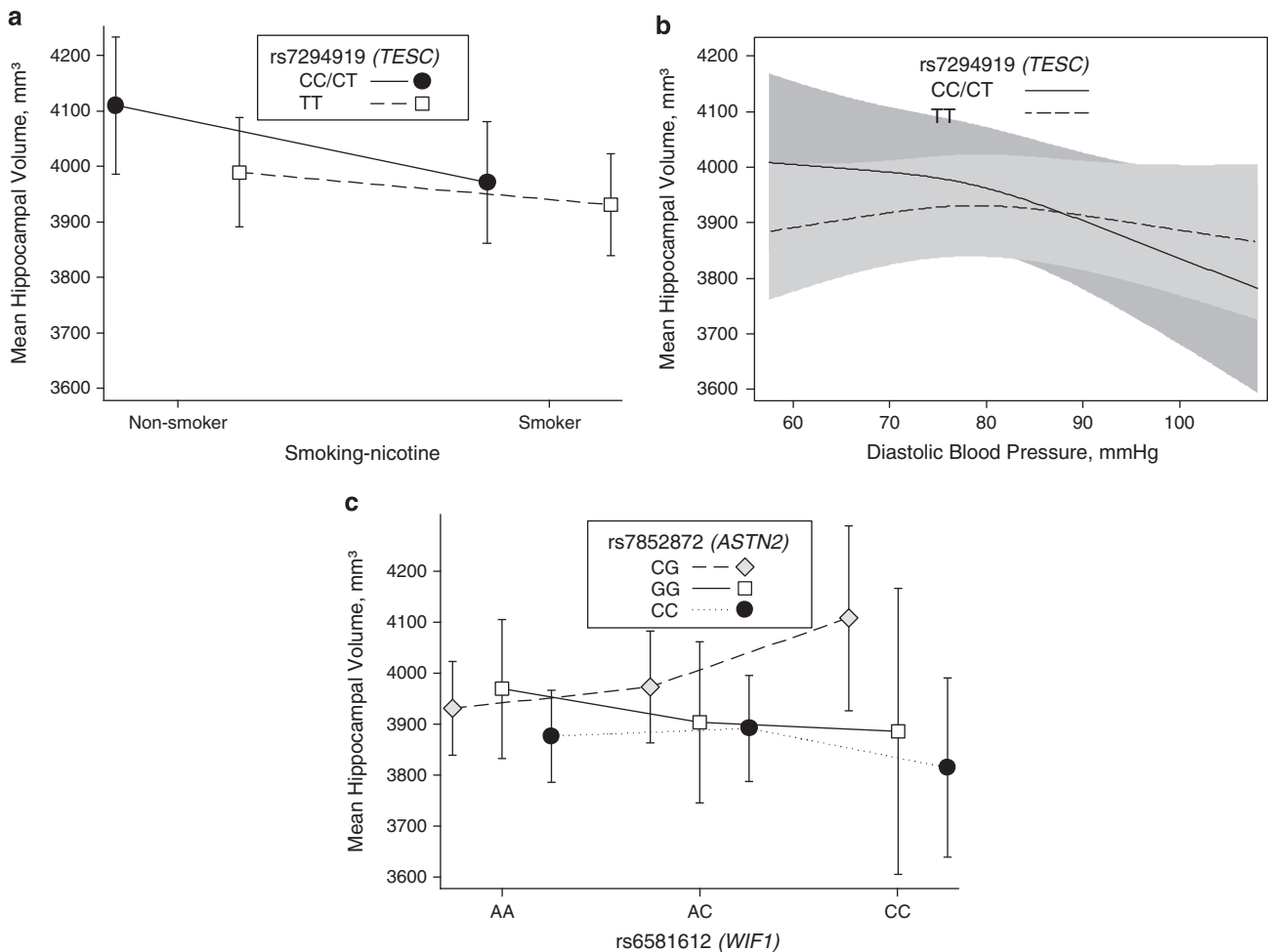


Figure 2. (a) The interaction between rs7294919 and smoking status for the mean hippocampal volume (mm³) is depicted. In this plot, an interaction becomes evident between carriers of the CC/CT genotype of rs7294919 and non-smoker for a larger hippocampal volume ($P=0.0473$). Number of subjects for each combination of genotype and smoking status are given below: rs7294919CT/CC and non-smoker $N=140$; rs7294919TT and non-smoker $N=537$; rs7294919CT/CC and smoker $N=204$; rs7294919TT and smoker $N=760$. (b) The interaction between rs7294919 and diastolic blood pressure for the mean hippocampal volume (mm³) is depicted. In this plot, an interaction becomes evident between carriers of the CC/CT genotype of rs7294919 and lower diastolic blood pressure for a larger hippocampal volume ($P=0.0447$). (c) Gene-gene interaction between rs7852872 (ASTN2) and rs6581612 (WIF1) is depicted for the mean hippocampal volume in mm³. This plot shows a considerable interaction for a larger hippocampal volume in CG-genotype carriers of rs7852872 and the CC-genotype carriers of rs6581612 ($P=0.0114$). Number of subjects for each combination of genotype are given below: rs6581612AA and rs7852872CC: $N=350$; rs6581612AA and rs7852872GG: $N=141$; rs6581612AA and rs7852872CG: $N=417$; rs6581612AC and rs7852872CC: $N=242$; rs6581612AC and rs7852872GG: $N=290$; rs6581612AC and rs7852872CG: $N=81$; rs6581612CC and rs7852872CC: $N=44$; rs6581612CC and rs7852872GG: $N=20$; rs6581612CC and rs7852872CG: $N=56$.

Associations of psychosocial and clinical factors with HV
The findings for education are in line with a study in healthy elders⁴⁹ and support the idea of an education-related biological and cognitive reserve (for review see Stern⁵⁰); higher reserve could counterbalance aging or pathological effects. The result for smoking is consistent with observations from Durazzo *et al.*^{51,52} who found smaller HVs in smokers compared with non-smokers. Although several studies indicate that nicotine induces neuronal cell loss, the exact pathomechanism of how nicotine affects brain tissue in general and the hippocampus in particular remains unclear. There is evidence that smoking affects the hippocampus by decreasing the regional cerebral blood flow.⁵³ Smoking was associated with atrophy compared with non-smoking in several brain regions in early stages of Alzheimer's.^{51,54} In animal studies, hippocampal neuronal damage after nicotine exposure has been demonstrated.^{55,56} Nicotine induced cell death in hippocampal progenitors.⁵⁷ Diastolic blood pressure was associated with HV, which is in line with previous studies.^{58–60} Hypertension is known

as a risk factor for Alzheimer's, although causality might be difficult to determine because of high prevalence of hypertension and brain atrophy while aging.⁶¹ Hypertension increases the risk of atherosclerosis that might lead to decreased blood flow in the hippocampus.⁶² In animal models, blood pressure-related cerebral changes promoted neuronal death and brain atrophy.^{63,64} This is consistent with Beauchet *et al.*⁶⁰ who concluded in their systematic review and meta-analysis that brain volume reduction in hypertension cannot solely be explained by age and use of hypertensive drugs. Several psychosocial and clinical factors were not confirmed to influence the HV in our study: waist circumference did not raise an association with HV. This result differs from other studies that could find associations between overweight/obese participants and smaller HV. In contrast to our study, their samples were not population-based or subjects were elderly.^{13,14} Effects of obesity on the HV may increase with age and in combination with other clinical determinants. Alcohol consumption was not associated with HV in contrast to the study from

Agartz *et al.*⁹ who investigated 52 hospitalized alcohol-addicted patients.

The effect of physical activity in our study was not convincing. The rather crude assessment with low threshold (>2 h per week) of physical activity in our study may have missed protective effects in subjects with higher physical activity. Furthermore, socially desired answers have possibly led to an overestimation of sportive activities.

Previous studies about HVs and major depression are contradictory: although there is some evidence for decreased HVs or decreased subregions,^{65–67} other studies did not find associations between depression and decreased brain volumes.^{68,69} Although we had data from clinical interviews (DSM-IV), we could not support any association for HV with depression. We neither analyzed severity or recurrence of depression nor measured hippocampal subregions. We controlled results for medication as effects from diseases on brain structures might be concealed or caused by the medication. Antidepressants have been demonstrated to induce hippocampal neurogenesis.^{70–72} Antidepressants—independent from the diagnosis of depression—did have negative impact on HV in our study. This result appears counterintuitive. Although the intake of antidepressants may largely be associated with depressive disorders, they are also prescribed for patients suffering from pain, anxiety or sleep disturbances. Among those participants suffering from depression, the use of antidepressants may indicate as well chronicity, recurrence or severity of depression, which lead to decrease of HV. In addition, loss of HV or reduction of hippocampal cell proliferation might not be a direct effect of antidepressants. Antidepressants could also be understood as a proxy for distress by pain, depression or anxiety, which lead to hippocampal volume loss. Effects of the chronic diseases might not be completely removed from the neural plastic and proliferative effects of antidepressants.

We could not confirm that diabetes or elevated cholesterol levels were associated with smaller HV. This is contradictory to previous findings of association of diabetes and total cholesterol levels and HV in a sample of older male subjects.¹²

Associations of genetic markers with HV

We confirmed not only rs7294919 but also the four loci discovered by Bis *et al.* (rs17178006, rs6741949, rs6581612 and rs7852872) as predictors for HV. These findings are consistent with our hypotheses to confirm all genetic loci named above.

Rs17178006 (*MSRB3*, methionine sulfoxide reductase B3) is a gene that encodes a protein involved in the reduction of methionine sulfoxide to methionine, preserving the biological activity of proteins after oxidative damage.^{33,34} Although pathophysiology is unclear, *MSRB3* seems to be cell-protective in the hippocampus and may prevent apoptosis leading to HV loss.^{73,74} As rs17178006 (*MSRB3*) and rs6581612 (*WIF1*) were in low linkage disequilibrium ($r^2=0.2$), we performed conditional analyses on their association on HV: both effects were attenuated but only the association with rs17178006 remained significant. Although both genes are excellent candidates of neural function and protection, this finding implies a single locus or complex mediation effects (for example, through gene transcription modulation or protein networks). A pathway analysis revealed a putative connection between *MSRB3* and *WIF1* via leptin and APOB (via text mining; Supplementary Figure 1).

Rs6741949 (*DPP*) was not involved in interactions or pathways but in a genetic association. In animal models, *DPP-4* inhibitors moderated brain mitochondrial function showed improved cognitive behavior³⁵ and reduced deposits of amyloid in Alzheimer models.^{75,76}

Interactions between clinical or psychosocial factors and genetic markers

Three interactions were associated with HV: the two interactions with rs7294919 are in line with both GWAS.^{25,26} C-allele carriers had larger HVs than non-carriers, but only in non-smokers or participants with low diastolic blood pressure. The intergenic variant rs7294919 comprises the genes *TESC*, *HRK* and *FBXW8*. *TESC* encodes a protein signaling complex⁷⁷ and has an important role in cell proliferation and brain development.^{26,27} *HRK* is a regulator of apoptosis,^{29,78} which exhibits death-inducing activity in cells.^{28,29} *FBXW8* encodes a member of the F-box protein family, which modifies neuronal dendrite growth and configuration.³⁰

Interactions between genetic markers

Carriers of CC genotype of rs6581612 (*WIF1*) and the CG genotype of rs7852872 (*ASTN2*) were associated with larger HVs. *ASTN2* is expressed in the hippocampus and is involved in neuronal migration.³⁶ *WIF1* also known as WNT inhibitory factor 1 is a protein that is encoded by the *WIF1* gene^{79,80} that inhibits WNT proteins from triggering signals and affects neuronal functions.^{31,32} The WNT signaling pathway is part of the cell–cell communication between neurons and astrocytes, which may be disturbed in neurodegenerative disorders.⁸¹ At the physiological level, an interaction between mechanisms of neuronal migration and cell–cell communication seems conceivable.

Landmarks of this study provide: the joint analysis of potential factors affecting HV in a large general population sample, applying the same sampling methods and investigated in the same scanner with identical protocols. Modeling with restricted cubic splines made it possible to detect nonlinear associations with HV. The statistical model for interaction analysis comprised all main effects and interaction terms that lead to robust adjustment for all confounding effects and circumvent the problem of multiple testing.

Limitations of our study are: first, our results may have a limited generalizability as distributions of risk/protective factors in different populations vary and lead to different associations. Second, selection bias because of MRI participation cannot be excluded. Third, unmeasured confounding and information bias could have influenced our results. Fourth, we did not measure hippocampal subregions. Some studies demonstrated that volume loss may rather be a process affecting hippocampal subregions without showing significant change in the whole hippocampus. Fifth, the cross-sectional nature of our study limits causal inference. Last, additional cognitive tests may be helpful to underlie the functional differences in HV.

This study confirmed the impact of education, diastolic blood pressure, smoking and genetic variants on HV. Complex interactions between these factors seem to have an important role in HV changes. We suggest composite scores for the individual estimate of HV and the biological reserve capacity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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