

A *CYP46* T/C SNP modulates parahippocampal and hippocampal morphology in young subjects

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Abstract

There is evidence that brain cholesterol metabolism modulates the vulnerability for Alzheimer's disease (AD). Previous data showed that brain β -amyloid load in elderly subjects with the *CYP46* (cholesterol 24S-hydroxylase) TT-positive genotype was higher than in *CYP46* TT-negative elderly subjects. We investigated effects of the *CYP46* T/C polymorphism on parahippocampal and hippocampal grey matter (GM) morphology in 81 young subjects using structural magnetic resonance imaging based morphometry. We found that young TT-homozygotes exhibited smallest and CC-homozygotes largest parahippocampal and hippocampal GM volumes with the volumes of the CT-heterozygotes ranging in between. Parahippocampal and hippocampal volumes were positively correlated with delayed memory performance in C-carriers and negatively with immediate memory performance in TT-homozygotes. It has been shown that the brain cholesterol metabolism in general modulates dendrite outgrowth, synaptogenesis, and neuron survival, and it was suggested that *CYP46* indirectly influences β -amyloid metabolism. *CYP46* C-carriers are privileged both in terms of β -amyloid metabolism and in terms of brain reserve due to their larger parahippocampal and hippocampal structures. The exact cellular mechanisms that translate the *CYP46* allelic variation into volumetric brain differences in the parahippocampal gyrus and hippocampus are still unknown and need to be further investigated.

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1. Introduction

The cytochrome 450 pigment 46 enzyme (*CYP46*, cholesterol 24S-hydroxylase) is a subfamily of cytochrome P450 proteins. This protein is anchored in the endoplasmatic reticulum. The most important function of cholesterol is to provide eukaryotic plasma membranes both stability and flexibility. *CYP46* degrades cholesterol mainly to 24S-hydroxycholesterol (24-OHC). The conversion of cholesterol

to 24-OHC is a mechanism for the elimination of excess cholesterol from and maintenance of cholesterol homeostasis in the brain (Björkhem et al., 1997). This mechanism is modulated by age (Lütjohann et al., 1996). There is no cholesterol import into the brain; hence, brain cholesterol availability depends entirely on local production. Being a constituent of myelin and neural cell membranes, cholesterol is important for brain function. Studies also suggest that cholesterol plays an important role in regulating β -amyloid (A β) metabolism (Papassotiropoulos et al., 2003; Puglielli et al., 2003; Simons et al., 1998; Wolozin, 2003). Therefore, a loss of *CYP46* activity might produce a corresponding increase in A β accumulation that may lead to neurodegeneration.

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Although preliminary findings point toward an influence of this cholesterol turnover mechanism on the vulnerability for Alzheimer's disease (AD), it is still disputed whether this SNP (rs754203 on intron 2) in the *CYP46* gene (on chromosome 14q32.1) is a risk factor for AD. *CYP46* TT-positive patients with AD were found to have increased cholesterol levels in cerebrospinal fluid (CSF) compared with *CYP46* TT-negative patients with AD (Papassotiropoulos et al., 2003), albeit only on a trend level toward significance ($p=0.07$). These increased cholesterol levels presumably coincide with a loss of *CYP46* activity (Papassotiropoulos et al., 2003). The majority of studies found the TT-genotype to be the risk variant for AD, while this was the case for the CC-genotype only in a few studies (Helisalmi et al., 2006). Most of the studies on this *CYP46* SNP and its association with AD were meta-analyzed by Helisalmi et al. (2006). An interaction of the *CYP46* T/C SNP with the $\epsilon 4$ allele of the apolipoprotein E (*ApoE*) gene synergistically increases the risk for AD (Papassotiropoulos et al., 2003). Recently, Reiman et al. used 18 fluoro-2-deoxyglucose (FDG) positron emission tomography (PET) to measure regional cerebral blood flow (rCBF) and showed that cholesterol-related genes modulate brain metabolism. These authors revealed hypometabolism in AD-affected brain regions in healthy elderly subjects who are carriers of the negative allelic variant of several cholesterol-related genes including the *CYP46* TT-genotype, even when controlling for the effects of *ApoE* $\epsilon 4$ gene dose (Reiman et al., 2008).

Also, there is accumulating and converging evidence that the brain cholesterol metabolism plays an important role in dendrite outgrowth, synaptogenesis, and neuronal survival. First, it has been shown that cholesterol complexed to *ApoE*-containing lipoproteins controls synaptogenesis in the central nervous system (Mauch et al., 2001). Second, it has been reported that cholesterol deficiency causes a selective inhibition of dendrite outgrowth due to the decreased stability of microtubules as a result of inhibition of microtubule-associated protein 2 (MAP2) phosphorylation (Fan et al., 2002). Third, inhibition of cholesterol production in the brain tissue cultures induced neuronal cell death (Michikawa and Yanagisawa, 1999). Fourth, it has been suggested that the deleterious effects of ethanol on the developing brain might be due, at least in part, to an effect on cholesterol homeostasis (Guzzetti and Costa, 2007). Last, there are inborn defects in cholesterol metabolism such as the Smith-Lemli-Opitz syndrome (Tint et al., 1994), a failure in cholesterol production accompanied by microcephaly, and the Niemann-Pick type C disease, a failure in cholesterol degradation accompanied by neurodegeneration (Sévin et al., 2007). Taken together, there is good evidence that brain cholesterol and its metabolism are important for the formation and maintenance of neural tissue as well as for neural functions. Because neuropathologic changes in Alzheimer's disease precede the occurrence of the first cognitive symptoms by decades (Mondadori et al., 2006), we anticipated that there might be structural brain differences in the medial tempo-

ral lobe already in young healthy subjects with the *CYP46* TT-genotype.

Hence, we hypothesized that the *CYP46* T/C SNP affects neural tissue, preferentially in brain structures with a high metabolic turnover such as the hippocampus and in fact the whole medial temporal lobe (MTL), which is first affected by Alzheimer's pathology. Here, we report first findings of the influence of the *CYP46* T/C SNP (rs754203) on parahippocampal and hippocampal morphology in healthy young Swiss subjects of Caucasian origin, while simultaneously controlling for the allelic variation in other genes with known influences on brain morphology and memory functions such as the gene that encodes for the brain-derived neurotrophic factor and the apolipoprotein gene that is associated with AD.

2. Methods

2.1. Subjects

The 81 participants were drawn from a larger sample of 354 healthy, young Swiss subjects of Caucasian origin (de Quervain et al., 2003). The 354 volunteers were university students and employees/trainees recruited at the university of Zurich and via advertisements in local newspapers. The 81 subjects already participated in two functional magnetic resonance imaging studies that investigated brain activity elicited by memory tasks between different apolipoprotein E genotypes (Mondadori et al., 2007) and between different prion protein genotypes (Buchmann et al., 2008). Our sample is homogenous with respect to age, education, and memory performance. The subjects reported no past or current psychiatric or neurological problems and denied taking illegal drugs or medication. All subjects gave written informed consent to participate in the study after the nature and possible consequences of the study had been explained. The local ethics committee approved the experiment. The subjects were paid for participating in the study.

2.2. Genotyping

Information on polymorphic sites was derived from the database of single nucleotide polymorphisms (dbSNP) (<http://www.ncbi.nlm.nih.gov/SNP/index.html>). Genotyping is described in detail elsewhere: for the cholesterol 24S-hydroxylase (*CYP46*; rs754203) gene (Papassotiropoulos et al., 2003), for the brain-derived neurotrophic factor (*BDNF*; rs6265) gene (Egan et al., 2003), for the apolipoprotein E (*ApoE*) gene (Nauck et al., 2000), for the serotonin 2a receptor (*5-HT2a*; rs6314) gene (de Quervain et al., 2003), and for the prion protein (*PrnP*; rs1799990) gene (Papassotiropoulos et al., 2005a). We also controlled for nonrandom genetic heterogeneity (hidden population structures) by genotyping each subject for 27 unlinked SNPs located in non-genic regions distributed over all autosomes (Papassotiropoulos et al., 2005b).

2.3. Neuropsychology

Memory performance (immediately after stimulus presentation as well as at delays of 5 min and 24 h) was assessed during two consecutive days using the Rey-15-figures free recall test (Rey, 1958), the recurring figures recognition test (Kimura, 1963), and an in-house developed verbal free recall test of 30 common German words, a test already applied and described in other publications (Papassotiropoulos et al., 2005a; de Quervain et al., 2003). The difficult in-house verbal free recall test had been developed to avoid ceiling effects that commonly occur when investigating cognitively high functioning subjects such as university students with other verbal memory tests. On the first day, subjects viewed six sets of semantically unrelated nouns (five nouns per set) presented at a rate of 1 word per second with the instruction to learn the words for immediate free recall after each series. In addition, subjects underwent an unexpected delayed free recall test of the learned words after 5 min and again after 24 h. Both delayed free recall tests reflect episodic memory (Squire and Alvarez, 1995).

In addition to the verbal memory test, subjects performed a modified version of the Rey-15-figures free recall test (Rey, 1958), which included the presentation of 15 figures in sequence—each figure for 2 s—with the instruction to learn them for immediate recall. In addition, subjects underwent an unexpected delayed free recall test of the learned figures after 5 min and again after 24 h.

In addition to the two free recall memory tests, we also applied a non-verbal recognition memory test using 13 complex figures of the recurring figures recognition test (Kimura, 1963). This test requires the presentation of geometric or irregular nonsense figures—each figure for 2 s—that have to be learned for later recognition at delays of 5 min and 24 h.

All reported memory scores are hits minus false alarms. Maximal scores are 15 for the Rey-Figures Test; 13 for the Recurring Figures Test; and 30 for the list of common words.

Additionally, we applied three different subscales of the revised Wechsler adult intelligence scale (WAIS-R) (Wechsler, 1981; German version: Tewes, 1991) to assess working memory capacity (Digit span, forward and backward), visuospatial cognition (Block design), and verbal reasoning (Similarities). A summary of the neuropsychological test results between the three different *CYP46* genotypes can be found in Table 1.

2.4. Magnetic resonance imaging data acquisition

Magnetic resonance imaging (MRI) scans were acquired on a 3T Philips Intera whole body scanner (Philips, Best, the Netherlands) equipped with a commercial eight-element head coil array. A T1-weighted gradient echo (fast field echo) sequence was applied with a measured spatial resolution of 1.0 mm × 1.0 mm × 1.0 mm (acquisition matrix 224 × 224 pixels) and a reconstructed resolu-

tion of 0.9 mm × 0.9 mm × 0.8 mm, echo-time TE = 2.3 ms, repetition-time TR = 20 ms, flip angle FA θ = 20°.

2.5. Preprocessing of magnetic resonance imaging data

Image preprocessing was done with Statistical Parametric Mapping (SPM5) software (<http://www.fil.ion.ucl.ac.uk/spm>) using voxel-based morphometry (VBM) (Ashburner and Friston, 2000) implemented in the VBM5 toolbox (<http://dbm.neuro.uni-jena.de/vbm/>). A Hidden Markov Random Field (HMRF) model was applied to enhance the accuracy of the tissue class segmentation process (<http://dbm.neuro.uni-jena.de/vbm/markov-random-fields/>). Customized a priori maps from the normalized, segmented, HMRF weighted, unsmoothed GM, white matter (WM), and CSF images of the subjects were constructed. Native images were again segmented with the customized a priori maps resulting in normalized, segmented, Jacobian modulated, and HMRF weighted GM images which were smoothed with a 9 mm Gaussian kernel. Images were subjected into a voxel-wise analysis on one hand and the unsmoothed versions were used for the volumes-of-interest (VOIs) approach on the other hand. VOIs for the parahippocampal gyri and for the hippocampi were derived from the PickAtlas (<http://www.fmri.wfubmc.edu/cms/software#PickAtlas>). These VOIs were originally drawn by others (Tzourio-Mazoyer et al., 2002) on the MNI single-subject brain (Holmes et al., 1998). We also normalized and segmented the MNI single-subject brain to obtain the warping parameters to adjust this brain onto the customized a priori maps. We then applied these transformations to the VOIs to adjust them onto the customized a priori maps, multiplied the VOIs by the GM images, and computed the volumes within these VOIs. Note that both the voxel-based and the VOI-based approach are fully automated procedures and therefore they are operator-independent.

2.6. Statistical analyses

We used SPM5 and SPSS 14 (<http://www.spss.com/>) to analyze voxel- and VOI-based data, respectively. Partial correlations between *CYP46* allelic frequency and parahippocampal or hippocampal volumes controlling for total GM volume (TGMV) and *BDNF* genotype as well as analysis of covariance (ANCOVA) models were applied. In the ANCOVAs, the allelic variant was the independent variable and parahippocampal and hippocampal volumes the dependent variables while TGMV and *BDNF* genotype were used as covariates. Gender and TGMV are collinear variables (Spearman correlation: $r = -0.657$, $p = 2.82 \times 10^{-11}$, male coded as 1, female coded as 2), i.e., TGMV explains most of the gender-related variance in brain morphology; hence, gender was not included in our statistical models to preserve statistical power. Education, handedness, and age showed no significant influence on morphology (tested in multiple regression analyses together with TGMV and *BDNF* genotype) and were therefore not modeled in both analyses. We

Table 1

Demographic characteristics, memory performance, and brain tissue volumes of *CYP46* genotypes. Memory measures are hits minus false alarms.

Measures	TT-homozygotes (n = 33)				CT-heterozygotes (n = 38)				CC-homozygotes (n = 10)				Probability ^a
	Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.	
Age (years)	22.9	3.5	18.7	39.0	23.2	2.3	19.7	31.0	24.0	3.4	20.0	32.1	0.613
Education (years)	14.5	1.7	10.5	18.0	15.4	1.8	12.0	19.0	15.5	1.1	13.5	17.0	0.104
TGMV (cm ³)	781.6	70.4	648.5	947.2	778.8	74.5	657.1	920.6	778.7	79.1	665.2	946.2	0.613
TWMV (cm ³)	420.4	46.5	320.9	499.1	428.4	49.6	348.8	584.2	417.2	67.1	353.9	592.2	0.616
TCSFV (cm ³)	405.5	63.3	296.1	595.1	412.4	50.6	296.8	516.7	410.3	43.8	350.0	506.5	0.912
ICV (cm ³)	1,607.5	143.2	1,313.7	1,909.7	1,619.7	132.8	1,386.4	1,936.3	1,606.1	163.5	1,387.7	1,958.6	0.924
Kimura figures short delay	6.6	3.0	0	12	7.1	3.0	0	13	6.0	2.9	1	9	0.544
Kimura figures long delay	5.3	3.0	−1	11	4.9	2.7	−3	11	5.2	2.7	1	8	0.860
Rey figures short delay	5.6	3.0	1	12	5.2	2.8	−1	12	6.0	4.1	1	12	0.674
Rey figures long delay	3.9	3.4	−7	9	2.4	3.2	−6	−8	3.8	4.6	−2	12	0.178
30 words immediate	22.4	4.0	14	29	22.7	2.9	15	29	22.0	3.7	17	27	0.818
30 words short delay	5.8	3.9	−3	14	6.0	3.5	0	14	5.7	2.4	2	9	0.979
30 words long delay	5.0	3.9	−8	11	3.6	4.2	−6	11	2.3	2.5	−3	5	0.122
Digit span ^b (WAIS-R)	15.6	3.8	9	25	15.3	3.6	8	21	16.5	3.9	10	23	0.651
Block design (WAIS-R)	42.8	6.9	27	51	43.8	5.4	33	51	43.8	4.5	36	50	0.776
Similarities (WAIS-R)	28.3	2.7	20	32	28.4	2.6	23	32	27.4	4.0	19	32	0.602
Gender (f/m)	Frequency 21/12				Frequency 18/20				Frequency 7/3				0.755
Handedness (r/l/a)	28/3/2				32/4/2				9/1/0				0.624
<i>BDNF</i> val66met	Frequency of amino acids/alleles 22 val/val 10 val/met 1 met/met				Frequency of amino acids/alleles 21 val/val 16 val/met 1 met/met				Frequency of amino acids/alleles 7 val/val 3 val/met 0 met/met				0.526
<i>5-HT2a</i> his452tyr	24 his/his 9 his/tyr 0 tyr/tyr				29 his/his 9 his/tyr 0 tyr/tyr				5 his/his 5 his/tyr 0 tyr/tyr				0.255
<i>PrnP</i> met129val	13 met/met 12 val/met 7 val/val				16 met/met 13 val/met 9 val/val				5 met/met 3 val/met 2 val/val				0.986
<i>ApoE</i> epsilon4 vs not epsilon4	10 epsilon 4 19 not epsilon 4				6 epsilon 4 32 not epsilon 4				3 epsilon 4 7 not epsilon 4				0.195
<i>GenHet</i> 2 clusters	15 cluster 1 18 cluster 2				20 cluster 1 18 cluster 2				6 cluster 1 4 cluster 2				0.682

Abbreviations: TGMV, total grey matter (GM) volume; TWMV, total white matter (WM) volume; TCSFV, total cerebrospinal fluid (CSF) volume; ICV, intracranial volume; WAIS-R, Wechsler adult intelligence scale—revised; f, females; m, males; r, right; l, left, a, ambidexter; *BDNF*, brain-derived neurotrophic factor; *5-HT2a*, 5-hydroxy-tryptamine 2a; *ApoE*, apolipoprotein E; *PrnP*, prion protein; GenHet, genetic heterogeneity; val, valine; met, methionine; his, histidine; tyr, tyrosine; S.D., standard deviation.

^a Confirmed by analysis of variance and covariance (two-tailed) and by χ^2 (two-tailed).

^b Forward and backward.

controlled for SNPs with published influences on brain morphology such as found in the *BDNF* gene (Egan et al., 2003; Hariri et al., 2003; Pezawas et al., 2004; Szesko et al., 2005; Bueller et al., 2006) and *5-HT2a* gene (Filippini et al., 2006), and for SNPs with potential influences on morphology such as found in the *ApoE* gene (Mondadori et al., 2007) and *PrnP* gene (Papassotiropoulos et al., 2005a), as well as for nonrandom genetic heterogeneity, i.e., hidden, genetic population structure (Papassotiropoulos et al., 2005b). Allelic frequencies of the SNPs with potential influences and the *5-HT2a* SNP were not significantly different between the *CYP46* genotypes and were not considered further. Although the allelic frequency of the *BDNF* SNP was not statistically different between the three *CYP46* genotypes it was included in our statistical models as a covariate because of its relevance on medial temporal lobe morphometry as well as memory functions (Egan et al., 2003; Hariri et al., 2003; Pezawas et al., 2004; Szesko et al., 2005; Bueller et al., 2006). VBM results were thresholded with an error probability of $p < 0.001$ (uncorrected for multiple comparisons) combined with a cluster extent threshold of $k = 50$ voxels (uncorrected). In order to account for the multiple comparisons problem we applied a small volume correction (SVC) that restricts the statistical analysis to the medial temporal lobe. This SVC was combined with a false discovery rate (FDR) of $p = 0.05$. For the VOI-based data, we used a common threshold of $p < 0.05$ (uncorrected). We correlated the parahippocampal and hippocampal volumes with memory performance and with the *CYP46* T-allele frequency and the latter also with memory performance. We applied two different types of correlations, a partial product-moment correlation according to Pearson that controls for TGMV and *BDNF* genotype, and a rank correlation according to Spearman. The rank correlations were based on residual values after the effects of TGMV

and *BDNF* genotype on hippocampal and parahippocampal volumes were regressed out.

3. Results

Demographic characteristics, memory performance, indices of intelligence, and global brain tissue volumes are shown in Table 1. There were neither significant differences nor trends toward significance ($0.05 < p < 0.1$) between the *CYP46* genotypes for the variables age, years of education, TGMV, total WM volume, total CSF volume, intracranial volume, indices of intelligence, and for any of the free recall and recognition memory measure as revealed by AN(C)OVAs. Nor were there significant differences in the distribution of *BDNF*, *5-HT2a*, *ApoE*, *PrnP*, *GenHet* allele frequencies, gender, and handedness between *CYP46* genotypes as assessed with χ^2 -tests. As expected, *CYP46* genotype distribution was under Hardy–Weinberg equilibrium conditions.

We found volumetric GM differences in the medial temporal lobe (MTL) structures between the *CYP46* genotypes (Figs. 1 and 3) as a result of both the correlations (Fig. 1 left) and ANCOVAs (Fig. 1 right). Voxel-wise (Figs. 1 and 2) and VOI-based (Fig. 3 and Table 2) analyses revealed that both parahippocampal gyri and both hippocampi were smaller in TT-homozygotes than in CT-heterozygotes and in turn than in CC-homozygotes. In the voxel-wise analyses (Fig. 1), the left parahippocampal cluster size is 7984 voxels ($p < 0.001$ uncorrected; $p = 0.003$ FDR corrected) as revealed by correlation and 2322 voxels ($p < 0.001$ uncorrected; $p = 0.024$ FDR corrected) as revealed by ANCOVA. The right parahippocampal cluster size is 785 voxels ($p < 0.001$ uncorrected; $p = 0.004$ FDR corrected) as yielded by the correlation analysis. In the VOI-based analyses (Fig. 3), the differences

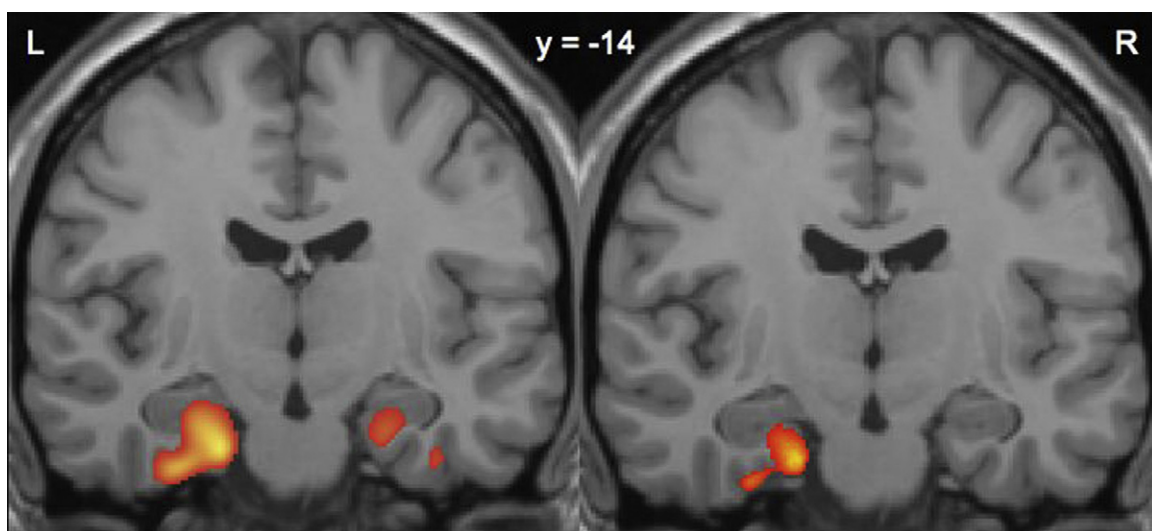


Fig. 1. Main effect of *CYP46* T-alleles on parahippocampal and hippocampal volumes revealed by voxel-wise analyses using partial correlation (left) and analysis of covariance (right). Total grey matter volume and *BDNF* allele frequency were used as covariates. Less T-alleles mean larger volumes. Clusters of volumetric differences are overlaid on the Montreal Neurological Institute (MNI) single-subject brain. Abbreviations: L, left; R, right; y, y-coordinate in MNI space.

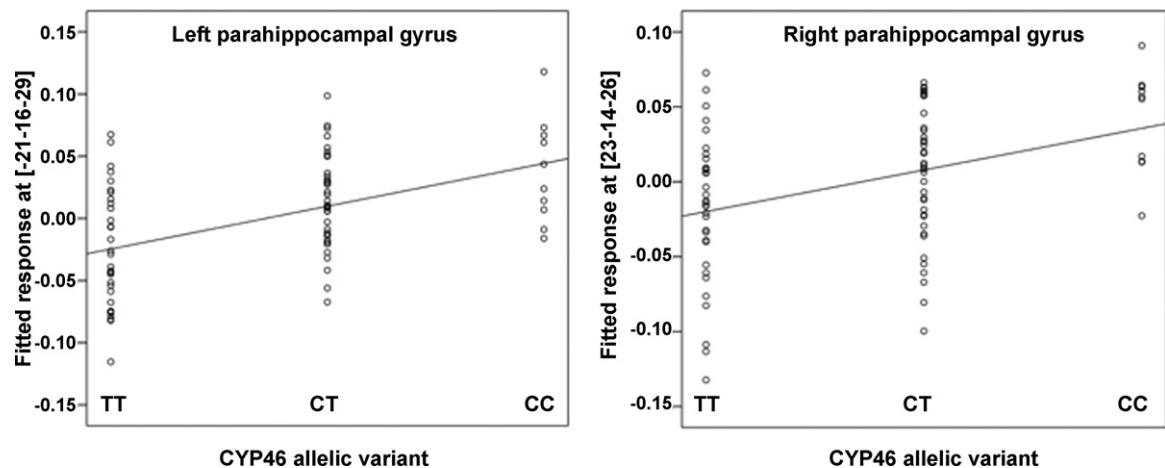


Fig. 2. Correlations between *CYP46* genotypes and parahippocampal volumes. Influences of total grey matter volume and *BDNF* allele frequency were regressed out. Less T-alleles go with larger volumes. Plots are shown for two local maxima located in the left and right entorhinal cortex. Coordinates are in Montreal Neurological Institute (MNI) space. The y-axis represents the beta-values of the general linear model.

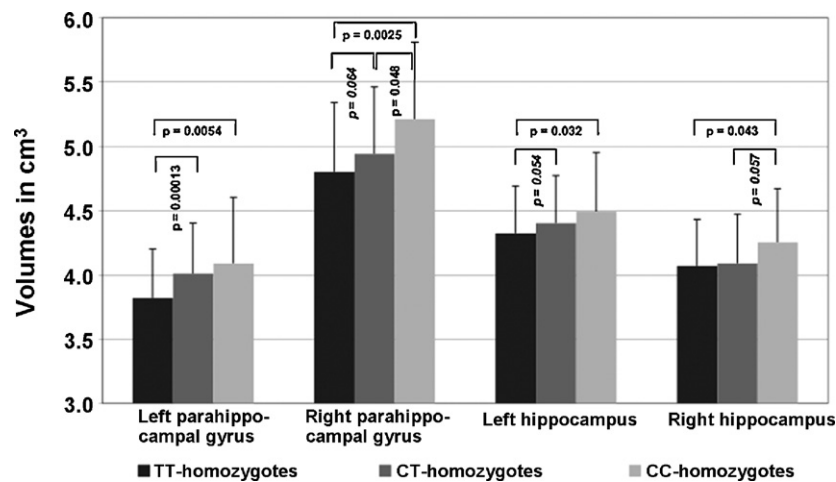


Fig. 3. Parahippocampal and hippocampal volumes of the different *CYP46* genotype groups. Error probabilities are corrected for total grey matter volume and *BDNF* genotype. Mean volumes and the error bar represents the standard deviation. Statistical trends ($0.05 < p < 0.1$) are printed in italic.

of the parahippocampal and hippocampal volumes between TT- and CC-homozygotes were all significant, whereas the volumetric differences between the homozygotes and the CT-heterozygotes showed only a trend toward significance (see Fig. 3). As stated above in Section 2, we

did not model gender in our analyses because gender is collinear to TGMV. Nevertheless, we also analyzed our data with TGMV and gender as simultaneous covariates. These analyses revealed qualitatively and quantitatively similar results.

Table 2
Correlations between T-allele frequency and parahippocampal or hippocampal volumes.

Structures— <i>CYP46</i> genotype correlation ($n = 81$)	Partial correlation ^a			Spearman correlation ^a		
	<i>r</i>	<i>p</i>	<i>d</i>	<i>r</i>	<i>p</i>	<i>d</i>
Left parahippocampal gyrus	0.43	0.00004	0.95	0.39	0.0003	0.85
Right parahippocampal gyrus	0.36	0.001	0.78	0.33	0.002	0.71
Left hippocampus	0.26	0.010	0.52	0.24	0.032	0.49
Right hippocampus	0.23	0.021	0.46	0.20	0.068	0.42

Less T-alleles (more C-alleles) mean larger volumes. Abbreviations: *CYP46*, cholesterol 24S-hydroxylase; *d*, Cohen's effect size; *p*, error probability; *r*, correlation coefficient.

^a Partial correlations corrected for total grey matter volume and brain-derived neurotrophic factor (*BDNF*) allele frequency. Probabilities $p < 0.05$ are in bold, statistical trends towards significance ($0.05 < p < 0.1$) are in italic.

Table 3

Correlations between memory performance and parahippocampal or hippocampal volumes in the *CYP46* genotype groups.

Structures—memory tests ^a	Partial correlation ^b			Spearman correlation ^b		
	<i>r</i>	<i>p</i>	<i>d</i>	<i>r</i>	<i>p</i>	<i>d</i>
TT-homozygotes (<i>n</i> = 33)						
Left hippocampus—30 words immediate recall	−0.58	0.001	1.44	−0.51	0.003	1.17
Right hippocampus—30 words immediate recall	−0.45	0.011	1.01	−0.40	0.020	0.88
Left parahippocampal g.—30 words immediate recall	−0.34	0.061	0.72	−0.24	0.185	0.49
Right parahippocampal g.—30 words immediate recall	−0.45	0.011	1.01	−0.45	0.009	1.00
CT-heterozygotes (<i>n</i> = 38)						
Right hippocampus—30 words short delay (5 min)	0.30	0.080	0.62	0.32	0.048	0.68
Right parahippocampal g.—30 words short delay (5 min)	0.34	0.045	0.71	0.28	0.092	0.58
Right hippocampus—30 words long delay (5 min)	0.28	0.104	0.57	0.27	0.106	0.55
Right parahippocampal g.—30 words long delay (24 h)	0.47	0.004	1.08	0.45	0.005	1.00
CC-homozygotes (<i>n</i> = 10)						
Left hippocampus—Rey figures long delay (24 h)	0.52	0.187	1.22	0.47	0.171	1.06
Right hippocampus—Kimura figures short delay (5 min)	0.30	0.474	0.62	0.23	0.527	0.47
Left parahippocampal g.—Rey figures long delay (24 h)	0.28	0.509	0.57	0.18	0.625	0.36
Right parahippocampal g.—30 words long delay (24 h)	0.53	0.177	1.25	0.53	0.116	1.25

The whole pattern of correlations can be found in the Supplementary Table S1 online. Abbreviations: *d*, Cohen's effect size; g., gyrus; min, minutes; h, hours; *p*, error probability; *r*, correlation coefficient.

^a All memory measures are hits minus false alarms.

^b Correlations are corrected for total grey matter volume and brain-derived neurotrophic factor (*BDNF*) allelic variant. Probabilities $p < 0.05$ are in bold, statistical trends towards significance ($0.05 < p < 0.1$) are in italic.

Furthermore, we obtained negative findings with respect to an influence of the *BDNF* and the *5-HT2a* SNP on hippocampal volumes. This is in contrast with the literature where the *BDNF* val66met-allele (Egan et al., 2003; Hariri et al., 2003; Pezawas et al., 2004; Szeszko et al., 2005; Bueller et al., 2006) and the *5-HT2a* his452tyr-allele (Filippini et al., 2006) were associated with smaller hippocampal volumes. Nevertheless, there was a statistical trend in our sample pointing towards an association between *BDNF* val66met-allele and smaller hippocampal and parahippocampal volumes. When *CYP46* genotypes were added as a second factor to a *BDNF*-MANCOVA with both hippocampi and parahippocampal gyri as dependent variables, *CYP46* genotypes explained most of the variance in these volumes (*BDNF*: $F = 1.17$, $p = 0.327$; *CYP46*: $F = 1.75$, $p = 0.045$). When the hippocampal and parahippocampal volumes were analyzed separately, all medial temporal lobe volumes were comparable between the different *BDNF* and *5-HT2a* genotypes: left hippocampus (*BDNF*, $p = 0.216$; *5-HT2a*, $p = 0.459$), right hippocampus (*BDNF*, $p = 0.133$; *5-HT2a*, $p = 0.639$), left parahippocampal gyrus (*BDNF*, $p = 0.114$; *5-HT2a*, $p = 0.279$), and right parahippocampal gyrus (*BDNF*, $p = 0.063$; *5-HT2a*, $p = 0.765$).

Memory performance in the *CYP46* genotypes is listed in Table 1. We correlated memory performance with the parahippocampal and hippocampal GM volumes derived from the VOIs (Table 3). When considering only the significant correlations, a dissociation was found in the direction of these correlations: while TT-carriers showed only negative correlations between immediate memory performance and parahippocampal and hippocampal volumes, CT- and CC-carriers showed only positive correlations between short-

and long-delay memory performance and parahippocampal and hippocampal volumes. However, when looking also at the insignificant correlations there is no further support for such a kind of dissociation. The whole pattern of correlations between the different memory measures and parahippocampal and hippocampal volumes across the different *CYP46* genotypes can be found in the Supplementary Table S1 online. There were no significant correlations between the measures of memory performance and the frequency of *CYP46* T-alleles.

4. Discussion

We found that the *CYP46* T/C SNP (rs754203), a possible risk factor for AD, modulates parahippocampal and hippocampal volumes in young, healthy Swiss subjects of Caucasian origin. These structures were smaller in TT-homozygotes than in CT-heterozygotes and in turn than in CC-homozygotes. The MRI T1-weighted intensity profiles, on which morphometric MRI studies are based, have been shown to be best explained by a weighted sum of cytoarchitectonic and myeloarchitectonic profiles (Eickhoff et al., 2005), suggesting that the volumetric differences originate in differences in neuronal and synaptic compartments, which are very important for brain functions, rather than differences in the stroma (including interstitial fluid) or edema.

The significant correlations of parahippocampal and hippocampal GM volumes with various measure of memory showed a dissociation between TT-carriers and CT-/CC-carriers. Correlations between parahippocampal or hippocampal volumes and immediate recall of 30 words

were *negative* for TT-homozygotes. Although traditional views do not account for a role of MTL structures in short-term memory, there is mounting evidence that the MTL supports short-term memory as well, particularly with high mnemonic load and with associative learning tasks (Ranganath and Blumenfeld, 2005). The negative correlation between parahippocampal or hippocampal volumes and immediate recall of 30 words in TT-carriers is difficult to explain given current knowledge. CT-heterozygotes and CC-homozygotes exhibited *positive* correlations between parahippocampal or hippocampal volumes and performance in tests of delayed recognition. Associations of larger volumes with better memory performance indicates that neurons in the measured volumes were functionally relevant and therefore carry the potential to keep memory functions up at an advanced age when pathological deposits start to disturb neural function locally. This relationship in the carriers of the favorable C-allele are interpretable in the light of the theory of brain reserve capacity (Katzman et al., 1988; Mori et al., 1997) as a protective factor against memory decline due to neurodegeneration at an advanced age. This “volume advantage” affords the individual greater physical resistance against neural degeneration. Thus, *CYP46* T-alleles might mediate a risk for AD (Helisalmi et al., 2006; Papassotiropoulos et al., 2003). The suggestion of a protective role of the *CYP46* C-alleles (SNP rs754203) is supported by recent findings in a Chinese population. Fu and colleagues showed that elderly subjects with the T allele are more likely to deteriorate in cognitive functioning over a two years period compared with those without the T allele (Fu et al., 2009).

Although several morphometric imaging studies found support for an association of the *BDNF* val66met SNP (rs6265) with volumetric differences in MTL structures and memory performance (Egan et al., 2003; Hariri et al., 2003; Pezawas et al., 2004; Szesko et al., 2005; Bueller et al., 2006), we were unable to replicate the finding of smaller hippocampal volumes in *BDNF* val66met carriers compared with val66val carriers within our dataset. Possible explanations include the diversity of methodological approaches applied to measure the volume of interest, the restricted age range of our subjects, the high level of education of our academic sample, the fact that we controlled for genetic variation in additional SNPs as well as background genetic heterogeneity, and/or the different numbers of *BDNF* met66met carriers between our and the other studies.

Evidence is accumulating showing that brain cholesterol metabolism plays an important role in dendrite outgrowth (Fan et al., 2002), synaptogenesis (Mauch et al., 2001), and neuronal survival (Michikawa and Yanagisawa, 1999). Inborn defects in cholesterol metabolism such as the Smith-Lemli-Opitz syndrome (Tint et al., 1994) and the Niemann-Pick type C disease (Sévin et al., 2007) disturb brain development profoundly. The exact molecular and cellular mechanisms that translate the *CYP46* allelic variation into volumetric brain differences in the parahippocampal gyrus and hippocampus are still unknown. However, intronic

SNPs have been shown to modulate genetic risk for AD (Dermaut et al., 2002; Wang et al., 2002) possibly through alternative splicing, altered RNA stability, differences in the distribution of the enzyme inside the neural cell, or through a linkage disequilibrium with other so far unknown loci in the *CYP46* gene. Recently, it has been shown that three other SNPs in the *CYP46* gene (rs7157609, rs4900442, and rs3742376) influence the risk for AD (Kölsch et al., 2009; Fu et al., 2009).

If the found structural differences should turn out to persist in older persons, one might speculate that structures may be larger because *CYP46* C-alleles favor the non-amyloidogenic amyloid precursor protein cleavage pathway by inhibiting the beta-site APP-cleaving enzyme (BACE) as suggested by Wolozin (2003), meaning that less A β might be produced and deposited during lifetime. Less A β production and deposition result in less cell destruction and preserved neural functionality. Therefore, reduced atrophy and hence better memory functions in older age can be expected in *CYP46* C-carriers compared with T-carriers. Taken together the *CYP46* T/C SNP might modulate the vulnerability for AD twofold: through its direct effects on the formation of parahippocampal and hippocampal tissue, neuronal survival, and synaptogenesis in early life and through its indirect role in regulating β -amyloid metabolism at an advanced age.

Conflict of interest

The authors reported no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.neurobiolaging.2009.07.001](https://doi.org/10.1016/j.neurobiolaging.2009.07.001).

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