The effect of the APOE genotype on individual *BrainAGE* in normal aging, mild cognitive impairment and Alzheimer’s disease

**Dissertation**

for the graduation to doctor medicinae (Dr. med.)

**Presented to the council of the Medical Faculty**
**of the Friedrich-Schiller-University of Jena**

from Luise Christine Löwe
born the 27th of April, 1988 in Meißen
Reviewer:

1. Prof. Dr. Christian Gaser,
   Hans Berger Kliniken, Klinik für Psychiatrie und Psychotherapie,
   Klinik für Neurologie, Universitätsklinikum Jena
2. PD Dr. med. Carsten Klingner,
   Klinik für Neurologie, Universitätsklinikum Jena
3. PD Dr. Stefan Klöppel,
   Klinik für Psychiatrie und Psychotherapie, Universitätsklinikum Freiburg

Date of the defense of the doctor's thesis: 3rd of May 2016
# TABLE OF CONTENTS

## LIST OF ABBREVIATIONS ......................................................................................... 2
## ABSTRACT ................................................................................................................. 4
  Context.................................................................................................................. 4
  Objective ............................................................................................................. 4
  Methods .............................................................................................................. 4
  Results ............................................................................................................... 4
  Conclusions ....................................................................................................... 5
## ZUSAMMENFASSUNG ............................................................................................... 6
  Kontext............................................................................................................... 6
  Zielsetzung ....................................................................................................... 6
  Methodik .......................................................................................................... 6
  Ergebnisse ....................................................................................................... 6
  Schlussfolgerung .............................................................................................. 7
## INTRODUCTION ....................................................................................................... 8
## OBJECTIVE ........................................................................................................... 12
## METHODS ........................................................................................................... 13
  ADNI database .................................................................................................. 13
  Subjects ............................................................................................................. 13
    Training Sample .............................................................................................. 13
    Longitudinal Sample ..................................................................................... 14
    Cross-sectional Sample .................................................................................. 17
  MRI Data Preprocessing and Data Reduction ..................................................... 19
  Estimation of BrainAGE scores ........................................................................ 19
  Statistical Analysis ............................................................................................ 20
## RESULTS ................................................................................................................. 22
  Longitudinal sample .......................................................................................... 22
  Cross-sectional sample ..................................................................................... 30
    Conversion of MCI patients ............................................................................ 31
    Prediction of Conversion ................................................................................. 31
  Accuracy of Prediction of Conversion ................................................................ 34
## DISCUSSION ........................................................................................................... 38
  Longitudinal sample .......................................................................................... 38
  Cross-sectional sample ..................................................................................... 40
  Limitations .......................................................................................................... 41
## CONCLUSIONS ....................................................................................................... 45
## REFERENCES ........................................................................................................ 46
## REFERENCES ....................................................................................................... 46
## APPENDICES ....................................................................................................... 56
  Acknowledgement .............................................................................................. 56
  List of Tables ...................................................................................................... 57
  List of Figures ..................................................................................................... 58
  Declaration of authenticity .................................................................................. 59
  Ehrenwörtliche Erklärung ................................................................................... 60
## LIST OF ABBREVIATIONS

| Abbreviation | Full Form |
|--------------|-----------|
| AD           | Alzheimer’s Disease |
| ADAS         | Alzheimer’s Disease Assessment Scale |
| ADNI         | Alzheimer’s Disease Neuroimaging Initiative |
| ANOVA        | Analyses of variance |
| APOE         | Apolipoprotein E |
| AUC          | Area under the curve |
| BrainAGE     | Brain age gap estimation |
| C            | Refers to group of carriers |
| CDR-SB       | Clinical Dementia Rating Scale Sum of Boxes |
| CI           | Confidence interval |
| CSF          | Cerebrospinal fluid |
| MCI          | Mild cognitive impairment |
| MMSE         | Mini-Mental State Examination |
| MRI          | Magnetic resonance images |
| PCA          | Principal Component Analysis |
| PET          | Positron Emission Tomography |
| pMCI         | Progressive MCI patients |
| pMCI_early   | Early converting (<12 months) progressive MCI patients |
| NC           | Refers to group of non-carriers |
| pMCI_late    | Late converting (>12 months) progressive MCI patients |
| NO           | Cognitively normal subjects |
| Abbreviation | Full Form |
|--------------|-----------|
| ROC          | Receiver operating characteristic |
| RVM          | Relevance vector machine |
| SD           | Standart deviation |
| sMCI         | Stable MCI patients |
| SVM          | Support vector machine |
ABSTRACT

Context
In our aging society, diseases in the elderly come more and more into focus. An important issue in research is Mild Cognitive Impairment (MCI) and Alzheimer’s Disease (AD) with their causes, diagnosis, treatment, and disease prediction.

Objective
In this work, the Brain Age Gape Estimation (BrainAGE) method was applied to examine the impact of the Apolipoprotein E (APOE) genotype on cognitive deterioration and MCI and AD development. We examined whether a combination of BrainAGE and APOE status could improve diagnostic accuracy and prediction of cognitive decline in MCI patients.

Methods
We analyzed structural magnetic resonance images (MRIs) of 405 subjects from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database with the BrainAGE framework. Hereby we tested for differences between carrier and non-carrier of APOE ε4 within the diagnostic groups during follow-up time and for estimated longitudinal changes up to 1600 days after baseline. The influence of the APOE status on conversion from MCI to AD was analyzed within all allelic subgroups as well as for carriers and non-carriers, and for a special risk group of MCI patients.

Results
The BrainAGE method differed significantly between normal controls, stable MCI (sMCI) and progressive MCI (pMCI) as well as AD patients. Differences between APOE ε4 carriers and non-carriers were seen with BrainAGE changing rates over time and for NO / sMCI vs. pMCI / AD. During baseline and follow-up, BrainAGE scores correlated significantly with cognitive scores for carriers and non-carriers throughout the whole sample, and for subgroups of pMCI and AD patients. Prediction of conversion was most accurate using the BrainAGE score compared to cognitive scores, even when the patient’s APOE status was unknown.
Conclusions
Diagnosing AD and its preliminary phases, as well as predicting conversion from MCI to AD, the BrainAGE method is a useful and accurate tool even if there is information of the patient’s APOE status missing.
ZUSAMMENFASSUNG

Kontext
Der demografische Wandel ist ein präsentes Thema in unserer Gesellschaft, und Erkrankungen des Alters rücken immer mehr in den Fokus des klinischen Alltags und der Wissenschaft. Ein Forschungsschwerpunkt in diesem Kontext liegt seit den 80iger Jahren auf der Diagnose und Verlaufsprognose der Milden Kognitiven Beeinträchtigung (Mild Cognitive Impairment, MCI) und der Alzheimer Demenz (AD) sowie deren Therapiemöglichkeiten als auch pathophysiologischen Ursachen.

Zielsetzung
In der vorliegenden Arbeit wurde die 2010 veröffentlichte BrainAGE-Methode angewandt und mit den etablierten kognitiven Tests MMSE, CDR-SB und ADAS verglichen. Dabei wurden die Verfahren auf Genauigkeit der diagnostischen Zuordnung zu gesunden Kontrollen (NO), stabilen und progressiven MCI- (sMCI und pMCI) und AD-Patienten, sowie die Vorhersage des Krankheitsverlaufs von MCI-Patienten hinsichtlich ihrer Konversion zu AD untersucht. Von besonderem Interesse war hierbei, ob der Einschluss des genetischen Status der Probanden hinsichtlich des Alzheimer-Risikogenes Apolipoprotein E (APOE) die diagnostische und prognostische Zuordnung des BrainAGE und der kognitiven Scores verbessert.

Methodik
Wir untersuchten strukturelle Magnetresonanztomogramme (MRT-Bilder) von insgesamt 405 Probanden aus der Datenbank der Alzheimer’s Disease Neuroimaging Initiative (ADNI) mit dem BrainAGE Framework. Wir testeten die BrainAGE-Werte auf signifikante Unterschiede zwischen APOE-Trägern und Nicht-Trägern während der Erst- und Folge-Untersuchungen sowie im vorhergesagten zeitlichen Verlauf. Von besonderem Interesse war der Einfluss des Risikogenes auf die mögliche Konversion von leicht kognitiv beeinträchtigten Patienten hin zu Alzheimer Demenz, welche für alle allelischen Subgruppen einzeln, als auch für Träger und Nicht-Träger von APOE ε4 untersucht wurde.

Ergebnisse
Die BrainAGE Methode zeigt, ebenso wie die kognitiven Tests, signifikante Unterschiede zwischen den diagnostischen Gruppen auf. APOE ε4-Träger und Nicht-Träger unterscheiden sich allerdings nur signifikant, wenn die diagnostischen
Gruppen NO / sMCI versus pMCI / AD kombiniert oder die Entwicklung des BrainAGE scores im zeitlichen Verlauf betrachtet werden. Während der Erst- und den Folgeuntersuchungen korreliert der BrainAGE score für Träger und Nicht-Träger der gesamten Kohorte, sowie für einen Großteil der pMCI- und AD-Patienten signifikant mit den kognitiven Scores. Der BrainAGE score erreichte die größte Genauigkeit in der Konversionsvorhersage von MCI zu AD im Vergleich zu den kognitiven Scores, sowohl ohne als auch unter Einschluss des APO-Status. Hierbei verbesserte die Hinzunahme des Trägerstatus die Modellgüte des BrainAGE scores in Tendenzen, jedoch nicht signifikant. Beste Vorhersagewerte werden in der Gruppe der ε4-positiven, progressiven MCI-Patienten beobachtet, die binnen eines Jahres zu AD konvertieren.

Schlussfolgerung
Die BrainAGE Methode hat sich als zuverlässige und akkurate, vollautomatische Anwendung zur Diagnostik und Prädiktionsvorhersage der Alzheimer Demenz und ihren Vorstadien erwiesen. Der APOE Status kann dabei helfen die Vorhersagegenauigkeit der Krankheitsverschlechterung in MCI-Patienten zu verbessern, und ist besonders nützlich bei der Hochrisikogruppe der Schnellkonvertierenden. Unsere Ergebnisse sprechen für ein allgemein erhöhtes Risiko der APOE ε4-Träger an Alzheimer Demenz zu erkranken. Wir fanden interessanterweise verlängerte Konversionszeiten für ε4-Träger im Vergleich zu ε3-Trägern, wobei erstere, einmal zu AD fortgeschritten, den schnellsten kognitiven Verfall und höchste Mortalitätsraten aufwiesen.
INTRODUCTION

During the last 20 years structural brain imaging was more and more integrated into research and diagnosis of neurological disorders (Hinrichs et al. 2009). It became part of the diagnostic workflow to assure clinical diagnosis, to clarify differential diagnoses (Bigler et al. 2000) or to obtain longitudinal data for patient’s follow-up. Brain imaging is also increasingly used as diagnostic marker for abnormal brain atrophy processes such as in Alzheimer’s Disease (AD) (Jack et al. 2010, Walhovd et al. 2010, Davatzikos et al. 2011). AD is of great importance for research since it is the most common cause of dementia late in life, affecting approximately 1% of the population of 60-65 years, and 10-35% of 85 years and older (Small et al. 1997, Kester and Scheltens 2009).

Many AD patients suffer from Mild Cognitive Impairment (MCI) before fully developing all symptoms of AD. MCI is seen as prodromal state of AD (Jack et al. 1999, Albert et al. 2011) or transitional state between normal aging and AD (Petersen 2004, Kovacevic et al. 2009). General criteria for MCI were presented in a first report of the International Working Group on Mild Cognitive Impairment (Winblad et al. 2004). The key symposium summarized the characteristics for MCI patients as: Not normal- not demented, but suffering from cognitive decline. The person must show impairment on objective tasks (self and/or reported by an informant) and a decline over time on these. Meanwhile, basic activities of daily living and the majority of complex instrumental functions should be preserved.

In the case of cognitive impairment and dementia, the pattern and dimension of brain atrophy correlate strongly with the current and future extent of the disease (Chetelat et al. 2002, Chan et al. 2003, Jack et al. 2004, Karas et al. 2004, Pennanen et al. 2005, Davatzikos et al. 2009, Spulber et al. 2010). Generally, whole brain atrophy rates are estimated to be -0.98% per year in patients with very mild AD compared to -0.45% in nondemented elderly (Fotenos et al. 2005), and approximately 2% per year for gray matter volume in AD patients compared to controls (Anderson et al. 2012).

In the last years, many methods have been developed to diagnose and predict conversion from MCI to AD. Some of them are based on MR imaging, since it is easy applicable in clinics and widely available, has low costs and low invasiveness. MRI data can be also easily used for further analysis and calculations, since automated
computer programs can be run everywhere. A large part of these tools work with computer based machine learning systems. Some of them use Relevant Vector Machines (RVMs; Stonnington et al. 2010), but the majority is based on Support Vector Machines (SVMs), like in the case of the structural phenotypic score (Misra et al. 2009), the SPARE-AD index (Davatzikos et al. 2009), the subspace ensemble method for multiple classifier (Liu et al. 2012) or the classification framework (Hinrichs et al. 2009).

Recently, Franke et al. (2010) presented a novel approach for estimating the individual neuroanatomical age based on structural magnetic resonance imaging (MRI) and a machine-learning pattern recognition method. A Relevance Vector Regression (RVM) was applied to model neuroanatomical aging in a large sample of healthy subjects. Analyzing the local patterns of brain atrophy and matching them to the chronological age of the subject, a reliable biomarker based on the estimation of a person’s *brain age gap estimation* (*BrainAGE*) score was obtained. Applying the *BrainAGE* approach to clinical samples, this score discriminated those MCI subjects that converted to AD (pMCI – progressive MCI) within 36 months from those who remained stable (sMCI – stable MCI) (pMCI vs. sMCI; Gaser et al. 2013). It was shown, that MCI and AD patients already have a 6 to 7 years higher *BrainAGE* score at their baseline MRI scan. During a follow-up of period up to 4 years they also revealed advanced brain aging of 1 additional year per follow-up year in MCI and 1.5 extra years per follow-up year in AD compared to controls and sMCI (Franke and Gaser 2012). These findings go along with other publications, which revealed abnormal high brain atrophy for MCI and AD (Karas et al. 2004, Fotenos et al. 2005, Driscoll et al. 2009, Henneman et al. 2009, Sluimer et al. 2009).

The scope of the present study was to investigate, whether conversion from MCI to AD and changes in cognitive abilities can be better predicted by MRI-based measurements when taking into account the patient’s apolipoprotein E (APOE) genotype. The APOE gene is located on chromosome 19q13.2 (Strittmatter et al. 1993), is polymorphic and the 3 most common allelic isoforms are ε2, ε3, ε4 (Corder et al. 1994, Nalbantoglu et al. 1994, Farrer et al. 1997).

It is well known, that APOE ε4 is a dose-dependant risk factor for developing late-onset AD (Corder et al. 1993, Saunders 2000, Bertram and Tanzi 2004, Brouwers et al. 2008, Bertram et al. 2010). Risk estimations vary from a 3 times (Bertram and
Tanzi 2004) to a more than 4 times elevated risk per APOE ε4 allele (Corder et al. 1994, Bertram et al. 2010). Thus, in homozygote carriers the AD affection would be around 8 to 15 times more likely as compared to non-carriers (Corder et al. 1993, Bertram and Tanzi 2004, Deuschl and Maier 2009).

APOE ε4 also influences the clinical course of AD (Hoyt et al. 2005, Martins et al. 2005, Cosentino et al. 2008), provoking an earlier onset of dementia (Corder et al. 1993, Roses et al. 1994, Kurz et al. 1996b, Jack et al. 1998, Saunders 2000, Liddell et al. 2001, Brouwers et al. 2008, Glodzik-Sobanska et al. 2009), a higher degree of brain atrophy (Honea et al. 2009), and a faster cognitive decline (Martins et al. 2005).

The mechanism of how exactly the gene locus has an impact on cognitive decline is still not surely known. However, APOE is a multifunctional molecule, potentially intervening in cholesterol and lipid metabolism (Utermann et al. 1980, Zhang et al. 1992, Brouwers et al. 2008), glucose metabolism (Reiman et al. 2004), oxidative stress (Miyata and Smith 1996) and immune modulation (Saunders 2000), as well as influencing cellular brain processes like amyloid deposition (Jack et al. 1998) and clearance (Saunders 2000), microtubule stability, and intracellular signaling (Mahley and Rall 2000, Saunders 2000). Most studies agree about the negative influence on severity of AD pathology by APOE ε4, manifesting in the deposit of diffuse and neuritic plaques (Schmechel et al. 1993, Olichney et al. 1996, Mortimer et al. 2009) or neurofibrillary tangles (Mortimer et al. 2009). Some studies also approved, that APOE ε4 carriers have lower temporal (Geroldi et al. 1999, Hua et al. 2009, Sluimer et al. 2009), hippocampal (Lehtovirta et al. 1996, Bigler et al. 2000, Mori et al. 2002, Honea et al. 2009, Hua et al. 2009, Manning et al. 2014), amygdala volumes (Lehtovirta et al. 1996, Basso et al. 2006) and significant thinner cortices (Querbes et al., 2009) in comparison to non-carriers.

In contrast, the APOE ε2 isoform is supposed to have a protective effect for MCI and AD (Corder et al. 1994, Talbot et al. 1994, Farrer et al. 1997, Saunders 2000, Brouwers et al. 2008, Berlau et al. 2009, Genin et al. 2011), e.g. manifesting in lower incidences or older age of onset. Further, the occurrence of an APOE ε2 allele is suggested to slow down cognitive decline processes in AD patients when compared to APOE ε2 non-carriers (Martins et al. 2005).
In this work, we examined if a combination of BrainAGE and APOE status could strengthen diagnostic accuracy for a conversion from MCI to AD. We also investigated if including the APOE carrier status would utilize to predict cognitive decline in MCI patients.
OBJECTIVE

Objective of this study was to investigate the relation between APOE genotype and individual BrainAGE score, compared to the cognitive scores Alzheimer’s Disease Assessment Scale (ADAS), Mini-Mental State Examination (MMSE) and Clinical Dementia Rating Scale Sum of Boxes (CDR-SB).

First, we wanted to find out if there were differences between carriers and non-carriers among the 4 diagnostic groups of cognitively normal subjects, patients with Mild Cognitive Impairment (MCI), subdivided into those who do not deteriorate (stable MCI – sMCI) and those who convert to Alzheimer’s Disease (AD) (progressive MCI – pMCI), and AD patients.

Also, we aimed to analyze effects of having particular allelic isoforms of APOE (i.e., $\varepsilon2/\varepsilon3$, $\varepsilon3/\varepsilon3$, $\varepsilon2/\varepsilon4$, $\varepsilon3/\varepsilon4$, $\varepsilon4/\varepsilon4$) on patient characteristics and score results.

Additionally, we wanted to test how much the BrainAGE score is correlated to other scores, namely ADAS, MMSE and CDR-SB, regarding diagnostic groups and carrier status at baseline and follow-up visits.

To better understand the trajectory of BrainAGE and cognitive scores, we wanted to analyse longitudinal changes over time and estimated up to 1600 days from baseline.

Second, we focused on a high-risk-group of patients, the MCI sample, to examine their conversion to AD. We wanted to compare days to conversion for APOE carrier and non-carrier, and additionally divided into illelic isoforms.

For a cross-sectional sample we asked, if there was a gain in prediction accuracy when incorporating the APOE $\varepsilon4$ status in predicting individual conversion from MCI to AD based on BrainAGE score or disease-specific cognitive scales. We wanted to know if the BrainAGE score in combination with the APOE carrier status could especially help to distinguish early progressing MCI patients (progression to AD within 12 months) from late progressing ones (progression after 12 months).

Finally, we aimed to compare the BrainAGE score with chronological age, as well as the ADAS, MMSE and CDR-SB score, to test for highest accuracy, sensitivity and specificity in predicting conversion from MCI to AD.
METHODS

ADNI database
Data from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (http://www.loni.ucla.edu/ADNI) were used for this study. The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial MRI, Positron Emission Tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, M.D., VA Medical Center and University of California–San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research—approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years, and 200 people with early AD to be followed for 2 years. Further up-to-date information, including detailed eligibility criteria, is available on the ADNI information website (http://www.adni-info.org).

Subjects

Training Sample
To train the BrainAGE framework (Franke et al. 2010), $T_1$-weighted MRI data of 560 healthy subjects aged 20–86 years (mean age 48.6±16.5 years) from the publicly accessible IXI cohort (http://www.brain-development.org; downloaded in September 2011) were used. Data were collected on 3 different scanners (Philips 1.5T, General Electric 1.5T, Philips 3.0T).
Additionally, MRI data of 126 healthy subjects from the publicly accessible database OASIS (http://www.oasis-brains.org; downloaded in June 2009) aged 51–94 years (mean age 71.3±11.8 years) were also included in the training sample.

**Longitudinal Sample**

To investigate the longitudinal pattern of *BrainAGE* changes as a function of the APOE ε4 status, the test sample included all subjects from the ADNI database (www.loni.ucla.edu/ADNI), for whom the APOE ε4 status as well as a baseline MRI scan and at least 1 follow-up MRI scan (1.5T) were available, resulting in a sample size of 405 subjects (Table 1). For the exact procedures of data collection and up-to-date information, see www.adni-info.org. Subjects were grouped as (1) NO (normal control group), if subjects were diagnosed cognitively healthy at baseline and remained so during the 3 years follow-up (n= 107); (2) sMCI (stable MCI), if subjects were diagnosed MCI patients at baseline and remained so during the 3 years follow-up (n=36), (3) pMCI (progressive MCI), if subjects were diagnosed MCI patients at baseline but classified AD at some point during follow-up, without reversion to MCI or NO (n=112), (4) AD, if subjects were diagnosed AD patients at baseline and remained so at any follow-up (n=150).

The following cognitive scales, administered at baseline and follow-up examinations, were used to evaluate the grade of cognitive decline: Alzheimer’s Disease Assessment Scale (ADAS, ranging from 0 to 85, with higher test scores indicating worse cognitive functioning; Mohs and Cohen 1988), global Clinical Dementia Rating Scale Sum of Boxes (CDR, ranging from 0 to 3, with 0 indicating NO, 0.5 denoting MCI, 1 and more indicates stages of AD; Morris 1993), Mini-Mental State Examination (MMSE, ranging from 0 to 30, with 30 indicating best cognitive function, 0 worse cognitive function; Cockrell and Folstein 1988).
Table 1. Characteristics of the longitudinal test sample.

|                     | NO (n = 107) | sMCI (n = 36) | pMCI (n = 112) | AD (n = 150) | F-statistics |
|---------------------|--------------|---------------|----------------|--------------|--------------|
|                     | ε4 carriers  | ε4 non-       | ε4 carriers    | ε4 non-      | ε4 carriers  | ε4 non-      | diagnostic    | ε4 status    | diagnostic     |
|                     | (ε2/ε4;     | non-carriers  | (ε2/ε4;       | non-carriers | (ε2/ε4;     | non-carriers | group         | (carriers     | group x ε4     |
|                     | ε3/ε4;     | (ε2/ε3;       | ε3/ε4;       | (ε2/ε3;       | ε3/ε4;     | (ε2/ε3;       |              | vs. non-      | status         |
|                     | ε4/ε4)      | ε3/ε4)       | ε4/ε4)       | ε3/ε4)       | ε4/ε4)     | ε4/ε4)       |              | carriers)     | status         |
| No. of subjects    | 26 (1 / 21 / 4) | 81 (16 / 65) | 14 (0 / 12 / 2) | 22 (3 / 19) | 78 (5 / 52 / 21) | 34 (2 / 32) | 101 (4 / 66 / 31) | 49 (4 / 45) | - | - | - |
| Age mean in years  | 75.0 (5.1) | 75.9 (4.9) | 77.3 (5.6) | 76.8 (6.5) | 74.1 (6.5) | 75.5 (9.3) | 74.1 (6.8) | 75.7 (8.9) | 1.08 | 1.06 | 0.23 |
| (SD)               |             |              |              |              |              |              |              |              | [p=0.36]     | [p=0.30]     | [p=0.88]     |
| MMSE mean (SD)     | 29.3 (0.8) | 29.2 (0.9) | 27.7 (1.7) | 27.2 (2.0) | 26.7 (1.8) | 26.4 (1.7) | 23.4 (2.0) | 23.5 (1.9) | 212.38 | 0.90 | 0.46 |
|                    | (0.0)       | (0.1)        | (0.6)        | (0.6)        | (1.0)       | (1.1)       | (1.5)       | (1.7)       | [p<0.001]    | [p=0.34]     | [p=0.71]     |
| CDR-SB mean (SD)   | 0.0 (0.0) | 0.0 (0.1) | 1.3 (0.6) | 1.1 (0.6) | 1.9 (1.0) | 1.9 (1.1) | 4.2 (1.5) | 4.3 (1.7) | 264.52 | 0.01 | 0.10 |
|                    | (0.0)       | (0.1)        | (0.6)        | (0.6)        | (1.0)       | (1.1)       | (1.5)       | (1.7)       | [p<0.001]    | [p=0.92]     | [p=0.96]     |
| ADAS mean (SD)     | 8.3 (3.9) | 8.9 (3.8) | 17.3 (5.3) | 17.3 (6.3) | 21.8 (5.8) | 21.8 (5.4) | 28.7 (7.2) | 29.0 (9.1) | 176.27 | 0.07 | 0.04 |
|                    | (3.9)       | (3.8)        | (5.3)        | (6.3)        | (5.8)       | (5.4)       | (7.2)       | (9.1)       | [p<0.001]    | [p=0.79]     | [p=0.99]     |
| BrainAGE score in  | -0.11 (6.79) | -1.35 (6.45) | -0.88 (6.13) | 0.09 (4.93) | 5.83 (6.44) | 5.54 (9.68) | 5.76 (7.68) | 6.20 (9.52) | 18.86 | 0.00 | 0.27 |
| years (SD)         |             |              |              |              |              |              |              |              | [p<0.001]    | [p=0.97]     | [p=0.85]     |
| Follow-up No. of   | 5.0 (0.7) | 5.0 (0.8) | 5.9 (1.0) | 5.8 (0.8) | 5.1 (1.5) | 5.4 (1.1) | 3.5 (0.8) | 3.4 (0.7) | 98.64 | 0.03 | 0.38 |
| scans (SD)         | (0.7)       | (0.8)        | (0.8)        | (0.8)        | (1.1)       | (1.1)       | (0.8)       | (0.7)       | [p<0.001]    | [p=0.87]     | [p=0.76]     |
|                          | 1171 (234) | 1197 (270) | 1121 (283) | 1110 (222) | 967 (381) | 974 (309) | 616 (223) | 595 (221) | 84.91 | 0.00 | 0.13 |
|--------------------------|------------|------------|------------|------------|-----------|-----------|-----------|-----------|-------|------|------|
| **Follow-up duration in days (SD)** |            |            |            |            |           |           |           |           |       |      |      |
| Age at last scan (SD)    | 78.2 (5.1) | 79.1 (5.0) | 80.4 (5.4) | 79.9 (6.5) | 76.7 (6.7) | 78.1 (9.4) | 75.8 (6.9) | 77.4 (9.1) | 3.12  | 1.03 | 0.21 |
| MMSE at last scan (SD)   | 28.5 (1.6) | 29.2 (1.1) | 26.7 (2.8) | 27.4 (2.6) | 21.4 (4.1) | 21.9 (4.7) | 19.2 (5.8) | 19.2 (5.3) | 95.81 | 1.05 | 0.04 |
| CDR-SB at last scan (SD) | 0.2 (0.5)  | 0.2 (0.5)  | 1.9 (1.0)  | 1.7 (1.2)  | 5.5 (2.6)  | 5.2 (2.5)  | 7.6 (3.7)  | 7.5 (3.7)  | 143.71| 0.16 | 0.04 |
| ADAS at last scan (SD)   | 10.0 (5.7) | 10.2 (5.4) | 18.0 (7.1) | 17.4 (6.2) | 32.1 (8.0) | 33.8 (12.2)| 38.9 (12.2)| 36.6 (12.1)| 160.06| 0.04 | 0.86 |
| BrainAGE at last scan (SD)| -0.16 (7.94) | -1.40 (6.06) | -0.01 (6.05) | -0.64 (4.77) | 8.68 (7.24) | 7.34 (10.29)| 8.30 (8.03) | 7.67 (10.14) | 30.56 | 1.00 | 0.05 |
| **Changing rates (per follow-up year)** |            |            |            |            |           |           |           |           | 31.10 | 0.77 | 0.24 |
| MMSE                    | -0.17      | -0.01      | -0.26      | 0.10       | -2.20      | -1.83      | -2.42      | -2.47      |       |      |      |
| CDR-SB                  | 0.06       | 0.03       | 0.19       | 0.24       | 1.40       | 1.32       | 1.81       | 1.82       | 53.47 | 0.01 | 0.04 |
| ADAS                    | 0.51       | -0.04      | -0.11      | -0.06      | 3.80       | 4.31       | 5.62       | 4.16       | 35.79 | 0.66 | 1.33 |
| BrainAGE                | -0.01      | 0.03       | 0.20       | -0.13      | 1.13       | 0.61       | 1.68       | 0.90       | 16.27 | 5.22 | 1.39 |

**Bold type= significant test results.**
Cross-sectional Sample

To explore the performance of the BrainAGE framework in predicting conversion from MCI to AD in APOE ε4 carriers and non-carriers, all MCI subjects were included for whom baseline MRI data (1.5T), at least moderately confident diagnoses (i.e. confidence >2), and test scores in certain cognitive scales (i.e. ADAS, CDR-SB, MMSE) were available. The MCI subjects (n=193) were grouped as (i) sMCI (stable MCI), if diagnosis was MCI at all available time points, but at least for 36 months (n=62); (ii) pMCI_early (progressive MCI), if diagnosis was MCI at baseline but converted to AD within the first 12 months, without reversion to MCI or cognitive normal (NO) at any available follow-up (n=57); (iii) pMCI_late, if diagnosis was MCI at baseline and conversion to AD was reported after the first 12 months (i.e. at 18, 24, or 36 months follow-up), without reversion to MCI or NO at any available follow-up (n=74). Details of the characteristics of the ADNI test sample are presented in Table 2. The subjects were further grouped according to their APOE ε4 status, resulting in ε4 carriers groups (sMCI^C, pMCI^C_early, pMCI^C_late) and non-carriers groups (sMCI^NC, pMCI^NC_early, pMCI^NC_late).
**Table 2.** Baseline characteristics of the cross-sectional MCI samples used for prediction.

|                         | ε4 carriers (n=117) | ε4 non-carriers (n=76) | F-statistics |
|-------------------------|---------------------|------------------------|--------------|
|                         | sMCI\(^C\) | pMCI\(^C\) - early | pMCI\(^C\) - late | sMCI\(^NC\) | pMCI\(^NC\) - early | pMCI\(^NC\) - late | Diagnostic group | ε4 status | Group x ε4 status |
| No. subjects            | 26 | 33 | 58 | 36 | 24 | 16 | - | - | - |
| Males / Females         | 23 / 3 | 20 / 13 | 36 / 22 | 26 / 10 | 13 / 11 | 11 / 5 | - | - | - |
| Age mean (SD)           | 76.5 (5.2) | 72.9 (6.0) | 75.0 (6.4) | 76.2 (6.8) | 75.3 (8.3) | 76.4 (10.0) | 1.64 [p=0.20] | 1.26 [p=0.26] | 0.61 [p=0.55] |
| Education years mean (SD) | 16.3 (2.7) | 15.7 (2.6) | 15.9 (3.0) | 16.6 (2.5) | 15.0 (3.4) | 16.1 (2.6) | 2.15 [p=0.12] | 0.00 [p=0.97] | 0.49 [p=0.61] |
| MMSE mean (SD)          | 28.0 (1.4) | 26.5 (2.0) | 26.8 (1.5) | 27.5 (2.0) | 26.4 (1.8) | 26.6 (1.7) | 9.01 [p<0.001] | 0.96 [p=0.33] | 0.33 [p=0.72] |
| CDR-SB mean (SD)        | 1.4 (0.7) | 2.1 (0.9) | 1.7 (0.9) | 1.3 (0.6) | 1.9 (0.9) | 2.0 (1.1) | 8.54 [p<0.001] | 0.03 [p=0.87] | 0.87 [p=0.42] |
| ADAS mean (SD)          | 17.1 (5.2) | 23.7 (6.6) | 20.6 (4.4) | 15.7 (6.1) | 23.1 (5.9) | 19.7 (4.2) | 24.32 [p<0.001] | 1.39 [p=0.24] | 0.07 [p=0.93] |
| BrainAGE mean (SD)      | 0.0 (4.4) | 9.0 (6.3) | 5.7 (6.0) | 1.2 (4.0) | 8.0 (9.2) | 5.0 (7.7) | 8.96 [p<0.001] | 0.64 [p=0.42] | 0.97 [p=0.38] |

**Bold** type= significant test results.
MRI Data Preprocessing and Data Reduction

Preprocessing of the T1-weighted images was done using the SPM8 package (http://www.fil.ion.ucl.ac.uk/spm) and the VBM8 toolbox (http://dbm.neuro.uni-jena.de), running under MATLAB. All T1-weighted images were corrected for bias-field inhomogeneities, then spatially normalized and segmented into gray matter, white matter, and cerebrospinal fluid (CSF) within the same generative model (Ashburner and Friston 2005). The segmentation procedure was further extended by accounting for partial volume effects (Tohka et al. 2004), by applying adaptive maximum a posteriori estimations (Rajapakse et al. 1997), and by using a hidden Markov random field model (Cuadra et al. 2005). Following the pipeline proposed by Franke et al. (2010) the images were processed with affine registration. Data reduction was performed by applying Principal Component Analysis (PCA) utilizing the “Matlab Toolbox for Dimensionality Reduction” (http://ict.ewi.tudelft.nl/~lvandermaaten/Home.html). PCA was performed on the training sample only. The estimated transformation parameters were subsequently applied to the test sample. No further data reduction or region pre-selection was accomplished.

Estimation of BrainAGE scores

The BrainAGE framework utilizes a machine-learning pattern recognition method, namely relevance vector regression (RVR; Tipping 2001), to estimate individual brain ages based on T1-weighted MR images (Franke et al. 2010). The brain age of each test subject can be estimated using the individual tissue-classified MRI data, aggregating the complex, multidimensional aging pattern across the whole brain into 1 single value (Figure 1A). The difference between estimated and true chronological age will reveal the individual Brain Age Gap Estimation (BrainAGE) score. Consequently, the BrainAGE score directly quantifies the amount of acceleration or deceleration in brain aging. For example, if a 70 years old individual has a BrainAGE score of +5 years, this means that this individual shows the typical atrophy pattern of a 75 year old individual (Figure 1B). Recent work has demonstrated that this method provides reliable and stable estimates, with a correlation of r=0.92 between the estimated and the chronological age and a mean absolute error of 5 years in healthy subjects aged 20–86 years (Franke et al., 2010). Additionally, BrainAGE scores
calculated from 2 shortly delayed scans produced an intraclass correlation coefficient (ICC) of 0.93 (Franke et al. 2012).

Within this study, the BrainAGE model was trained with preprocessed whole brain structural MRI data of, including all cognitively healthy subjects of the IXI sample and the OASIS database. For training the model as well as for predicting individual brain ages, we used “The Spider” (http://www.kyb.mpg.de/bs/people/spider/main.html), a freely available toolbox running under MATLAB. For an illustration of the most important features (i.e., the importance of voxel locations for regression with age) that were used by the RVR to model normal brain aging and more detailed information please refer to Franke et al. (2010).

**Figure 1.** Depiction of the BrainAGE concept.

*Notes:* (A) The model of healthy brain aging is trained with the chronological age and preprocessed structural MRI data of a training sample (left, with an exemplary illustration of the most important voxel locations that were used by the age regression model). Subsequently, the individual brain ages of previously unseen test subjects are estimated, based on their MRI data (blue, picture modified from (Schölkopf and Smola 2002)). (B): The difference between the estimated and chronological age results in the BrainAGE score, indicating abnormal brain aging. [Image reproduced from (Franke and Gaser 2012), with permission from Hogrefe Publishing, Bern]

**Statistical Analysis**

First, the longitudinal changes in individual BrainAGE scores, which were corrected for age and gender, were fitted against days from baseline with a linear regression model. Scores at baseline, last visit, and longitudinal changes were calculated for BrainAGE as well as for the other cognitive scores (i.e. MMSE, CDR-SB and ADAS). To compare score results among the 4 diagnostic groups as a function of the APOE ε4 carrier status analyses of variance (ANOVA) were used. Post-hoc analyses (with Bonferroni adjustment to compensate for multiple comparisons) were conducted to further explore significant group differences.
Additionally, the effects of the particular allelic isoforms (i.e., $\varepsilon2/\varepsilon3$, $\varepsilon3/\varepsilon3$, $\varepsilon2/\varepsilon4$, $\varepsilon3/\varepsilon4$, $\varepsilon4/\varepsilon4$) on BrainAGE were analyzed.

The relationship between BrainAGE scores and cognitive scales (i.e. MMSE, CDR-SB, ADAS) was explored using Pearson’s linear correlation coefficients.

In the second part of the study, prediction of conversion from MCI to AD in APOE $\varepsilon4$ carriers and non-carriers based on baseline BrainAGE scores was studied. All analyses within this cross-sectional cohort were performed separately for the APOE $\varepsilon4$ carrier and non-carrier groups. Receiver operating characteristics (ROC) for discriminating MCI subjects who converted to AD from those who remained stable during follow-up were computed in early converting, and all MCI subjects together, resulting in the area under the (ROC) curve (AUC), also known as C-statistics or c-index. The AUC shows the quality of classification, with 1.0 indicating a perfect discrimination and 0.5 indicating a result obtained by chance only. In order to test whether the resulting AUC derived from ROC analysis based on BrainAGE scores is statistically greater than the AUCs of the cognitive scores, one-tailed z-tests were performed. Additionally, the McNemar test for paired data was performed in order to statistically test whether predictions of conversion based on baseline BrainAGE scores are significantly better than predictions based on cognitive scores.

Within both MCI samples (progressive and stable MCIs), univariate Cox regression was used to estimate the hazard rate for conversion to AD, adjusting for age, gender, and education years. The time-to-event variable was time from baseline visit to first visit with AD diagnosis for pMCI subjects. For sMCI subjects, the duration of follow-up was truncated at 3 years. The main predictor was the baseline BrainAGE score as a continuous variable initially and with median split subsequently. Additionally, Cox regression was performed with baseline cognitive scores as main predictors. Furthermore, it was tested whether including the individual APOE status into the Cox regression model would significantly improve the model performance. As checked by log-minus-log-plots of survival, the assumption of proportional hazards was met for all Cox proportional hazard models. Cox regression was performed using SPSS. All other statistical testing was performed using MATLAB.
RESULTS

Longitudinal sample

BrainAGE scores and cognitive tests at baseline and follow-up were analyzed in all diagnostic groups (NO, sMCI, pMCI, AD) according to APOE ε4 carrier status (i.e., carrier & non-carrier; Table 1) and particular allelic isoform (i.e., ε2/ε3, ε3/ε3, ε3/ε4, ε4/ε4; Table 3). The allelic combination of ε2/ε2 was not represented in this sample. Orientating on other studies, we assigned APOE ε2/ε4 positive patients to the ε4 carrier group with dose-dependent risk (Nalbantoglu et al. 1994, Khachaturian et al. 2004, Hoyt et al. 2005, Glodzik-Sobanska et al. 2009).

Baseline BrainAGE scores differed significantly among all 4 diagnostic groups (F=18.86, p<0.001; Table 1). Post-hoc t-tests showed significant differences in BrainAGE scores between NO / sMCI vs. pMCI / AD (p<0.05) (Figure 2A), suggesting neuroanatomical changes in pMCI and AD patients that show patterns of advanced brain aging. There was no significant effect regarding APOE ε4 status (F=0.00, p=0.97) or interaction between diagnostic group and APOE ε4 status (F=0.27, p=0.85). Additionally, no significant effect was found for allelic isoforms (F=0.01, p=0.99; Table 3), which may be due to the very small number of patients for some allelic isoforms.
Figure 2. BrainAGE scores at baseline (A) and at the last visit (B) for non-carriers and carriers of APOE-ε4 (ε4 as distinctive feature). Non-carriers: ε2/ε3 & ε3/ε3. Carriers include ε2/ε4, ε3/ε4 & ε4/ε4.

Shown are boxplots which are presenting the distribution of the BrainAGE scores for the four diagnostic groups NO, sMCI, pMCI and AD. Figure 2A shows values from the first baseline scan, figure 2B from the last scan after maximum 36 months. Significant differences in BrainAGE score values were shown between the diagnostic groups at baseline (F= 18.86, p<0.001) and at follow-up scans (F= 30.56, p<0.001), as well as for NO/sMCI vs. pMCI/AD at baseline and last visit (p<0.05).

The boxes include values between the 25th and 75th percentiles and the median (red line). The lines extending the boxes below and above include data within 1.5 times the interquartile range. All outliers are symbolized with a red plus. The width of the box stands for the group size.
Table 3. Mean BrainAGE scores at baseline and last follow-up apportioned to APOE genotypes within the four diagnosis groups NO, sMCI, pMCI and AD.

| No. subjects | NO baseline (SD) | NO last follow-up (SD) | sMCI baseline (SD) | sMCI last follow-up (SD) | pMCI baseline (SD) | pMCI last follow-up (SD) | AD baseline (SD) | AD last follow-up (SD) |
|--------------|------------------|------------------------|-------------------|--------------------------|-------------------|--------------------------|------------------|-----------------------|
| e2/e3       | -2.66 (5.32)     | -3.01 (5.42)           | +1.95 (6.92)      | +2.71 (5.45)             | +3.43 (5.29)      | +9.24 (2.10)             | +8.80 (4.86)     | +11.31 (6.16)         |
| e2/e4       | -1.03 (6.69)     | -1.01 (6.18)           | -0.21 (4.73)      | -1.16 (4.60)             | +5.67 (9.93)      | +7.22 (10.60)            | +5.97 (9.82)     | +7.35 (10.40)         |
| e3/e4       | 1+3.28 (0.00)    | 1+11.72 (0.00)         | —                  | —                        | +3.39 (6.72)      | +7.25 (6.05)             | +2.10 (10.60)    | +3.29 (7.97)          |
| e4/e4       | 1-1.42 (6.44)    | 1-1.28 (8.07)          | -0.15 (6.28)      | +0.47 (6.45)             | +5.38 (5.85)      | +7.40 (7.03)             | +6.19 (8.74)     | +8.45 (8.60)          |
| e3/e4       | 4-3.44 (4.44)    | 2-2.75 (4.94)          | -5.29 (2.93)      | -2.85 (0.59)             | +7.52 (7.64)      | +12.18 (7.12)            | +5.33 (5.18)     | +8.63 (6.69)          |
At last MRI scan, BrainAGE scores differed significantly among the diagnostic groups (F=30.56, p<0.001; Table 1), without showing a significant effect for APOE ε4 status (F=1.00, p=0.32) or interaction between diagnostic group and APOE ε4 status (F=0.05, p=0.98). Again, post-hoc t-tests showed significant differences between NO / sMCI vs. pMCI / AD (p<0.05) (Figure 2B).

As mentioned above, patients with the allelic isoform ε2/ε4 were assorted to carriers. Since there were no representatives of this isoform in the sMCI cohort, we subsequently examined the possible effect of falsification by excluding all patients with a combination of ε2/ε4 from our longitudinal sample. However, test results showed no substantial changes (F-statistics at baseline for diagnostic group: F=9.22, p<0.001; APOE ε4 status: F=0.01, p=0.99; interaction: F=0.6, p=0.79; follow-up scan for diagnostic group: F=16.35, p<0.001; APOE ε4 status: F=0.62, p=0.60; interaction: F=0.74, p=0.67).

Correlation between BrainAGE and cognitive scores were analyzed for baseline and follow-up visits (up to 4 years later). Throughout the whole sample, BrainAGE scores correlated significantly with each of the cognitive scores in function of the carrier status (correlation coefficients for APO ε4 carriers of baseline BrainAGE scores and baseline MMSE: -0.34, p<0.001, CDR-SB: 0.29, p<0.001, ADAS: 0.33, p<0.001; at last scan: MMSE: -0.44, p<0.001, CDR-SB: 0.40, p<0.001, ADAS: 0.43, p<0.001. Correlation coefficients for APO ε4 non-carriers of baseline BrainAGE scores and baseline MMSE: -0.52, p<0.001, CDR-SB: 0.50, p<0.001, ADAS: 0.50, p<0.001; at last scan: MMSE: -0.59, p<0.001, CDR-SB: 0.57, p<0.001, ADAS: 0.58, p<0.001). Within the group of pMCI and AD patients, there were partly found linear correlations between BrainAGE and cognitive scores in dependency of the APOE carrier status (correlation coefficients in AD patients for APO ε4 carriers of BrainAGE scores at last scan and MMSE at last scan: -0.38, p<0.001; for APO ε4 non-carriers of baseline BrainAGE scores and baseline MMSE: -0.62, p<0.001, CDR-SB: 0.60, p<0.001, ADAS: 0.52, p<0.001; at last scan: MMSE: -0.66, p<0.001, CDR-SB: 0.59, p<0.001, ADAS: 0.56, p<0.001; Table 4).
Table 4. Correlation coefficients between baseline BrainAGE score and cognitive functioning (ADAS scores) as well as disease severity (MMSE & CDR-SB scores) for each diagnostic group and the whole test sample, separated into carriers and non-carriers.

| Correlation with | NO | sMCI | pMCI | AD | Whole sample |
|------------------|----|------|------|----|--------------|
|                  | ≤4 carriers | ≤4 non-carriers | ≤4 carriers | ≤4 non-carriers | ≤4 carriers | ≤4 non-carriers | ≤4 carriers | ≤4 non-carriers |
| No.              | 26 | 81   | 14   | 22 | 78          | 34          | 101         | 49          |
| MMSE score at last scan | 0.04 | -0.21 | 0.45 | -0.08 | -0.08 | -0.29 | -0.28** | -0.62*** | -0.34*** | -0.52*** |
| CDR-SB score at last scan | -0.04 | -0.15 | -0.12 | 0.02 | 0.27* | 0.13 | 0.10 | 0.60*** | 0.29*** | 0.50*** |
| ADAS score at last scan | -0.06 | 0.04 | -0.45 | -0.07 | 0.27* | 0.32 | 0.19 | 0.52*** | 0.33*** | 0.50*** |

| Correlation with | NO | sMCI | pMCI | AD | Whole sample |
|------------------|----|------|------|----|--------------|
|                  | ≤4 carriers | ≤4 non-carriers | ≤4 carriers | ≤4 non-carriers | ≤4 carriers | ≤4 non-carriers | ≤4 carriers | ≤4 non-carriers |
| MMSE score at last scan | 0.00 | -0.17 | 0.30 | -0.12 | -0.21 | -0.33 | -0.38*** | -0.66*** | -0.44*** | -0.59*** |
| CDR-SB score at last scan | -0.07 | 0.00 | -0.07 | 0.09 | 0.38** | 0.23 | 0.21* | 0.59*** | 0.40*** | 0.57*** |
| ADAS score at last scan | -0.06 | 0.04 | -0.34 | 0.09 | 0.38** | 0.38* | 0.25* | 0.56*** | 0.43*** | 0.58*** |

***p<0.001; **p<0.01; *p<0.05.
To follow the course of scores from the patients’ first to their last MRI scan, changing rates of BrainAGE and cognitive scores were calculated (Table 1). Between the diagnostic groups, changing rates of BrainAGE and cognitive scores differed significantly during follow-up (BrainAGE: $F=16.27$, $p<0.001$; MMSE: $F=31.10$, $p<0.001$; CDR-SB: $F=53.47$, $p<0.001$; ADAS: $F=35.79$, $p<0.001$). Changing rates of the BrainAGE score differed significantly between carriers and non-carriers, with carriers showing higher changing rates than non-carriers.

To further investigate how BrainAGE scores will change for carriers and non-carriers in each diagnostic group, longitudinal changes were estimated for 1600 days after baseline examination. BrainAGE scores of NO and sMCI patients remained relatively stable during the follow-up period (ranging between -0.13 and 0.20), suggesting no, respectively normal age-related brain-atrophy (Franke and Gaser, 2012). BrainAGE scores of pMCI and AD patients changed much stronger during follow-up (Figure 3).
Figure 3. Longitudinal changes in BrainAGE scores for (A) NO, (B) sMCI, (C) pMCI and (D) AD patients. The thin lines are standing for the individual development of the BrainAGE score over time. The thick lines show the estimated average regression lines for non-carriers (red) and carriers (blue).
In the post-hoc t-tests, BrainAGE changing rates for ε4 carriers as well as for non-carriers differed significantly between NO / sMCI vs. pMCI / AD (p< 0.05; Figure 4).

Figure 4. Estimated longitudinal changes in BrainAGE scores for the four diagnostic groups NO (light blue), sMCI (green), pMCI (red) and AD (blue), subdivided into non-carriers and carriers.

Post-hoc t-tests showed significance for carriers as well as non-carriers for NO & sMCI vs. pMCI & AD (p<0.05).

For all pMCI patients (n=112) days to conversion from MCI to AD diagnosis were calculated. In general, there was a tendency (F=3.14; p=0.08) that APOE ε4 carriers took about 3 months longer to convert to AD (560±280 days) as compared to non-carriers (471±233 days). Split into allelic subgroups, homozygous ε4 carriers (with 591.48 mean days to conversion) range in the midfield between slowest conversion in patients with a genetic combination of a protective ε2 allele with either ε3 (757.67 days) or ε4 (756.20 days) and the fast converting homozygous ε3 carriers (447.65 days) (Figure 5).
Figure 5. Mean days to conversion from MCI to AD subdivided into all allelic combinations of APOE.

Presented are the mean (SD) days to conversion within the given allelic combinations of APOE (F=3.14; p=0.08): ε2/ε3 [n=2]: 758 (356), ε3/ε3 [n=32]: 448 (210), ε2/ε4 [n=5]: 756 (201), ε3/ε4 [n=52]: 534 (292), ε4/ε4 [n=21]: 591 (246). The boxplots include values between the 25th and 75th percentiles and the median (red line). The lines extending the boxes below and above include data within 1.5 times the interquartile range. The width of the box stands for the group size.

Cross-sectional sample

In addition, effects of APOE ε4 status on prediction of conversion from MCI to AD were explored. The prediction subsample included 193 MCI patients who were diagnosed MCI at baseline. The cohort included 117 APOE ε4 carriers and 76 non-carriers, sub-sampled in pMCI_early, pMCI_late and sMCI. Chronological age and education years did not differ between groups.

Baseline BrainAGE scores differed significantly between stable, early and late converting MCI patients (F= 8.96, p<0.001; Table 2), as did the cognitive scores. There weren’t any effects for the APOE ε4 status or for interactions between diagnostic group and APOE ε4 status (Table 2).
Conversion of MCI patients

Within the prediction subsample of 193 MCI patients a total number of 91 (out of 117) carriers and 40 (out of 73) non-carriers converted to AD during the 36 months of follow-up. That corresponds to a pre-test probability of 78% in carriers and 53% in non-carriers, respectively. In carriers, 28% of the MCI subjects converted to AD within the first 12 months after baseline examination, whereas 50% converted to AD after the first year of follow-up. In non-carriers, 32% of the MCI subjects converted to AD within the first 12 months after baseline examination, whereas 21% converted to AD after the first year of follow-up.

Prediction of Conversion

Cox regression analysis for the prediction subsample was based on baseline BrainAGE scores and resulted in a highly significant association of higher baseline BrainAGE scores with higher risk of converting to AD for carriers and non-carriers together ($\chi^2=53.88$, $p<0.001$; Table 5). Compared with subjects having a BrainAGE score below the median (4.5 years), subjects with a BrainAGE score above median had a nearly 4 times greater risk of converting to AD (hazard ratio [HR]: 3.76, $p<0.001$; Table 5). Including the APOE $\varepsilon$4 status into the Cox regression model, the quality of the prediction model tended to improve, but lacked statistical significance ($\chi^2=3.23$, $p=0.07$). If no additional information about a subject’s APOE status is available, the quality of the BrainAGE score’s prediction of conversion is equally high.

In patients with a BrainAGE score above the median, it is 3.58 times more likely for carriers to convert to AD ($p=<0.001$) than for non-carriers, when the BrainAGE score increases per 1 unit. Within this group of patients, carriers compared to non-carriers generally tend to convert 1.41 times more likely ($p=0.08$) to AD. Multiplied with the above named Hazard Ratio of 3.58, they do have an approximately 5 times higher risk than non-carriers to convert to AD and can be considered a group of high risk patients.

The Cox regression model based on baseline BrainAGE scores outperformed models based on baseline MMSE, CDR-SB, or ADAS scores, even when including the APOE $\varepsilon$4 status into these models (Table 5, Figure 6).
Table 5. Cox Regression values for cumulative AD incidence in non-carriers/carriers in the BrainAGE score, MMSE, CDR-SB and ADAS alone and in combination with the APOE ε4 carrier status, based on a median split.

|                      | Test of overall model | Change from previous model | Hazard ratio [HR] | Confidence interval [CI] | Significance |
|----------------------|-----------------------|-----------------------------|------------------|--------------------------|--------------|
| **BrainAGE**         | 53.88 p < 0.001       |                             | 3.76             | [2.58–5.48]              | p < 0.001    |
| & APOE               | 56.79 p < 0.001       | 3.23 p = 0.07               | 3.58             | [2.44–5.24]              | p < 0.001    |
| MMSE                 | 18.46 p < 0.001       | -                           | 2.37             | [1.58–3.55]              | p < 0.001    |
| & APOE               | 27.68 p < 0.001       | 9.62 p < 0.01               | 2.42             | [1.61–3.63]              | p < 0.001    |
| CDR-SB               | 12.91 p < 0.001       | -                           | 2.05             | [1.37–3.05]              | p < 0.001    |
| & APOE               | 19.61 p < 0.001       | 6.95 p < 0.01               | 1.97             | [1.32–2.93]              | p < 0.001    |
| ADAS                 | 22.57 p < 0.001       | -                           | 2.35             | [1.63–3.38]              | p < 0.001    |
| & APOE               | 26.62 p < 0.001       | 4.27 p < 0.05               | 2.22             | [1.54–3.20]              | p < 0.001    |
|                      |                       |                             | 1.48             | [1.01–2.18]              | p < 0.05     |

**Bold type** = significant; **marked type** = best performance of all models.
Figure 6. Cumulative probability for MCI patients of remaining AD-free, divided into patients with the score of interest below the median (light lines) and above it (dark lines). Non-carriers of the APOE-ε4 gene are painted in blue, carriers in red.

Shown are Kaplan-Meier survival curves based on Cox regression, comparing the cumulative AD incidence in carriers and non-carriers in (A) BrainAGE score quartiles: $\chi^2=56.8$, $p<0.001$; hazard ratio [HR] for median split: 3.58, $p<0.001$; HR for ε4-carriers vs. non-carriers: 1.41, $p=0.08$; (B) MMSE values: $\chi^2=27.7$, $p<0.001$; HR median split: 2.42, $p<0.001$; HR ε4: 1.91, $p<0.01$; (C) CDR-SB values $\chi^2=19.6$, $p<0.001$; HR median split: 1.97, $p<0.001$; HR ε4: 1.72, $p<0.01$ and (D) ADAS values: $\chi^2=26.6$, $p<0.001$; HR median split: 2.22, $p<0.001$; HR ε4: 1.48, $p<0.05$. The follow-up duration, after MCI diagnosis at baseline, is limited to 1250 days.
Accuracy of Prediction of Conversion

The effect of APOE ε4 status on prediction accuracy was further examined with ROC analyses. By varying the threshold applied to the BrainAGE score, ROC curves were constructed for a binary discrimination between MCI subjects who remained stable during 3 years follow-up from those who converted to AD.

For the discrimination of pMCI_early from sMCI, ROC analyses at baseline BrainAGE scores resulted in an AUC (or c-index) of 0.88 with an accuracy rate of 85% in APOE ε4 carriers. In APOE ε4 non-carriers, prediction performances were slightly lower with an AUC of 0.75 and an accuracy rate of 78% (Figure 7).

Figure 7. ROC curves for pMCI_early subjects, analyzing individual subject classification based on baseline BrainAGE score in either remaining at a MCI stage or changing to AD, subdivided into non-carriers and carriers.

Achieved accuracies (sensitivity/ specificity) for predicting progression from MCI to AD for non-carriers: 78% (0.71/ 0.83), for carriers: 85% (0.79/ 0.92).
For discriminating early and late converting MCI from sMCI patients, the AUC resulted in 0.82 for APOE ε4 carriers and 0.71 for non-carriers. Achieved accuracies for APOE ε4 carriers were 75%, for APOE ε4 non-carriers 74% (Figure 8).

Figure 8. ROC curves of non-carriers vs. carriers in relation with the BrainAGE score in all MCI patients.

Achieved accuracies (sensitivity/specificity) for predicting progression from MCI to AD in this time span for non-carriers: 74% (0.65/0.83) and for carriers: 75% (0.70/0.92).

Furthermore, the McNemar test was applied to explore whether predictions of future conversion to AD in pMCI patients based on baseline BrainAGE scores were significantly better than predictions based on chronological age and cognitive tests. Compared to chronological age and cognitive scores in APOE ε4 carriers (Table 6), and compared to chronological age, MMSE and CDR-SB scores in APOE ε4 non-carriers (Table 7) the BrainAGE score showed significantly better results in predicting conversion. The BrainAGE score showed best performance concerning accuracy, sensitivity and specificity compared to the other scores in APOE ε4 carriers.
Table 6. Results for predicting conversion to AD in MCI subjects (APOE ε4 carriers).

|                          | sMCI vs. pMCI<sup>C</sup>_early | sMCI vs. pMCI<sup>C</sup> (all) |
|--------------------------|---------------------------------|---------------------------------|
|                          | Accuracy [CI] | Sensitivity [CI] | Specificity [CI] | McNemar test | Accuracy [CI] | Sensitivity [CI] | Specificity [CI] | McNemar test |
|                          | Error rate [CI] |  |  |  | Error rate [CI] |  |  |  |  |
| BrainAGE score           | 0.85 [0.76-0.94] | 0.79 [0.68-0.89] | 0.92 [0.85-0.99] | 0.15 [0.06-0.22] |  |  |  |  | 0.75 [0.67-0.83] | 0.70 [0.62-0.79] | 0.92 [0.87-0.97] | 0.25 [0.17-0.33] |
|                          |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Chronological age        | 0.39 [0.27-0.51] | 0.39 [0.27-0.52] | 0.92 [0.85-0.99] | 0.61 [0.49-0.73] | 17.79 [p<0.001] | 0.54 [0.45-0.63] | 0.35 [0.26-0.44] | 0.88 [0.83-0.94] | 0.46 [0.37-0.55] |  |  | 7.78 [p<0.01] |
| MMSE score               | 0.46 [0.33-0.58] | 0.52 [0.39-0.64] | 0.85 [0.75-0.94] | 0.54 [0.42-0.67] | 8.53 [p<0.01] | 0.23 [0.15-0.31] | 0.68 [0.60-0.77] | 0.65 [0.57-0.74] | 0.77 [0.69-0.85] |  |  | 40.01 [p<0.001] |
| CDR-SB score             | 0.49 [0.36-0.62] | 0.70 [0.58-0.81] | 0.81 [0.71-0.91] | 0.51 [0.38-0.64] | 14.28 [p<0.001] | 0.26 [0.18-0.34] | 0.52 [0.43-0.61] | 0.81 [0.74-0.88] | 0.74 [0.65-0.81] |  |  | 46.12 [p<0.001] |
| ADAS score               | 0.69 [0.58-0.81] | 0.79 [0.68-0.89] | 0.69 [0.57-0.81] | 0.31 [0.19-0.42] | 3.20 [n.s.] | 0.43 [0.34-0.52] | 0.71 [0.63-0.80] | 0.69 [0.61-0.78] | 0.57 [0.48-0.66] |  |  | 22.44 [p<0.001] |

**Bold type** = significant; **marked type** = best performance of all models.

n.s. = not significant
Table 7. Results for predicting conversion to AD in MCI subjects (APOE ε4 non-carriers).

|                          | sMCI vs. pMCINC _early | sMCI vs. pMCI( all) |
|--------------------------|------------------------|---------------------|
|                          | Accuracy [CI] | Sensitivity [CI] | Specificity [CI] | McNemar test | Accuracy [CI] | Sensitivity [CI] | Specificity [CI] | McNemar test |
|                          | Error rate [CI] | $\chi^2$ |                |              | Error rate [CI] | $\chi^2$ |              |              |
| BrainAGE score           | 0.78 [0.68-0.89] | 0.71 [0.59-0.82] | 0.83 [0.74-0.93] | 0.22 [0.11-0.32] | - | 0.74 [0.64-0.84] | 0.65 [0.54-0.76] | 0.83 [0.75-0.92] | 0.26 [0.16-0.36] | - |
| Chronological age        | 0.50 [0.37-0.63] | 0.83 [0.74-0.93] | 0.31 [0.19-0.42] | 0.50 [0.37-0.63] | 8.53 [p<0.01] | 0.47 [0.36-0.59] | 0.15 [0.07-0.23] | 0.97 [0.93-1.00] | 0.53 [0.41-0.64] | 6.81 [p<0.01] |
| MMSE score               | 0.60 [0.48-0.72] | 0.79 [0.69-0.89] | 0.58 [0.46-0.71] | 0.40 [0.28-0.52] | 4.17 [p<0.05] | 0.47 [0.36-0.59] | 0.78 [0.68-0.87] | 0.58 [0.47-0.69] | 0.53 [0.41-0.64] | 9.00 [p<0.01] |
| CDR-SB score             | 0.67 [0.55-0.79] | 0.58 [0.46-0.71] | 0.75 [0.64-0.86] | 0.33 [0.21-0.45] | 1.64 [n.s.] | 0.51 [0.40-0.63] | 0.53 [0.41-0.64] | 0.75 [0.65-0.85] | 0.49 [0.37-0.60] | 8.53 [p<0.01] |
| ADAS score               | 0.68 [0.57-0.80] | 0.92 [0.85-0.99] | 0.58 [0.46-0.71] | 0.32 [0.20-0.43] | 0.93 [n.s.] | 0.64 [0.54-0.75] | 0.90 [0.83-0.97] | 0.58 [0.47-0.69] | 0.36 [0.25-0.46] | 1.20 [n.s.] |

Bold type = significant
n.s. = not significant

37
DISCUSSION

This study explored the effects of individual APOE ε4 status on the performance of a novel MRI-based biomarker based on the BrainAGE framework (Franke et al., 2010, Franke & Gaser, 2012) in (1) recognizing advanced brain aging in a longitudinal design and (2) predicting prospective cognitive decline and conversion to AD on an individual subject level.

**Longitudinal sample**

F-statistics for BrainAGE and cognitive scores revealed significant differences between the diagnostic groups NO, sMCI, pMCI and AD. Since diagnosis is based on cognitive tests, these results prove reliability of the BrainAGE method as a diagnostic tool.

BrainAGE scores at follow-up were at minimum -1.40 years in APOE ε4 negative healthy subjects, indicating slower brain aging in healthy elderly compared to the population’s average. Progressive, APOE ε4 positive MCIs showed highest BrainAGE scores at last scan with +8.68. They might have highest average BrainAGE scores compared to the other diagnostic groups, since they convert to AD anyway and their structural brain changes precede clinical evolution. The detection of preceding morphological changes using MRI was shown when examining volume loss in predefined antero-temporal regions (Smith et al. 2012) and atrophy patterns summarized with the SPARE-AD score (Davatzikos et al. 2011), in a time frame of up to 10 years before appearance of clinical symptoms (Tondelli et al. 2012). Generally, progressive MCIs and AD patients presented a similar score development over follow-up time, signifying equal neuroanatomical changes. Other studies came to the same conclusions using different methods, e.g. the SPARE-AD score (Davatzikos et al. 2011) or the MRI-based Index, which could not find any significant differences between MCIs with AD-like patterns and AD patients (Aguilar et al. 2014). Evidences of similar hippocampal atrophy rates were found in progressive MCI and AD, as well as in stable MCI and normal controls (Leung et al. 2013). The BrainAGE score as well revealed comparable scores in NOs and sMCI, which goes along with an absence of clinical disease aggravation within follow-up time in sMCI at least during our follow-up time.
Therefore the BrainAGE method might be of special benefit to detect patients at risk, namely progressive MCIs, which present accelerated brain aging, even years before AD-like morphologic brain changes and AD symptoms occur. A distinction between pMCIs from sMCIs was also considered in other studies with different methods and success, resulting in an AUC of 0.76 ±0.04 using the normalized cortical thickness index, measuring severity of brain atrophy (Querbes et al. 2009); a local MRI based Index with an AUC: 0.74 (SENS 72% /SPEZ 65%) (Chincarini et al. 2011); a Multi-Method Analysis achieving a (SENS 67%/SPEZ 69%) (Wolz et al. 2011); and not being successful when performing a ROI (region of interest) analysis of hippocampal volume changes over time (Franko and Joly 2013). Taking into account the patients APOE genotype revealed significant differences within the MCI groups at baseline and follow-up measurements in a study, using a MRI-based index for diagnosis and prediction of conversion (Aguilar et al. 2014). In our study, there are no significant differences between carriers and non-carriers for any score at baseline or follow-up. But when taking into account changing rates in BrainAGE during follow-up time or estimated longitudinal changes up to 1600 days from baseline, carriers clearly showed worse BrainAGE scores than non-carriers for sMCI, pMCI and AD patients. This goes along with the idea that APOE ε4 carriers are supposed to suffer from faster pathologic processes than non-carriers (Yu et al., 2013) and therefore have higher atrophy rates (Jack et al. 2008). The BrainAGE score itself reflects these APOE ε4 related, atrophic brain processes, which are connected to advanced brain aging. NOs are probably excluded from this effect, since they do not have any disease related changes.

Highly significant correlations between BrainAGE and cognitive scores were found within the group of carriers and non-carriers throughout the whole sample, and within most of carriers and non-carriers in the diagnostic groups of pMCI and AD. In pMCI patients, strongest correlation with the BrainAGE score showed ADAS, which focuses on cognition. In AD patients, strongest correlation was found for BrainAGE score and MMSE, which especially measures disease severity in AD. Both key aspects were equally reflected by the BrainAGE score, pointing to the high potential of the BrainAGE concept as diagnostic tool in clinical routine.
Cross-sectional sample

Analysing the effect of APOE on the risk of conversion to AD, we compared carriers and non-carriers within the MCI sample. A total of 78% of carriers converted to AD within 3 years of follow-up, compared to only 53% in non-carriers, underlining a higher risk for carriers to convert to AD. Within this sample, ε4-carriers tend to convert slower than non-carriers. The APOE ε4 genotype is said to be associated with a faster cognitive decline (Hirono et al. 2003) and clinical progression of AD (Martins et al. 2005). Other studies doubt accelerated deterioration in APOE ε4 carriers (Corder et al. 1995, Growdon et al. 1996, Holmes et al. 1996, Kurz et al. 1996a, Stern et al. 1997, Hoyt et al. 2005, Martins et al. 2005). APOE ε4 homozygosity is even suggested to slow down disease progression, since biological processes involved in AD onset and disease progression are of a different nature (Hoyt et al. 2005).

Our results suggest, that the APOE ε4 status is not proportionally associated with a faster conversion from MCI to AD since having at least 1 ε4 allele in comparison to homozygous ε3/ε3 carriers slows down conversion of about 3 to over 4 months. In our sample, slowest conversion times showed heterozygous ε2 carriers. Since there were no subjects with the allelic combination of ε2/ε2 we can not draw any solution about average conversion times of the assumed protective allelic combination. Apart we can say, that ε4 carrier do not convert fastest, but once been converted to AD, show the fastest cognitive decline and highest mortality rate as proved by shorter follow-up duration and worse BrainAGE and cognitive scores. In this context, some studies conclude that survival rates in AD patients mainly depend on their age at onset than on their APOE genotype (Corder et al. 1994, Corder et al. 1995).

In predicting conversion from MCI to AD using the APOE ε4 status, MMSE, CDR-SB and ADAS scores improved accuracy, while the BrainAGE score gave best significant prediction values alone. Nevertheless, a trend can be seen in further improving prediction performance when including the carrier status into the BrainAGE model. Besides, for APOE ε4 carriers as well as for non-carriers prediction of conversion was the most accurate when using baseline BrainAGE scores as compared to chronological age and most cognitive tests. In APOE ε4 carriers, prediction accuracy achieved by the BrainAGE method was higher for than for non-carriers within the MCI sample, going along with results of another study which
took decreases in hippocampal volume as correlate to disease progression (Apostolova et al. 2014).

These results underline the high benefit of using the BrainAGE approach for screening MCI patients in aiming to find those who are in a special high risk of conversion to AD in comparison to patients, who primarily remain at a stable cognitive level. Detecting the quickly progressing subjects as early and secure as possible could help to prepare them best for their probable illness progression and supply them early with potential disease programs, cognitive training and medical treatments (Sperling et al. 2011, Franko and Joly 2013).

Summarizing, we can say that a genetical test for APOE cannot replace any part of the clinical diagnostic procedure (Kurz et al. 1996b), but has the potential to improve specificity in individuums who meet clinical MCI or AD criteria by complementing other diagnostic tests such as memory tests or MR imaging (Tierney et al. 1996, Mayeux et al. 1998, Bertram and Tanzi 2004, Petersen 2004, Boissonneault 2010). In predicting future conversion of MCI patients to AD, it can improve accuracy (Susanto et al. 2015). A genetic test for APOE cannot and should not be used alone to give a secure diagnosis or prediction of disease progression (Alzheimer'sAssociationWorkingGroup 1996, Tsuang et al. 1999). This statement can be underlined by postmortem examinations, which only showed a sensitivity of 65% and a specificity of 68% for APOE ε4 towards AD diagnosis (Mayeux et al. 1998). It is furtermore not possible to predict if an asymptomatic individual with a certain APOE genotype will develop cognitive impairment or AD, nor to draw secure conclusions if or when a homozygote ε4 carrier will get AD (Henderson et al. 1995, Myers et al. 1996, Liddell et al. 2001, Aguilar et al. 2014).

**Limitations**

The present study focused on the influence of APOE status on individual BrainAGE scores of MCI and AD patients. Therefore we divided our cohort in different APOE carrier types, based on the 3 allele haplotypes of the Apolipoprotein E gene, composed of ε2, ε3 and ε4. The distribution was 4.3% for ε2, 61.5% for ε3 and 34.2% for ε4. In caucasians, frequencies of the 3 allelic types were previously estimated 11% for ε2, 72% for ε3 and 17% for ε4 (Zannis et al. 1981), respectively 8% for ε2,
77% for ε3 and 15% for ε4 (Utermann et al. 1980, Thakkinstian et al. 2006). The underrepresentation of ε2 and ε3, and the overrepresentation of ε4 in our sample could be due to a sort of preselection in the ADNI database. Homozygous APOE ε4/ε4 carriers form about 1% to 2% of the general population (Roses et al. 1994, Saunders 2000), whereas our sample included 14.3%. Besides, we found a relative overrepresentation of ε4 within the group of AD patients in our sample (ε4/ε4 in ADs: 20.7%, compared to 3.7% in NOs), which was also reported in other studies (Strittmatter et al. 1993, Kurz et al. 1996b, Myers et al. 1996). In comparison the frequency of ε2 was lower (ε2/ε3 in ADs: 2.7%, compared to 15.0% in NOs), and again shown in previous studies as well (Corder et al. 1994, Kurz et al. 1996b).

The ADNI cohort, which was used for this study, may differ from the general population, since it only includes individuals from memory clinics, patient registries and people recruited in public media campaigns and other forms of public advertisements. There could also exist differences in the North American population in comparison to that in Central Europe. This might also explain the differences in APOE isoform frequencies within our sample and the estimations for the general Caucasian population. This bias may probably diminish when including community based, healthy test subjects.

Then, the clinical follow-up for our ADNI cohort was done in average 1.66 years in ADs, 2.86 years in MCIs and 3.24 years in NOs. We cannot make any statement if some sMCI patients would have converted later on.

Besides, group sizes of some allelic subgroups were limited to a very small number (e.g., 14 carriers and 22 non-carriers in sMCIs) due to low prevalences of some APOE isoforms or limiting selection criterions for our study. It would of interest to repeat the study in some years, to be able to work with a grown ADNI cohort.

Within the ADNI cohort and in clinics in general misdiagnosis of prodromal states of AD and AD can never been excluded due to the possibility of mixed dementia forms with 1 dominating entity or overlaying physical illness (with a pathological postmortem confirmation of diagnosed MCI due to AD in only about 70% of cases, (Jicha et al. 2006)). Especially in very elderly persons it is also sometimes difficult to assess physiological functional loss of cognition caused by age (Albert et al. 2011, Peltz et al. 2011). Besides, cognitive impairment is a continuous process, and it is not always easy to securely classify the grade of disorder (Albert et al., 2011). But a
combination of different assessment tools so far used in clinical practice makes an assignment to prodromal and more severe stages of AD more certain and reduces the rate of misdiagnosis.

Furthermore, it would be interesting to examine age specific effects of the APOE genotype on BrainAGE and cognitive scores, respective disease burden. It would be interesting to verify, if the APOE ε4 genotype really is associated with an earlier age of onset (Corder et al. 1993), or in contrast if it maybe is not influencing the probability succumbing to AD but only the time point of disease’s outbreak (Meyer et al. 1998, Liddell et al. 2001, Khachaturian et al. 2004). That idea claims that there is an age specific risk getting demented, which is markedly earlier for APOE ε4 carriers than for non-carriers, who might mostly already been passed away at the disease's maximum risk point. Examining age specific effects also has the potential to test, if the positive association of APOE ε4 to AD risk and gross brain morphology diminishes in very old age (Corder et al. 1994, Sobel et al. 1995, Farrer et al. 1997, Bigler et al. 2000). This could be a possible focus of another study, but would have gone beyond the scope of our study.

In addition, when forecasting an individuum’s progression from MCI to AD via BrainAGE score analysis, we can never take into account neither all possible risk factors nor all influencing variables like comorbidities or cognitive reserve (Stern 2002) to securely presee their progress. The variability of disease progression, documented through the different tests, can be seen in the strong alternations of slopes for the longitudinal BrainAGE changes. Generally, abnormal brain atrophy could also been proved in asymptomatic subjects, whose sufficient cognitive reserve or well adapted coping methods delayed dementia appearing (Stern 2002, Stern et al. 2003, Winblad et al. 2004, Davatzikos et al. 2008, Querbes et al. 2009, Aguilar et al. 2014). This in turn provokes a strong divergency between anatomical and clinical findings. But since we examine flexible biological systems we will only be able to give estimations and never certainty in diagnosis and progression.

Beside APOE there are also other genetic risk factors known which influence MCI and AD pathogenesis. But since the inheritance of preposition for AD is very complex and gene polymorphism and mutations are interacting with each another as they do with non-genetic factors, only 4 genes could be securely detected playing a role in in a pool of 100 potential candidate genes so far analyzed in literature (Bertram and
Tanzi 2004). Next to APOE there is evidence, that the amyloid precursor protein gene (APP) and presenilin 1 and 2 genes (PSEN1 and PSEN 2) (Mueller et al. 2005, Brouwers et al. 2008) have an effect, even though it is considered relatively small (Bertram et al. 2010, Albert et al. 2011) or respectively limited to the rare early-onset form of AD (Bertram and Tanzi 2004, Mueller et al. 2005). In contrast, it was even claimed, that genetic testing might only be of little more use than generating family pedigrees to predict someone's risk for getting AD (Liddell et al. 2001). To prove or disprove further genetic influences goes beyond the scope of this work, but it could provide topics for further research work.
CONCLUSIONS

Summing up, this work did show, that the BrainAGE method could be a very useful and accurate tool in completing clinical diagnosis and predicting AD related cognitive decline. Due to its easy handling by giving only 1 global classifier to evaluate brain age status, the BrainAGE method might be helpful in every day clinical application. It provides more accurate results in predicting conversion from MCI to AD than the already well established MMSE, CDR-SB and ADAS tests. Thereby the patients’ APOE genotype is not necessarily of need to improve statements significantly, but tended to improve the prediction performance. Since genetical testing might become more and more usual in everyday clinical practice in the future, we recommend to include APOE genotype into medical assessment to support diagnosis, and disease progression estimation.

Since APOE ε4 related genetic risk groups were best discriminated when taking the BrainAGE changes over time, we also recommend regular follow-up MRI scans. Follow-up data for rate-of-change measurements compared to single-time-point-measures were shown to significant improve risk prediction (McEvoy et al. 2011, Aguilar et al. 2014). Having patients in careful monitoring also provides the possibility to evaluate the effect of disease-modifying drugs within a short time (McEvoy et al. 2011) and to learn more about patterns of AD pathology.
REFERENCES

Aguilar C, Muehlboeck JS, Mecocci P, Vellas B, Tsolaki M, Kloszewska I, Soininen H, Lovestone S, Wahlund LO, Simmons A, Westman E. 2014. Application of a MRI based index to longitudinal atrophy change in Alzheimer disease, mild cognitive impairment and healthy older individuals in the AddNeuroMed cohort. Front Aging Neurosci, 6:145.

Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH. 2011. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement, 7 (3):270-279.

Alzheimer'sAssociationWorkingGroup. 1996. Apolipoprotein E genotyping in Alzheimer's disease. National Institute on Aging/Alzheimer's Association Working Group. Lancet, 347 (9008):1091-1095.

Anderson VM, Schott JM, Bartlett JW, Leung KK, Miller DH, Fox NC. 2012. Gray matter atrophy rate as a marker of disease progression in AD. Neurobiol Aging, 33 (7):1194-1202.

Apostolova LG, Hwang KS, Kohannim O, Avila D, Elashoff D, Jack CR, Jr., Shaw L, Trojanowski JQ, Weiner MW, Thompson PM. 2014. ApoE4 effects on automated diagnostic classifiers for mild cognitive impairment and Alzheimer's disease. Neuroimage Clin, 4:461-472.

Ashburner J, Friston KJ. 2005. Unified segmentation. Neuroimage, 26 (3):839-851.

Basso M, Gelernter J, Yang J, MacAvoy MG, Varma P, Bronen RA, van Dyck CH. 2006. Apolipoprotein E epsilon4 is associated with atrophy of the amygdala in Alzheimer's disease. Neurobiol Aging, 27 (10):1416-1424.

Berlau DJ, Corrada MM, Head E, Kawas CH. 2009. APOE epsilon2 is associated with intact cognition but increased Alzheimer pathology in the oldest old. Neurology, 72 (9):829-834.

Bertram L, Tanzi RE. 2004. The current status of Alzheimer's disease genetics: what do we tell the patients? Pharmacol Res, 50 (4):385-396.

Bertram L, Lill CM, Tanzi RE. 2010. The genetics of Alzheimer disease: back to the future. Neuron, 68 (2):270-281.

Bigler ED, Lowry CM, Anderson CV, Johnson SC, Terry J, Steed M. 2000. Dementia, quantitative neuroimaging, and apolipoprotein E genotype. AJNR Am J Neuroradiol, 21 (10):1857-1868.

Boissonneault GA. 2010. MCI and dementia: diagnosis and treatment. JAAPA, 23 (1):18, 21-12.

Brouwers N, Sleegers K, Van Broeckhoven C. 2008. Molecular genetics of Alzheimer's disease: an update. Ann Med, 40 (8):562-583.
Chan D, Janssen JC, Whitwell JL, Jenkins R, Frost C, Rossor MN, Fox NC. 2003. Change in rates of cerebral atrophy over time in early-onset Alzheimer's disease: longitudinal MRI study. Lancet, 362 (9390):1121-1122.

Chetelat G, Desgranges B, De La Sayette V, Viader F, Eustache F, Baron JC. 2002. Mapping gray matter loss with voxel-based morphometry in mild cognitive impairment. Neuroreport, 13 (15):1939-1943.

Chincarini A, Bosco P, Calvini P, Gemme G, Esposito M, Olivieri C, Rei L, Squarcia S, Rodriguez G, Bellotti R, Cerello P, De Mitri I, Retico A, Nobili F. 2011. Local MRI analysis approach in the diagnosis of early and prodromal Alzheimer's disease. Neuroimage, 58 (2):469-480.

Cockrell JR, Folstein MF. 1988. Mini-Mental State Examination (MMSE). Psychopharmacol Bull, 24 (4):689-692.

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science, 261 (5123):921-923.

Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC, Jr., Rimmler JB, Locke PA, Conneally PM, Schmader KE, et al. 1994. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet, 7 (2):180-184.

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Jr., Rimmler JB, Locke PA, Conneally PM, Schmader KE, Tanzi RE, et al. 1995. Apolipoprotein E, survival in Alzheimer's disease patients, and the competing risks of death and Alzheimer's disease. Neurology, 45 (7):1323-1328.

Cosentino S, Scarmeas N, Helzner E, Glymour MM, Brandt J, Albert M, Blacker D, Stern Y. 2008. APOE epsilon 4 allele predicts faster cognitive decline in mild Alzheimer disease. Neurology, 70 (19 Pt 2):1842-1849.

Cuadra MB, Cammoun L, Butz T, Cuisenaire O, Thiran JP. 2005. Comparison and validation of tissue modelization and statistical classification methods in T1-weighted MR brain images. IEEE Transactions on Medical Imaging, 24 (12):1548-1565.

Davatzikos C, Fan Y, Wu X, Shen D, Resnick SM. 2008. Detection of prodromal Alzheimer's disease via pattern classification of magnetic resonance imaging. Neurobiol Aging, 29 (4):514-523.

Davatzikos C, Xu F, An Y, Fan Y, Resnick SM. 2009. Longitudinal progression of Alzheimer's-like patterns of atrophy in normal older adults: the SPARE-AD index. Brain, 132 (Pt 8):2026-2035.

Davatzikos C, Bhatt P, Shaw LM, Batmanghelich KN, Trojanowski JQ. 2011. Prediction of MCI to AD conversion, via MRI, CSF biomarkers, and pattern classification. Neurobiol Aging, 32 (12):2322 e2319-2327.
de Leon MJ, DeSanti S, Zinkowski R, Mehta PD, Pratico D, Segal S, Clark C, Kerkman D, DeBernardis J, Li J, Lair L, Reisberg B, Tsui W, Rusinek H. 2004. MRI and CSF studies in the early diagnosis of Alzheimer's disease. J Intern Med, 256 (3):205-223.

Deuschl G, Maier W. 2009. S3-Leitlinie Demenzen. Deutsche Gesellschaft für Psychiatrie, Psychotherapie und Nervenheilkunde (DGPPN), Deutsche Gesellschaft für Neurologie (DGN).

Driscoll I, Davatzikos C, An Y, Wu X, Shen D, Kraut M, Resnick SM. 2009. Longitudinal pattern of regional brain volume change differentiates normal aging from MCI. Neurology, 72 (22):1906-1913.

Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. 1997. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA, 278 (16):1349-1356.

Fotenos AF, Snyder AZ, Girton LE, Morris JC, Buckner RL. 2005. Normative estimates of cross-sectional and longitudinal brain volume decline in aging and AD. Neurology, 64 (6):1032-1039.

Franke K, Gaser C. 2012. Longitudinal Changes in Individual BrainAGE in Healthy Aging, Mild Cognitive Impairment, and Alzheimer's Disease. GeroPsych, 25 (4):235-245.

Franke K, Gaser C, for the Alzheimer's Disease Neuroimaging Initiative. 2012. Longitudinal changes in individual BrainAGE in healthy aging, mild cognitive impairment, and Alzheimer’s disease. GeroPsych: The Journal of Gerontopsychology and Geriatric Psychiatry, 25 (4):235 - 245.

Franke K, Ziegler G, Klöppel S, Gaser C, for the Alzheimer's Disease Neuroimaging Initiative. 2010. Estimating the age of healthy subjects from T1-weighted MRI scans using kernel methods: Exploring the influence of various parameters. NeuroImage, 50 (3):883-892.

Franko E, Joly O. 2013. Evaluating Alzheimer's disease progression using rate of regional hippocampal atrophy. PLoS One, 8 (8):e71354.

Gaser C, Franke K, Kloppel S, Koutsouleris N, Sauer H. 2013. in Mild Cognitive Impaired Patients: Predicting the Conversion to Alzheimer's Disease. PLoS One, 8 (6):e67346.

Genin E, Hannequin D, Wallon D, Slaeegers K, Hiltunen M, Combarros O, Bullido MJ, Engelborghs S, De Deyn P, Berr C, Pasquier F, Dubois B, Tognoni G, Fievret N, Brouwers N, Bettens K, Arosio B, Coto E, Del Zompo M, Mateo I, Epelbaum J, Frank-Garcia A, Helisalmi S, Porcellini E, Pilotto A, Forti P, Ferri R, Scarpini E, Siciliano G, Solfrizzi V, Sorbi S, Spalletta G, Valdivieso F, Vepsalainen S, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossu P, Hanon O, Piccardi P, Annoni G, Seripa D, Galimberti D, Licastro F, Soininen H, Dartigues JF, Kamboh MI, Van Broeckhoven C, Lambert JC,
Amouyel P, Campion D. 2011. APOE and Alzheimer disease: a major gene with semi-dominant inheritance. Mol Psychiatry, 16 (9):903-907.

Geroldi C, Pihlajamaki M, Laakso MP, DeCarli C, Beltramello A, Bianchetti A, Soininen H, Trabucchi M, Frisoni GB. 1999. APOE-epsilon4 is associated with less frontal and more medial temporal lobe atrophy in AD. Neurology, 53 (8):1825-1832.

Glodzik-Sobanska L, Pirraglia E, Brys M, de Santi S, Mosconi L, Rich KE, Switalski R, Saint Louis L, Sadowski MJ, Martiniuk F, Mehta P, Pratico D, Zinkowski RP, Blennow K, de Leon MJ. 2009. The effects of normal aging and ApoE genotype on the levels of CSF biomarkers for Alzheimer's disease. Neurobiol Aging, 30 (5):672-681.

Growdon JH, Locascio JJ, Corkin S, Gomez-Isla T, Hyman BT. 1996. Apolipoprotein E genotype does not influence rates of cognitive decline in Alzheimer's disease. Neurology, 47 (2):444-448.

Henderson AS, Easteal S, Jorm AF, Mackinnon AJ, Korten AE, Christensen H, Croft L, Jacomb PA. 1995. Apolipoprotein E allele epsilon 4, dementia, and cognitive decline in a population sample. Lancet, 346 (8987):1387-1390.

Henneman WJ, Sluimer JD, Barnes J, van der Flier WM, Sluimer IC, Fox NC, Scheltens P, Vrenken H, Barkhof F. 2009. Hippocampal atrophy rates in Alzheimer disease: added value over whole brain volume measures. Neurology, 72 (11):999-1007.

Hinrichs C, Singh V, Mukherjee L, Xu G, Chung MK, Johnson SC. 2009. Spatially augmented LPboosting for AD classification with evaluations on the ADNI dataset. Neuroimage, 48 (1):138-149.

Hirono N, Hashimoto M, Yasuda M, Kazui H, Mori E. 2003. Accelerated memory decline in Alzheimer's disease with apolipoprotein epsilon4 allele. J Neuropsychiatry Clin Neurosci, 15 (3):354-358.

Holmes C, Levy R, McLoughlin DM, Powell JF, Lovestone S. 1996. Apolipoprotein E: non-cognitive symptoms and cognitive decline in late onset Alzheimer's disease. J Neuropsychiatry Clin Neurosci, 15 (3):354-358.

Honea RA, Vidoni E, Harsha A, Burns JM. 2009. Impact of APOE on the healthy aging brain: a voxel-based MRI and DTI study. J Alzheimers Dis, 18 (3):553-564.

Hoyt BD, Massman PJ, Schatschneider C, Cooke N, Doody RS. 2005. Individual growth curve analysis of APOE epsilon 4-associated cognitive decline in Alzheimer disease. Arch Neurol, 62 (3):454-459.

Hua X, Lee S, Yanovsky I, Leow AD, Chou YY, Ho AJ, Gutman B, Toga AW, Jack CR, Jr., Bernstein MA, Reiman EM, Harvey DJ, Kornak J, Schuff N, Alexander GE, Weiner MW, Thompson PM. 2009. Optimizing power to track brain degeneration in Alzheimer's disease and mild cognitive impairment with tensor-based morphometry: an ADNI study of 515 subjects. Neuroimage, 48 (4):668-681.
Jack CR, Jr., Petersen RC, Xu YC, O'Brien PC, Waring SC, Tangalos EG, Smith GE, Ivnik RJ, Thibodeau SN, Kokmen E. 1998. Hippocampal atrophy and apolipoprotein E genotype are independently associated with Alzheimer's disease. Ann Neurol, 43 (3):303-310.

Jack CR, Jr., Petersen RC, Xu YC, O'Brien PC, Smith GE, Ivnik RJ, Boeve BF, Waring SC, Tangalos EG, Kokmen E. 1999. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. Neurology, 52 (7):1397-1403.

Jack CR, Jr., Weigand SD, Shiung MM, Przybelski SA, O'Brien PC, Gunter JL, Knopman DS, Boeve BF, Smith GE, Petersen RC. 2008. Atrophy rates accelerate in amnestic mild cognitive impairment. Neurology, 70 (19 Pt 2):1740-1752.

Jack CR, Jr., Shiung MM, Gunter JL, O'Brien PC, Weigand SD, Knopman DS, Boeve BF, Ivnik RJ, Smith GE, Cha RH, Tangalos EG, Petersen RC. 2004. Comparison of different MRI brain atrophy rate measures with clinical disease progression in AD. Neurology, 62 (4):591-600.

Jack CR, Jr., Wiste HJ, Vemuri P, Weigand SD, Senjem ML, Zeng G, Bernstein MA, Gunter JL, Pankratz VS, Aisen PS, Weiner MW, Petersen RC, Shaw LM, Trojanowski JQ, Knopman DS. 2010. Brain beta-amyloid measures and magnetic resonance imaging atrophy both predict time-to-progressio from mild cognitive impairment to Alzheimer's disease. Brain, 133 (11):3336-3348.

Jicha GA, Parisi JE, Dickson DW, Johnson K, Cha R, Ivnik RJ, Tangalos EG, Boeve BF, Knopman DS, Braak H, Petersen RC. 2006. Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. Arch Neurol, 63 (5):674-681.

Karas GB, Scheltens P, Rombouts SA, Visser PJ, van Schijndel RA, Fox NC, Barkhof F. 2004. Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. Neuroimage, 23 (2):708-716.

Khachaturian AS, Corcoran CD, Mayer LS, Zandi PP, Breitner JC. 2004. Apolipoprotein E epsilon4 count affects age at onset of Alzheimer disease, but not lifetime susceptibility: The Cache County Study. Arch Gen Psychiatry, 61 (5):518-524.

Kovacevic S, Rafii MS, Brewer JB. 2009. High-throughput, fully automated volumetry for prediction of MMSE and CDR decline in mild cognitive impairment. Alzheimer Dis Assoc Disord, 23 (2):139-145.

Kurz A, Egensperger R, Haupt M, Lautenschlager N, Romero B, Graeber MB, Muller U. 1996a. Apolipoprotein E epsilon 4 allele, cognitive decline, and deterioration of everyday performance in Alzheimer's disease. Neurology, 47 (2):440-443.

Kurz A, Altland K, Lautenschlager N, Zimmer R, Busch R, Gerundt I, Lauter H, Muller U. 1996b. Apolipoprotein E type 4 allele and Alzheimer's disease: effect on age at onset and relative risk in different age groups. J Neurol, 243 (6):452-456.
Lehtovirta M, Soininen H, Laakso MP, Partanen K, Helisalmi S, Mannermaa A, Ryynanen M, Kuikka J, Hartikainen P, Riekkinen PJ, Sr. 1996. SPECT and MRI analysis in Alzheimer's disease: relation to apolipoprotein E epsilon 4 allele. J Neurol Neurosurg Psychiatry, 60 (6):644-649.

Leung KK, Bartlett JW, Barnes J, Manning EN, Ourselin S, Fox NC. 2013. Cerebral atrophy in mild cognitive impairment and Alzheimer disease: rates and acceleration. Neurology, 80 (7):648-654.

Liddell MB, Lovestone S, Owen MJ. 2001. Genetic risk of Alzheimer's disease: advising relatives. Br J Psychiatry, 178 (1):7-11.

Liu M, Zhang D, Shen D. 2012. Ensemble sparse classification of Alzheimer's disease. Neuroimage, 60 (2):1106-1116.

Mahley RW, Rall SC, Jr. 2000. Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet, 1:507-537.

Manning EN, Barnes J, Cash DM, Bartlett JW, Leung KK, Ourselin S, Fox NC. 2014. APOE epsilon4 is associated with disproportionate progressive hippocampal atrophy in AD. PLoS One, 9 (5):e97608.

Martins CA, Oulhaj A, de Jager CA, Williams JH. 2005. APOE alleles predict the rate of cognitive decline in Alzheimer disease: a nonlinear model. Neurology, 65 (12):1888-1893.

Mayeux R, Saunders AM, Shea S, Mirra S, Evans D, Roses AD, Hyman BT, Crain B, Tang MX, Phelps CH. 1998. Utility of the apolipoprotein E genotype in the diagnosis of Alzheimer's disease. Alzheimer's Disease Centers Consortium on Apolipoprotein E and Alzheimer's Disease. N Engl J Med, 338 (8):506-511.

McEvoy LK, Holland D, Hagler DJ, Jr., Fennema-Notestine C, Brewer JB, Dale AM. 2011. Mild cognitive impairment: baseline and longitudinal structural MR imaging measures improve predictive prognosis. Radiology, 259 (3):834-843.

Meyer MR, Tschanz JT, Norton MC, Welsh-Bohmer KA, Steffens DC, Wyse BW, Breitner JC. 1998. APOE genotype predicts when--not whether--one is predisposed to develop Alzheimer disease. Nat Genet, 19 (4):321-322.

Misra C, Fan Y, Davatzikos C. 2009. Baseline and longitudinal patterns of brain atrophy in MCI patients, and their use in prediction of short-term conversion to AD: results from ADNI. Neuroimage, 44 (4):1415-1422.

Miyata M, Smith JD. 1996. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nat Genet, 14 (1):55-61.

Mohs RC, Cohen L. 1988. Alzheimer's Disease Assessment Scale (ADAS). Psychopharmacol Bull, 24 (4):627-628.

Mori E, Lee K, Yasuda M, Hashimoto M, Kazui H, Hirono N, Matsui M. 2002. Accelerated hippocampal atrophy in Alzheimer's disease with apolipoprotein E epsilon4 allele. Ann Neurol, 51 (2):209-214.
Morris JC. 1993. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology, 43 (11):2412-2414.

Mortimer JA, Snowdon DA, Markesbery WR. 2009. The effect of APOE-epsilon4 on dementia is mediated by Alzheimer neuropathology. Alzheimer Dis Assoc Disord, 23 (2):152-157.

Mueller SG, Weiner MW, Thal LJ, Petersen RC, Jack CR, Jagust W, Trojanowski JQ, Toga AW, Beckett L. 2005. Ways toward an early diagnosis in Alzheimer's disease: the Alzheimer's Disease Neuroimaging Initiative (ADNI). Alzheimers Dement, 1 (1):55-66.

Myers RH, Schaefer EJ, Wilson PW, D'Agostino R, Ordovas JM, Espino A, Au R, White RF, Knoefel JE, Cobb JL, McNulty KA, Beiser A, Wolf PA. 1996. Apolipoprotein E epsilon4 association with dementia in a population-based study: The Framingham study. Neurology, 46 (3):673-677.

Nalbantoglu J, Gilfix BM, Bertrand P, Robitaille Y, Gauthier S, Rosenblatt DS, Poirier J. 1994. Predictive value of apolipoprotein E genotyping in Alzheimer's disease: results of an autopsy series and an analysis of several combined studies. Ann Neurol, 36 (6):889-895.

Olichney JM, Hansen LA, Galasko D, Saitoh T, Hofstetter CR, Katzman R, Thal LJ. 1996. The apolipoprotein E epsilon 4 allele is associated with increased neuritic plaques and cerebral amyloid angiopathy in Alzheimer's disease and Lewy body variant. Neurology, 47 (1):190-196.

Peltz CB, Corrada MM, Berlau DJ, Kawas CH. 2011. Incidence of dementia in oldest-old with amnestic MCI and other cognitive impairments. Neurology, 77 (21):1906-1912.

Pennanen C, Testa C, Laakso MP, Hallikainen M, Helkala EL, Hanninen T, Kivipelto M, Kononen M, Nissinen A, Tervo S, Vanhanen M, Vanninen R, Frisoni GB, Soininen H. 2005. A voxel based morphometry study on mild cognitive impairment. J Neurol Neurosurg Psychiatry, 76 (1):11-14.

Petersen RC. 2004. Mild cognitive impairment as a diagnostic entity. J Intern Med, 256 (3):183-194.

Querbes O, Aubry F, Pariente J, Lotterie JA, Demonet JF, Duret V, Puel M, Berry I, Fort JC, Celsis P. 2009. Early diagnosis of Alzheimer's disease using cortical thickness: impact of cognitive reserve. Brain, 132 (Pt 8):2036-2047.

Rajapakse JC, Giedd JN, Rapoport JL. 1997. Statistical approach to segmentation of single-channel cerebral MR images. IEEE Transactions on Medical Imaging, 16 (2):176-186.

Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, Saunders AM, Hardy J. 2004. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. Proc Natl Acad Sci U S A, 101 (1):284-289.
Roses AD, Strittmatter WJ, Pericak-Vance MA, Corder EH, Saunders AM, Schmechel DE. 1994. Clinical application of apolipoprotein E genotyping to Alzheimer's disease. Lancet, 343 (8912):1564-1565.

Saunders AM. 2000. Apolipoprotein E and Alzheimer disease: an update on genetic and functional analyses. J Neuropathol Exp Neurol, 59 (9):751-758.

Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD. 1993. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci U S A, 90 (20):9649-9653.

Schölkopf B, Smola A. 2002. Learning with Kernels: Support vector machines, regularization, optimization, and beyond. Cambridge: MA: MIT.

Sluimer JD, van der Flier WM, Karas GB, van Schijndel R, Barnes J, Boyes RG, Cover KS, Olabariaga SD, Fox NC, Scheltens P, Vrenken H, Barkhof F. 2009. Accelerating regional atrophy rates in the progression from normal aging to Alzheimer's disease. Eur Radiol, 19 (12):2826-2833.

Smith CD, Andersen AH, Gold BT. 2012. Structural brain alterations before mild cognitive impairment in ADNI: validation of volume loss in a predefined antero-temporal region. J Alzheimers Dis, 31 Suppl 3:S49-58.

Sobel E, Louhija J, Sulkava R, Davanipour Z, Kontula K, Miettinen H, Tikkanen M, Kainulainen K, Tilvis R. 1995. Lack of association of apolipoprotein E allele epsilon 4 with late-onset Alzheimer's disease among Finnish centenarians. Neurology, 45 (5):903-907.

Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR, Jr., Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH. 2011. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement, 7 (3):280-292.

Spulber G, Niskanen E, MacDonald S, Smilovici O, Chen K, Reiman EM, Jauhiainen AM, Hallikainen M, Tervo S, Wahlund LO, Vanninen R, Kivipelto M, Soininen H. 2010. Whole brain atrophy rate predicts progression from MCI to Alzheimer's disease. Neurobiol Aging, 31 (9):1601-1605.

Stern Y. 2002. What is cognitive reserve? Theory and research application of the reserve concept. J Int Neuropsychol Soc, 8 (3):448-460.

Stern Y, Zarahn E, Hilton HJ, Flynn J, DeLaPaz R, Rakitin B. 2003. Exploring the neural basis of cognitive reserve. J Clin Exp Neuropsychol, 25 (5):691-701.

Stern Y, Brandt J, Albert M, Jacobs DM, Liu X, Bell K, Marder K, Sano M, Albert S, Del-Castillo Castenada C, Bylsma F, Tycko B, Mayeux R. 1997. The absence of an apolipoprotein epsilon4 allele is associated with a more aggressive form of Alzheimer's disease. Ann Neurol, 41 (5):615-620.
Stonnington CM, Chu C, Kloppel S, Jack CR, Jr., Ashburner J, Frackowiak RS. 2010. Predicting clinical scores from magnetic resonance scans in Alzheimer's disease. Neuroimage, 51 (4):1405-1413.

Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD. 1993. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A, 90 (5):1977-1981.

Susanto TA, Pua EP, Zhou J. 2015. Cognition, brain atrophy, and cerebrospinal fluid biomarkers changes from preclinical to dementia stage of Alzheimer's disease and the influence of apolipoprotein e. J Alzheimers Dis, 45 (1):253-268.

Talbot C, Lendon C, Craddock N, Shears S, Morris JC, Goate A. 1994. Protection against Alzheimer's disease with apoE epsilon 2. Lancet, 343 (8910):1432-1433.

Thakkinstian A, Bowe S, McEvoy M, Smith W, Attia J. 2006. Association between apolipoprotein E polymorphisms and age-related macular degeneration: A HuGE review and meta-analysis. Am J Epidemiol, 164 (9):813-822.

Tierney MC, Szalai JP, Snow WG, Fisher RH, Tsuda T, Chi H, McLachlan DR, St George-Hyslop PH. 1996. A prospective study of the clinical utility of ApoE genotype in the prediction of outcome in patients with memory impairment. Neurology, 46 (1):149-154.

Tipping ME. 2001. Sparse bayesian learning and the relevance vector machine. Journal of Machine Learning Research, 1 (3):211-244.

Tohka J, Zijdenbos A, Evans A. 2004. Fast and robust parameter estimation for statistical partial volume models in brain MRI. Neuroimage, 23 (1):84-97.

Tondelli M, Wilcock GK, Nichelli P, De Jager CA, Jenkinson M, Zamboni G. 2012. Structural MRI changes detectable up to ten years before clinical Alzheimer's disease. Neurobiol Aging, 33 (4):825 e825-836.

Tsuang D, Larson EB, Bowen J, McCormick W, Teri L, Nochlin D, Leverenz JB, Peskind ER, Lim A, Raskind MA, Thompson ML, Mirra SS, Gearing M, Schellenberg GD, Kukull W. 1999. The utility of apolipoprotein E genotyping in the diagnosis of Alzheimer disease in a community-based case series. Arch Neurol, 56 (12):1489-1495.

Utermann G, Langenbeck U, Beisiegel U, Weber W. 1980. Genetics of the apolipoprotein E system in man. Am J Hum Genet, 32 (3):339-347.

Walhovd KB, Fjell AM, Brewer J, McEvoy LK, Fennema-Notestine C, Hagler DJ, Jr., Jennings RG, Karow D, Dale AM. 2010. Combining MR imaging, positron-emission tomography, and CSF biomarkers in the diagnosis and prognosis of Alzheimer disease. AJNR Am J Neuroradiol, 31 (2):347-354.

Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, Nordberg A, Backman L, Albert M, Almkvist O, Arai H, Basun H, Blennow K, de Leon M, DeCarli C, Erkinjuntti T, Giacobini E, Graff C, Hardy J, Jack C, Jorm A, Ritchie
K, van Duijn C, Visser P, Petersen RC. 2004. Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med, 256 (3):240-246.

Wolz R, Julkunen V, Koikkalainen J, Niskanen E, Zhang DP, Rueckert D, Soininen H, Lotjonen J. 2011. Multi-method analysis of MRI images in early diagnostics of Alzheimer's disease. PLoS One, 6 (10):e25446.

Zannis VI, Just PW, Breslow JL. 1981. Human apolipoprotein E isoprotein subclasses are genetically determined. Am J Hum Genet, 33 (1):11-24.

Zhang SH, Reddick RL, Piedrahita JA, Maeda N. 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science, 258 (5081):468-471.
APPENDICES

Acknowledgement
I would like to thank Christian Gaser and Katja Franke for their ongoing support and kind encouragement throughout my dissertation work.
I am also grateful to my husband Markus for his loving and unconditional support.

This work was supported by a grant from the European Community FP7 HEALTH, Project 279281 (BrainAge).

The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the BMBF or the European Community. No potential conflicts of interest to this work were reported.

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI, National Institutes of Health Grant U01 AG024904; http://adni.loni.ucla.edu). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following organizations (also available at http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgment_List.pdf): Abbott; Alzheimer’s Association; Alzheimer’s Drug Discovery Foundation; Amorfix Life Sciences Ltd.; AstraZeneca; Bayer HealthCare; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals Inc.; Eli Lilly and Company; F. Hoffmann-La Roche Ltd. and its affiliated company Genentech, Inc.; GE Healthcare; Innogenetics, N. V.; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research provides funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer’s Disease Cooperative Study at the University of California, San Diego. ADNI data are
disseminated by the Laboratory for NeuroImaging at the University of California, Los Angeles. This research was also supported by NIH grants P30 AG010129 and K01 AG030514.

List of Tables

| Title                                                                 | Page     |
|----------------------------------------------------------------------|----------|
| **Table 1** Characteristics of the longitudinal test sample         | 15, 16   |
| **Table 2** Baseline characteristics of the cross-sectional MCI samples used for prediction | 18       |
| **Table 3** Mean *BrainAGE* scores at baseline and last follow-up apportioned to APOE genotypes within the four diagnosis groups NO, sMCI, pMCI and AD | 24       |
| **Table 4** Correlation coefficients between baseline *BrainAGE* score and cognitive functioning (ADAS scores) as well as disease severity (MMSE & CDR-SB scores) for each diagnostic group and the whole test sample, separated into carriers and non-carriers | 26       |
| **Table 5** Cox Regression values for cumulative AD incidence in carriers/ non-carriers in the *BrainAGE* score, MMSE, CDR-SB and ADAS alone and in combination with the APOE ε4 carrier status, based on a median split | 32       |
| **Table 6** Results for predicting conversion to AD in MCI subjects (APOE ε4 carriers) | 36       |
| **Table 7** Results for predicting conversion to AD in MCI subjects (APOE ε4 non-carriers) | 37       |
## List of Figures

| Title                                                                 | Page |
|----------------------------------------------------------------------|------|
| Figure 1 Depiction of the *BrainAGE* concept                        | 20   |
| Figure 2 *BrainAGE* scores at baseline (A) and at the last visit (B) for non-carriers and carriers of APOE-ε4 | 23   |
| Figure 3 Longitudinal changes in *BrainAGE* scores for (A) NO, (B) sMCI, (C) pMCI and (D) AD patients | 28   |
| Figure 4 Longitudinal changes in *BrainAGE* scores for (A) NO, (B) sMCI, (C) pMCI and (D) AD patients | 29   |
| Figure 5 Mean days to conversion from MCI to AD subdivided into all allelic combinations of APOE | 30   |
| Figure 6 Cumulative probability for MCI patients of remaining AD-free, divided into patients with the score of interest below the median and above it, as well as in non-carriers and carriers of the APOE-ε4 gene | 33   |
| Figure 7 ROC curves for pMCI_early subjects, analyzing individual subject classification based on baseline *BrainAGE* score in either remaining at a MCI stage or changing to AD, subdivided into non-carriers and carriers | 34   |
| Figure 8 ROC curves of non-carriers vs. carriers in relation with the *BrainAGE* score in all MCI patients | 35   |
Declaration of authenticity

I hereby certify

that I am knowing about the doctorate regulations of the Medical Faculty, of the Friedrich-Schiller-University Jena,

that this dissertation has been composed by me and is based on my own work, unless stated otherwise,

that the following persons helped me in composing this dissertation:

KATJA FRANKE, Ph.D.\textsuperscript{a}
CHRISTIAN GASER, Ph.D.\textsuperscript{a,b}

\textsuperscript{a}Structural Brain Mapping Group. Department of Psychiatry, University of Jena, Germany
\textsuperscript{b}Department of Neurology, University of Jena, Germany

that I was not accepting help from a Ph.D. supervisor and that no other persons received directly or indirectly money for the work they did in the context of this dissertation,

that this work has not been submitted as examination paper or for any other degree

and that I did not submit this work, a very similar one or another thesis at any other university as dissertation work.

Place, date \hspace{2cm} author’s signature
Ehrenwürdige Erklärung

Hiermit erkläre ich, dass mir die Promotionsordnung der Medizinischen Fakultät der Friedrich-Schiller-Universität bekannt ist,

ich die Dissertation selbst angefertigt habe und alle von mir benutzten Hilfsmittel, persönlichen Mitteilungen und Quellen in meiner Arbeit angegeben sind,

mich folgende Personen bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskripts unterstützt haben:

Dr. phil. KATJA FRANKE\textsuperscript{a}

Prof. Dr. CHRISTIAN GASER\textsuperscript{a,b}

\textsuperscript{a} Structural Brain Mapping Group. Abteilung für Psychiatrie, Universitätsklinikum Jena, Deutschland.
\textsuperscript{b} Abteilung für Neurologie, Universitätsklinikum Jena, Deutschland.

die Hilfe eines Promotionsberaters nicht in Anspruch genommen wurde und dass Dritte weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen,

dass ich die Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht habe und

dass ich die gleiche, eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung nicht bei einer anderen Hochschule als Dissertation eingereicht habe.

Ort, Datum

Unterschrift des Verfassers