Quantitative EEG analysis in Alzheimer’s disease

Young Alzheimer patients show distinct regional changes of oscillatory brain dynamics

H. de Waal, C. J. Stam, W. de Haan, E.C.W. van Straaten, P. Scheltens and W. M. van der Flier PhD

Abstract

Objective
To study the differences in oscillatory brain dynamics in AD according to age at onset using quantitative EEG.

Method
We examined resting state EEGs of 320 probable AD patients and 246 controls, both categorised into a young (≤65 years) and old (>65 years) group. Relative power in four different frequency bands was calculated. The effect of age on global and regional relative power was examined.

Results
Globally, young AD patients showed lower alpha- and higher delta-power than old AD patients. Regional analysis showed that these differences were most pronounced in the parieto-occipital region. Young AD patients had lower beta- and higher theta-power than old patients in all but the temporal regions. In controls, there was no age effect on global relative power in any frequency band.

Conclusion
Young AD patients present with more severe slowing of spontaneous oscillatory activity than old AD patients, which is most pronounced in the posterior brain areas. This finding supports the hypothesis that early onset AD presents with a distinct endophenotype.
Introduction

Large-scale neuronal activity can be measured with electroencephalography (EEG). The hallmark oscillatory brain dynamics finding in AD patients is a diffuse slowing of activity,¹ with a decrease in alpha power and an increase in delta power.²⁻⁴ In addition, a reduced reactivity of alpha power on eye opening has been reported in mild AD patients.⁵ However, within the spectrum of AD there is a large variation in severity of EEG abnormalities. In a former study in our memory clinic cohort, visually rated EEG in AD patients varied from a completely normal EEG to a combination of both focal and diffuse abnormalities.⁶

There is a growing body of evidence suggesting that age-at-onset is related to manifestation of the disease. Differences between young and old patients may point at different underlying disease pathways. Understanding these different pathways may provide new targets for treatment. Young patients have a clinical profile with more focal disturbances like aphasia and apraxia, in contrast to old patients where memory impairment is most pronounced.⁷⁻¹¹ In addition, young patients often show a different pattern of atrophy, with posterior regions being more affected.¹²,¹³ In a recent study, we assessed the influence of age-at-onset on EEG abnormalities. Severity of EEG abnormalities was rated on a simple, 4-point scale ranging from no abnormalities to severe abnormalities. We found that young AD patients had more frequent and severe EEG abnormalities than old AD patients.¹⁴ In this study we did not quantify the abnormalities, nor could we say anything about regional distribution. Using quantitative EEG, a higher power in the theta band and a more ‘abnormal’ EEG with a younger age at onset has been reported, but only in small samples or using only a few electrode positions.¹⁵,¹⁶

In the present study we used quantitative EEG to compare severity and location of oscillatory brain dynamics changes in a large sample of young and old AD patients and controls in order to gain more insight in the heterogeneity of pathophysiological differences within Alzheimer’s disease. We hypothesize that young AD patients will have a different regional vulnerability than old AD patients, with more severe slowing in the posterior brain regions.
Methods

Subjects

We included 320 AD patients and 246 controls. All patients had been referred to the memory clinic of the Alzheimer center of the VU university medical center, Amsterdam, the Netherlands between September 2003 and June 2009. Standardised dementia screening included a history and, when available, an informant based history, a standard neurological examination, a cognitive examination including Mini Mental State Examination (MMSE), electroencephalography (EEG), Magnetic Resonance Imaging (MRI) of the brain, neuropsychological evaluation and laboratory tests. Patients were diagnosed with probable AD according to the NINCDS-ADRDA criteria during a multidisciplinary consensus meeting. The control group consisted of patients who presented at our memory clinic with subjective complaints, but who had normal clinical investigations and did not have significant cognitive deficits (i.e. MCI criteria were not fulfilled) or major psychiatric disorder. Both groups were categorised in young (65 years or younger; AD n=113; controls n=174) and old (older than 65 years; AD n=207; controls n=72). The ethical review board of the VU University Medical Center has approved the study. All patients gave written informed consent to use their clinical data for research purposes.

EEG recording

All EEGs were recorded using the OSG digital equipment (Brainlab®; OSG b.v., Rumst, Belgium) from 21 electrodes at the positions of the 10-20 system: Fp2, Fp1, F8 F7, F4, F3, A2, A1, T4, T3, C4, C3, T6, T5, P4, P3, O2, O1, Fz, Cz, Pz, with an average reference which included all electrodes, except Fp2, Fp1, A2 and A1. Sample frequency was 500 Hz. Electrode impedance was below 5kΩ. Initial filter settings were: time constant 1s; low pass filter, 70 Hz. Patients were seated in a slightly reclined chair in a sound attenuated room. Patients sat mainly with eyes closed, EEG technicians were alert on keeping patients awake by sound stimuli.
Four 10-second epochs of artefact free data (containing no eye-blinks, slow eye-movements, excess muscle activity, ECG artefacts, etc.) were selected from each EEG (HdW). Relative power of all frequency bands (delta 0.5-4 Hz, theta 4-8 Hz, alpha 8-13 Hz, beta 13-30 Hz and gamma 30-48 Hz) was calculated for all EEG channels using Fast Fourier Transformation. A flat window was applied over the full 4096 samples of each epoch. The power in the frequency bands of interest was determined by averaging over the four epochs (with Brainwave software, version 0.8.60, developed by CS; further information: http://home.kpn.nl/stam7883/brainwave.html). By averaging the relative power of all electrodes a mean relative power for each frequency band was obtained. Our group used this method in previous studies. Gamma band was not included in further analyses, because of the possible admixture of muscle artefacts in this frequency band.18,19 Channels were clustered into four regions of interest: frontal, temporal, central and parieto-occipital. The frontal cluster was formed by channels Fp1, Fp2, F7, F8, F3, F4 and Fz; the temporal cluster was formed by channels A1, A2, T3, T4, T5 and T6; the central cluster was formed by channels C4, C3 and Cz; and the parieto-occipital cluster was formed by channels P3, P4, Pz, O1 and O2. For further statistical analysis the mean relative power values were log transformed (x = log [x/1-x]) to obtain a more Gaussian distribution.20

Statistics

PASW Statistics 18.0 for Mac was used for statistical analyses. Differences between groups for baseline characteristics were investigated with t-tests and \( \chi^2 \)-tests where appropriate. First, the effect of age (young vs. old) on relative power was tested in both AD patients and controls, for each frequency band separately, using two-way analysis of variance (ANOVA). The mean relative power was used as dependent variable, age (young vs. old) and diagnosis were entered in the model as binary fixed factors, sex was entered as covariate. Secondly, the effect of age on relative power in different brain regions was examined, using ANOVA’s for repeated measures. Age was used as between-subjects factor and brain area (4 regions: frontal, temporal, central and parieto-occipital) was used as within-subjects factor. Relative power was the dependent variable. Sex was entered as covariate. Separate models for each frequency band were used; analyses were done separately for AD patients and controls.
Results

Baseline characteristics are summarized in table 1. The young AD group was on average a few years older than the young control group (p<.05) and the old AD group was also a few years older than the old controls (p<.05). The young and old AD patients had comparable gender distribution, MMSE and disease duration. In the young and old AD group a comparably small percentage of patients used acetylcholinesterase inhibitors.

We used two-way ANOVA to assess the effect of age on the global power spectrum in AD patients and controls. Global relative power values for AD and controls stratified by age are given in table 1. There was a main effect of diagnosis in all frequency bands [alpha: F(1,561) = 64.03, p < 0.001; beta: F(1,561) = 36.07, p < 0.001; theta: F(1,561) = 162.72, p < 0.001; delta: F(1,561) = 14.61, p < 0.001], as AD patients had a higher delta and theta and lower alpha and beta power than controls. In the alpha and delta band we found a main effect of age [alpha: F(1,561) = 12.16, p = 0.001; delta: F(1,561) = 15.40, p < 0.001] and an interaction between age and diagnosis [alpha: F(1,561) = 4.25, p = 0.040; delta: F(1,561) = 3.60, p = 0.58]. Young AD patients had a lower global alpha power and higher delta power than old AD patients, whereas in controls there were no differences between the young and old. In the beta and theta band no age effects or interactions were found. When we repeated the analyses after exclusion of acetylcholinesterase inhibitor users, the results did not change essentially, except for the interaction between age and diagnosis in the alpha band, which lost significance.

Subsequently, we explored regionally specific effects of age in AD patients and controls separately using ANOVA for repeated measures (figure 1; table 2). For AD patients there was a main effect of age [F(1,317) = 19.59, p < 0.001] and area [F(2.78,881.30) = 3.83, p = 0.012] and an interaction between age and area [F(2.78,881.30) = 3.83, p = 0.012] in the alpha band. We found a lower relative power in young AD patients as compared to old patients in all brain regions, but the difference between young and old patients was largest in the parieto-occipital region. In the beta band, there was no main effect of age, but there was a main effect of area [F(2.82,893.83) = 30.76, p < 0.001] and an interaction between age and area [F(2.82,893.83) = 3.58, p = 0.016]. Young AD patients had a lower relative power than old patients in the frontal, central and parie-
to-occipital region, while old patients had lower relative power in the temporal regions. In the theta band, there was no main effect of age, but there was a main effect of area \([F(2.83,897.25) = 6.26, p < 0.001]\) and an interaction between age and area \([F(2.83,897.25) = 6.92, p < 0.001]\), which mirrored the pattern observed in the beta band. Young AD patients had a higher relative theta power in the frontal, central and parieto-occipital regions, but old patients had a higher relative power in the temporal regions. In the delta band, there were main effects of age \([F(1,317) = 19.392, p < 0.001]\) and area \([F(2.87,910.48) = 35.38, p < 0.001]\) and an interaction between age and area \([F(2.87,910.48) = 4.43, p = 0.005]\). Young AD patients had a higher relative delta power than old AD patients, which was most pronounced in the frontal and parieto-occipital regions. When we repeated the analyses after exclusion of acetylcholinesterase inhibitor users, the results did not change essentially.

For controls, there was a main effect of area \([\text{alpha}: F(2.66,645.63) = 26.35, p < 0.001; \text{delta}: F(2.68,650.75) = 39.29, p < 0.001]\), but no main effect of age nor an interaction between age and area in the alpha and delta band. In the beta band there was no main effect of age, but there was a main effect of area \([F(2.89,701.97) = 34.79, p < 0.001]\) and an interaction between age and area \([F(2.89,701.97) = 11.41, p < 0.001]\). Old controls had lower beta power in the temporal and parieto-occipital regions than young controls, while such an effect was not observed in other brain regions. In the theta band there was no main effect of age, but there was a main effect of area \([F(2.94,714.42) = 9.33, p < 0.001]\) and an interaction between age and area \([F(2.94,714.42) = 8.93, p < 0.001]\). Old controls had higher theta power than young controls in all regions, except for the central region, where there was no difference between groups.
Discussion

The main finding of this study is that young AD patients had relatively less power in the faster alpha band and more power in the slower delta band than old AD patients and that this difference was greatest in the posterior regions. Moreover, in the beta band and theta band we also found a different regional distribution between young and old AD patients. In controls, there was no difference in global relative power between age groups. We did find some specific regional differences between young and old controls in the beta and theta band, but these were smaller than the differences in AD. Previous EEG studies have shown a decrease of power in the fast frequency bands and increase of power in slower frequency bands in AD patients compared to controls.\(^1\)\(^-\)\(^4\) We confirm these former findings as the entire group of AD patients had more delta and theta power and less alpha and beta power than controls. Variability in severity of EEG abnormalities in AD has been shown before. In a study including 35 AD patients and 35 controls, quantitative EEG discriminated between patients and controls, but within the group of AD patients there was a large, unexplained, variation in the ratios between fast and slow activity.\(^2\) In a former study on EEG in a memory clinic population, we found that AD patients more often show EEG abnormalities than nondemented individuals. Within the group of AD patients however, EEG’s ranged from completely normal to both focal and diffuse abnormalities.\(^6\) As yet, it remains unclear what causes this heterogeneity. Severity of the disease and the use of acetylcholinesterase inhibitors have been found to influence these differences,\(^21\)\(^-\)\(^23\) but this does not explain the entire picture.

In the current study, we focused on age-at-onset as a determinant of severity and distribution of oscillatory brain dynamics. Our findings are in line with the few previous studies on quantitative EEG analysis in young and old AD patients.\(^15\)\(^,\)\(^16\)\(^,\)\(^24\)\(^-\)\(^26\) In a study using visual classification of power spectra, a more abnormal EEG was correlated with a younger age of onset, but regional differences were not taken into account.\(^16\) In a very small cohort of 9 young and 10 old AD patients compared with healthy controls, maximal group differences between young AD patients and controls were found in the right posterior temporal lobe, whereas maximal group differences between old AD patients and
their controls were found in the midfrontal and bilateral anterior areas.\textsuperscript{26} A direct comparison between early onset and late onset AD was not made however.

We found more slowing (relatively less alpha and more delta power) in young than in old AD patients. Moreover, we observed a different regional distribution of EEG abnormalities, with relatively more disturbed activity in the parieto-occipital regions in young AD, while in old AD patients activity was relatively more disturbed in the temporal regions. A study using source localization has provided evidence that EEG findings are a marker of underlying brain pathology.\textsuperscript{27} Furthermore, these findings coincide with results from studies in other modalities and suggest a different regional vulnerability in young onset patients. AD is typically characterized by memory impairment accompanied by atrophy of the medial temporal lobe. In patients with early onset however, the cognitive profile is often dominated by nonmemory problems such as apraxia, aphasia and visuospatial dysfunction, and atrophy is often observed in parietal and posterior brain regions.\textsuperscript{10,12,28-32} Furthermore, in early onset AD reduced metabolism has been found in the parietal and posterior association cortices.\textsuperscript{33-38}

Strengths of the present study are the large population of consecutive patients and controls, who all received an EEG as part of the standard work up. We measured relative power, a well-known measure of quantitative EEG analysis, which allowed us to study regional changes in oscillatory brain activity in a reliable and detailed way. Possible limitations are the fact that we used persons with subjective complaints as control group, as these patients are known to have an increased risk of progression to dementia. The main question in this study involved a comparison between early and late onset AD however, which is not influenced by the control group.

In this study we focused on regional differences in oscillatory activity between early and late onset AD. Regional analyses do not take into account connections between neurons. AD has been termed a disconnection syndrome, with evidence for disrupted functional and structural connectivity in AD patients.\textsuperscript{39,40} Future studies should focus on functional networks, as it can be hypothesized that these networks are differentially compromised in early onset and late onset AD.
In conclusion, we found that young AD patients present with more severe slowing of spontaneous oscillatory activity than old AD patients, which is most pronounced in the posterior brain areas. This finding supports the hypothesis that early onset AD presents with a distinct endophenotype. A picture emerges that there may be different pathways leading to AD in young and old patients. These pathways may in turn be linked to differences in prognosis and response to therapy. Furthermore, the biological underpinnings of differences between old and young patients may lead to new targets for therapy.

Acknowledgements

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Disclosure Statement

- H. de Waal reports no disclosures.
- Dr. Stam reports no disclosures.
- W. de Haan reports no disclosures.
- Dr. van Straaten reports no disclosures.
- Dr. Scheltens serves/has served on the advisory boards of: Genentech, Novartis, Roche, Danone, Nutricia, Baxter and Lundbeck. He has been a speaker at symposia organised by Lundbeck, Merz, Danone, Novartis, Roche and Genentech. For all his activities he receives no personal compensation. He serves on the editorial board of Alzheimer’s Research & Therapy and Alzheimers Disease and Associated Disorders, is a member of the scientific advisory board of the EU Joint Programming Initiative and the French National Plan Alzheimer.
- Dr. van der Flier reports no disclosures.
References


### Table 1. Subject characteristics

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<tr>
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<th>AD</th>
<th>Control</th>
</tr>
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<tr>
<td></td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>N</td>
<td>113</td>
<td>207</td>
</tr>
<tr>
<td>Age, years</td>
<td>59 (5) *</td>
<td>75 (5) #</td>
</tr>
<tr>
<td>Sex, female</td>
<td>62 (55%)</td>
<td>94 (45%)</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>3.8 (1.9)</td>
<td>3.4 (2.3)</td>
</tr>
<tr>
<td>Using AChEI</td>
<td>9 (8%)</td>
<td>10 (5%)</td>
</tr>
<tr>
<td>MMSE</td>
<td>20 (5)</td>
<td>21 (5)</td>
</tr>
<tr>
<td>Alpha band, relative power a,b,c</td>
<td>.224 (.12)</td>
<td>.284 (.13)</td>
</tr>
<tr>
<td>Beta band, relative power a</td>
<td>.157 (.07)</td>
<td>.165 (.09)</td>
</tr>
<tr>
<td>Theta band, relative power a</td>
<td>.228 (.09)</td>
<td>.226 (.12)</td>
</tr>
<tr>
<td>Delta band, relative power a,b</td>
<td>.333 (.13)</td>
<td>.274 (.12)</td>
</tr>
</tbody>
</table>

Data are mean(SD) or n(%). For EEG, raw data are shown, but statistical analyses were performed on log-transformed data. AChEI=Acetylcholinesterase inhibitor

* Early onset AD group versus young controls: p<.05  
# Late onset AD group versus old controls: p<.05  
  a Main effect of diagnosis: p<.05  
  b Main effect of age group: p<.05  
  c Interaction between age group and diagnosis: p<.05
**Table 2. Regional relative power according to diagnosis and age group**

<table>
<thead>
<tr>
<th></th>
<th>Alpha band</th>
<th>Beta band</th>
<th>Theta band</th>
<th>Delta band</th>
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<tr>
<td><strong>AD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Young</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal</td>
<td>0.180 (0.11)</td>
<td>0.142 (0.07)</td>
<td>0.213 (0.09)</td>
<td>0.399 (0.14)</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.224 (0.12)</td>
<td>0.157 (0.07)</td>
<td>0.234 (0.10)</td>
<td>0.309 (0.12)</td>
</tr>
<tr>
<td>Central</td>
<td>0.227 (0.12)</td>
<td>0.215 (0.11)</td>
<td>0.232 (0.10)</td>
<td>0.271 (0.12)</td>
</tr>
<tr>
<td>Parieto-occipital</td>
<td>0.281 (0.17)</td>
<td>0.143 (0.08)</td>
<td>0.240 (0.11)</td>
<td>0.305 (0.15)</td>
</tr>
<tr>
<td><strong>Old</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal</td>
<td>0.237 (0.13)</td>
<td>0.156 (0.08)</td>
<td>0.213 (0.11)</td>
<td>0.335 (0.13)</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.274 (0.12)</td>
<td>0.155 (0.08)</td>
<td>0.243 (0.12)</td>
<td>0.263 (0.11)</td>
</tr>
<tr>
<td>Central</td>
<td>0.284 (0.12)</td>
<td>0.228 (0.12)</td>
<td>0.223 (0.12)</td>
<td>0.224 (0.11)</td>
</tr>
<tr>
<td>Parieto-occipital</td>
<td>0.360 (0.17)</td>
<td>0.150 (0.10)</td>
<td>0.229 (0.13)</td>
<td>0.235 (0.13)</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Young</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal</td>
<td>0.292 (0.16)</td>
<td>0.173 (0.08)</td>
<td>0.117 (0.05)</td>
<td>0.357 (0.13)</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.332 (0.14)</td>
<td>0.206 (0.08)</td>
<td>0.138 (0.07)</td>
<td>0.258 (0.09)</td>
</tr>
<tr>
<td>Central</td>
<td>0.330 (0.16)</td>
<td>0.263 (0.11)</td>
<td>0.137 (0.07)</td>
<td>0.224 (0.11)</td>
</tr>
<tr>
<td>Parieto-occipital</td>
<td>0.444 (0.19)</td>
<td>0.202 (0.10)</td>
<td>0.112 (0.06)</td>
<td>0.214 (0.12)</td>
</tr>
<tr>
<td><strong>Old</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal</td>
<td>0.299 (0.15)</td>
<td>0.179 (0.07)</td>
<td>0.126 (0.07)</td>
<td>0.326 (0.13)</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.356 (0.12)</td>
<td>0.180 (0.07)</td>
<td>0.158 (0.09)</td>
<td>0.245 (0.09)</td>
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<tr>
<td>Central</td>
<td>0.344 (0.14)</td>
<td>0.263 (0.10)</td>
<td>0.137 (0.07)</td>
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<tr>
<td>Parieto-occipital</td>
<td>0.460 (0.16)</td>
<td>0.188 (0.09)</td>
<td>0.124 (0.07)</td>
<td>0.200 (0.11)</td>
</tr>
</tbody>
</table>
Table 2 (p. 55)
Shown are mean relative power values, mean(SD), for all frequency bands in AD and control. Please note that raw data are shown, while statistical analyses were performed on log-transformed data.

Figure 1

A. AD patients
**B. Controls**

**Figure 1 (p. 56 / 57)**

Differences in relative power between young and old. Colors reflect the amount of difference in relative power between young and old. Blue means less power in young than in old, red means more power in young than in old. **A. Differences** in relative power between early onset and late onset AD in four different brain regions. Interaction between Area and Age was significant (p<.05) in all frequency bands. **B. Differences in** relative power between young and old controls in four different brain regions. Interaction between Area and Age group was significant in the beta and theta band (p<.05).
Quantitative EEG analysis in Alzheimer’s disease

APOE ε4 non-carrying Alzheimer patients show more severe slowing of oscillatory brain activity

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Abstract

Objective
The objective of this study was to quantitatively assess the relationship between Apolipoprotein (APOE) genotype and EEG oscillatory brain dynamics in AD patients and controls and its regional distribution.

Method
We obtained resting-state EEGs of 320 AD patients and 246 controls, categorized into APOE-ε4-carriers and non-carriers. Peak frequency and relative power in four different frequency bands were calculated. We tested the associations between APOE genotype and relative power in four brain regions.

Results
Peak frequency was comparable in APOE ε4 carrying and non-carrying controls, but lower in APOE ε4 non-carrying AD patients. In controls, APOE ε4 carriers had a different regional distribution of alpha power than non-carriers. We found no APOE effect in beta, delta, and theta bands. In AD, APOE ε4 non-carriers had lower alpha and higher delta power than carriers. This difference was most pronounced in the parieto-occipital region. In the theta band, APOE ε4 non-carriers had a different regional distribution of power compared to carriers.

Conclusion
In conclusion, the most pronounced effect of genotype was seen in AD patients, where APOE e4 non-carriers showed slower activity, especially in parieto-occipital regions.
Introduction

In Alzheimer’s disease (AD) there is a distinct dysfunction of neuronal activity, most likely prior to the occurrence of neuronal degeneration. Cognitive deficits in AD are associated with this neuronal dysfunction. Electroencephalography (EEG) allows the measurement of large-scale neuronal activity; characteristic findings in AD include a shift of the power spectrum to slower frequencies. Within the spectrum of AD however, there is a wide variety in severity and pattern of these EEG abnormalities, of which the specific cause is unknown.

The Apolipoprotein E ε4 (APOE ε4) genotype is a risk factor for AD. A considerable proportion of patients develop AD however without an APOE ε4 allele and it has been identified that these patients present with a different profile than APOE ε4 carriers. They demonstrate a faster decline and altered EEG functional connectivity. To date, little is known about the influence of APOE on oscillatory brain activity measured by EEG. The few available studies have conflicting results, some report increased slowing in ε4 carrying AD patients, while another reports no difference according to ε4 carrier status.

In a previous study on the association between APOE and visual EEG analysis, we found more severe EEG abnormalities in APOE ε4 non-carrying patients. These analyses did not allow quantification of the differences, nor could we investigate the regional distribution. The aim of the present study was to quantitatively assess the influence of APOE genotype on oscillatory brain dynamics in AD patients and controls, taking into account the regional configuration of these differences.

Methods

Subjects

We included 320 AD patients and 246 controls in this study from the memory clinic of the Alzheimer center of the VU University Medical Center between April 2003 and May 2009. The standardised diagnostic work-up included: a history,
an informant based history (when available), a standard neurological exam, a
cognitive examination including Mini Mental State Examination (MMSE), elec-
troencephalography (EEG), Magnetic Resonance Imaging (MRI) of the brain,
neuropsychological evaluation and laboratory tests. Diagnoses were made
during a multidisciplinary consensus meeting. AD patients fulfilled the clinical
criteria of the National Institute on Aging-Alzheimer’s Association (NIA-AA) di-
agnostic guideline.20 The control group consisted of patients who presented
at our memory clinic with subjective complaints, but did not have interference
in Instrumental Activities of Daily Living (IADL). They had a neuropsychological
evaluation, MRI scan and EEG within normal limits (i.e. Mild Cognitive Impair-
ment [MCI] criteria were not fulfilled) and they did not have a major psychiatric
disorder. The ethical review board of the VU University Medical Center has ap-
proved the study. All patients provided written informed consent to use their
clinical data for research purposes.

**APOE genotyping**

APOE genotyping was performed after DNA isolation from 10 ml EDTA blood,
with the Light Cycler APOE mutation detection method (Roche Diagnostics
GmbH, Mannheim, Germany). APOE ε4 carrier status was dichotomized in car-
rrier (at least one ε4 allele) and non-carrier (AD: APOE ε4 carriers n=216; APOE
ε4 non-carriers n=104; Control: APOE ε4 carriers n=86; APOE ε4 non-carriers
n=160).

**EEG**

All EEG’s were recorded using the OSG digital equipment (Brainlab®; OSG b.v.,
Rumst, Belgium) at the positions of the 10-20 system: : Fp2, Fp1, F8 F7, F4, F3,
A2, A1, T4, T3, C4, C3, T6, T5, P4, P3, O2, O1, Fz, Cz, Pz, with an average refer-
ence which included all electrodes, except Fp2, Fp1, A2 and A1. Sample fre-
quency was 500 Hz. Electrode impedance was below 5kΩ. Initial filter settings
were: time constant 1s; low pass filter, 70 Hz. Patients were seated in a slightly
reclined chair in a sound attenuated room. Patients sat with eyes closed, EEG
technicians were alert on keeping patients awake by sound stimuli.
Four 10-second non-overlapping epochs of artefact free (containing no eye-blks, slow eye movements, excess muscle activity, ECG-artefacts, etc.), eyes-closed, resting state data were selected from each EEG. Relative power of all frequency bands (delta 0.5-4 Hz, theta 4-8 Hz, alpha 8-13 Hz, beta 13-30 Hz and gamma 30-48 Hz) was calculated for all EEG channels, using Fast Fourier Transformation (with Brainwave software, version 0.8.68, developed by C.J. Stam; further information and free available software: http://home.kpn.nl/stam7883/brainwave.html). Peak frequency was calculated within the 4-13 Hz range. No window was applied over the full 4096 samples of each epoch. Before averaging the four epochs of each patient the raw relative power values of each electrode were transformed by means of logit transformation ($x = \log [x/1-x]$) to obtain a more Gaussian distribution.

By averaging the logit mean relative power of all electrodes, a global relative power in each frequency band was obtained. Gamma band was not used in further analyses, because of the possible admixture of muscle artefacts in this frequency band. The average peak frequency of posterior channels was calculated (P4, P3, O2, O1) and for a further regional analysis of relative power results, channels were clustered into four regions of interest: frontal, formed by channels Fp1, Fp2, F7, F8, F3, F4 and Fz; temporal, formed by channels A1, A2, T3, T4, T5 and T6 (A1 and A2 (left and right earlobe) were reference electrodes, however not electrically inactive and therefore included in the analyses); central, formed by channels C4, C3 and Cz; parieto-occipital, formed by channels P3, P4, Pz, O1 and O2 (in line with our previous study).

**Statistics**

PASW Statistics 18.0 for Mac was used for statistical analysis. Differences between groups in baseline characteristics were tested with t-tests and $\chi^2$-tests where appropriate. First, we studied the influence of diagnosis (AD vs. control) and APOE genotype (ε4 carriers vs. non-carriers) on the peak frequency by means of two-way ANOVA. Sex and age were entered as covariates. Subsequently, the effect of APOE genotype on relative power in four brain regions (frontal, temporal, central, parieto-occipital) was assessed, using ANOVA for repeated measures, in AD patients and controls separately. APOE genotype was used as between-subjects factor, brain area as within-subjects factor and
logit transformed relative power as dependent variables (separate models for each frequency band). Sex and age were entered as covariates. Since it has been suggested that the APOE ε4 effect is modulated by age at onset of AD, we considered the interaction between age and apoe. Because there was no significant interaction between age at onset and APOE ε4 genotype in any of the models, the interaction term was not included.

Results

Baseline characteristics are summarized in **table 1**. AD patients were on average older than controls, but in both groups there was no difference in age between APOE ε4 carriers and non-carriers. There was no gender difference between patients and controls, but APOE ε4 carriers were more often female in AD patients. AD patients had a lower MMSE than controls, but there was no difference within diagnosis groups between APOE ε4 carriers and non-carriers. AD patients had a lower alpha and beta power and a higher theta and delta power than controls. Two-way ANOVA revealed main effects of both diagnosis and APOE ε4 genotype (resp. p < 0.01 and p < 0.05) on peak frequency. Furthermore, there was an interaction between diagnosis and genotype (p = 0.06). Post-hoc analysis, stratified by diagnostic group, using one-way ANOVA showed that within the group of controls APOE genotype was not related to peak frequency (p = 0.60), while in the group of AD patients APOE ε4 non-carriers had a lower peak frequency than APOE ε4 carriers (p < 0.01) (**table 1**).

Subsequently, we used ANOVA for repeated measures to assess the effect of APOE genotype on regional oscillatory brain activity in controls and AD patients separately. **Figure 1** provides a visual representation of the differences in relative power according to APOE genotype in controls, with the raw values shown in **table 2**. In all frequency bands we found a main effect of area (all p < 0.01). Alpha power was highest in the parieto-occipital region, beta power in the central region, theta power in the temporo-central region and delta power in the frontal region. In the alpha band we did not find a main effect of APOE, but we found an interaction between APOE genotype and area (p < 0.05), implicating that APOE ε4 carriers had a different distribution of power.
than non-carriers, with less frontal and central alpha power. In the beta band we found a main effect for APOE genotype (p < 0.05), but no interaction between APOE genotype and brain area. APOE ε4 non-carriers globally had a slightly lower beta power than carriers. In the theta and delta band we found neither a main effect for APOE genotype, or interactions between APOE genotype and brain area.

In AD patients we found a main effect of area in all frequency bands as well, with the distribution of power between brain areas comparable to that in controls (p < 0.01). Alpha power was highest in the parieto-occipital region, beta power in the central region, theta power in the temporal region and delta power in the frontal region. For the difference between APOE ε4 carriers and non-carriers in AD patients we observed a different pattern than in controls (figure 2; table 3). Non-carriers had a lower alpha power than APOE ε4 carriers and this difference was most pronounced in the parieto-occipital region (main effect APOE genotype, interaction area by APOE, both p < 0.01). In the beta band there was neither a main effect of APOE genotype, nor an interaction between APOE genotype and area. In the theta band we found a different regional distribution of power between carriers and non-carriers. APOE ε4 non-carriers had a higher power in frontal and parieto-occipital areas than carriers (no main effect APOE; interaction area by APOE, p < 0.05). APOE ε4 non-carriers had a higher delta power than APOE ε4 carriers, which was most pronounced in the parieto-occipital region (main effect APOE, interaction area by APOE, both p < 0.01).

Discussion

In this study, we showed that APOE genotype modulates the regional distribution of oscillatory brain activity of AD patients and controls. In controls, we found no difference in peak frequency and subtle differences in regional distribution of power in the alpha band according to APOE genotype. In AD patients we found more pronounced differences. APOE ε4 non-carriers had a slower peak frequency than carriers. Furthermore, they had more power in the slower frequency bands than carriers and less power in the faster alpha band. These differences were most pronounced in the parieto-occipital region.
We found no association between APOE genotype and global relative power in controls, but on closer regional inspection we did find subtle differences between APOE ε4 carriers and non-carriers in the distribution of power. It is known that several EEG measures are highly heritable and APOE could be one of the genes involved.\(^2^6\) In our previous study on visually analysed EEG abnormalities we did not find a difference according to APOE genotype in occurrence of EEG abnormalities in controls,\(^1^9\) comparable to our present finding of equal peak frequency. Two former quantitative EEG studies on healthy controls and healthy AD family members did not find any differences in resting-state EEG according to APOE genotype either.\(^1^6,2^7\) The first study did find a difference between APOE ε4 carrying and non-carrying relatives of AD patients in a hyperventilation condition, where the carriers had more slow wave activity during hyperventilation than non-carriers. None of these studies examined regional differences between carriers and non-carriers, whereas we found a subtle difference in regional distribution of alpha power. An influence of APOE genotype on brain activity in healthy controls is in line with connectivity studies using EEG and other modalities. APOE ε4 carriers have been reported to have higher functional connectivity in lower and upper alpha band in an EEG study examining functional connectivity.\(^1^4\) Different default mode and salience networks,\(^2^8\) and differences in functional brain networks as compared to APOE ε3 carriers.\(^2^9\)

In the group of AD patients we extend on our former finding of more severe EEG abnormalities in APOE ε4 non-carrying AD patients by showing that this difference consists of slowing of the background rhythm, particularly in posterior brain regions.\(^1^9\) Our results contrast with several former studies examining quantitative EEG in relation to APOE genotype.\(^1^5-1^7\) These studies reported more slow activity in APOE ε4 carrying AD patients as opposed to our finding of more slowing in non-carrying AD patients. In one of these studies,\(^1^5\) the group of APOE ε4 carriers was significantly younger than the group of non-carriers. Since previous studies have shown that young AD patients show more EEG abnormalities than old patients, it is not clear whether the slowing of the background rhythm in this study is an effect of APOE or age.\(^2^5,3^0,3^1\) Furthermore, these former studies analysed small samples and neither specified regional differences in relative power.\(^1^5,1^6\) A study using an estimation of cortical sources of EEG rhythms,\(^1^7\) shows that amplitude of alpha 1 and alpha 2 sources was higher in non-carriers than in carriers. The reason for these different findings is not ea-
sily explained. We are confident however, since in our group the characteristic EEG findings of decreased alpha and beta power and increased theta and delta power and decreased peak frequency in AD patients compared to controls are replicated, that our results are robust. A possible explanation for the difference in findings between the two studies could be differences in sample selection. Our center is a tertiary referral clinic that might draw a somewhat different sample of AD patients than in non-academic clinics. Our study is also a single center study as opposed to the multicentric approach of Babiloni et al. A multicentric study has the advantages of obtaining a larger and possibly more diverse study population, however it has the disadvantage of inducing variability due to EEG hardware, recording protocols, sample frequency, among others, which does not play a role in our sample.

Our finding of particularly posterior slowing in APOE ε4 non-carriers is largely in line with studies in other modalities. APOE ε4 non-carriers have been shown to have different regional patterns of brain atrophy, with more whole brain atrophy than carriers, while the carriers had more hippocampal and amygdaloid atrophy. They also had different structural connectivity loss and lower gradient of regional changes of neuritic plaque deposition. A recent study by our group showed a differential effect of APOE genotype on different aspects of the disease, as APOE ε4 non-carrying AD patients had increased uptake of amyloid in the frontal cortex, while APOE ε4 carriers had more severe posterior hypometabolism than APOE ε4 non-carriers. These results show that genetic make-up has complex influences on the pathological pathways leading to AD. Studies incorporating multiple modalities of imaging in the same patients are needed to shed light on the exact mechanisms.

The present study is one of the few studies to investigate the association between APOE genotype and oscillatory brain dynamics. The large population, in which all patients underwent EEG, render our findings quite robust. For the EEG analysis we used relative power that is considered a well-known method in EEG research. A possible limitation of this study is the relatively young age of our population, which may render our results less representative for the general AD population. For research purposes, this younger population has the advantage of being a more pure form of AD having less comorbidity than an older cohort.
to age, but in our study we did not find an interaction between age and APOE genotype. Our study seems to point to two different properties of APOE. Carriers of the ε4 allele generally display an earlier onset of disease, attributable to the increased deposition of amyloid beta.\textsuperscript{36} The role of APOE genotype in direct brain activity may be completely independent of this process and for example related to the association of APOE with cholesterol, which has an important role in synaptogenesis and neurotransmission.\textsuperscript{37}

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**Disclosure statement**

- H. de Waal reports no disclosures.
- Dr. Stam reports no disclosures.
- Dr. W. de Haan reports no disclosures.
- Dr. van Straaten reports no disclosures.
- Dr. Blankenstein has received speaker honoraria from Abbott and Ferring and serves as an Associate Editor for Annals of Clinical Biochemistry.
- Dr Scheltens serves/has served on the advisory boards of: Genentech, Novartis, Roche, Danone, Nutricia, Baxter and Lundbeck. He has been a speaker at symposia organised by Lundbeck, Merz, Danone, Novartis, Roche and Genentech. For all his activities he receives no personal compensation. He serves on the editorial board of Alzheimer’s Research & Therapy and Alzheimers Disease and Associated Disorders, is a member of the scientific advisory board of the EU Joint Programming Initiative and the French National Plan Alzheimer.
- Dr. van der Flier reports no disclosures.

The medical ethical committee of the VU University Medical Center approved the study
Former studies have suggested that the effect of APOE ε4 differs according


Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>APOE ε4 non-carriers</td>
</tr>
<tr>
<td>N</td>
<td>320</td>
<td>104</td>
</tr>
<tr>
<td>Age years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70 (9)**</td>
<td>69 (10)</td>
</tr>
<tr>
<td>Sex, female</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>156 (49%)</td>
<td>41 (39%)*</td>
</tr>
<tr>
<td>MMSE</td>
<td>21 (5)**</td>
<td>21 (5)</td>
</tr>
<tr>
<td>Alpha power</td>
<td>-0.50 (0.30)**</td>
<td>-0.59 (0.27)*</td>
</tr>
<tr>
<td>Beta power</td>
<td>-0.76 (0.22)**</td>
<td>-0.75 (0.27)</td>
</tr>
<tr>
<td>Theta power</td>
<td>-0.57 (0.28)**</td>
<td>-0.54 (0.27)</td>
</tr>
<tr>
<td>Delta power</td>
<td>-0.41 (0.26)**</td>
<td>-0.35 (0.25)*</td>
</tr>
<tr>
<td>Peak frequency (Hz)</td>
<td>8.26 (1.44)**</td>
<td>7.91 (1.43)*</td>
</tr>
</tbody>
</table>

Data are mean(SD) or n(%).
* APOE ε4 non-carriers vs. APOE ε4 carriers: p<.05.
** AD patients vs. controls: p<.05.
Table 2. Regional relative power according to APOE ε4 carriers status in controls.

<table>
<thead>
<tr>
<th>Control</th>
<th>APOE ε4</th>
<th>Alpha band</th>
<th>Beta band</th>
<th>Theta band</th>
<th>Delta band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>non-carriers</td>
<td>0.32 (0.17)</td>
<td>0.17 (0.07)</td>
<td>0.12 (0.06)</td>
<td>0.34 (0.13)</td>
</tr>
<tr>
<td>Frontal</td>
<td>carriers</td>
<td>0.28 (0.14)</td>
<td>0.19 (0.07)</td>
<td>0.11 (0.04)</td>
<td>0.34 (0.13)</td>
</tr>
<tr>
<td></td>
<td>non-carriers</td>
<td>0.35 (0.14)</td>
<td>0.20 (0.07)</td>
<td>0.14 (0.07)</td>
<td>0.25 (0.10)</td>
</tr>
<tr>
<td>Temporal</td>
<td>carriers</td>
<td>0.34 (0.13)</td>
<td>0.21 (0.07)</td>
<td>0.14 (0.06)</td>
<td>0.24 (0.08)</td>
</tr>
<tr>
<td></td>
<td>non-carriers</td>
<td>0.34 (0.16)</td>
<td>0.26 (0.10)</td>
<td>0.14 (0.06)</td>
<td>0.22 (0.11)</td>
</tr>
<tr>
<td>Central</td>
<td>carriers</td>
<td>0.33 (0.14)</td>
<td>0.28 (0.10)</td>
<td>0.13 (0.06)</td>
<td>0.22 (0.10)</td>
</tr>
<tr>
<td></td>
<td>non-carriers</td>
<td>0.46 (0.20)</td>
<td>0.20 (0.10)</td>
<td>0.11 (0.06)</td>
<td>0.21 (0.12)</td>
</tr>
<tr>
<td>Parieto-occipital</td>
<td>carriers</td>
<td>0.45 (0.17)</td>
<td>0.21 (0.10)</td>
<td>0.11 (0.05)</td>
<td>0.20 (0.10)</td>
</tr>
</tbody>
</table>

Shown are mean relative power values, mean (± SD), for all frequency bands. Please note that raw data are shown, while all statistical analyses are performed on logit transformed data. We used ANOVA for repeated measures to assess the effect of APOE genotype on regional oscillatory brain activity. Main effect of Area in all frequency bands (p < 0.01). Alpha band: no main effect of APOE, interaction between Area and APOE (p < 0.05); Beta band: main effect APOE (p < 0.05), no interaction between Area and APOE; Theta band: no significant effects; Delta band: no significant effects.
Table 3. Regional relative power according to APOE ε4 carriers status in AD patients.

<table>
<thead>
<tr>
<th>Area</th>
<th>Alpha band</th>
<th>Beta band</th>
<th>Theta band</th>
<th>Delta band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-carriers</td>
<td>0.18 (0.09)</td>
<td>0.15 (0.08)</td>
<td>0.22 (0.11)</td>
<td>0.37 (0.13)</td>
</tr>
<tr>
<td>carriers</td>
<td>0.23 (0.13)</td>
<td>0.15 (0.08)</td>
<td>0.21 (0.11)</td>
<td>0.34 (0.13)</td>
</tr>
<tr>
<td>Temporal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-carriers</td>
<td>0.22 (0.10)</td>
<td>0.16 (0.08)</td>
<td>0.25 (0.11)</td>
<td>0.30 (0.12)</td>
</tr>
<tr>
<td>carriers</td>
<td>0.27 (0.12)</td>
<td>0.15 (0.07)</td>
<td>0.24 (0.11)</td>
<td>0.26 (0.11)</td>
</tr>
<tr>
<td>Central</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-carriers</td>
<td>0.23 (0.10)</td>
<td>0.23 (0.12)</td>
<td>0.24 (0.11)</td>
<td>0.26 (0.12)</td>
</tr>
<tr>
<td>carriers</td>
<td>0.28 (0.13)</td>
<td>0.22 (0.11)</td>
<td>0.23 (0.11)</td>
<td>0.23 (0.11)</td>
</tr>
<tr>
<td>Parieto-occipital</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-carriers</td>
<td>0.28 (0.15)</td>
<td>0.15 (0.10)</td>
<td>0.25 (0.13)</td>
<td>0.29 (0.13)</td>
</tr>
<tr>
<td>carriers</td>
<td>0.36 (0.17)</td>
<td>0.15 (0.09)</td>
<td>0.23 (0.12)</td>
<td>0.24 (0.13)</td>
</tr>
</tbody>
</table>

Shown are mean relative power values, mean (± SD), for all frequency bands. Please note that raw data are shown, while all statistical analyses are performed on log-transformed data. We used ANOVA for repeated measures to assess the effect of APOE genotype on regional oscillatory brain activity. Main effect of Area in all frequency bands (p < 0.01). Alpha band: main effect APOE (p < 0.01), interaction between APOE and area (p < 0.01); beta band: no main effect APOE, no interaction between APOE and area; theta band: no main effect APOE, interaction effect between APOE and area (p < 0.05); delta band: main effect APOE (p < 0.01), interaction between APOE and area (p < 0.01).
Figure 1

Beta band
Main effect APOE genotype, p < 0.05

Delta band

Alpha band
Interaction Area*APOE, p < 0.05

Theta band

non-carriers > carriers

Difference in Relative Powervalues

non-carriers < carriers
Chapter 3.2 | APOE e4 non-carriers show more severe slowing of oscillatory brain dynamics

Figure 2
**Figure 1 (p. 75)**
Differences in relative power between APOE ε4 carrying and non-carrying controls. We used ANOVA for repeated measures to assess the effect of APOE on regional oscillatory brain activity. In the alpha band carriers had a higher relative power in frontal brain regions than non-carriers. In the beta band the non-carriers had less power than the carriers in all brain regions. No differences were observed in theta and delta band.

**Figure 2 (p. 76)**
Differences in relative power between APOE ε4 carrying and non-carrying AD patients. We used ANOVA for repeated measures to assess the effect of APOE on regional oscillatory brain activity. APOE ε4 non-carriers had less alpha power in all brain regions as compared to carriers, this difference was most pronounced in the parieto-occipital region. In the beta band no differences in relative power were observed between carriers and non-carriers. In the theta band we found an interaction between APOE ε4 genotype and area. Non-carriers had more theta power in parieto-occipital regions. In the delta band APOE ε4 non-carriers had more power than carriers, with the most pronounced difference in the parieto-occipital region.