Quantitative EEG during normal aging: association with the Alzheimer's disease genetic risk variant in PICALM gene

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A B S T R A C T

Genome-wide association studies have identified novel risk variants for Alzheimer’s disease (AD). Among these, a gene carrying one of the highest risks for AD is PICALM. The PICALM rs3851179 A allele is thought to have a protective effect, whereas the G allele appears to confer risk for AD. The influence of the PICALM genotype on brain function in nondemented subjects remains largely unknown. We examined the possible effect of the PICALM rs3851179 genotype on quantitative electroencephalography recording at rest in 137 nondemented volunteers (age range: 20—79 years) subdivided into cohorts of those younger than and those older than 50 years of age. The homozygous presence of the AD risk variant PICALM GG was associated with an increase in beta relative power, with the effect being more pronounced in the older cohort. Beta power elevation in resting-state electroencephalography has previously been linked to cortical disinhibition and hyperexcitability. The increase in beta relative power in the carriers of the AD risk PICALM GG genotype suggests changes in the cortical excitatory-inhibitory balance, which are heightened during normal aging.

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1. Introduction

Genetic predisposition and increasing age are the greatest known risk factors for AD. Mutations of the APP, presenilin-1 (PSEN1), and presenilin-2 (PSEN2) genes are causative factors for early onset AD (Goate et al., 1991; Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995). Polymorphisms of the apolipoprotein E (ApoE) gene are the most prevalent genetic risk factors for late onset AD in Caucasian ethnic groups, including the Russian population (Farrer et al., 1997; Rogaev, 1999; Saunders et al., 1993; Schmechel et al., 1993). Recent genome-wide association studies have identified novel risk variants for AD (Benitez et al., 2014; Chauhan et al., 2015; Harold et al., 2009; Lambert et al., 2009; Liu et al., 2013; Naj et al., 2014). The putative epistatic interaction of the novel risk variants with the APOE ε4 variant in the risk for AD has been demonstrated (Golenkina et al., 2010; Naj et al., 2014). Among the identified genes, phosphatidylinositol clathrin assembly lymphoid-myeloid leukemia (PICALM, chr11q14) is currently one of the top 6 risk genes for AD in the AlzGene database (http://www.AlzGene.org).

Understanding how the genes identified in association studies influence AD pathogenesis can potentially contribute to the earlier prediction of AD and the use of personalized prevention strategies in individuals at risk for AD.

The PICALM protein has been implicated in clathrin-mediated endocytosis (Ford et al., 2001). Clathrin-mediated endocytosis is responsible for the internalization of receptors, the recycling of membrane components, and the regulation of autophagic processes (Xu et al., 2015). Convincing data indicate that genetically directed changes in PICALM function lead to alterations in APP processing through endocytic pathways, amyloid beta (Aβ) production (Xiao et al., 2012) and Aβ clearance into the bloodstream (Ando et al., 2013; Baig et al., 2010; Parikh et al., 2014). PICALM may also contribute to AD development due to defects in autophagy and the clearance of tau, which is an autophagy substrate (Moreau et al., 2012).
The PICALM protein is associated with neurofibrillary tangles and is abnormally cleaved in Alzheimer’s brains (Ando et al., 2013). PICALM may cause synaptic perturbations by modulating the abundance of the glutamate receptor subunit GluR2 (Harel et al., 2011; Harold et al., 2009) and may influence AD risk by disrupting iron homeostasis and lipid metabolism (Xu et al., 2015).

The influence of the PICALM genotype on hippocampal volume and the thickness of the entorhinal cortex was found in patients with AD and mild cognitive impairment (MCI), and in normal controls (Biff et al., 2010). Gene-wide scoring highlighted PICALM as the most significant gene associated with entorhinal cortical thickness (Furney et al., 2011).

However, the mechanism by which PICALM polymorphisms influence brain function in nondemented subjects remain largely unknown. The SNP rs3851179, which was the first PICALM SNP associated with AD risk (Harold et al., 2009), is located in a non-coding region ~80 kb 5’ of PICALM. There are several PICALM SNPs (e.g., rs543293, rs659023, rs7110631, rs7941541, and rs3851179) in linkage disequilibrium with each other and associated with AD (Harold et al., 2009; Raj et al., 2012; Xu et al., 2016). Hence, the variant rs3851179 investigated in the present study is tagging the AD-associated haplotype.

Electroencephalography (EEG) is a powerful and cost-effective method for studying alterations in brain function during normal and pathological aging. EEG reflects the integrated synaptic activity of large populations of neurons, which progressively deteriorates in normal and physiological aging (Buzsaki, 2006). Recent data showed that EEG may be a valuable biomarker of the development of the pathologic processes in AD (Babiloni et al., 2006a; Jeong et al., 2004; Prichep et al., 2006; Moretti et al., 2012). Such biomarkers can be helpful to estimate the effect of potential therapies to prevent or delay the onset of neurodegenerative diseases (Ilizaroskin et al., 2004; Masdeu et al., 2012).

The primary EEG abnormalities in AD patients consist of a shift of the power spectrum to lower frequencies and a decrease in the coherence of fast rhythms (Jelic et al., 1997; Jeong, 2004). AD patients exhibit a slowing of the dominant EEG frequency, increased delta and theta power, and decreased alpha power compared to healthy age-matched controls (Babiloni et al., 2014; Rossini et al., 2007, van Straaten et al., 2014). MCI, which is in most cases a prodromal stage of AD, has EEG characteristics intermediate of those of normal subjects and AD patients (Babiloni et al., 2006a). Longitudinal studies have revealed EEG-based predictors of future decline in MCI patients and even in healthy elderly subjects (Babiloni et al., 2014; Prichep et al., 2006; Van der Hiele et al., 2008).

Healthy aging is associated with an amplitude decrease in the posterior alpha rhythm, with the anteriorization of the alpha activity and the slowing of alpha frequency (Babiloni et al., 2006b; Klimesch, 1999; Ponomareva et al., 2013; Tsuno et al., 2002; Volf and Gluhih, 2011). Several, but not all, studies also reported a decrease in delta activity and an increase in beta activity during physiological aging (Babiloni et al., 2006b; Vlahou et al., 2014).

EEG patterns have been suggested as promising tools to assess endophenotypes—basic heritable quantitative biological traits that more directly reflect the influence of specific genetic abnormalities than a complex disorder. Resting-state EEG characteristics are among the most heritable traits in humans, and the heritability of the spectral power of different EEG bands is in the range 70%–90% (van Beijsterveldt et al., 1996). EEG endophenotypes might help to clarify the role of genetic variants in brain function and disease development (De Geus, 2010).

Recent studies have demonstrated an association between EEG characteristics and AD risk variants in the ApoE and CLU genes in AD and MCI patients and even in healthy adults (Babiloni et al., 2006a; Jelic et al., 1997; Lee et al., 2012; Lehtovirta et al., 2000; Ponomareva et al., 2008, 2012, 2013; Stam et al., 2003).

The effect of the PICALM genotype on EEG characteristics has not been previously investigated.

The present study aimed to determine whether the PICALM genotype influences EEG characteristics in nondemented adults, and to estimate whether this possible effect is modified over the course of aging.

2. Materials and methods

2.1. Participants

The enrolled cohort included 137 nondemented individuals (47 men and 90 women; age range: 20–79 years).

The subjects were of Russian descent from Moscow and the Moscow region. The participants underwent a neurologic examination and cognitive screening. The recruited subjects were free of dementia and other medical, psychiatric, and neurologic conditions. The exclusion criteria included a history of neurologic and psychiatric diseases, any type of memory impairment, signs of clinical depression or anxiety, physical brain injury or other medical conditions (e.g., hypertension, diabetes, cardiac disease, or thyroid disease), or a personal history of drug or alcohol addiction.

The Spielberger State-Trait Anxiety Inventory (Spielberger et al., 1983) and the Hamilton Rating Scale for Depression (Hamilton, 1960) were used to examine anxiety and depression. The subjects were evaluated with the mini-mental state examination and Clinical Dementia Rating scale (Hughes et al., 1982). Only subjects with mini-mental state examination scores of 28 or more and Clinical Dementia Rating scores of 0 were included in the study. All subjects were right-handed.

Written informed consent was obtained from all the participants. The experimental protocol for this study was approved by the local ethics committee. ApoE genotyping was performed for all participants, and the effect of the ApoE genotype on the EEG characteristics was statistically controlled.

All subjects were divided into subgroups according to the PICALM (PICALM AA and AG and PICALM GG) polymorphisms. The PICALM AA and AG group included subjects with the homozygous PICALM AA or heterozygous PICALM AG genotypes. The PICALM GG group consisted of subjects with the homozygous PICALM GG genotype. Each group was further subdivided into cohorts of those younger than and those older than 50 years of age (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger cohort age range (20–50)</th>
<th>Older cohort age range (51–79)</th>
<th>All participants age range (20–79)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PICALM AA and AG</td>
<td>PICALM GG</td>
<td>PICALM AA and AG</td>
</tr>
<tr>
<td>N</td>
<td>50</td>
<td>23</td>
<td>39</td>
</tr>
<tr>
<td>Age (y)</td>
<td>34.4 ± 1.4</td>
<td>37.3 ± 2.3</td>
<td>63.6 ± 1.3</td>
</tr>
<tr>
<td>Sex (men/women; men%)</td>
<td>18/32; 36%</td>
<td>8/15; 34.8%</td>
<td>14/25; 35.9%</td>
</tr>
</tbody>
</table>

The data are presented as the means and standard errors.
2.2. EEG procedure

The registration and evaluation of EEG was carried out in accordance with International Pharmaco-EEG Society guidelines (Jobert et al., 2013; Versavel et al., 1995). All recordings were obtained in the afternoon from 3 to 4 PM. EEGs were recorded for 3 minutes during the resting state, with the subjects sitting comfortably in a chair. They were asked to close their eyes and relax, but to stay awake during the recording. To maintain a constant level of vigilance, an experimenter monitored the subject, and the EEG traces on-line and verbally alerted the subject any time when there were signs of behavioral and/or EEG drowsiness.

EEGs were recorded on Nihon Kohden 4217G EEG (Japan) using a time constant of 0.3 seconds. The high frequency cutoff was 45 Hz. The 14 Ag/AgCl electrodes were placed according to the international 10–20 system at the O2, O1, P4, P3, F4, C3, F3, Fp2, Fp1, T4, T3, F8, and F7 positions. Linked ears served as the reference. The electrode impedance did not exceed 10 kΩ. During the recordings, 180 seconds of EEG at rest was simultaneously sampled at 256 Hz per channel and stored on a computer for further analysis offline. The EEG was reviewed visually for artifacts, which were eliminated from the subsequent analysis. After the artifacts were eliminated, segments of the resting EEG of 120 seconds in duration were selected for further analysis.

2.3. EEG analysis

Frequencies below 2 Hz and above 35 Hz were eliminated using digital filtering. Thirty 4-s artifact-free resting EEG epochs were processed using fast Fourier transform. The relative powers (% of the total EEG power) of the delta (2.00–3.99 Hz), theta (4.00–7.99), alpha (8.00–12.99), beta 1 (13.00–19.99) and beta 2 (20.00–30.00) bands and the occipital (O2, O1), parietal (P4, P3), central (C4, C3), frontal 1 (F4, F3), frontal 2 (Fp2, Fp1), mid-temporal 2 (T4, T3), and anterior temporal (F8, F7) regions were calculated. Log transformations of the relative power of the various bandwidths in each derivation were calculated to compensate for data skewness, according to the recommendations of John et al. (1980), using the formula \( \log(x[1 - x]) \), where \( x \) is the fraction of total power for each 4-s sample. The average log relative power for each frequency band was then calculated. The details of the spectral analysis procedures have been previously described (Ponomareva et al., 2008, 2012, 2014).

2.4. Genetic analysis

Genomic DNA was isolated from peripheral venous blood using the standard phenol-chloroform extraction method or a Qiagen DNA isolation kit. Genotyping was performed using polymerase chain reaction (PCR), followed by restriction fragment length polymorphism analysis. Amplification was performed according to the manufacturer’s instructions using both the Tercyc DNA amplifier (DNA technology, Russia) and the GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems).

The restriction fragment length polymorphism analysis was developed to genotype the rs3851179 polymorphism of PICALM. We designed oligonucleotide primers flanking the polymorphic region of the PICALM gene that contains the rs3851179 polymorphism primers: PICALM F sequence 5’-3’ CTCCCTAACACGTGTCCT and PICALM-R sequence 5’-3’ TGCTACCCATGTCATCCT.

PCR was performed using the isogen “Core” kits according to the manufacturer's instructions. Each assay contained 0.2 μM of each primer and 30–100 ng of genomic DNA and was run on a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems). The PCR reaction conditions were as follows: primary denaturation at 95 °C for 1 minute, 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 57 °C for 45 seconds, and extension at 72 °C for 1 minute, followed by a terminal extension at 72 °C for 10 minutes. The PCR product was digested using the SspI restriction enzyme (SibEnzyme) at 37 °C overnight. The resulting restriction fragments were analyzed using electrophoresis with 2% agarose gels (Fig. 1).

For the G allele, when SspI cannot digest the PCR product, we identified one 480 bp band. Otherwise, the SspI restriction enzyme digests the PCR product and generates 2 bands, 392 bp and 88 bp, and this is the A allele.

ApoE genotyping was performed according to previously described methods (Golenkina et al., 2010).

2.5. Statistical analysis

Differences in the demographic and neuropsychological scores between the groups (PICALM AA and AG young, PICALM GG young, PICALM AA and AG old, and PICALM GG old) were compared using an analysis of variance (ANOVA) in the case of the normally distributed continuous variables (age and education), and the Mann-Whitney U test for the categorical variables (sex).

The EEG parameters from each group were tested for normal distribution using the Shapiro-Wilk test, and in none of the cases were the data found to be skewed.

The significance of the differences between the log-transformed EEG parameters was estimated using repeated measures of ANOVA in the general linear model (GLM) with genotype (PICALM AA and AG vs. PICALM GG) and age cohort (old vs. young) as the between-subject factors, and bands as a within-subject factor. Gender and ApoE genotype were included as fixed factors. Post hoc comparisons for between-subject effects and within-subject effects were analyzed using the Duncan test, and the level of significance was set to \( p < 0.05 \) for the post hoc comparisons.

Fig. 1. Gel electrophoresis of the PICALM gene restriction products. M is the SibEnzyme 100-bp marker. C is the uncleaved PCR product, which is equivalent to the G allele and used as a negative control. Samples 3, 5, 6, 8, 11, 14, and 16 are GG homozygotes; samples 1, 9, 10, 12, and 15 are AG heterozygotes; and samples 2, 4, 7, and 13 are AA homozygotes. In the case of the G allele, when SspI cannot digest the PCR product, one 480-bp band is visible. Otherwise, SspI digests the PCR product to 2 bands, 392 bp and 88 bp, and this is the A allele. Abbreviation: PCR, polymerase chain reaction.
We combined the groups with the PICALM AA and PICALM AG genotypes into a single group because the group with the PICALM AA genotype was relatively small (22 subjects) and because the control ANOVA analysis with genotype (PICALM AA vs. PICALM AG) as the between-subject factor did not show significant differences in EEG parameters between the PICALM AA and PICALM AG groups.

3. Results

Table 1 shows the demographic information for the participants. There were no differences in age and sex between the PICALM GG and PICALM AA and AG subgroups in either the young or the old subgroups, or in the whole sample ($p > 0.05$). There were no significant differences in sex between the young and the old subgroups with the same PICALM genotype.

Statistical examination of the normalized EEG relative power values yielded a significant main effect for the PICALM genotype [$F(1,131) = 4.09, p = 0.045$]. Post hoc comparisons revealed that in the entire sample, the beta 1 and beta 2 relative power in the subjects with a homozygous PICALM GG genotype was higher than that in the subjects with the PICALM AA and AG genotypes ($p = 0.039$ for beta 1 and $p = 0.033$ for beta 2; Fig. 2).

Histograms of the distribution of the values of the beta 1 and beta 2 relative power in the carriers of the different PICALM genotypes are presented in Fig. A (Supplementary Materials). The data confirmed higher values of beta 1 and beta 2 relative power in the subjects with the PICALM GG genotype than in the individuals with the PICALM AA and AG genotypes despite other overlap among the groups.

Moreover, the distribution of these EEG parameters in the 3 genotype groups (PICALM AA, PICALM AG, and PICALM GG) indicated that the PICALM AA homozygotes had the lowest beta 1 and beta 2 relative power compared to the non-PICALM AA carriers (Fig. B, Supplementary Materials). However, the number of PICALM AA homozygotes was insufficient for an ANOVA to examine differences of the EEG parameters in the 3 genotype groups.

Fig. C (Supplementary Materials) shows examples of the EEGs in the subjects with the different PICALM genotypes. A marked increase in beta activity was revealed in the carriers of the PICALM GG genotype.

The ANOVA revealed a significant interaction between the factors age and EEG bands [$F(4,496) = 3.48, p = 0.008$] in the whole sample. Post hoc comparisons demonstrated significant differences in the delta and beta 1 relative power between the older and the younger subjects. In the older subjects, the log-transformed delta relative power was $-2.24 \pm 0.07$, whereas in the younger individuals, this parameter was significantly higher ($-2.05 \pm 0.06, p = 0.045$). The log-transformed beta power in the older cohort was $-1.80 \pm 0.05$, whereas in the younger cohort it was significantly lower ($-2.05 \pm 0.05, p = 0.01$).

Post hoc comparisons showed that in the subjects with the PICALM GG genotype, the beta 1 and beta 2 power was significantly higher in the older cohort compared to the younger cohort ($p = 0.035$ for beta 1 and $p = 0.046$ for beta 2). In the older carriers of the PICALM GG genotype, beta 2 relative power was increased compared to the older carriers of the PICALM AA and AG genotypes (Fig. 3).

4. Discussion

This study shows that the PICALM rs3851179 polymorphism is associated with beta relative power in the resting-state EEG of nondemented adults. An increase in beta 1 and beta 2 relative power was observed in the carriers of the homozygous AD risk variant PICALM GG compared to the carriers of the protective PICALM A allele (PICALM AA and AG genotypes).

This study also showed that the main effect of age was significant for the relative power in the delta and beta 1 frequency bands. Delta power was decreased, and beta power was increased in the older compared to the younger cohort. This age-related decrease in delta relative power is consistent with the results of previous studies (Babiloni et al., 2006b; Leirer et al., 2011; Vlahou et al., 2014). An increase of beta relative power during aging was previously found in many (Rossiter et al., 2014; Vlahou et al., 2014; Vyasata et al., 2012), but not all, studies (Babiloni et al., 2006b).

Our results showed that the increase in relative beta power during aging was significant only in the PICALM GG genotype carriers; however, similar tendency was observed in all groups. The genetic heterogeneity of the samples may have caused the discrepancies in the results of previous studies. Accounting for genetic factors may improve the homogeneity of the results of future studies in this area.

The beta rhythm is one of the essential functional features of the brain. This rhythm has been associated with the coordination among many parts of the neocortex and the mediation of signals transmitted from higher-order structures to the lower-order structures (Siegel et al., 2012). Beta oscillations have been reported to be involved in regulating attention, motor planning and control, multisensory integration, and language processing (Donner et al., 2009; Leocani et al., 1997, Siegel et al., 2012; Spitzer and Blankenburg, 2011; Tallon-Baudry et al., 2004; von Stein et al., 1999).

The beta rhythm is generated in cortico-cortical, cortico-hippocampal, and cortico-basal ganglia-thalamo-cortical networks (Cannon et al., 2014, for review). Beta oscillations within these networks are dependent on the balance between excitatory glutamatergic pyramidal cells and inhibitory interneurons, with GABAergic cells playing the role of pacemakers (Bibbig et al., 2002; Yamawaki et al., 2008). A significant genetic linkage has been found for the beta frequency in resting EEG with a cluster of GABA_A receptor genes on chromosome 4 (Porjesz et al., 2002).

The increase in beta relative power in the resting-state EEG can be interpreted as a sign of hyperarousal, central nervous system disinhibition, and/or hyperexcitability (Enoch et al., 2008; Fernandez-Mendoza et al., 2016; Jaworska et al., 2012; Riemann et al., 2010). Elevated beta power was found to be associated with alterations of inhibitory controls, insomnia, or anxiety (Porjesz and
The association of the PICALM GG genotype with neurophysiological signs of hyperexcitability or disinhibition implies that carrier of this genotype may be prone to stress reactions. Previous studies have reported that long-standing distress in midlife increases the risk of AD (Johansson et al., 2014; Landfield et al., 2007; Lupien et al., 2007; Sousa et al., 2000).

Given that PICALM GG was reported to have genetics associated with the risk for AD, the observed GG-associated neurophysiological alterations may have a potentially causative contribution to an increased risk for AD, or alternatively, may be associated with the early physiological changes that occur decades before AD symptoms. Previous research has revealed the influence of the PICALM rs3851179 genotype on PICALM expression (Parikh et al., 2014). Because the PICALM protein modulates glutamatergic synaptic transmission (Harel et al., 2011) and glutamatergic mechanisms are essential for beta rhythm generation, it is possible that the EEG beta alterations found in the carriers of the PICALM GG genotype are mediated by the changes in glutamatergic neurotransmission.

The neurophysiological signs of hyperexcitability and/or cortical disinhibition in the PICALM GG carriers revealed in the present study may be related to the accumulation of pathogenic Aβ assemblies many years before the manifestation of AD (Ando et al., 2013; Baig et al., 2010; Parikh et al., 2014), which may elicit aberrant excitatory network activity and epileptiform discharges that could lead to network disinhibition (Laferla, 1995; Palop and Mucke, 2009). Tau accumulation in the carriers of the PICALM GG genotype may also contribute to neuronal hyperexcitability (Devos et al., 2013; Moreau et al., 2014). These processes may be related to the early signs of degeneration in the hippocampus and entorhinal cortex in nondemented adults that have been associated with the PICALM SNP rs3851179 (Biffl et al., 2010). Further studies are needed to verify these hypotheses.

EEG alterations in the carriers of the PICALM GG genotype differ from those in the ApoE ε4 allele carriers; however, both of these genetic risk variants underlie cerebral disinhibition and/or hyperexcitability. In a previous study, we found that the presence of the ApoE ε4 allele in cognitively normal younger adults was associated with neurophysiological signs of hyperexcitability, which was characterized by the manifestation of synchronous high-voltage delta and theta activity and sharp waves under hyperventilation. It was suggested that aberrant network hyperactivity contributes to cognitive decline through changes in the expression of neuronal gene products and the remodeling of neuronal circuits (Palop and Mucke, 2010; Sanchez et al., 2012) (Bakker et al., 2015). Promising results have been obtained in recent studies demonstrating that the use of low doses of antiepileptic drugs can rescue neurophysiological alterations and cognitive deficits in mouse models of AD (Sanchez et al., 2012), and in patients with amnestic MCI (Bakker et al., 2015).

In conclusion, this study found an increase in beta relative power associated with the AD risk PICALM GG genotype in nondemented adults, with the effect being more pronounced in subjects older than 50 years. This increase suggests cortical disinhibition and/or neuronal hyperexcitability in the carriers of the AD risk PICALM GG genotype, which is heightened during normal aging.

**Disclosure statement**

The authors have no actual or potential conflicts of interest.
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