SHORT COMMUNICATION

Neural hyperactivation in carriers of the Alzheimer's risk variant on the clusterin gene

Thomas M. Lancaster, Alison Baird, Claudia Wolf, Margaret C. Jackson, Stephen J. Johnston, Rossen Donev, Johannes Thome, David E.J. Linden

School of Medical Sciences, Bangor University, Bangor, LL57 2AS, UK
Laboratory of Molecular Psychiatry and Pharmacology, Institute of Life Science, School of Medicine, Swansea University, Singleton Park, Swansea, SA2 8PP, UK
Wolfson Centre for Cognitive and Clinical Neuroscience, School of Psychology, Bangor University, Bangor, LL57 2AS, UK
School of Social Sciences, Brunel University, Uxbridge, UK
MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, Cardiff, UK

Received 20 December 2010; received in revised form 1 February 2011; accepted 3 February 2011

KEYWORDS
Dementia;
Genetic imaging;
Working memory;
Clusterin;
Hippocampus;
Prefrontal cortex

Abstract

Recent GWAS identified a risk variant for Alzheimer’s disease (AD) at a locus (rs11136000) of the clusterin gene (CLU). Here we use functional magnetic resonance imaging (fMRI) during working memory to probe the effect of the risk variant on brain activation in healthy individuals. Participants with the CLU risk genotype had higher activity than participants with the protective allele in frontal and posterior cingulate cortex and the hippocampus, particularly during high memory demand. These results inform pathophysiological models of the preclinical progression of AD.

© 2011 Elsevier B.V. and ECNP. All rights reserved.

1. Introduction

Functional imaging studies have suggested that carriers of the apolipoprotein E (APOE) ε4 risk allele for Alzheimer’s disease (AD) present with higher levels of neural activity during cog-
Corneveaux et al., 2010; Harold et al., 2009; Lambert et al., 2009). Although clusterin has been implicated in the pathophysiology of AD (Bertram and Tanzi, 2010), little is known about how the gene and its protein product contribute to the manifestation of the disease. CLU levels have previously been shown to be associated with symptom severity, entorhinal/hippocampal cortex atrophy and amyloid-beta (Aβ) burden (Thambisetty et al., 2010). Imaging studies have also demonstrated that the risk locus is associated with variations in cortical morphology (Biffi et al., 2010). However, the functional differences between individuals with risk and protective genotypes have not yet been studied in pre-clinical cases.

In the present study we trace the effects of the risk variant on brain activation in a young healthy population using a visual working memory task with functional neuroimaging previously described (Jackson et al., 2009). We used an ‘emotional faces’ working memory task as testing memory for faces has been linked to higher risk (homozygous, CC) (Harold et al., 2009). We studied 43 healthy subjects (age range 18–51, median age 29.1, 22 males, 21 females, 3 left handed, and 40 right handed). All subjects were of Caucasian ethnicity because ethnic matching is critical in genetic imaging and association studies (Hariri and Weinberger, 2003). Participants and relatives had no history of neuropsychiatric, neurological or neurodegenerative disease. Participants also had no chronic somatic illness or history of substance abuse. Subjects were tested using a robust face working memory paradigm for functional magnetic resonance imaging (fMRI) as previously described (see supplementary material). Data were from a subsample of the participants of a larger genetic imaging study (Wolf et al., 2011), for whom information about theCLU SNP data was available. Subjects were genotyped forCLU rs11136000 (CC: 13, CT: 24, TT=6) and pooled according to hypothesised risk allele (RISK Carriers: CC, Non-Risk CT/TT (Harold et al., 2009)). Hardy–Weinberg Equilibrium was checked withχ2-test (α-level = .05; DF=2) and independent-samples t-test (2-tailed) determined no differences in gender (p=.212) and age (p= .174).

### 2. Experimental procedures

We studied 43 healthy subjects (age range 18–51, median age 29.1, 22 males, 21 females, 3 left handed, and 40 right handed). All subjects were of Caucasian ethnicity because ethnic matching is critical in genetic imaging and association studies (Hariri and Weinberger, 2003). Participants and relatives had no history of neuropsychiatric, neurological or neurodegenerative disease. Participants also had no chronic somatic illness or history of substance abuse. Subjects were tested using a robust face working memory paradigm for functional magnetic resonance imaging (fMRI) as previously described (see supplementary material). Data were from a subsample of the participants of a larger genetic imaging study (Wolf et al., 2011), for whom information about theCLU SNP data was available. Subjects were genotyped forCLU rs11136000 (CC: 13, CT: 24, TT=6) and pooled according to hypothesised risk allele (RISK Carriers: CC, Non-Risk CT/TT (Harold et al., 2009)). Hardy–Weinberg Equilibrium was checked withχ2-test (α-level = .05; DF=2) and independent-samples t-test (2-tailed) determined no differences in gender (p=.212) and age (p= .174).

### 3. Results

A main effect of genotype, reflecting higher activation for the risk group, was observed in the right dorso-lateral prefrontal cortex (rDLPFC) (F1A), the right hippocampus/entorhinal cortex (hippocampal formation, rHF) (F1B) and the dorsal posterior cingulate (dPC) (F1C) (cluster threshold of 1000 voxels). All three clusters also survived a threshold p<.01 (cluster threshold of 100 voxels), but not a more conservative threshold of p<.001.

The rDLPFC (F1A) and dPC (F1C) showed an interaction between genotype and load in which increased brain activity in the risk group was particularly marked under high memory load (loads 3 and 4). We therefore also performed t-tests for whole brain group differences at just the higher loads (WM loads 3 and 4). For areas demonstrating a significant genotype×load interaction at higher loads (rDLPFC and dPC) clusters demonstrated a more pronounced effect with higher significance (Table 1B and C). There were no differences in the magnitude of genotype main effect between all four WM loads and High WM loads in the rHF, supported by the absence of a genotype×load interaction (Table 1B).

### Table 1

Talairach coordinates for peak voxel in clusters from Fig. 1 as determined by Talairach Daemon (Lancaster et al., 2000). F and p values (cluster mean) for main effects of genotype, load×genotype interactions and high load main effects (loads 3 and 4).

<table>
<thead>
<tr>
<th>Region</th>
<th>Talairach coordinates</th>
<th>CLU risk main effect F(1,41)</th>
<th>Load×CLU risk interaction F(3,123)</th>
<th>Main effect (WM loads 3–4) t(84)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hemisphere X Y Z</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Dorsolateral prefrontal cortex (BA9)</td>
<td>R 40 15 35</td>
<td>9.84, p&lt;.01</td>
<td>4.957, p&lt;.01</td>
</tr>
<tr>
<td>B</td>
<td>Hippocampal formation (BA35)</td>
<td>R –30 –29 –7</td>
<td>9.496, p&lt;.01</td>
<td>Not sig</td>
</tr>
<tr>
<td>C</td>
<td>Dorsal posterior cingulate (BA31)</td>
<td>– 3 –26 –40</td>
<td>9.213, p&lt;.01</td>
<td>3.240, p&lt;.05</td>
</tr>
</tbody>
</table>
These effects were further corroborated by a dose dependent rs11136000 genotype effect, which was documented in the rDLPFC and rHF (Fig. 2). In these regions, there was additive up-regulation of brain activation associated with the C allele, where homozygous expression of the C allele was associated with highest mean beta values (CC > CT > TT) as reflected in pairwise comparisons (Table 2).

4. Discussion

Healthy individuals with the AD risk genotype on the CLU gene activated several brain areas (DLPFC, hippocampus, and cingulate) that were not active in the controls. Their performance on the WM task equaled that of controls, and one interpretation is thus that the carriers of the protective allele performed the task with more efficient use of neural resources. Correlations between AD risk (APOE isoform status) and neural hyperactivation have previously been reported in the right dorsolateral prefrontal cortex (Wishart et al, 2006) and hippocampus (Bookheimer et al, 2000).

This finding of functional changes in young healthy individuals who may have a slightly increased risk of developing AD would conform to neuropathological models where cellular changes of AD can precede the clinical phenotype by several decades (Donev et al, 2009). It is of note that the hyperactive areas included some of those implicated relatively early in the cascade of AD pathology such as HF and PC (Braak and Braak, 1998). This hyperactivation conforms to the pathophysiological models of AD vulnerability which posit an initial left-shift of brain activation in response to cognitive demand, resulting in higher activation during early stages of AD pathology and for difficult tasks, followed by hypoactivation once compensation mechanisms have collapsed and the disease manifests itself clinically (Prvulovic et al, 2005).

In keeping with this model, the hyperactivation of risk carriers in DLPFC and cingulate was more marked at memory loads 3 and 4 than 1 and 2. These loads were supra-capacity because the limit for face WM is commonly thought to be at two faces (Jackson et al., 2009).

What then are the mechanisms through which the risk genotype may lead to compensatory hyper-activation? It could be argued that as CLU belongs to the same protein family as APOE that it may have similar pathophysiological effects, which may explain the similar presentation of compensatory neuronal resources. CLU encodes an extracellular multifunctional glycoprotein that may interact with itself, amyloid proteins and lipids, as well as assisting in synapse turnover (Bertram and Tanzi, 2009) in a similar manner to APOE. It has a potential role in the pathogenesis of AD including the hallmark features of Aβ deposition, aggregation and fibrillogenesis (Bertram and Tanzi, 2009). The cellular mechanisms of neural hyperactivation in carriers of AD risk genes are unknown. The exaggerated calcium

Figure 1  A, B and C: Coronal slices from whole brain analysis determining the impact of genotype on the emotional working memory paradigm. Post-hoc analysis (a, b and c) demonstrating mean beta values across WM loads (1–4). Risk genotypes represent the ‘CC’ genotype (n=13), the ‘Non-Risk’ genotype represents the CT and TT genotypes pooled together (n=30).

Figure 2  Mean neural activity during the whole task. Significant clusters and their respective cortical areas separated according to genotype (rs11136000: CC (n=13), CT (n=24), and TT (n=6)).
signalling observed in association with several AD risk genes and implicated in AD neuropathology (Cowburn et al., 2007; Cheung et al., 2008; Small et al., 2000) may be a factor, but further work on the cellular biology of the CLU risk variant is needed to pursue such a hypothesis. Another possibility is that AD risk is associated with dysregulation of neurovascular coupling, as has been suggested for clinical AD (Girouard and Iadecola, 1985).

The genetic mechanism underlying the association between the specific variant (rs11136000) and AD also remains unknown. It is possible that rs11136000 directly influences gene expression or splicing, that it is in linkage disequilibrium with another variant that does, or that risk is conferred by some other mechanism. However it seems clear from our data that whatever mechanism is involved, impacts on brain function occur many years before the onset of dementia and can be detected by subtle effects on activation in fMRI experiments.

A limitation of the present study was the sample size, although it was within the standard range for current genetic imaging studies (Rasch et al., 2010). Furthermore, although effects were significant at a cluster-level thresholded level of p < .05, they did not withstand more rigorous corrections for multiple comparisons, which calls for replication in larger samples.

The current genetic imaging approach can guide further invasive work into the specific pathological mechanisms underlying the effects of risk alleles and serve as additional vulnerability marker. The development of such vulnerability markers of AD-related pathology is important for the early intervention and prevention of dementia.

Role of the funding source

This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC) grant BB/G021538 and the Wales Institute of Cognitive Neuroscience (WICN). The authors are grateful to Professor Michael Owen for comments on a previous version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.euroneuro.2011.02.001.

References


Table 2  Significant main effects of genotype in all 3 regions with additive, dose-dependent effects of genotype in rDLPFC and rHF. Pair-wise comparisons suggest significant difference in neural activity increases in a dose-dependent manner (*p < .05, **p < .01).

<table>
<thead>
<tr>
<th>Region</th>
<th>Talairach coordinates</th>
<th>Rs11136000 F(2,40)</th>
<th>Load × rs11136000 F(3,123)</th>
<th>Pairwise comparisons (LSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Dorsolateral Prefrontal cortex (BA9)</td>
<td>R</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>B</td>
<td>Hippocampal formation (BA35)</td>
<td>R</td>
<td>−30</td>
<td>−29</td>
</tr>
<tr>
<td>C</td>
<td>Dorsal posterior cingulate (BA31)</td>
<td>–</td>
<td>3</td>
<td>−26</td>
</tr>
</tbody>
</table>


