The impact of brain-derived neurotrophic factor Val66Met polymorphism on cognition and functional brain networks in patients with intractable partial epilepsy

Running title: BDNF Val66Met and cognitive impairment in mTLE

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SUMMARY

INTRODUCTION

Medial temporal lobe epilepsy (mTLE) is the most common refractory focal epilepsy in adults. Around 30-40% of patients have prominent memory impairment and experience significant post-operative memory and language decline after surgical treatment. BDNF Val66Met polymorphism has also been associated with cognition and variability in structural and functional hippocampal indices in healthy controls and some patient groups.

AIMS

We examined whether BDNF Val66Met variation was associated with cognitive impairment in mTLE.

METHODS

In the present study, we investigated the association of Val66Met polymorphism with cognitive performance (n = 276), post-operative cognitive change (n = 126) and fMRI activation patterns during memory-encoding and language paradigms in two groups of patients with mTLE (n = 37 and 34).

RESULTS

mTLE patients carrying the Met allele performed more poorly on memory tasks and showed reduced medial temporal lobe activation and reduced task-related deactivations within the default mode networks in both the fMRI memory and language tasks than Val/Val patients.

CONCLUSIONS

Although cognitive impairment in epilepsy is the result of a complex interaction of factors, our results suggest a role of genetic factors on cognitive impairment in mTLE.

Keywords: temporal lobe epilepsy**,** cognitive decline, BDNF, fMRI, biomarker

1-INTRODUCTION

Medial temporal lobe epilepsy (mTLE) is the most common medically intractable focal epilepsy. Around 40% of patients show memory impairment and 30-60% of those undergoing left anterior temporal lobe resection (ATLR) to control their seizures experience a substantial post-operative memory decline [1,2].

Wide variability is observed in pre- and post-operative memory function in individuals with mTLE dependent on several factors including type of pathology, hippocampal volume, age at onset of epilepsy, epilepsy duration and seizure frequency [1,3-5]. Both genetic and environmental factors are known to also influence cognitive functions in healthy individuals. Twin studies have estimated that genetic factors account for approximately 50% of the variability in human memory capacity [6]. Disruption of cognition in epilepsy is probably the consequence of interacting genetic, epigenetic, developmental and environmental factors in addition to factors related to the epilepsy syndrome.

The brain-derived neurotrophic factor (BDNF) gene has a well-documented role in brain development, synaptic plasticity, learning and memory [7,8]. In view of its role in modulating excitatory and inhibitory synaptic transmission, BDNF has been investigated as a gene candidate for mechanisms of epileptogenesis [9]. Its most commonly investigated genetic variant Val66Met, and in particular the presence of the Met allele, has been associated with structural abnormalities in the hippocampus, poorer cognitive performance, and decreased activation in medial temporal structures during memory tasks in neurologically healthy controls and in various nervous system disorders [10-13]. To date, cognitive function relative to BDNF Val66Met allele has not been examined in individuals with mTLE having surgical resections.

In the present study, we explored the impact of Val66Met polymorphisms on cognition, and functional brain networks in mTLE. Given that some studies support the role for BDNF in recovery from brain injury [14] [15] [16] we also investigated the predictive value of Val66Met polymorphism for post-operative memory outcome.

Efforts to identify early risk factors for poor cognitive function and recovery, especially for these disorders that already affect cognition, are fundamental as this

would improve prognosis and facilitate other type of interventions or therapies specifically tailored to prevent or reduce dysfunction.

2 MATERIALS AND METHODS

2.1 Subjects

All individuals were selected from the epilepsy surgery database of the National Hospital for Neurology and Neurosurgery (NHNN), London, UK. These patients were referred to NHNN for the pre-surgical evaluation of medically intractable epilepsy between 1988 and 2011. Inclusion criteria were medically refractory mTLE, English as a first language and/or known European ancestry, neuropsychological assessment and/or language or memory fMRI data, in addition to available genome-wide genotyping data [17].

A total of 276 patients with medically refractory mTLE (194 Val/Val, 70 Val\Met, 12 Met\Met) including 126 who had post-operative data collected a year after anterior temporal lobe resection, had genotyping of BDNF Val66Met (rs6265) and neuropsychological data. Table 1 includes details on patient demographics, clinical diagnosis and medication.

The genotype frequencies of the polymorphism in each studied group were in Hardy–Weinberg equilibrium ($P > 0.05$). Given that the Met/Met genotype is rare in the general population (comprising only 4% of people in Caucasian populations) (Shimizu *et al.*, 2004) Met/Met homozygous and Val/Met heterozygous individuals were grouped together as Met carriers.

In each of the data sets, genotype groups did not differ by age, age onset, epilepsy duration, aetiology, antiepileptic drugs (AED) taken, gender, verbal or performance IQ or laterality ($P > 0.01$) (Table 1). Age, gender and IQ were variables that significantly affected memory performance and were therefore included in the regression model.

Functional MRI was investigated in two independent groups of patients. There was no significant difference in age at onset of epilepsy, epilepsy duration, right and left hippocampal volumes and cognitive performance between Val/Val and Val/Met patients in either of the two fMRI patient groups studied ($p > 0.01$) (Table 2).

This study was approved by the National Hospital for Neurology and Neurosurgery and the UCL Institute of Neurology Joint Research Ethics Committee. Written informed consent was obtained from all participants.

2.2 Genotyping

Most of the patients (91.6 %) included in this study had been previously genotyped. Details of sample collection and genotyping quality control steps have been previously described [17].

Patients with refractory mTLE who had previously participated in an fMRI study and have not been genotyped (n = 23) were recruited to the investigation via telephone and DNA was extracted from saliva after obtaining consent. Oragene DNA Self-Collection kit (DNAgenotek®) was used to collect and extract DNA. Samples were then genotyped for the polymorphism rs6265 using optimized TaqMan Gene Expression Assays (Applied Biosystems) on a 7900HT Fast Real-Time PCR System thermal cycler (Applied Biosystems). The details of BDNF rs6265 genotyping have been previously described [18]. Paired blood and saliva samples were compared from 15 study participants with 100% concordance. The feasibility of using DNA extracted from saliva for high throughput genotyping has been shown previously [19].

2.3 Neuropsychological testing

All patients underwent cognitive assessments as part of their surgical investigations. All had IQ measures derived from the Wechsler Intelligence Scales. Memory performance was measured using the Story Recall, List Learning and Design Learning subtests from the BIRT Memory and Information and Processing Battery (BMIPB) or its forerunner the Adult Memory and Information Processing Battery [20].

Raw scores from memory tests were converted into standardized (z) scores. The tests were subsequently grouped into four conceptual memory domains relating to the time of recall (immediate versus delayed) and modality (verbal versus visual). Each domain consisted of the summed average *z* scores. Immediate visual memory was based on the immediate retention of the figure and immediate verbal memory was based on the immediate recall of the story, the verbal learning trials and the recall of List B. Delayed visual memory was composed of figure retention at 30 minutes and the immediate/delayed recall % retained. Delayed verbal memory was

based on the delayed recall of the story, the immediate/delayed % retained and the delayed recall of the list (Trial 6). The sub-tests included are summarized in Table 3. Domain groupings are similar to those described in previous literature [21].

Post-operative cognitive data was available a year after ATLR. Post-operative memory decline was classified as significant if test scores fell at least one standard deviation from preoperative testing (derived from normative data set). The patients were then categorized as either having declined or not declined.

2.4 Functional imaging

Functional MRI was investigated in two independent groups of patients. The two patient groups were not combined due to differences in scanning parameters described below. Two fMRI tasks, a memory encoding and verbal fluency task were employed.

2.4.1 Magnetic Resonance data acquisition

Both groups were imaged using a 3T General Electric Excite HDx MRI scanner with a gradient strength of 40 m Tm^{-1} and slew rate 150Tm-1s-1.

2. 4.2 Memory encoding

Group 1

For the memory fMRI, gradient-echo echo planar images were acquired, providing blood oxygen level dependent (BOLD) contrast. Each volume comprised 36 contiguous oblique axial slices, slice thickness 2.5 mm (0.3 mm gap), field of view 24 cm, matrix 96 x 96 interpolated to 128 x 128 during image reconstruction, in-plane resolution 2.5, SENSE factor 2.5, Echo time (TE) 25 ms, TR 2.75 s. The field of view was positioned to cover the temporal and frontal lobes with the slices aligned with the long axis of the hippocampus on the sagittal view.

Group 2

Each memory fMRI volume comprised 44 contiguous 1.5 mm oblique axial slices through the temporal and frontal lobes, with a 24 cm field of view, 128 x 128 matrix and in-plane resolution of 1.88 x 1.88 mm; TE 30 ms and TR 4.5 s. The field of view was positioned to cover only the temporal lobe with the anterior–posterior axis

aligned with the long axis of the hippocampus on sagittal views, and with the body of the hippocampus in the centre.

Paradigm

Group 1

Two material types, visual stimuli (faces) and verbal stimuli (words) were visually presented to patients during a single scanning session.

Black and white photographs of non-famous faces unfamiliar to the subjects and single concrete nouns were presented on an MR compatible screen viewed via a mirror [22] . Each item was presented for 3 seconds in 60 seconds blocks. Each block consisted of 10 faces and 10 words followed by 15 seconds cross hair fixation. We presented a total of 10 blocks (100 faces and 100 words). Participants were explicitly instructed to memorise items for subsequent out of scanner recall. A deep encoding task [23] which involved a subjective decision on whether each stimulus was pleasant or unpleasant, using a joystick was performed.

Group 2

Group 2 performed a very similar task except an additional material type of pictures was presented [24].

2.4.3 Verbal Fluency

Each volume comprised 58 contiguous 2.5 mm oblique axial slices, through the temporal and frontal lobes with a 24 cm field of view, 96 x 96 matrix, reconstructed to 128 x 128 for an in-plane resolution of 1.88 x 1.88 mm. TE 30 ms and TR 4.5 s. The field of view was positioned to maximize coverage of the frontal and temporal lobes in both patient groups.

Paradigm

A blocked experimental design with 30 seconds activation blocks alternating with 30 seconds of cross-hair fixation over 5.5 minutes was employed in both patient groups. Subjects were instructed to covertly generate different words beginning with a visually presented letter (A, S, W, D, and E) during the activated phase contrasted by crosshair fixation as rest condition.

2.4 Statistics

2.4.1 Cognition

Analyses were carried out using SPSS v18.0 and R. Analysis of variance (ANOVA) was performed to investigate differences between the BDNF Val66Met genotype groups in quantitative demographics variables and cognitive scores (z-scores).

A linear regression was also used to test for association between z-scores and BDNF Val66Met genotypes. The effects of a number of covariates (gender, age, IQ, laterality) on z-scores were tested for significance and added to the regression model when significant. Chi-square and Fisher's exact test were performed to test for differences in frequency or proportion (e.g. gender, antiepileptic drug treatment, aetiology).

All P-values were corrected for multiple comparisons using the False Discovery Rate (P = 0.05).

2.4.2 Functional MRI

Memory encoding

Analysis was performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/). The imaging time series were realigned, normalized into standard anatomical space (using a scanner specific template created from 30 healthy controls, 15 patients with left hippocampal sclerosis (HS) and 15 patients with right HS using the high resolution whole brain EPI) and smoothed with a Gaussian kernel of 8mm full-width at half maximum.

The task positive network irrespective of material type was investigated by creating regressors of interest for faces and words in group 1 and faces, words and pictures in group 2 convolved with the canonical HRF. Movement parameters were included as confounds and parameter estimates for the regressors were calculated for each voxel. Task related deactivations irrespective of material type within the default mode network (DMN) were investigated across all material types in both groups by investigating the corresponding negative contrasts.

These task positive activations and task negative contrast images were used for the second-level analysis. A random effects analysis was performed at the second level. Subjects were divided into two groups: Val/Val and Val/Met groups. A one-sample t-test was performed to examine the group effect of each contrast. A two-sample t test was performed to quantitatively assess statistically different brain activations [22] between the Val/Val and Val/Met patients. This was done for both fMRI groups. Importantly differences between activation and deactivation between the groups were explicitly tested by masking the analysis with either group activation or deactivation masks respectively.

Verbal encoding

Similar to the encoding task, a blocked design was used to form a regressor of interest by creating a box-car function for the activation phase of the paradigm. A contrast was generated for both the task activations and task related deactivations. Both the activation and deactivation contrasts were entered into the second level analysis as detailed above.

All results for the main effects are shown corrected for multiple comparisons (family wise error (FWE)), $p< 0.05$ unless otherwise stated. Quantitative group differences are shown at $p<0.001$, uncorrected.

3 RESULTS

3.1 Cognitive Measures

There was not a significant difference between the performance of the Val/Val group (n = 194) and Met carriers (n = 82) for immediate memory (P = 0.04). When the immediate memory category was subdivided into visual and verbal recall, Met carriers showed poorer performance in immediate visual memory than the Val/Val group (P = 0.01) but no difference was found in the verbal modality (P = 0.41).

For delayed memory performance, subjects with Met allele had a significantly poorer performance (P = 0.002) than the Val/Val group (Table 2). Performance differences persisted when delayed memory was subdivided into visual and verbal modalities (P = 0.016 and P = 0.031 respectively).

In linear regression analyses, even after adjusting for age, gender and IQ, BDNF Met allele remained significantly associated with impaired delayed memory performance (P = 0.004).

There was no significant difference in phonemic verbal fluency performance between Met carriers (n = 74) and Val/Val homozygotes (n = 174) (P = 0.92).

The proportion of patients showing verbal and visual memory decline one year post-operatively, was not significantly different between the Val/Val group (n = 85) or Met carriers (n = 41) (P = 0.15 and P = 0.58 respectively).

3.2 Functional MRI

Significant differences observed between Val/Val and Met carriers (Val/Met) patients across both functional MRI tasks in both patient groups are detailed below.

3.2.1 Verbal Fluency fMRI

In group 1 there were 24 Val/Val patients (12 RTLE) and 13 Val/Met (4 RTLE) and in group 2 there were 21 Val/Val (14 RTLE) and 13 Val/Met (5 RTLE). When RTLE and LTLE patients were analysed separately, changes in both activations and task related deactivations were noted bilaterally. As laterality of pathology had no bearing on the side of changes seen, LTLE and RTLE patients were combined to explore the changes between Val/Val and Val/Met patients.

Activations

No differences within the verbal fluency activation maps were seen between Val/Val and Val/Met patients in both Groups 1 and 2.

Task related deactivations

Group1: Task related deactivation within the DMN in Val/Val and Val/Met patients are shown in Fig 1. Val/Met patients had less DMN deactivations within the right postcentral, angular, inferior temporal, medial orbitofrontal and left middle occipital gyrus compared to Val/Val patients. Group 2: Deactivation within the DMN in Val/Val and Val/Met patients are shown in Fig. 1. As in group 1, Val/Met patients had less

deactivations within the left post-central gyrus, medial orbitofrontal cortex, right pre-central gyrus and middle occipital gyrus (Fig. 1, Supplementary Table 1).

3.2.2 Memory fMRI

In group 1 there were 24 Val/Val patients (12 RTLE) and 13 Val/Met (4 RTLE) and in group 2 there were 15 Val/Val (11 RTLE) and 12 Val/Met (4 RTLE). Differences between Val/Val and Val/Met patients were analysed separately in both RTLE and LTLE patients in groups 1 and 2.

Group 1: In LTLE patients there was no significant difference between Val/Val and Val/Met patients whilst in RTLE patients greater activations in the left insula, superior temporal gyrus and anterior cingulum were seen in Val/Val patients compared to val/met patients during memory encoding.

Group 2: In LTLE patients Val/Val patients significantly activated the amygdala bilaterally compared to Val/Met patients whilst no significant difference was seen between Val/Val and Val/Met patients in the RTLE group.

As there was no consistent effect of the side of pathology on either the Val/Val or Val/Met patients, RTLE and LTLE patients were combined to explore the changes between Val/Val and Val/Met patients in both groups 1 and 2.

Activations

Group1: Val/Met patients showed significantly less activations within the right inferior frontal and superior temporal gyri. At a lower threshold, Val/Met patients also showed reduced bilateral amygdala activation.

Group 2: Val/Met patients showed significantly less memory encoding activations within the amygdala and hippocampi bilaterally (Fig. 2, Supplementary Table 2).

Task related deactivations

Group1: Val/Met patients had less deactivation within the DMN within the right lingual gyrus, posterior cingulum and left precuneus (Figure 3). Group 2: Val/Met patients had less deactivation within the DMN within the left angular, supramarginal, superior occipital gyrus and left precuneus (Figure 3, Supplementary Table 3).

4 DISCUSSION

This is the first study to assess the impact of the BDNF Val66Met polymorphism on cognition, and brain function in a large cohort of patients with refractory temporal lobe epilepsy.

Consistent with our hypothesis we found that in patients with mTLE, the Met allele was associated with poorer memory performance. No differences were evident for verbal fluency and post-operative memory decline.

Two separate groups of mTLE patients also showed concordant functional MRI findings. Patients with BDNF Met allele showed reduced medial temporal lobe activations during memory encoding and showed significantly reduced task-related deactivation during both memory encoding and verbal fluency tasks compared to Val/Val patients indicating functional disruption of cognitive networks.

Cognition and Val66Met polymorphism in refractory mTLE

Studies of the BDNF gene polymorphism have reported episodic memory deficits associated with the Met allele in healthy controls [10,12,25,26] and in a range of neurological and psychiatric disorders [10,25,27]. In particular, the presence of the Met allele has been associated with poorer performance on tests of episodic delayed memory [28,29], especially for measures of prose recall [10,25,27]. Consistent with these studies we found that in patients with mTLE, the Met allele was associated with poorer delayed recall.

The effect of the Met allele on immediate memory has not always been replicated [25,28,29]. We found a significant association between the BDNF Val66Met polymorphism and one visual immediate recall task. No association was found when the verbal modality was used. It is possible that the inconsistent effect of the Met allele in immediate memory recall and the stronger effect in delayed memory are related with the conversion of brief cellular changes into stable alterations in brain function, which constitute the cellular mechanisms of memory. Since Met carriers may have weak or unstable early cellular changes, this may further compromise the consolidation of the memory trace over time. BDNF not only acts by strengthening existing connections but also participates in the formation of new synaptic contacts,

therefore if the efficacy and number of synaptic connections are already fragile, the maintenance of this altered state will be more difficult.

We did not find an association between BDNF Val66Met genotype and phonemic fluency. The evidence for an effect of Met allele in prefrontal cortex function is less convincing. Few studies report association [26,30] and the largest studies failed to find an association [31].

Given the role of BDNF in the development, survival and functional maintenance of neurons, we expected that genetic variability in BDNF could account for some of the inter-subject variability in post-surgical functional decline. However we did not find an association between BDNF Val66Met genotype and post-surgical memory decline. BDNF has been reported to be neuroprotective in models of excitotoxicity, axotomy and cerebral ischemia [32]. Furthermore BDNF Met allele has been associated with poorer recovery and greater disability post-stroke as well as with worsening of physical disability and cognitive function [14]. ATLR differs from other brain injuries as it is employed to have a positive benefit, to control seizures and does so in a large proportion of patients. Cessation of seizures has been associated with better cognitive performance.

Some studies have suggested that genetic differences are not apparent at baseline but emerge as an interaction with experience and training. For example, following cervical spinal lesion, improvements of motor performance have been reported to be significant when BDNF treatment was combined with training, but not when two treatments were applied alone [33]. In a different but related study, a brief period of motor training enhanced corticospinal output and motor map reorganization in Val/Val but not Met carriers [34]. It would be interesting to determine if there are differences in mTLE in how BDNF genotypes interact with cognitive training pre and post-operatively. Future studies, with larger sample size, are needed to further explore this intriguing possibility that could have clinical implications such as guiding personalized treatment.

Functional activation, brain network and Val66Met polymorphism in mTLE

In both groups 1 and 2, Val/Met patients showed reduced medial temporal

activations compared to Val/Val patients. Our findings are in keeping with these studies showing differences in medial temporal lobe structure activation during encoding tasks [12,35]. Patients in group 1 additionally showed areas of reduced activation within the dorsolateral prefrontal cortex (inferior frontal gyrus) and superior temporal gyrus. In group 2, memory encoding was imaged within a smaller field of view that only incorporated the temporal lobes. Therefore, no further comment can be made about frontal activations in this group.

We additionally report novel findings of significant reduction in task related deactivations within the DMN in patients with mTLE and Met allele across two separate cognitive tasks in two separate groups of mTLE patients. Concurrent suppression of activity in a distinct set of brain areas, the DMN, together with distributed patterns of cerebral activation is believed to be functionally relevant for cognition. Supporting this suggestion, deactivation of regions considered to be part of the DMN during encoding of novel information have been found to be associated with successful retrieval of the learned information while a reduction or failure to deactivate DMN regions has been linked with worsened memory performance [36]. Failure to deactivate DMN regions has also been reported in several neurological disorders that impact cognitive function such as AD [37,38] and depression [39,40]. Our findings reinforce the view that there are differences between BDNF Val66Met genetic groups within cognitive networks.

All patients with mTLE assessed were taking antiepileptic drugs, which may have cognitive side-effects [41]. In this study however, the type and number of AEDs were not different between genotype groups. Some structural MRI studies have also reported reduced hippocampal and cerebral neocortex volume in subjects with the Met allele. We report no differences in hippocampal volume in either of the cohorts studied here.

We conclude that genetic factors may contribute to cognitive performance in those with mTLE, and this knowledge may help treatment stratification. Early identification of risk factors for poor cognitive function is essential; as such knowledge would facilitate appropriate educational and psychological intervention especially for those with disorders that already affect cognition.

Acknowledgements

We thank all patients, and their families for participating in this study. We would like to thank AROS Applied Biotechnology AS company laboratories and Dr. Lee Stanyer for their valuable input. We also thank Prof Sanjay Sisodiya for providing us with some of the *BDNF* genotypes.

This work was supported by The Wellcome Trust (programme grant no 083148 and 084730), the Marie Curie International Reintegration Grant (FP7-PEOPLE-2009-RG grant No 256545), and Epilepsy Research UK (F1206), The Epilepsy Society MRI scanner was supported by the Wolfson Trust and Epilepsy Society. This work was undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme. The Epilepsy Society and the Wolfson Trust support MRI scanner.

Disclosure:

None of the authors has any conflict of interest to disclosure. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figure 1: Real time whole brain fMRI processing images. Verbal fluency task related deactivations within the default mode network in Val/Val (VV) and Val/Met (VM) patients in groups 1 (upper panel) and 2 (lower panel) at $p<0.001$. Qualitatively VM patients have reduced deactivations within the default mode. Quantitative activation differences are shown (VM<VV: Less deactivation within the default mode in Val/Met compared to Val/Val patients) in both patient groups. Maps are shown at $p<0.001$.

Figure 2: Memory encoding activation differences were observed in Val/Val (VV) and Val/Met (VM) patients in groups 1 (upper panel) and 2 (lower panel). Quantitatively VM patients have reduced activations within the medial temporal lobes in both patient groups and additional reduced activations within the prefrontal cortex and superior temporal gyrus in group1. Maps are shown at $p<0.01$.

Figure 3: Memory encoding task related deactivations within the default mode network in Val/Val (VV) and Val/Met (VM) patients in groups 1 (upper panel) and 2 (lower panel). Qualitatively VM patients have reduced deactivations within the default mode. Quantitative activation differences are shown (VM<VV: Less deactivation within the default mode in Val/Met compared to Val/Val patients) in both patient groups. Maps are shown at $p<0.001$.