

Effect of Variation in Diacylglycerol Kinase Eta (*DGKH*) Gene on Brain Function in a Cohort at Familial Risk of Bipolar Disorder

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Several lines of evidence indicate that the diacylglycerol kinase eta (*DGKH*) gene is implicated in the etiology of bipolar disorder (BD). However, the functional neural mechanisms of *DGKH*'s risk association remain unknown. Therefore, we examined the effects of three haplotype-tagging risk variants in *DGKH* (single nucleotide polymorphisms rs9315885, rs1012053, and rs1170191) on brain activation using a verbal fluency functional magnetic resonance imaging task. The subject groups consisted of young individuals at high familial risk of BD ($n=81$) and a comparison group of healthy controls ($n=75$). Individuals were grouped based on risk haplotypes described in previous studies. There was a significant risk haplotype*group interaction in the left medial frontal gyrus (BA10, involving anterior cingulate BA32), left precuneus, and right parahippocampal gyrus. All regions demonstrated greater activation during the baseline condition than sentence completion. Individuals at high familial risk for BD homozygous for the *DGKH* risk haplotype demonstrated relatively greater activation (poor suppression) of these regions during the task vs the low-risk haplotype subjects. The reverse pattern was seen for the control subjects. These findings suggest that there are differential effects of the *DGKH* gene in healthy controls vs the bipolar high-risk group, which manifests as a failure to disengage default-mode regions in those at familial risk carrying the risk haplotype. *Neuropsychopharmacology* (2012) **37**, 919–928; doi:10.1038/npp.2011.272; published online 2 November 2011

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INTRODUCTION

Bipolar disorder (BD) is a highly debilitating condition marked by recurrent episodes of depression and mania. Epidemiological research has shown that BD has a strong genetic component, with heritability estimates ranging from 59 to 93% (Bertelsen *et al*, 1977; McGuffin *et al*, 2003). Genetic linkage and candidate gene association studies indicate that BD is likely to be a complex polygenic disorder with individual genes conferring small influences to overall risk of disorder (Barnett and Smoller, 2009).

One of the first susceptibility genes for BD to be identified by the genome-wide association study (GWAS) approach in two independent samples of European origin was diacylglycerol kinase eta (*DGKH*) (Baum *et al*, 2008). Association was found for three single nucleotide polymorphisms (SNPs) located in the first intron of the *DGKH* gene (rs9315885, rs1012053, and rs1170191). Although some studies have not confirmed this association (Sklar *et al*, 2008; Tesli *et al*, 2009; Yosifova *et al*, 2009), numerous lines

of evidence indicate that *DGKH* might be involved in the etiology of the disorder. First of all, *DGKH* is located in the region 13q14 that has been linked to BD in previous studies (Badner and Gershon, 2002; Detera-Wadleigh and McMahon, 2006). Further, an association of *DGKH* with BD has been replicated in a Sardinian as well as a Chinese sample at the haplotype level (Zeng *et al*, 2011; Squassina *et al*, 2009). A recent study also reported an association of *DGKH* with BD, as well as unipolar depression and adult attention deficits/hyperactivity disorder (Weber *et al*, 2011). In addition, SNP rs9315885 has been associated with BD in a Finnish family cohort (Ollila *et al*, 2009), and the GWAS carried out by the Wellcome Trust Case Control Consortium (The Wellcome Trust Case Control Consortium, 2007) indicated association for several SNPs near and within *DGKH* (Manchia *et al*, 2009; Robbins and Arnsten, 2009). Finally, gene expression level of *DGKH* in the prefrontal cortex has been reported to be significantly increased in BD patients (Moya *et al*, 2010). The *DGKH* gene is also of particular interest as it encodes the η isoform of diacylglycerol kinase that is involved in the phosphoinositol pathway through which lithium, one of the most effective pharmacological treatments for BD, is thought to exert its therapeutic effects (Manji and Lenox, 1999).

Together, these findings suggest the involvement of the *DGKH* gene in the pathophysiology of BD. To the best of our knowledge, however, no study has examined association

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between genetic variation in the *DGKH* gene on brain activation. Therefore, we investigated the effects of three SNPs of the *DGKH* gene that have been associated with BD (Baum *et al*, 2008) on brain activation during a functional magnetic resonance imaging (fMRI) paradigm in healthy controls and a group of individuals at high familial risk of the disorder. The advantage of studying brain activation patterns in individuals who are unaffected by BD themselves but have a close relative suffering from the disorder is that findings are likely to reflect the influence of shared risk genes for the disorder, excluding confounding effects of chronic illness or medication.

We examined the effects of the *DGKH* haplotype in those at familial risk for the disorder in relation to healthy controls. We hypothesized that as *DGKH* has been associated with risk for BD, the effects of the low-risk *vs* high-risk haplotype may differ in those who have an already present genetic loading for the disorder *vs* those without. As there is limited literature regarding this particular susceptibility gene in terms of regional brain activation, we hypothesized that these differences would occur in regions previously implicated in the disorder, specifically the prefrontal cortex. We employed the verbal initiation section of the Hayling Sentence Completion Test (Burgess and Shallice, 1996) involving verbal fluency and word production to test this hypothesis. The task was chosen as it has been shown to differentiate BD patients and those at increased familial risk for the disorder from healthy controls with respect to differing brain activation patterns (McIntosh *et al*, 2008b; Whalley *et al*, 2011). It has also been found to activate left superior and middle prefrontal regions (Allen *et al*, 2010), anterior cingulate areas (Nathaniel-James *et al*, 1997), as well as the striatum (McIntosh *et al*, 2008b), which are considered to be involved in the pathophysiology of BD (Strakowski *et al*, 2005; Cerullo *et al*, 2009). Moreover, it probes neuropsychological deficits in verbal initiation and verbal fluency commonly observed in BD (Arts *et al*, 2008).

MATERIALS AND METHODS

Subjects

Individuals at high genetic risk of BD I (BDI) because of a close family history of the disorder and control subjects were recruited as part of the Scottish Bipolar Family Study that has been described in detail elsewhere (Sprooten *et al*, 2011; Whalley *et al*, 2011). To identify high-risk participants, caseloads of psychiatrists across Scotland were searched for individuals diagnosed with BDI. Diagnoses were confirmed with the Structural Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I) (First *et al*, 2002) or the symptom checklist of the Operational Criteria (McGuffin *et al*, 1991). Subsequently, subjects affected by BDI were asked to identify a first- or second-degree relative aged 16–25 years not suffering from the disorder. These unaffected individuals were invited to participate in this study providing that they had at least one first-degree, or two second-degree relatives suffering from BDI. It should be noted, owing to the age of the subjects, the high-risk cohort will still be within the risk period for development of the disorder, hence this group will likely contain those at high

risk who will remain well and those who will subsequently become unwell. The results should be considered in this light. Control subjects with no personal history of BD or family history of a mood disorder in first-degree relatives were identified from the social networks of the bipolar high-risk subjects. Only unrelated individuals were included in the current analysis. Exclusion criteria for all groups included a personal history of major depression, mania or hypomania, psychosis, or any major neurological or psychiatric disorder, a history of substance dependence, a history of learning disability or any history of head injury that included loss of consciousness, and any contraindications to fMRI. A total of 81 bipolar high-risk subjects and 75 controls provided suitable fMRI data along with genetic information. All participants provided written informed consent, and the study was approved by the multicentre research ethics committee for Scotland.

Genotyping

Genomic DNA was extracted from venous blood samples. The genotyping was conducted by the Wellcome Trust Clinical Research Facility, Edinburgh, UK (www.wtcrf.ed.ac.uk), and used standard TaqMan assays, by the TaqMan PCR-based method (TaqMan, AssayByDesign, Applied Biosystems, Foster City, California). Subjects were typed at SNPs rs9315885, rs1012053, and rs1170191 that have been previously shown to be associated with BDI (Baum *et al*, 2008). As there was evidence of linkage disequilibrium (LD) between the SNPs (rs9315885 and rs1012053 $D' = 0.999$, $p < 0.001$; rs9315885 and rs1170191 $D' = 0.642$, $p < 0.001$), we studied the effects of genetic variation of the SNPs combined as a three-marker haplotype in line with a previous study (Squassina *et al*, 2009). In this previous study, the most common haplotype was T-A-C (for rs9315885, rs1012053, and rs1170191, respectively). This haplotype was the only one that showed significant association with BD, remaining significant after permutation testing (Squassina *et al*, 2009). Subjects in the current study were therefore grouped according to the presence of this presumed risk haplotype (T-A-C). If they were homozygous for this risk haplotype they are referred to as 'RISK+', if they were not carriers of the risk haplotype or heterozygous they are referred to as 'RISK-'. Differences from the Hardy-Weinberg equilibrium (HWE) and assessments of linkage disequilibrium were performed using the genetics package in 'R' (version 2.12.1, the R Foundation for Statistical Computing).

Clinical Assessments

All participants were interviewed by one of the two experienced psychiatrists (AMM and JES) using the SCID (First *et al*, 2002) to confirm the absence of any lifetime axis I disorders. Current manic and depressive symptoms were rated using the Young Mania Rating Scale (Young *et al*, 1978) and Hamilton Rating Scale for Depression (HAM-D) (Hamilton 1960). Estimates of temperamental variations in minor affective symptoms were assessed using the Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire (TEMPS-A) (Akiskal *et al*, 2005), a validated self-rated questionnaire that provides measures

of cyclothymic, depressive, hyperthymic, irritable, and anxious temperament. Statistical analysis of demographic characteristics was conducted using independent *t*-tests or χ^2 tests. For the clinical assessments and measures of temperament, comparison of groups was conducted using Mann–Whitney *U*-tests.

Experimental Paradigm

Subjects performed the verbal initiation section of the Hayling Sentence Completion Test (Burgess and Shallice, 1997) in the scanner (Whalley *et al*, 2004). This task is considered an extension of the verbal fluency task, where constraint is based on sentence context rather than letter or semantic category. Briefly, subjects were shown sentences with the last word missing and asked to silently think of an appropriate word to complete the sentence and press a button when they had done so. The task has four levels of difficulty, according to the range of suitable completion words suggested by the sentence context. This design allows both a standard subtraction analysis (sentence completion *vs* baseline) and the more constrained parametric analysis (examining areas of increasing activation with increasing task difficulty). Sentences were presented in blocks of fixed difficulty. Each block lasted 40 s and included eight sentences. Sentences were presented for a period of 3 s followed by a fixation cross for 2 s. The baseline condition consisted of viewing a screen of white circles on a black background for 40 s. The order of the blocks was pseudo-random, and each block was repeated four times using different sentences. Standardized verbal instructions were given before scanning.

Immediately after scanning, subjects were given the same sequence of sentences on paper and requested to complete each sentence with the word they first thought of in the scanner. ‘Word appropriateness’ scores were determined from the word frequency list of sentence completion norms (Bloom and Fischler, 1980). A score of one was given to the most frequently produced word in the word frequency list, a score of two for the next most frequently produced word, etc.

Scanning Procedure

Imaging was carried out at the Brain Imaging Research Centre (BIRC) for Scotland on a GE 1.5 T Signa scanner (GE Medical, Milwaukee, WI, USA). The functional imaging protocol consisted of axial gradient-echo planar images (EPI) (TR/TE = 2000/40 ms; matrix = 64 × 64; field of view (fov) = 24 cm) acquired continually during the experimental paradigm. Twenty-seven contiguous 5 mm slices were acquired within each TR. Each EPI acquisition was run for 404 volumes, the first four of which were discarded. The T1 sequence yielded 180 contiguous 1.2 mm coronal slices (matrix = 192 × 192; fov = 24 cm; flip angle = 8°). Visual stimuli were presented using a screen (IFIS, MRI Devices, Waukesha, WI, USA) placed in the bore of the magnet.

Image Processing and Analysis

The EPI and T1 images were reconstructed into nifti format (Mayo Foundation, Rochester, MN, USA) using DICOM

convert functions available in SPM5 (Statistical Parametric Mapping: The Wellcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London) running in Matlab (The MathWorks, Natick, MA, USA). Images were preprocessed using standard protocols available in SPM5. All EPI images were realigned to the mean volume in the series. The functional images were then normalized according to the standard co-registration procedures using the individual’s structural scan. Finally, all realigned and normalized images were smoothed with an 8 × 8 × 8 mm full-width half-maximum Gaussian filter.

First-level statistical analysis was performed using the general linear model approach. At the individual subject level, the data were modeled with four conditions corresponding to the four difficulty levels each modeled by a boxcar convolved with a synthetic hemodynamic response function. Estimates of the subject’s movement during the scan were entered as ‘covariates of no interest’. Before fitting the model, the participants data were filtered in the time domain using high pass filter (128 s cutoff), and serial correlations were accounted for by using the autoregressive (AR(1)) model. Contrasts were constructed to examine all four sentence completion conditions *vs* baseline, and areas of increasing activation with increasing task difficulty (the parametric contrast).

Second-Level Analysis

All second-level statistical analyses were conducted in SPM5. In order to directly test our hypothesis of differential effects of the risk haplotype between the controls and bipolar high-risk groups, our approach was to examine regions where there was a significant genotype × group interaction. This was followed by analyzing pair-wise comparisons of the genotype groups within the controls and high risk separately to explore the origin of these interaction effects. For each contrast of interest (sentence completion *vs* baseline and parametric effects), one contrast image per subject was entered into a second-level random effects analysis. Haplotype × group interactions were examined using a full factorial ANOVA model comparing the bipolar high-risk group against the healthy controls. Haplotype and group were entered as two factors in the design matrix with two levels of genotype (risk haplotype homozygotes *vs* the remainder) and two diagnostic groups (healthy controls *vs* individuals at high risk of BD). Where significant interactions were found, pair-wise group comparisons were explored and the effect of haplotype within each diagnostic group was examined.

Statistical maps were thresholded at a level of $p < 0.005$ (uncorrected), and regions were considered significant at a cluster level of $p < 0.05$, corrected for multiple comparisons. All coordinates are quoted in Montreal Neurological Institute (MNI) convention (<http://www.mni.mcgill.ca>), and images are overlaid onto standard brain in MNI space using Mango software package (<http://ric.uthscsa.edu/mango>). Based on our previous hypothesis, small volume corrections were applied for the prefrontal cortex, created using the WFU PickAtlas (Tzourio-Mazoyer *et al*, 2002; Maldjian *et al*, 2003).

RESULTS

Demographic, Clinical, and Behavioral Measures

Controls. Thirty control subjects were homozygous for the risk haplotype (T-A-C), referred to as RISK+. Of the remainder ($n=45$), referred to as RISK-, 38 individuals carried one copy of the (T-A-C) haplotype.

Bipolar high risk. For the bipolar high-risk group, 35 subjects were homozygous for the risk haplotype. Of the remainder ($n=46$), 39 individuals carried one copy of the (T-A-C) haplotype.

For each of the SNPs individually, none of the allele frequencies differed from HWE, and the haplotype frequencies did not differ between the groups ($\chi^2=0.16$, $p=0.69$). Measures of linkage disequilibrium are provided in the Materials and Methods. Further details describing the individual haplotype frequencies are presented in Supplementary Table. These frequencies were consistent with those reported previously (Squassina *et al*, 2009).

Demographic details are presented in Table 1. There were no significant differences between the haplotype groups or between high-risk subjects and controls in terms of age, gender, handedness, or verbal intelligence as estimated with the National Adult Reading Test (Nelson 1982).

For the clinical measures, there were significant differences between controls and high-risk subjects on measures of depression and cyclothymia as measured with the HAM-D (Hamilton 1960) and a short version of the TEMPS-A (Akiskal *et al*, 2005), respectively with the high-risk subjects having higher scores on both measures. These findings have been described previously in this sample (Sprooten *et al*, 2011; Whalley *et al*, 2011). There were, however, no significant differences within the groups based on haplotype.

Statistical analyses revealed no significant differences in Hayling Sentence Completion Test reaction time or word appropriateness scores between the two haplotype groups or between the controls and high-risk subjects.

Table 1 Demographic, Clinical, and Behavioral Characteristics

	Controls ($n=75$)				Bipolar high-risk subjects ($n=81$)				Between-group comparison	
	RISK- ($n=45$)	RISK+ ($n=30$)	Within-group comparison		RISK- ($n=46$)	RISK+ ($n=35$)	Within-group comparison			
<i>Demographics</i>										
	Mean/n (SD)	Mean/n (SD)	T/χ^2	p	Mean/n (SD)	Mean/n (SD)	T/χ^2	p	T/χ^2	p
Age (years)	20.99 (2.35)	20.53 (2.50)	0.81	0.42	21.00 (2.52)	21.75 (3.08)	1.21	0.23	1.23	0.22
Gender (male:female)	20:25	13:17	0.01	0.92	21:25	19:16	0.59	0.44	0.45	0.50
Handedness (right:other)	41:1	27:3	2.16	0.14	42:4	31:4	1.33	0.51	1.62	0.45
Verbal IQ (NART)	110.49 (5.91)	109.67 (9.41)	0.46	0.64	109.24 (7.25)	111.60 (6.17)	1.55	0.13	0.09	0.93
<i>Clinical characteristics</i>										
	Median (IQR)	Median (IQR)	U	p	Median (IQR)	Median (IQR)	U	p	U	P
YMRS sum score	0 (0)	0 (0)	0.12	0.90	0 (0)	0 (0)	0.19	0.19	1.67	0.10
HAM-D sum score	0 (1)	0 (1)	1.33	0.18	0 (2)	1 (2)	1.54	0.12	3.72	<0.01
<i>Temperament characteristics (TEMPS-A)</i>										
	Median (IQR)	Median (IQR)	U	p	Median (IQR)	Median (IQR)	U	p	U	p
Cyclothymia	1.00 (2.75)	1.00 (2.75)	0.02	0.99	2.00 (4.00)	2.00 (3.00)	0.01	0.99	2.91	<0.01
Depressive	0.00 (2.00)	0.00 (1.00)	0.16	0.87	0.00 (2.00)	0.00 (1.00)	0.20	0.85	0.95	0.34
Imitability	1.00(2.00)	1.00 (2.00)	0.39	0.69	1.00 (1.00)	1.00 (2.00)	1.54	0.12	1.70	0.09
Hyperthymia	2.00 (2.00)	1.50 (4.00)	0.48	0.64	2.00 (2.00)	1.00 (3.50)	0.72	0.47	0.55	0.58
Anxious	0.50 (1.00)	0.50 (2.00)	0.09	0.93	0.00 (1.00)	1.00 (2.00)	0.99	0.32	0.17	0.86
<i>Behavioral characteristics (Hayling sentence completion test)</i>										
	Mean (SD)	Mean (SD)	T	p	Mean (SD)	Mean (SD)	T	p	T	p
Reaction time (s)	2466 (584)	2440 (543)	0.71	0.81	2505 (679)	2471 (543)	0.25	0.81	0.33	0.74
Word Appropriateness	3.01 (0.51)	3.02 (0.64)	0.17	0.96	2.86 (0.561)	2.91 (0.56)	0.40	0.69	1.25	0.22

Abbreviations: HAM-D, Hamilton Rating Scale for Depression; IQR, interquartile range; NART, National Adult Reading Test; RISK-, non-risk haplotype carriers; RISK+, risk haplotype homozygotes; TEMPS-A, Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire; YMRS, Young Mania Rating Scale.

Task-Related Brain Activation Patterns

All subjects demonstrated the expected patterns of brain activation and behavioral responses indicating subjects were performing the tasks appropriately in the scanner (see Supplementary Figure) (Whalley *et al*, 2004; McIntosh *et al*, 2008a; Whalley *et al*, 2011). Regions activated across the groups for the sentence completion *vs* baseline contrast included the left medial and lateral prefrontal regions, left lateral temporal cortex, subcortical structures, left lateral parietal cortex, occipital lobes bilaterally, and right cerebellum. For the parametric contrast, all groups demonstrated activation in similar areas including left lateral and medial prefrontal cortex, left lateral temporal cortex, and right cerebellum. Regions of reduced activation during the task (greater activation during baseline) involved anterior medial prefrontal regions, precuneus, posterior cingulate cortex, and parahippocampal regions.

Haplotype-Dependent Differences in Brain Activation

Sentence completion *vs* baseline. For sentence completion *vs* baseline, there were significant haplotype*group interactions in several clusters in the left medial frontal gyrus extending to the anterior cingulate ($p=0.04$, $K_E=470$, $Z=3.76$, $x=-10$, $y=54$, and $z=4$, with a small volume correction for the prefrontal cortex), in the left precuneus extending to posterior cingulate ($p<0.01$, $K_E=1149$, $Z=3.80$, $x=-6$, $y=-32$, and $z=50$, at the whole brain level), and in the right parahippocampal region ($p=0.04$, $K_E=707$, $Z=3.86$, $x=16$, $y=-42$, and $z=2$, at the whole brain level), see Figure 1, Table 2. For all regions, there was relatively greater activation during task conditions in the high-risk RISK+ group *vs* the high-risk RISK- group, with the reverse seen in the control groups, see Figure 2.

Post-hoc pair-wise significance tests between RISK- and RISK+ within the control and high-risk groups separately indicated these clusters were also significantly different between the haplotype groups (controls RISK- *vs* RISK+: $p<0.05$, $p<0.01$, $p<0.01$; high-risk RISK- group *vs* RISK+ group: $p<0.01$, $p<0.05$, $p<0.05$ for prefrontal, precuneus, and parahippocampal clusters, respectively).

Parametric contrast. For the parametric contrast, there was a significant haplotype*group interaction in the posterior cingulate cortex ($p<0.01$, $K_E=1317$, $Z=4.07$, $x=4$, $y=-66$, and $z=20$, at the whole brain level, see Figure 3, Table 2). This interaction was in the direction of a decreased gradient of activation with increasing sentence difficulty in RISK+ *vs* RISK- high-risk subjects. The reverse pattern was seen in the control group.

Post-hoc pair-wise significance tests between RISK- and RISK+ within the control and high-risk groups separately indicated this cluster was also significantly different between haplotype groups (controls RISK- *vs* RISK+: $p<0.01$; high-risk RISK- *vs* RISK+ subjects: $p<0.05$, for posterior cingulate cluster).

Correlation Analyses

We examined correlations across all subjects between data extracted from the clusters of difference reported above

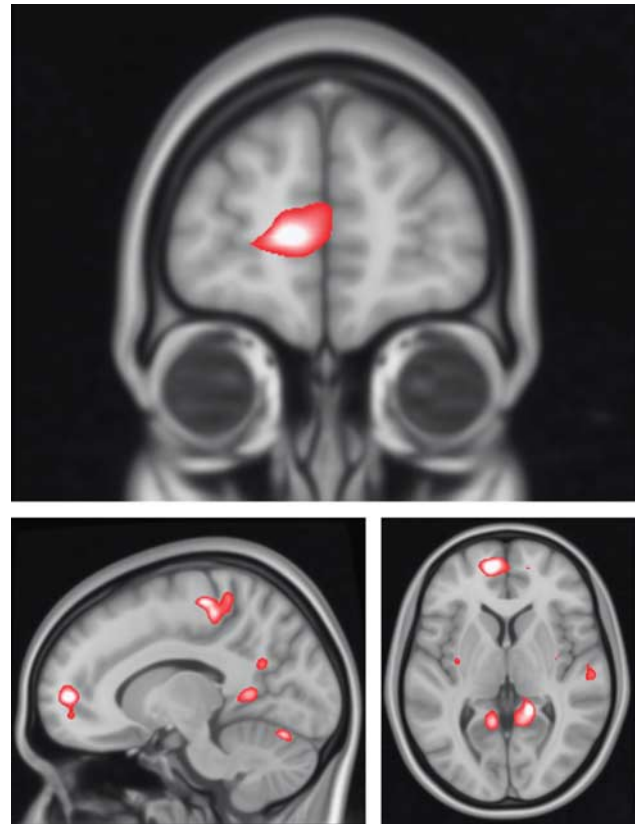


Figure 1 Haplotype*group interactions for *DGKH* for sentence completion *vs* baseline. Images are overlaid onto standard brain in MNI space using Mango software package (<http://ric.uthscsa.edu/mango>). Map represents F-statistic images thresholded at $F\geq 8$, equivalent to p uncorrected = 0.005, see Materials and Methods for further details.

(medial prefrontal cortex, precuneus, parahippocampal gyrus, and posterior cingulate) and the clinical data (HAM-D score, Young Mania Rating Scale score, and individual components of the TEMPS-A). None remained significant after controlling for multiple comparisons.

DISCUSSION

In the current study, we have demonstrated differential effects of *DGKH* haplotypes on brain activation in the medial prefrontal cortex, precuneus, parahippocampus, and posterior cingulate between healthy controls and a cohort at familial risk of BD during a verbal initiation task. The task was found to engage typical language-related regions, as well as demonstrating reduced activation in regions typically disengaged during cognitive paradigms as previously described (Raichle *et al*, 2001; Whalley *et al*, 2004; Whalley *et al*, 2011), and see Supplementary Figure 1. The advantage of studying such effects in a high genetic risk group allows the examination of the impact of the gene on brain function in the absence of confounding disease and medication effects associated with studies on patient groups.

From Figure 2, the origin of the haplotype*group interactions for the clusters originating from the sentence

Table 2 Significant Haplotype*Group Interactions

Contrast	Region	MNI coordinates x, y, z (mm)	Cluster size (k)	Z	p
Sentence completion vs baseline	Left medial frontal gyrus	-10, 54, 4	470	3.76	<0.05
	Left precuneus	-6, -32, 50	1149	3.80	<0.01
	Right parahippocampus	16, -42, 2	707	3.86	<0.05
Parametric	Right posterior cingulate	4, -66, 20	1317	4.07	<0.01

Abbreviations: MNI, Montreal Neurological Institute; k, number of voxels.

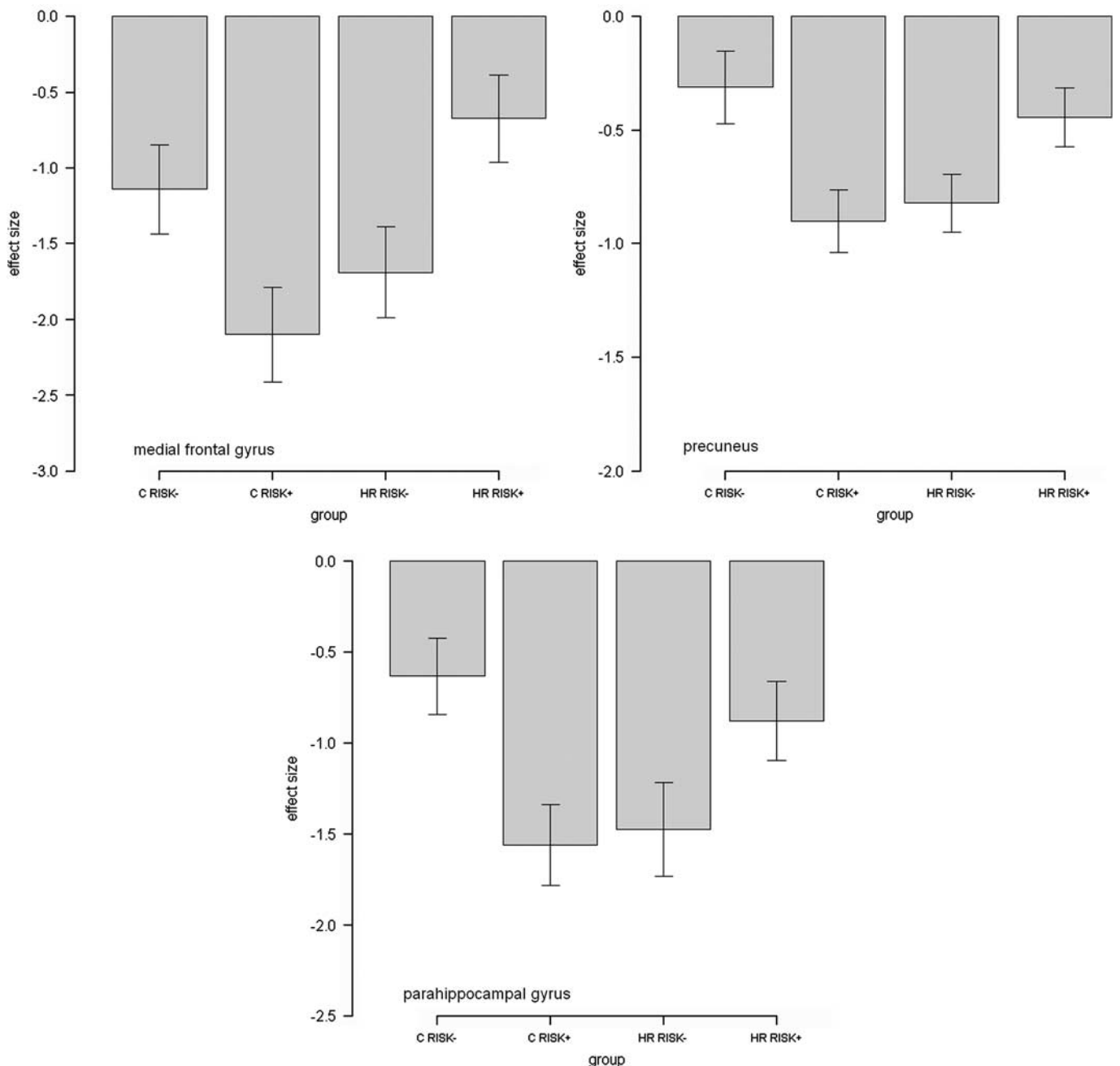


Figure 2 Graph of effect size for clusters of significant interaction. For sentence completion vs baseline, RISK- represents non-risk haplotype carriers and RISK+ represents risk haplotype homozygotes. The Y axis denotes BOLD effect size.

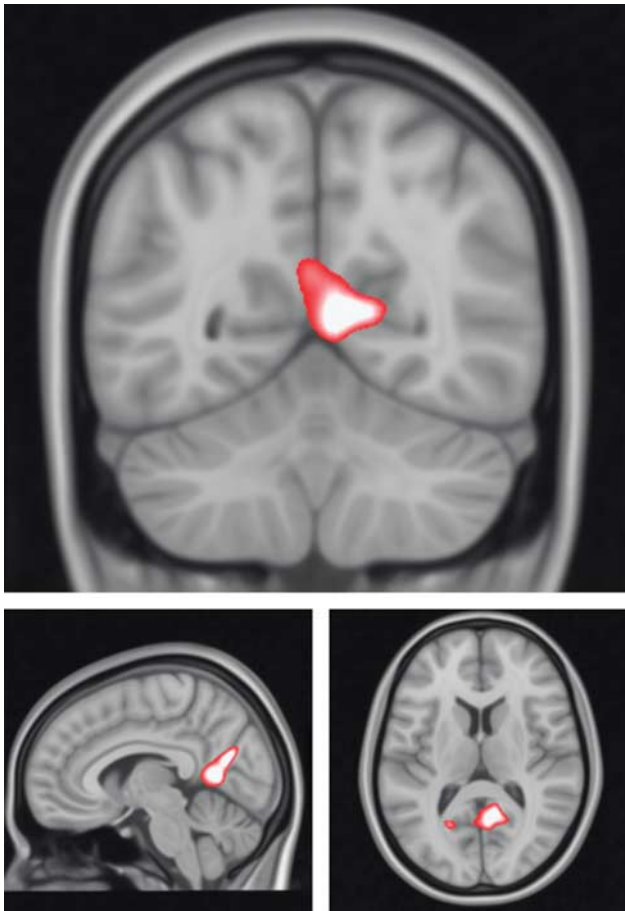


Figure 3 Haplotype*group interactions for *DGKH* for the parametric contrast. Images are overlaid onto standard brain in Montreal Neurological Institute space using Mango software package (<http://ric.uthscsa.edu/mango>). Map represents F-statistic images thresholded at $F \geq 8$, equivalent to p uncorrected = 0.005.

completion *vs* baseline contrast appeared to be a reduced disengagement, or failure to suppress activation in the high-risk RISK+ haplotype group *vs* high-risk RISK- during task conditions. The reverse was demonstrated in the control group, Figure 2 (ie., there was greater suppression in the control RISK+ group *vs* the control RISK- group). The clusters demonstrating interaction effects for this contrast, the medial prefrontal cortex, parahippocampal gyrus, precuneus, and posterior cingulate are indeed typically associated with default-mode patterns of activation. The default-mode network refers to a set of regions that consistently demonstrate synchronous task-independent deactivation across a range of cognitive tasks compared with baseline conditions (Raichle *et al*, 2001; Greicius *et al*, 2003; Raichle and Snyder, 2007). The posterior cingulate cortex was also found to demonstrate significant interaction effects for the parametric contrast, Figure 4. This contrast focusses on a gradation of neural activity in relation to task difficulty, rather than the sentence completion contrast, which relates activation across all levels of task condition to a simple visual baseline. In the posterior cingulate for the parametric contrast, the RISK- high-risk subjects and RISK+ controls demonstrate small increases in activation with increasing task difficulty. The RISK+ high-risk

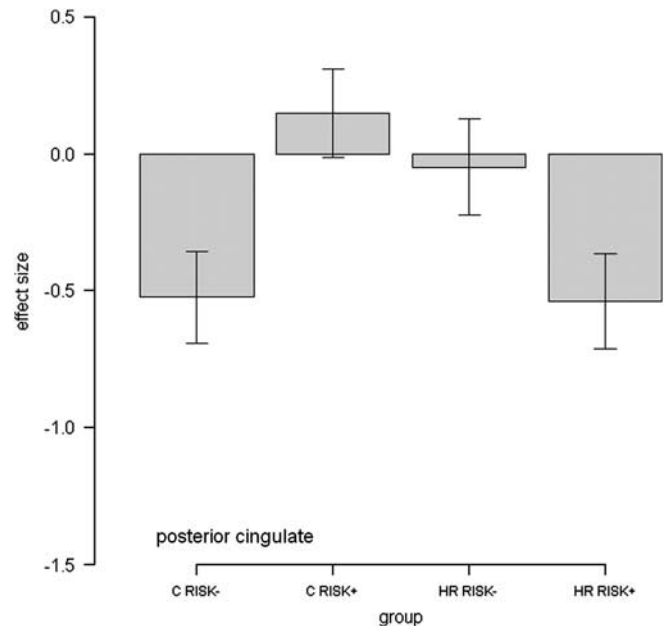


Figure 4 Graph of effect size for cluster of significant interaction. For parametric contrast, RISK- represents non-risk haplotype carriers, RISK+ represents risk haplotype homozygotes. The Y axis denotes BOLD effect size.

subjects, however, demonstrated decreases in activation with increasing task difficulty. In summary therefore, in the posterior cingulate, the RISK+ high-risk subjects have poor suppression when looking across all task conditions; however, when looking at the relationship between activation and task load, the RISK+ high-risk subjects demonstrate a greater decrease in activation with increasing task difficulty.

Default-mode regions are considered to subservise self-referential mental activity, autobiographical memory, and aspects of internal emotion processing (Gusnard and Raichle, 2001; Cabeza *et al*, 2002). Failure to deactivate these regions has been linked to performance deficits in healthy subjects (Weissman *et al*, 2006; Mason *et al*, 2007; Eichele *et al*, 2008). It has also been suggested that abnormalities within this network may underlie deficits in cognitive and affective processing seen in psychiatric disorders (Ongur *et al*, 2010). Indeed, abnormalities in the default-mode network have been reported in BD patients (Calhoun *et al*, 2008; Ongur *et al*, 2010; Costafreda *et al*, 2011). Further, the default-mode network is also considered to be strongly influenced by genetic factors (Glahn *et al*, 2010).

As the specific molecular role of *DGKH* is not yet known, the precise mechanisms of action of *DGKH* variation in conferring risk for the disorder is as yet unclear. All SNPs contributing to this haplotype are indeed located within intronic regions and are therefore noncoding. We would argue, however, that this does not necessarily mean that they are nonfunctional, rather that any function is currently unknown. We could speculate that, given previous evidence for the involvement of the gene in BD, they may be involved in gene regulation, or may be in LD with another as yet unknown functional variant of the gene, which may have

downstream influence on BOLD signal. In the context of the current findings, they suggest that there is an influence of the *DGKH* gene on function in default-mode brain regions, and this differs between those at risk for the disorder and healthy controls. In other words the risk haplotype appears to alter the ability to disengage regions involved in self-referential and internal emotional processing during cognitive tasks in the bipolar high-risk group, as compared to healthy controls. One possible explanation for this difference is that additional genetic or environmental risk factors interacting with the effects of *DGKH* in the high-risk group. Presumably, controls carrying the risk haplotype are able to compensate and allocate cognitive resources more efficiently during task conditions than the bipolar high-risk subjects carrying the risk haplotype, who also importantly carry additional background risk factors. We interpret these results to suggest therefore that the relationship between *DGKH* genotype and functional architecture in the high-risk group may differ from controls based on an already present genetic loading for the disorder.

In terms of its biological relevance, *DGKH* is an upstream regulator of protein kinase C (PKC). It catalyzes the conversion of diacylglycerol to phosphatidic acid, thereby leading to reductions in diacylglycerol. The latter is essential for activating several isoforms of PKC; a family of enzymes that is heterogeneously distributed in the brain and has a pivotal role in regulating pre- and post-synaptic neurotransmission (Casabona 1997; Catapano and Manji, 2008). Accordingly, it has been proposed that variations in the *DGKH* gene might result in reductions of diacylglycerol kinase activity, thereby leading to increased PKC signaling (Robbins and Arnsten, 2009). Numerous lines of evidence suggest the importance of PKC in the pathophysiology and treatment of BD. Investigations of peripheral blood and postmortem studies indicate altered PKC levels in BD, and lithium interacts with the PKC pathway resulting in decreasing downstream PKC levels and activity (Catapano and Manji, 2008). Similarly, the mood stabilizer valproate inhibits PKC, suggesting a commonality in the pathway of therapeutic effect (Catapano and Manji, 2008). Moreover, the PKC antagonist tamoxifen has been shown to reduce manic symptoms in BD patients (Yildiz *et al*, 2008). Hence, *DGKH* is predicted to have a role in lithium response and in mood control and therefore in the etiology of the disorder.

Although there is limited literature relating specifically to the effects of *DGKH* on brain functioning, there is evidence of increased gene expression of *DGKH* in the prefrontal cortex of patients with BD *vs* controls (Moya *et al*, 2010). There are also several lines of evidence suggesting that PKC impacts on cognitive functioning. Research has focussed on its role in mediating the effects of chronic stress on the prefrontal cortex (Hains *et al*, 2009), in regulating optimal prefrontal activity during working memory performance (Birnbaum *et al*, 2004), and in association with age-related cognitive decline (Brennan *et al*, 2009). Although speculative, these may fit with neurobiological models of BD where there is altered prefrontal modulation of other cortical and subcortical structures involved in emotional processing leading to impaired emotional regulation and increased mood fluctuation in the disorder (Phillips *et al*, 2003).

In summary, these findings suggest that variation in the *DGKH* gene is associated with differential modulation of default network suppression during a sentence completion paradigm in bipolar high-risk individuals when compared with healthy controls. Differential effects of *DGKH* haplotypes in those at high familial risk of BD *vs* controls supports previous evidence that the *DGKH* gene may be involved in the pathogenesis of BD. Further replication in patient groups and/or during resting state scanning may help confirm the nature of these effects.

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