Title: Pinpointing resilience in Bipolar Disorder

1. AIM OF THE RESEARCH AND BRIEF BACKGROUND

Bipolar disorder (BD) is a mood disorder characterised by episodes of depression and mania. It ranks as one of the ten most significant causes of disability worldwide. Genetic factors account for up to 85% of the liability for BD[1]. Genome-wide association studies (GWAS) have successfully identified several single nucleotide polymorphisms (SNPs) and genes associated with increased risk for BD[2]. However, the single most significant predictor of BD expression remains genetic proximity to affected individuals; with first-degree relatives of BD patients showing a 10-fold increase in their risk to develop BD[3]. Nevertheless, the path from genetic risk to the BD clinical phenotype is multifaceted and not clearly mapped. The majority of first-degree relatives (around 60%) of BD patients do not develop any psychiatric disorder[4]. As a result research up to this point has focused almost exclusively on factors which increase risk of developing BD without similar attention being directed to factors associated with resilience. This study plans to address this issue within the framework of resilient adaptation; resilient adaptation in the sense that brain features have adapted in order to avert psychopathology despite expressed genetic predisposition to BD.

Neuroimaging studies have provided evidence for disorder-associated neural phenotypes in BD relating to abnormalities in the circuitry underpinning working memory (WM) processing[5,6,7]. WM processing abnormalities have been found to be present in first-degree relatives of BD patients[8,9,10] and have been identified as heritable endophenotypes1[1,12,13,14,15]. We are now faced with the challenge of finding an explanatory framework of how genetic risk might affect complex-related traits and impact upon adaptive responses that prevent BD. Identification of the adaptive brain responses associated with avoidance of psychopathology in individuals with increased genetic predisposition to BD would be a key step in understanding resilient adaptation.

Dr Dima has published extensively in this area. First was a demonstration of the importance of the concept of resilience in identifying the contribution of brain regions and connectivity measures that would not have been predicted by models of risk for disease expression[16]. Secondly she has defined functional networks of effective connectivity for processing paradigm[17] in healthy participants.

Furthermore, neuroimaging and genetic studies have shown that human brain structure and function are highly heritable and influenced by multiple genes some of which are associated with BD[9,13,14]. The CACNA1C rs1006737 and ANK3 rs10994336 SNPs from GWAS have received the strongest support for their association with BD[18,19]. Dr Dima has shown that both risk-variants independently modulate connectivity of the face network during affect processing independent of diagnosis[20]. These results confirm that GWAS-supported genes for BD have measurable, and possibly converging, functional consequences for neural connectivity during cognitive processing.

Although these data validate the usefulness of genetic imaging, single SNP analyses alone do not address the overall genomic or “polygenic” architecture of BD as the amount of

phenotypic variation explained by each GWAS-supported SNP is small whereas the number of SNPs/regions underlying risk for the illness is thought to be very large[2]. The polygenic risk score models the aggregate effect of alleles associated with disease status present in each individual and allows us to utilize the power of large GWAS to be applied robustly in small samples[2,18,19]. It has been successfully used in the study of brain structure in individuals at high risk of mood disorders[21,22]. Resilient (healthy) relatives may have a low polygenic risk, as polygenic theory implies that, despite relatives of BD patients having on average higher scores than the general population, considerable variation in polygenic score does exists between relatives[21,22,23]. Additionally, recent whole genome DNA methylation ('methylomic') studies have found differences in the brains of depressed patients, BD patients and schizophrenic patients relative to controls[24,25]. Furthermore, studies have identified methylomic signatures which differentiate between controls and major psychoses patients in blood, and between mood disorder subtypes (bipolar disorder I and bipolar disorder II)[26,27]. Altered DNA methylation profiles may underlie the resilience of affected relatives, in essence correcting for the effects of increased genetic risk.

This Psychiatry Research Trust application is for a pilot project that aims to address these questions by:

(1) Defining the models of connectivity during WM processing present in patients with BD in respect of clinical relevance and in resilient relatives of BD patients,

(2) Defining adaptive changes in effective connectivity during WM processing in resilient relatives of BD patients, which will help us understand why BD does not manifest despite genetic predisposition,

(3) Testing whether the genetic contribution to connectivity in BD and in resilient relatives of BD is best described as a function of polygenic risk score,

(4) Profiling genome-wide patterns of DNA methylation in BD patients and their resilient relatives,

(5) Identifying interactions between DNA methylation, genotype and effective connectivity which could help describe resilient adaption.

Identifying mechanisms of adaptive resilience to BD will offer new insights for the prevention and treatment of BD.

2. MATERIALS & METHOD

2.1 Study Sample: All participants took part in the Vulnerability Indicators to Bipolar Disorders (VIBES) study sample[28]. Forty-one euthymic bipolar disorder patients, twenty-five unaffected/resilient first-degree relatives, and forty-six healthy individuals participated in the fMRI study. BD patients fulfilled criteria for BD type I. The manic and the depressed psychopathology was investigated by using the Hamilton Depression Rating Scale (HDRS), the Young Mania Rating Scale (YMRS) and the Brief Psychiatric Rating Scale (BPRS). At the

day of the scanning, all the participants were tested for the current full scale IQ by using the Wechsler Adult Intelligence Scale 3rd Edition (**Table 1**).

Table 1	**BD patients (n = 41)**	**Controls (n = 46)**	**Resilient Relatives (n=25)**
Age	44.3	40.3	39.7
Sex (Male/Female)	20/21	25/21	13/12
Educational level	3.5 (1.0)	3.6 (1.0)	3.6 (0.8)
IQ	117.9	112.6	115.8
HDRS total score	4.8 (5.3)	0.1 (0.5)	0.14 (0.4)
YMRS total score	1.4 (3.0)	0.2 (0.6)	0.0 (0.0)
BPRS total score	27.5 (4.0)	24.3 (0.7)	24.1 (0.4)
Age of onset (y)	24.7 (8.0)	-	-
Duration of illness	20.2 (10.5)	-	-
Depressive episodes	5.7 (7.5)	-	-
Manic episodes (n)	5.6 (7.7)	-	-

2.2 fMRI acquisition: The N-back verbal working memory task was presented as an alternating block paradigm incorporating active conditions (1-, 2-, 3-back) and a baseline (0-back) condition[17]. Participants were instructed to respond to target letters by button press. In the baseline condition participants responded to the X letter. In the 1-, 2-, and 3-back conditions participants responded when the letter currently presented matched the one presented in the preceding 1, 2, or 3 trials. There were 18 epochs in all, each lasting 30s. Each letter was presented for 2s. Performance was evaluated in terms of response time to target letters and accuracy. Both anatomical and functional imaging data were acquired during the same session using a 1.5T. For the working memory paradigm, 180 T2*-weighted MR images reporting blood-oxygenation level-dependent contrast were acquired. A high-resolution T1 weighted structural image was acquired for each participant for co-registration.

2.3 DNA Extraction: DNA from buccal swabs has already been extracted from all participants and is stored at the MRC SGDP Centre, Institute of Psychiatry, King's College London. In addition, this DNA has been subjected to vacuum filtration and suspension to increase the concentration and molecular weight of the DNA available. Buccal swab DNA is thought to be a superior source of DNA for methylation experiment due to the reduction of cellular heterogeneity and has been found to perform well in various studies[29,30].

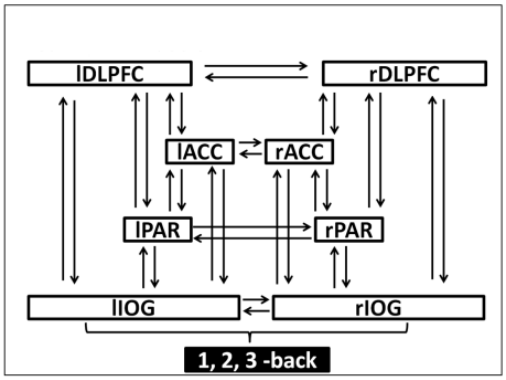
New analyses:

2.4 DNA Genotyping: DNA will be genotyped using the BRC Genomics and Biomarkers theme core Illumina microarray facility at the MRC Social, Genetic and Developmental Psychiatry Centre on the PsychChip (the PsychChip is an Illumina GWAS + exome + 50K custom chip designed by the Psychiatric Genomics Consortium). Sample results will be subjected to quality control and SNPs adjusted for population stratification using principal components analysis. Genotyped will be imputed with 1000 genomes and the UK10K using standard pipelines (IMPUTE2 or miniMACH).

2.5 Methylation arrays: In collaboration with the Epigenetics group at SGDP DNA will be subject to the Infinium HD DNA Restoration Protocol (originally design for FFPE samples) and purified DNA will undergo bisulfite conversion and be applied to the Illumina Infinium Human Methylation 450K array, which generates a quantitative measurement of DNA methylation for >480,000 CpG sites spanning all annotated genes and other functional motifs across the genome.

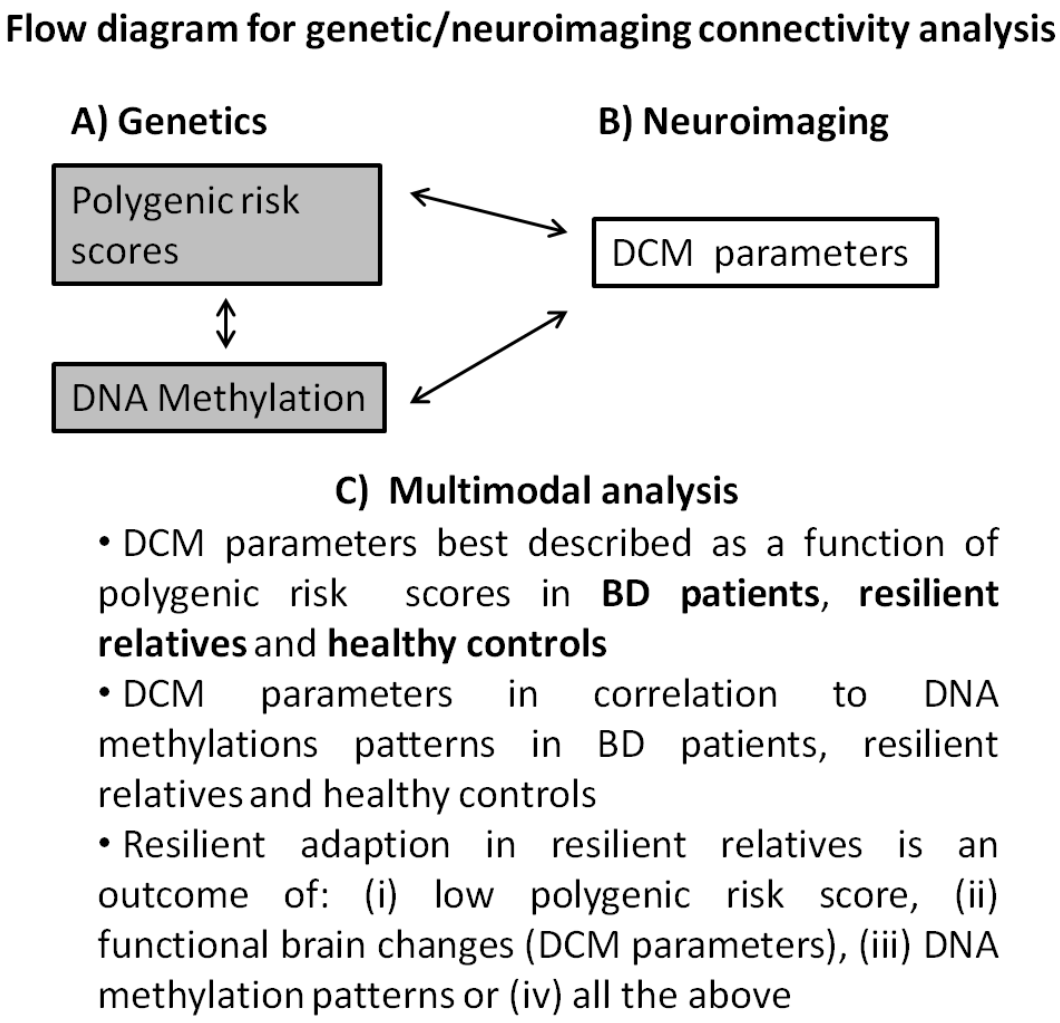
3. STATISTICAL TREATMENT OF RESULTS

3.1 fMRI processing and connectivity analysis: All analyses will be implemented using Statistical Parametric Mapping software (www.fil.ion.ucl.ac.uk/spm). *Dynamic Causal Modelling:* In a previous study of ours[17] we detail our strategy for determining the most parsimonious model for WM processing. In summary an eight-area DCM will be defined for all participants with the left and right inferior occipital gyrus (lIOG and rIOG), parietal cortex (lPAR and rPAR), anterior cingulate cortex (lACC and rACC), and dorsolateral prefrontal cortex (lDLPFC and rDLPFC) as volumes of interests. Within each hemisphere we will define bidirectional connections between these regions. Bidirectional connections will also be specified between homologous regions in each hemisphere. Thirty-two endogenous connections will be specified in total with the main effect of memory as the driving input entering the lIOG and rIOG. This architecture served as our base model which was then elaborated systematically to produce 32 alternative variants for each experimental condition to test how the 3-back memory load could modulate the 32 connections. In total 32 models were constructed, fitted and compared for all participants. To summarise the strength of effective connectivity, we will use random effects Bayesian Model Averaging to obtain average DCM connectivity parameters across all models for each participant.



3.2 Polygenic Risk Score Determination: Polygenic risk scores for each participant will be generated using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink following the methods by Purcell et al (2009)[2]. Estimates of the log of the odds ratios of case/control allelic association tests will be obtained from the GWAS data from the bipolar subgroup of the Psychiatric Genomics Consortium (https://pgc.unc.edu/), of which Dr Breen is a member. Linkage disequilibrium clumping will be applied to further select SNPs in approximate linkage equilibrium with each other. The primary analyses will focus on SNPs associated with case-control status at significance threshold of $p \leq 0.5$ as this most reliably differentiates BD patients from controls.

3.3 Association between DCM Parameters and Polygenic Risk Score: Associations between polygenic risk scores with group-interaction imaging effects and DCM parameters will be performed.



3.4 Methylation arrays analysis: For the detection of the DNA methylation patterns, optimal pre-processing and normalization methods for our array data will be selected based on results from the wateRmelon package in R (bioConductor). [31] Array data will be corrected for age, sex, current medication use, duration of illness, population stratification (utilizing GWAS data) and array batch effects. Our aim is to investigate epigenetic differences between groups in two genes (CACNA1C and ODZ4) that have received the strongest genome-wide significant evidence in BD[19].

3.5 Association between DNA methylation, DCM parameters and polygenic risk score: Associations between DNA methylation differences with group-interaction imaging effects, DCM parameters and polygenic risk scores will be performed.