

CNRI Gene is Associated with High Neuroticism and Low Agreeableness and Interacts with Recent Negative Life Events to Predict Current Depressive Symptoms

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Cannabinoid receptor 1 (CB1) gene (CNRI) knockout mice are prone to develop anhedonic and helpless behavior after chronic mild stress. In humans, the CB1 antagonist rimonabant increases the risk of depressed mood disorders and anxiety. These studies suggest the hypothesis that genetic variation in CB1 receptor function influences the risk of depression in humans in response to stressful life events. In a population sample ($n = 1269$), we obtained questionnaire measures of personality (Big Five Inventory), depression and anxiety (Brief Symptom Inventory), and life events. The CNRI gene was covered by 10 SNPs located throughout the gene to determine haplotypic association. Variations in the CNRI gene were significantly associated with a high neuroticism and low agreeableness phenotype (explained variance 1.5 and 2.5%, respectively). Epistasis analysis of the SNPs showed that the previously reported functional 5' end of the CNRI gene significantly interacts with the 3' end in these phenotypes. Furthermore, current depression scores significantly associated with CNRI haplotypes but this effect diminished after covariation for recent life events, suggesting a gene \times environment interaction. Indeed, rs7766029 showed highly significant interaction between recent negative life events and depression scores. The results represent the first evidence in humans that the CNRI gene is a risk factor for depression—and probably also for co-morbid psychiatric conditions such as substance use disorders—through a high neuroticism and low agreeableness phenotype. This study also suggests that the CNRI gene influences vulnerability to recent psychosocial adversity to produce current symptoms of depression.

Neuropsychopharmacology (2009) **34**, 2019–2027; doi:10.1038/npp.2009.19; published online 25 February 2009

Keywords: CNRI; haplotype; personality; depression; epistasis; gene \times environment interaction

INTRODUCTION

Accumulating evidence suggests that the endocannabinoid (eCB) system plays an important role in the etiology of depression through control of emotional behavior (Martin *et al*, 2002; Hill and Gorzalka, 2005a; Vinod and Hungund, 2006; Serra and Fratta, 2007). In humans, the acute effects of cannabis include euphoria and relaxation, but early and chronic use has been associated with increased risk of psychotic illness and deterioration of bipolar disorder (Hall and Solowij, 1998; Ashton *et al*, 2005). Rimonabant, a cannabinoid receptor 1 (CB1) antagonist, administered to humans as a weight-loss drug, has been shown to increase the risk of depressed mood disorder, and anxiety even

though depressed mood in the history was an exclusion criteria in these trials (Christensen *et al*, 2007). In animal studies, anxiogenic-like or anxiolytic effects of cannabinoids depend on the strain, the behavioral test used, and the drug dosage (Martin *et al*, 2002; Haller *et al*, 2004a). Rimonabant produces antidepressant-like effects in different species and models (Witkin *et al*, 2005) but an eCB uptake inhibitor (AM404) and a potent CB1 receptor agonist (HU-210) also elicit antidepressant-like effects in the forced swim test in rats (Hill and Gorzalka, 2005b). Animals with genetic deletion of the CB1 receptor (CB1 $-/-$) exhibit anxiogenic-like responses in different behavioral models (Haller *et al*, 2004a) and a tendency to develop a depressive-like state (anhedonia) during the exposure to the chronic unpredictable mild stress paradigm (Martin *et al*, 2002; Valverde *et al*, 2005). Thus, taken as a whole, the current evidence supports the theory that reduction in CB1 receptor signaling induces increased anxiety and depression-like behavior (Hill and Gorzalka, 2005a).

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Received 25 November 2008; revised 12 January 2009; accepted 27 January 2009

The human CB1 receptor gene (CNRI) is located at chromosome 6q14–15, but the exact structure of the gene and its effect on the CB1 receptor function are still unclear (Zhang *et al*, 2004). This genetic region has been linked to schizophrenia (Levinson *et al*, 2000; Leroy *et al*, 2001), but this has not been replicated, although recently the CNRI gene has been associated with specific symptoms or with non-responder status for antipsychotic drugs rather than with schizophrenia as a disorder (Chavarria-Siles *et al*, 2008; Hamdani *et al*, 2008). There are more convincing results on the association of the CNRI gene and substance abuse disorders (Zhang *et al*, 2004; Zuo *et al*, 2007; Chen *et al*, 2008), although there is a negative replication study (Herman *et al*, 2006). Regarding depression, two studies with significantly smaller sample sizes failed to find any association with depression using the (AAT)_n polymorphism (Barrero *et al*, 2005; Tsai *et al*, 2001), although the former found that a significant association emerged when only the depressed patients with Parkinson's disease were considered (Barrero *et al*, 2005).

Given that the CNRI gene has been implicated in a broad spectrum of psychiatric disorders, it is plausible that it affects dimensions of psychopathology (eg, specific personality factors) that are risk factors for these disorders (Widiger and Sankis, 2000; Khan *et al*, 2005). Twin studies have demonstrated that personality factors are highly influenced by genes. Using measures of Big Five-factor models of personality, the heritability has been estimated as follows: extraversion 0.54, agreeableness 0.42–0.52, conscientiousness 0.40–0.49, neuroticism 0.48–0.58, and openness 0.52–0.57 (Bouchard and McGue, 2003). Furthermore, twin studies have also suggested that the correlations between personality factors and psychiatric disorders are mainly due to shared genetic factors (Kendler *et al*, 2006; Fanous and Kendler, 2005). However, we could not find a systematic investigation of the association between the CNRI gene and different personality factors. Impulsivity, which is one facet of neuroticism, has been associated with polymorphisms in the region of the CNRI gene in an Indian population (Ehlers *et al*, 2007). Furthermore, two recent studies demonstrated association between striatal response to happy faces and variations of the CNRI gene and suggested that this gene represents a risk factor for depression through subcortical hypo-responsiveness to social reward stimuli (Chakrabarti *et al*, 2006; Domschke *et al*, 2008).

We hypothesized that the CNRI gene influences personality factors that predispose to specific psychiatric conditions, especially anxiety and depressive symptoms. Most of the animal studies suggest that stress plays crucial role in the effects of the eCB system, so we also investigated the role of adverse life events. We used a population study design with independent individuals and applied haplotypic association analysis to investigate the effect of the CNRI gene on these phenotypes.

MATERIALS AND METHODS

Subjects

Subjects aged 18–60 years predominantly from Greater Manchester, UK, were recruited through general practices and the NewMood website (<http://www.newmood.co.uk>).

Almost 4500 packs were sent out to individuals on two GP practice lists in the Manchester area, 30% of people responded, and about half of these agreed to participate. The NewMood website was launched in August 2005 and almost 2000 information/questionnaire packs were sent out to people enquiring about the study through the website and over 50% of those participated. Altogether more than 2000 people completed and returned the NewMood questionnaire (39% through general practices and 61% through NewMood website). Of the sample, 92% were white, 68% were women (which reflects the gender ratio of people with depression), 48% had a history of depression (28% in the last year), 40% had had antidepressant treatment in the past, 35% had a family history of depression, and 84% were willing to give DNA.

Participants who returned the signed consent form to provide DNA ($n = 1679$) were then sent a genetic sampling kit. Of these subjects, $n = 1520$ returned the kit (35% from general practices and 65% from NewMood website). Women (78 vs 71% of men; $p = 0.001$) and those who had a history of depression (55 vs 42% of those who reported no depression in the past; $p < 0.001$) were more likely to send a DNA sample.

We excluded those reporting manic or hypomanic episodes, psychotic symptoms, obsessive-compulsive disorder and those of non-Caucasian origin, but we did not exclude those with a self-reported history of depression or any other anxiety disorder, or substance misuse (all information based on background questionnaire data); thus 1269 participants were included in the analysis. Population details are shown in Table 1.

This study was approved by the local ethics committees and was carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Questionnaires

We used brief standard questionnaires that were easy for participants to complete and return to us by post. The booklet included questions covering background information (age, ethnicity, and family circumstances), personal and family psychiatric history, and questionnaires covering current mood and anxiety, personality, life events, and childhood trauma.

To assess personality, we used the Big Five Inventory (BFI-44) (John and Srivastava, 1999). For analysis, BFI factor scores were calculated using a continuous weighted dimension score (sum of items scored divided by the number of items completed). Although the NEO PI-R (Costa and McCrae, 1992) is the best validated Big Five questionnaire battery, the BFI-44 Inventory has the advantage of being short, easy to understand, and shows a high cross-instrument convergent validity correlations with other five-factor personality questionnaires (eg with NEO, mean $r = 0.73$) (John and Srivastava, 1999). In total, 142 of our participants filled out both the NEO PI-R and BFI-44 questionnaires for validation purposes and the results showed highly significant ($p < 0.001$) correlations between the two instruments (Pearson's correlation: extraversion $R = 0.79$; agreeableness $R = 0.59$; conscientiousness $R = 0.75$; neuroticism $R = 0.81$; and openness $R = 0.66$).

Table 1 Population Details for Those who Fulfilled the Inclusion Criteria for the Study ($n = 1269$)

Demographics	
Sex	
Women	883 (70%)
Men	386 (30%)
Age (years) (mean \pm SEM)	34.04 \pm 0.28
Personal psychiatric history	
Reported depression	676 (53%)
Single episode	181 (14%)
Recurrent episodes	495 (39%)
Reported suicide	190 (15%)
Reported anxiety	368 (29%)
Reported substance use disorder	85 (7%)
Family psychiatric history	
Reported depression in immediate blood relatives	449 (35%)
Personality scores (range 1–5)	
BFI extraversion (mean \pm SEM)	3.22 \pm 0.03
BFI agreeableness (mean \pm SEM)	3.78 \pm 0.02
BFI conscientiousness (mean \pm SEM)	3.66 \pm 0.02
BFI neuroticism (mean \pm SEM)	3.31 \pm 0.03
BFI openness (mean \pm SEM)	3.63 \pm 0.02
Symptom scores (range 0–4)	
BSI depression (mean \pm SEM)	1.03 \pm 0.03
BSI anxiety (mean \pm SEM)	0.94 \pm 0.03
Adversities	
Recent negative life events (mean \pm SEM)	1.29 \pm 0.04
Childhood adversity (mean \pm SEM)	3.53 \pm 0.10

BFI, Big Five Inventory; BSI, Brief Symptom Inventory.

Depressive symptoms were measured using the depression subscale plus additional items, and anxiety symptoms using the anxiety subscale from the 53-item Brief Symptom Inventory (BSI) (Derogatis, 1993). A continuous weighted dimension score (sum of item scores on the dimension divided by the number of items completed) was calculated. Mood and anxiety symptoms of 140 participants were also rated by independent trained investigators using the Montgomery Asberg Depression Rating Scale (Montgomery and Asberg, 1979) and the Clinical Anxiety Scale (Snaith *et al*, 1982). The results showed highly significant ($p < 0.001$) correlations between the self-reported symptom scores and the independent ratings (Pearson's correlation: depression $R = 0.79$; anxiety $R = 0.80$) in our population.

The recent negative life events questionnaire was adapted from the list of life threatening experiences (Brugha *et al*, 1985) and asked participants to identify negative life events related to intimate relationships, financial difficulties, illnesses/injuries, and network problems occurring in the last year. The sum of life event items was used in the analysis.

Childhood adversity questions related to emotional and physical abuse, and emotional and physical neglect were derived from the Childhood Trauma Questionnaire (Bernstein *et al*, 1994). An additional question asked about parental loss during childhood. The sum of item scores was used in the analysis.

Genotyping

Buccal mucosa cells were collected using cytology brush (Cytobrush plus C0012; Durbin PLC) and 15-ml plastic tube containing 2.0 ml of collection buffer. Genomic DNA was extracted according to a protocol suggested by Freeman *et al* (2003).

HaploView software package (<http://www.broad.mit.edu/personal/jcbarret/haploview/>) was employed to identify haplotype tag SNPs (htSNP; minimum pairwise correlation (r^2) to select htSNPs was 0.8) (Barrett *et al*, 2005; Gabriel *et al*, 2002) based on the AFD_EUR_PANEL (AFD_EUR_18-MAY-2004) population data of PERLEGEN (<http://genome.perlegen.com/>) (Hinds *et al*, 2005) and the CEPH population data of the International HapMap Project (<http://www.hapmap.org>; Phase I. June 2005). Previously identified possibly functional htSNPs in the 5' direction from the CNR1 gene were also examined (Zhang *et al*, 2004).

The chosen SNPs were genotyped using the Sequenom[®] MassARRAY technology (Sequenom, San Diego). The Iplex[™] assay was followed according to the manufacturer's instructions (<http://www.sequenom.com>) using 25 ng of DNA. Genotyping was blinded with regard to phenotype. All laboratory work was performed under the ISO 9001:2000 quality management requirements.

Statistical Analysis

PLINK v1.04 (<http://pngu.mgh.harvard.edu/purcell/plink/>) was used for testing the association of different genetic models (dominant, recessive, and additive; linear regression model covariation with age and sex), interactions between htSNPs (epistasis, linear regression model based on allele dosage for tested SNPs), and with life events (linear regression model, covariation with age and sex). Quanto 1.2 version (<http://hydra.usc.edu/gxe>) was employed to calculate the power of the recruited populations. Hardy-Weinberg and haplotype analyses were performed using HelixTree[™] 6.4.1 (Golden Helix, USA).

For haplotypic association analysis, we used haplotype trend regression. Only haplotypes with a frequency greater than 5% were used in the analysis. In all cases, data were adjusted for age and sex. We used linear regression in HelixTree to identify variance in the dependent variable explained by age and sex (the 'reduced model'). We then determined whether adding haplotype frequencies to the model (now the 'full model') explained significantly more variance than the reduced model using a variance ratio F-test. To remove the influence of multiple testing, we used a permutation test, randomly grouping the sample 10 000 times. The permuted p -values were the fraction of permuted tests that gave an improved p -value.

Bonferroni correction assumes independent outcome variables and this assumption is not true of personality factors and symptoms (depression and anxiety); therefore,

this method unnecessarily increases stringency (Westfall and Young, 1993). Thus, we used false discovery rate (FDR) calculation at a level of 5% (Qvalue: <http://genomics.princeton.edu/storeylab/qvalue/>) to adjust p -values according to the number of hypothesis tested (Storey and Tibshirani, 2003). We report q -value, which is a measure of significance of each test of many tests performed simultaneously.

RESULTS

The CNRI gene identified from the current National Center for Biotechnology Information (NCBI, March 2007) database was covered by seven htSNPs; in addition, we genotyped three reported SNPs located 5' from the gene (see Figure 1). The latter SNPs have a possible effect on the mRNA expression of this gene (Zhang *et al.*, 2004). All of the genotyped SNPs were in Hardy–Weinberg equilibrium. Allele and genotype frequencies can be seen in Supplementary Table S1. On the basis of the method published by Gabriel *et al.* (2002), we identified two blocks of linkage disequilibrium (LD; haploblock 1: rs806379, rs1535255, and rs2023239; haploblock 2: rs806369, rs1049353, rs4707436, rs12720071, rs806368, and rs806366), whereas rs7766029 just partially belonged to the second haploblock (for LD data, see Supplementary Table S2). This haplotype structure is in close agreement with the results previously reported on the European American population (Zuo *et al.*, 2007).

Single Marker Association with Personality and Symptoms

Detailed single marker association data (additive (add), dominant (dom), and recessive (rec) model) can be seen in

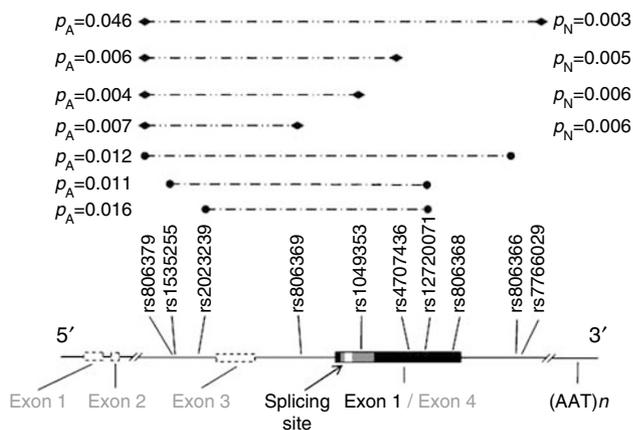


Figure 1 Schematic figure of the CNRI gene and the position of the genotyped SNPs. Exon numbers in gray are proposed by Zhang *et al.* (2004). The black bar represents the exonic region according to the National Center for Biotechnology Information (NCBI, March 2007) and the University of California at Santa Cruz Browser. The gray region within the black bar corresponds to the coding region and the splicing site is in white. Dashed lines above the rs numbers refer to the significant epistasis between the two haploblocks; p -values represented raw p data without correction for multiple testing. (p_A : significant epistasis p -values on agreeableness; p_N : significant epistasis p -values on neuroticism; ◆: relevant SNP pairs in the epistasis calculation that showed significant p -values on both phenotypes; ●: relevant SNP pairs in the epistasis calculation that showed significant p -values only on agreeableness).

Supplementary Table S3. In summary, rs1535255 G allele ($p_{rec} = 0.023$), rs2023239 C allele ($p_{rec} = 0.046$), and rs806366 C allele ($p_{add} = 0.024$ and $p_{dom} = 0.009$) showed significant positive association with agreeableness; rs4707436 A allele negatively associated with conscientiousness ($p_{rec} = 0.043$); neuroticism positively associated with rs1049353 A allele ($p_{add} = 0.042$ and $p_{dom} = 0.039$) and negatively associated with rs806366 C allele ($p_{add} = 0.017$ and $p_{dom} = 0.038$); and openness negatively associated with rs806368 C allele ($p_{dom} = 0.044$) as did rs7766029 T allele ($p_{dom} = 0.040$). Finally, rs806369 T allele showed negative association with depression ($p_{rec} = 0.038$) and positive association with anxiety ($p_{dom} = 0.019$), as did rs7766029 T allele ($p_{dom} = 0.038$). All of the reported p -values are nominal significance values and none of them survived correction for multiple testing.

For a continuous trait such as neuroticism, using an independent individual study design and assuming a linear model relating the phenotype to genotype, we would require 1045 individuals to detect a polymorphism (with an allele frequency > 10%) that explained 1% of the variance of the trait at the 5% two-tailed significance level with 90% power. As we included 1269 participants, our study was adequately powered.

Haplotypic Association with Personality

Haplotype trend regression showed significant association with neuroticism ($p = 0.0043$) and agreeableness ($p = 0.00003$; see Table 2). This association remained significant after permutation and FDR correction (neuroticism: $q = 0.0025$ and agreeableness: $q = 0.0002$). The variations in the CNRI gene explained 2.5% variance in agreeableness and 1.5% variance in neuroticism.

SNP × SNP Interaction (Epistasis) in Relation to Neuroticism and Agreeableness

Previously, Zhang *et al.* (2004) showed that haplotypic combination of rs806379, rs1535255, and rs2023239 influenced the expression of CNRI mRNA. In our study, we tested the hypothesis that the effects of SNPs in the 5' end haploblock 1 can be modified by the SNPs in the haploblock 2. Indeed, in our study, rs806379 showed significant epistasis with several SNPs on both neuroticism and agreeableness after covariation for age and sex, whereas rs1535255 and rs2023239 showed significant epistasis on agreeableness (Figure 1). Results where $p < 0.04$ remained significant after FDR correction for multiple testing.

Haplotypic Association with Depressive and Anxiety Symptoms

Using haplotypic trend regression, with age and sex as covariates, depressive symptoms were significantly associated with CNRI haplotypes (explained variance 1.1%, $p = 0.037$, permuted $p = 0.039$, FDR $q = 0.018$; Table 3). Figure 2 shows significant haplotypic effects on depression scores, neuroticism, and agreeableness. There was no significant haplotypic association with anxiety symptoms (explained variance 0.7%, $p = 0.281$; Table 3).

Table 2 Haplotypic Trend Regression Results for Personality Factors Measured by the Big Five Inventory (BFI)

Haplotypes	Frequency (%)	Extraversion		Agreeableness		Conscientiousness		Neuroticism		Openness	
		Beta	<i>p</i>	Beta	<i>p</i>	Beta	<i>p</i>	Beta	<i>p</i>	Beta	<i>p</i>
A,T,T,T,G,G,A,T,C,T	18.34	0.078	0.625	0.144	0.169	0.069	0.562	-0.343	0.0193	0.040	0.705
A,T,T,C,A,A,A,T,T,C	15.27	-0.454	0.0145	-0.486	<0.0001	-0.238	0.087	0.530	0.0019	0.054	0.661
T,T,T,C,G,G,A,T,C,C	8.30	-0.012	0.982	<i>0.589</i>	<i>0.089</i>	-0.059	0.881	<i>-0.895</i>	<i>0.065</i>	-0.047	0.893
T,T,T,T,G,G,A,T,C,T	8.10	-0.057	0.839	-0.378	0.0394	-0.355	0.090	0.353	0.168	0.047	0.801
T,G,C,C,A,A,A,T,T,C	7.31	-0.173	0.641	0.634	0.0094	0.060	0.829	0.210	0.538	-0.133	0.587
A,T,T,C,G,G,A,C,T,T	7.06	-0.434	0.276	-0.352	0.187	-0.172	0.572	0.253	0.498	0.082	0.761
T,T,T,C,A,A,A,T,T,C	5.38	0.578	0.185	0.225	0.434	-0.165	0.614	-0.600	0.135	0.296	0.291
Rare haplotypes	30.23										
Full vs reduced model <i>p</i>			0.342		<0.0001		0.413		0.0043		0.938
Permutated <i>p</i>			0.337		0.0001		0.409		0.0028		0.939
FDR <i>q</i>			0.088		0.0002		0.092		0.0025		0.190

FDR *q*, false discovery rate significance value.

Age and sex were covariates in all calculations and the order of the htSNPs in the haplotypes corresponds to the SNP order in Figure 1.

Bold values indicate significant results ($p < 0.05$), italic values indicate trends ($0.05 < p < 0.01$).

Table 3 Haplotypic Trend Regression Results for Symptom Scores Measured by the Brief Symptom Inventory (BSI)

Haplotypes	Frequency (%)	Anxiety		Depression		Depression ^a		Depression ^b	
		Beta	<i>p</i>	Beta	<i>p</i>	Beta	<i>p</i>	Beta	<i>p</i>
A,T,T,T,G,G,A,T,C,T	18.34	-0.324	0.039	-0.421	0.011	-0.352	0.029	-0.354	0.020
A,T,T,C,A,A,A,T,T,C	15.27	0.250	0.169	0.403	0.037	0.339	0.071	0.456	0.010
T,T,T,C,G,G,A,T,C,C	8.30	-0.781	0.131	-0.398	0.467	-0.365	0.493	-0.321	0.525
T,T,T,T,G,G,A,T,C,T	8.10	0.210	0.444	0.259	0.373	0.261	0.355	0.174	0.515
T,G,C,C,A,A,A,T,T,C	7.31	-0.066	0.857	<i>0.661</i>	<i>0.086</i>	<i>0.727</i>	<i>0.052</i>	0.774	0.029
A,T,T,C,G,G,A,C,T,T	7.06	-0.058	0.884	0.129	0.761	0.208	0.612	0.011	0.977
T,T,T,C,A,A,A,T,T,C	5.38	-0.234	0.585	-0.366	0.419	-0.286	0.516	-0.153	0.713
Rare haplotypes	30.23								
Full vs reduced model <i>p</i>			0.281		0.037		0.071		0.012
Permutated <i>p</i>			0.278		0.039		0.074		0.012
FDR <i>q</i>			0.084		0.018		0.027		0.007

FDR *q*, false discovery rate significance value.

^aAfter covariation for recent negative life events ($p < 0.001$).

^bAfter covariation for childhood adversity ($p < 0.001$).

Age and sex were covariates in all calculations and the order of the htSNPs in the haplotypes corresponds to the SNP order in Figure 1.

Bold values indicate significant results ($p < 0.05$), italic values indicate trends ($0.05 < p < 0.01$).

The magnitude of the haplotypic effect on depression scores remained unchanged after excluding subjects with reported substance use disorder (explained variance 1.3%, $p = 0.026$, permutated $p = 0.027$) and with reported suicide attempt (explained variance 1.3%, $p = 0.047$, permutated $p = 0.053$).

CNRI × Environment Interaction in Relation to Depression Scores

The significant CNRI haplotype association with depressive symptoms became nonsignificant (explained variance 0.9%, $p = 0.071$, permutated $p = 0.074$; Table 3) after controlling for recent negative life events scores. It is possible that the

CNRI gene might have an effect either on experiencing repeated negative life events through personality-dependent life choices or through modifying the interpretation of a specific life event (McGue and Bouchard, 1998; Bouchard and McGue, 2003).

Indeed, rs7766029 showed highly significant interaction with recent negative life events on depression scores ($p_{\text{add}} = 0.0004$, $p_{\text{rec}} = 0.0007$), which remained significant after FDR correction for multiple testing ($q_{\text{add}} = 0.004$, $q_{\text{rec}} = 0.004$; age, sex, and recent negative life events were covariates in the analysis; Figure 3). Two other SNPs also showed tendencies to interact with recent negative life events on depression scores (rs806369 $p_{\text{dom}} = 0.028$; rs1049353 $p_{\text{dom}} = 0.052$), but these associations did not

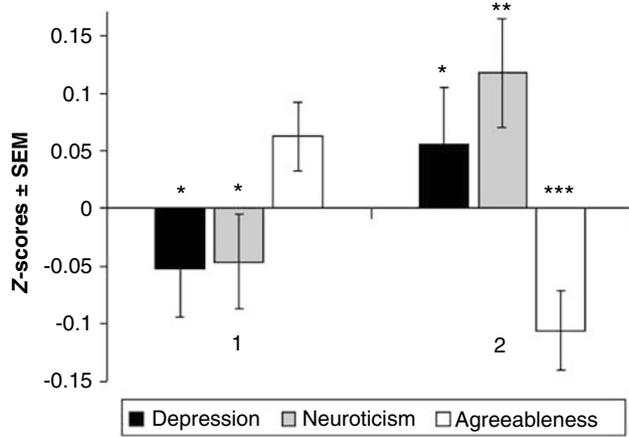


Figure 2 Effects of haplotype 1 (A,T,T,T,G,G,A,T,C,T; population frequency: 18.34%) and haplotype 2 (A,T,T,C,A,A,A,T,T,C; population frequency: 15.27%) on depression, neuroticism, and agreeableness scores. Z-scores were calculated as follows: (haplotype mean score – total population mean)/SD of haplotype mean. SEM: standard error of mean. Significant haplotypic effects: * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0001$.

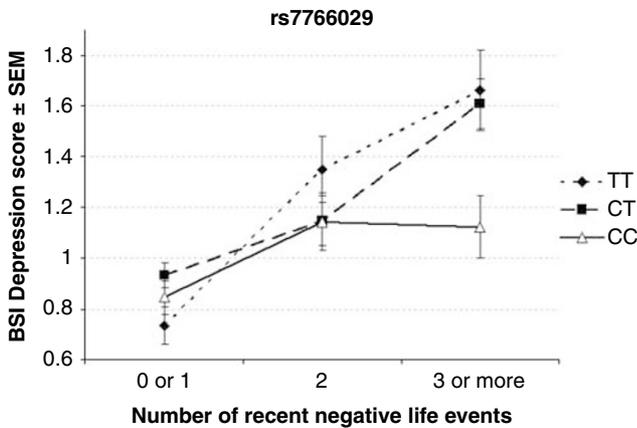


Figure 3 Significant interaction between rs7766029 and recent negative life events on depression scores ($p_{add} = 0.0004$, $p_{rec} = 0.0007$, after false discovery rate (FDR) correction for multiple testing $q_{add} = 0.004$, $q_{rec} = 0.004$). Age, sex, and recent negative life events were covariates in this calculation. T allele carriers are more vulnerable to negative life events than CC genotype carriers (numbers in subgroups according to negative life events and genotype: 0 or 1: TT = 174, CT = 387, CC = 207; 2: TT = 54, CT = 96, CC = 72; and 3 or more: TT = 37, CT = 97, CC = 60).

survive FDR correction (for detailed results, see Supplementary Table S4).

Finally, we used childhood adversity scores as covariates in the haplotypic trend regression. Haplotypic association between the CNR1 gene and depression scores became more significant (explained variance 1.1%, $p = 0.012$, permuted $p = 0.012$, FDR $q = 0.007$; Table 3), suggesting that childhood adversity acts independently from the CNR1 gene. This model remained significant after reintroducing recent negative life events scores into it (explained variance 1.0%, $p = 0.035$, permuted $p = 0.029$, FDR $q = 0.013$). Failure to include childhood adversity in variance models could be an important confounding variable in association studies of CNR1 and possibly other genes in depression.

DISCUSSION

This is the first study to demonstrate a significant association between the CNR1 gene and a high neuroticism/low agreeableness phenotype. Haplotype had a statistically significant effect on neuroticism and agreeableness, although single markers showed only weak associations. This observation directed our interests toward interactions between SNPs. Using epistasis calculations on agreeableness and neuroticism, we observed interaction between SNPs located at the 5' end and SNPs in the second haploblock. Previously, Zhang *et al* (2004) demonstrated that combinations of rs806379, rs1535255, and rs2023239 influenced the transcription of the CNR1 gene. Furthermore, in our study, we found that the same haploblock 1 variation (ATT in haplotypes 1 and 2) showed an opposite effect on agreeableness, neuroticism, and depression depending on the variation in haploblock 2 (haplotype 1: TGGATCT and haplotype 2: CAAATTC).

Our data emphasize the point that although separate SNPs probably explain less than 1% variance in these phenotypes (Flint *et al*, 2008; Shifman *et al*, 2008), genetic risk factors can be revealed in gene \times gene or gene \times environment interactions. Shifman *et al* (2008) in a recent whole genome association study failed to find a susceptibility locus for neuroticism in the CNR1 genetic region but they had only a 50% power to detect loci accounting for 1% of the variance. Furthermore, based on HapMap CEU data set, the SNPs covering this region using Affymetrix 500K mapping arrays are in low LD with the SNPs in our study (we found only one common SNP: rs806369).

Depression symptom scores showed significant association with the CNR1 gene after controlling for the effect of childhood adversity. This observation suggests that the CNR1 gene does not modify the influence of early trauma on current levels of depression. In contrast, the CNR1 gene significantly interacted with negative life events over the past 12 months in explaining depression scores. On the basis of this result, we propose that the CNR1 gene modifies the depressive effect of experienced negative life events in humans, which parallels the finding that CB1 $^{-/-}$ mice are more sensitive than wild-type controls to 4 weeks of chronic mild unpredictable stress in triggering learned helplessness and anhedonia (Martin *et al*, 2002; Valverde *et al*, 2005; Gorzalka *et al*, 2008).

Neuroticism can be defined as the ease with which emotions are aroused. It is a well-known predictor for lifetime depression and acts by magnifying the triggering effect of life events on the onset of depression (Kendler *et al*, 2004). About 50% of the genetic vulnerability to depression is shared with genetic risk factors for neuroticism (Kendler *et al*, 2006). Hitherto, the main identified genetic influence is the short-long polymorphism in the promoter region of the serotonin transporter gene (5HTTLPR) (Lesch, 2004). The less active short allele of the 5HTTLPR is associated with higher neuroticism (Greenberg *et al*, 2000) and it affects both the structure and function of an endophenotypic corticolimbic circuit underpinning emotion regulation (Hariri *et al*, 2006). Although the 5HTTLPR polymorphism accounts for only a few percent of the variation in neuroticism, it nevertheless shares the characteristic of

increasing sensitivity to life events in triggering depression (Caspi *et al*, 2003; Jacobs *et al*, 2006; Uher and McGuffin, 2008; Lazary *et al*, 2008). The fact that CNR1 variants are not only associated with neuroticism but also interact with recent life events to predict current depressive symptoms suggests the variants act on the core endophenotypic emotion regulation processes of neuroticism.

Neuroticism has been shown to be a strong risk factor also for anxiety (Bienvenu *et al*, 2004; Kendler *et al*, 2007). In our study, we could not demonstrate significant effect of the CNR1 gene on anxiety symptoms, although both human (Christensen *et al*, 2007) and animal (Haller *et al*, 2004a,b) data suggest that impaired CB1 receptor signaling induces anxiety. This negative result raises the possibility that interaction with other genes or environmental factors is necessary for the anxiogenic effect of the CB1 gene. Indeed, CB1^{-/-} animals showed state-dependent increase of anxiety: they behaved similarly to the wild-type animals in non-/mildly stressful paradigms in contrast to the acute highly stressful paradigms where anxiety symptoms markedly increased compared with wild-type animals (Haller *et al*, 2004a). Further studies are needed to test CNR1 gene \times gene and CNR1 gene \times environment interactions in humans.

Much less is known about the role of agreeableness in depression. In general, people who score highly for agreeableness (reflecting a prosocial cooperative nature) are more likely to cope by seeking support and using less confrontation (O'Brien and DeLongis, 1996). Most studies investigating the role of personality in depression have not reported any major effects of agreeableness (Bienvenu *et al*, 2004; Kendler *et al*, 2006). However, indirect evidence suggests that lower agreeableness predicts the development of depression in various patient groups including adolescents who suffered severe burns in early childhood (Liber *et al*, 2008), adults undergoing interferon-alpha treatment for hepatitis C (Lotrich *et al*, 2007), and patients with chronic kidney disease (Hoth *et al*, 2007). Finch and Graziano (2001) demonstrated that agreeableness exerts its effects on depression exclusively through social exchange such as social support and social conflict. In CB1^{-/-} animals, social contacts were reduced in the social interaction test (Haller *et al*, 2004a) and this is usually taken as an index of anxiety, but could indicate a lack of response to social reinforcement that might underpin the association between agreeableness and CNR1 gene variations. Furthermore, although infrequently cited, the 5HTTLPR short allele has also been associated with lower agreeableness (Greenberg *et al*, 2000). Therefore, it is possible that a high neuroticism/low agreeableness phenotype has a stronger predictive value for genetic vulnerability to depression than neuroticism alone. Further studies are needed to explore this hypothesis.

High neuroticism and low agreeableness are also risk factors for several other psychiatric conditions that are frequently co-morbid with depression. Independent of the clusters, high neuroticism and low agreeableness were consistent dimensional characteristics of personality disorders in young adults (Moran *et al*, 2006). Substance use disorders, especially smoking (Terracciano and Costa, 2004), alcohol (Malouff *et al*, 2007), marijuana (Terracciano *et al*, 2008), and polysubstance abuse (McCormick *et al*,

1998) have also been associated with a high neuroticism/low agreeableness phenotype. The CNR1 gene has been associated with substance use disorders (Zhang *et al*, 2004; Zuo *et al*, 2007) and this association might be driven by the common personality structure influenced by this gene, as we have demonstrated in this study. Another study did not find association between substance use and CNR1 gene, but they investigated only a limited part of the CNR1 gene (Herman *et al*, 2006). We found a significant interaction to exist between the two haploblocks of this gene (epistasis), and this may have an impact on the expression of CB1 receptor (Zhang *et al*, 2004). Schizophrenia has also been associated with the CNR1 gene, although with conflicting results (Levinson *et al*, 2000; Leroy *et al*, 2001; Zhang *et al*, 2004; Chavarria-Siles *et al*, 2008; Hamdani *et al*, 2008). It is important to note that schizophrenic patients compared with depressed subjects, score just as high for neuroticism but even lower for agreeableness (Bagby *et al*, 1997), particularly if they have co-morbid substance abuse disorder (Reno, 2004).

In conclusion, our results provide evidence that CNR1 gene is implicated in determining a personality phenotype (high neuroticism and low agreeableness), which has been associated with, and may be a vulnerability factor for, a variety of psychiatric conditions, including major depression and substance misuse. In addition, our finding that current depressive symptoms can be partly explained by an interaction between recent life events and variation in the CNR1 gene is further support for the eCB system playing a role in the development of depressive symptoms. Further studies are needed to evaluate the mechanism by which CNR1 gene expression and translation are controlled.

ACKNOWLEDGEMENTS

We are grateful to Heaton Mersey Medical Practice and Cheadle Medical Practice for their assistance in the recruitment. This study was supported by the Sixth Framework Program of the EU, NewMood, LSHM-CT-2004-503474.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)