Original article

The D₂ dopamine receptor (DRD2) gene is associated with co-morbid depression, anxiety and social dysfunction in untreated veterans with post-traumatic stress disorder

Bruce R. Lawford, M.D. a,b, Ross Young, Mc.D. Ph.D. b,c, Ernest P. Noble, Ph.D. M.D. b,d,*, Burnett Kann, M.D. e, Terry Ritchie, Ph.D. b

a Greenslopes Private Hospital, Brisbane, Qld., Australia
b Alcohol Research Centre, Neuropsychiatric Institute, UCLA, Los Angeles, CA, USA
c School of Psychology and Counselling, Queensland University of Technology, Carseldine, Brisbane, Qld., Australia
d Brain Research Institute, UCLA, Los Angeles, CA, USA
e Nambour Hospital, Nambour, Qld., Australia

Received 19 August 2004; accepted 14 January 2005
Available online 24 March 2005

Abstract

Objective. – To identify clusters of patients with post-traumatic stress disorder (PTSD) according to symptom profile and to examine the association of the A1 allele of the D₂ dopamine receptor (DRD2) gene with these clusters.

Method. – Fifty-seven untreated Caucasian Vietnam veterans with PTSD were administered the General Health Questionnaire-28 (GHQ) and the Mississippi Scale for combat-related PTSD. DRD2 allelic status was determined by PCR.

Results. – Subjects with the DRD2 A1 allele compared to those without this allele had significantly higher scores on GHQ 2 (anxiety/insomnia), GHQ 3 (social dysfunction) and GHQ 4 (depression). Cluster analysis of the GHQ data identified two primary groups. A high psychopathology cluster (cluster 3), featured by high co-morbid levels of somatic concerns, anxiety/insomnia, social dysfunction and depression, and a low psychopathology cluster (cluster 1), manifested by the reverse pattern. Scores in each of the four GHQ groups were significantly higher in cluster 3 than cluster 1, as was Mississippi Scale PTSD score. DRD2 A1 allele veterans compared to those without this allele were significantly more likely to be found in the high than the low psychopathology cluster group.

Conclusions. – DRD2 variants are associated with severe co-morbid psychopathology in PTSD subjects.

Keywords: PTSD; DRD2 polymorphism; Anxiety; Depression; Social dysfunction

1. Introduction

Post-traumatic stress disorder (PTSD) does not develop in all persons subjected to traumatic stress indicating considerable individual differences in susceptibility to this disorder [39]. Equally, the symptom profile in those that develop PTSD varies considerably. A genetic influence on symptomatology remains significant after accounting for the extent of combat exposure and contributes 13–34% of the variance in liability for PTSD symptoms [43]. The risk of developing PTSD following combat trauma in Vietnam was higher in those with a history of parental depression [43]. A family history of psychiatric illness is found more frequently in combat veterans with co-morbid depression and PTSD than in those with PTSD alone [24]. This co-morbidity is not associated with
stressor severity or number of traumatic events [24]. These data suggest a possible genetic pre-morbid risk in the development of co-morbid disorders in those with PTSD.

Combat-related PTSD is a highly debilitating condition with a chronic course. The quality of life of PTSD patients is frequently compromised by a variety of co-morbid conditions including social anxiety disorder, panic disorder, generalised anxiety disorder, dysthymia and major depressive disorder [17,49,31]. Both animal [38,13] and human [37,22] studies implicate the D2 dopamine receptor (DRD2) gene in the pathogenesis of anxiety and depression. Taq1A variants of the DRD2 gene are associated with marked differences in D2 receptor density and are therefore good candidates to investigate the genetic contributions to the symptoms of PTSD.

An in vitro post-mortem study using (\(^{3}\)H)spiperone [30] as a D2 dopamine receptor ligand, found a significant decrease in the number of D2 dopamine receptors in the brains of those with DRD2 A1+ (A1A1 and A1A2 genotypes) allelic status than in those with A1– (A2A2 genotype) status. Similarly, an in vivo autoradiographic study [41] using (\(^{3}\)H)raclopride as a D2 dopamine receptor ligand confirmed this finding, as have subsequent positron emission tomography (PET) studies [34,18]. The developmental impact of A1+ status is evident early in life. The A1+ allele is associated with social dysfunction in children [27]. In adolescents, an interaction between A1+ allelic status and family stress results in compromised visuo-spatial functioning [4].

As differences in D2 dopamine receptor density have been associated with depression, anxiety and impaired social function and Taq1A variants are also associated with differences in D2 dopamine receptor density, we examined whether Taq1A allelic status was associated with symptom severity (anxiety, depression and social function) in a sample of combat veterans with PTSD. The association of these variants with PTSD per se was not investigated.

2. Method

2.1. Subjects

Fifty-seven unrelated male Caucasian patients diagnosed using DSM IV criteria for PTSD were recruited for study. All subjects were Vietnam combat veterans who had served in the Australian Defence Force. None were being treated with psychotropic medication. Patients were excluded from the study if they had a diagnosis of psychosis, bipolar disorder, obsessive–compulsive disorder, or organic brain syndrome including dementia. All subjects had sufficient comprehension of English and could understand the administered questionnaires.

Patients were assessed for PTSD by a consultant psychiatrist (B.L.) or a senior psychiatric registrar (B.K.) using Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria. PTSD diagnosis was confirmed for all subjects. Furthermore, every patient exceeded the clinical cut-off score of 94 on the Mississippi Scale for combat-related PTSD [49]. The validity and reliability of the Mississippi scale are well established in veterans [20]. Patients then provided a clinical history. Demographic data including ethnic background were obtained.

The General Health Questionnaire-28 (GHQ) was administered. The GHQ measures four psychopathological factors relevant to PTSD co-morbidity: somatic concerns (GHQ1), anxiety/insomnia (GHQ2), social dysfunction (GHQ3) and depression (GHQ4). The GHQ has been widely validated internationally as a means of detecting psychiatric caseness and is sensitive to change [32]. The GHQ has utility as a follow-up measure of veteran mental health following exposure to combat [9] and is sensitive to changes in combat-related PTSD symptoms [47]. It has been used to assess symptom severity [12,25].

After the procedure had been fully explained, all participants provided written informed consent. They were able to terminate their involvement at any time during the interview without prejudice. Institutional ethics approval was obtained from the Greenslopes Private Hospital.

2.2. Genotyping

A 10 ml blood sample was drawn from each patient. Genomic DNA was extracted employing standard techniques and used as a template for determination of TaqIA DRD2 alleles by the polymerase chain reaction [14]. The amplification of DNA was carried out using a Perkin Elmer GeneAmp 9600 Thermocycler. Approximately 500 ng of amplified DNA was digested with five units of TaqI restriction enzyme (New England Biolabs) at 65 °C overnight. The resulting products were separated by electrophoresis in a 2.5% agarose gel containing ethidium bromide and visualised under ultraviolet light. Three genotypes are obtained: the A1A2 (A1– allele subjects have the A1A1 or A1A2 genotype; A1– allele subjects have the A2A2 genotype only.

2.3. Analysis

Information coded from the interviews, GHQ, Mississippi Scale and genotyping results were entered into a computer database. Nominal data were analysed by Yates corrected \(\chi^2\) test and continuous data were analysed by analysis of variance (ANOVA). A “joining tree” cluster analysis was employed to identify various symptom cluster groups [11]. \(P\) values \(\leq 0.05\) were considered statistically significant.

3. Results

The mean age of the 57 participants was 51.4 years; (S.D. = 5.3). They had the following genotypes: A1A2 (A1+
allele), n = 21; and A2A2 (A1– allele), N = 36. There were no patients with the A1A1 genotype. This genotype distribution did not deviate from Hardy–Weinberg equilibrium ($\chi^2 = 2.86, df = 1, P = 0.09$). There was no significant difference in age between A1+ and A1– allele subjects (50.9 years (S.D. = 3.6) vs. 51.6 years (S.D. = 6.0), $F = 0.30, df = 1, 55$, $P = .59$).

GHQ and allelic data are shown in Table 1. ANOVA did not reveal a significant difference in GHQ 1 scores between A1+ and A1– allele patients. However, significantly higher scores in A1+ compared to A1– allele patients were found in GHQ 2 ($F = 7.32, df = 1,55, P = .009$), GHQ 3 ($F = 7.75, df = 1,55, P = 0.007$) and GHQ 4 ($F = 12.9, df = 1,55, P = .0007$) scores. These differences remain significant after Bonferroni correction.

The GHQ data were analysed using “joining tree” cluster analysis [11] in order to determine subgroups of patients that differed according to co-morbid symptom profile. This analysis was conducted blind to allelic status. Four clusters were evident, with the GHQ factor scores in each of the clusters shown in Table 2. Table 3 contains $Z$-transformed GHQ cluster scores. Cluster 1 was characterised by low scores on all four GHQ factors; cluster 2 revealed low scores on somatic concerns and social dysfunction. Cluster 3 was characterised by raised scores on all GHQ factors, whereas cluster 4 showed raised somatic concerns and anxiety/insomnia scores but lower social dysfunction and depression scores. Only two clusters, the low psychopathology cluster 1 and the high psychopathology cluster 3 had sufficient numbers of patients for statistical analyses. ANOVA revealed that cluster 3 had significantly higher scores in GHQ factor scores in each of the clusters differed according to co-morbid symptom profile. This analysis was conducted blind to allelic status. Four clusters were evident, with the GHQ factor scores in each of the clusters shown in Table 2. Table 3 contains $Z$-transformed GHQ cluster scores. Cluster 1 was characterised by low scores on all four GHQ factors; cluster 2 revealed low scores on somatic concerns and social dysfunction. Cluster 3 was characterised by raised scores on all GHQ factors, whereas cluster 4 showed raised somatic concerns and anxiety/insomnia scores but lower social dysfunction and depression scores. Only two clusters, the low psychopathology cluster 1 and the high psychopathology cluster 3 had sufficient numbers of patients for statistical analyses. ANOVA revealed that cluster 3 when compared to cluster 1 had significantly higher $Z$ scores in GHQ 1 ($F = 36.2, df = 1,46, P = 2.7 \times 10^{-5}$), GHQ 2 ($F = 193, df = 1,46, P < 10^{-10}$), GHQ 3 ($F = 56.5, df = 1,46, P = 1.5 \times 10^{-9}$) and GHQ 4 ($F = 82.1, df = 1,46, P < 10^{-10}$).

The Mississippi scale score for combat-related PTSD of each of the four psychopathology clusters. The four cluster groups scores were significantly different ($F (3,52) = 6.62, P = 7.0 \times 10^{-6}$), with cluster 3 having a significantly higher score than cluster 1 ($F (1,45) = 18.8, P = 8.2 \times 10^{-5}$).

The final analysis examined the allelic status of subjects within the low and high psychopathology clusters. In the low psychopathology cluster 1, eight patients had the A1+ allele, whereas 24 patients carried the A1– allele. On the other hand, in the high psychopathology cluster 3, 12 patients had the

Table 1
GHQ scores in patients with the DRD2 A1+ and A1– allele*

\[
\begin{array}{lcc}
\text{GHQ number} & \text{A1+ allele} & \text{A1– allele} \\
\text{(N = 21)} & \text{(N = 36)} & \\
\hline
\text{GHQ 1 (somatic concerns)} & 11.3 \pm 1.1 & 10.2 \pm 0.7 & \text{NS} \\
\text{GHQ 2 (anxiety/insomnia)} & 14.4 \pm 1.1 & 11.0 \pm 0.7 & P = 0.009 \\
\text{GHQ 3 (social dysfunction)} & 14.4 \pm 1.0 & 11.3 \pm 0.6 & P = 0.007 \\
\text{GHQ 4 (depression)} & 13.1 \pm 1.2 & 7.8 \pm 0.9 & P = 0.0007
\end{array}
\]

* A1+ allele represents A1A1 or A1A2 genotype A1– allele represents A2A2 genotype.

Table 2
GHQ cluster scores

\[
\begin{array}{lcccc}
\text{GHQ number} & 1 (N = 32) & 2 (N = 3) & 3 (N = 16) & 4 (N = 6) \\
\text{GHQ 1 (somatic concerns)} & 7.91 \pm 0.62 & 10.00 \pm 1.16 & 14.31 \pm 0.86 & 15.33 \pm 1.26 \\
\text{GHQ 2 (anxiety/insomnia)} & 8.53 \pm 0.37 & 13.67 \pm 2.33 & 17.88 \pm 0.59 & 16.50 \pm 0.56 \\
\text{GHQ 3 (social dysfunction)} & 10.19 \pm 0.60 & 9.00 \pm 1.53 & 17.38 \pm 0.61 & 13.00 \pm 0.68 \\
\text{GHQ 4 (depression)} & 6.59 \pm 0.70 & 17.67 \pm 0.67 & 16.38 \pm 0.58 & 4.67 \pm 1.38
\end{array}
\]

Table 3
GHQ cluster scores ($Z$-transformed)

\[
\begin{array}{lcccc}
\text{GHQ number} & 1 (N = 32) & 2 (N = 3) & 3 (N = 16) & 4 (N = 6) \\
\text{Cluster scores} & & & & \\
\text{GHQ 1 (somatic concerns)} & -0.581 \pm 0.133 & -0.129 \pm 0.249 & 0.803 \pm 0.186 & 1.023 \pm 0.271 & P < 10^{-6} \\
\text{GHQ 2 (anxiety/insomnia)} & -0.767 \pm 0.077 & 0.288 \pm 0.479 & 1.153 \pm 0.121 & 0.870 \pm 0.116 & P < 10^{-6} \\
\text{GHQ 3 (social dysfunction)} & -0.516 \pm 0.138 & -0.788 \pm 0.350 & 1.131 \pm 0.140 & 0.129 \pm 0.157 & P < 10^{-6} \\
\text{GHQ 4 (depression)} & -0.524 \pm 0.118 & 1.331 \pm 0.112 & 1.115 \pm 0.097 & -0.846 \pm 0.232 & P < 10^{-6}
\end{array}
\]
A1+ allele, whereas four patients carried the A1– allele. Yate’s corrected \( \chi^2 \) statistic revealed that cluster 3 had a significantly higher prevalence of A1+ allele participants than cluster 1 (\( \chi^2 = 9.01, df = 1, P < .003 \)).

4. Discussion

The A1+ allele of the DRD2 was associated with increased GHQ anxiety, depression and social dysfunction scores in untreated Veterans with PTSD. The association between GHQ depression scores and DRD2 status is consistent with a large body of research that supports the association between dopamine deficiency and mood disorders [33]. Equally, the association between the GHQ scores of social dysfunction and anxiety with A1+ allelic status, and thus low D2 dopamine receptor density, in PTSD, is consistent with social anxiety disorder and detachment being linked to dopamine hypofunction [36]. More broadly, the association of A1+ allelic status with the processing of environmental rewards has been postulated [48]. Previous research has proposed an association between the A1+ allele and harm avoidance, a personality trait composed of worry, pessimism and shyness [16].

In addition to the associations of the A1+ allele with individual GHQ scores, A1+ allelic status was also strongly over-represented with a severe psychopathology PTSD cluster featuring more intense co-morbid symptoms of somatic problems, anxiety, social dysfunction, and depression. The two main symptom clusters found in this study are similar to those identified in other research of veterans mental health. For example, Hallman et al. [15] studied 1161 Gulf War veterans using cluster analysis and identified a group reporting good health and few moderate or severe symptoms. The second group was characterised by fair or poor health and increased frequency of moderate or severe symptoms. Equally two of the three PTSD clusters identified by Miller et al. [28] were a low psychopathology cluster and a cluster characterised by severe anxiety and depressive symptoms.

Epidemiological studies and clinical experience show that the co-morbidity of anxiety and depressive disorders is frequent [5]. There is considerable evidence that genetic risk for anxiety and depression is probably accounted for by similar or substantially overlapping genes [29]. The association of A1+ status with a cluster of severe co-morbid depression, anxiety and social dysfunction symptom individuals in a clinical sample with PTSD suggests that the Taq 1A polymorphism of the DRD2 is one of these genes.

A1+ allelic status is associated with decreased D2 dopamine receptor binding [30,41,34,18]. In non-clinical subjects, a significant relationship was found between low D2 dopamine receptor binding and the NEO personality trait of depression [21]. Regarding clinical depression, depressed patients exhibit a hypersensitive response to dextroamphetamine with severity of depression correlating highly with the rewarding effects of this agent [42]. In the laboratory, subjects administered intravenous methylphenidate experience reward that is inversely proportional to D2 dopamine receptor binding [44,45]. This suggests that depression is associated with decreased brain D2 dopamine receptor density. Similarly, subjects with schizophrenia treated with typical antipsychotic drugs show a worsening of depressive symptoms with increasing D2 dopamine receptor occupancy [7]. Moreover, a PET study of patients with schizophrenia treated with the atypical antipsychotics olanzapine or risperidone demonstrated that D2 dopamine receptor occupancy was proportional to subjective experience of depression [8].

There is considerable overlap between the pharmacotherapeutic treatment of depression and anxiety disorders and the beneficial effects of many of these agents may be mediated via the D2 dopamine receptor. For example, selective serotonin reuptake inhibitors are effective treatments for both anxiety and depressive disorders [3,35]. Fluoxetine causes an increase in nucleus accumbens shell D2 dopamine receptor mRNA resulting in increased D2 dopamine receptor postsynaptic binding in the nucleus accumbens [1]. Similarly, the SSRI citalopram increases D2 dopamine receptor mRNA in the striatum and nucleus accumbens [10]. This effect of citalopram on increased transcription of the DRD2 gene is likely to be mediated by increased serotonin levels induced by SSRIs, as 5-hydroxytryptophan also increases D2 dopamine receptor mRNA transcription [19]. D2 dopamine receptor binding increases in SSRI treatment responders and decreases in non-responders [23] suggesting that induction of D2 dopamine receptor gene expression is likely to be an important mechanism by which SSRIs exert therapeutic effects. This is a possible explanation for the finding that PTSD patients with the A1+ allele treated with the SSRI paroxetine, showed more significant improvements in social functioning than PTSD A1– allele patients [25].

Dopamine receptor ligands also have both anxiolytic and antidepressant effects [2,40]. A double-blind placebo controlled study [26] examined the effects of a DRD2 agonist, bromocriptine (BRO) and placebo (PLA) on treatment outcome in alcoholism. The results showed that in the four groups of alcoholics studied (BRO A1+ allele, BRO A1– allele, PLA A1+ allele, PLA A1– allele) the greatest and most significant decreases in craving and anxiety were found in A1+ allele alcoholics treated with bromocriptine (BRO A1+ allele). Bromocriptine has similar efficacy as imipramine and amitriptyline for the treatment of endogenous depression [6,46]. The pharmacogenetic application of SSRI’s and D2 dopamine receptor agonists may have considerable potential in the future treatment of PTSD and other anxiety/depressive disorders in A1+ allele individuals.

Although this study has a number of strengths there are also some weaknesses. The numbers of patients recruited were small; however the differences between cluster groups in terms of DRD2 allelic status were marked. The current study recruited male patients only and the results may not be generalisable to female patients or patients who were exposed to trauma other than combat (for example, trauma related to sexual abuse). The age range of patients was also restricted...
and there was a period of several decades between traumatic exposure and recruitment for this research. The implications of DRD2 A1+ allele status for the acute response to trauma are yet to be established.

In conclusion, considerable evidence emphasises the importance of D2 dopamine receptor functioning in both the aetiology and treatment of anxiety and depression. This study extends this work by confirming that the A1 allele of the DRD2 gene is specifically associated with co-morbid severe anxiety, depression and social dysfunction in combat veterans with PTSD. Furthermore, A1+ allelic status may confer a “general” vulnerability to anxiety and depression. Investigation of this polymorphism in other DSM-IV anxiety/depressive disorders is warranted as A1+ allelic individuals may score highly on anxiety/depressive symptoms regardless of actual DSM-IV diagnosis. The identification of a core genetic psychoarchitecture underlying the anxiety/depressive disorders would improve understanding of the pathophysiology of these states and may eventually lead to targeted pharmacogenetic treatment interventions.

References


[3] Berk M. Selective serotonin reuptake inhibitors in mixed anxiety–depressive disorders is warranted as A1+ allelic individuals may score highly on anxiety/depressive symptoms regardless of actual DSM-IV diagnosis. The identification of a core genetic psychoarchitecture underlying the anxiety/depressive disorders would improve understanding of the pathophysiology of these states and may eventually lead to targeted pharmacogenetic treatment interventions.


