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Association between serotonin transporter promoter polymorphisms and psychological distress in a diabetic population

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ABSTRACT

Investigations into serotonin transporter and anxiety and depression have shown an association between stress, depression onset and genotype. We investigated the relationship between 5-HTTLPR genotype and depression and anxiety in a population with diabetes mellitus, a condition associated with high rates of stress and depression. Participants were classified according to 'S' and 'L' alleles as well as the modification of the single nucleotide polymorphism (SNP) rs25531. The 5-HTTLPR low-expression genotype group (S or L_G allele carriers) had significantly higher psychological distress (K10) scores ($N=234$, $P=0.047$). Subsequent analysis revealed that the effect of genotype was related to anxiety symptoms rather than depression symptoms. Furthermore, the main effect of genotype was not observed when the modification of the SNP polymorphism was not taken into account. Findings suggest that 5-HTTLPR/rs25531 genotype is associated with psychological distress in a sample of subjects with diabetes.

1. Introduction

The relationship between the common genetic polymorphism (5-HTTLPR) in the promoter region of the human serotonin transporter gene (*SLC6A4*) and response to stress, first reported by Caspi et al. (2003), has led to considerable interest in examining the relationship between variants of the serotonin transporter and proneness to stress adaptation and depression onset. The functional 5-HTTLPR polymorphism has two primary alleles, short (S) and long (L), with the short allele being associated with lower transcriptional efficiency and thereby decreased serotonin transporter (5-HTT) expression (Lesch et al., 1996) although additional minor insertion/deletion alleles have been reported (Nakamura et al., 2000). The S and L alleles are further modified by an A/G SNP polymorphism (rs25531) in the 5-HTTLPR that also affects gene expression, such that the L_G allele shows low expression, nearly equivalent to the S allele (Wendland et al., 2006; Hu et al., 2006).

The gene (serotonin transporter genotype) × environment (stressful life events or SLEs) interaction has been replicated in subsequent studies investigating a variety of populations and stressors (reviewed in Uher and McGuffin, 2008). However, debate over the robustness of the association was triggered by publication of recent meta-analyses. Risch et al. (2009), in a meta-analysis of 14 studies, concluded there was no evidence that 5-HTTLPR genotype alone, or in interaction with SLEs, was associated with elevated risk of depression in males, females alone or both sexes. Munafo et al. (2009) reported in a meta-analysis of five studies that the interaction between 5-HTTLPR genotype and SLEs on risk for depression was negligible. However, Karg et al. (2011) noted that the Caspi et al. (2003) study reported an interaction between 5-HTTLPR and two types of stressor: (a) SLEs such as employment, financial, housing, health and relationship stressors and (b) childhood maltreatment stress. They conducted a meta-analysis of all 54 published studies, similarly stratified according to the type of stressor and reported a strong association for specific stressors such as childhood maltreatment and medical illness and a marginal association for SLEs. As no association was found when Karg et al. included only the studies

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analysed by Munafo et al. and Risch et al., they concluded that the negative findings were due to the exclusive focus on studies of SLE, as opposed to specific stressors. It was suggested that the weaker association for SLEs was due to potential biases in the SLE study design, including variable effects of the heterogeneous life events and inconsistent participant recall of stressors. Accordingly, Karg et al. reported stronger evidence among studies which assessed SLEs with objective measures than those which used self-report (see also Caspi et al., 2010; Rutter, 2010, for review).

In an attempt to avoid such problems, recent studies have examined gene × environment (g × e) interactions in populations experiencing substantial homogenous stressors associated with high rates of depression. Positive associations have been reported in populations experiencing specific medical illnesses including Parkinson's disease, migraine, coronary disease, stroke, hip fracture, and interferon treatment (Mossner et al., 2001; Lenze et al., 2005; Nakatani et al., 2005; Ramasubbu et al., 2006; Gonda et al., 2007; Otte et al., 2007; Kohen et al., 2008; Bull et al., 2009; see Karg et al., 2011, for review).

Depression is twice as common in persons with diabetes as in non-diabetic controls (Anderson et al., 2001) and meta-analysis indicates greater odds of depression (Ali et al., 2006) and generalised anxiety disorder (Grigsby et al., 2002) amongst people with type 2 diabetes than those without. Diabetes is characterised by daily 'hassles' such as strict dietary and treatment regimens, which often include frequent injections, threat of hypoglycaemic events, medical complications and frustration of poor control despite strict adherence. The associated stress is thought to contribute to high rates of anxiety and depression found in people with diabetes (Aikens et al., 2009). Despite the high prevalence of depression in diabetes, to date no research has investigated the role of the serotonin transporter genotype.

The relationship between diabetes and depression is complex, whereby depression itself is a risk factor for the onset of type 2 diabetes (Knol et al., 2006), potentially mediated via cortisol dysregulation and insulin resistance (Pan et al., 2008). However, diabetes-related distress has been shown to be predictive of depressive symptomatology one year after diagnosis, even when controlling for baseline depressive symptoms (Skinner et al., 2010), suggesting that the stress of the illness itself is a contributor to the higher incidence of depression, over and above pre-existing risk factors. Furthermore, Aujla et al. (2010) reported no significant association between impaired glucose regulation and depressive symptoms in people newly diagnosed with Type 2 diabetes, suggesting that it is the stress of experiencing diabetes after diagnosis, rather than the intrinsic impaired glucose regulation, that contributes to the higher incidence of depression.

The study described in this paper aimed to examine the relationship between serotonin transporter genotype and depression, anxiety and anxiety-related traits in a population with diabetes mellitus. Expression assays of 5-HTTLPR/rs25531 genotypes show co-dominant allele action and low, nearly equivalent expression for the L_G and S alleles (Hu et al., 2006). We hypothesised that in the presence of a chronic stressor (i.e. having diabetes), individuals with the S and L_G alleles would be more likely to report depressive and anxiety disorders as well as higher levels of symptoms of depression, neuroticism and psychological distress.

2. Method

2.1. Participants

Participants were recruited from Diabetes Clinics at St. Vincent's Hospital and the Prince of Wales Hospital, Sydney. Patients were approached in the waiting room by study investigators and provided with a participant information sheet and flyer. These patients were attending a clinic for people with established diabetes, often with complications, rather than newly diagnosed diabetes. Written informed consent was obtained from patients, who were eligible to participate if they had either type 1 or type 2 diabetes, were aged 18 or over and were able to read and write in English. Exclusion criteria included cognitive impairment, psychotic illness, severe depressive illness or the occurrence of a severe life event in the past month.

Approximately 60% of those approached agreed to participate in the study. The remainder fell roughly into three groups: those who did not meet the exclusion criteria and two groups of 'refusers'. The 'refusers' were more likely to be young or middle-aged males who stated (i) they were already "involved with too many people at the clinic" or (ii) they were "in a rush" to go to work or other commitments.

A total of 254 participants aged between 23 and 84 ($M=57.4$, S.D.$=13.5$) were recruited between July 2006 and March 2008. One hundred and forty five (57.1%) participants were male and 66 (26.3%) had Type 1 diabetes.

2.2. Measures

Diagnosis of current anxiety and depressive disorder was made using the Patient Health Questionnaire (PHQ) (Spitzer et al., 1999). The PHQ is a 60-item self-report diagnostic questionnaire used to diagnose eight current disorders using DSM-IV criteria. These include Major Depressive Disorder (MDD), Panic Disorder (PD) and Other Anxiety Disorder (OAD). Participants were classified as having an anxiety disorder if they met criteria for PD or OAD. It is suggested that these diagnoses are confirmed at a clinical interview (see below). The validity of the PHQ as a measure of current MDD has been well established with structured clinical interviews, including the Structured Clinical Interview for DSM disorders (SCID) as the validation criterion (Grafe et al., 2004).

The PHQ-9 comprises the nine-item depression scale of the PHQ. Participants are asked to rate whether nine symptoms of depression applied to them '*not at all*', '*several days*', '*more than half the days*', or '*nearly every day*' over the past two weeks. Responses can be used in two ways: (i) to make a tentative diagnosis of major depression, if a question about role dysfunction is endorsed and four or more symptoms are rated as present '*more than half the days*' or '*nearly every day*' (except for suicidal ideation which is included if any response other than '*not at all*' is endorsed') and (ii) to calculate a depression severity score, where '*not at all*'=0 and '*nearly every day*'=3, to help select and monitor treatment. The instrument is intended to be presented to the clinician for further clarification. It has been found to be useful in the detection of major depression (Spitzer et al., 1999) and assessment of depression severity and has established criterion, construct and external validity (Kroenke et al., 2001).

A clinical interview was conducted with one of the two study psychiatrists (JR or KW) in the diabetes clinic immediately after completion of self-report measures. Responses to the PHQ were reviewed at this interview and participants were then asked a series of structured questions and shown a list of psychotropic medications to act as a memory prompt. The aim was to: (i) assess current diagnoses and treatment, (ii) clarify physical health issues and (iii) assess past mental health and treatment history.

Neuroticism was measured with the relevant sub-scale from the 60 item NEO Five Factor Inventory (NEO-FFI) (Costa and McCrae, 1992). Participants are asked to rate the applicability of statements to themselves on a five point Likert type scale where 1=*strongly disagree* and 5=*strongly agree*.

The 10-item Kessler psychological distress scale (K10) (Kessler et al., 2002) was used to measure psychological distress experienced '*over the past 30 days*'. It is suitable for screening for cases in the general population and there is a strong association between a high K10 score and current Composite International Diagnostic Inventory (CIDI) diagnosis of anxiety or affective disorder (Andrews and Slade, 2001).

2.3. DNA extraction and genetic analysis

Genomic DNA was extracted from cheek swabs according to the method described in Wilhelm et al. (2006). Phase-known 5-HTTLPR and rs25531 genotypes were determined by a modification of the procedure by Wendland et al. (2006). Genomic DNA was amplified using the QIAGEN Multiplex PCR Kit (QIAGEN Pty Ltd., Victoria, Australia) with the following primers: forward, 5′-TCCTCCGGTTTGGCGCCTCTTCC-3′; reverse, 5′-TGGGGGTTGCAGGGGAGATCCTG-3′. PCR amplification conditions were as follows: 94 °C for 15 min; 38 cycles of 94 °C for 30 s, 66 °C for 45 s and 72 °C for 60 s; followed by 72 °C for 10 min. Amplicons were digested with *Hpa*II, and fragments were separated by agarose gel electrophoresis. The forward primer introduces an additional *Hpa*II site to enable efficient digestion of the amplicon. Fragment sizes (in bp) for each allele were therefore as follows: L_A, 506+6; L_G, 396+110+6; S_A, 463+6; S_G, 396+67+6. All genotypes were scored independently by two researchers.

To account for the effect of the A/G SNP rs25531 polymorphism, we grouped people according to their predicted levels of 5-HTT expression: 'Low' (S_AS_A, S_AS_G, S_AL_G, S_GL_G and L_GL_G genotypes), 'Medium' (S_AL_A, S_GL_A and L_AL_G genotypes), or 'High' (L_AL_A genotypes). Of the 254 participants, 72 (28.3%) were classified as 'Low', 129 (50.8%) as 'Medium' and 49 (19.3%) as 'High'. Four participants (1.6%) had ambiguous genotypes and were not included in the statistical analyses.