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Short Communication

CTLA-4 single-nucleotide polymorphisms in a Caucasian population with schizophrenia

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ABSTRACT

Associations between a single-nucleotide polymorphism (SNP) in exon 1 of the cytotoxic T-lymphocyte antigen-4 (*CTLA4*) gene and schizophrenia in a Korean population have been previously described. The current study investigated whether a similar link occurs in a Caucasian population with schizophrenia. One hundred and twenty-two age- and sex-matched pairs of people with DSM-III-R diagnosis of schizophrenia and healthy controls were included in this study. Three previously described SNPs (from the promoter, exon 1 and 3′ UTR) of the *CTLA4* gene were analysed. In the entire sample, we detected no allelic or genotypic association for any of the three SNPs. Given documented gender differences in incidence of schizophrenia, we conducted separate analyses of male and female participants. In males, both the promoter region SNP (−318C/T) and the 3′ UTR SNP demonstrated nominally significant association with schizophrenia. The 3′ UTR SNP remained significant following correction for multiple testing (permuted $P = 0.046$). In addition, all possible haplogenotypes showed significant association with disease in males with two – both containing the 3′ UTR SNP – remaining significant following correction for the genotypic tests of all SNPs and haplogenotypes in males. These results suggest a role for the 3′ UTR SNP and/or variants in high linkage disequilibrium with this SNP in the pathogenesis of schizophrenia.

1. Introduction

Schizophrenia (SZ) is a chronic debilitating psychiatric disorder that affects ~1% of the global population. It is characterised by persistent disruptions in language, thought and perception with associated functional impairment. The aetiology of this disorder is unknown. However, aberrant immune function has long been reported (Rothermundt et al., 2001) and SZ shares several features with known autoimmune diseases. An immune or autoimmune mechanism may thus be involved in at least some cases of SZ (Jones et al., 2005).

A critical component of appropriate immune response is the T-cell system, the regulation of which is necessary for peripheral tolerance (Janeway et al., 2001). T-cell-associated dysfunction, particularly dysregulation of interleukins (IL)-2, -6 and -10 (Potvin et al., 2008) has commonly been reported in SZ. Therefore, genes controlling T-cell responses may play a role in SZ.

CTLA4 maps to 2q33 and encodes a membrane protein of the immunoglobulin superfamily which is expressed on activated T cells (Converse and Hamosh, 2006). Upon binding its costimulatory ligands, the CTLA-4 protein transmits an inhibitory signal to downregulate T-cell activation and reactivity, and helps to establish and maintain peripheral T-cell tolerance. Mutations in *CTLA4* have been associated with several autoimmune diseases (Kristiansen et al., 2000; Ueda et al., 2003).

In view of *CTLA4*'s central role in immune system function, the association of *CTLA4* SNPs with autoimmune diseases and the potential autoimmune basis for SZ, one study has investigated *CTLA4* SNPs in SZ. Jun et al. (2002) reported significant association of a SNP (A49G) in exon 1 with SZ in a Korean population.

In our study, we conducted association analyses of three *CTLA4* SNPs in 122 Caucasian SZ cases and 122 healthy, matched controls. These SNPs were selected based on their association with autoimmune or immune-mediated diseases (Kristiansen et al., 2000; Ueda et al., 2003). We aimed to determine whether the previously reported SZ association could be replicated in an ethnically different population and also assess the association of two other *CTLA4* SNPs in SZ. We also aimed to determine whether any association would

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be different between genders given the difference in incidence of SZ (McGrath et al., 2004).

2. Methods

2.1. Subjects

This study was conducted using 122 Australian Caucasian matched sample pairs, consisting of 122 cases with a DSM-III-R (American Psychiatric Association, 1987) diagnosis of SZ and 122 age- and gender-matched controls. The case and control groups each contained 81 men and 40 women. These samples were selected from a larger cohort ($n = 310$) initially recruited for the 2002 Australian national prevalence study of psychosis (McGrath et al., 2002), for which diagnoses were assigned using the Diagnostic Interview for Psychosis (DIP) (Castle et al., 2006). Individuals were included in the current study if they were Caucasian, had a confirmed diagnosis of SZ and a viable DNA sample available at the time of testing. The mean age of the study participants was 41.6 ± 12.3 years for cases and 42.0 ± 13.1 for controls. All participants provided written informed consent prior to participation, and the appropriate Institutional Ethics committees approved this study.

2.2. DNA preparation

DNA was extracted from immortalised lymphoblastoid cell lines (LCLs) derived from case and control blood samples, using an adapted version of the salting out technique (Miller et al., 1988). The DNA samples were then stored at −80 °C in 50 ng/μL aliquots until sequenced.

2.3. SNP sequences and primers

The SNPs analysed in this study are given in Table 1. SNP sequences were determined using the SEQUENOM RealSNP™ Assay Database and the NCBI Reference SNP (RefSNP) database. Primers were designed by the Australian Genome Research Facility (AGRF) (St. Lucia, Queensland, Australia) using the published SNP sequence and 50 bp overlap.

2.4. SNP genotyping

SNP genotyping was conducted by the AGRF using the Homogenous MassExtend™ and iPlex™ assays (Oeth et al., 2005) and PCR products were then analysed on the SEQUENOM Autoflex™ mass spectrometer (Sequenom Inc., San Diego, America).

2.5. Association analysis

Association analysis of individual SNPs and multi-locus haplotypes was performed using the UNPHASED (Dudbridge 2008) program. *P* values corrected for the multiple markers or haplotypes tested were calculated using a permutation test (1000 replicates) in which the "case" and "control" labels were reassigned. A *P* value of less than 0.05 was considered statistically significant.

3. Results

Genotype frequencies for each of the SNPs were calculated in controls and found to be in Hardy–Weinberg equilibrium ($P > 0.05$). Allelic tests of association showed no significant association of any of the three SNPs with SZ (Table 2). However, the promoter region SNP (−318C/T transition) yielded a *P* value approaching significance (pointwise $P = 0.055$; permuted $P = 0.13$), with increased frequency of the C allele in affected individuals. Genotypic tests (Table 2) detected nominal association of this SNP ($P = 0.045$), with an increased frequency of the CC genotype in cases. However, this association did not survive correction for testing multiple SNPs (permuted $P = 0.15$).

Evidence for association was also assessed for all possible multimarker haplotypes constructed from the three *CTLA4* SNPs. In this sample, we observed three of the four possible 2-marker haplotypes and four of the eight possible 3-marker haplotypes. No observed haplotype showed evidence of association at nominal $P < 0.05$.

Since SZ occurs more frequently in males than in females, we conducted separate association tests in male and female participants (Table 3). In allelic tests, the promoter region SNP showed nominally significant association in males (pointwise $P = 0.027$; permuted $P = 0.055$), with an increased frequency of the C allele in affected individuals. In genotypic tests, nominally significant association was detected for the promoter SNP ($P = 0.02$), which showed increased frequency of the CC genotype and decreased frequency of the CT genotype in cases. Nominally significant association was also observed for the 3′ UTR SNP ($P = 0.017$), with decreased frequency of the two homozygote genotypes (AA and GG) and increased frequency of the heterozygote genotype (AG) in cases. The 3′ UTR SNP remained significant following correction for testing the three SNPs in males (permuted $P = 0.046$).

Evidence for association in males was not improved by constructing haplotypes from individual SNP alleles. However, nominally significant association was observed in all four haplotypic tests incorporating genotypes of the three SNPs (three 2-marker tests and one three-marker test) (Table 4). Globally significant association (nominal $P < 0.012$, representing the permuted 5% quantile, incorporating permutation-based correction for all SNP

Table 1
CTLA4 SNP details

SNP No.	SNP[a]	Position[b] (bp)	Gene region	Alleles
1	rs5742909	−318	Promoter	C/T
2	rs231775	+49	Exon 1	A/G
3	rs3087243	+6230	3′ UTR	A/G

[a] Identification code in the SEQUENOM RealSNP™ assay database or NCBI refSNP database.
[b] Position in base pairs (bp) relative to the translation initiation site of *CTLA4*.

Table 2
Results of allelic and genotypic tests for the three *CTLA4* SNPs

	Allele/genotype	Schizophrenia[a]	Control[a]	*P*
Promoter −318 C/T				
Allelic	C	226	217	0.055
	T	12	23	
Genotypic	CC	107	97	0.045
	CT	12	23	
	TT	0	0	
Exon 1 +49 A/G				
Allelic	A	141	137	0.71
	G	101	105	
Genotypic	AA	40	39	0.88
	AG	61	59	
	GG	20	23	
3′ UTR A/G				
Allelic	A	107	97	0.36
	G	133	143	
Genotypic	AA	21	22	0.25
	AG	65	53	
	GG	34	45	

[a] Allele/genotype counts in case and control groups.