The C/C genotype of the C957T polymorphism of the dopamine D2 receptor (DRD2) is associated with schizophrenia.

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Abstract

The T allele of the human dopamine D2 receptor (DRD2) gene C957T polymorphism is associated with reduced mRNA translation and stability. This results in decreased dopamine induced DRD2 upregulation and decreased in-vivo D2 dopamine binding. Conversely, the C allele of the C957T polymorphism is not associated with such changes in mRNA leading to increased DRD2 expression. PET and post-mortem binding studies show that schizophrenia is often associated with increased DRD2 availability. We report that on the basis of comparing the frequencies of the C/C and T/T genotypes of 153 patients with schizophrenia and 148 controls that schizophrenia is associated with increased DRD2 availability. We report that on the basis of comparing the frequencies of the C/C and T/T genotypes of 153 patients with schizophrenia and 148 controls that schizophrenia is associated with increased DRD2 availability. The C957T shows a population attributable risk for schizophrenia of 24% and an attributable risk in those with schizophrenia of 42%. Increased expression of D2 receptors associated with the C allele is likely to be important in the underlying pathophysiology of at least some forms of schizophrenia. Enhanced understanding of schizophrenia afforded by this finding may lead to advances in treatment and prevention.

Author Keywords: Association; Dopamine; D2 receptor; Genetic; Schizophrenia, C957T.
Schizophrenia is a common, chronic, disabling illness with an incidence of 15 new cases per 100,000 population per year with an onset typically in late adolescence or early adulthood (Kelly et al., 2003). Additionally, first-degree relatives show cognitive deficits from childhood (Niendam et al., 2003) into adulthood (MacDonald, 2003) indicating the presence of pre-morbid markers of the disorder. Siblings of schizophrenic patients exhibit an abnormal fMRI response in the dorsolateral prefrontal cortex resulting in information processing deficits (Callicott et al., 2003). Both those with schizophrenia and their unaffected siblings show reductions in hippocampal volume and a hippocampal shape deformity (Tepest et al., 2003). Decreased temporoparietal P300 amplitude and increased frontal P300 amplitude are also found in both schizophrenic patients and their siblings (Winterer et al., 2003).

Twin study data reveal that the heritability of schizophrenia is about 80% (Sullivan et al., 2003). The high genetic risk for schizophrenia has led to considerable research efforts aimed at the identification of susceptibility genes, including large linkage studies (for example; Mowry et al., 2004). Significant linkages with specific chromosomal regions have been identified (Owen et al., 2004). Despite this progress, the conclusive identification of specific molecular genetic aetiological factors in the pathogenesis of schizophrenia that account for significant risk has not yet occurred.

Several lines of evidence implicate the dopamine D2 receptor (DRD2) gene as a candidate gene for susceptibility to schizophrenia. All antipsychotic medications are either antagonists or partial agonists of the dopamine D2 receptor, which is the primary site of action for these medications (Miyamoto et al., 2004). Schizophrenic symptoms are ameliorated by a reduction in dopamine D2 receptor function. Additionally, neuroimaging evidence indicates that schizophrenic patients have increased brain dopamine D2 receptor density (Seeman & Kapur, 2000). For example, untreated patients with schizophrenia and controls pharmacologically depleted of endogenous dopamine have been studied using in-vivo measurements of D2 receptor availability made both before and after dopamine depletion (Abi-Dargham, et al., 2000). The schizophrenic subjects demonstrated a larger increase in D2 receptor availability post-dopamine depletion than controls. These data suggest that schizophrenia is characterized by a physiological state of increased D2 receptor availability.
Association studies of polymorphisms of the DRD2 have generally yielded results that are just significant, are inconsistent across different ethnic groups, or fail to reach significance (see Table 1 for a summary of studies published in English). The most consistent association identified in Table 1 is between the A2 allele of the Taq 1A polymorphism of the DRD2 gene and schizophrenia (Dubertret, et al., 2001, 2004) however this association is of a small magnitude. The -141 C Ins/del has shown a significant association with schizophrenia; however these associations have been in the opposite direction in Japanese and Scandinavian participants (Arinami, et al., 1997; Ohara et al., 1998; Jonsson et al., 1999) when compared to their British counterparts (Breen et al., 1999).

A synonymous polymorphism in the DRD2 gene, the C957T is in linkage disequilibrium with Taq 1A. The C957T has been associated with in vitro functional effects (Duan et al., 2003) that have considerable potential implications for genetic risk for schizophrenia. The T allele is associated with decreased translation of DRD2 mRNA, decreased DRD2 mRNA stability and markedly diminished dopamine induced upregulation of D2 receptors. Furthermore, the C957T affects striatal dopamine D2 binding in healthy subjects (Hirvonen et al., 2004). Subjects homozygous for the C allele (C/C) have the highest striatal binding. Heterozygous individuals (C/T) have intermediate binding whilst individuals homozygous for the T allele (T/T) have the lowest binding. As schizophrenia is associated with increased D2 availability we postulated that the C/C genotype, which is associated with a greater expression of D2 receptors, would be found more frequently in schizophrenia. Conversely, the T/T genotype would be protective against schizophrenia and have a decreased frequency amongst schizophrenic patients. The C/T genotype, with intermediate binding, was postulated to be found at the same frequency in both schizophrenic and control subjects.

2. Method

2.1. Subjects

To evaluate the frequency of the C and T alleles of the C957T polymorphism in unrelated Caucasian patients with schizophrenia, we genotyped 153 patients with schizophrenia and 148 Caucasian controls. To avoid population stratification bias both control and clinical subjects were of mixed Northern European origin from the same breeding population. The study is more than sufficiently powered with an N = 301 to detect a small-medium effect in allelic frequency between the two groups. For example, to detect a real difference in allelic frequency with a power of 0.8 (α = 0.05), d.f. = 1, Lambda = 7.88, and an effect size of 0.2; a minimum sample of 197 would be necessary (Cohen, 1988).

Inclusion criteria for patients were being aged between 18 and 65 years and having a stable DSM IV diagnosis of schizophrenia. These patients had no other psychiatric disorder, including schizoaffective disorder, major depressive episode with psychotic features, or substance misuse. There were 20 females and 133 males in the group diagnosed with schizophrenia. They had a mean age of 36.2 years (s.d. ± 12.3 years). Patients were being treated at the Royal Brisbane and Women's Hospital, The Park...
Psychiatric Unit and the Valley Community Mental Health Centre. No patients were treated with regular antidepressant, anxiolytic or mood stabilising psychotropic medication. The sample was composed of 69 inpatients and 84 outpatients.

While the patients did not meet criteria for a substance dependence disorder self-report data indicated that 47 patients reported binge drinking (defined as any drinking in the last 12 months where alcohol consumption exceeded 40 grams per day for women and 60 grams per day for men). A significant number of patients (N=74) also described severe past psychological distress as indicated by a suicide attempt. A total of 121 patients were able to provide information on self-reported biological family history of psychiatric illness with 82 patients reporting a positive history of schizophrenia. As such, the sample represents a group with schizophrenia who have minimal psychiatric comorbidity but contains significant proportions of individuals with a relatively severe history and/or a familial risk for psychosis.

There were 43 females and 105 males in the control group with a mean age of 36.8 years (s.d. ±12.8 years). The control group was composed of hospital nursing and medical staff as well as university staff and students. Ethics approval was obtained from the various institutions involved.

2.2. Genotyping

Genotyping was performed by kinetic real-time PCR using the Applied Biosystems 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA). Sequence specific primers were designed for the C allele (5′-ATGGTCTCCACAGCACTCTC-3′), the T allele (5′-ATGGTCTCCACAGCACTCTT-3′) and a common reverse primer (5′-CATTGGGCATGGTCTGGATC-3′). A total of 5-10 ng of genomic DNA was amplified in 1 x SYBR green PCR master mix (Applied Biosystems) containing 0.4 µM of allele specific forward primer and 0.4 µM of common reverse primer in a 25 µL volume. Amplification conditions were; 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. A cycle time (Cₜ) value was obtained by setting the threshold during geometric phase of amplification and scored relative to the ΔCₜ generated between the matched and mismatched primer pairs.

2.3 Statistical analysis

Chi-squared statistic was employed to evaluate differences in frequency of the C and T alleles of the C957T polymorphism found in the control and schizophrenic groups.

3. Results

The observed frequency of the C and T alleles in schizophrenic and control individuals showed a significant difference in allele frequency between the two groups with the C allele being found more frequently in the schizophrenia group (χ²=11.219, p=0.0008, Table 2). The genotypes of both groups are displayed in Table 3. As the maximum functional difference in binding occurs between the C/C and T/T genotypes the frequency of C and T alleles in homozygotes were compared in the schizophrenia and control groups. The C allele was significantly associated with
schizophrenia ($\chi^2=20.46$, $p<0.00001$). Upon inspection of Table 3 it is evident that there is no difference in the frequency of C/T heterozygotes.

Both schizophrenic and control groups are in Hardy-Weinberg equilibrium based on the respective allele frequencies of each group. Assuming a prevalence of schizophrenia of 1% of the population, the population attributable genetic risk for this polymorphism is 24% (CI=10%-35%). The attributable risk in the schizophrenic population is 42% (CI=20%-58%)(Abramson & Gahlinger, 2001)

Table 2. Allele frequencies of the C and T alleles of the C957T polymorphism in schizophrenic and control groups.

<table>
<thead>
<tr>
<th>Allele Frequency</th>
<th>C</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls n=296</td>
<td>124 (41.9%)</td>
<td>172 (58.1%)</td>
</tr>
<tr>
<td>Schizophrenia* n=306</td>
<td>171 (55.5%)</td>
<td>137 (44.5%)</td>
</tr>
</tbody>
</table>

* $\chi^2 = 11.219; P = 8 \times 10^{-4}$

Table 3. Genotype frequencies of the C/C, C/T, and T/T alleles of the C957T polymorphism in schizophrenic and control groups.

<table>
<thead>
<tr>
<th>Genotype frequency</th>
<th>C/C</th>
<th>C/T</th>
<th>T/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls n=148</td>
<td>27 (18.2%)</td>
<td>70 (47.3%)</td>
<td>51 (34.5%)</td>
</tr>
<tr>
<td>Schizophrenia n=153</td>
<td>48 (31.2%)</td>
<td>75 (48.7%)</td>
<td>31 (20.1%)</td>
</tr>
</tbody>
</table>

4. Discussion

We report an association between C957T variants and schizophrenia that exceeds the magnitude of other findings examining DRD2 polymorphisms (see Table 1). These results suggest that in addition to the D2 receptor being centrally involved in the action of antipsychotic medication (Kapur & Mamo, 2003), genetically determined differences in D2 upregulation are at least partially involved in the underlying pathophysiology of schizophrenia.

When our results are considered together with functional studies of the C957T polymorphism (Duan et al., 2003) a scientifically plausible link can be found between
gene function and the underlying pathophysiology of schizophrenia. The decreased translation and stability of mRNA and consequent reduction in dopamine induced upregulation of DRD2 expression produced by the T/T genotype appears to result in a lower likelihood of developing schizophrenia. The C/C genotype may constitute a risk factor for this disorder. These findings are consistent with the known association of both schizophrenia and the C/C genotype with increased striatal D2 receptor availability (eg, Miyamoto et al., 2004; Seeman & Kapur, 2000; Hirvonen et al., In press).

A meta-analysis of post-mortem and in-vivo studies of D2 receptor density in brains of schizophrenic subjects conducted between 1980 and 1996 revealed that increased D2 receptor binding could only be found in 70% of cases. In 30% of patients D2 receptor binding could not be distinguished from that found in normal controls (Zakzanis & Hansen, 1998). Although increased binding is found in the majority of people with schizophrenia a significant proportion do not exhibit this finding. Furthermore, in a recent study of ten drug naïve patients with schizophrenia, thalamic D2 receptor binding was reduced when compared with that of unaffected controls (Yasuno et al., 2004). In our sample, 20.1% of patients with schizophrenia have the T/T genotype and, based on in vitro (Duan et al, 2003) and in-vivo data (Hirvonen et al., 2004) have decreased D2 binding. Equally 79.9% will have intermediate or increased binding (C/C and C/T genotypes). In the control group, 34.5% have decreased binding with 65.5% having intermediate or increased binding. These data are consistent with the observation that although D2 receptor binding tends to be increased in schizophrenia this marker is not specific for the illness in general. Given this lack of specificity, neuroimaging studies of small numbers of patients and controls have the potential to report unreliable results depending on the proportion of genotypes selected.

Our data also suggest involvement of other mechanisms and genes in the pathogenesis of schizophrenia. Evidence is accumulating that in addition to hyperstimulation of striatal D2 receptors, deficient stimulation of prefrontal D1 receptors and N-methyl-d-aspartate (NMDA) hypofunction are all associated with this disorder (Laurelle, et al., 2003). These findings are not mutually exclusive, as functional links exist between the systems involved. Upregulation of prefrontal D1 receptors may result from dopamine deficiency in the cortex leading to the negative and cognitive symptoms of schizophrenia whilst subcortical excess of dopamine is postulated to be responsible for positive symptoms. Furthermore, dopamine D2 receptors inhibit NMDA induced glutamate release in the substantia nigra (Marti, et al., 2002) and transactivate a receptor tyrosine kinase to inhibit NMDA receptor transmission in pyramidal neurons (Kotecha, et al 2002). Increased translation and stability of DRD2 mRNA and consequent augmentation of in-vivo dopamine D2 expression in subjects with the C/C genotype may result in NMDA hypofunction. Conversely the T/T genotype, which is associated with decreased in vitro translation and stability of DRD2 mRNA, as well as reduced in-vivo dopamine D2 receptor expression, can produce reduced inhibition of NMDA receptors. This allele is found less commonly in patients with schizophrenia. The tendency for high dopamine D2 binding to be associated with schizophrenia may be at least partly responsible for the NMDA hypofunction found in this disorder.

A recent review describes the lack of convergence between studies of gene expression conducted to date and neuropathology in schizophrenia (Harrison & Weinberger, In
press). Specifically, the link between gene function and the psychopathophysiology of schizophrenia has not been demonstrated with a range of putative susceptibility genes including neuregulin, dysbindin, DISC1, RGS4, GRM3, G72. Harrison & Weinberger (in press) state that, with the possible exception of COMT, no causative mechanism to explain why these genes predispose to schizophrenia has been identified. Our findings offer a scientifically plausible and theory driven link between gene function and the known psychopathophysiology associated with schizophrenia. Indeed, Harrison & Weinberger (in press) state that "characterisation of a core molecular pathway and a "genetic cytoarchitecture" would be a profound advance in the understanding of schizophrenia which may also have equally significant therapeutic implications".

Antipsychotic medication depends on dopamine D2 antagonism and may be more efficacious in C/C patients who have an enhanced capacity for dopamine upregulation compared to T/T homozygous patients. This marker may be a useful focus for pharmacogenetic research. Environmental influences such as illicit drug use and stress are also known to be important in the aetiology of schizophrenia and stimulate dopamine release (Howes et al., 2004). Individuals with the C/C genotype may be more susceptible to such environmental influences as a result increased dopamine D2 receptor expression and as such this genotype may be a useful marker for studies examining the development of schizophrenia. Improving the identification of those who may be at increased risk for this disorder, or a specific subgroup of these individuals, would allow for the development of effective preventative strategies aimed at minimizing such environmental risk. Knowledge of parental and sibling C/C or T/T genotypes is likely to assist family counselling for those with an affected family member (Hodgkinson et al., 2001).

Limitations of this study include that the findings cannot be generalised to other psychotic disorders, for example schizoaffective disorder or mood disorders with psychotic features. The strength of the results is tempered by small sample size and this finding needs further replication in a larger sample. Future research should also examine whether specific phenotypic characteristics, such as symptom profile, age of onset and treatment response is associated with C957T polymorphisms and examine the generalisation of this to other ethnic groups.

5. Conclusions.
The proportion of attributable risk indicates that the C/C genotype of the C957 polymorphism is a major factor in the heritability of schizophrenia, or for a large subgroup of those with schizophrenia. This highly significant result, which is consistent with both known in vitro and in-vivo gene function and binding studies, indicates likely aetiological pathophysiological significance and is likely to enable major advances in diagnosis and treatment.

6. Acknowledgements
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Competing Interests Statement
The authors declare that they have no competing financial interests.
References


Table 1. Association Studies of DRD2 polymorphisms and schizophrenia

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Study Years</th>
<th>Sample Size</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq 1A</td>
<td>Dubertret et al, 2001</td>
<td>50 patients, 50 controls</td>
<td>A2 Significant (only in those with an onset over 20 years of age)</td>
</tr>
<tr>
<td></td>
<td>Dubertret et al, 2004</td>
<td>103 patients, 83 controls</td>
<td>A2 Significant</td>
</tr>
<tr>
<td>Ser311Cys</td>
<td>Itokawa et al 1993</td>
<td>50 patients, 110 controls</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td>Aranami, et al, 1994</td>
<td>156 patients, 300 controls</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>Laurent et al, 1994</td>
<td>113 patients, 184 controls</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td>Hattori, et al 1994</td>
<td>100 patients, 100 controls</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td>Kaneshima et al, 1997</td>
<td>78 patients, 112 controls</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td>Hori, et al, 2001</td>
<td>241 patients, 201 controls</td>
<td>Not significant</td>
</tr>
<tr>
<td>-141C Ins/del</td>
<td>Arinami et al, 1997</td>
<td>260 patients, 312 controls</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>Stober et al, 1998</td>
<td>260 patients, 290 controls</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td>Ohara et al, 1998</td>
<td>170 patients, 120 controls</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>Tallerico, Upian &amp; Liu, 1999</td>
<td>50 patients, 51 controls</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td>Breen et al, 1999</td>
<td>439 patients, 437 controls</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>Jonsson et al, 1999</td>
<td>129 patients, 179 controls</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>Hori et al, 2001</td>
<td>241 patients, 241 controls</td>
<td>Not significant</td>
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