Association between schizophrenia and homozygosity at the dopamine D3 receptor gene

M-A Crocq, R Mant, P Asherson, J Williams, Y Hode, A Mayerova, D Collier, L Lannfelt, P Sokoloff, J-C Schwartz, M Gill, J-P Macher, P McGuffin, M J Owen

Abstract
Disturbances in dopamine neurotransmission have been postulated to underlie schizophrenia. We report data from two independent studies of a BalI polymorphism in the dopamine D3 receptor gene in patients with schizophrenia. In both studies, more patients than controls were homozygous (p = 0.005, p = 0.008). When pooled data were analysed, this difference was highly significant (p = 0.0001) with a relative risk of schizophrenia in homozygotes of 2.61 (95% confidence intervals 1.60-4.26).

Evidence from family, twin, and adoption studies suggests that there is an important genetic contribution to the aetiology of schizophrenia.1 However, the mode of inheritance, like that of other common disorders, is complex and non-mendelian. Liability to develop the disease probably reflects variation at several (oligogenic) or many (polygenic) genetic loci in combination with a number of different environmental contributors, although genes of major effect may be operating, particularly in some multiply affected families.2

Disturbances in dopamine neurotransmission and dopamine receptors have long been postulated to underlie schizophrenia.3 Until recently, it was widely accepted that dopamine affects its target cells by interaction with only two receptor subtypes, known as D1 and D2, which differ from each other in pharmacological specificity and in having the opposite effect upon adenylate cyclase.4 It also appeared that the therapeutic effects of antipsychotic drugs were related to their high affinity for the D2 receptor. However, recently three further dopamine receptors have been discovered, known as D3, D4, and D5. 5-7 The D3 and D4 receptors are structurally related to D2 and, though distinguishable pharmacologically, both bind D2 selective ligands, including antipsychotic drugs, with high affinity.8 In addition, expression of the D3 receptor gene appears to be restricted to limbic areas of the brain implicated in schizophrenia in contrast to that of the D2 receptor gene which is more widespread.9 These findings suggest that a plausible role can be the for involvement of the D3 receptor in the pathogenesis of schizophrenia.

The human D3 receptor gene has been localised to chromosome 3q13.3 by in situ hybridisation.4 It contains a polymorphic site in the first exon that gives rise to a glycine to serine substitution in the N-terminal extracellular domain. This results in the creation of a BalI restriction enzyme site.4 The failure of ourselves and others to show genetic linkage between schizophrenia and this polymorphism in multiply affected families (unpublished data) makes it unlikely that a mutation in D3 is a major factor predisposing to illness in the majority of such families. However, we have also tested the hypothesis that variation at this locus might be associated with more subtle differences in liability to develop schizophrenia by comparing allele and genotype frequencies in patients and controls. We present data from two independent studies carried out in the UK and France.

Materials and methods
In the UK study, 68 unrelated patients with schizophrenia (25 female and 43 male) were recruited. All patients satisfied the DSMIII-R criteria for schizophrenia. Sixty-eight controls (29 female and 39 male) were recruited from among the married in members of families seeking DNA diagnosis in the University of Wales College of Medicine Department of Medical Genetics. All patients and controls were Caucasians of Western European descent. The age of the controls ranged between 20 and 83 years with a mean of 45-90 (SD 14-98). The age range in the affected subjects was between 19 and 72 with a mean of 43-15 (SD 13-77) and an average duration of illness of 17 years. Age of onset was defined as age at first admission.

In the French study, 73 unrelated inpatients (53 male and 20 female) with chronic schizophrenia according to DSMIII-R criteria were recruited from a single hospital in Alsace. Seventy-one controls were recruited from among the staff and students of the same hospital. All the patients and controls were West European Caucasians of French or Alsatian ancestry. The age of the controls ranged between 20 and 65 years with a mean of 33-9 (SD 8-9), and that of the patients between 20 and 69 years with a mean of 38-4 (SD 12-0).

High molecular weight genomic DNA was extracted from whole blood or transformed lymphoblasts according to routine procedures. The BalI polymorphism was typed by restriction enzyme digestion after amplification of genomic DNA by polymerase chain reaction (PCR). PCR was carried out with the following primers flanking exon 1 of the D3 receptor gene: 5'-GCTCTATCTCCAAACTCTCAACA-3' and 5'-AAGTCTACTCACCTCAGGTATA-3'. The PCR products were then
digested with restriction enzyme before agarose gel electrophoresis and staining with ethidium bromide. The undigested PCR product was 462 bp in size. Digestion produced constant bands of 111 and 47 bp. The presence of a further 304 bp band denoted a 1-1 homozygote, bands at 206 and 98 bp a 2-2 homozygote, and bands at 304, 206, and 98 bp a heterozygote.

Results
Allele frequencies and genotype counts are shown in the table. Data were analysed by the method of Woolf.2 No significant differences between the groups were observed in allele frequencies in either the UK ($\chi^2 = 1.03$, df = 1, NS) or the French study ($\chi^2 = 0.29$, df = 1, NS) or when pooled data from both studies were analysed ($\chi^2 = 1.20$, df = 1, NS). There was a modest tendency for the 1-1 genotype to be more common in the patients than controls which achieved statistical significance only in the UK study ($\chi^2 = 4.22$, df = 1, $p = 0.04$), but was also significant when the pooled data were analysed ($\chi^2 = 7.01$, df = 1, $p < 0.008$; relative risk = 1.91, 95% confidence intervals = 1.18-3.08). However, there was a stronger tendency for the patients to be homozygotes of either type more often than controls. This was present in both the data from the UK ($\chi^2 = 7.77$, df = 1, $p = 0.005$) and France ($\chi^2 = 6.96$, df = 1, $p = 0.008$) and was highly significant in the combined data ($\chi^2 = 14.68$, df = 1, $p = 0.0001$). The relative risk for schizophrenia in homozygotes was 2.61 (95% confidence intervals = 1.60-4.26). The number of each genotype observed in the control groups did not differ significantly from the values expected under Hardy-Weinberg equilibrium ($\chi^2$, with 0.25 continuity correction = 3.61, df = 1, $p = 0.06$). However, the observed and expected values did differ significantly in the patients ($\chi^2$, with 0.25 continuity correction = 10.67, df = 1, $p = 0.001$).

We are aware of one other study of this polymorphism in schizophrenia (Nothen et al, submitted). Although not statistically significant, this study also found an excess of homozygotes in schizophrenics. When the data were pooled with ours, the excess of homozygotes remained highly significant ($\chi^2 = 12.04$, df = 1, $p = 0.0005$; relative risk = 2.01, 95% confidence interval 1.35-2.97) with no evidence for heterogeneity ($\chi^2 = 3.14$, df = 2, $p = 0.21$).

Discussion
The finding of excess homozygosity in schizophrenic patients as compared with controls would appear to reflect departure from Hardy-Weinberg equilibrium in the patients and cannot be attributed to differences in allele frequencies between the groups. One possibility is that these observations reflect non-random mating. However, we have studied a number of other, unlinked polymorphisms in the UK sample and have not observed departures from Hardy-Weinberg equilibrium10 (unpublished observations). Rather, our findings suggest that persons who are homozygous for the Ball polymorphism have an approximately two-fold increased risk of schizophrenia.

This would appear to represent an example of heterozygote advantage,11 perhaps because the presence of two different molecular forms of the receptor results in an increased ability to respond adaptively to variations in the environment occurring either during neural development or in adult life. The polymorphism studied is unlikely either to alter the ability of the D3 receptor to bind ligands, such as dopamine or antipsychotic drugs, or to affect signal transduction, but may change the way in which the protein is inserted in the membrane. Alternatively, it might not itself be of functional significance but be in linkage disequilibrium with another genetic variant that is. This could be located either elsewhere in the coding sequence or in the regulatory regions of the gene. It is important to stress that homozygosity at the Ball site is associated with only a modest relative risk of schizophrenia, and probably contributes only a small amount to the liability of developing the disorder. However, it is possible that homozygosity at other loci also influences genetic susceptibility to schizophrenia, and that we have identified a particular example of a mechanism of more general relevance to the disorder.

If homozygosity at the Ball site does influence susceptibility to schizophrenia then this should result in non-random allele sharing by affected sib pairs. However, the fact that homozygosity at D3 is likely to make only a modest contribution to the aetiology of the disorder, together with low informativity, will reduce power and a large sample will probably be necessary.

Given the history of false positive findings in psychiatric genetics, it would be prudent to regard the present results as preliminary, in spite of the independent replication and the high degree of statistical significance when the combined data are analysed. We are therefore currently attempting further replication in a new group of patients and controls. We are also carrying out experiments to determine whether the observed differences in homozygosity are specific to schizophrenia or are present in other major psychiatric disorders.

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Allele frequencies and genotype counts for the patients and controls in the two studies.

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3 Carlsson A. The current status of the dopamine hypothesis of schizophrenia. Neupharmacology 1988;31:179-86.
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