

LETTERS TO THE EDITOR

Excess of homozygosity at the dopamine D3 receptor gene in schizophrenia not confirmed

We read with interest the paper by Crocq *et al*¹ who reported an association between schizophrenia and homozygosity of a polymorphic site in the first exon of the dopamine D3 receptor gene. The polymorphic site which gives rise to a glycine to serine substitution in the N-terminal extracellular domain of the protein was investigated in two independent samples of patients and controls from France and the UK. After pooling the results of both studies the overall relative risk was 2.61 for schizophrenia in homozygotes.

As noted by the authors, we have studied this polymorphism in an independent series of schizophrenic patients (n=60) of German origin and also observed a mild but not statistically significant trend towards an overrepresentation of homozygotes (table).² The finding by Crocq *et al*¹ prompted us to study another 51 schizophrenic patients of German descent, including 25 index patients from highly loaded schizophrenic pedigrees collected for linkage studies, to see whether our initial trend remains stable in an extended sample. The results of the new patient group show a genotype distribution which is similar to the expected distribution according to Hardy-Weinberg equilibrium ($\chi^2=0.07$, df=1, p=0.79) (table). Subgrouping of patients with regard to a family history of schizophrenia had no influence on the genotype distribution (results not shown). Our combined data on 111 schizophrenic patients do not provide evidence of a significant association between schizophrenia and homozygosity at the dopamine D3 receptor gene ($\chi^2=0.38$, df=1, p=0.54, relative risk=1.19) (table). Moreover, our healthy German controls (n=100), who were investigated as part of another study, showed a genotype distribution no different from the expected values according to Hardy-Weinberg equilibrium (table).³ Our results suggest that if the excess of homozygotes in the French and English samples is indeed a real finding it cannot easily be applied to patients of German descent.

Finally, it should be noted that the genotype distribution in the French and the UK control samples showed an overrepresentation of heterozygotes which, in addition to the excess of homozygotes in the patients, accounts for the observed relative risk. In the

French control group this deviation from Hardy-Weinberg equilibrium was significant ($\chi^2=5.47$, df=1, p=0.019). Although our controls did not show a similar deviation from Hardy-Weinberg equilibrium, the significant overrepresentation of heterozygotes in the healthy controls from France, and to a lesser extent also in the UK controls, is an interesting finding, which might reflect a heterozygote advantage.

In the view of (1) our failure to replicate the finding of an association between schizophrenia and homozygosity at the dopamine D3 receptor gene and (2) the finding of an overrepresentation of heterozygotes in the control groups studied by Crocq *et al*,¹ it will be necessary to attempt replication in independent samples of patients as well as in well characterised controls deriving from the same ethnic populations where the initial positive findings were obtained.

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- 1 Crocq MA, Mant R, Asherson P, *et al*. Association between schizophrenia and homozygosity at the dopamine D3 receptor gene. *J Med Genet* 1992;29:858-60.
- 2 Nöthen MM, Körner J, Lannfelt L, *et al*. Lack of association between schizophrenia and alleles of the dopamine D1, D2, D3 and D4 receptor loci. *Psychiatr Genet* (in press).
- 3 Rietschel M, Nöthen MM, Lannfelt L, *et al*. A serine to glycine substitution at position 9 in the extracellular N-terminal part of the dopamine D₃ receptor protein. No role in the genetic predisposition to bipolar affective disorder. *Psychiatr Res* 1993;46:253-9.

Association between schizophrenia and homozygosity at the dopamine D3 receptor gene

Recently, Crocq *et al* (*J Med Genet* 1992;29:858-60) reported a statistically sig-

nificant excess in homozygosity among schizophrenics for a polymorphism in exon 1 of the dopamine D3 receptor gene. They offered two possible explanations for this observation: (1) that the deviation from Hardy-Weinberg equilibrium among the affected population is the result of assortative mating; (2) that the excess of homozygotes among patients reflects heterozygote advantage. The authors favour the second explanation for reasons they discuss in the paper. I would like to offer a third: (3) that there is an excess of persons *hemizygous* for the dopamine D3 receptor gene markers among the patient population surveyed.

The assay that the authors used relies on the ability to PCR amplify the first exon, and then resolve restriction fragments of the amplified DNA on an agarose gel. However, subjects who lack one or more of the PCR primer sites owing to a point mutation or deletion of one allele would be misclassified as homozygous for the *other* allele in this system. This phenomenon is known as allelic dropout, and it is one of the pitfalls of PCR genotyping, especially when assaying candidate genes for mutant alleles. A similar situation was described by Fujimura *et al*¹ when typing for alleles of cystic fibrosis by PCR.

The hypothesis of allelic dropout has several advantages in explaining the observation of Crocq *et al*. It accounts for why there should be an excess of *either* of the homozygotes among the affected population. It also does not require that various unspecified mutant alleles be in linkage disequilibrium with two different polymorphisms at the locus, nor does it require that there be a functionally significant polymorphism among the dopamine D3 receptor gene products. I strongly encourage them to assay their patients for changes either in the primer sequences or in copy number at the dopamine D3 receptor locus.

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- 1 Fujimura FK, Northrup H, Beaudet AL, *et al*. Genotyping errors with the polymerase chain reaction. *N Engl J Med* 1990;322:61.

These letters were shown to Dr Owen *et al* who reply as follows.

Nothen *et al* report that they are unable to replicate our finding of excess homozygosity at the dopamine D3 receptor gene in their extended sample of 111 patients. We too have extended our initial observations by studying a further 66 patients with DSM IIIR schizophrenia and 98 healthy controls (unpublished observations). Once again we found excess homozygosity though the effect was not as strong as in our first study and did not achieve statistical significance on its own (patients *v* controls $\chi^2=2.35$, p=0.06, one tailed; patients' Hardy-Weinberg $\chi^2=2.67$, p=0.05, one tailed). This led us to explore the possibility that there might be important differences between patients in our two studies and that excess homozygosity might be a characteristic of a particular subgroup of schizophrenic patients. Our preliminary unpublished observations suggest that the effect is consistently at its strongest in those patients who have a very high familial loading and in those who have a good response to neuroleptic treatment. We also observed that the proportion of good responders sampled

Genotype and allele frequencies of the *BalI* polymorphism at the dopamine D3 receptor gene in schizophrenic patients and healthy controls of German origin.

	Genotypes*†			Allele frequencies†	
	1-1	1-2	2-2	1	2
Patients					
60 ²	31 (29.4)	22 (25.2)	7 (5.4)	0.70	0.30
51	28 (28.7)	20 (19.1)	3 (3.2)	0.75	0.25
111 (total)	59 (57.5)	42 (44.8)	10 (8.7)	0.72	0.28
Controls					
100 ³	49 (49)	42 (42)	9 (9)	0.70	0.30

* Expected numbers according to Hardy-Weinberg equilibrium are given in brackets.

† Allele 1 corresponds to absence of *BalI* restriction site and allele 2 to its presence.