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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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.

in our second study (25%) was markedly lower than that sampled in our first study (75%). It is possible that this may have contributed to the reduction of effect in the second study. Nothen *et al* found that family history of schizophrenia had no effect on genotype distributions. However, the possibility remains that their patients differed from ours with respect to treatment response or to some other as yet undetermined variable.

Nothen *et al* point out correctly that the controls in the French sample were not in Hardy-Weinberg equilibrium and showed an excess of heterozygotes (though χ^2 is in fact 5.47 and not 8.7). However, the Cardiff controls did not show significant departure from Hardy-Weinberg equilibrium and nor did the further set of 98 controls typed in Cardiff. Moreover, we should remember that the significant excess of homozygotes seen in the patients as compared with Hardy-Weinberg expectations is not dependent upon the distribution of genotypes seen in the controls. Furthermore, the difference between patients and controls remains significant when the French controls are removed from the analysis ($\chi^2 = 6.8$, $p = 0.009$). In view of the fact that the French controls were all members of hospital staff, whereas the controls in the first Cardiff study were married in members of families seeking DNA diagnosis for non-psychiatric diseases, we too wondered whether these differences might reflect heterozygote advantage. However, when we compared a sample of members of staff from our Institute, all of whom are graduates ($n = 37$), with our other controls ($n = 129$), no significant homozygosity effect ($p = 0.66$) or other differences were found and no significant departures from Hardy-Weinberg equilibrium were observed (both p values = 0.9).

Morell is correct to point out that our findings could have resulted from allelic dropout. We have also considered this possibility and have retyped our sample with a second set of primers placed internally to the first. The results we obtained were identical to those obtained from the first set of primers.

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Severity of chest disease in CF patients in relation to their genotypes

Al-Jader *et al* (*J Med Genet* 1992;29:883-7) assessed severity of lung disease by calculating % predicted values for FEV₁ and FVC

and comparing single values in subjects of different ages, as did Johansen *et al*¹ in a similar study. Although in widespread use, this method has deficiencies, especially when analysing lung function data over time. The widely held assumption that a given % predicted value means the same for subjects of different ages is untrue; older subjects will have a lower % predicted value than younger ones with comparable lung function.²

Furthermore, older survivors with any CF genotype are likely to have milder disease and better lung function. This source of bias influences the plot of FEV₁ against time presented by Al-Jader *et al* and Johansen *et al*.¹ In each case the plot first shows a linear fall in FEV₁ with time followed by an upturn in the curve for older patients. On the basis of this, Johansen *et al*¹ claim the $\Delta F508$ heterozygotes have less severe lung disease. However, if the three most extreme data points from the oldest patients are excluded from the graph in both studies, then the relation between FEV₁ and time becomes approximately linear, and the difference between heterozygotes and homozygotes is no longer apparent.

We have examined longitudinal lung function data in Grampian CF patients.³ Forty-four patients were $\Delta F508$ homozygotes, nine were G551D/ $\Delta F508$ compound heterozygotes, and 18 patients had other genotypes, including nine heterozygotes for $\Delta F508$ and an unidentified mutation. Annual spirometric data were retrospectively collected from case notes. A regression line was constructed for each patient's lung function data over time, and a slope value calculated. A logarithmic transformation was applied to linearise the data, and mean slope values were calculated for each group. Mean slope values of the groups were not significantly different. Therefore, we too were unable to show differences in severity of lung disease in CF patients with different mutations.

Al-Jader *et al* suggest that patients with the genotype 621 + 1G → T/ $\Delta F508$ may have more severe disease and R117H heterozygotes milder disease, but differences in lung function data were not significant. Moreover, only four patients with R117H were studied, so it is not valid to make any general inference about that mutation. There is a need for different centres to pool data before firm conclusions can be made about clinical features of CF patients with rare mutations.

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- Packe GE, Miedzybrodzka ZH, Russell G, *et al*. Comparison of clinical features in cystic fibrosis patients with different genotypes. *Thorax* 1992;47:866P.

This letter was shown to Dr Al-Jader *et al* who reply as follows.

We were interested to learn that Packe *et al*¹ were unable to show differences in severity of lung disease with different mutations by analysis of longitudinal data; this finding is in keeping with ours² by analysis of cross sectional data.

The observation that, "older subjects have lower % predicted values for respiratory function tests than younger ones with comparable lung function"³ was unknown to us. However, the paper would seem to refer to an adult population (30 to 70 years) in whom the objective was to define abnormality. We see no problem with the use of this method (FEV₁ and FVC, % predicted by vitalograph) versus age in our cross sectional study of patients aged 5 to 31 years. In addition to the use of respiratory function tests, we also scored chest x ray appearances (by Crispin-Norman score) in four age bands: <5.0 years, 5.0-9.9, 10.0-14.9, and >15.0 years. Patients homozygous and heterozygous for $\Delta F508$ showed similar declines in score; from this, similar declines in lung function could be inferred.⁴

We question whether it is valid to remove the three most extreme data points from the oldest patients as plotted on the FEV₁ % predicted graphs of Johansen *et al*¹ and from our graphs and to consider the remaining data without these points. As we discussed in our paper,² the upturn in the curves for the heterozygous patients could relate to the death of the more severely affected patients and survival of those with a more advantageous genotype. Further work will establish whether this is so.

Regarding our patients who are heterozygous for 621 + 1G → T ($n = 7$) and heterozygous for R117H ($n = 4$), we accept that differences in lung function are not significant. We agree that there is a need for multicentre studies to examine the clinical features of CF patients with unusual mutations.

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