

the percent of the variance of IQ attributable to the *CHRM2* gene of 1% may seem small, it is very likely that IQ is a true polygenic trait due to the additive effect of many genes, each with a small effect. In our studies of over 200 phenotype–genotype correlations with behavioral traits we have found that even when the association is significant, in the majority of cases the percent of the variance attributable to each gene is less than 3% and averages 1.2%.<sup>8</sup>

We have replicated these findings using a quantitative TDT method developed by Abecasis *et al*<sup>9</sup> in 230 parent-child trios from the MTFSS. While a marginally significant association was found between *CHRM2* and total IQ, after stratifying parental origin of transmission, there was a highly significant association for paternal transmission ( $P=0.007$ ).

Although in need of replication, we believe these preliminary results are consistent with a role of the *CHRM2* gene in cognitive processes in humans, as assessed by both total IQ and years of education.

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- 1 Bouchard TJ Jr, McGue M. *Science* 1981; **212**: 1055–1059.
- 2 Chorney MJ *et al.* *Psychol Sci* 1998; **9**: 159–166.
- 3 Baxter MG, Chiba AA. *Curr Opin Neurobiol* 1999; **9**: 178–183.
- 4 Everitt BJ, Robbins TW. *Annu Rev Psychol* 1997; **48**: 649–684.
- 5 Iacono WG *et al.* *Development and Psychopathology* 1999; **11**: 869–900.
- 6 Orita M *et al.* *Proc Natl Acad Sci USA* 1989; **86**: 2766–2770.
- 7 Calabresi P *et al.* *Eur J Neurosci* 1998; **10**: 3020–3023.
- 8 Comings DE *et al.* *Clin Genet* 2000; **57**: 178–196.
- 9 Abecasis GR *et al.* *Am J Hum Genet* 2000; **66**: 279–292.

## No association of alcohol dependence with a NMDA-receptor 2B gene variant

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SIR – Alcohol dependence is a disorder with strong genetic influences and heritability estimates ranging between 40 and 60%.<sup>1</sup> Recent results have demonstrated the importance of ethanol-induced glutamatergic neurotransmission for the development of alcohol dependence. Ethanol-induced glutamatergic neurotransmission has been shown to influence pathophysiological mechanisms central to the development of

alcohol dependence, including tolerance, withdrawal symptoms, craving and ethanol-related neurotoxicity.<sup>2</sup> We performed an association study of alcohol dependence and a silent single nucleotide polymorphism (SNP) of the *N*-methyl-d-aspartate (NMDA) receptor 2B (NR2B) subunit leading to a C to T exchange at position 2873 of the gene (accession number XM 006636.2).<sup>3</sup> A total of 204 patients (157 males, 47 females) with alcohol dependence according to DSM-IV criteria and 258 unrelated control subjects (138 males, 120 females) were included in the study.

Ethanol acts specifically by inhibiting NMDA receptors. It inhibits NMDA evoked release of various neurotransmitters.<sup>4</sup> In case of acute withdrawal from ethanol, increased expression of NMDA receptors results in a heightened excitability, which is thought to contribute to clinical symptoms such as seizures, delirium tremens and neuronal cell death leading to brain atrophy.<sup>5</sup> NR2B and NR2A are subunits that confer a high sensitivity to ethanol-induced inhibition.<sup>6</sup> Ethanol withdrawal signs in mice have been suppressed by treatment with the selective NR2B-containing NMDA receptor antagonist, ifenprodil.<sup>7</sup> In addition, the pharmacological action of the anticraving drug acamprosate is thought to be mediated by its interaction with a polyamine site particularly on the NMDA-receptor 2B subunit.<sup>8</sup> These findings suggest a direct, but not exclusive, involvement of the NR2B subunit in alcohol dependence. The goal of the present study was to analyse if an association of alcohol dependence with the NR2B polymorphism can be detected.

All patients were admitted for an in-patient alcohol withdrawal therapy and fulfilled the DSM-IV criteria for alcohol dependence. Symptoms related to alcohol dependence were assessed using the Michigan alcoholism screening test (MAST).<sup>9</sup> Patients and controls were analysed using the SCID and the CIDI interview.<sup>10</sup> Alcohol-related problems in past and present history were ruled out in the control sample. The study was approved by the local ethics committee. The NR2B genotype was determined by sequencing analysis using an ABI 377 sequencer.

The observed genotype frequencies (Table 1) did not significantly deviate from those expected under Hardy–Weinberg equilibrium. Our result reveals that alcohol dependence is not associated with the NR2B genotype ( $P=0.604$ ). It is supported by the allele distribution, showing no significant association with alcohol dependence ( $P=0.606$ ) (Table 1). An increased risk for alcohol dependence can be ruled out for an OR > 1.076 (Exact confidence intervals: 0.777–1.490;  $P=0.766$ ) in our population. No interaction was found between sex and genotype (data not shown). We were interested to perform an exploratory analysis of phenotypes presumed to carry a high genetic load with the NR2B genotype. Thus, we tested for an association of the NR2B genotype with early age of onset, an increased SCIDII score for antisocial personality traits, existence of a positive family history, and withdrawal-related items of the MAST: Item 18 ('Do you ever drink before noon?') and item

**Table 1** Genotype<sup>a</sup> and allele<sup>b</sup> frequencies in patients and controls

	Total	CC	CT	TT	C	T
Patients	204	103 (50.49%)	84 (41.18%)	17 (8.33%)	290 (0.711)	118 (0.289)
Controls	258	135 (52.33%)	105 (40.70%)	18 (6.98%)	375 (0.727)	141 (0.273)
Total	462	238	189	35	658	259

<sup>a</sup> $\chi^2$ -test for deviance from the Hardy–Weinberg equilibrium:  $\chi^2 < 0.0001$ ,  $df = 1$ ,  $P = 0.983$  [patients];  $\chi^2 = 0.157$ ,  $df = 1$ ,  $P = 0.691$  [controls]; genotype–phenotype association: Cochran–Armitage test  $Z = 0.540$ ,  $P = 0.604$ .

<sup>b</sup>Cochran–Armitage test (two-sided);  $Z = 0.536$ ,  $P = 0.606$  (patients vs controls).

20 ('Have you ever had delirium tremens or seizures, severe shaking, heard voices or seen things that were not there after heavy drinking?'). None of these analyses yielded a significant association with the NR2B genotype. We performed additional subdivisions of our sample with respect to sex and psychiatric comorbidity, including affective disorders, anxiety disorders and substance abuse other than alcohol dependence. No significant association with the NR2B genotype was observed (Cochran–Armitage test, data not shown).

In conclusion, our results show no increased risk for alcohol dependence associated with a C2873T-SNP genotype of the NR2B subunit, and show no significant association with alcohol-dependent phenotypes presumed to carry a high genetic load. Although the NR2B subunit remains an important candidate gene for alcoholism, our results suggest that the C2873T polymorphism is not a promising lead to be followed up in larger patient samples. A systematic assessment of all the informative genetic variations of the NR2B subunit for an association with alcohol dependence is warranted.

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- Schuckit MA. *Am J Addict* 2000; **9**: 103–112.
- Fadda F *et al. Prog Neurobiol* 1998; **56**: 385–431.
- Nishiguchi N *et al. Am J Psychiatry* 2000; **157**: 1329–1331.
- Fink K *et al. Naunyn Schmiedebergs Arch Pharmacol* 1996; **354**: 312–319.
- Tsai G *et al. Am J Psychiatry* 1995; **152**: 332–340.
- Masood K *et al. Mol Pharmacol* 1994; **45**: 324–329.
- Narita M *et al. Eur J Pharmacol* 2000; **401**: 191–195.
- Williams K *et al. Mol Pharmacol* 1994; **45**: 803–809.
- Selzer ML. *Am J Psychiatry* 1971; **127**: 1653–1658.
- Wittchen HU. *J Psychiat Res* 1994; **28**: 57–84.

## Clozapine-induced reduction of glutamate transport in the frontal cortex is not mediated by GLAST and EAAC1

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SIR – Glutamate (Glu), the main excitatory neurotransmitter in the cerebral cortex, has been implicated in several neuropsychiatric disorders, including schizophrenia.<sup>1</sup> The effects of Glu are determined, among others, by the action of high-affinity Glu transporters (GLTs).<sup>2</sup> To date, five GLTs have been characterized: GLT-1, GLAST, EAAC1, EAAT4, and EAAT5.<sup>2,3</sup>

Clozapine, a highly effective antipsychotic,<sup>4</sup> increases the extracellular levels of Glu in the rat frontal cortex.<sup>5,6</sup> Recently, we showed that clozapine reduces cortical expression of GLT-1 and decreases total Glu uptake by 60% in the rat frontal cortex,<sup>6</sup> suggesting that the clozapine-induced increase in Glu levels depends on GLT-1 down-regulation. In the neocortex, EAAT4 and EAAT5 expression is poor or absent<sup>3</sup> and therefore their contribution to total cortical Glu uptake is extremely small, whereas GLAST and EAAC1 are robustly expressed<sup>7,8</sup> and mediate a sizeable fraction of total Glu uptake.<sup>9</sup> Whether the expression of GLAST and EAAC1 is altered by clozapine is unknown.

Thus, we used immunocytochemical and Western blotting methods to investigate whether clozapine