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SCIENTIFIC CORRESPONDENCE

Association between PRODH and schizophrenia is not confirmed

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SIR – It has recently been reported that genetic variants of proline dehydrogenase (PRODH) increase susceptibility to schizophrenia.[1] This is of interest since PRODH maps within the velo-cardio-facial syndrome (VCFS) region of 22q11, deletion of which is one of the strongest known risk factors for schizophrenia.[2] We have examined this claim using two samples each with high power (>99%) to detect the effects reported. Our data suggest that genetic variation at PRODH is unlikely to be associated with even modestly increased risk of schizophrenia, and that the earlier report is the result of chance.

Liu *et al*[1] reported association between various permutations of SNPs and haplotypes constructed from markers at positions 1766, 1945, and 2026 of PRODH in a small sample ($n=107$) of European-American schizophrenic proband–parent trios, in a second sample of 26 trios where the proband had a childhood onset illness, and in a third small sample of 109 adult Afrikaners and 75 controls. Their analysis suggested an association between schizophrenia and a '221' haplotype at three loci 1766/1945/2026, defined by alleles G, T, and C, respectively, and this effect was strongest for disease of early onset.

We have tested this in a large, well-matched Caucasian DSM IV schizophrenia case ($n=677$) and control (679) sample ascertained in the UK and the Republic of Ireland (for details of ascertainment and diagnostic methodology, see Anney *et al*[3]). In total, 49 of the subjects had juvenile onset schizophrenia (JOS) defined by hallucinations, delusions or grossly disorganised behaviour at age 17 years or less (mean age at onset 15.8 y, s.d. = 1.9 years). We also studied a sample of 55 unrelated parent–JOS proband–parent trios from Bulgaria (25 male; mean age at onset 16 years (range 9–17). Assessment and diagnostic methods used were the same as for the UK/Irish sample.

Tests for haplotype association between schizophrenia and detected SNPs were performed using EHPLUS[4] and PMPLUS.[5] The Bulgarian sample was analysed using eTDT software.[6] It was possible to use this method for haplotypes as the LD structure of these three markers allows all parental and proband haplotypes to be unambiguously assigned by hand.

The frequency of the 221 putative risk haplotype in our case–control sample was actually lower (0.11) in cases than in controls (0.13), although not significantly so (OR = 0.86, 95% CI = 0.68–1.09, $\chi^2=1.6$, 1 df, $P=0.21$). When the UK JOS sample was considered separately, again the risk haplotype was reduced in frequency (0.07), but not significantly (OR = 0.54, 95% CI = 0.25–1.17, $\chi^2=2.4$, 1 df, $P=0.12$). We also found no evidence for excess transmission of the risk haplotype (17 transmitted, 19 nontransmitted, $P=0.74$) in the Bulgarian JOS sample.

Although there is no *a priori* hypothesis, in order to fully explore our data sets, we undertook global tests of haplotype association, and tested the SNPs individually for genotypic and allelic association in the UK case–control sample. Genotypic and allelic data are given in Table 1. We found not even a trend ($P<0.15$) for association with any allele, genotype or haplotype in the sample as a whole, or in the UK or Bulgarian JOS samples alone (data not shown). All genotypes were in Hardy–Weinberg equilibrium, and the LD relationships between markers were identical in the UK and Bulgarian samples.

Our case–control sample has a power of 99% to detect the effect described[1] in European-American adult samples at $\alpha=0.01$, yet we found not even a trend for association. As the upper ends of the 95% confidence interval for the OR for the risk haplotype is 1.09, we can exclude even small effects in our sample. Similarly, although our JOS samples are small, each has greater than 99% power to detect

Table 1 Genotype and allele counts (and frequencies) of PRODH SNP1945 and 2026 in UK/Eire case–control sample

SNP		11	12	22	χ^2	P	1	2	χ^2	P
1945	Case (*n*=668)	448(0.67)	196(0.29)	24(0.04)	0.07	0.97	1092(0.82)	244(0.18)	0.00	0.99
	JOS (*n*=49)	36(0.73)	13(0.27)	0	2.19	0.34	85(0.87)	13(0.13)	1.57	0.21
	Control (*n*=668)	445(0.67)	200(0.30)	23(0.03)	/	/	1090(0.82)	246(0.18)		
2026	Case (*n*=677)	585(0.86)	89(0.13)	3(0.01)	2.36	0.31	1259(0.93)	95(0.07)	2.07	0.15
	JOS (*n*=49)	43(0.88)	6(0.12)	0	0.36	0.83	92(0.94)	6(0.06)	0.04	0.85
	Control (*n*=679)	605(0.89)	71(0.10)	3(0.01)	/	/	1281(0.94)	77(0.06)		

The data from SNP1766 are not included as this SNP is in complete LD with SNP2026. Significance (χ^2 and *P* values) is based upon comparison with controls. JOS data are data from subjects with onset of schizophrenia before the age of 18 years.

the larger effect size attributable to this group[1] at $\alpha = 0.01$. However, again, no significant associations were present, and the upper 95% CI for the risk haplotype also excludes effect sizes to OR = 1.17.

Liu *et al* also reported that amino acid changes in exon 11 act independent of the haplotype as risk factors for schizophrenia. In order to test this, we sequenced this exon in all 49 UK/Irish JOS cases, and in 45 controls. We also sequenced this exon in 44 unrelated subjects with VCFS and with FISH confirmed deletions of 22q11, 12 of whom had a DSM IV diagnosis of schizophrenia (27%). Details of this sample are given elsewhere.[7] None of the amino-acid changes displayed a pattern in the samples suggestive of association. The variants that Liu *et al* reported to be in particular excess in the European-American cases were arg453cys and ala472thr variants. In contrast, we found the 453cys risk allele in only a single subject, who had VCFS but not psychosis, while the 'risk' 472thr was found in 6/58 schizophrenic subjects compared with 7/75 nonschizophrenic subjects. These findings do not suggest either variant confers susceptibility to psychosis. In order to pursue the hypothesis further, we have also undertaken detailed mutation and association analysis of PRODH and its pseudogene. None of the variants we have detected show evidence for association with schizophrenia (manuscript in preparation).

Given the high power our samples have to replicate the findings of Liu *et al* we believe that the most likely explanation for the discrepancy between our data and those of the earlier study is that the former represents a type I error. Given the apparent consistency of the findings of Liu *et al* across three samples, type I error may seem unlikely. However, in the earlier study[1] the susceptibility haplotype was not independently associated in all three samples despite the use of a one-tailed test for one sample, and it is not clear that even after phenotypic stratification, the specific risk haplotype was associated independently in each of the three JOS samples. Thus, the previous findings are less robust than they initially appear.

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1 Liu H *et al. Proc Natl Acad Sci* 2002; **99**: 3717–3722.
2 Murphy KC, Jones LA, Owen MJ. *Schizophr Res* 2000; **41**: 29.
3 Anney RJ *et al. Mol Psychiatry* 2002; **7**: 493–502 (1359–4184).
4 Xie X, Ott J. *Am J Hum Genet* 1993; **53**: 1107.
5 Zhao JH, Curtis D, Sham PC. *Hum Hered* 2000; **50**: 133–139.
6 Sham PC, Curtis D. *Ann Hum Genet* 1995; **59**: 323–336.
7 Murphy KC, Jones LA, Owen MJ. *Arch Gen Psychiatry* 1999; **56**: 940–945.