Polymorphic variants and differential expression of *DPYSL2* in schizophrenia

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Introduction

- We identified *DPYSL2* as a candidate gene for schizophrenia from a microarray analysis of the prefrontal cortex of a rodent phencyclidine model of the cognitive deficits of schizophrenia[1,2].
- *DPYSL2* maps to human chromosome 8p21, a schizophrenia locus replicated in genome-wide linkage studies and supported by meta-analyses of these data.
- However, genetic association is ambiguous with several positive associations in different populations but also some reports showing no association[3-7] (Figure 1).
- DPYSL2 protein has shown to be differentially expressed in the frontal cortices and hippocampus of schizophrenia patients[8-9].
- The exact role of DPYSL2 in disease pathophysiology is unknown but its normal expression during neurodevelopment and in adult brain and its participation in the regulation of axonal outgrowth render it a promising candidate gene for schizophrenia.

Methods

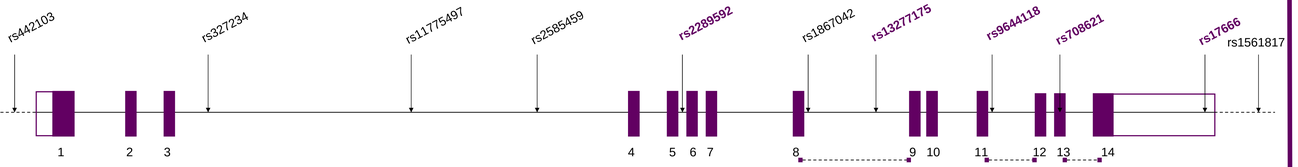


Figure 1: Genomic structure of *DPYSL2*
The genomic region of *DPYSL2* spans approximately 100kb, containing 14 exons (purple boxes). The most abundant brain transcripts show alternate splicing at the 5' end of the gene with an alternative first exon.
The 11 tag SNPs used in this study are shown (black arrows). SNPs highlighted in purple show weak association in this study (Figures 2 and 3). Several of these SNPs have been used in other studies; no association with schizophrenia was found with rs442103 in a European population[6], or with rs327234 and rs2289592 in a Chinese Han population[7], or with rs708621 in Japanese[3] or Chinese Han[7] populations. However association with schizophrenia was found with rs17666 and its haplotype in Japanese (T risk allele)[3], Caucasian (C risk allele)[4] and Ashkenazi Jewish (haplotype)[5] populations but not in African Americans[4] or Chinese Han[7].
The cross-exon real-time PCR TaqMan assays are shown for exons 8+9, exons 11+12 and exons 13+14 (▪----▪).

Genotyping:

- Tagged SNPs spanning *DPYSL2* were selected based on haplotype block patterns using the HapMap CEU data[10] and allele frequency (MAF>20%) (ABI SNPBrowser[11] and dbSNP[12]) (Figure 1).
- 500 Caucasian schizophrenia cases with DSM-IV diagnosis and 500 unrelated control DNA samples (North European origin, collected from the West of Scotland and from the University College London Schizophrenia Case Control set) were genotyped blind to diagnosis, using TaqMan SNP genotyping assays (ABI).
- Statistical analyses were carried out using 'haplo.stats'[13] and 'genetics' R suites. Haplotype predictions were made using the *haplo.em* algorithm employed in haplo.stats. In some analyses, the best predicted phase for each individual was kept (>70% likelihood); in others all phase predictions for each individual were utilised probabilistically.

Quantitative real-time PCR:

- Total RNA was isolated from post-mortem DLPFC (Brodmann area 9 and 10) from 16 schizophrenia patients and 15 healthy controls (Qiagen RNeasy Lipid Midi kit plus DNaseI). The integrity of the total RNA was assessed using the Agilent 2100 Bioanalyzer Nano LabChip and by inter- and intra- exonic PCR of several genes to confirm gDNA removal.
- First strand cDNAs were synthesised from 1μg (Agilent Nanodrop) of total RNA using random hexamers and SuperScript® III Reverse Transcriptase (VILO™ cDNA synthesis kit, Invitrogen), following the manufacturer's protocol.
- The cDNAs were genotyped for two exonic SNPs, rs708621 and rs17666, using TaqMan SNP genotyping assays (ABI).
- TaqMan gene expression assays (Figure 1, ABI) were used to quantitate relative *DPYSL2* expression using the ΔCt between *DPYSL2* and *GAPD*. The data were analysed using a General Linear Model (MINITAB) with diagnosis, brain region and genotype as independent variables.
- Comparisons between schizophrenia cases and controls were also made using ANCOVA (MINITAB) with *GAPD* as the covariate.

Results

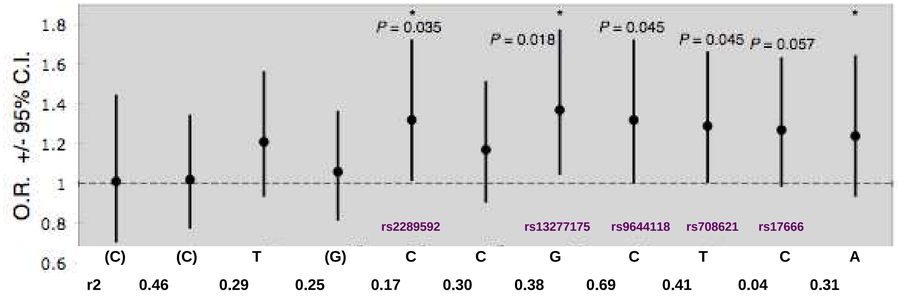
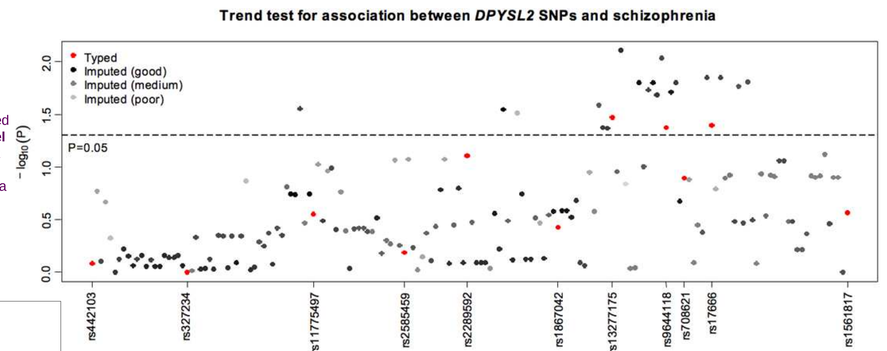


Figure 2: Weak association with several SNPs at 3' end of *DPYSL2*

The Odds Ratio (OR) for the 'risk' alleles are shown (SNPs 5'-3' as in Figure 1) from a logistic regression analysis under a **dominant model**. () indicates risk allele is arbitrary as O.R.=~1. Logistic regression P values are given for SNPs with P<0.05 (**rs2289592, rs13277175**, **rs9644118, rs708621** and **rs17666**). LD between adjacent markers is given as r^2. No evidence for a variety of Haplotype effects.
* SNP not in Hardy-Weinberg equilibrium in controls (HWE $\chi 2$, 1 d.f., $0.017 < P < 0.045$).

Figure 3: Association with 3 typed SNPs and several imputed SNPs at 3' end of *DPYSL2*

Logistic regression association analysis of typed and imputed SNPs spanning *DPYSL2*. A trend test was applied to **model additive allelic** effects at each site. HAPMAP v.2 CEU data and IMPUTE v.0.5 were used for imputation. Points above the dotted line have individual P<0.05. The colour of the data points indicates confidence of imputation.
rs13277175, rs9644118 and **rs17666** show a weak significant association under the additive model test.



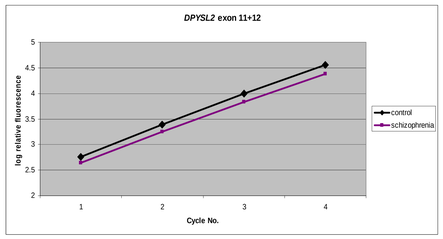
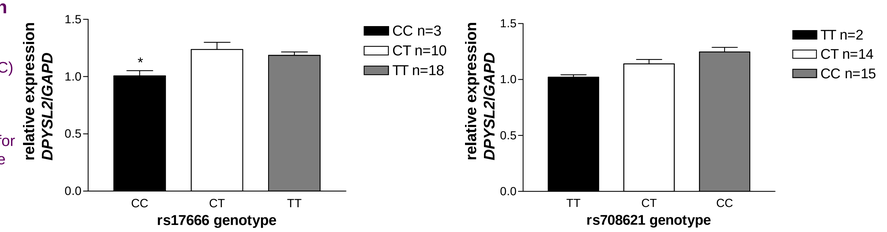


Figure 4: Decreased *DPYLS2* expression in BA10 of the DLPFC of schizophrenia patients

The significant decrease in rat *Dpysl2* expression observed in the prefrontal cortex of rats treated with phencyclidine[2] was mirrored in the dorsolateral prefrontal cortex of schizophrenia patients (ANCOVA[14] with *GAPD* as covariate, P=0.02). A custom assay designed to amplify the same region of *Dpysl2* as represented on the Affymetrix RGU34A GeneChip (spanning exons 11+12) was utilised for the quantitative real-time PCR. Data points were taken from 4 cycles during the linear phase of the amplification (X axis). Controls n=9 and cases n=9.

Figure 5: Decreased *DPYSL2* expression in subjects homozygous for risk alleles

There was a significant decrease in expression of *DPYSL2* in homozygotes for the rs17666 risk allele (CC) in the entire cohort (Tukey's post hoc, P<0.05) when a gene expression assay spanning exons 8 and 9 was utilised to quantitate relative expression. A trend for a decrease was also observed in subjects homozygous for the rs708621 risk allele (TT) when measuring the same transcripts. Decreased *DPYSL2* expression in homozygote subjects for these risk alleles was also observed when a gene expression assay spanning exons 13 and 14 was used (data not shown).



Summary

- We present weak association of several SNPs at the 3'end of *DPYSL2* with schizophrenia and decreased expression of the gene in the DLPFC of patients and in subjects homozygous for some of the risk alleles.
- The effect of these alleles is currently under investigation using fluorescent allele-specific PCR in heterozygote subjects.
- These data are consistent with the hypothesis that decreased *DPYSL2* expression in the prefrontal cortex contributes to disease aetiology.

References
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Acknowledgements
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