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Muscarinic M1 receptor gene sequence: Preliminary investigations into its effects on cognition and gene expression.

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Abstract:

Objective: It has been shown that people with schizophrenia who are homozygous for c.267C>A cholinergic muscarinic M1 receptor perform less well at the Wisconsin Card Sorting Test compared to *CHRM1* heterozygotes suggesting *CHRM1* sequence may be associated with impaired executive function in schizophrenia. *Method:* To confirm and extend the hypothesis that *CHRM1* sequence could affect cognition in subjects with schizophrenia we sequenced the *CHRM1* in peripheral DNA from 97 subjects with schizophrenia who completed the Wisconsin Card Sorting Test (WCST), Verbal Fluency test and National Adult Reading Test (NART). Clinical severity was assessed using the Positive and Negative Symptom Scale. To determine if such an association could be due to an effect of changes in *CHRM1* sequence on CNS gene expression sequenced *CHRM1* using post-mortem tissue, from another cohort, and measured levels of cortical *CHRM1* mRNA in tissue from that cohort. *Results:* Participants with schizophrenia performed less well on WCST and Verbal Fluency, but not the NART – compared to what – nothing in methods section. Subjects with schizophrenia who were 267C/C made significantly more perseverative errors ($p<0.05$) and perseverative responses ($p<0.05$) than those who were 267C/A but variation in gene sequence was not associated with any symptom domain, NART or Verbal Fluency scores. Although cortical *CHRM1* mRNA levels were lower in subjects with schizophrenia compared that in age / sex matched controls ($p<0.001$), levels of *CHRM1* mRNA did not vary with gene sequence. *Conclusions:* The study of peripheral DNA in this study supports the proposal that *CHRM1* sequence is associated with changes in cognitive processes but the CNS data does not suggest this is simply related to changes in levels of CNS *CHRM1* expression. Our results highlights that complex gene and environment interactions are likely to have a role in the underlying pathophysiology of schizophrenia.

Keywords: Acetylcholine, psychiatric disorders, human, cognition, postmortem, *CHRM1*.

Introduction:

Schizophrenia is diagnosed on the basis of a constellation of symptoms and is currently defined by the presence of positive symptoms (delusions and hallucinations), negative symptoms (social withdrawal, lack of emotion) and cognitive deficits (reduced attention, disorganised thoughts and memory problems) {American Psychiatric Association, 2000 1591 /id}. Notably, antipsychotic drugs are efficacious at reducing the positive symptoms but have little or no effect on either negative symptoms or cognitive deficits {Tandon, 2008 2250 /id}. The failure of antipsychotic drugs to ameliorate cognitive deficits is significant because almost every person who has the disorder displaying some degree of cognitive impairment {Peuskens, 2005 1956 /id}.

It is now acknowledged that some individuals experience cognitive dysfunction from illness onset, whilst others may be relatively unaffected and that areas of cognition are impaired in nearly all people with schizophrenia to differing extents {Heinrichs, 1998 2072 /id} and impairments differ according to stage of illness {Pantelis, 2009 3003 /id}. Cognitive domains affected include speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving {Nuechterlein, 2004 1619 /id}. The impact of cognitive dysfunction on daily functioning of people with schizophrenia is now being fully realised as there are strong relationship between levels of cognitive impairments and social or vocational functioning. At a more quantitative level, deficits in vigilance, secondary verbal memory and performance on the Wisconsin Card Sorting Test appear to predict the participants' ability to acquire and retain skills necessary to function independently within the broader community {Green, 1996 2071 /id}. Critically, cognitive deficits in schizophrenia are predominantly independent of the severity of positive and negative symptoms {Gold, 2004 1621 /id} suggesting an independent pathophysiology

which in turn explains their resistance to current medications targeting positive symptom-related pathways {Tandon, 2008 2250 /id}.

Given the current lack of understanding of the underlying pathophysiology it is significant that it has been reported that people with schizophrenia who are homozygous (C267C) at *CHRM1* c.267C>A (rs2067477) perform poorly on the Wisconsin Card Sort Test (WCST) compared to subjects with disorder who are heterozygotes (C267A) {Liao, 2003 1363 /id}. Unfortunately, this sequence variation is synonymous at the level of *CHRM1* protein {Lucas, 2001 1285 /id}, the potential mechanism underlying the association between *CHRM1* sequence and cognitive functioning is not clear. However at the behavioural level the finding is particularly significant because the WCST is considered a measure of executive functioning, including concept formation and cognitive flexibility {Green, 1996 2071 /id} even though its usefulness as a specific predictor of prefrontal function has been challenged {Barcelo, 1999 2990 /id}. However, what is perhaps more important, outcomes on the WCST have been shown to predict the ability of patients with schizophrenia to acquire the skills necessary for social reintegration {Green, 1996 2071 /id}.

Whilst the exact relationship between *CHRM1* sequence variation and *CHRM1* function is not clear there is a large body of data showing that the cholinergic system is critical in modulating processes that underpin cognition {Gold, 2003 2574 /id}. In humans this is highlighted by the observation that the anticholinergic load of some medications prescribed to patients with schizophrenia is associated with a higher degree of impairment in attention and selective impairment of memory and recall {Minzenberg, 2004 2062 /id}. Such data have underpinned the hypothesis that the muscarinic cholinergic system is a primary target for the development of drugs designed to improve cognitive function {Gray, 2007 2151

/id}{Harvey, 2009 2568 /id}. Moreover, data from *chrml^{-/-}* mice showing selective deficits in a behavioural paradigm proposed to relate to working memory {Anagnostaras, 2003 1495 /id}, a domain commonly affected in subjects with schizophrenia {Nuechterlein, 2004 1619 /id}, highlights the importance of that receptor in maintaining cognitive functioning. At the therapeutic level, the findings that CHRM1/CHRM4 agonist xanomeline {Bymaster, 1997 1958 /id} improved cognitive functioning (specifically in verbal learning and short-term memory) in a small group of medication-free, treatment-resistant patients with schizophrenia {Shekhar, 2008 2260 /id} supports a potential role for *CHRM1* in the cognitive deficits of schizophrenia. It is also significant that postmortem CNS studies have reported decrease *CHRM1* expression in cortex of subjects with schizophrenia {Dean, 2002 1282 /id}{Mancama, 2003 1643 /id}. Thus, it could be possible that changes in gene sequence, although synonymous at the levels of CHRM1 protein could be affecting gene transcription (Johansen et al PNAS December 1, 1984 vol. 81 no. 24 7698–7702).

Taking into account the potential effects of *CHRM1* c.267C>A on cognitive function we genotyped the *CHRM1* in a cohort of participants with schizophrenia and assessed their performance on the WCST, Verbal Fluency and the National Adult Reading Test (NART). To determine if *CHRM1* c.267C>A might affect levels of gene expression we compared *CHRM1* sequence and cortical levels of mRNA in different cohorts of subjects with schizophrenia and age/sex matched controls.

Methods:

Clinical Study:

Approval for this study was obtained from the Mental Health Human Research Ethics Committee of Melbourne Health.

Inclusion criteria for the clinical study were that subjects must be aged 18-60 years and meet the clinical criteria (DSM-IV {American Psychiatric Association, 1994 26 /id}) for schizophrenia or schizoaffective disorder. Exclusion criteria included a history of neurological disorder, depression, major head injury, hypoxia, concurrent substance abuse and ECT in the last 12 months. People currently undergoing treatment with anticholinergics or with a diagnosis of premorbid intellectual disability (NART IQ < 70) were also excluded. After a complete description of the study to the subjects, written, informed consent was obtained.

Using these criteria 97 subjects with schizophrenia (86) or schizoaffective disorder (11) were recruited from the psychiatric clinics of the Northern Area Mental Health Service in Melbourne, Australia (see table 1 for demographic information). The majority of participants were inpatients (62%), had a gender ratio of 65 males : 32 females and a mean age of 35.27 yrs (SD = 9.06, range 18 – 58). The clinical status of the subjects were assessed by a trained psychologist (AK-D) using the Positive and Negative Syndrome Scale (PANSS) {Kay, 1986 823 /id}. All subjects completed the computerised 64 card WCST (PAR Inc) and provided a blood for genetic testing, 93 were assessed for Verbal Fluency and 91 had NART. The WCST, Verbal Fluency and NART data from subjects from this tour were compared to that from 200 people with no history of psychiatric illness recruited for a separate study (Testa & Bennet, submitted). The mean age of the control group (30.7 ± 9.1 yrs) was significantly lower than that of the schizophrenia group (35.4 ± 9.0 years, $t = -4.12$, $df = 295$, $p < 0.0001$, see Table 1). ? sex ratio?

Post-mortem study:

Approval for this study was obtained from the Ethics Committee of the Victorian Institute of Forensic Medicine and the Mental Health Research and Ethics Committee of Melbourne Health. Subjects used in this study had previously had DNA extracted from the cerebellum and the *CHRM1* sequence determined {Scarr, 2009 2206 /id}.

Brodmann's Area 9 (BA 9) was excised from 76 subjects diagnosed as having schizophrenia according to DSM-IV criteria {Roberts, 1998 309 /id} using the Diagnostic Instrument for Brain Studies {Hill, 1999 25 /id} and 73 subjects with no history of psychiatric illness (controls; see table 2 for demographic details). Following case history reviews, duration of illness (DOI: time from first hospital admission to death) was calculated. Comprehensive medication histories were obtained and the most recently prescribed doses of antipsychotic drugs were converted to a standardized drug dose {Remington, 1999 1588 /id} (see table 2).

Cadavers were refrigerated within 5 hours of being found and CNS tissue was rapidly frozen to -70°C , within 30 minutes of removal at autopsy. Where death was witnessed, the time between death and autopsy was taken as the postmortem interval (PMI). When death was not witnessed, PMI was taken as the interval mid-way between the donor last being seen alive and being found dead. The pH of CNS tissue was determined {Kingsbury, 1995 15 /id}.

Gene sequencing

DNA was extracted from whole blood or cerebellum using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and the sequence determined essentially as described previously {Scarr, 2009 2206 /id}. Briefly, the sequence of the *CHRM1* was determined after amplification with primers: M1-F1 5' ggtgatgactttcccctgag and M1-R1 5' ctgggaatagcgaagtctgg, designed from the *CHRM1* sequence (GenBank Accession Number NM_000738). The PCR product, consisting of the single coding exon and flanking intronic sequence of *CHRM1* was sequenced on an ABI3130xl genetic analyser with POP7 polymer using the PCR primers and a third internal primer (M1-F2 5' ggagacagagaaccgagcac) permitting the genotype at each polymorphic site to be determined.

In situ hybridization

In situ hybridization was performed as described previously {Scarr, 2007 2047 /id}. Briefly, 6 x 10µm sections were cut from the BA 9 of each subject, fixed in 4% paraformaldehyde, placed in fresh 0.25% acetic anhydride in 0.1 M triethanolamine HCl (pH 8.0), dehydrated, delipidated and equilibrated in 95% ethanol before being dried.

To increase specificity, the oligonucleotide probe contained a mix of 3 antisense sequences, the probes were complementary to bases 4-51, 721-768 and 811-853 of human *CHRM1* mRNA (accession #: X52068) {Dean, 2004 1608 /id}. The oligonucleotides were labelled with [³⁵S]ATP using the NEN Oligonucleotide 3' end labelling system and purified using a NENSORB™ 20 purification cartridge (Perkin Elmer).

For hybridization, the probe was prepared at 1×10^6 dpm of [^{35}S]ATP per 50 μl of hybridization buffer and incubated with tissue sections for 20 hours at 37°C, in a humidified chamber. Two sections were incubated with the radioactive oligonucleotide probe (TB) whilst two sections were incubated with radioactive probes in the presence of excess non-radioactive probes (NSB). Hybridization to mRNA was confirmed by incubating two sections, pre-incubated in 0.002% RNaseA, with the radioactive probe (negative control).

After hybridisation, all sections were washed {Loiacono, 1999 888 /id} and dried before being apposed to BAS-SR2025 imaging plates (Fujifilm), with [^{14}C]microscales™ (GE Healthcare) for 4 weeks before being scanned in the BAS 5000 high resolution phosphoimager (Fujifilm). The signal intensity of the phosphoimages were measured against those on the [^{14}C]microscales™ using AIS image analysis software. Results were expressed as TB minus NSB in dpm $\times 10^3$ /mg estimated wet weight tissue equivalents (ETE).

Statistical Analyses:

Clinical data:

The WCST data was analysed using an Australian normative cohort as the reference group (Testa & Bennet, Submitted). Patients and controls were compared using t-tests, and where the assumption of equal-variance was not supported, a degrees-of-freedom correction was made. The influence of age and NART was subsequently assessed by including those variables in an analysis-of-covariance. The only exception to this was in the comparison of the number of successfully completed categories, which was compared using Mann-Whitney U. In participants with schizophrenia, the impact of the 267C>A SNP on WCST performance, NART and Verbal Fluency were determined in a similar fashion.

Post-mortem study:

The D'Agostino & Pearson omnibus normality test showed that data generated by the *in situ* hybridization study were skewed (K2 range 13.19-66.73); the data was thus transformed by taking the natural log. The transformed data was analysed using a 3-way ANOVA, with diagnosis, cortical layer and sequence (no SNPS vs. all SNPs) as factors, followed by post-hoc tests with Bonferroni correction for multiple testing. A secondary analysis specifically to determine the effect of the 267C>A SNP on levels of *CHRM1* mRNA was completed using a 3-way ANOVA, with diagnosis, cortical layer and sequence (267C/A vs. all other sequences) as factors (SPSS version 15). The association between potential confounding factors and levels of mRNA were determined using Spearman correlations.

Results:

Clinical Study:

There was no significant differences between NART scores between the control group (107.9 ± 7.9) and the group with schizophrenia (109.9 ± 10.3 , $t = -1.62$, $df = 140.8$, $p = 0.107$), suggesting that overall IQ did not differ with diagnoses. The subjects with schizophrenia showed a significant lower number of correct responses (control: 44.1 ± 11.2 vs. schizophrenia: 30.3 ± 10.2 , $t = 10.10$, $df = 288$, $p < 0.0001$) and increased number of errors (control: 1.46 ± 1.6 vs. schizophrenia: 2.93 ± 2.7 , Mann-Whitney $U = 6092$, medians = 1.0 (control) and 2.0 (schizophrenia) $p < 0.0001$) for their Verbal Fluency. The people with schizophrenia had total PANSS scores of 73.95 ± 16.53 with a total positive symptom score

of 18.07 ± 5.66 , a total negative symptom score of 17.62 ± 5.57 and a general score of 37.11 ± 9.2).

In the WCST, people with schizophrenia had fewer correct trials ($p < 0.0001$), fewer xxxx concept responses ($p < 0.0001$) as well as completing fewer categories ($p < 0.0001$) and trials to 1st category ($p < 0.0001$) (Table 1?). However, after correcting for age and premorbid IQ, there were no significant differences between the groups on errors, perseverative response, perseverative errors or failure to maintain.

c.267C>A and cognitive measures:

CHRM1 sequencing showed that people with schizophrenia had 5 of the 13 SNPs already reported in the *CHRM1* sequence {Lucas, 2001 1285 /id}, no novel SNPs were identified. Overall, the combinations of gene sequence variations gave 8 haplotypes (see supplementary table 1). At c.267C>A, 83 of the 97 subjects were homozygous for the major allele (C267C) giving C267A a frequency of 0.144. The heterozygous and homozygous groups did not differ in age, duration of illness, years of education, chlorpromazine equivalents or PANSS ratings (see Table 1).

Participants with schizophrenia who were homozygous (C267C) had significantly more perseverative responses (C267C: 16.0 ± 11.1 vs. C267A: 9.2 ± 6.0 , $p = 0.03$) and perseverative errors (C267C: 13.9 ± 8.6 , vs. C267A: 8.3 ± 5.4 , $p = 0.021$) compared to heterozygotes (See Figure 1). There were no significant differences in the number of correct trials, errors, completed categories, concept response, trials to complete first category or failure to maintain. Genotype had no effect on NART scores (C267C = 111.0 ± 10.8 vs. C267A =

109.5 ±10.2, $t = -0.50$, $df = 87$, $p = 0.619$) or Verbal Fluency (correct responses (30.3 ±10.3 vs. 29.5 ± 7.7, $t = 0.26$, $df = 89$, $p = 0.8$), number of errors (\pm vs. 1.95 ±, $t = df = p =$) or repeated responses (\pm vs. \pm , $t =$, $df = p =$)).

Effect of diagnoses on cortical *CHRM1* mRNA:

In situ hybridization showed distinct layers of *CHRM1* mRNA in BA 9 (see Figure 2a). Comparisons to cresyl violet stained sections showed the most intense hybridization to mRNA occurred in deep laminae V (layer B), with lesser hybridization occurring in laminae I to upper V (layer A) and laminae VI (layer C).

A 3-way ANOVA, with diagnosis and predicted sequence vs. all sequence variants as between subject factors and layer of hybridization as a within subject factor, on the log transformed mRNA data found significant differences in means due to diagnosis ($F = 5.975$, $df = 1, 145$, $p = 0.016$), layer of hybridization ($F = 44.66$, $df = 2, 290$, $p < 0.0001$) but not sequence (predicted sequence against all SNPs; $F = 0.679$, $df = 1, 145$, $p = 0.411$). There were no significant interactions between any of the factors. *Post-hoc* Bonferroni analyses found that the variance with diagnosis was due to lower levels of mRNA in layer A of the cortex from subjects with schizophrenia (2.366 ± 1.4 vs. 2.901 ± 0.99 , $t = 2.903$, $p < 0.05$; see Figure 2b). The variance of mRNA between cortical layers was due to higher levels of mRNA in layer B compared to layers A (controls: 3.331 ± 0.78 vs. 2.901 ± 0.99 , $p < 0.05$; schizophrenia: 2.935 ± 0.86 vs. 2.366 ± 1.4 , $p < 0.01$) and C in both diagnoses (controls: 3.331 ± 0.78 vs. 2.766 ± 1.3 , $p < 0.01$; schizophrenia: 2.935 ± 0.86 vs. 2.366 ± 1.29 , $p < 0.01$). ? was mRNA decreased in each layer in schizophrenia?

There were no strong correlations between levels of cortical mRNA and age, PMI, brain pH, DOI, chlorpromazine equivalents (Spearman r range: 0.01 to -0.23).

Effect of *CHRM1* sequence on cortical *CHRM1* mRNA:

The majority of subjects from who tissue had been collect post-mortem were homozygous (C267C); 10 control subjects and 11 subjects with schizophrenia were C267A giving frequencies of 0.137 and 0.145, respectively). Gender ration and suicide did not differ with genotype.

A 3-way ANOVA, with diagnosis and 267 C/C vs. 267 C/A as between subject factors and layer of hybridization as a within subject factor, on log transformed data found confirmed that subjects with schizophrenia had significantly lower levels of mRNA compared to that in control subjects ($F=4.03$, $df=1,145$, $p=0.047$). Layer of hybridization was also significant ($F=25.62$, $df=2,290$, $p<0.001$) but genotype was not ($F=2.38$, $df=1,145$, $p=0.125$; See Figure 2c). There were no significant interactions between any of the factors. [How is this different to previous section that shows changes with diagnoses and layer but not genotype?

Discussion:

This study confirms a previous report that subjects with schizophrenia and the *CHRM1* C267A had fewer perseverative errors than those who were C267C {Liao, 2003 1363 /id}. In addition, we found the subjects with C267A had fewer perseverative responses, whereas the earlier study found these subjects had more correct conceptual responses and completed more categories. Together, these studies strongly suggest that the people with schizophrenia who

are C267A perform better on the WCST than C267C. Whilst not an ideal construct, we were able to compare data from subjects with schizophrenia with data from a cohort of control subjects who were not *CHRM1* genotyped. These comparisons showed that differences in WCST are not clearly apparent at the level of diagnoses. More tentatively, such comparisons showed that subjects with schizophrenia who were heterozygous (C267A) had less perseverative responses than the control group. Thus, it is possible that the lower number of perseverative responses and errors in the C267A group counterbalanced the deficits present in the C267C group at the diagnostic level.

Our comparison of data from subjects with schizophrenia to our controls seems to have face validity because we confirmed findings from previous studies that subject with that disorder perform less well on the WCST {Braff, 1991 2985 /id}{Goldstein, 1996 2986 /id}. These earlier studies showed a link between increased perseveration and poor performance on the verbal component of the Wechsler Adult Intelligence Scale, especially in patients exhibiting primarily negative symptomatology {Goldstein, 1996 2986 /id}. In our study we failed to show any association between *CHRM1* genotype and the Verbal Fluency test, which also involves executive function {Rende, 2002 2989 /id}, suggesting variation in *CHRM1* sequence was not affecting all aspects of executive functioning. These data not only add to the hypothesis that the executive system can be fractionated into a number of component skills, which have varying degrees of inter-dependence {Alvarez, 2006 2993 /id} but add a possible biological correlate to explain some of the underlying pathophysiology of cognitive deficits in schizophrenia.

The WCST and Verbal Fluency are suggested to assess different a set of executive functions that may have minimal association, despite being within the executive system. The

specific association with performance on the WCST is in agreement with the previous study, which found no associations between scores on the Mini-Mental Status Examination and this SNP {Liao, 2003 1363 /id}. In neither study was there an association between c.267C>A and symptom severity or other factors that might indicate prognosis. Thus, this SNP appears to be specifically associated with *factors* that determine performance on the WCST.

The finding of a decrease in *CHRM1* mRNA in tissue from subjects with schizophrenia is in agreement with previous studies {Dean, 2002 1282 /id}{Mancama, 2003 1643 /id}, adding further support for altered *CHRM1* expression having a role in the pathophysiology of the disorder. However, levels of *CHRM1* mRNA did not vary with c.267C>A, this suggests the SNP, which is known to be synonymous, does not have a significant effect on levels of *CHRM1* mRNA in the cortex of subjects with schizophrenia. Thus, it would appear that poor performance on the WCST cannot simply be credited to a change in expression of cortical *CHRM1*. Given these data it is possible that C267A is in linkage disequilibrium with a SNP in another gene and it is changes in the expression / function of that gene that is causing the decrease in cognitive ability in subjects with schizophrenia. However, our data confirming an association *CHRM1* sequence with aspects of cognitive functioning increases the probability that this marker may be a useful biomarker allowing the early detection of subjects with schizophrenia at risk of cognitive deficits.

In conclusion, our data suggest that homozygosity at c.267C>A *CHRM1* is associated with worse performance on certain components of the WCST in subjects with schizophrenia. Our postmortem CNS data, albeit on a separate cohort of subjects, does not appear to be mediated by an increased expression of the receptor at the transcript level. Whilst this conclusion must be made with caution, such a comparison gains credibility from the fact that the frequency of

homozygosity is not significantly different in subjects with schizophrenia from whom blood or CNS was collected. Importantly, *CHRM1* genotype only appears to be associated with a limited number of components of the WCST and is not associated with the number of years of education, Mini-Mental Status Examination {Liao, 2003 1363 /id}, NART or Verbal Fluency scores. Significantly, whilst WCST and Verbal Fluency test both require the activating of the dorsolateral prefrontal, temporal and parietal cortices WCST also activates the basal ganglia, ventromedial, orbitofrontal and occipital cortices whereas the Verbal Fluency test activates thalamus and anterior cingulate (VF) {Alvarez, 2006 2993 /id}. This raises the possibility that variation in *CHRM1* sequence may be associated with impaired integration of specific aspects of cognition mediated by specific combinations of cortical or subcortical regions. One limitation of this study is the sizes of the diagnostic cohorts however, this is of lesser concern given that at the level of peripheral DNA this is a replication study. However, it is clear that further research is required to determine i) the validity of our finding in a large cohort of subjects with schizophrenia, ii) where there is a familial aspect to the association of *CHRM1* sequence and cognitive deficits and iii) whether a combination of studying *CHRM1* sequence and neuroimaging can highlight abnormalities of CNS function which might expand our understanding of how a synonymous change in gene sequence that does not seem to affect gene expression can result in increased cognitive dysfunction in some subjects with schizophrenia.

References

Figure Legends:

Figure 1: Graphical representation of perseverative response, errors and non-perseverative responses for the Wisconsin Card Sort Test separated by sequence at the c.267C>A single nucleotide polymorphism. P = perseverative; N-P = non-perseverative; * p<0.05.

Figure 2: a) Autoradiograph showing the different layers of hybridization to *CHRM1* mRNA.

b) Graphical representation of the normalized levels of *CHRM1* mRNA in Brodmann's Area 9 from controls and subjects with schizophrenia. * p<0.05.

c) Graphical representation of the normalized levels of *CHRM1* mRNA in Brodmann's Area 9 from controls and subjects with schizophrenia, separated by sequence at the c.267C>A single nucleotide polymorphism.

Table 1: Demographic and psychometric data for the clinical participants.

	Control participants	Participants with schizophrenia		
		Combined (97)	267C/C (73)	267C/A (14)
Age (years)	30.7 ± 9.1	35.4 ± 9.0	35.6 ± 9.4	33.4 ± 6.4
Gender (M/F)	/	68/33	59/28	9/5
Education (years completed)	±	11.5 ± 2.3	11.5 ± 2.3	11.5 ± 2.1
Years since worked	±	4.45 ± 5.8	4.8 ± 5.9	2.6 ± 4.3
NART	107.9 ± 7.9	109.9 ± 10.3	111.0 ± 10.8	109.5 ± 10.2
VF correct	44.1 ± 11.24	30.3 ± 10.2	30.4 ± 10.6	29.5 ± 7.7
VF error	1.55 ± 1.6	2.93 ± 2.7	2.97 ± 2.7	2.61 ± 2.5
Duration of illness (years)	N/A	11.6 ± 8.7	11.2 ± 8.9	14.4 ± 7.6
Chlorpromazine Equivalents (mg)	N/A	547.0 ± 264.9	524.3 ± 273.6	694.0 ± 145.4
PANSS Total		73.95 ± 16.53	74.30 ± 16.96	72.00 ± 14.35
PANSS Positive		18.1 ± 5.7	18.2 ± 5.9	17.3 ± 4.4
PANSS Negative		17.6 ± 5.6	17.6 ± 5.5	17.9 ± 6.1
PANSS General		37.1 ± 9.2	37.2 ± 9.4	36.8 ± 8.1

Data is expressed as mean ± SD.

Table 2: Demographic data for the postmortem cohort.

	Control subjects			Subjects with schizophrenia		
	Combined (73)	267C/C (63)	267C/A (10)	Combined (76)	267C/C (65)	267C/A (11)
Age (years)	45.6 ± 16.6	45.3 ± 16.3	47.5 ± 19.7	44.7 ± 16.7	43.7 ± 16.5	50.1 ± 17.9
Gender (M/F)	53/20	50/13	7/3	54/22	48/17	6/5
Suicide (Y/N)	0/75	0/65	0/10	36/40	33/32	3/8
Brain pH	6.3 ± 0.2	6.3 ± 0.2	6.3 ± 0.2	6.2 ± 0.3	6.3 ± 0.26	6.2 ± 0.28
Post mortem interval	40.7 ± 13.8	40.6 ± 13.8	41.1 ± 14.4	40.9 ± 12.6	40.2 ± 11.5	44.9 ± 18.2
Duration of illness (years)	N/A	N/A	N/A	18.2 ± 13.7	17.9 ± 14.0	20.6 ± 11.5
Chlorpromazine Equivalents (mg)	N/A	N/A	N/A	649.2 ± 608.0	643.5 ± 574.2	687.5 ± 844.7

Data is expressed as mean ± SD.

Figure 1:

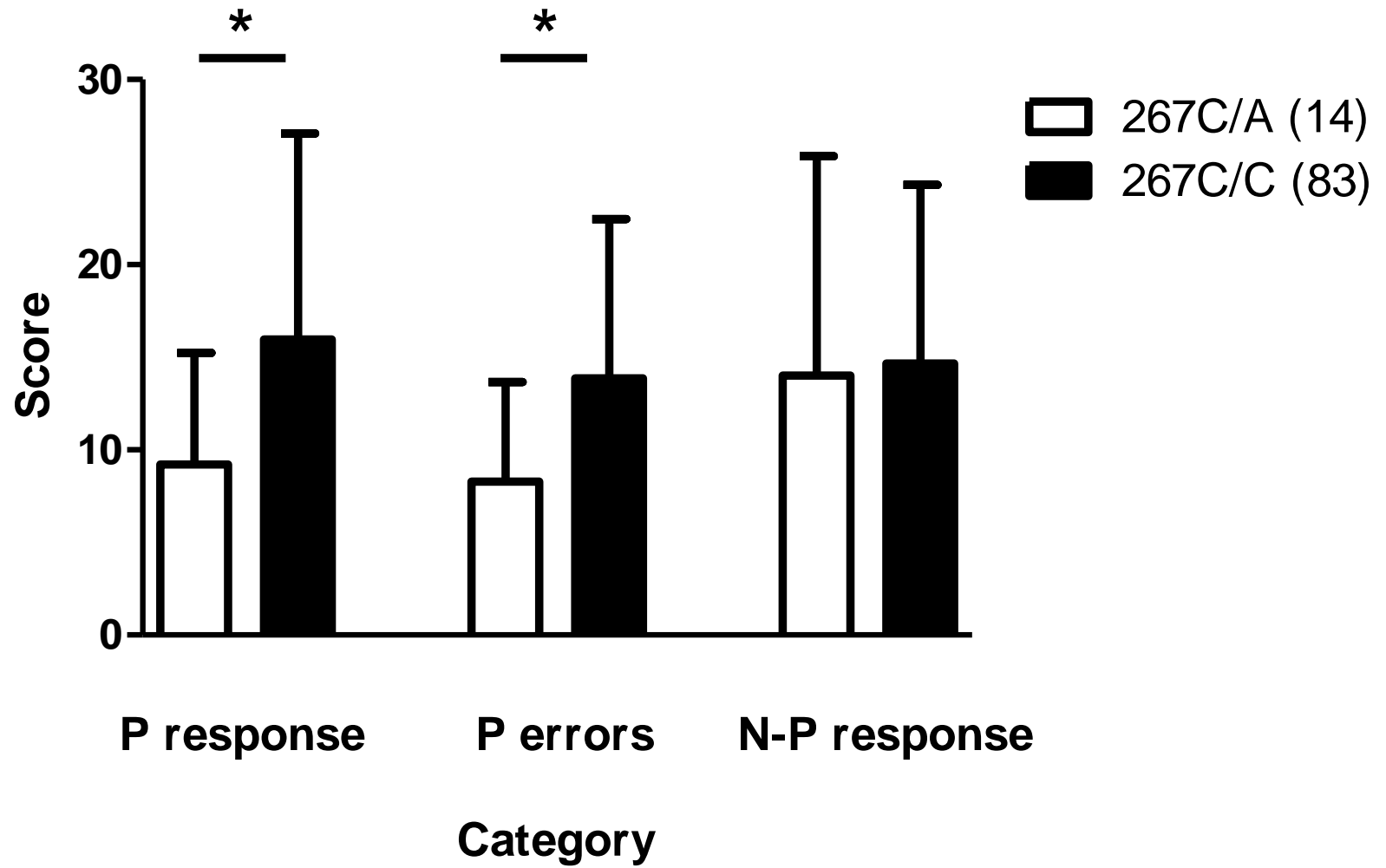
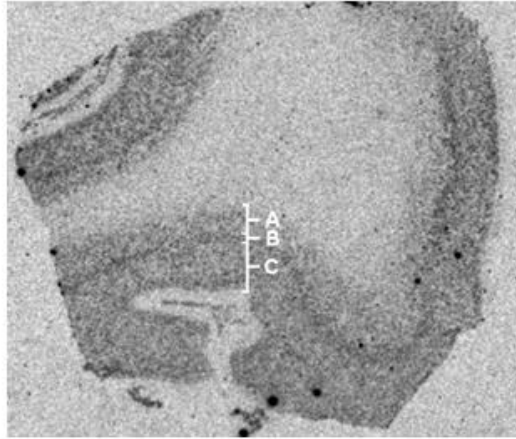
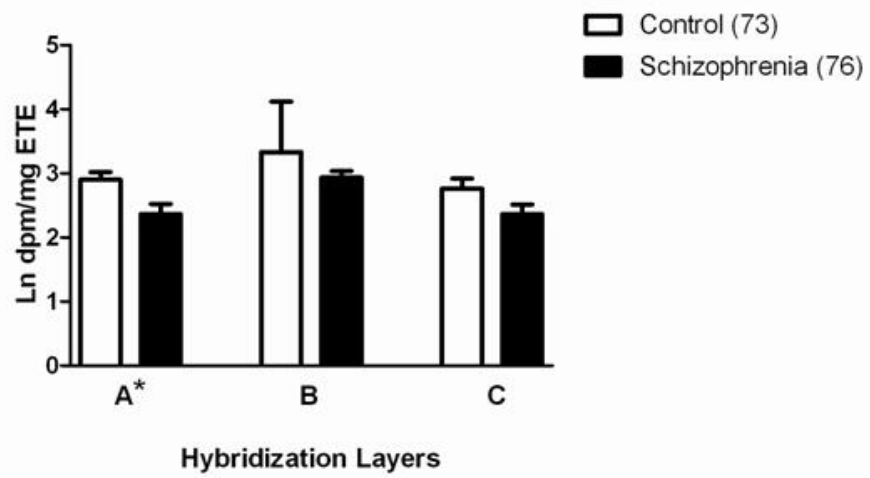


Figure 2:

A



B



* Significant difference between diagnosis ($t = 2.90$, $df = 145$, $p < 0.05$)

C

