The effects of a muscarinic receptor 1 gene variant on executive and non-executive cognition in schizophrenia spectrum disorders.

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1. Introduction

Previous results from association, pharmacological intervention and animal-model studies has suggested an abnormal central muscarinic system, particularly the muscarinic M1 receptor (CHRM1) in the facilitation of clinical and cognitive symptoms in schizophrenia spectrum disorders (SSD; Carruthers et al., 2015). Two independent studies have reported that individuals with schizophrenia who are C-allele homozygotes at the CHRM1 c.267C > A (rs2067477) single nucleotide polymorphism (SNP) exhibit more pronounced executive functioning deficits on the Wisconsin Card Sorting Test (WCST) compared to those who are 267C/A heterozygous (Liao et al., 2003; Scarr et al., 2012). However, rs2067477 genotype variation in these studies had no association with premorbid IQ, symptom severity, illness-related factors or verbal fluency and did not confer altered risk for schizophrenia.

Associations between the rs2067477 genotype and psychomotor speed, working and visual memory in SSD were detected in a large sample (N=447) as part of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) Study (Need et al., 2009); however, no directions were reported. Cropley et al. (2015) showed that C/C homozygosity in SSD had no influence on working memory, verbal fluency, visuospatial-construction and attention, however could not comment on perseveration or cognitive flexibility due to an absence of WCST data. It was also revealed that C/C homozygosity in SSD was associated with reduced grey matter volume in a large cluster within the right precentral gyrus, incorporating the dorsal and ventral aspects of the premotor cortex; a structural change that was not detected in the cortical thickness or surface area of patients in a follow-up study (Carruthers et al., 2018). Previous research has implicated the premotor cortex in the set-shifting and response execution stages of the WCST (Abe and Hanakawa, 2009). Taken together, it appears that rs2067477 genotype variation in schizophrenia is linked to processes specific to performance on the WCST; particularly perseveration or cognitive flexibility. The

aim of the present study was to further investigate the association between rs2067477 genotype variation and cognition. As previous research investigating the association between rs2067477 genotype variation and performance on the WCST has been restricted only to patients with a diagnosis of schizophrenia, we sought to explore the previously reported association amongst a group of SSD patients cross-diagnostically in a combined patient-healthy control group and more broadly using a multidimensional neuropsychological test battery.

1. Method

Data from a combined total of 147 participants with SSD (duration of illness 18.0±9.3 years) and 294 healthy controls (HC) were obtained from the Cognitive and Genetic Explanations of Mental Illnesses (CAGEMIS) and Cooperative Research Centre (CRC) for Mental Health bio-databanks. Ninety-six participants with a confirmed diagnosis of schizophrenia, 27 with schizoaffective disorder and 173 HCs completed the 128-card computerised version of the WCST (Version 4; Heaton, 1993). Sixty-five participants with a confirmed diagnosis of schizophrenia, 23 with schizoaffective disorder and 225 HCs completed the MATRICS Consensus Cognitive Battery (MCCB; Nuechterlein et al., 2008). All participants had given prior informed consent for the analysis of their stored data and were recruited from metropolitan-based outpatient community clinics in Australia. Participants were fluent in English, between the ages of 18 and 65 years old, and had an estimated premorbid IQ > 70, as scored by the Wechsler Test of Adult Reading (Wechsler, 2001). Participants with significant visual or verbal impairments, a known neurological disorder and/or current substance/alcohol abuse or dependence were excluded. At time of testing, all patients were on stable doses of anti-psychotic medication. Patient symptomology was assessed with the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987).

Items N4 and G16 were not included as reports from patient primary care worker/family were not available. See Supplementary Material for genotyping methodology. Groups were compared on demographic and neuropsychological variables using analysis of variance (ANOVA) or Chi-square analysis as appropriate. Brown-Forsythe F-ratio was used when appropriate. To correct for multiple corrections, a conservative α-value of $p \leq .01$ was considered statistically significant.

2. Results

The frequencies of the c.267C > A CHRM1 genotypes in the patient-only group were 77.6% (*n*=114) C/C and 22.4% (*n*=33) C/A, which were in Hardy-Weinberg equilibrium and similar to that reported in previous studies (Cropley et al., 2015; Liao et al., 2003; Scarr et al., 2012). No significant SSD diagnosis differences or SSD diagnosis-by-genotype interactions were detected (see Supplementary Table S1, S2). For the combined SSD-HC group, the frequencies were 77.6% (*n*=342) C/C and 22.4% (*n*=99) C/A, which were in Hardy-Weinberg equilibrium. Given the small number of participants homozygous for the minor allele (A/A), these cases were combined with the C/A group. Groups did not differ on demographic or clinical characteristics. For both the combined SSD-HC group analysis and the SSD-only analysis, no significant genotype effects were detected for any of the WCST or MCCB variables (Table 1).

3. Discussion

The present study sought to further examine the influence the CHRM1 c.267C > A SNP had on executive and non-executive cognitive functions in a sample of SSD patients and cross-diagnostically in a combined SSD-HC group. Despite previous reports of a significant association between rs2067477 genotype and executive function in schizophrenia (Liao et

al., 2003; Need et al., 2009; Scarr et al., 2012), the present study failed to detect any such link between the CHRM1 SNP and performance on the WCST in a cross-diagnostic SSD sample and combined SSD-HC group. Two previous studies have reported that the rs2067477 genotype is specifically linked to perseveration/set-shifting processes in schizophrenia, without having any associations with symptom severity or premorbid IQ (Liao et al., 2003; Scarr et al., 2012). Consistent with the previous research, rs2067477 genotype variation was not related to any demographic or clinical characteristics (Cropley et al., 2015). However, we were unable to replicate the reported significant association between homozygosity at c.267C > A and impaired perseveration/set-shifting on the WCST in a SSD sample. Consistent with Scarr et al. (2012) and Cropley et al. (2015), we were unable to detect any signifiacnt genotype effects on non-executive cognitive function. The lack of significant genotype effects detected here, paired with previous reports suggests that the rs2067477 genotype is not associated with non-executive cognitive function in SSD. Despite the null-findings reported here, previous research suggests that the muscarinic system is involved in facilitating both executive and non-executive cognition (Carruthers et al., 2015). Therefore, further investigation of genetic markers associated with the CHRM1 and their influence on cognition should be considered with a broader focus than that employed here.

Acknowledments

See Supplementary Material

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Table 1. Results Summary

Wisconsin Card Sorting Test

	Combined Patient-Healthy Control Group				Patient-only Group			
	267C/C (*n*=226)	267C/A-A/A (*n*=70)	η^2	*p*	267C/C (*n*=94)	267C/A-A/A (*n*=29)	η^2	*p*
TA	103.7±24.3	103.5±23.3	0.00	0.95	118.9±16.7	120.6±16.8	0.00	0.66
TC	66.6±13.8	66.9±11.7	0.00	0.86	63.5±18.0	65.6±15.6	0.00	0.58
TE	37.1±27.6	36.6±27.4	0.00	0.89	55.4±26.2	55.0±24.3	0.00	0.94
PR	22.0±20.9	21.3±18.8	0.00	0.81	33.8±23.0	33.8±19.9	0.00	0.99
PE	19.0±16.2	18.9±15.9	0.00	0.98	28.5±17.2	29.7±16.4	0.00	0.73
NPE	17.9±14.8	17.8±15.3	0.00	0.99	26.4±15.5	25.3±14.6	0.00	0.74
CC	4.4±2.2	4.2±2.3	0.00	0.50	3.0±2.3	2.6±2.1	0.00	0.46
FMS	0.8±1.3	1.0±1.3	0.00	0.09[#]	1.1±1.3	1.6±1.7	0.03	0.09[#]
TFC	27.8±33.5	30.10±34.1	0.00	0.62	43.3±45.7	48.0±44.3	0.00	0.63

MATRICS Consensus Cognitive Battery

	Combined Patient-Healthy Control Group				Patient-only Group			
	267C/C (*n*=244)	267C/A-A/A (*n*=69)	η^2	*p*	267C/C (*n*=70)	267C/A-A/A (*n*=18)	η^2	*p*
SoP	51.4±12.9	51.8±10.8	0.00	0.79	40.6±11.9	44.0±13.6	0.01	0.30
AV	44.9±10.6	47.5±9.9	0.01	0.08	39.8±12.8	39.9±10.7	0.00	0.97
WM	51.1±10.3	50.7±11.2	0.00	0.77	43.5±10.3	38.2±10.2	0.04	0.06
VerL	45.7±10.5	47.0±10.2	0.00	0.35	37.9±8.5	40.3±8.8	0.01	0.29
VisL	50.0±12.1	49.3±11.6	0.00	0.68	41.0±13.4	41.8±14.1	0.00	0.82
SC	45.3±12.1	44.2±12.6	0.00	0.50	42.0±10.9	36.9±12.6	0.03	0.12
RP	49.6±10.8	50.6±10.9	0.00	0.48	42.5±9.1	44.4±8.9	0.00	0.77

Note: Data is presented as mean ± SD; [#] Brown-Forsythe Test performed; TA, trials administered; TC, total correct; PR, perseverative responses; PE, perseverative errors; NPE, non-perseverative errors; CC, categories completed; FMS, failure to maintain set; TFC, trials to first category; SoP, speed of processing; AV, attention-vigilance; WM, working memory; VerL, verbal learning; VisL, visual learning; SC, social cognitions; RP, reasoning and problem solving

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Supplementary Material

Method

CHRM1 Genotyping

DNA was extracted from whole blood using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden Germany) and SNP assays were designed using the Agena Assay Design Suite 1.0 software (Agena San Diego CA). Genotyping for rs2067477 (CC, CA, AA) was carried out using the MassARRAY system (Agena, San Diego CA) as per the manufacturer's standard protocols. The MassArray platform relies on a primer extension reaction in combination with a mix of mass-tagged dideoxy-nucleotides (iPlex Gold chemistry) to generate a pool of oligo products that are analysed by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Adherence to Hardy-Weinberg equilibrium and allele frequency were assessed to ensure validity of the results.

Table S1. WCST performance across diagnosis and genotype.

WCST		267C/C (n = 94)	267C/A-A/A (n = 29)	Total		Diagnosis	Diagnosis x Genotype
				Schizophrenia (n = 96)	Schizoaffective Disorder (n = 27)		
TA	Sz	121.7 (14.4)	120.7 (17.0)	121.4 (15.0)	111.9 (22.8)	$F_{1,122} = 6.6, p \leq 0.05$	$F_{1,123} = 1.4, p = 0.23$
	SaD	110.0 (23.7)	120.2 (17.4)				
TC	Sz	63.1 (18.9)	65.0 (16.0)	63.5 (18.2)	65.6 (14.8)	$F_{1,122} = 0.3, p = 0.59$	$F_{1,123} = 0.4, p = 0.85$
	SaD	64.9 (15.0)	68.9 (15.1)				
PR	Sz	35.7 (23.4)	33.1 (19.1)	35.1 (22.3)	29.4 (21.6)	$F_{1,122} = 1.4, p = 0.24$	$F_{1,123} = 0.9, p = 0.34$
	SaD	27.7 (20.9)	36.8 (25.8)				
PE	Sz	29.8 (17.1)	29.0 (15.5)	29.6 (16.6)	25.8 (17.8)	$F_{1,122} = 1.0, p = 0.31$	$F_{1,123} = 1.0, p = 0.32$
	SaD	24.2 (17.0)	32.8 (21.8)				
NPE	Sz	28.1 (15.9)	26.7 (14.5)	27.8 (15.5)	20.5 (12.7)	$F_{1,122} = 5.0, p \leq 0.05$	$F_{1,123} = 0.0, p = 0.94$
	SaD	20.9 (12.5)	18.8 (15.2)				
CC	Sz	2.74 (2.3)	2.6 (2.1)	2.7 (2.2)	3.6 (2.2)	$F_{1,122} = 3.4, p = 0.07$	$F_{1,123} = 0.5, p = 0.51$
	SaD	2.8 (2.2)	2.8 (2.3)				
FMS	Sz	1.2 (1.4)	1.6 (1.5)	1.3 (1.4)	1.0 (1.5)	$F_{1,122} = 1.0, p = 0.33$	$F_{1,123} = 1.2, p = 0.27$
	SaD	0.7 (1.0)	2.0 (2.8)				
TFC	Sz	47.9 (47.9)	42.4 (43.5	46.8 (46.6)	36.1 (39.5)	$F_{1,122} = 1.7, p = 0.28$	$F_{1,123} = 3.5, p = 0.07$
	SaD	28.5 (34.6)	69.8 (46.4)				

Note WCST, Wisconsin Card Sorting Test; TA, trials administered; TC, total correct; PR, perseverative responses; PE, perseverative errors; NPE, non-perseverative errors; CC, categories completed; FMS, failure to maintain set; TFC, trials to first category; Sz, schizophrenia; SaD, schizoaffective disorder; Bonferroni corrected to $p \leq .01$.

Table S2. MCCB performance across diagnosis and genotype.

MCCB		267C/C (n = 70)	267C/A-A/A (n = 18)	Total		Diagnosis	Diagnosis x Genotype
				Schizophrenia (n = 65)	Schizoaffective Disorder (n = 23)		
SoP	Sz	40.5 (12.1)	41.6 (12.8)	40.8 (12.1)	42.9 (12.6)	$F_{1,87} = 0.5, p = 0.47$	$F_{1,88} = 1.8, p = 0.19$
	SaD	40.9 (11.6)	52.3 (14.7)				
AV	Sz	39.0 (13.4)	39.7 (9.0)	39.2 (12.5)	41.6 (11.9)	$F_{1,78} = 0.6, p = 0.45$	$F_{1,79} = 0.1, p = 0.80$
	SaD	41.9 (10.9)	40.5 (17.0)				
WM	Sz	43.3 (11.1)	37.7 (10.7)	42.1 (11.2)	43.4 (8.1)	$F_{1,86} = 0.3, p = 0.61$	$F_{1,87} = 0.0, p = 0.83$
	SaD	44.2 (7.9)	40.0 (9.3)				
VerL	Sz	36.7 (8.6)	39.9 (7.9)	37.4 (8.5)	41.6 (8.1)	$F_{1,86} = 4.0, p = 0.05$	$F_{1,87} = 0.3, p = 0.57$
	SaD	41.5 (7.2)	41.8 (12.8)				
VisL	Sz	39.4 (13.4)	40.9 (14.5)	39.7 (13.5)	45.4 (12.7)	$F_{1,86} = 3.0, p = 0.08$	$F_{1,87} = 0.1, p = 0.82$
	SaD	45.4 (12.8)	45.0 (14.3)				
SC	Sz	40.6 (10.9)	36.6 (14.1)	39.7 (11.7)	44.0 (10.2)	$F_{1,73} = 2.2, p = 0.14$	$F_{1,74} = 0.3, p = 0.61$
	SaD	45.4 (10.2)	47.8 (8.7)				
RP	Sz	49.4 (8.9)	42.0 (8.9)	42.2 (8.9)	45.1 (9.3)	$F_{1,83} = 1.7, p = 0.19$	$F_{1,84} = 0.4, p = 0.52$
	SaD	44.2 (9.7)	49.0 (6.7)				

Note: MCCB, MATRICS Consensus Cognitive Battery; SoP, speed of processing; AV, attention-vigilance; WM, working memory; VerL, verbal learning; VisL, visual learning; SC, social cognitions; RP, reasoning and problem solving; Sz, schizophrenia; SaD, schizoaffective disorder; Bonferroni corrected to $p \leq .01$.

Acknowledgements

This work was supported by Australian Postgraduate Awards (S.P.C, P.J.S) and by the Australian National Health and Medical Research Council (NHMRC; fellowships to C.G (ID: 5467262), T.V.R (1088785), E.J.T (1142424) and C.P (628386 & 1105825); and a project grant to S.L.R (ID: 1060664)). The authors acknowledge the support of the Monash Alfred Psychiatry Research Centre and the financial support of the Cooperative Research Centre (CRC) for Mental Health. The CRC programme is an Australian Government Initiative. The authors wish to acknowledge the CRC Scientific Advisory Committee, in addition to the contributions of study participants, clinicians at recruitment services, staff at the Murdoch Children's Research Institute, staff at the Australian Imaging, Biomarkers and Lifestyle Flagship Study of Aging, and research staff at the Melbourne Neuropsychiatry Centre, including coordinators Phassouliotis, C., Merritt, A., and research assistants, Burnside, A., Cross, H., Gale, S., and Tahtalian, S. None of the funding sources played any role in the study design; collection, analysis or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. Participants for this study were sourced, in part, through the Australian Schizophrenia Research Bank (ASRB), which is supported by the National Health and Medical Research Council of Australia (Enabling Grant ID: 386500), the Pratt Foundation, Ramsay Health Care, the Viertel Charitable Foundation and the Schizophrenia Research Institute. We thank the Chief Investigators and ASRB Manager: Carr, V., Schall, U., Scott, R., Jablensky, A., Mowry, B., Michie, P., Catts, S., Henskens, F., Pantelis, C., Loughland, C. We acknowledge the help of Jason Bridge for ASRB database queries. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.