ORIGINAL ARTICLE

Effects of *GRASP* Variation on Memory in Psychiatrically Healthy Individuals and Cognitive Dysfunction in Schizophrenia

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Abstract

Mechanistic studies indicate general receptor for phosphoinositides-1-associated scaffold protein (GRASP, also referred to as Tamalin) is involved in neurite development, proliferation, and branching in hippocampal neurons, but its physiological effects on cognitive function is mostly unknown. Cognitive impairment is a core feature of schizophrenia, and recent reports indicate increased abundance of GRASP proteins in postmortem schizophrenia cortex and hippocampus, both of these structures being highly relevant to cognitive processes. We therefore assessed the effects of a single nucleotide polymorphism (SNP) rs10876227 [G>A] in *GRASP* on eight different domains of cognitive function in a well-established Caucasian case-control cohort for schizophrenia. In 261 control individuals (166 males), strong effects of rs10876227 were observed on immediate memory, delayed memory, and working memory, with the major G allele associated with worse memory performance on each test. Additional analyses including 249 patients with schizophrenia (174 males) indicated that the G allele of rs10876227 was also able to distinguish male schizophrenia participants with severe cognitive deficits (CD; 81 males) from male schizophrenia participants with relatively spared cognitive function (CS; 91 males). However analyses of the effects of *GRASP* variation on individual cognitive domains in the combined sample showed no interactive effects of clinical status and rs10876227 variation. These findings converge with prior mechanistic and postmortem studies to strongly support contribution of *GRASP* variation to memory function, and general cognitive ability in men with schizophrenia, likely via GRASP-directed plasticity. The implications of these findings extend to other disorders where cognitive function is a core component, such as dementia, autism and mental retardation.

Keywords:

GRASP; general receptor for phosphoinositides-1-associated scaffold protein; Tamalin; memory; schizophrenia; case-control analysis; postmortem brain.

Introduction

The General Receptor for phosphoinositides 1-Associated Scaffold Protein (GRASP; also referred to as the alias Tamalin) is a putative regulator of cognitive function by modulation of neuronal plasticity (Yanpallewar et al., 2012). The main function of GRASP is to form macromolecular complexes in the postsynaptic density (PSD) by binding multiple signaling partners *via* its protein-interacting domains (Kitano et al., 2003). Importantly, GRASP clusters the major scaffold proteins PSD-95 and GKAP proteins (Kitano et al., 2003), which facilitate widespread protein-protein interactions in the PSD (Kitano et al., 2003). GRASP also facilitates cell surface expression and intracellular movement of inhibitory and excitatory G-protein coupled neurotransmitter receptors, such as the gamma aminobutyric acid (GABA) receptor and mGluR1/5 (Kitano et al., 2002).

Organization of GABA and glutamate receptors in time and space is critical for modulation of inhibitory:excitatory neurotransmission and plasticity (Lewis, 2014), suggesting an important role for *GRASP* in the modulation of cognitive function. This hypothesis is supported in mice, with a deficiency in *GRASP* expression blocking electroconvulsive shock therapy (ECS)-induced neurite proliferation, development and dendritic arborization in adult progenitor cells from the dentate gyrus (Yanpallewar et al., 2012). Knockout of *GRASP* in rat hippocampal cultures was also shown to reduce dendritic outgrowth and arborization (Mo et al., 2012), together supporting that *GRASP* critically contributes to the modulation of dendritic development and consequently may influence activity-dependent plasticity. *GRASP*$^{-/-}$ knockout mice showed no difference in neurodevelopment or behaviors involving sensorimotor functions (including prepulse inhibition) or emotion (light/dark transition, elevated plus maze, social interactions or forced swim tests); however, the effects of *GRASP* knockout on cognitive functions was limited (Ogawa et al., 2007).

Cognitive dysfunction is a core symptom of the severe neuropsychiatric disorder, schizophrenia. Cognition is moderately to severely impacted in the disorder across several domains, including learning and memory (Green, 2006). This cognitive decline is arguably the most debilitating and least well-treated aspect of schizophrenia, considering the severity of impairment is closely linked with the level of disability and long-term functional outcomes (Green, 2006; Heinrichs, 2005; Jablensky, 2006; Keefe and Harvey, 2012). However cognitive impairments are not uniformly severe among schizophrenia patients, as demonstrated by several recent studies that have delineated two subtypes of schizophrenia on the basis of cognitive profile. The

existence of these two subtypes of schizophrenia have been replicated in large case-control cohorts and their family members, representing cases with severe cognitive deficits (CD) as distinct from those with relatively spared cognitive function (CS) (CS; Green et al., 2013; Hallmayer et al., 2005; A. Jablensky, 2006). Further study of genetic variants specific to cognitive subtypes may assist in understanding how genetic variation contributes to cognitive impairment in schizophrenia.

Despite converging findings indicating a role for *GRASP* in human cognition, this remains unconfirmed. The present study therefore aimed to explore the role of genetic variation in *GRASP* in human cognition, and its potential contribution to cognitive dysfunctions observed in schizophrenia. To our knowledge, there have been no studies examining single nucleotide polymorphisms (SNP) in *GRASP* within human cohorts. We therefore selected a variant, rs10876227, on the basis of its location near the 5' untranslated region (5' UTR) of the gene (Figure 1), which is a genomic region critical for the regulation of protein translation (Chorev and Carmel, 2012; Hughes, 2006), and relevant in light of recent postmortem reports indicating increased GRASP protein levels in schizophrenia (Matosin et al., 2015a, 2015b). Rs10876227 was tested for its association with eight measures of cognitive function in a Caucasian cohort, consisting of 268 healthy control participants and 268 matched schizophrenia cases. A separate postmortem human brain cohort was also genotyped to assess the possible impact of the chosen variants on GRASP protein levels. It was hypothesized that the examined genetic variant in *GRASP* would be associated with cognitive function in healthy controls and participants with schizophrenia. Considering the position of rs10876227 near the 5' UTR, we also hypothesized this variant would be associated with the levels of GRASP protein levels in the brains of individuals with schizophrenia.

Materials and Methods

Participants

Samples were obtained from the Australian Schizophrenia Research Bank (ASRB), a national bank of biological specimens. Subjects were selected from the bio bank using strict criteria:

(1) Schizophrenia patients diagnosed with schizophrenia according to the DSM-IV;
(2) Control subjects were required to have no personal or family history of mental disorder;
(3) Control subjects were selected to match schizophrenia cases according to sex and age (Table 1);
(4) All participants were required to be of Caucasian ethnicity, fluent in English and have no history of an organic brain disorder, post-traumatic amnesia, mental retardation, movement disorder, or substance dependence;
(5) Participants who received electroconvulsive therapy in the six months prior to testing were excluded from selection.

Trained researchers performed the Diagnostic Interview for Psychosis (DIP) for all participants. Extensive details regarding the clinical and demographic characterization, sampling frameworks and consent procedures for samples from the ASRB are published elsewhere (Loughland et al., 2010). This study was approved by and conducted according to the guidelines of the University of Wollongong Human Research Ethics Committee (HE10/161) and the University of New South Wales Human Ethics Committee (HC12658).

Neuropsychological measures and cognitive subtyping

Standardized measures of premorbid and current intelligence quotient (IQ) were obtained using the Wechsler Abbreviated Scale of Intelligence test (WASI) and the Wechsler Test for Adult Reading (WTAR) (Wechsler, 2001, 1997). The Letter Number Sequencing test (LNS) was used to assess working function (Spreen, 1998; Wechsler, 1997). Indices of attention, delayed memory, immediate memory, visuospatial construction and language construction were derived using the Repeatable Battery of Neuropsychological Status (RBANS) (Randolph, 1998). A large subgroup of 247 schizophrenia patients were classified as either

displaying a general cognitive deficit (CD) or displaying relatively spared cognitive function (CS), based on previous Grade of Membership (GoM) analyses of the ASRB sample previously described in detail (Green et al., 2013). Briefly, continuous indices for individual cognitive performance domains were converted to categorical performance measures (poor, moderate or good), and entered into the GoM analysis along with descriptive and demographic information including (but not limited to) sex, age of onset, mode of onset, psychosocial stressors, drug abuse, illness course, and family history of schizophrenia (Green et al., 2013). Breakdowns of the demographics for this sample are provided within Table 1 and 4.

Postmortem samples

A postmortem sample was used to assess whether the studied variants influence expression of GRASP proteins. Human postmortem brain samples from the hippocampal CA1 region of 39 participants (20 healthy controls and 19 schizophrenia cases) were obtained from the New South Wales Brain Bank Network (Sydney, Australia). GRASP protein levels in these samples was previously determined by quantitative immunoblot (Matosin et al., 2015b). Genomic DNA was extracted from brain tissues using the QIAamp DNA Mini Kit (Qiagen, Australia). This experiment was approved by the Human Research Ethics Committees at the University of Wollongong (HE 10/161 and HE99/222).

Genotyping

The rs10876227 [G>A] SNP was selected based on its MAF, which was greater than 20% in a Caucasian population according to the National Centre for Biotechnology Information (NCBI) database. High-throughput SNP genotyping was performed using the MassARRAY ® genotyping assay from Sequenom Inc. (San Diego, CA, USA). Analyses were subsequently performed using matrix-assisted laser desorption and ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Polymerase Chain Reactions and extension primer design, selection and multiplexing were performed using Sequenom MassARRAY ® Designer Software.

Statistical methods

Analyses were performed using SPSS v21, and Holm-Bonferroni correction was applied to adjust for multiple comparisons. Genotypic distribution of rs10876227 was firstly assessed for deviation from Hardy-Weinberg Equilibrium (HWE; $p>0.05$). χ^2 analyses were then used to assess the allele and genotype frequencies between the case and control groups. Subjects were grouped into all minor allele carriers and compared to major allele homozygotes due to the low frequency of minor homozygote genotypes across the cohort. All analyses in the ASRB sample were performed sex-wise, to address possible sex-specific effects which are prevalent in schizophrenia (Goldstein et al., 2013).

A separate mixed design multiple analyses of variance (MANOVA) was conducted in the total cohort, to analyze group-by-genotype effects of rs10876227 and clinical status on cognitive performance, with two levels of within-subject factors for genotype (minor allele carriers: major allele homozygotes) and two levels of between-subject factors of group (control/schizophrenia). Significant interactions were further assessed using post-hoc pairwise comparisons. The capacity of rs10876227 to predict membership in GoM-derived cognitive phenotypes was conducted using a series of multinominal logistic regressions, where categorical GoM classes were set as dependent variables. This analysis was performed in two steps, first using healthy controls as the reference category (Model 1), and second using the CS as the reference category (Model 2; Table 5). Lastly, the postmortem sample was used to assess whether the studied variants influence protein abundance of GRASP. Analysis of Covariance (ANCOVA), using diagnosis as a covariate, was implemented to test the effects of rs10876227 genotypes on GRASP protein abundance in all subjects. This was performed irrespective of diagnosis owing to the limited sample size.

Results

Genotypic and allelic frequencies in schizophrenia compared to healthy controls.

Allele frequency of rs10876227 in the total cohort did not deviate from HWE (χ^2=0.293, p=0.588), and the control group displayed a pattern similar to those reported for Caucasian populations according to the NCBI database. There was no difference in allele frequency (χ^2=0.182, p=0.669, Odds Ratio [OR]=1.058; Table 2) of rs10876227 in patients with schizophrenia compared to controls. Exploratory examination of sex-specific effects was also performed, but no significant differences were observed for either variant in male (χ^2=0.294, p=0.588; OR=1.099) or female subsets (χ^2=0.002, p=0.961; OR=0.9762; Table 2).

Effects of genotypes on cognitive function in control and schizophrenia participants

MANOVAs were used to assess the effects of group (control:schizophrenia), genotype (minor allele carriers: major allele homozygotes) and group-by-genotype interactions on cognitive performance scores assessed across the eight cognitive domains. Schizophrenia subjects showed significantly lower scores in all cognitive assessments compared to healthy controls ($F_{1,460}\geq37.77$, $p\leq0.001$; Table 3). Main effects of rs10876227 genotypes were also assessed across several domains, independent of clinical group-status (Table 3a): working memory (LNS; $F_{1,454}$=11.635, p=0.001; d=0.279), immediate memory ($F_{1,454}$=6.877, p=0.009, d=0.204) and delayed memory ($F_{1,454}$=10.289, p=0.001, d=0.260), with major G allele homozygotes having worse performance on these tests compared to minor A allele carriers. Sex-specific analyses revealed the significant effects of rs10876227 on LNS and delayed memory appeared to be driven by males, with male major G allele homozygotes having lower scores across these cognitive domains compared to male minor allele carriers ($F_{1,299}\geq10.771$, $p\leq0.001$, $d\geq0.330$; Table 3a). There were no group-by-genotype interactions detected across any tested cognitive domains.

Effects of genotypes on cognitive function in individual diagnostic groups

Considering the main-effects of rs10876227 on working memory, immediate memory and delayed memory observed in the combined sample (schizophrenia + controls), it was unclear whether the schizophrenia subjects were driving a whole-group main effect or if these associations were representative of the “general

population". We therefore explored the main effects of rs10876227 in the control and schizophrenia group separately with independent one-way analyses of variance (ANOVA) using genotype [major allele homozygotes: minor allele carriers] as the fixed factor and cognitive domains as the dependent variable, followed by Tukey's post-hoc tests (Table 3b-c).

In the control group, significant effects of rs10876227 were observed on working memory (determined by LNS; $F_{1,242}$=5.996; p=0.005, d=0.329), immediate memory ($F_{1,242}$=9.058; p=0.003, d=0.404) and delayed memory ($F_{1,242}$=6.500; p=0.011, d=0.343) in the control group alone, with major G allele homozygotes having reduced performance on these tests relative to minor A allele carriers. The effects of rs10876227 were upheld in male subjects only for working memory ($F_{1,153}$=6.803, p=0.010, d=0.445) and delayed memory ($F_{1,153}$=7.986, p=0.005, d=0.482) and in females only for immediate memory ($F_{1,153}$=13.876, p<0.001, d=0.821).

There were no main effects of rs10876227 genotypes in both genders combined, males or females, when the schizophrenia group was analyzed alone (Table 3c).

Prediction of cognitive phenotypes (CD/CS) in schizophrenia

Table 4 summarizes sample characteristics of the cognitive subtypes of schizophrenia (CS vs CD). Table 5 summarizes the results of multinominal logistic regressions to determine the capacity of rs10876227 to distinguish patients displaying severe cognitive deficits from those with relatively spared cognitive function and/or healthy controls. In Model 1, rs10876227 did not predict membership of the CD or CS group relative to healthy controls (χ^2=4.43, df=2, p=0.109, Exp B=0.735); however, sex-specific analyses revealed that variation in rs10876227 distinguished the CS group from healthy controls when males were examined separately (χ^2=8.60, df=2, p=0.014, Exp B=0.546), with major G allele homozygotes more highly represented in the CS group relative to the HC group; however the contribution of CS to the model did not reach the reduced significance after Holm-Bonferroni correction (B=-0.61, p=0.030; 95% CI=0.32-0.94). In Model 2, rs10876227 did not distinguish schizophrenia patients in the CD group relative to the CS group after correction for multiple comparisons (χ^2=4.30, df=1, p=0.038, Exp B=1.814). However this analyses reached significance when the male subgroup was examined alone (model significance: χ^2=7.945, df=1, p=0.005, Exp B=2.646; B=0.353, p=0.006; 95% CI=1.33-5.281), with more major G allele homozygous genotypes in the CD group relative to the CS group.

Effects of genetic variation on protein abundance

ANCOVA assessing the effects of genetic variation on protein abundance indicated no association of rs10876227 on GRASP protein levels in the hippocampus (GG: N=25, GA/AA: N=14; $F_{1,39}$=00.465, p=0.500, d=0.325; Figure 3). Examination of genotype-by-diagnosis interactions could not be performed owing to small sample sizes.

Discussion

In this study, we show association of common variation in *GRASP* with three different types of human memory only in psychiatrically healthy subjects, but not in individuals with schizophrenia. Specifically, healthy controls who were carriers of the major (G) allele of rs10876227 showed worse memory function on tests of immediate memory, working memory and delayed memory, relative to minor allele carriers, in healthy participants. Variation in rs10876227 was not however associated with individual cognitive performance indicators in the schizophrenia group alone, or relative to controls. In male participants only, the major G allele of rs10876227 was able to delineate a subgroup of schizophrenia subjects characterized by severe cognitive deficits from those with relatively spared cognitive function. Rs10876227 was not associated with GRASP protein expression in postmortem brain samples.

The association of rs10876227 genotypes with memory processes specifically in psychiatrically healthy participants (not schizophrenia) is interesting, considering the lack of association with several other domains of cognitive performance (i.e., intelligence quotients, attention, visuo-spatial construction, and language construction), and considering that these three different types of memory (delayed, working and immediate) are functionally independent and involve different brain structures (Hales and Clark, 2015). Delayed memory is mediated by the medial temporal lobe (Lee et al., 2005), while immediate memory and working memory are dependent on the dorsolateral prefrontal cortex and temporo-parietal lobes, respectively (Baddeley, 2003; Barbey et al., 2013). These structures all have remarkable plastic potential, and thus the common denominator linking *GRASP* with these different types of memory is not the structures themselves, but rather the mechanisms that occur within them (Kandel, 2009). Accordingly, *GRASP* is abundantly expressed in brain structures required for these three different types of memory (Kitano et al., 2002), and GRASP itself has emerged as a critical regulator of neuroplasticity *in vivo* (Yanpallewar et al., 2012) and neuronal dendritic development *in vitro* (Mo et al., 2012). The present study now provides support that *GRASP* is involved in cognitive function in humans, and specifically that rs10876227 variation is involved in the modulation three types of memory function.

GRASP-mediated neuronal plasticity has been studied in (or in neurons from) the hippocampus (Mo et al., 2012; Yanpallewar et al., 2012), which is an integral component of the brain systems that support explicit memory formation (Hales and Clark, 2015). However, procedural memories, such as immediate and working

memory, rely heavily on other structures including the cortex (Hales and Clark, 2015). The present results thus suggest that the influence of GRASP on neuroplasticity are widespread, and likely extend to the prefrontal and temporoparietal cortices where non-declarative memories are mediated. The mechanism by which rs10876227 effects human memory *via* neuronal plasticity does not appear to be by influence of GRASP protein abundance, at least in the CA1 region, which was not associated with rs10876227 variation in the complimentary postmortem work conducted in this study. This was contrary to our initial hypothesis, considering this variant is located near the 5' UTR of *GRASP*, a genomic region with regulatory function of gene and protein (Chorev and Carmel, 2012; Hughes, 2006). It should be considered that there are many regulatory steps between gene and protein which are differentially modulated according to brain region, neuronal population, and based upon the experience/activity-dependent requirements; rs10876227 could influence transcript or protein expression in other brain structures, or in specific neuronal populations. Further, the studied postmortem sample might have been limited by the inclusion of control and schizophrenia cases (despite co-varying for diagnosis). Rs10876227 might also operate in the context of additional genetic variation in the *GRASP* gene or other genes with which it operates in epistasis, or in response to environmental enrichment or experiences that are known to influence plasticity (Nithianantharajah and Hannan, 2006). The possibility that this SNP has functional properties that contribute to neuronal plasticity cannot be fully excluded, and *in vitro* characterization of rs10876227 and its investigation in the context of the whole genome are important avenues for future studies.

Considering cognitive dysfunction is a central feature of schizophrenia, we hypothesized that differences in the effects of the *GRASP* variants on cognitive function would exist in schizophrenia pathology relative to the healthy control scenario. The results indicated no group-by-genotype interactions when assessing the effects of *GRASP* variation on individual cognitive domains, but the major G allele of rs10876227 delineated a group of males with schizophrenia characterized by having significant cognitive dysfunction, relative to schizophrenia males with relatively spared cognition. These cognitive subtypes were derived from a previous study using a clustering method known as Grade of Membership (GoM) (Green et al., 2013), on the basis that accumulating evidence suggests the existence of cognitive subphenotypes of schizophrenia (Jablensky, 2006). Considering no group-by-genotype effects on individual cognitive domains were observed in this study, it is important to consider that there are likely other contextual effects at play that were not

examined in the scope of this work, such as epistatic effects. Another possibility is the contribution of different cognitive domains (e.g., long-term, episodic or semantic memories) to the severe cognitive sub-phenotype of schizophrenia.

Multiple sex-specific effects of rs10876227 variation were also observed. In controls, the effects of rs10876227 on working and delayed memories were upheld only in males, while the effects of immediate memory were seen only in the female subject subset. This is not surprising considering inherent differences in general memory ability between males and females are established (Halpern, 2013). Male dominant findings were observed in the analyses examining the schizophrenia cognitive subtypes. This could reflect inherent sex differences in etiology and symptoms (Goldstein et al., 2013). Sex-specific biological distinctions have also been found between brain structures of CD and CS types (Gould et al., 2014), consistent with the sex-specific differences between the GoM-derived cognitive subtype classes observed in this study. It is therefore possible that rs10876227 may operate sex-specifically, however, replication in a larger case-control cohort with greater power for sex-specific analyses will be required.

Other limitations of this study include that possible effects of medication or medication responses could not be analyzed, due to limited medication details collected during patient testing. Additionally, testing candidate SNPs precludes the contextual effects from the entire genome, omitting the possibility of epistatic interactions. With regards to our postmortem study, the sample was not big enough to separate by case and control, and cases might have obscured potential significant associations. Replication in a larger postmortem cohort of healthy control participants would be necessary. Characterization of the function of rs10876227 variation in the context of the whole genome would be beneficial, *in vitro* but also in animal models where *GRASP* could be manipulated and its effects on cognitive processes could be assessed.

In summary, the findings of this study suggest that *GRASP* variation plays a role in human memory in psychiatrically healthy individuals, possibly *via* its reported ability to modulate neuronal plasticity. The major G allele of rs10876227 was also able to delineate a cognitive subphenotype of male schizophrenia cases characterized by having severe cognitive dysfunction. These findings are consistent with evidence of contribution of GRASP to neuroplasticity. Considering the widespread associations of *GRASP* variation with human memory, these findings may have wider implications for other disorders with cognitive and memory impairments, including dementia, autism, and mental retardation. Future studies should aim to replicate these

findings and determine the functional characteristics of the rs10876227 polymorphism in *in vitro* and *in vivo* models.

Contributors

Author NM conducted experiments, data analyses, interpreted results and wrote the manuscript. Author MJG contributed cognitive data, performed analysis and assisted with data interpretation and manuscript preparation. Author KN contributed to data interpretation and manuscript preparation. Author FF designed the study, conducted experiments, performed data analysis, assisted with data interpretation and manuscript preparation and management. All authors contributed and approved the final manuscript.

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Figures

Figure 1. Schematization of *GRASP* and rs10876227 (located in exon 2). **Abbreviations**: bp, base pair; SNP, single nucleotide polymorphism; rs, reference SNP.

Figure 2. Bar graphs showing difference in different memory functions between major allele homozygotes (GG) and minor allele carriers (GA/AA) of rs10876227 in healthy participants. Major allele homozygotes displayed significantly lower memory function relative to minor allele carriers in each case (*$P \leq 0.01$). Bars represent means + standard error of the mean for each respective test.

Figure 3. Representative western blots of GRASP protein abundance for four subjects with the following genotypes: 1, AA homozygotes; 2 AG heterozygotes; 3 and 4 GG homozygotes. There was no significant difference in protein levels between genotypes (A carriers vs GG homozygyotes). **Abbreviations**: kDa: kilodaltons.

Tables

Table 1. Sample characteristics of control and schizophrenia subjects.

	Control	**Schizophrenia**	F/χ^2	P-value
Gender (males, females)	166, 95	174, 75	0.159[b]	0.08
Age (mean, SD)	38.6, 12.55	39.94, 10.99	0.077[a]	0.78
Years of education (mean, SD)	15.52, 2.978	13.33, 2.72	74.246[a]	<0.001
GAF score (mean, SD)	84.82, 8.219	52.70, 12.49	969.153[a]	<0.001
NES score (mean, SD)	3.65, 3.409	8.80, 6.87	110.853[a]	<0.001
Positive symptoms (mean, SD)*	-	10.423, 4.01	-	-
Negative symptoms (mean, SD)†	-	27.85, 17.57	-	-
DIP onset age (<25 years, >25years)	-	184, 65	-	-
Family history of schizophrenia (yes, no)	-	80, 197	-	-
DIP lifetime cannabis (yes, no)	4, 85	96, 143	38.941[b]	<0.001
DIP lifetime alcohol (yes, no)	5, 87	89, 151	32.82[b]	<0.001
Cognitive scores				
WTAR scores (mean, SD)	40.84, 5.97	36.37, 7.75	53.644[a]	<0.001
WASI (mean, SD)	118.01, 10.27	102.69, 13.94	201.050[a]	<0.001
LNS score (mean, SD)	12.39, 2.75	9.54, 2.77	138.488[a]	<0.001
RBANS total score (mean, SD)	508.85, 41.44	426.74, 53.48	377.640[a]	<0.001
RBANS construction (mean, SD)	98.84, 15.66	86.13, 15.27	85.686[a]	<0.001
RBANS language (mean, SD)	105.38, 11.02	94.04, 11.56	128.312[a]	<0.001
RBANS attention (mean, SD)	105.27, 15.55	83.69, 16.89	225.393[a]	<0.001
RBANS immediate memory (mean, SD)	101.16, 14.25	80.38, 17.33	219.313[a]	<0.001
RBANS delayed memory (mean, SD)	98.2, 11.50	82.44, 15.93	164.833[a]	<0.001

DIP, Diagnostic Interview for Psychosis; GAF, Global Assessment of Functioning; NES, Neurological Evaluation Scale; SD, standard deviation. [a]ANOVA (analyses of variance). [b] χ^2 test. *derived from DIP hallucination and delusion items. †derived from the Scale for the Assessment of Negative Symptoms (SANS).

Table 2. Allele frequency of rs10876227 major G allele (%), A allele (%) in control compared to schizophrenia subjects.

	Control	Schizophrenia (all)	χ^2	P value
All subjects	391 (80.5%), 95 (19.5%)	357 (79.3%), 93 (20.7%)	0.182	0.669
Male	248 (80.5%), 60 (19.5%)	241 (78.8%), 65 (21.2%)	0.294	0.588
Female	143 (80.3%), 35 (19.7%)	116 (80.6%), 28 (19.4%)	0.002	0.961

Table 3. Summary of rs10876227 genotype effects on cognitive performance measures analysed by (a) multiple analyses of variance in the total cohort, irrespective of diagnosis, and one-way analyses of variance in (b) control and (c) schizophrenia participants only.

Rs10876227		Degrees of Freedom	WTAR *IQ estimate*	WASI *IQ scale*	LNS *Working memory*	RBANS *Immediate memory*	RBANS *Delayed memory*	RBANS *Attention*	RBANS *Construction*	RBANS *Language*
(a) Total cohort (controls + schizophrenia)	**All**	1, 461	F=4.003 P=0.046 d=0.175	F=2.977 P=0.085 d=0.130	**F=11.635** **P=0.001** **d=0.279**	**F=6.877** **P=0.009** **d=0.204**	**F=10.289** **P=0.001** **d=0.260**	F=0.512 P=0.475 d=0.040	F=0.017 P=0.898 d=0.022	F=2.542 P=0.112 d=0.133
	Male	1, 302	*F=5.933* *P=0.015* *d=0.253*	*F=6.537* *P=0.011* *d=0.206*	**F=15.648** **P<0.001** **d=0.367**	F=3.445 P=0.064 d=0.136	**F=13.471** **P<0.001** **d=0.330**	F=1.598 P=0.207 d=0.079	F=0.021 P=0.884 d=0.020	F=2.852 P=0.092 d=0.146
	Female	1, 158	F=0.002 P=0.960 d=0.009	F=0.413 P=0.522 d=0.039	F=0.074 P=0.786 d=0.089	F=3.283 P=0.072 d=0.352	F=0.147 P=0.702 d=0.094	F=0.426 P=0.515 d=0.047	F=0.121 P=0.729 d=0.027	F=0.181 P=0.671 d=0.121
(b) Controls	**All**	1, 242	F=1.241 P=0.266 d=0.150	F=2.357 P=0.126 d=0.206	**F=5.996** **P=0.005** **d=0.329**	**F=9.058** **P=0.003** **d=0.404**	**F=6.500** **P=0.011** **d=0.343**	F=0.179 P=0.673 d=0.057	F=0.073 P=0.787 d=0.036	F=4.111 P=0.044 d=0.272
	Male	1, 151	F=2.290 P=0.132 d=0.258	F=2.867 P=0.092 d=0.289	**F=6.803** **P=0.010** **d=0.445**	F=1.218 P=0.272 d=0.188	**F=7.986** **P=0.005** **d=0.482**	F=0.196 P=0.659 d=0.076	F=0.008 P=0.927 d=0.016	F=2.490 P=0.117 d=0.269
	Female	1, 86	F=0.082 P=0.776 d=0.063	F=0.172 P=0.776 d=0.091	F=0.276 P=0.601 d=0.116	**F=13.876** **P<0.001** **d=0.821**	F=0.166 P=0.685 d=0.090	F=0.013 P=0.910 d=0.025	F=0.016 P=0.900 d=0.028	F=1.245 P=0.268 d=0.246
(c) Schizophrenia	**All**	1, 219	F=1.528 P=0.218 d=0.173	F=1.273 P=0.261 d=0.157	F=5.246 P=0.023 d=0.320	F=0.622 P=0.431 d=4.580	F=3.633 P=0.058 d=0.266	F=1.697 P=0.194 d=0.182	F=0.008 P=0.927 d=0.013	F=0.071 P=0.790 d=0.037
	Male	1,148	F=1.907 P=0.169 d=0.233	F=2.184 P=0.142 d=0.330	F=1.114 P=0.293 d=0.445	F=1.755 P=0.187 d=0.224	F=0.976 P=0.325 d=0.386	F=0.003 P=0.953 d=0.383	F=0.013 P=0.911 d=0.050	F=3.023 P=0.084 d=0.132
	Female	1,65	F=0.183 P=0.670 d=0.047	F=1.835 P=0.180 d=0.246	F=1.701 P=0.197 d=0.023	F=0.012 P=0.914 d=0.075	F=0.043 P=0.836 d=0.043	F=0.054 P=0.816 d=0.174	F=0.063 P=0.802 d=0.092	F=0.755 P=0.388 d=0.081

Abbreviations: IQ, intelligence quotient; LNS, Letter Number Sequencing; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; WASI, Wechsler Abbreviated Scale for Intelligence; WTAR, Wechsler Test of Adult Reading. Effect size is presented as Cohen's *d*. Significant associations (P<0.01) are highlighted in bold. Borderline significant associations are italicized.

Table 4. Sample characteristics of cognitive schizophrenia subtypes (cognitively spared [CS] and cognitive deficits [CD]).

	CS subtype	**CD subtype**	F/χ^2	P-value
Gender (males, females)	81, 27	91, 48	0.125	0.07
Age (mean, SD)	38.00, 10.17	39.64, 11.50	0.366[a]	0.24
Years of education (mean, SD)	14.14, 2.68	12.29, 2.43	31.475[a]	<0.001
GAF score (mean, SD)	55.41, 11.48	49.05, 12.82	16.564[a]	<0.001
NES score (mean, SD)	7.13, 5.58	11.10, 7.80	21.000[a]	<0.001
Positive symptoms (mean, SD)*	11.009, 4.04	9.527, 3.87	6.167[a]	0.01
Negative symptoms (mean, SD)†	26.55, 16.39	29.23, 18.99	1.331[a]	0.25
DIP onset age (<25 years, >25years)	99, 40	83, 25	0.993[b]	0.32
Family history of schizophrenia (yes, no)	47, 91	32, 75	0.475[b]	0.49
DIP lifetime cannabis (yes, no)	40, 65	56, 76	0.455[b]	0.50
DIP lifetime alcohol (yes, no)	38, 67	51, 82	0.116[a]	0.73
Cognitive scores				
WTAR scores (mean, SD)	40.35, 4.76	31.25, 7.88	126.059[a]	<0.001
WASI (mean, SD)	110.50, 10.40	92.34, 10.91	177.421[a]	<0.001
LNS score (mean, SD)	10.73, 2.16	7.91, 2.68	84.231[a]	<0.001
RBANS total score (mean, SD)	459.65, 37.91	384.19, 39.23	233.674[a]	<0.001
RBANS construction (mean, SD)	90.32, 14.75	80.78, 14.41	25.860[a]	<0.001
RBANS language (mean, SD)	97.93, 11.21	89.05, 10.13	41.357[a]	<0.001
RBANS attention (mean, SD)	91.88, 14.37	73.11, 13.92	106.177[a]	<0.001
RBANS immediate memory (mean, SD)	89.09, 14.89	69.29, 13.58	115.720[a]	<0.001
RBANS delayed memory (mean, SD)	90.57, 10.75	71.96, 15.53	122.983[a]	<0.001

DIP, Diagnostic Interview for Psychosis; GAF, Global Assessment of Functioning; NES, Neurological Evaluation Scale; SD, standard deviation. [a]ANOVA (analyses of variance). [b] χ^2 test. *derived from DIP hallucination and delusion items. †derived from the Scale for the Assessment of Negative Symptoms (SANS).

Table 5. Summary of logistic regressions predicting membership to cognitive phenotypes (CS/CD), and odds ratios calculated on the basis of *GRASP* genotype.

		Model significance	Pseudo R^2 (Cox and Snell)	B	s.e.	P	(95% CI)	Exp (B)
Model 1 (*reference category = HC*)								
Predicting CS subtype	all	χ^2=4.433, df=2, P=0.109	0.010	-0.307	0.223	0.17	0.475-1.139	0.735
	male	**χ^2=8.604, df=2, P=0.014**	0.028	-0.606	0.278	0.03	0.316-0.941	0.546
	female	χ^2=0.426, d=2, P=0.808	0.003	0.245	0.383	0.52	0.603-2.705	1.278
Predicting CD subtype				0.288	0.265	0.28	0.793-2.243	1.334
				0.367	0.323	0.26	0.767-2.718	0.767
				0.03	0.475	0.95	0.406-2.613	1.03
Model 2 (*reference category = CS type*)								
Predicting CD subtype	all	χ^2=4.303, df=1, P=0.038	0.019	1.814	0.29	0.04	1.027-3.204	1.814
	male	**χ^2=7.945, df=1, P<0.005**	**0.052**	**0.973**	**0.353**	**<0.01**	**1.325-5.281**	**2.646**
	female	χ^2=0.166, df=1, P=0.683	0.002	-0.215	0.526	0.68	0.288-2.261	0.806

Abbreviations: CD: schizophrenia subjects displaying a severe cognitive deficit; CI: confidence interval; CS: schizophrenia subjects with relatively spared cognition; HC: healthy controls; NS: not significant; OR: odds ratio. Significances are highlighted in bold.