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Original article

Effect of glutamate transporter EAAT2 gene variants and gray matter deficits on working memory in schizophrenia

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ABSTRACT

Glutamate is the major excitatory neurotransmitter in the brain, with up to 40% of all synapses being glutamatergic. An altered glutamatergic transmission could play a critical role in working memory deficts observed in schizophrenia and could underline progressive changes such as grey matter loss throughout the brain. The aim of the study was to investigate if gray matter volume and working memory could be modulated by a genetic polymorphism related to glutamatergic function. Fifty schizophrenia patients underwent magnetic resonance and working memory testing outside of the scanner and were genotyped for rs4354668 EAAT2 polymorphism. Carriers of the G allele had lower gray matter volumes than T/T homozygote and worse working memory performance. Poor working memory performance was associated with gray matter reduction. Differences between the three genotypes are more relevant among patients showing poor performance at the 2-back task. Since glutamate abnormalities are known to be involved in excitotoxic processes, the decrease in cortical thickness observed in schizophrenia patients could be linked to an excess of extracellular glutamate. The differential effect of EAAT2 observed between good and poor performers suggests that the effect of EEAT2 on gray matter might reveal in the presence of a pathological process affecting gray matter.

1. Introduction

Glutamate is the major excitatory neurotransmitter in the brain, with up to 40% of all synapses being glutamatergic [13]. Accumulation of excess extracellular glutamate and subsequent overstimulation of glutamatergic receptors increases the production of reactive and excitotoxic oxygen/nitrogen species, which induce oxidative stress leading to neuronal death [22]. Dysfunction of the finely tuned system of glutamatergic signalling has been proposed as a major mechanism in the schizophrenia pathogenesis [14,27,29] and several neurochemical, neurodevelopmental and genetic data corroborate this view [11,24,33].

Many of the genes recently associated with an increased risk for schizophrenia can influence the function of modulatory sites on the NMDA receptor or intracellular-receptor interacting proteins that link glutamate receptors to signal transduction pathways [18,35]. Exposure to NMDAr, glutamate and glycine antagonists, such as phencyclidine or ketamine, induces negative symptoms and cognitive dysfunction similar to that of schizophrenia [23,44]. Blood levels of antibodies against NMDAr in patients with systemic autoimmune disease were associated with impairment of particular cerebral functions, namely learning and memory deficits, depressed mood, and others neuropsychiatric disturbances [39]. Data suggest that an altered glutamatergic transmission could play a critical role in the working memory deficits observed in schizophrenia and its levels are dysregulated in DLPFC [5]. The PFC is an essential component of a neural circuit for working memory [2,16], impaired in schizophrenia patients and their relatives [15,45,46]. Finally it has been suggested that glutamate dysfunction could underline the progressive changes such as grey matter loss throughout the brain found by structural neuroimaging studies [38].

The inactivation of glutamate is handled by a series of molecular glutamate transporter (EAATs) which are membrane-bound pumps that closely resemble ion channels. These transporters play the important role of regulating concentrations of glutamate in the extracellular space, maintaining it at low physiological levels that promote biological function without promoting toxicity [9]. Five human excitatory amino acid

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transporters have been cloned, among them, EAAT2 is responsible for up 95% of extracellular glutamate clearance [41]. Impaired glutamate uptake by dysfunction or reduced expression of EAAT2 has been implicated in the pathogenesis of various diseases like multiple sclerosis and Alzheimer disease [8,28,31,40].

The human EAAT2 gene is located on 11p13-12 [34]. Mallolas et al. [30,34] have found an T-to-G polymorphism at –181 bp from the transcription start site of the EAAT2 gene. The mutant genotype abolishes a putative regulatory site for activator protein-2 and creates a new binding site for the transcription repressor factor GC-binding factor 2, resulting in less transporter expression and with the G allele inducing a 30% reduction in promoter activity compared with the T allele. Changes in EAAT2 expression in schizophrenia brains may be an important contributor to glutamate dysfunction [25,32,42]. Decreased expression of EAAT2 [36] and less glycosylation of EAAT2 which might reflect decreased glutamate reuptake, have been observed in DLPFC of schizophrenia patients [4]. Significant increases of mRNA expression of EAAT1 and EAAT2 have been reported in the thalamus of schizophrenics [43] while a significant decrease of EAAT2 mRNA expression was observed in the parahippocampal gyrus [37], thus confirming that abnormal homeostatic regulation of glutamate synaptic levels could be associated with schizophrenia. The homologous of EAAT2 in the rat, GLT-1, plays critical roles in LTP induction, a critical mechanism for memory functioning, through regulation of extracellular levels of glutamate [21], thus suggesting that EAAT2 changes could play a role in mediating the relationship between working memory deficits and gray matter abnormalities in schizophrenia.

No study so far investigated if gray matter regional volume and working memory could be modulated by a genetic polymorphism related to glutamatergic function.

2. Material and methods

Fifty patients (34 males and 16 females) with chronic schizophrenia were recruited at the psychiatric ward of San Raffaele Turro Hospital in Milan from 2009 to 2012. Exclusion criteria were mental retardation, lifetime clinically relevant substance abuse including cannabis, history of major unstable physical illness and other psychiatric co-morbidities. Patients were biologically unrelated, clinically stabilized outpatients meeting The Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria for chronic schizophrenia and were responders to typical and atypical antipsychotics in monotherapy (clozapine $n = 15$, risperidone $n = 13$, aripiprazole $n = 2$, haloperidol $n = 7$, paliperidone $n = 6$, olanzapine $n = 7$). Doses had been stable in the 3 months before enrollment. Schizophrenia diagnosis was made by trained psychiatrists using the SCID-I questionnaire and mental retardation was assessed by a trained psychologist through WAIS-R. After complete description of the study to the subjects, a written informed consent was obtained. The local ethical committee approved the study protocol.

All patients underwent magnetic resonance and working memory testing outside of the scanner and were genotyped for rs4354668 EAAT2 polymorphism. Moreover, all patients were administered the Brief Assessment of Cognition in Schizophrenia (BACS) to evaluate global cognitive performance (for a complete description of BACS see Anselmetti et al. [1]). For each subtest an equivalent score has been obtained which show if the performance is good (score of 2, 3 or 4) or if it is poor (score of 0 or 1). A global cognitive index has been calculated as the mean equivalent score of all subtests of the BACS. Neuropsychological assessment (BACS and N-back) has been performed the same day of the scan.

2.1. Working memory assessment

2.1.1. N-back task

In the N-back task as instantiated here, a number between 1 and 4 is shown randomly on a screen. For the 0-back, subjects respond to the number currently showing on the screen by pressing the appropriate button; for the 1-back, subjects respond to the previous number on the screen, and so on for 2-back conditions. Each number is shown for 160 ms, with an interval of 1640 ms between numbers (and 3000 ms between blocks). The task imposes a parametric load on working memory, and the version we used is relatively demanding [7]. The primary performance measure is accuracy (correct responses); we also measured reaction time (RT).

2.2. Genotyping

DNA was extracted from whole blood by a manual extraction, using the "Illustra blood genomic Prep Midi Flow kit" (GE Healthcare, Milan, Italy).

To identify the polymorphism rs4354668T/G (DNA forward strand), a standard Polymerase Chain Reaction (PCR) was carried with the following primers: 5′-GCC ACC TGT GCT TTG CTG-3′ and 5′-TGA TGT CAG CTC TCG ACG AA-3′.

The PCR was carried out in a 10 μl volume containing 150 ng genomic DNA, 1 μl of 1× Hot Master Taq Buffer with Mg++ (Eppendorf), 0.1 μl of each primer [50 uM], 1 μl of deaza-dNTPs [10 mM], 0,5 μl of Dimethyl sulfoxide (DMSO) solution (Sigma-Aldrich, Milan, Italy) and 0.1 μl of Hot Master Taq [5 U/ul] (Eppendorf).

After an initial step of 5 min at 94 °C, 35 cycles of amplification (35 s at 94 °C, 35 s at 58 °C, 45 s at 70 °C) and a final extension step of 10 min at 70 °C were performed.

An aliquot of PCR product was digested using Msp I (20 U/ul) (New England Biolabs, England, UK) and incubated at 37 °C for 8 h; fragments were separated in agarose gels.

Depending on the presence of two or three restriction Msp I sites, either three fragments (allele T) or four fragments (allele G) were produced.

2.3. Brain imaging

Brain imaging volumetric T1-weighted sequences were acquired on a 3.0 Tesla scanner (Gyroscan Intera, Philips, The Netherlands) using a 6-channel SENSE head coil using a T1-weighted MPRAGE sequence (TR 25.00 ms, TE 4.6 ms, field of view = 230 mm, matrix = 256 × 256, in-plane resolution 0.9 × 0.9 mm, yielding 220 transversal slices with a thickness of 0.8 mm). Images were analyzed using STATISTICAL PARAMETRIC MAPPING software (SPM8, Wellcome Department of Imaging Neuroscience, Institute of Neurology and the National Hospital for Neurology and Neurosurgery, London, UK) and the voxel-based morphometry (VBM) toolbox (VBM 5.1; http://dbm.neuro.uni-jena.de/vbm/) implemented in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/), which combines tissue segmentation, bias correction and spatial normalization into a unified model. We used the optimized VBM procedure, which segments grey and white matter and normalizes GM segmented images to a standard space by matching them to their template [3]. The procedure yielded modulated GM normalized images: modulated parameters were used to test for voxel-wise differences in the relative volume of GM by compensating for the effects of warping, to ensure that the total amount of GM in a region is the same before and after spatial normalization [17]. The voxel size for all images was resliced to 1 × 1 × 1 mm.

We realigned the scans to correct for head movement. Images were then normalized to the standard EPI template volume of the Montreal Neurological Institute (MNI) reference brain and smoothed using a 8-mm full-width at half-maximum isotropic Gaussian kernel because smaller smoothing kernels are appropriate to detect the volume reductions typical of schizophrenia in small structures [20].

2.4. Statistical analysis

More that 25% of correct responses were considered as a good performance at the n-back task. 2-back was chosen for the analysis as scores were better distributed between genotype groups. To assess the statistical significance of group differences, data were analyzed within the context of the General Linear Model (GLM). Based on previous literature showing that both homozygotes and heterozygotes carriers of the mutant genotype show greater excitotoxic damage after stroke compared to wild type [30], we pooled together G carriers genotype. Firstly an analysis of variance was performed to investigate interaction between EAAT2 polymorphism and working memory; 2-back scores and genotype were entered as factors and medication load, duration of illness and mean cognitive performance as nuisance covariates.

2.4.1. Voxel-based morphometry

Structural modulated images were entered into a second level analysis of variance (ANOVA) with genotype and performance (good and poor) as factors. This procedure allowed for the regions where both factors significantly influenced brain volume to be explored (conjunction analysis, as implemented in the SPM8 statistical software package), and to test the levels of significance of the main effects of EAAT2 and of working memory performance one by one. Moreover, we performed an interaction analysis. We included as covariates the total intracranial volume to adjust for global atrophy and identify regions with differences that cannot be explained by the total GM differences, medication load, duration of illness and mean cognitive performance as possible confounding factors. The total intracranial volume was calculated as the sum of the volumes of GM, white matter and cerebrospinal fluid, as estimated by the MATLAB get totals script implemented for SPM (http://www.cs.ucl.ac.uk/staff/g.ridgway/vbm/get totals.m). Statistical threshold was $P < 0.05$ corrected for multiple comparisons with whole-brain family-wise error (FWE) correction.

Using the Wake Forest PickAtlas software (Wake Forest University, USA; www.fmri.wfubmc.edu), statistical maps were limited to priori regions of interest (ROIs). The mask included inferior, middle, medial and superior frontal gyrus.

3. Results

Observed genotype frequencies were as follows: T/T 19/50 (38%), T/G 18/50 (36%) and G/G 13/50 (13%). Allelic frequencies (T 56% and C 42%) were slightly different from those observed in normal subjects [30]. The sample was in Hardy–Weinberg equilibrium ($\chi 2 = 3.63$; df = 1; $P = 0.056$). Clinical and demographic characteristics of the sample are presented in Tables 1 and 2; no difference among genotypic groups reached significance (Table 2) and only performance in executive functions was significantly different between good performers and poor performers (Table 1). Both total intracranial volume and brain volumes of GM, white matter and cerebrospinal fluid did not significantly differ among genotype groups (Table 2) nor in relation to performance (Table 1).

The analysis of variance showed a significant effect of genotype on working memory performance (G carrier 9.74 ± 5.57; T/T 6.73 ± 3.75; F = 4.45, $P = 0.040$) with G carriers showing worse performance compared to T/T subjects ($\beta = 0.31$; t = 2.11; $P = 0.040$). No significant effect medication load (F = 0.19, $P = 0.067$), duration of illness (F = 0.30, $P = 0.58$) and mean cognitive performance (F = 1.62, $P = 0.21$) was found.

At the VBM analysis, the combined effects of genotype and working memory performance (conjunction analysis) survived the statistical threshold in two main clusters in Broadmann Area 9, one of 98 mm^3 in left middle frontal gyrus (at MNI coordinates −38 8 36; F = 17.87, Z = 5.5, p-FWE = 0.001) and one of 43 mm^3 in right inferior frontal gyrus (at MNI coordinates 39 24 38 F = 15.29, Z = 5.15, p-FWE = 0.005) (Fig. 1).

Table 1
Clinical and demographic characteristics of the sample as a whole and divided according to working memory performance. Medication load refers to chloropromazine equivalent dosages.

	2-back		
	Good performers $n = 28$ (T/T = 4; T/C = 13; C/C = 11)	Bad performers $n = 22$ (T/T = 10; T/C = 6; C/C = 6)	
	Mean ± SD	Mean ± SD	T/P
Age	34.66 ± 9.45	36.59 ± 8.13	−0.75/0.45
Onset (years)	23.96 ± 7.16	25.64 ± 5.96	−0.87/0.38
Education (years)	12.04 ± 2.99	12.05 ± 2.98	−0.01/0.99
Duration of illness (years)	10.70 ± 8.84	10.95 ± 5.87	−0.11/0.91
Medication	313.26 ± 205.17	302.84 ± 186.84	0.18/0.85
IQ	84.04 ± 7.11	79.95 ± 7.33	1.88/0.07
N° hospitalizations	4.22 ± 4.43	4.04 ± 2.66	0.16/0.87
Total intracranial volume (ml)	1538.53 ± 192.68	1510.99 ± 176.54	0.51/0.61
Gray matter (ml)	655.66 ± 103.27	646.46 ± 84.36	0.33/0.74
White matter (ml)	546.81 ± 68.49	515.65 ± 75.31	1.51/0.13
Cerebrospinal fluid (ml)	336.06 ± 68.69	348.88 ± 62.36	−0.68/0.5
PANSS positive	17.29 ± 6.56	15.76 ± 3.39	0.96/0.34
PANSS negative	19.83 ± 3.72	20.86 ± 4.42	−0.84/0.40
PANSS general	36.58 ± 5.47	36.71 ± 6.84	−0.07/0.94
PANSS total	73.71 ± 11.47	73.33 ± 12.26	0.10/0.92
Verbal memory	45.52 ± 10.63	46.33 ± 14.37	−0.23/0.82
Working memory	16.89 ± 4.43	16.23 ± 3.90	0.54/0.59
Psychomotor coordination	67.04 ± 4.43	65.5 ± 17.76	0.32/0.75
Verbal fluency	37.81 ± 10.63	34.63 ± 12.96	0.94/0.35
Selective attention	40.88 ± 10.38	37.27 ± 11.07	1.17/0.24
Executive functions	14.22 ± 3.97	12 ± 3.61	2.03/0.048

Table 2
Clinical and demographic characteristics of the sample as a whole and divided according to rs4354668 genotype. Medication load refers to chloropromazine equivalent dosages.

	Total sample	GG ($n = 13$)	EAAT2		
			GT ($n = 18$)	TT ($n = 19$)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	F/*P*
Age	35.56 ± 8.76	35.15 ± 8.91	34.89 ± 8.06	36.47 ± 9.65	0.16/0.85
Onset (years)	24.62 ± 6.60	25.54 ± 5.17	25.61 ± 7.43	23.05 ± 6.67	0.86/0.43
Education (years)	11.96 ± 2.97	11.61 ± 3.47	12.33 ± 2.22	11.84 ± 3.34	0.23/0.79
Duration of illness (years)	10.82 ± 7.58	9.61 ± 7.18	8.82 ± 5.88	13.42 ± 8.73	1.94/0.15
Medication	314.41 ± 197.54	335.57 ± 211.51	309.72 ± 186.12	304.36 ± 207.93	0.10/0.90
IQ	82.17 ± 7.38	81.7 ± 5.91	81.28 ± 8.17	83.33 ± 7.53	0.36/0.69
Nº hospitalizations	4.1 ± 3.68	3.61 ± 2.81	4.17 ± 4.42	4.37 ± 3.59	0.16/0.85
% 0-back	89.92 ± 20.45	91.38 ± 8.46	89.77 ± 23.93	89.05 ± 23.39	0.05/0.95
% 1-back	51.44 ± 27.83	44.31 ± 29.55	47.11 ± 27.83	60.42 ± 25.57	1.68/0.19
% 2-back	31.59 ± 18.9	24.31 ± 17.73	28.94 ± 15.33	38.94 ± 22.30	2.76/0.07
Total intracranial volume (ml)	1523.83 ± 183.1	1573.62 ± 182.59	1525.94 ± 159.81	1487.76 ± 204.27	0.84/0.43
Gray matter (ml)	649.55 ± 94.48	670.65 ± 96.14	655.19 ± 96.82	629.75 ± 92.29	0.76/0.47
White matter (ml)	532.99 ± 71.84	549.67 ± 80.35	538.39 ± 58.3	516.46 ± 77.57	0.9/0.41
Cerebrospinal fluid (ml)	341.29 ± 65	353.3 ± 51.29	332.35 ± 63.26	341.54 ± 76.05	0.38/0.68
PANSS positive	16.58 ± 5.26	17.27 ± 4.29	17.05 ± 7.28	15.64 ± 2.91	0.42/0.66
PANNS negative	20.22 ± 4.05	21.36 ± 3.14	18.94 ± 4.66	20.82 ± 3.73	1.55/0.22
PANNS general	36.56 ± 6.03	38.27 ± 7.88	35.44 ± 4.78	369.65 ± 5.97	0.74 ± 0.48
PANNS total	73.53 ± 11.71	76.91 ± 12.62	73.56 ± 9.33	71.44 ± 13.13	0.73/0.48
Verbal memory	45.82 ± 12.15	47.75 ± 15.31	46.95 ± 12.88	43.33 ± 8.88	0.6/0.55
Working memory	16.64 ± 4.14	17.69 ± 3.86	15.95 ± 5.17	16.61 ± 3.05	0.67/0.51
Psychomotor coordination	66.22 ± 16.58	69.23 ± 13.92	62.79 ± 19.96	67.66 ± 14.54	0.68/0.51
Verbal fluency	36.14 ± 11.73	34.85 ± 11.93	39.31 ± 12.19	33.72 ± 10.95	1.16/0.32
Selective attention	39.26 ± 10.74	38.61 ± 10.15	41.42 ± 11.37	37.35 ± 10.65	0.66/0.52
Executive functions	13.22 ± 3.94	13.38 ± 4.29	13.79 ± 4.25	12.47 ± 3.37	0.5/0.6

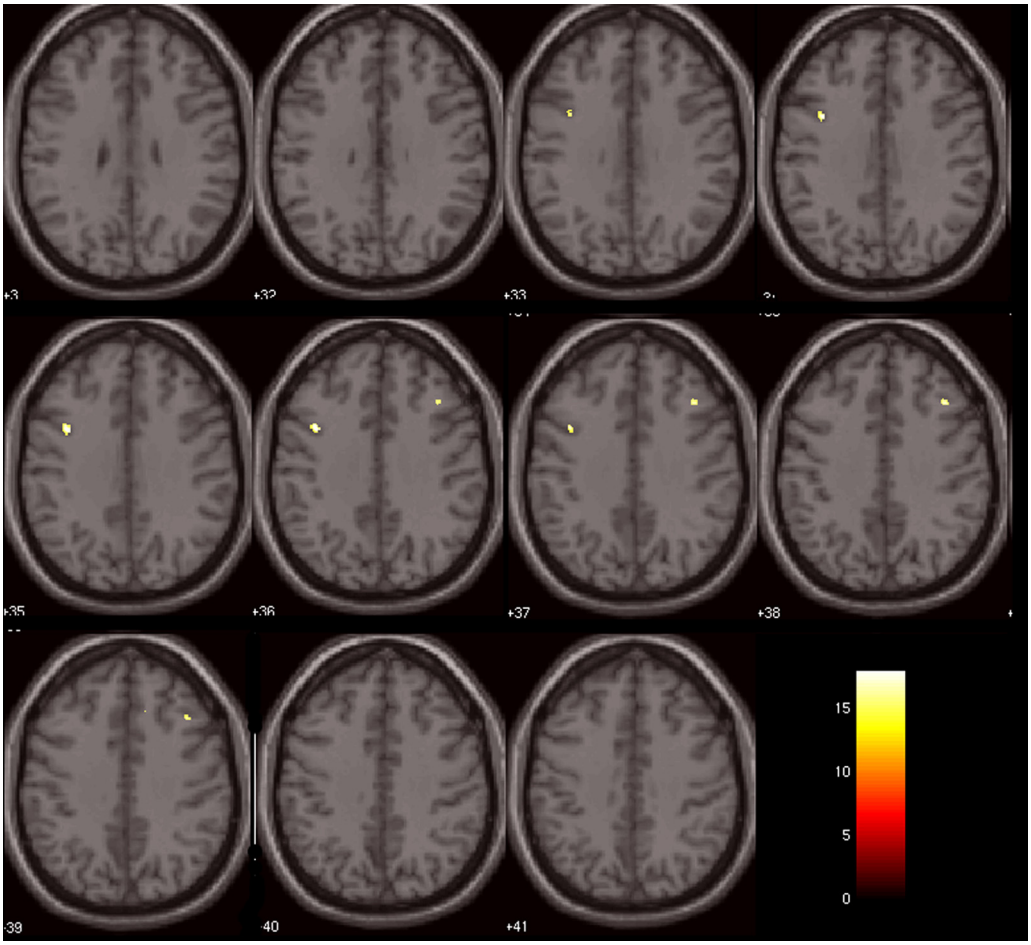

Fig. 1. Localization of grey matter areas where rs4354668 and working memory performance influenced grey matter volume at a statistical threshold of whole-brain $P = 0.05$ FWE corrected.

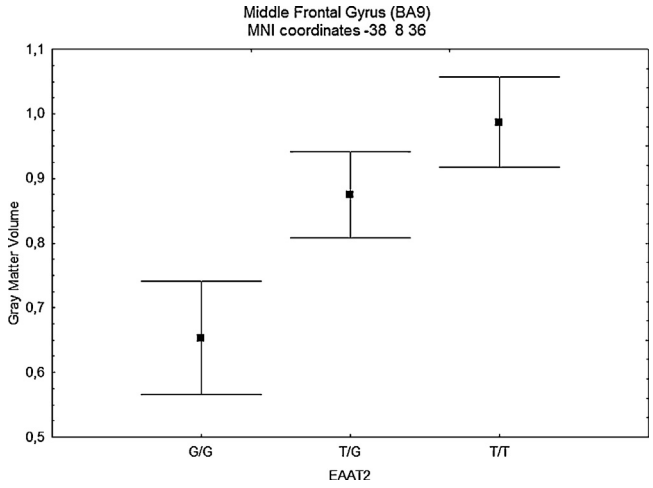

Fig. 2. Global effect of genotype. Direction and size effect of the observed difference in left middle frontal gyrus (MNI coordinates –38, 8, 36). Bars are means, whiskers are standard errors.

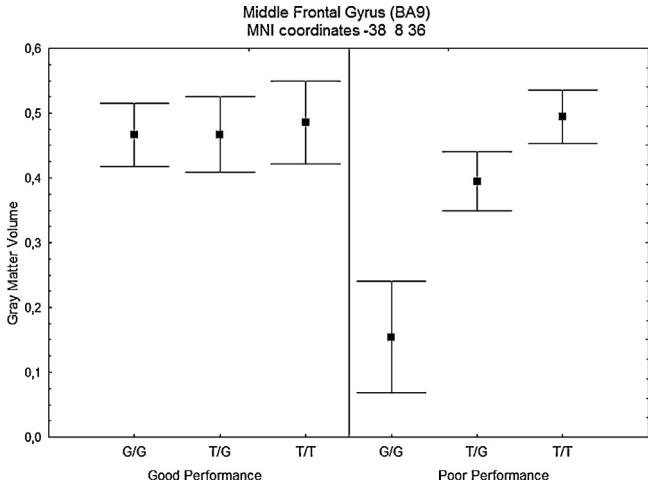

Fig. 4. Interaction genotype × performance. Direction and size effect of the observed difference in left middle frontal gyrus (MNI coordinates –38, 8, 36). Bars are means, whiskers are standard errors.

The analysis of the main effect of the two factors in these clusters showed that in left middle frontal gyrus (Fig. 2) patients with T/T polymorphism had increased GM volumes compared to patients with G/G, with heterozygotes showing an intermediate pattern (F = 11.51, Z = 3.68 p-FWE = 0.002); and that patients poor performers had significantly lower volumes than good performers (t = 4.23, Z = 3.81, p-FWE = 0.001) (Fig. 3). In right inferior frontal gyrus, the main effect of genotype did not survived correction for multiple comparison, but patients poor performers had significantly lower volumes than good performers (t = 3.91, Z = 3.57, p-FWE = 0.003).

The effect of genotype was driven by significant differences between genotype groups among poor performers, while among good performers genotype had no effect on gray matter volume (Fig. 4). An ANOVA test confirmed the significance of the interaction between these two factors (Fig. 2) (MNI coordinates: –40, 9 37) (Z = 4.70, PFWE = 0.026).

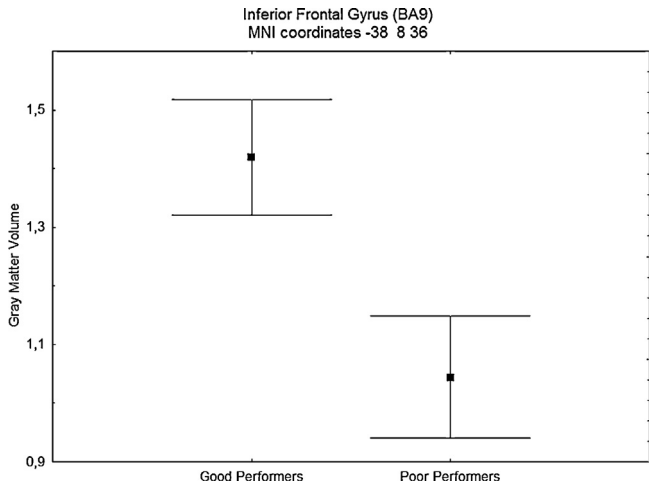

Fig. 3. Global effect of performance. Direction and size effect of the observed difference in left middle frontal gyrus (MNI coordinates –38, 8, 36). Bars are means, whiskers are standard errors.

4. Discussion

This is the first study associating a genetic variant of a polymorphism involved in glutamate clearance, working memory performance and gray matter volume in schizophrenia patients. Carriers of the rs4354668 G allele, which is associated with less transporter expression and a 30% reduction in promoter activity compared with the T allele, reported lower GM volumes than T/T homozygotes and worse working memory performance. Poor working memory performance was associated with GM reduction (Fig. 1). Interestingly differences between the three genotypes are definitely more relevant among patients showing poor performance at the 2-back task.

Major limitations of this study are the lack of a healthy control group and a small sample size when stratifying good vs poor performers in accordance to EAAT2 polymorphism. Other limitations of the present study, which is retrospective, uncontrolled and correlational in nature, include issues of generalizability, previous medications, non drug-naïve, no placebo control, no standardized treatments, population stratification, no evaluation for compliance, varying treatment periods, without consideration of gene-environment interactions.

The G allele is associated with lower EAAT2 expression which leads to decreased protein level. This may affect glutamate recycling at the synaptic cleft and contribute to reported alteration of glutamatergic function in schizophrenia patients [14]. Moreover, EAATs are also involved in functions other than glutamate clearance, such as attenuating NMDA receptor function [6], which has been found to be altered in schizophrenia patients [39]. Since glutamate is known to be involved in the production of reactive and excitotoxic oxygen/nitrogen species, which induce oxidative stress leading to neuronal death, the decrease in cortical thickness observed in schizophrenia patients could be linked to an excess of extracellular glutamate.

Our results are consistent with the study of Egan et al. who previously found lower EAAT2 expression and impaired prefrontal cognitive functions among subjects carrying the high-risk mGluR3 associated with schizophrenia [12]. Indeed the presence of the G allele may result in a disruption of the control mechanisms necessary to maintain extracellular glutamate levels below the excitotoxic threshold concentration in the prefrontal cortex. The impaired EAAT2 expression could determine a prefrontal neuronal

damage with a consequent disadvantageous effect on cognitive functions. This effect could be especially relevant to working memory as prodromal studies found WM deficits similar to those marking affected patients, but of lesser magnitude [19,26,47].

The differential effect of EAAT2 polymorphism observed between good and poor performers suggests that the effect of EEAT2 on gray matter might reveal in the presence of a pathological process affecting gray matter. In analogy with the observation of worse damage in G carriers after stroke [30] we found the detrimental effect of G allele in patients who had poor working memory performance associated with schizophrenia, but not in patients with preserved neuropsychological performance thus suggesting a major effect for other variables (environmental stress, drugs, neurotrophic factors) interacting with glutamate function.

These observations seem to suggest that the brains of patients with schizophrenia may be disadvantaged in their ability to maintain adequate connections between neurons, to effectively control programmed cell death and cell proliferation, and to adapt to changes in their environment and defend against various physiological insults.

Therefore it could be postulated that in poor performers, there are no resilience factors that could counteract the detrimental effect of glutamate excitotoxicity.

Recently the glutamate system, thanks to the several possibilities of modulation it offers, has become the target of search trends for augmentation strategies [24] of antipsychotic treatments. Two different pharmacological mechanisms are involved: modulation of receptor activity or glutamate release inhibition. Results on human subjects are controversial but seem to suggest that glutamatergic agonists like glycine and D-serine and antagonists like memantine could improve positive, negative and cognitive symptoms [10]. In particular, the use of memantine in the early stages of schizophrenia may block the glutamate excitotoxicity correlated to high glutamate levels, slowing the progression of negative symptoms associated to more advanced stages of the illness [10].

These data seem to further support our results showing an association between a genetic variant of a polymorphism involved in glutamate clearance, cognitive performance and gray matter.

Further research on the interaction between glutamate and other genetic and environmental factors as well as longitudinal studies on the long term effects of carrying the rs4354668 G allele are needed to clarify this issue.

5. Conclusions

Schizophrenia patients have been shown to have decreased expression and less glycosylation of EAAT2 [36] which might reflect decreased glutamate reuptake. In our study, we demonstrated an association between this polymorphism and working memory in influencing gray matter volume in these patients. Carriers of the rs4354668 G allele, which is associated with less transporter expression and a 30% reduction in promoter activity compared with the T allele, had lower GM volumes than T/T homozygote and worse working memory performance. Alterations in glutamate concentration could compromise structural connectivity and integrity, programmed cell death and cell proliferation, and the ability to adapt to changes in the environment and defend against various physiological insults.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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