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Letter to the editor

Genetic variability of glutamate reuptake: Effect on white matter integrity and working memory in schizophrenia

Elena Mazza [b,1], Marco Spangaro [a,b,*,1], Sara Poletti [b], Roberto Cavallaro [a,b], Francesco Benedetti [a,b]

[a] IRCCS San Raffaele Scientific Institute, Department of Clinical Neurosciences, Milan, Italy
[b] Vita-Salute San Raffaele University, Milan, Italy

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Working memory (WM) deficit is a core feature of schizophrenia, associated with impairments in overall cognitive functioning, functional outcome, and course of illness (Lett et al., 2014). Glutamate is the major central neurotransmitter, and alterations of its regulation mechanisms have been largely associated with cognitive and structural impairments in schizophrenia (Moghaddam and Javitt, 2012). The excitatory amino acid transporter 2 (EAAT2), is responsible for >90% of glutamate uptake, playing a crucial role preventing excitoxic damage, modulating synaptic activity and contributing to preserve an efficient energy metabolism. A functional SNP located in the gene promoter, rs4354668 (−181 T/G), modulates EAAT2 expression, influencing both cerebral activity and structure (Mallolas et al., 2006; Zhang et al., 2015). We previously reported that patients with schizophrenia carrying the G allele, associated with lower EAAT2 expression, show impaired WM performance and reduced WM improvements after cognitive rehabilitation therapy (Spangaro et al., 2014, 2018a). Moreover, we also observed reduced frontal cortical volumes among EAAT2 G carriers, associated with poor WM performance, thus hypothesizing the presence of an underling excitoxic process (Poletti et al., 2014). In order to further investigate the relationship between EAAT2 genetic variability, cognition and brain structure in schizophrenia, in the present study we aimed to investigate possible effect of rs4354668 on white matter integrity, as well as possible association of Diffusion Tensor Imaging (DTI) measures with WM performance.

The study included 39 clinically stabilized patients (26 males and 13 females) with a diagnosis of schizophrenia (DSM-IV-TR criteria). Exclusion criteria were: age > 65 years, intellectual disability, psychiatric comorbidities, substance abuse, neurological disorders.

All subjects provided informed consent to a protocol approved by the local Ethical Committee following the principles of the Declaration of Helsinki.

WM performance was evaluated with the N-Back test (Callicott et al., 2003).

For genotyping and DTI acquisition/analysis technical details please see our previous works (Spangaro et al., 2018a,b).

Analysis of covariance (ANCOVA) was used to evaluate differences in WM between genotype groups considering N-Back results as dependent variables, EAAT2 genotype as categorical factor and age as covariate. Consistently with literature, we grouped subjects homozygous for the T allele (19 subjects) vs. G carriers (22 subjects).

A *t*-test on DTI measures of white matter microstructure across the white matter skeleton was performed between genotype groups. We then performed an interaction analysis between genotype and WM, and post hoc analyses to evaluate effect direction. We accounted for the effects of nuisance covariates potentially influencing white matter structure: age, sex, onset, and antipsychotics dosage (chlorpromazine equivalents).

T/T patients showed better WM performances (ANCOVA; 1-back: $F = 6.30$, $p = .017$; 2-back: $F = 4.86$, $p = .034$) and a widespread higher white matter integrity, as indicated by the observation of lower Axial Diffusivity (AD), Mean Diffusivity (MD), and Radial Diffusivity (RD) in different areas including internal capsule, corpus callosum, bilateral superior longitudinal fasciculus, anterior thalamic radiation, cingulum, corticospinal tract and anterior/superior corona radiate.

Moreover, white matter integrity differently associated with WM performances between genotype groups, with a positive correlation between 2-back and Fractional Anisotropy (FA) and a negative correlation with AD, MD and RD in the above-mentioned structures among EAAT2 T/T subjects. (Fig. 1).

* Corresponding author at: Department of Clinical Neurosciences, IRCCS San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Via Stamira d'Ancona 20, 20127 Milano, Italy.
E-mail address: spangaro.marco@hsr.it (M. Spangaro).
[1] These authors contributed equally to this work..

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This is the first study to report an association between genetic variability of glutamate reuptake and white matter integrity among patients with schizophrenia. We showed that EAAT2 G carriers had lower FA and higher AD, MD and RD than EAAT2 T/T subjects. In agreement with previous studies, G carriers also showed worse WM performance (Poletti et al., 2014; Spangaro et al., 2012; Zhang et al., 2015); and in the present study WM performance positively correlated with FA in EAAT2 T/T, thus suggesting that effects of EAAT2 gene variants on cognition could be influenced by effects on white matter microstructure.

Schizophrenia is characterized by a reduction in the number of oligodendrocytes and abnormal microstructure of myelin sheaths and axons in different white matter tracts and regions (Ellison-Wright and Bullmore, 2009; Takahashi et al., 2011). EAAT2 is transiently expressed in oligodendrocytes in the developing human brain, a phenotype

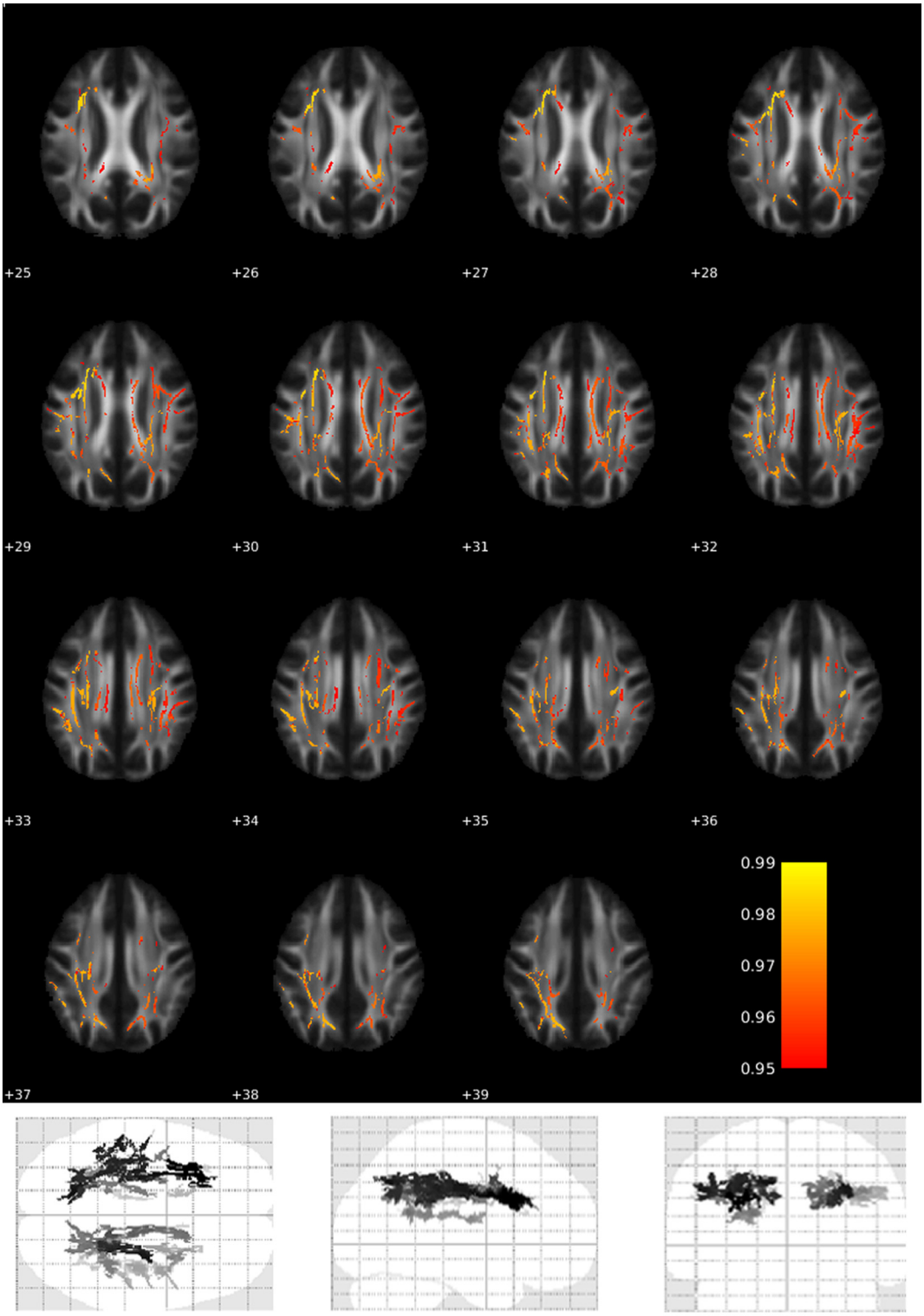

Fig. 1. White matter tracts where G carriers differently associated with working memory performances (2-back) compared to TT subjects (FA measure). Numbers are z MNI coordinates; colorbar refers to 1-*p* values for the observed differences. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

associated with the vulnerability of white matter to excitotoxic insults (Desilva et al., 2007), and dysregulated glutamate uptake by astrocytes results in oligodendroglial damage in animal models (Murugan et al., 2013). Glutamate is involved in neurodevelopment, neurotoxicity and neurotransmission in schizophrenia (Moghaddam and Javitt, 2012), and rs4354668 might then explain some of the heterogeneity of brain structure and cognitive function of schizophrenia, also representing a potential therapeutic target. In this context, EAAT2 rs4354668 could contribute to this pathway influencing white matter integrity. We can hypothesize that the presence of the disadvantageous G allele could lead to a twofold synergic effect on white matter microstructure. Indeed, reduced transporter expression on one side could determine an inefficient energy metabolism, impairing glutamate recycling (Robinson and Jackson, 2016), neurovascular coupling and astrocyte–neuron lactate shuttle. On the other, a lower EAAT2 activity could also lead to an increased glutamate spillover between synapses inducing excitoxic neuronal damage and loss of signal input specificity, thus impairing WM (Sheldon and Robinson, 2007; Spangaro et al., 2012). Indeed, several studies reported neuroprotective effects of compounds that raise glutamate reuptake by increasing EAAT2 expression, suggesting possible applications in the treatment of neurodegenerative disorders.

The results of our study have to be interpreted in the context of some limitations. Sample size is limited and results need to be replicated in larger samples. Limits of the present study also include generalizability, possible population stratification, effects of concomitant medications, and technical issues related to DTI acquisition and analysis.

Despite these limitations, this is the first study to report an effect of EAAT2 rs4354668 on white matter integrity in patients affected by schizophrenia, providing in vivo evidence for the importance of heritable variation in glutamate reuptake in white matter microstructure. Future studies should investigate possible influence of the transporter among healthy subjects as well, in order to investigate if the effect of EAAT2 rs4354668 on connectivity is specific to schizophrenia or rather affects general population.

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Declaration of interest

All authors declare that they have no conflict of interest.

Contributors

Authors Marco Spangaro, Elena Mazza and Francesco Benedetti elaborated the hypothesis and designed the study. Authors Marco Spangaro, Elena Mazza wrote the manuscript and performed statistical analysis. Author Sara Poletti performed the neuropsychological assessment. Authors Roberto Cavallaro and Francesco Benedetti supervised the study design and revised the manuscript. All authors contributed to and have approved the final manuscript.

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