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DGCR2 influences cortical thickness through a mechanism independent of schizophrenia pathogenesis

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ABSTRACT

We investigated the role of *DGCR2*, a corticogenesis-related gene, on schizophrenia (SZ) and its subphenotypes, including brain morphology. A total of 221 SZ patients, 263 controls and 70 antipsychotic-naïve first episode of psychosis (FEP) were genotyped for 17 *DGCR2* polymorphisms. While no association between *DGCR2* polymorphisms and SZ was found, the missense variant rs2072123 was associated to left rostral anterior cingulate thickness, showing that *DGCR2* seems not to be associated directly with the SZ but might be influencing the brain morphology. We also showed a *DGCR2* downregulation in SZ patients when compared to controls and FEP.

1. Introduction

Schizophrenia is a chronic and severe mental disorder that affects ~1% of the general population. Both genetic and environmental factors and their interaction have been implicated in its etiology. Among environmental factors, cannabis use, childhood adversity and migration seem to confer risk for the disorder (van Os et al., 2014). Numerous single-nucleotide polymorphisms (SNPs) and copy number variation (CNV) were associated to this psychotic disorder, and among the CNVs, the 22q11 deletion is one of the strongest risk factors (Bassett et al., 2017).

The deletion of this segment of chromosome 22 leads to 22q11.2 deletion syndrome (22q11.2DS), which affects ~1 in 4000 live births and can involve heart, immune system and psychiatric abnormalities, among others. One of the genes located at 22q11.2 region, the *DGCR2* (DiGeorge syndrome critical region 2 gene) encodes for an activity-dependent adhesion protein (Kajiwara et al., 1996) and is expressed in human brain tissues (GTEx - www.gtexportal.org), including during neurodevelopment (Brain Span - http://www.brainspan.org/). *DGCR2* common and rare variations have been associated with schizophrenia (Shifman et al., 2006; Xu et al., 2011). Its expression is increased in dorsolateral prefrontal cortex in patients with schizophrenia and in rats under treatment with antipsychotic drugs (Shifman et al., 2006). Recently, it was shown that DGCR2 regulates critical steps of early corticogenesis possibly through a Reelin-dependent mechanism (Molinard-Chenu and Dayer, 2018). This is in line with the neurodevelopmental hypothesis of schizophrenia, which suggests that pathologic processes, caused by both genetic and environmental factors, begin *in utero*, long before the onset of psychotic symptoms (Fatemi and Folsom, 2009). This hypothesis has been supported by neuroimaging, animal and

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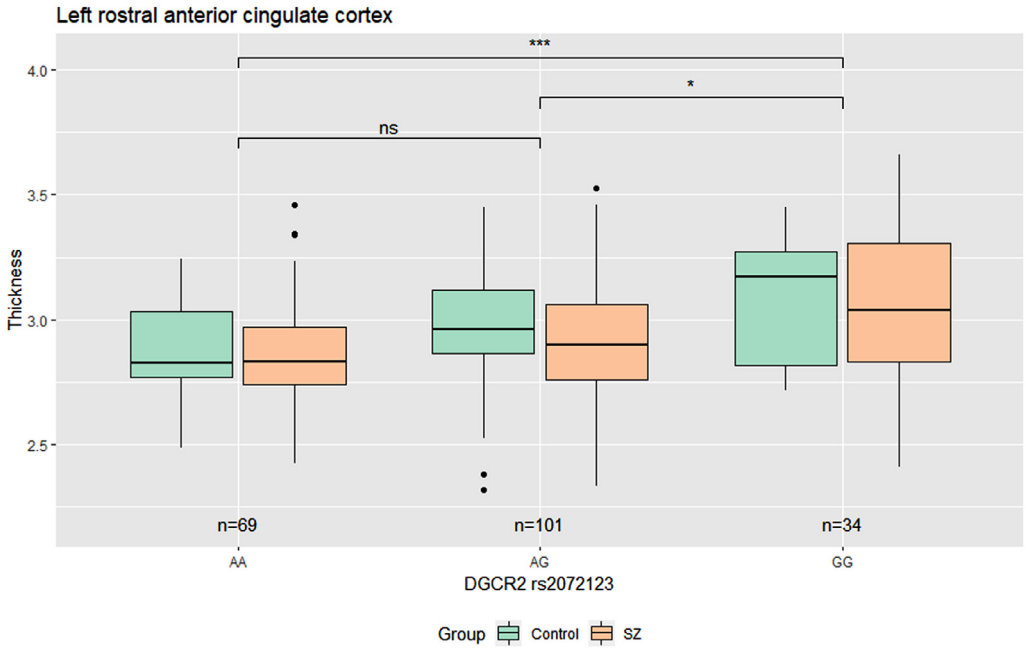

Fig. 1. Results of general linear model (GLM) comparing left rostral anterior cingulate thickness and DGCR2 rs2072123 genotypes. *$p = 0.003$; ***$p < 0.001$; ns: non-significant.

genetic studies.

Although there is evidence of an important role of *DGCR2* in schizophrenia, there is a lack of studies investigating its association with intermediate phenotypes, which are believed to better represent underlying pathophysiology than clinical diagnostic categories (Meyer-Lindenberg and Weinberger, 2006).

Considering the evidences of DGCR2 role on cortical development, we hypothesized that *DGCR2* could impact brain regions and cognitive related functions central for schizophrenia, i.e. brain associative regions and executive functions. Thus, we aimed to investigate the association of functional *DGCR2* polymorphisms with brain morphology, cognitive function and schizophrenia subphenotypes. Furthermore, we investigated whether there were differences in blood *DGCR2* mRNA levels between healthy controls, and patients in early stages (antipsychotic-naïve first episode of psychosis - FEP), and chronic schizophrenia.

2. Material and methods

Detailed description of the Methods is available in Supplementary Information. A total of 221 patients with schizophrenia, 263 healthy controls and 70 antipsychotic naïve FEP subjects were included in this study. FEP subjects were included only in the *DGCR2* expression analysis.

2.1. Genetic analyses

Chronic schizophrenia cases ($N = 221$) and controls ($N = 263$) collected blood for genotyping of 17 *DGCR2* SNPs using real-time PCR with TaqMan probes (Thermo Scientific, USA) or SNParray HumanOmniExpress (Illumina, USA).

For *DGCR2* expression analyses, we collected blood for a subsample of 154 patients with schizophrenia and 139 controls and, additionally we included 70 antipsychotic-naïve FEP patients to verify changes in gene expression unrelated to antipsychotic treatment. *DGCR2* expression was quantified by real-time PCR with TaqMan probes.

2.2. Neuropsychological tests and neuroimaging

Neuropsychological tests were performed in 115 chronic patients to evaluate inhibitory control, attention allocation and flexibility, working memory update, executive function, cognitive flexibility, abstraction, planning, verbal learning and memory.

Brain MRIs were obtained from 134 patients with schizophrenia and 70 controls using a 1.5T scanner [Magnetom Sonata (Maestro Class) Siemens AG, Medical Solutions, Erlangen, Germany] and analyzed using Freesurfer.

2.3. Statistical analysis

Association between each SNP and disease or treatment resistant schizophrenia (TRS) was tested using logistic regression. General linear model (GLM) was used to test association between SNP and age at onset, adding chronological age as covariate and sex as fixed factor. Association between cognitive variables/brain measures/*DGCR2* expression and genotypes was tested using GLM. Bonferroni correction was performed considering the number of analyses tested (number of SNPs * cognitive/brain measurements).

3. Results

3.1. Sample description

A brief description of the samples is provided in Supplementary Table S1.

3.2. Association between DGCR2 genotypes and haplotypes and schizophrenia

No SNP was associated with schizophrenia (Supplementary Table S2), TR schizophrenia or age at onset ($p > 0.05$). Also, no haplotype was associated with schizophrenia. Linkage disequilibrium plot is presented in Supplementary Fig. S1.

3.3. Investigating DGCR2 effects on brain structure and cognitive functioning

For these analyses, we selected 4 functional SNPs, such as described in Supplementary Information. Considering cognitive variables, no

association remained after Bonferroni correction. On the other hand, we observed a significant association between rs2072123 and left rostral anterior cingulate (adjusted p-value = 0.008, p-value corrected for 4 SNPs*166 brain regions comparisons), wherein this region is thicker in GG-carriers ($N = 34$) compared to both AG-carriers ($N = 101$; $p = 0.003$) and AA-carriers ($N = 69$; $p < 0.001$) (Fig. 1). These differences were found when we analyzed only patients (GG vs AG: $p = 0.001$; GG vs AA: $p = 0.001$); When we analyzed only controls, a significant difference was observed comparing both homozygotes (GG vs AA: $p = 0.009$). Notably, no association between group or the interaction of group and genotype (group*genotype) and this region thickness was observed ($p > 0.05$). Furthermore, no significant differences between cases and controls on left rostral anterior cingulate thickness was found when comparing each genotype separately ($p > 0.05$), suggesting that the main effect was due to rs2072123 genotypes. When we performed the same analysis considering each self-declared ethnicity (European, African or Asian), the same pattern could be found in all of them though no significant due to the small sample size (Supplementary Fig. S2).

3.4. DGCR2 expression in blood

Comparing blood *DGCR2* expression among healthy controls ($N = 139$), patients with chronic schizophrenia ($N = 154$) and antipsychotic-naïve FEP individuals ($N = 70$), we found a significant association ($p = 8.43 \times 10^{-6}$). Patients with schizophrenia showed lower *DGCR2* levels [ΔCrt mean = 4.94 (SD = 0.41)] than controls [$p = 0.003$, ΔCrt mean = 4.72 (SD = 0.42)] and FEP [$p < 0.001$, ΔCrt mean = 4.54 (SD = 0.35)]. ΔCrt is a measure negatively correlated with gene expression. No association between *DGCR2* expression and genotypes was found ($p > 0.05$); therefore, we hypothesize that the selected SNPs does not influence *DGCR2* expression in blood. Indeed, none of them were expression Quantitative Trait Loci (eQTLs) for *DGCR2* in whole blood in the GTEx portal.

4. Discussion

Our results showed no association between *DGCR2* SNPs and schizophrenia, though it seems to influence brain morphology, by increasing left rostral anterior cingulate thickness. Moreover, whole blood *DGCR2* expression was lower in patients with chronic schizophrenia than controls or antipsychotic-naïve FEP.

Shifman et al. (2006) suggested that antipsychotic drugs elevate the expression of *DGCR2* in the brain. Rats treated with olanzapine, haloperidol or clozapine, but not risperidone, exhibited higher levels of *Dgcr2* in frontal cortex (Shifman et al., 2006). Particularly, our previous studies have shown that *DGCR2* expression in blood is not associated to risperidone treatment or FEP (Ota et al., 2015) nor TRS compared to non TRS (Moretti et al., 2018), nor mania (Gouvea et al., 2016) and nor ultra-high risk for psychosis (UHR) individuals (Santoro et al., 2015). Herein, we observed that patients with chronic schizophrenia present a significant *DGCR2* downregulation in blood compared to antipsychotic-naïve FEP or healthy controls. Therefore, we could hypothesize that *DGCR2* expression levels might be affected by antipsychotic treatment, except for risperidone, and not by psychosis itself, being downregulated in blood and upregulated in brain tissues.

Although Shifman et al. (2006) observed a significant association between *DGCR2* SNPs, their findings were not confirmed by other investigators (Georgi et al., 2009; Ishiguro et al., 2008). On the other hand, rare mutations located in *DGCR2* were observed in patients with schizophrenia (Xu et al., 2011) and intellectual disability (Niederhoffer et al., 2016). Recently, it was suggested that *DGCR2* regulates early steps of corticogenesis (Molinard-Chenu and Dayer, 2018). Considering that schizophrenia is conceived as a neurodevelopmental disorder, this gene might be relevant for the disorder and brain morphology. Indeed, we found that the missense rs2072123 SNP was associated with differences in the left rostral anterior cingulate thickness, with GG-carriers showing an increased thickness compared to AA-carriers, in both patients and controls. We hypothesize that this SNP may be associated with this variable independently of the presence of schizophrenia. Previous studies revealed brain thickness, which might reflect cytoarchitectonic differences, is heritable (Winkler et al., 2010). Thus, *DGCR2*, especially rs2072123, may play a role in regional cortical thickness, but not necessarily directly in schizophrenia.

There is a plethora of evidence that suggest a role of *DGCR2* in the disorder and antipsychotic treatment; however, we did not observe a significant association between its SNPs and schizophrenia in our Brazilian sample, confirming some previous studies. The missense rs2072123 SNP was associated with left rostral anterior cingulate thickness and we found differences in *DGCR2* expression in blood of patients with schizophrenia compared to healthy controls and antipsychotic-naïve FEP. These results suggest that *DGCR2* may not be associated directly with the disorder but might be influencing brain morphology and its expression could be affected by antipsychotics treatment, corroborating previous studies.

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Conflict of interest

No conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.02.068.

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