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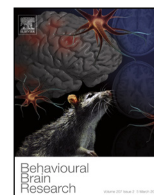

Research report

Association of functional genetic variation in PP2A with prefrontal working memory processing

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HIGHLIGHTS

- Rs959627, a SNP in *PPP2R2B* modulates postmortem prefrontal cortex gene expression.
- The same SNP affects prefrontal cortex function during Working Memory processing.
- Putative mechanism relies on modulation on D2 cAMP-independent dopamine signaling.

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ABSTRACT

Variation in prefrontal dopaminergic signaling mediated by D2 receptor has been implicated in cognitive phenotypes of schizophrenia, including working memory. Molecular cascades downstream of D2 receptor include a cAMP-dependent- and a cAMP-independent-pathway. Protein-Phosphatase-2A (PP2A) is a key partner of D2 receptor in cAMP-independent signaling. This enzyme comprises a regulatory subunit that is coded by *PPP2R2B* gene. Given the molecular relationship between PP2A and D2 signaling, we hypothesized genetic variation in *PPP2R2B* affecting mRNA expression of this gene in prefrontal cortex to be associated with prefrontal processing during working memory. In order to probe such a hypothesis we investigated SNPs associated with *PPP2R2B* expression in two independent samples of human *postmortem* prefrontal cortex. Then, we tested SNPs for which association was replicated as predictors of prefrontal activity during WM as probed by functional magnetic resonance (fMRI) in a sample of healthy humans. We found that a SNP associated with *PPP2R2B* expression (rs959627) predicted prefrontal activity during the N-Back working memory task. In particular, individuals carrying rs959627T allele, a condition associated with lower *PPP2R2B* expression in *postmortem* prefrontal cortex, showed greater activity in right inferior frontal gyrus (IFG) during N-Back compared to CC subjects. Furthermore, such an activity was negatively correlated with behavioral performance at the task.

Consistently with previous studies, these findings suggest reduced right IFG efficiency during working memory processing in rs959627 T-carriers, as indexed by their greater need to activate this brain region in order to achieve similar levels of behavioral proficiency as compared to CC individuals.

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1. Introduction

Different lines of research have implicated D2 Dopamine Receptor in modulation of neuronal activity during Working Memory (WM). Studies in non human primates have associated pharmacological modulation of D2 receptors with prefrontal activity during memory-guided oculomotor responses of a WM task [36]. Moreover, studies in humans have demonstrated that stimulation of D2 receptors modulates WM related prefrontal activity [16,15]. Furthermore, consistent findings indicate that genetic variation relevant to D2 signaling modulates prefrontal activity [9]. These results have been interpreted in terms of prefrontal efficiency, i.e., individuals with the 'risk' genotype need greater prefrontal resources to perform the WM task in spite of similar or lower behav-

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ioral proficiency compared with subjects having the 'favorable' genotype.

Different downstream molecular pathways mediate the function of dopamine D2 receptors. One operates through the regulation of cAMP and Protein Kinase A activity (cAMP-dependent pathway); alternatively, D2 signaling follows a cAMP-independent pathway, involving the kinase Akt1 and the Glycogene Synthase Kinase 3β (GSK3β). In previous studies with fMRI we used functional genetic variation to probe how D2 receptor and its signaling partners modulate prefrontal physiology. In other words, we characterized how physiological variation of the expression of *DRD2* and its signaling partners in the cAMP-independent pathway is associated with prefrontal activity in humans [5,6,8,7].

Among D2 receptor partners, the Protein Phosphatase 2A is a key mediator of D2 cAMP-independent signaling [3] involved in cell metabolism, growth and differentiation [29]. Previous findings have indicated that D2 receptor stimulation by dopamine induces the formation of a β-arrestin/PP2A complex, which allows dephosphorylation and inhibition of Akt1 [3,2,4]. A crucial effect of Akt1 activity is the phosphorylation and inhibition of the GSK3β. Thus, the net effect of the β-arrestin/PP2A complex catalyzed by stimulation of D2 receptors by dopamine is the activation of GSK3β, which has been implicated in neurodevelopment and cognition [21,7,27].

The regulatory subunit of PP2A exists in a number of different isoforms, which have tissue-specific distribution. The PR55β isoform is the most expressed in the brain [22] and is coded by the *PPP2R2B* gene (5q32), previously associated with spinocerebellar ataxia 12 [20]. Given PP2A close molecular relationship with the D2 cAMP-independent pathway and the crucial role of D2 signaling in prefrontal cognition, in the present study we hypothesized that genetic variation predicting prefrontal *PPP2R2B* expression levels would be associated with system level phenotypes related to prefrontal cognitive processing. In order to probe such a hypothesis, in a first exploratory step we investigated in two independent samples of *post mortem* human prefrontal cortex the association of single nucleotide polymorphisms (SNPs) with mRNA expression of *PPP2R2B*. Such an investigation identified rs959627 as a SNP predicting gene expression, i.e. an eQTL (expression Quantitative Trait Loci) of *PPP2R2B* gene, in two independent datasets of brain tissue. Thus, we considered this SNP as a proxy of *PPP2R2B* expression and probed its association with prefrontal processing during WM in a sample of healthy humans. We hypothesized that rs959627 would predict prefrontal activity during working memory because of the role of PP2A in signaling of neurotransmitters strongly involved in this cognitive process.

2. Materials and methods

2.1. Association of PPP2R2B variation with post mortem prefrontal PPP2R2B mRNA expression

2.1.1. Discovery sample

We used the Braincloud dataset available at http://braincloud.jhmi.edu [14] as a discovery sample to investigate the association between *PPP2R2 B* SNP genotypes and transcription levels in human *post mortem* pre-frontal cortex (BA – Brodmann Area – 46). In particular, we investigated 258 brains of Caucasian (N = 112) and African American (N = 146) non psychiatric individuals (males, N = 176; mean age, 27.9 years [SD = 22.2]; brain pH = 6.5 [SD = 0.3]; *post mortem* interval (PMI), 26.4 h [SD = 17.1]; RNA integrity number (RIN), 8.4 [SD = 0.9]). Details of tissue acquisition, handling, processing, dissection, clinical characterization, neuropathological examinations, RNA extraction and quality control measures were described previously [26,14].

RNA from prefrontal grey matter was analyzed using spotted oligonucleotide microarrays yielding data from 30176 gene expression probes and allowing us to focus on *PPP2R2B* mRNA expression. In particular, total RNA was extracted, amplified and fluorescently labeled. Reference RNA was pooled from all samples and treated identically to sample RNAs. After normalization [13], log2 intensity ratios were further adjusted to reduce the impact of known and unknown sources of systematic noise on gene expression measures using surrogate variable analysis [24,14]. Data on *PPP2R2B* expression levels relative to three different probes were available (hHA039595 [NM_004576]; hHA037283 [NM_181678]; hHA038394 [NM_181674]).

DNA from cerebellar tissue was studied with Illumina BeadChips producing 625,439 SNP genotypes called using the BeadExpress software for each subject as previously described [14].

Possible confounding effect of demographical variable (age, gender) and sample quality features (RIN, pH, PMI) on *PPP2R2B* mRNA *post mortem* expression levels were controlled for by using the Akaike Information Criterion (AIC) stepwise regression model. We employed a model including RIN, RIN squared, pH, PMI, age, and gender as confounding variables. Regression residuals of *PPP2R2B* expression were used for the eQTL detection. Extreme observations that could exert a disproportionate effect on the association tests and thus bias results were defined as those exceeding 2nd and 98th percentile and were excluded. After this procedure, the discovery sample included 248 subjects (105 Caucasians; 84 females; age: mean ± SD = 27 ± 22 years, range: 0.5 prenatal – 78 postnatal years). Shapiro-Wilk's test indicated that gene expression residuals deviated from the normal distribution ($p < 0.001$).

We selected 148 SNPs occurring within 100 kbp upstream and downstream of the *PPP2R2 B* gene available in Braincloud dataset and used the software SnpVariationSuite (SVS; GoldenHelix, Bozeman, Montana) to detect 75 tagging SNPs with low to moderate LD between each other ($R^2 > 0.5$) [28]

In order to assess genotype/mRNA expression association, we performed Kruskal-Wallis tests due to deviation from normal distribution of the expression data. In particular, genotype was used as the independent variable and regression residuals of *PPP2R2 B* mRNA expression as the dependent variable separately for each SNP. Results were corrected using False Discovery Rate (FDR) correction for multiple comparisons at $p < 0.05$.

2.1.2. Replication sample

We used BrainEAC (http://www.braineac.org) in order to replicate association between rs959627 and *PPP2R2 B* mRNA expression in a sample of Caucasian subjects. This dataset includes 137 brains of non psychiatric Caucasian individuals (males, N = 102; mean: age, 59 [SD = 8.5]; brain pH, 6.3 [SD = 0.21]; PMI, 43.7 [SD = 3]; RIN, 4.41 [SD = 1.57]) [34]. Frontal mRNA expression levels and SNPs genotypes were available for 123 brain samples. Details of tissue acquisition, handling, processing, dissection, clinical characterization, neuropathological examinations, RNA extraction and quality control measures were described previously [34].

As we did for the discovery sample, also in BrainEAC we focused on *PPP2R2 B* mRNA expression values (BrainEAC ID: t2880051) controlling for the effects of confounding variables and removing extreme observations using the same method described above. After this procedure, the replication sample included 119 subjects (33 females; age: mean ± SD = 58 ± 19 years, range: 16–102 years). Given deviation from a normal distribution of expression data (Shapiro-Wilk's test $p < 0.001$), we performed Kruskal-Wallis tests with rs959627 genotype as the independent variable and *PPP2R2B* mRNA expression values as the dependent variable. Post-hoc analysis was performed with multiple comparisons of mean rank. Given

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