Brain Structural Evidence of Epistasis between *RGS4* & *COMT* Variations in Schizophrenia

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Abstract

Background: Polymorphisms of the Catechol-O-methyl transferase (*COMT*) and the Regulator of G-protein Signaling 4 (*RGS4*) genes have independently been implicated in schizophrenia (SZ) genesis. Based on previous findings of statistical and functional evidence of epistatic interactions in the dorsolateral prefrontal cortex (DLPFC), we evaluated our samples for similar associations and, in addition, examined epistatic interactions of *RGS4* and *COMT* polymorphisms on grey matter volumes among SZ and healthy subjects (HS).

Methods: We attempted to replicate the previous findings by examining structural MRI scans from 21 first-episode, antipsychotic-naïve schizophrenia or schizoaffective disorder subjects and 19 healthy subjects using both hypothesis free and hypothesis driven tests for interaction using voxel-based morphometry to examine grey matter alterations associated with *COMT* and *RGS4* risk alleles both independently and interactively.

Results: At the whole brain level, we observed *RGS4* rs2842018 and *COMT* rs4818 interactions in the prefrontal, heteromodal association, and temporal regions. The most consistent finding among all interactions was grey matter reductions at the inferior and superior temporal gyri, corresponding to the heteromodal association areas.

Discussion: Our observations suggest that *RGS4* and *COMT* variations are independently and epistatically associated with grey matter reductions at the dorsal components of heteromodal association areas. Previous associations of functional interaction between *COMT* and *RGS4* with altered working memory performance and BOLD responses at the prefrontal cortex in SZ may be mediated by structural changes in the dorsal heteromodal association areas.

Background

Polymorphisms of the Catechol-O methyltransferase (*COMT*) and the Regulator of G-protein Signaling 4 (*RGS4*) genes are independently implicated in schizophrenia (SZ) genesis. Based on previous findings of statistical and functional evidence of epistatic interactions in the dorsolateral prefrontal cortex (DLPFC), we evaluated our samples for similar associations (Buckholtz et. al, 2007) and, in addition, examined epistatic interactions of *RGS4* and *COMT* polymorphisms on grey matter volumes among SZ and healthy subjects (HS)

Methods

Clinical Evaluations

- A cohort of 21 first episode antipsychotic naïve SZ and 19 healthy subjects were recruited.
- SZ was diagnosed using SCID and following consensus diagnostic meeting
- Significant substance use, presence of DSM IV defined mental retardation, neurological illness were exclusion criteria

Genotyping methods

A PCR-based multiplex assay was used to genotype the *RGS4* and *COMT* variations
Positive controls and negative controls were included in the assay plates for quality control
Genotypes clusters were examined on GeneMapper v 4.0
Allele calls were independently checked and confirmed by two investigators (KMP & Chowdari)

Imaging procedures

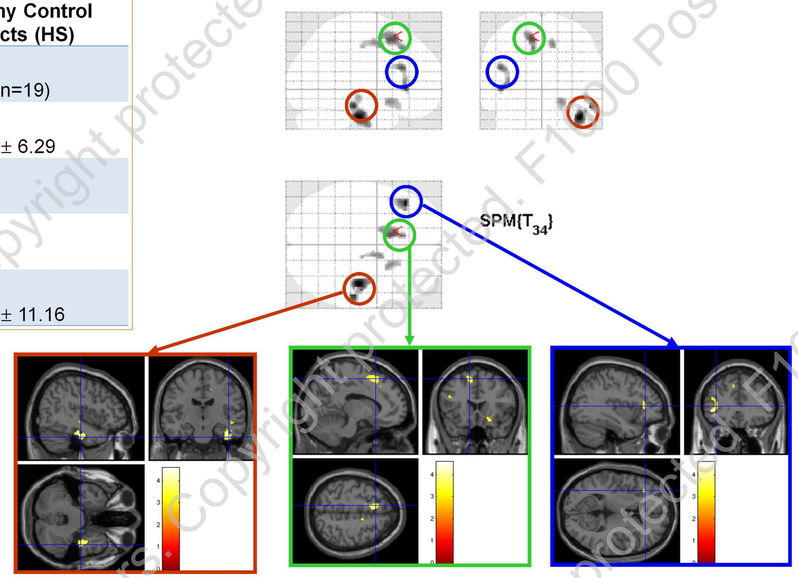
- MRI acquired on 1.5T GE Whole body scanner
- Scanning parameters: 3-dimensional spoiled gradient-recalled, steady-state pulse sequence (124 coronal slices, 1.5-mm thickness, TE=5 msec, TR=25 msec, acquisition matrix=256×192, field of view=24 cm, flip angle=40°)
- Images were analyzed using the Statistical Parametric Mapping (SPM 5) software (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/). The voxel-based morphometry (VBM) analysis was implemented on a Mac Pro workstation using the SPM5 package on Matlab 7 platform (Mathworks Inc, 2002).
- Initially, we examined the association of individual genotypes and grey matter changes followed by setting up the interaction analysis.
- A priori combined voxel extent threshold and cluster size significance level for the clusters were determined by running 10,000 Monte Carlo simulations using Alpha Sim to minimize false positives:
  - Simulations revealed that a cluster size of 68 voxels at a threshold of $p<0.005$ per voxel would provide a corrected significance of $p<0.05$ for the whole brain analysis
- To examine interactions, we analyzed the combined SZ and healthy subjects after controlling for the diagnosis effects in order to increase power

Results

Table: Sample Characteristics

	Schizophrenia (SZ)	Healthy Control Subjects (HS)
	Total (n=21)	Total (n=19)
Age (in years)[a] mean ± SD	24.16 ± 8.63	24.39 ± 6.29
Gender[b] Male	17	7
Female	4	1
Average SES[c]	41.38 ± 14.66	46.55 ± 11.16

Fig 1: rs2842018 (*RGS4*) & rs4818 (*COMT*) Interactions



- Significant interactions were observed mainly for the rs2842018 (*RGS4*) & rs4818 (*COMT*)
- Individuals homozygous for allele G on both SNPs had significantly *higher* grey matter volumes on the inferior temporal gyrus including the parahippocampal gyrus (red circle), prefrontal region within the Brodmann area 8 (green circle) and the inferior frontal region within the Brodmann area 46/47 (blue circle) *and* decreased GM volumes at the heteromodal association areas bilaterally (not shown)
- In addition, rs2842018 and rs6269 on *COMT*, rs2842018 and rs9265 on *COMT* interactions were noted at the middle temporal gyrus
- *RGS4* (rs951436) - *COMT* (rs16559) interactions were noted at the inferior temporal region

Conclusions

Our observations suggest that *RGS4* and *COMT* variations are independently and epistatically associated with grey matter reductions at the prefrontal and dorsal components of heteromodal association areas. Previous associations of functional interaction between *COMT* and *RGS4* with altered working memory performance and BOLD responses at the prefrontal cortex in SZ may be mediated by structural changes in the dorsal heteromodal association areas.

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Acknowledgements

This work was supported by research grants from the NIMH MH72995, APIRE-Lilly Award and NARSAD Young Investigator Award (KMP), MH63480 (VLN) and MH45156 to the Conte Center for the Neuroscience of Mental Disorders (Dr. Lewis).

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