

reported by Kinoshita *et al.*⁹ was 1.11, which is similar to that (OR=1.15) in the present study, although the ORs of the A allele of SNP2 and the second most common haplotype for schizophrenia were 1.0 in the Kinoshita *et al.*⁹ study and 1.09 and 1.10 in the present study, respectively. Recently, Yamada *et al.*⁷ reported a nominally significant association of SNP2 (CCS3, rs2461491) and a trend towards association of SNP1 (CC21, rs10108011) with schizophrenia in Japanese family based-association analysis.

The present study replicated the allelic and haplotypic associations of *PPP3CC* with schizophrenia. Thus, an association between genetic variations of *PPP3CC* and schizophrenia appears to exist in US and Japanese populations. However, the ORs of 1.10–1.15 observed in the present study indicate that the associations are weak and will be difficult to replicate without large sample sizes. Further studies are needed to evaluate whether alterations in calcineurin signaling contribute to the pathogenesis of schizophrenia.

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References

- Rusnak F, Mertz P. *Physiol Rev* 2000; **80**: 1483–1521.
- Miyakawa T, Leiter LM, Gerber DJ, Gainetdinov RR, Sotnikova TD, Zeng H *et al. Proc Natl Acad Sci USA* 2003; **100**: 8987–8992.
- Rushlow WJ, Seah YH, Belliveau DJ, Rajakumar N. *J Neurochem* 2005; **94**: 587–596.
- Klee CB, Ren H, Wang X. *J Biol Chem* 1998; **273**: 13367–13370.
- Falush D, Stephens M, Pritchard JK. *Genetics* 2003; **164**: 1567–1587.
- Barrett JC, Fry B, Maller J, Daly MJ. *Bioinformatics* 2005; **21**: 263–265.
- Yamada K, Gerber DJ, Iwayama Y, Ohnishi T, Ohba H, Toyota T *et al. Proc Natl Acad Sci USA* 2007; **104**: 2815–2820.
- Gerber DJ, Hall D, Miyakawa T, Demars S, Gogos JA, Karayiorgou M *et al. Proc Natl Acad Sci USA* 2003; **100**: 8993–8998.
- Kinoshita Y, Suzuki T, Ikeda M, Kitajima T, Yamanouchi Y, Inada T *et al. J Neural Transm* 2005; **112**: 1255–1262.
- Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I *et al. Am J Hum Genet* 2003; **73**: 34–48.

fMRI evidence for functional epistasis between COMT and RGS4

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COMT and RGS4 are promising candidate risk genes for schizophrenia¹ that impact on dopamine signaling^{2–4} and on prefrontal function.^{5,6} While, in general, convergent molecular pathways and neurophysiological effects implicate gene × gene interactions, there is growing evidence to support a specific direct interaction between risk alleles in COMT and RGS4. First, statistical epistasis between a number of putative schizophrenia risk genes, notably RGS4 and COMT, has been reported in risk for schizophrenia.⁷ Second, a recent study in human postmortem dorso-lateral prefrontal cortex demonstrated a significant correlation between both COMT val158met genotype and COMT enzyme activity and RGS4 mRNA levels, such that increased COMT enzyme activity (and Val allele load) predicted decreased RGS4 mRNA expression.⁸ Using an fMRI working memory task in healthy subjects that robustly engages DLPFC in a manner sensitive to variation in both COMT and RGS4, we employed a moderated multiple regression approach to examine interactions between the COMT (rs4680 G/A(val158met)) and the RGS4 (rs951436 A/C ('Chowdari SNP4')),⁹ SNPs most consistently associated with schizophrenia and with prefrontal activation. In line with earlier findings, we expected that the impact of genetic variation in RGS4 would be more pronounced on a COMT Val allele background, such that individuals with both RGS4 SNP4-A and COMT-Val would exhibit the most inefficient pattern of prefrontal cortical engagement (that is, greater prefrontal activation in the absence of performance differences).¹⁰

We studied 82 healthy Caucasian subjects using an N-back working memory task as described previously.¹⁰ Subjects were genotyped for RGS4 and COMT^{5,6} (COMT: 25 V/V, 43 V/M, 14 M/M; RGS4: 26 A/A, 36 A/C, 20 C/C). Since only two COMT Met/Met-RGS4 A/A subjects were available in this sample, V/M heterozygote and M/M homozygote subjects were combined. One-way analysis of variance (ANOVA) with five groups (COMT Val/Val, Met-carriers and RGS4 SNP4 A/A, A/C, C/C) revealed no significant between-group differences in performance (accuracy or reaction time), gender or age. Whole brain BOLD fMRI data were collected on a 3 T GE scanner (TE = 30 ms, TR = 2 s, flip angle = 90, FOV = 24 cm) while subjects performed an N-back working memory task (2-back and 0-back) as previously described.¹⁰ A second level moderated multiple regression was used to map the main effects for

each gene and the gene \times gene interaction across the brain. We used a random effects general linear model (GLM) multiple regression model on 2-back > 0-back individual activation maps, with age, gender, reaction time and performance as nuisance covariates in SPM (www.fil.ion.ucl.ac.uk/spm). Based on our specific *a priori* hypothesis regarding a meaningful gene \times gene interaction within DLPFC, we masked the regression analysis contrast using a DLPFC region of interest (ROI) with a statistical threshold corrected for multiple comparisons ($P < 0.05$, family-wise error (FWE)). In addition, we investigated effects within the wider WM network across the whole brain, using an exploratory uncorrected threshold of $P < 0.001$. As a final test, we extracted parameter estimates from the right DLPFC ROI and analyzed them using a GLM analysis of covariance (ANCOVA) with COMT and RGS4 genotypes as between-subject factors and age and gender as covariates (SPSS 13.0, Chicago, IL, USA).

As predicted, we found a significant interaction between COMT and RGS4 genotype within DLPFC during the N-back working memory task (peak within Talarach-Tournoux space at (38, 17, 43); $Z = 3.26$, cluster size (k) = 43 voxels, $P < 0.05$ FWE) (Figure 1a). ANCOVA confirmed a significant COMT \times RGS4 genotype interaction effect for right prefrontal BOLD signal ($F(2, 74) = 5.78$, $P < 0.005$). *Post hoc* analysis

using Fisher's least significant difference measure (LSD) showed significant differences ($P < 0.05$) between the following groups: Val/Val-A/A > Met-A/A, Val/Val-A/A > Met-A/C, Val/Val-AA > Val/Val-C/C, Val/Val-A/A > Met-C/C (Figure 1b). We also found a significant interaction between COMT and RGS4 in the midbrain (-4, -19, -4), although this finding did not survive whole brain voxelwise correction for multiple comparisons ($Z = 3.98$, $k = 10$, $P < 0.001$ uncorrected) (Figure 1c). ANCOVA as above using the extracted parameter estimates from this cluster showed a significant interaction ($F(2, 74) = 9.41$, $P < 0.0002$) (Figure 1d).

Consistent with our hypothesis, we found that COMT genotype modulated the impact of the RGS4 risk allele on prefrontal function. Val/Val subjects demonstrated a significant allele-dose effect of RGS4 on prefrontal BOLD, while the effect of RGS4 was attenuated in Met-carrying subjects. Thus, the impact of the RGS4 A allele, previously associated with abnormal prefrontal function,⁵ is magnified in Val/Val individuals and protected against in Met individuals. This relatively increased inefficiency seen in healthy subjects on an RGS4 A-Val/Val background is reminiscent of alterations in prefrontal function seen in schizophrenic patients, as might be expected from the interaction of two putative schizophrenia risk genes.

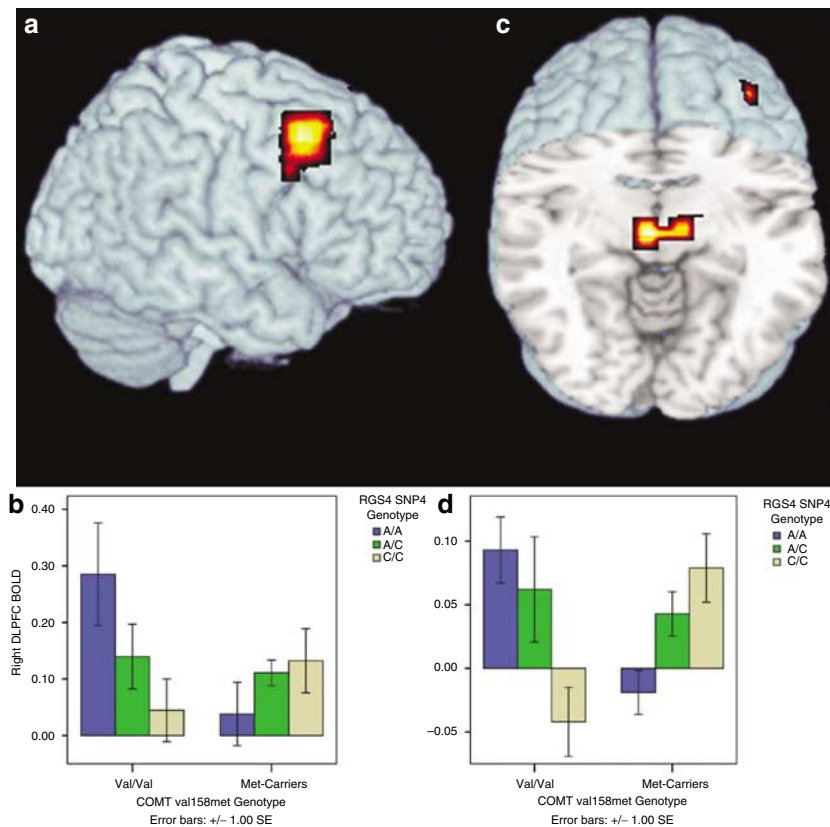


Figure 1 Statistical Parametric Maps depict the interaction of COMT and RGS4 risk alleles on brain activation in human dorsolateral prefrontal cortex (DLPFC) (a) and midbrain (c) during working memory. Bar graphs show percent fMRI signal change in DLPFC (b) and midbrain (d) for each genotype group.

These data accord with earlier studies demonstrating regulation of RGS4 expression by dopamine⁴ and specifically by COMT genotype.⁸ Lipska and colleagues showed significantly decreased RGS4 mRNA expression in the DLPFC of Val/Val subjects, relative to Met-carriers. These changes suggest that variation in cortical dopamine may alter RGS4-dependent signaling by regulating its transcription. Alternatively or in addition, genetic variation in RGS4 may become more manifest in COMT Val/Val subjects, whose cortical synaptic dopamine levels are lower. Our findings support earlier statistical genetic and neuropathological evidence for epistasis between COMT and RGS4 by demonstrating an interaction between these two putative risk genes on an *in vivo* measure of prefrontal function. These results bolster the notion that COMT val158met genetic background mediates the impact of other schizophrenia susceptibility genes.

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References

- Harrison PJ, Weinberger DR. *Mol Psychiatry* 2005; **10**: 40–68.
- Mattay VS, Goldberg TE, Fera F, Hariri AR, Tessitore A, Egan MF *et al. Proc Natl Acad Sci USA* 2003; **100**: 6186–6191.
- Meyer-Lindenberg A, Kohn PD, Kolachana B, Kippenhan S, McInerney-Leo A, Nussbaum R *et al. Nat Neurosci* 2005; **8**: 594–596.
- Taymans JM, Kia HK, Claes R, Cruz C, Leysen J, Langlois X. *Eur J Neurosci* 2004; **19**: 2249–2260.
- Buckholtz JW, Meyer-Lindenberg A, Honea R, Straub RE, Egan MF, Vakkalanka R *et al. J Neurosci* 2007; **27**: 1584–1593.
- Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE *et al. Proc Natl Acad Sci USA* 2001; **98**: 6917–6922.
- Nicodemus KK, Kolachana BS, Vakkalanka R, Straub RE, Giegling I, Egan MF *et al. Hum Genet* 2007; **120**: 889–906.
- Lipska BK, Mitkus S, Caruso M, Hyde TM, Chen J, Vakkalanka R *et al. Hum Mol Genet* 2006; **15**: 2804–2812.
- Chowdari KV, Mirmics K, Semwal P, Wood J, Lawrence E, Bhatia T *et al. Hum Mol Genet* 2002; **11**: 1373–1380.
- Callicott JH, Mattay VS, Verchinski BA, Marenco S, Egan MF, Weinberger DR. *Am J Psychiatry* 2003; **160**: 2209–2215.

Activated p38 MAPK is associated with decreased CSF 5-HIAA and increased maternal rejection during infancy in rhesus monkeys

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Recent data indicate that activation of the p38 mitogen-activated protein kinase (MAPK) signaling cascade by cytokines including interleukin (IL)-1 and tumor necrosis factor (TNF)- α increases the expression and activity of the serotonin transporter (SERT). Herein, we report that increased p38 activity, as manifested by an increased percentage of peripheral blood monocytes staining positive for intracellular phosphorylated p38 (p-p38), was associated with decreased cerebrospinal fluid (CSF) concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), and increased maternal rejection in 17 rhesus monkeys, 8 of whom were exposed to poor maternal care as infants. These data provide the first evidence of an *in vivo* relationship between p38 MAPK activation and brain serotonin metabolism in an animal model of early life stress and indicate that activation of p38 MAPK signaling pathways may participate in the contribution of early life stress to psychiatric morbidity.

Early life stress including physical/sexual abuse as well as neglect has been associated with a number of adverse health outcomes including increased anxiety and depression.¹ We recently reported that adolescent rhesus monkeys (*Macaca mulatta*) exposed to physical abuse and high levels of maternal rejection as infants exhibit increased distress and anxiety, delayed social development and reduced exploration, compared to non-abused animals.^{2,3} This early exposure to poor maternal care was also associated with reduced brain serotonergic function as reflected by decreased CSF concentrations of serotonin (5-HT) and the serotonin metabolite, 5-HIAA, that were in turn correlated with increased anxiety-like behavior during adolescence.⁴

Data indicate that stress, including early life stress, also can be associated with activation of innate immune responses including release of proinflammatory cytokines and activation of proinflammatory cytokine signaling cascades.^{5,6} Relevant to the impact of early life stress on serotonin metabolism, the cytokine signaling pathway, p38 MAPK, has been found to increase expression and activity of the SERT in both a rat embryonic raphe cell line