



ASSOCIATION AND LINKAGE ANALYSES OF *RGS4* POLYMORPHISMS IN SCHIZOPHRENIA

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Abstract

Gene expression analyses of postmortem cerebral cortex suggest that transcription of the Regulator of G-protein Signaling 4 (*RGS4*) is decreased in a diagnosis specific manner in subjects with schizophrenia (Mirnics K et al, 2001). We have shown using the TDT, significant transmission distortion in three samples ascertained independently in Pittsburgh, New Delhi and by the NIMH Collaborative Genetics Initiative at this locus (Chowdari K et al 2002). Among SNPs spanning approximately 300 kb, significant associations involved four SNPs localized to a 10 kb region at *RGS4*, but the associated haplotypes differed. Consistent with the TDT results, samples with affected siblings (NIMH, India) showed

higher levels of allele sharing, identical by descent, at *RGS4*. When the US patients were contrasted to two population-based control samples, however, no significant differences were observed. We also examined US families with Bipolar I Disorder (BD1) probands. This sample showed a trend for transmission distortion and cases differed significantly from the population-based controls for the four-SNP haplotypes tested in the other samples. The transmission distortion is unlikely to be due to chance. To understand these disparate findings, we have identified several novel SNPs localized in a 30 kb region upstream to the *RGS4* locus for further analysis in our samples. We are also re-sequencing genomic DNA from the post-mortem brain samples employed in the gene expression analyses.

1 Strategy

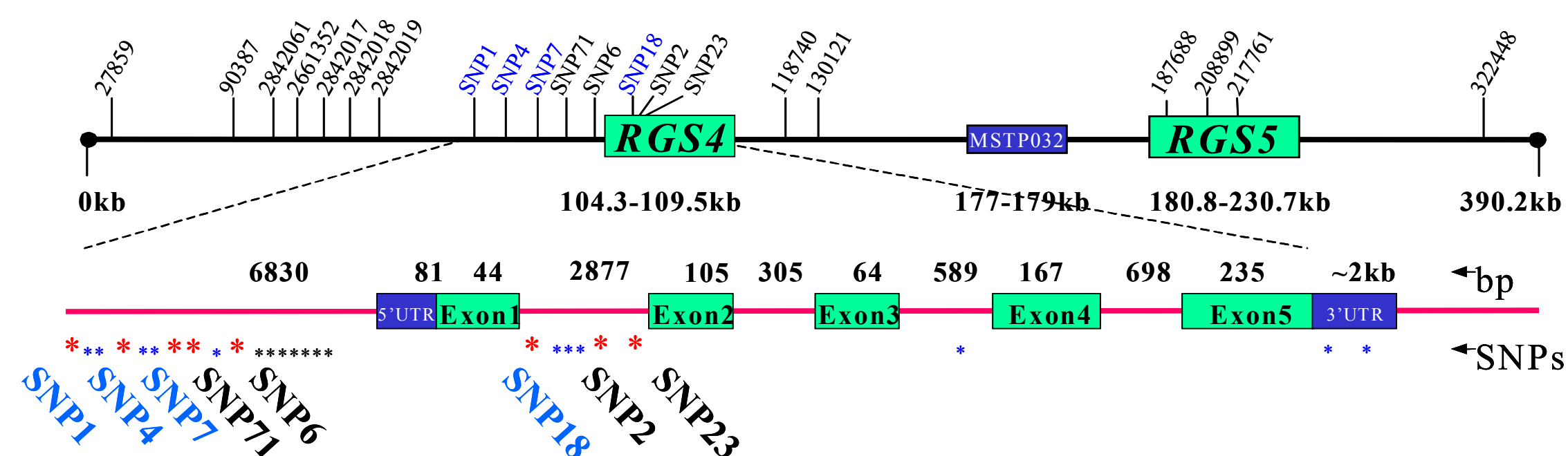
- Examine genes at the intersection of linkage and biology
- Strive for consistency
 - across samples
 - across analytical techniques
 - Linkage (affected sib-pairs)
 - Linkage & association (TDT)
 - Association (case-control)

2 *RGS4*

- Regulator of G Protein signaling
- Differentially expressed in schizophrenia (Mirnics et al. Mol Psych, 2001, 6, 293-301)
 - Localized to 1q21

A positional candidate?

3 Organization of *RGS4* and flanking regions



4 TDT results: Single locus/haplotypes

Pittsburgh families			NIMH families		
SNPs	T	T/NT	SNP	T	T/NT
SNP 1	o	53/35 (0.055)	SNP 1	•	30/13 (0.01)
SNP 4	•	51/33 (0.05)	SNP 4	o	22/6 (0.003)
			SNP 18	o	24/8 (0.005)

27859 – 90387 – 1-4-7-18-23 – 118740 – 130121 – 187688 – 208899 – 217761 – 322448

○●○●○●	●○●○●○
37/13 (P=0.0007)	19/3 (0.001)*

TRANSMIT:
 $\chi^2 = 16.6, 8 \text{ d.f.}, p = 0.035$ $\chi^2 = 18.8, 8 \text{ d.f.}, p = 0.010$

Caucasian SZ+SA Trios - Pitt: 93, NIMH: 39

- ◆ Possible explanation:
 - chance finding
 - General transmission distortion
 - True association

5 TDT analyses: SNPs 1, 4, 7, 18

Analysis	New Delhi SZ / SA	Pittsburgh BD1
SNPs	NS	NS
Haplotypes	* p = 0.055	Sig. under-transmission
TRANSMIT	p = 0.078	p = 0.108

New Delhi: SZ / SA; n = 197 trios
 Pittsburgh: BD1; n = 101; 39 trios, 62 duos.

6 Synthesis of multi-site TRANSMIT results

Sample	Individual sample		Cumulative		
	p	-2 log (p)*	df	p	
Pittsburgh SZ/SA	0.035	6.70	6.70	2	0.035
NIMH SZ/SA	0.010	8.27	14.97	4	0.0048
New Delhi SZ/SA	0.078	5.10	20.07	6	0.0027
Pittsburgh BD1	0.108	4.45	24.52	8	0.0019

*p-values follow a uniform distribution on the interval (0,1). The negative logarithm of the p-values follow a Chi-square distribution with two degrees of freedom (DF). The sum of N independent, transformed p-values follows a Chi-square with 2N DF.

7 Pittsburgh case-control analyses

Haplotype	Adult cont: n=89	Neonatal cont: n=85	SZ/SA cases	BD 1 cases
○-○-○-○	0.066	0.096	0.078	0.044
●-○-○-○	0.021	0.004	0.022	0.029
○-○-○-○	0.006	0.006	0	0
○-○-○-○	0	0	0.006	0.025
●-○-○-○	0.442	0.388	0.378	0.378
○-○-○-○	0.006	0	0	0
●-○-○-○	0	0	0.006	0
○-○-○-○	0.004	0	0	0.017
○-○-○-○	0.006	0	0	0
○-○-○-○	0.425	0.439	0.494	0.349
●-○-○-○	0.013	0.008	0	0.007
○-○-○-○	0.013	0.053	0.017	0.050
○-○-○-○	0.000	0.006	0	0.102*

Pittsburgh BD1 samples

SNP-EM Omnibus likelihood ratio test: p = 0.0002 (cases vs neonates or cases vs adults)

8 Ongoing work

- ◆ Replicate studies
- ◆ Investigate clinical sub-groups
- ◆ Re-sequenced 25kb upstream region
- ◆ Identified 17 SNPs, genotyped 5 SNPs
- ◆ Association localized to 12kb region

Conclusions

- ◆ Evidence for association / linkage at *RGS4*
- ◆ General transmission distortion unlikely

- ◆ Association not restricted to schizophrenia?
- ◆ Pathogenic SNPs / haplotypes uncertain