Population and family based association study on TPH1, TPH2 and ITGB3 genes indicate serotonergic system involvement in autism spectrum disorder

Asem Surindro Singh, Ph. D.
Email: asemsuren@gmail.com

Introduction

- ASD: childhood onset, genetic, neurodevelopmental disorder. (autism, asperger's syndrom, PDD-NOS)

- Behaviorally defined

1. Lack of social interaction
2. Lack of communication
3. Restricted, repetitive behaviors and interests.

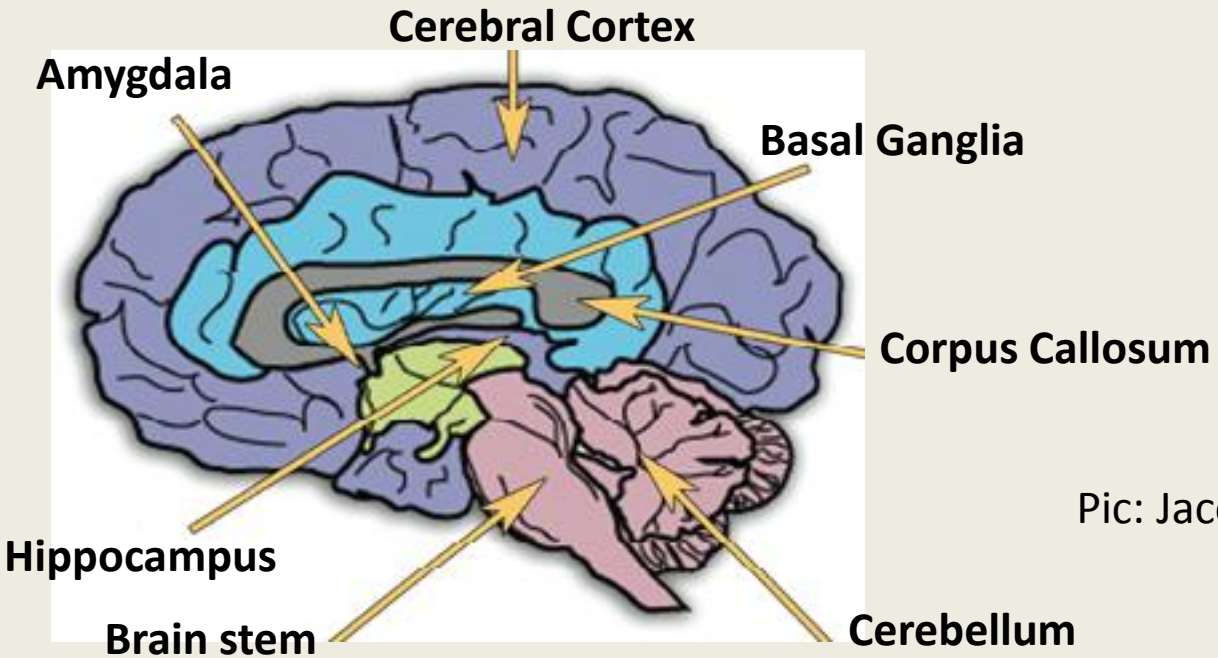


Figure 1. Parts of brain affected by autism

Pic: Jacob Bartnett

- High Prevalence: 1 in 68 children, (CDC, 2014).
- Prevalence and cure: Urgently needed, pathophysiology unclear.
- Concordance: 3 - 8% (dizygotic twins) and 69 – 95% (monozygotic twins) (Dawson G, 2008).
- Serotonin system abnormality in ASD: Brain and peripheral
- Serotonergic system: TPH1, TPH2, SLC6A4, SLC18A2, ITGB3.
- Serotonin abnormality: Synthesis, degradation, transport.
- Candidate gene association studies.
- Present study: TPH1, TPH2 and ITGB3 genes
- Two approaches: genetic association & gene-gene interaction analysis.

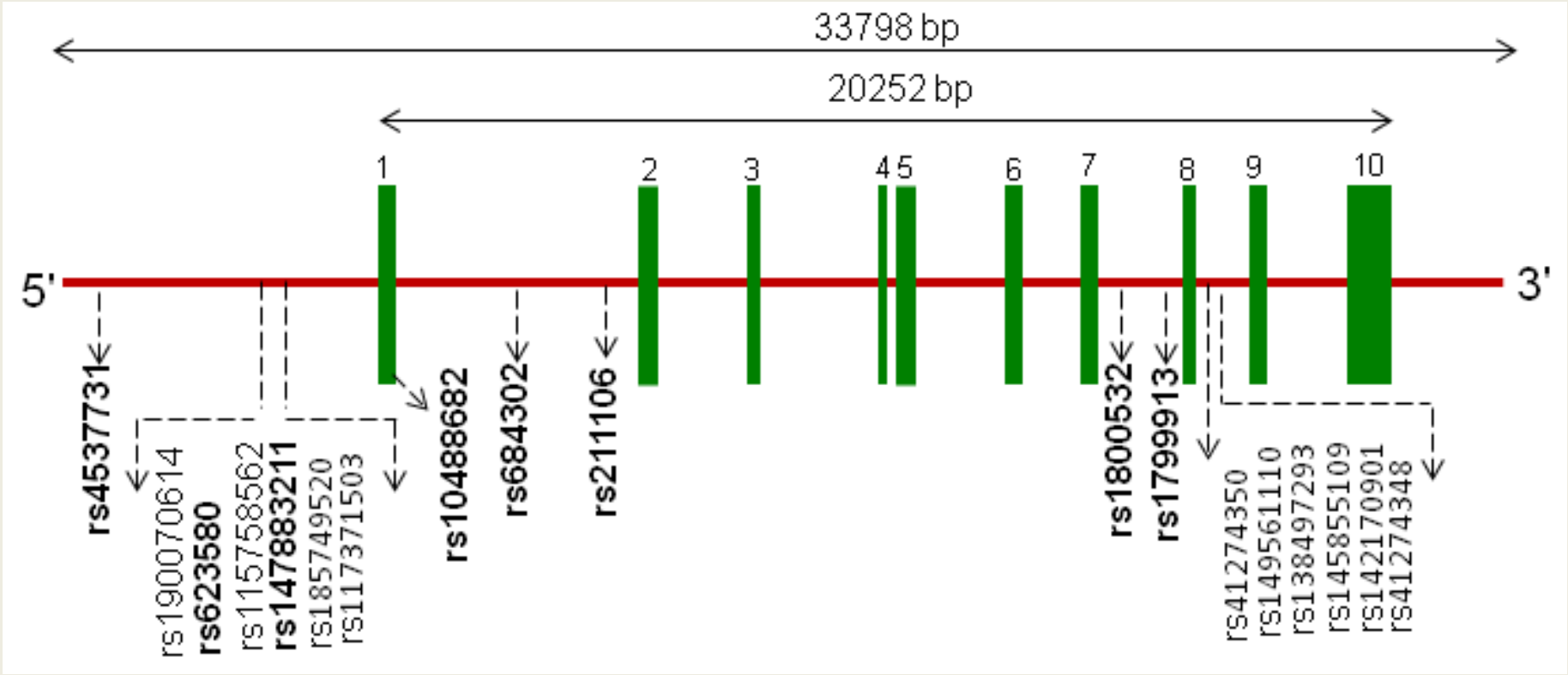
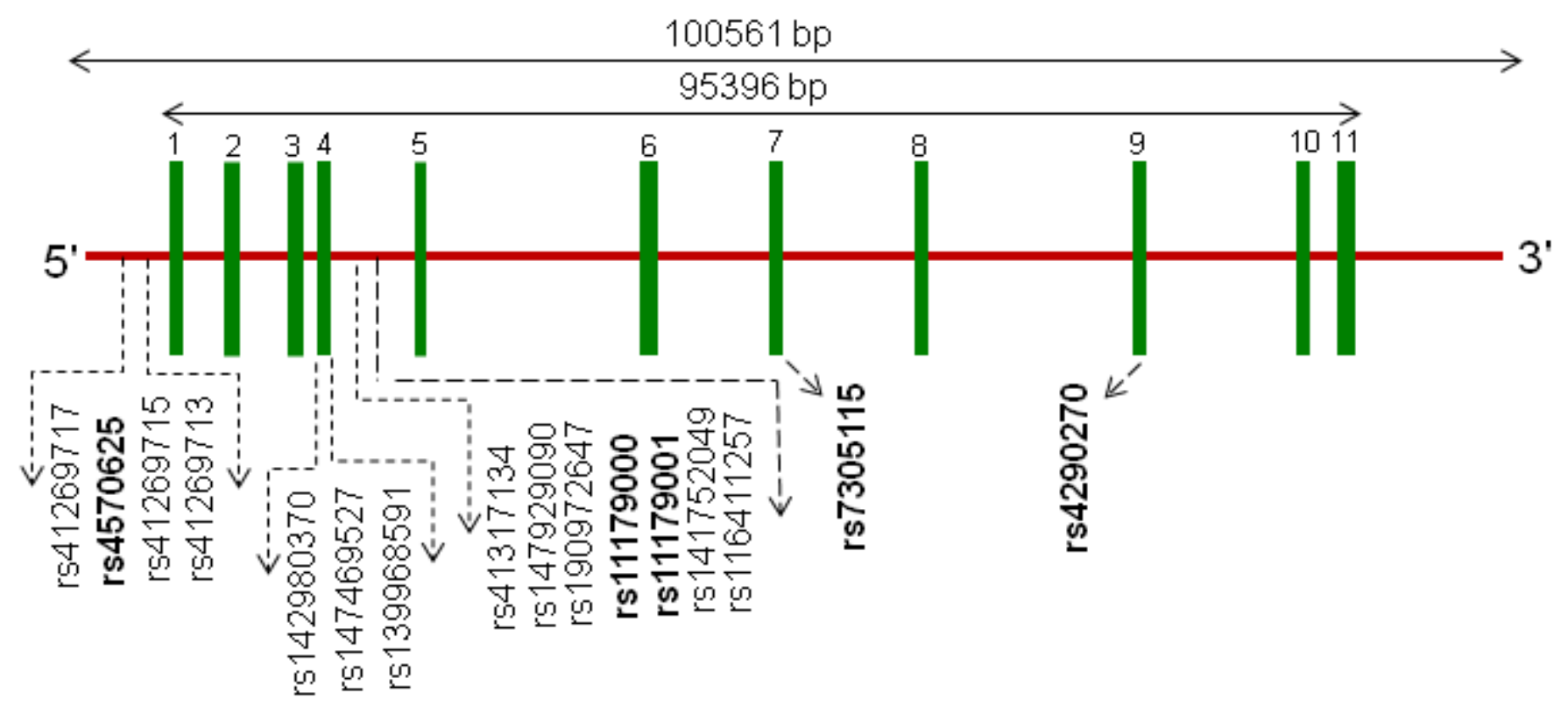
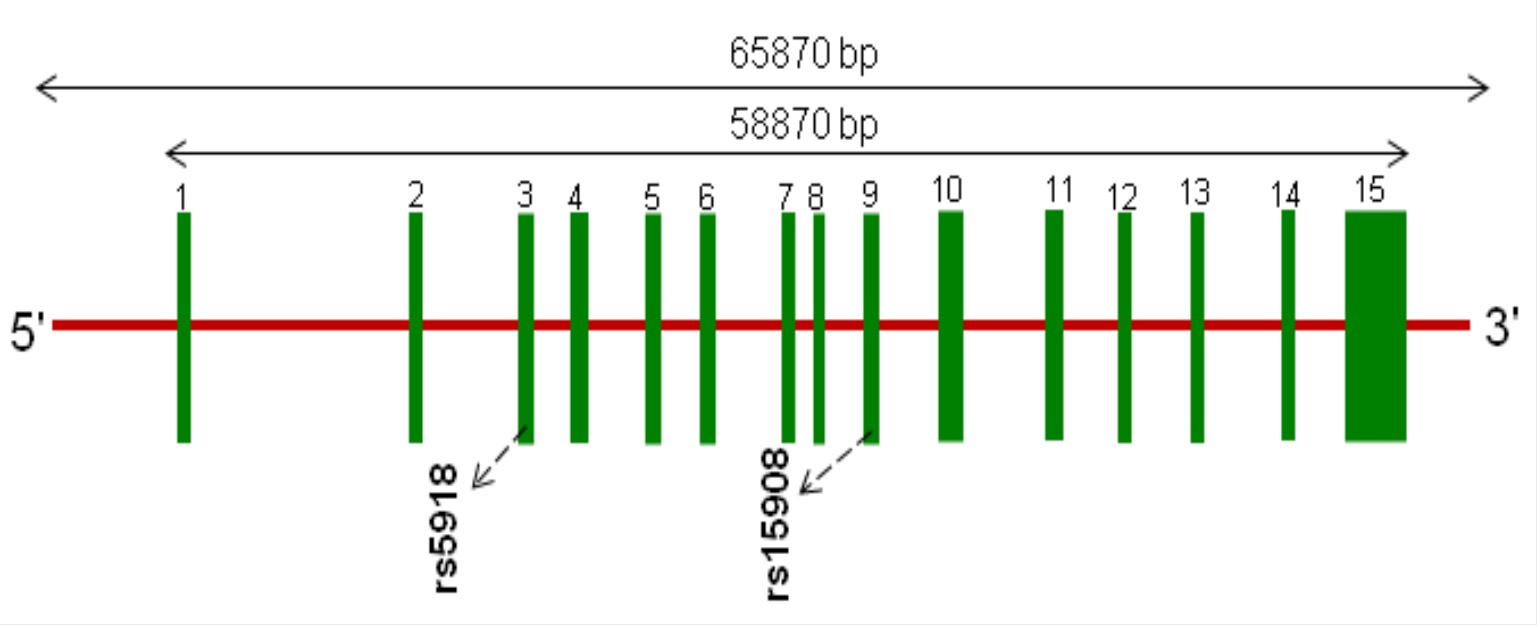


Figure 2. Diagrammatic representation of *TPH1* showing the location of the investigated SNPs.



Singh AS et al., *PROG NEURO-PSYCHOPH,* 2014

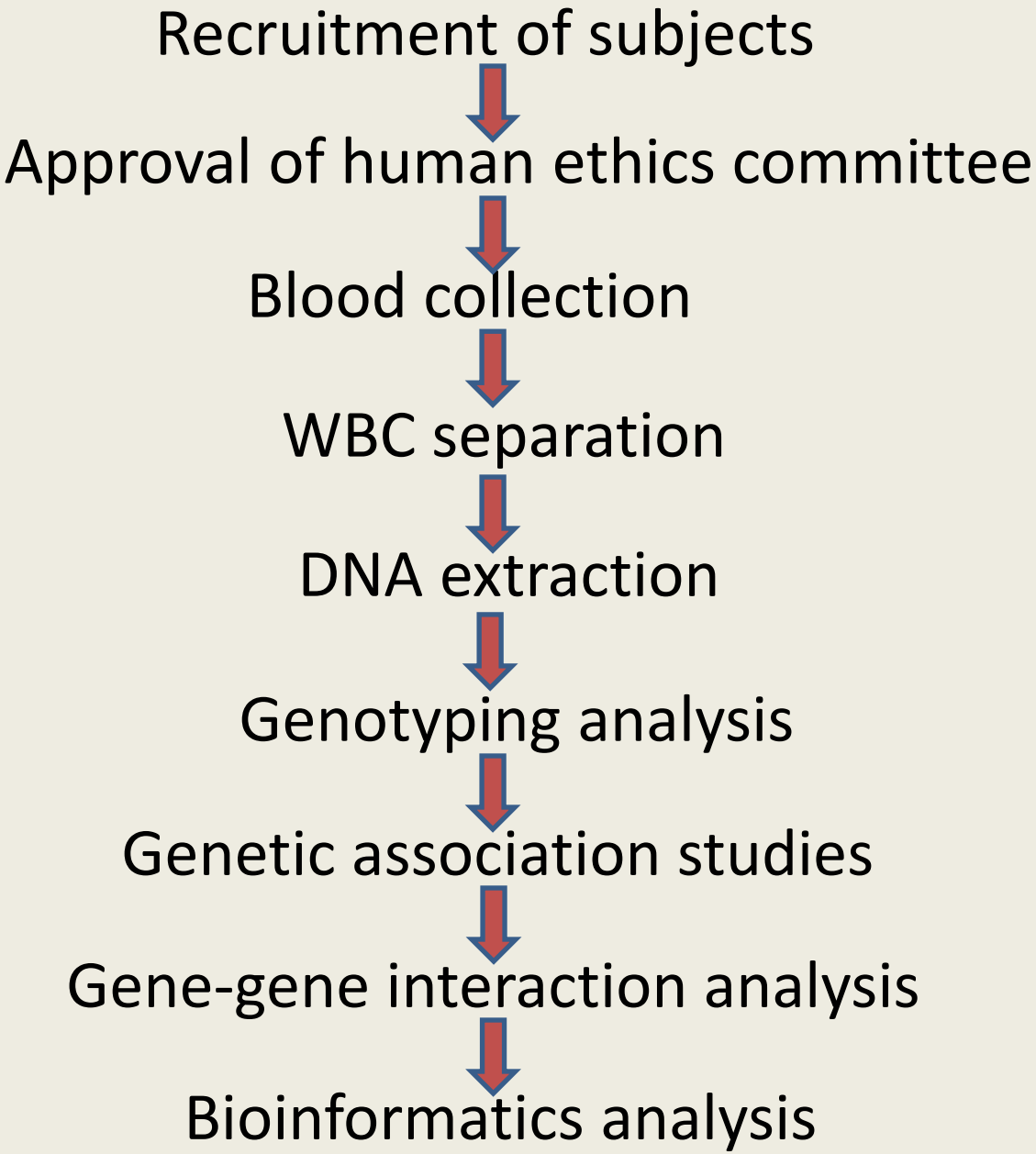
Figure 3. Diagrammatic representation of *TPH2* showing the location of the investigated SNPs.



Singh AS et al., *PROG NEURO-PSYCHOPH,* 2014

Figure 4. Diagrammatic representation of *ITGB3* showing the location of the investigated SNPs.

Study Designs



Results in brief

Table 1. Genotypic and allelic frequencies of *ITGB3* and *TPH2* Markers

SNP ID	Study group	Genotypic frequency			Allelic frequency		HWE χ^2	p_ value
			ITGB3					
rs15908		**CC**	**CA**	**AA**	**C**	**A**		
	Case (n = 139)	0.20	0.58	0.23	0.48	0.52	3.740	0.053
	Parent (n = 260)	0.22	0.48	0.31	0.45	0.55	0.413	0.520
	Control (n = 165)	0.24	0.56	0.21	0.52	0.48	2.112	0.146
rs5918		**TT**	**TC**	**CC**	**T**	**C**		
	Case (n = 139)	0.76	0.23	0.01	0.87	0.13	0.043	0.834
	Parent (n = 260)	0.81	0.18	0.01	0.90	0.10	0.148	0.700
	Control (n = 163)	0.76	0.23	0.01	0.87	0.13	0.141	0.707
			TPH2					
rs4570625		**GG**	**GT**	**TT**	**G**	**T**		
	Case (n = 136)	0.56	0.32	0.13	0.72	0.28	**6.892**	**0.008**
	Parent (n = 254)	0.48	0.45	0.07	0.71	0.29	2.068	0.150
	Control (n = 129)	0.57	0.36	0.07	0.75	0.25	0.299	0.584
rs11179000		**AA**	**AT**	**TT**	**A**	**T**		
	Case (n = 136)	0.46	0.38	0.15	0.65	0.35	3.416	0.064
	Parent (n = 252)	0.45	0.44	0.11	0.67	0.33	0.099	0.752
	Control (n = 131)	0.44	0.45	0.11	0.66	0.34	0.000	0.998
rs11179001		**GG**	**GA**	**AA**	**G**	**A**		
	Case (n = 136)	0.68	0.30	0.03	0.82	0.18	0.009	0.923
	Parent (n = 252)	0.71	0.25	0.04	0.84	0.16	2.208	0.137
	Control (n = 130)	0.68	0.30	0.02	0.83	0.17	0.256	0.612
rs4290270		**TT**	**TA**	**AA**	**T**	**A**		
	Case (n = 132)	0.33	0.46	0.20	0.56	0.44	0.540	0.462
	Parent (n = 245)	0.31	0.48	0.21	0.55	0.45	0.201	0.653
	Control (n = 125)	0.31	0.48	0.21	0.55	0.45	0.140	0.707
rs7305115		**GG**	**GA**	**AA**	**G**	**A**		
	Case (n = 131)	0.33	0.44	0.24	0.55	0.45	2.087	0.148
	Parent (n = 243)	0.29	0.51	0.21	0.54	0.46	0.071	0.788
	Control (n = 159)	0.25	0.51	0.24	0.51	0.49	0.040	0.841

Table 2. Population wise data on the allele frequencies obtained from the HapMap dbSNP database and present study population from India

Population	*TPH2*										*ITGB3*			
	rs4570625		rs11179000		rs11179001		rs7305115		rs4290270		rs15908		rs5918	
	G	**T**	**A**	**T**	**G**	**A**	**A**	**G**	**A**	**T**	**C**	**A**	**C**	**T**
ASW	0.675	0.325	NA	NA	0.868	0.132	0.360	0.640	NA	NA	0.342	0.658	0.123	0.877
CEU	0.792	0.208	0.784	0.216	0.912	0.088	0.357	0.643	0.285	0.715	0.336	0.664	0.137	0.863
CHB	0.482	0.518	0.488	0.512	0.836	0.164	0.478	0.522	0.511	0.489	0.554	0.456	0.007	0.993
CHD	0.445	0.555	NA	NA	0.839	0.161	0.583	0.417	NA	NA	0.628	0.372	0.005	0.995
GIH	0.782	0.218	NA	NA	0.851	0.149	0.460	0.540	NA	NA	0.431	0.569	0.089	0.911
JPT	0.504	0.496	0.456	0.544	0.894	0.106	0.434	0.566	0.411	0.589	0.504	0.496	0.011	0.989
LWK	0.560	0.440	NA	NA	0.873	0.127	0.514	0.486	NA	NA	0.545	0.455	0.145	0.855
MEX	0.675	0.325	NA	NA	0.836	0.164	0.509	0.491	NA	NA	0.379	0.621	0.112	0.888
MKK	0.580	0.420	NA	NA	0.939	0.061	0.478	0.522	NA	NA	0.641	0.359	0.173	0.827
TSI	0.750	0.250	NA	NA	0.911	0.089	0.475	0.525	NA	NA	0.426	0.574	0.162	0.838
YRI	0.619	0.381	0.548	0.452	0.894	0.106	0.337	0.663	0.587	0.413	0.493	0.507	0.128	0.872
Study from INDIA	**0.750**	**0.250**	**0.660**	**0.340**	**0.830**	**0.170**	**0.550**	**0.450**	**0.510**	**0.490**	**0.520**	**0.480**	**0.130**	**0.870**

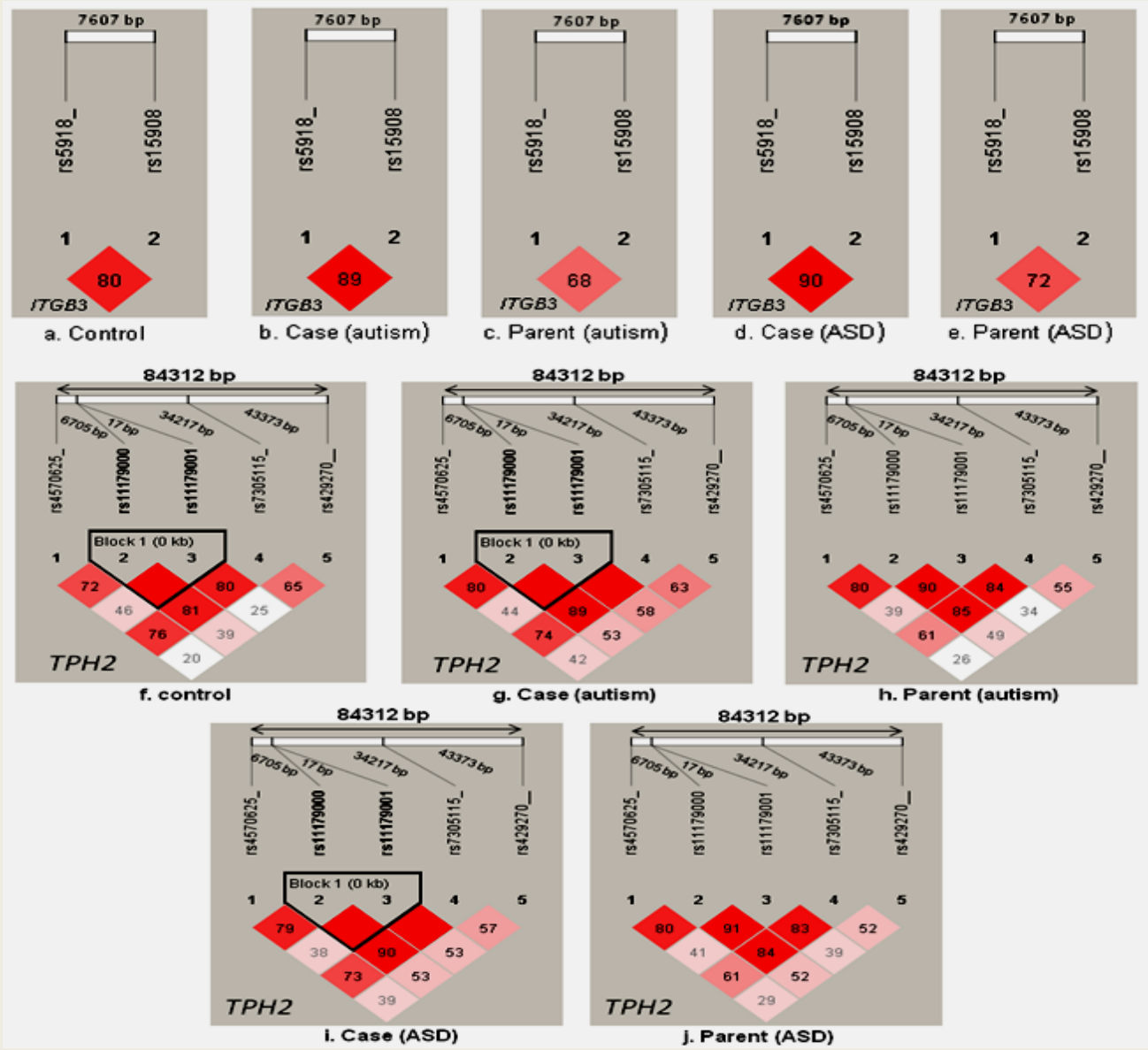


Figure 5. Linkage analysis.

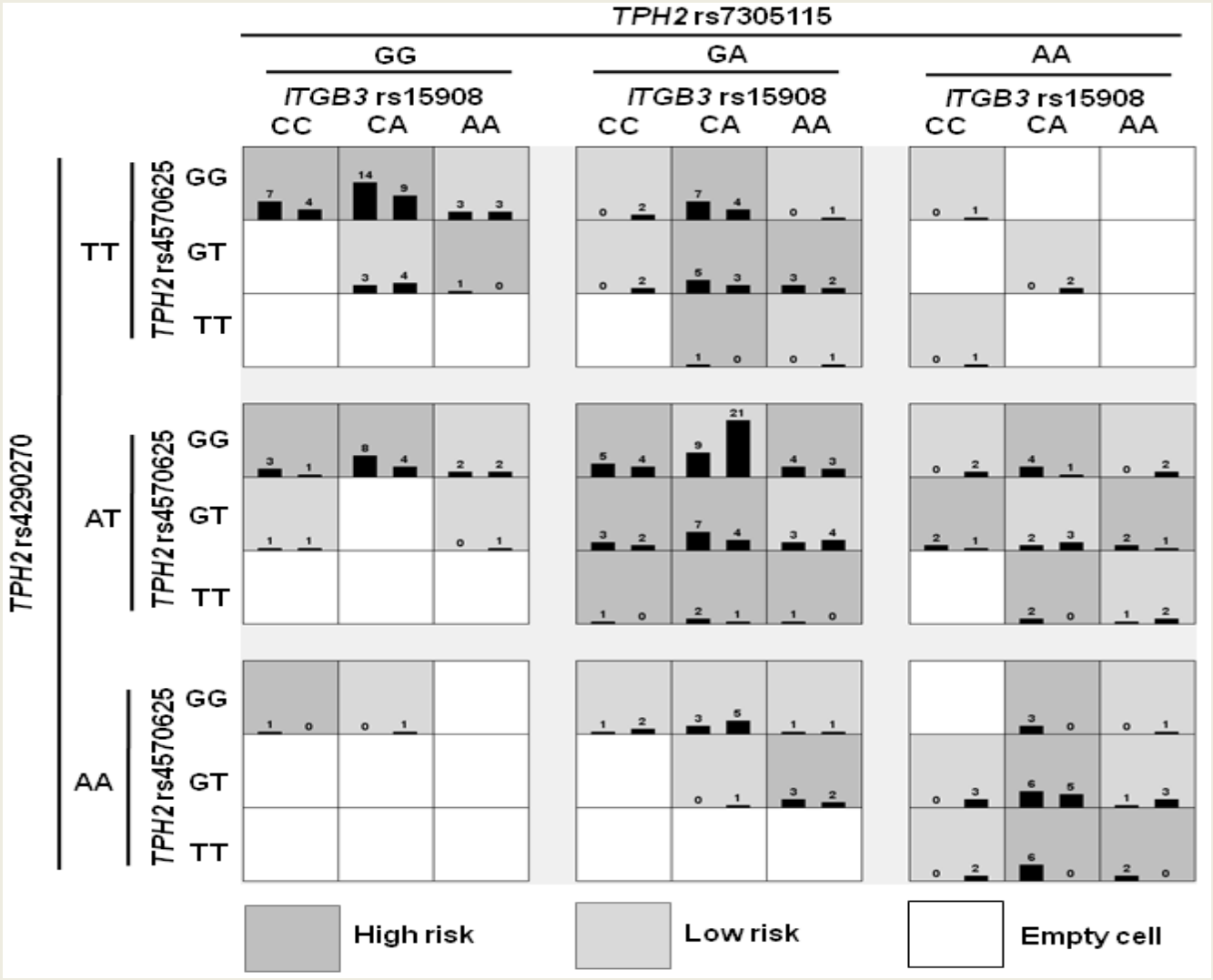


Figure 6. Multifactor dimensionality reduction test for case control

Table 3. MDR-phenomics analysis for *TPH1* and *TPH2* interaction using all trio families

Marker combination	HR		LR		M (Fix P)
	T	NT	T	NT	
rs4537731-rs211106	58	31	67	88	**37.1 (0.026)**
rs623580-rs10488682	80	51	48	75	**25.5 (0.043)**
rs623580- rs4570625	54	30	71	93	**31.0 (0.024)**
rs623580- rs4290270	78	47	50	78	**22.4 (0.050)**
rs623580-rs1799913- rs4570625	69	35	56	88	**52.7 (0.026)**
rs623580- rs1799913- rs4290270	69	32	57	90	**45.7 (0.041)**

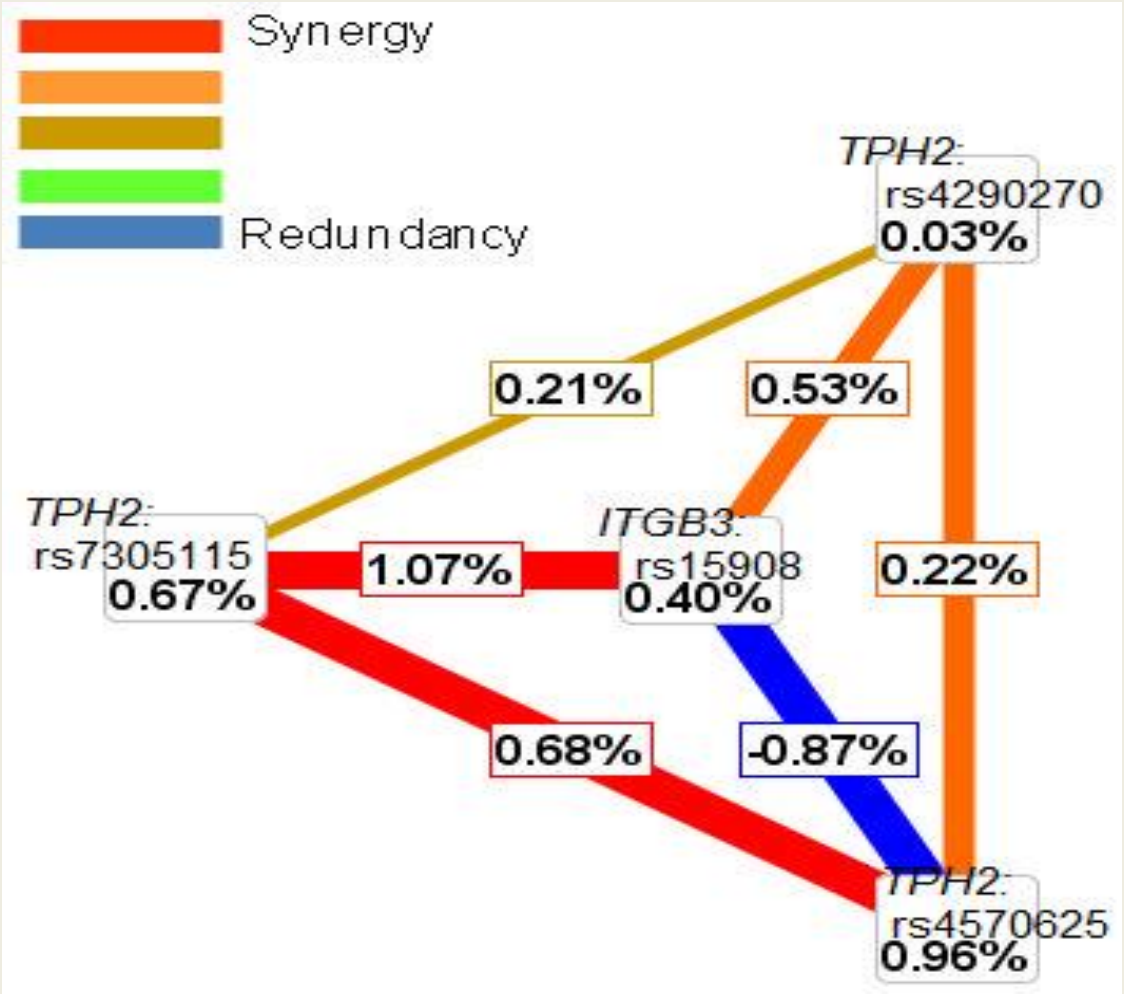


Figure 7. Radial diagram showing genetic interaction pattern between the SNPs of the genes.

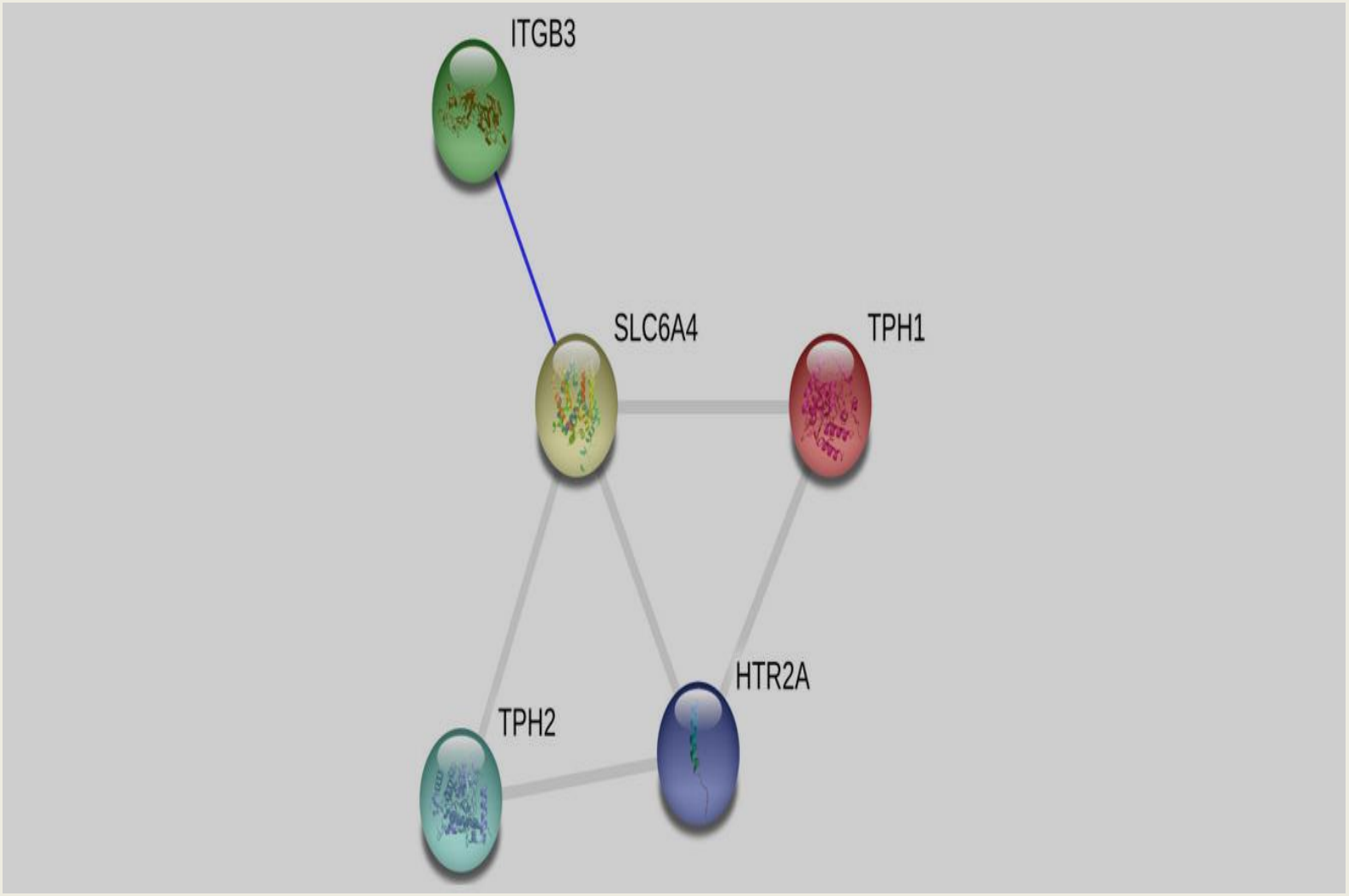


Figure 8. Bioinformatic analysis for protein-protein interaction

Table 4. Interacting proteins with the text mining scores resulted by online database resource Search Tool “STRING”

Interacting Proteins	Text mining score
SLC6A4 and TPH1	0.956
TPH2 and SLC6A4	0.889
HTR2A and TPH2	0.908
HTR2A and TPH1	0.963
HTR2A and SLC6A4	0.926
ITGB3 and SLC6A4	0.960

Table 5. Transcription factor and transcription factor binding site changes due to single nucleotide polymorphism

SNP ID	Alleles	TFB sites	TF	Score
rs4570625	G	TA**G**AATA ←	CdxA	85.7
	T	AATTA**T**A →	CdxA	98.6
		TA**T**AATA ←	CdxA	91.4
		TA**T**AATA →	CdxA	91.4
rs11179000	A	AC**A**CGTGT →	USF	94.1
		AC**A**CGTGT ←	USF	94.1
		GTAC**A**CGTGTTG →	N-Myc	90.8
		TGTAC**A**CGTGTTGA ←	USF	88.2
		TGTAC**A**CGTGTTGA →	USF	88.2
		GTAC**A**CGTGTTG ←	N-Myc	87.6
		GTAC**A**CGTGTTG →	c-Myc	86.8
		GTAC**A**CGTGTTG ←	MyoD	86.0
	T	NA	NA	NA
rs11179001	A	ACTGGAAGC**A** →	c-Ets-	90.2
	G	ACTGGAAGC**G** →	c-Ets-	89.2
		ACTGGAAGC**G** →	NRF-2	86.0

Summary of the result

- LD variation pattern was observed between the SNPs of case, control and parents.
- Pair-wise haplotype analysis showed under-expression of *TPH2.*
- Pair-wise haplotype analysis reveal over-transmission of*TPH1.*
- MDR and MDRP showed significant interaction of *TPH1* & *TPH2*
- Indirect interaction of TPH1,TPH2 and ITGB3 was shown by bioinformatic analysis; and the SNP markers were shown to have functional (from low to moderate) role in gene regulation.

Conclusion

- The present study using genetic association and gene-gene interaction analyses of *TPH1*, *TPH2* and *ITGB3* variants support the serotonergic abnormality in ASD.

- The three genes are likely involve in the etiology of the disorder.

- However further studies using cellular and animal models are required to validate the finding which may provide some important information in the disease pathophysiology and in the future therapeutic development of ASD.