

## Combined effect of polymorphic sites in the DTNBP1 and GRIN1 genes on schizophrenia

Hamid Galehdari,<sup>1</sup> PhD, Tahereh Ajam<sup>2</sup>, MSc., Atefeh Pooryasin<sup>3</sup>, MSc.  
Ali Mohammad Foroughmand,<sup>4</sup> PhD, Seyed Reza Kazeminejad,<sup>5</sup> PhD.

*Dept of Genetics, College of Sciences, Shahid Chamran University, Ahvaz, Iran.*

### Abstract

**Background:** Schizophrenia is a severe mental disorder and numerous genes and loci are believed to be involved in this disease. Recent studies have reported a strong genetic association between DTNBP1 (dystrobrevin-binding protein 1) gene variants and schizophrenia.

**Methods:** In this research, we used a case-control study to establish the possible association between the P1635 (rs3213207) polymorphism in the DTNBP1 gene and schizophrenia in an Iranian cohort of 200 unrelated patients and 200 controls. The allele and genotype frequencies of the polymorphism in the two groups were determined using PCR-RFLP and the data analyzed using logistic regression and Mantel-Haenszel chi-square tests.

**Results:** The additive effect of the P1635 polymorphism in DTNBP1 and the previously reported G1001C polymorphism in the GRIN1 gene were investigated. Analysis of data revealed a strong association between the P1635 polymorphism and schizophrenia (AG genotype: OR=0.39, 95% CI= 0.24-0.62, P<0.001).

**Conclusion:** Our results indicated that the coexistence of the A and C alleles from the two polymorphisms, P1635 and G1001C, increase the risk for schizophrenia.

**Keywords:** schizophrenia, DTNBP1 gene, dysbindin, NMDA receptor, GRIN1 gene, association study.

### Introduction

Schizophrenia is a serious psychotic disorder affecting 1% of general population. Family, twin and adoption studies have shown that schizophrenia has a predominant genetic basis with a high heritability [1,2]. The DTNBP1 gene, encoding dysbindin (dystrobrevin-binding protein 1), is widely expressed in the human brain and appears to play an important role in cognitive function and memory [3]. This gene is located at 6p22.3, a chromosomal region where positive linkage for schizophrenia has

been reported [4]. Dysbindin is 40 kD coiled coil-containing protein that binds to  $\gamma$ -dystrobrevin, a component of the dystrophin glycoprotein complex [5]. The dystrophin complexes are found in postsynaptic terminals in a number of brain areas [5,6]. Although dysbindin function is largely unknown, its localization has suggested that genetic variations in DTNBP1 might confer higher risk of schizophrenia via changes in postsynaptic structure and function [7].

Dysbindin functions in a soluble 200 kDa protein complex and is involved in protein traf-

1. **Corresponding author**, Associate Professor, Dept of Genetics, College of Sciences, Shahid Chamran University, Ahvaz, Iran. Fax: +98611 3338965, Tel: +98611 3338965, Email: galehdari187@yahoo.com.

2. MSc Student, Dept of Genetics, College of Sciences, Shahid Chamran University, Ahvaz, Iran.

3. MSc Student, Dept of Genetics, College of Sciences, Shahid Chamran University, Ahvaz, Iran.

4. Associated Professor, Dept of Genetics, College of Sciences, Shahid Chamran University, Ahvaz, Iran.

5. Assistant Professor, Dept of Genetics, College of Sciences, Shahid Chamran University, Ahvaz, Iran.

ficking and in the biogenesis of lysosome-related organelles complex-1 (BLOC-1) [8]. Disturbances of BLOC-1 function affect intracellular glutamate vesicle trafficking and reduce glutamate release [9-11]

The DTNBP1 gene is considered as one of the best validated candidate genes affecting schizophrenic diseases, and variation in this gene affects mRNA processing or expression [12]. Further studies have identified reduced dysbindin levels in the brain of individuals with schizophrenia [13]. Numerous polymorphisms have been identified in the DTNBP1 gene, and remarkably, most variations lie within introns [14]. Some investigations involving different populations have found a positive association between DTNBP1 and developing schizophrenia [7,9,15-21].

Another important gene in the etiology of schizophrenia is GRIN1, which encodes the NR1 subunit of the N-methyl-D-aspartate (NMDA) receptor. The NMDA receptor, a member of the family of ionotropic glutamate receptors, functions as a glutamate-gated cation channel [22]. Several lines of evidence have implicated NMDA receptors, especially the NR1 gene (GRIN1), as being involved with susceptibility for schizophrenia [23-27].

Recently, we reported a significant association between the GRIN1 G1001C polymorphism and the development of schizophrenia in an Iranian ethnic group [28]. In the present study, we investigated the effect of the P1635 polymorphism in DTNBP1 with risk for schizophrenia only and in combination with the GRIN1 G1001C polymorphism in a case-control study.

## Methods

**Subject:** For this case-control study, 200 unrelated patients with a mean age of 43.34 years (SD= 11.353) were recruited from hospitals in south and southwestern Iran. Of these, 117 were men (63 and 54 from the south and southwest, respectively) and 83 women (48 and 35 from

the south and southwest, respectively). The diagnosis of schizophrenia was based on DSM IV criteria. The control group consisted of 200 healthy blood donors with a mean age of 39.43 years (SD= 11.103), which were matched for gender and ethnicity to the patient cohort. Informed consent was obtained from all participants.

**Genotyping assay:** Genomic DNA was extracted from whole blood using the standard salting out method. The P1635 polymorphism in the DTNBP1 gene was screened using the PCR-RFLP method. Primer sequences were designed to amplify a 240 bp fragment using the publicly available Gene Fisher software (www.GeneFisher.com) and the ENSG00 000047579 gene sequence (www.Ensembl.org). The PCR reactions were carried out in 25µl, containing 10ng genomic DNA, 10mM Tris-HCl (pH:8.3), 50mM KCl, 2mM MgCl<sub>2</sub>, 200µM dNTPs, 0.5pmol each primer (for: 5-GCAGACCATG-TATTTGAAAAGC-3, rev: 5-GC CAGTTTC-CTCAAATTCC-3) and 0.25 units Taq DNA polymerase. Amplification conditions were as follows: denaturation at 94°C for 5 min, followed by 35 cycles at 95°C for 30 sec, 56°C for 30 sec, 72°C for 30 sec, and a final extension at 72°C for 5 min. PCR products were digested overnight at 65°C with 1 unit of BsrI restriction endonuclease (Biolabs), which cuts only the G allele, and final fragments subsequently separated on 12% polyacrylamide gel electrophoresis. Digestion specificity confirmed by retesting 20% of the samples, and no discrepancies were detected upon the testing replicate. The genotyping of the G1001C polymorphism has been previously described in detail [28].

**Statistical analysis:** The chi-square test was used to compare genotypes between the gender groups and determine genotypic frequencies. The association between P1635 genotypes and development of schizophrenia was examined using odd ratios (ORs) and 95% confidence intervals (CIs). The chi-square tests, ORs and their CIs were calculated using the SPSS 13 sta-

tistical package. Because the genotypic frequencies were different in the two provinces, Mantel-Haenszel weighted-odd ratios and chi-square tests were performed to analyse the entire sample cohort. EpiInfo 6 statistical software was used to carry out Mantel-Haenszel tests. Odd ratios and 95% CIs were calculated to compare possible pairs of genotype combination with additive effects of the GRIN1 G1001C and the DTNBP1 P1635 polymorphisms. Probabilities  $<0.05$  were considered statistically significant, and all P values had two-tailed.

### Results and Discussion

Study of socio-demographic features in this cohort identified that levels of marital status and education were significantly lower for the case group than the control group ( $P < 0.05$ ).

Control and patient groups were initially classified according to gender and geographical region. Gender groups were pooled, since statistical difference between their genotypic frequencies ( $P > 0.05$ ) did not exist. We analyzed samples from the south and southwest of Iran separately due to variation in the allelic and genotypic frequencies ( $P < 0.05$ ). The genotyping assay for the P1635 polymorphism did not identify the GG genotype in any of our samples (detailed results in table 1). Heterozygosity for the A allele was associated with decreased risk of schizophrenia (OR= 0.270, 95% CI= 0.48-0.52,  $P < 0.001$ ) in patients from the south. This was not the case for individuals from southwest Iran, for which we identified no association between heterozygosity of the A allele and schizophrenia ( $P > 0.05$ ). To increase statistical power, we analyzed the entire cohort from both regions using Mantel-Haenszel testing methods which have indicated that heterozygosity for the A allele was associated with decreased risk of schizophrenia (OR= 0.39, 95% CI= 0.24-0.62,  $P < 0.001$ ). These analyses also identified a significant linear trend in risk associated with one and two A alleles ( $P < 0.001$ ).

Since GRIN1 has also been shown in the previous report (Galehdari, 2009) to play a role in the development of schizophrenia in the Iranian population, but in only a small subgroup, we were interested to test if the combination of polymorphisms in the DTNBP1 and GRIN1 genes had a larger impact on our study cohort. The distribution of compound genotypes indicated a significant difference between controls and patients ( $\chi^2 = 41.064$ ,  $df = 5$ ,  $P < 0.001$ ). Table 2 shows the frequencies of the compound genotypes within the groups of controls and patients. Very few patients were identified in our cohort who had the CC genotype of the G1001C polymorphism. In order to increase statistical robustness for analysis of polymorphisms in the GRIN1 gene, we combined patient having the CC and the GC genotypes into one group. We also observed a significant trend for increasing risk with increasing number of one, two and three risk alleles ( $P < 0.001$ ). Our results indicated that coexistence of the A and C alleles from the P1635 and G1001C polymorphisms increased the risk of developing schizophrenia.

### Conclusion

The molecular mechanisms by which DTNBP1 acts in the brain, and their functional consequences in subjects with variants in the DTNBP1 gene are not completely understood. Nevertheless, studies on the brains of schizophrenic patients, post-mortem have shown low dysbindin expression at both the protein and mRNA levels in some areas of the schizophrenic patient's brains [10,13]. Decreased dysbindin protein and glutamate transmission have been reported in rats stably inducible expressing an shRNA for the DTNBP1 gene [9]. Taken together, these pieces of evidence support that this gene may play an important role in developing schizophrenia through the glutamatergic pathway. Our results provided statistical support for DTNBP1 as a susceptibility gene for schizophrenia, and indicated the A allele in the

	Controls	Cases
Allelic frequencies		
<i>South</i>		
<b>A</b>	0.73	0.88
<b>G</b>	0.27	0.12
<i>Southwest</i>		
<b>A</b>	0.86	0.89
<b>G</b>	0.14	0.11
Genotypic distributions		
<i>South</i>		
<b>AA</b>	52	85
<b>AG</b>	59	26
<i>Southwest</i>		
<b>AA</b>	65	71
<b>AG</b>	24	18

Table 1. Allelic frequencies and genotypic distribution of the P1635 polymorphism in DTNBP1 gene.

P1635 polymorphism is significantly associated with increased risk of developing schizophrenia ( $P < 0.001$ ). Consequently, the G allele might possess a protective role against schizophrenia development. Because P1635 is located in intron 4 of the DTNBP1 gene, it might influence DTNBP1 expression in a cis-acting manner [29]. Other reports have demonstrated this SNP is associated with the outcome of schizophrenia patients. Several studies have considered the A allele as a risk factor for schizophrenia [16,17,19], while other researchers have postulated the G allele to be linked with increasing risk for the disorder [7,9, 18]. Moreover, some independent studies have shown no association between this variant and schizophrenia [15,20,21,30]. These discrepancies in the reported results may be due to the genetic heterogeneity and different allelic frequencies in various ethnic populations. Additionally, interactions of the DTNBP1 gene with other

genes or even with environmental factors may influence the role it plays in regard to the development on schizophrenia [19]. The frequencies of DTNBP1 genotypes observed in our study deviate from those expected under Hardy-Weinberg equilibrium for controls and schizophrenia patients. As has been reported in previous works [9,15,20,30,31], the frequency of the GG genotype for p1635 polymorphic site is rare in different populations. Hence, the absence of the GG genotype, as we showed here for both controls and schizophrenic patients, is not surprising. The case-control study presented here is the first to be conducted in an Iranian population, and supports an association between the p1635 polymorphism and schizophrenia. The different results for individuals from the south and southwest regions may be due to population admixture [31]

In addition to investigating the association of the P1635 polymorphism in the DTNBP1 gene

	Controls			Cases			
	<i>GRIN1</i>	GG	GC	CC	GG	GC	CC
<b>DTNBP1</b>	AA	54	63	0	37	107	11

Table 2. Distribution of the combined G1001C and P1635 genotypes within the case and control groups.

Number of risk alleles	Genotype combinations	OR	(95% CI)	P value
1	AG/GG	1		
2	AA/GG or AG/GC	1.991	0.885- 4.477	0.096
3	AA/GC	5.571	2.468- 12.577	<0.001

Table 3. Odds ratios (ORs) and 95% confidence intervals (95% CI) calculated for each number of risk alleles of the G1001C and P1635 genotypes.

and schizophrenia, we analyzed the combined effect of P1635 and the recently published data for the influence of the G1001C polymorphisms on the risk of schizophrenia development [28]. In fact, the GRIN1 and DTNBP1 genes both appear to exert their influences through the glutamatergic pathway [10,12]. Dysbindin affects intracellular trafficking of glutamate vesicles and glutamate release in presynaptic terminals. NR1 is the main subunit in NMDA receptor which works as a glutamate-gated cation channel and plays crucial role in excitatory synaptic transmission [10,22]. Furthermore, dysbindin binds the dystrophin-associated protein complex (DPC) in the brain. Since DPC is concentrated at the post-synaptic density (PSD), dysbindin is thought to be involved in one or more PSD functions, which include trafficking and tethering of NMDA receptors [9,12]. In summary, we suggest that the coexistence of risk alleles in the two polymorphisms may affect signal transduction in an additive manner. The results we present here provide evidence that these two genes may be important in the etiology of schizophrenia, at least in the Iranian population.

## References

1. Cardno A.G., Gottesman I.I. Twin studies of schizophrenia: from bow-and-arrow concordances to Star Wars Mx and functional genomics. *Am J Med Genet* 2000; 97:12-17.
2. McGuffin P, Tandon K, Corsico A. Linkage and association studies of schizophrenia. *Curr Psychiatry Rep* 2003; 52: 121-127.
3. Owen M.J., Williams N.M., O'Donovan M.C. The molecular genetics of schizophrenia: new findings promise new insights. *Mol Psychiatry* 2004; 9: 14-27.
4. Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, and et al. Genome scan metaanalysis of schizophrenia and bipolar disorder, part II: schizophrenia. *Am. J. Hum. Genet* 2003;73:34-48.
5. Benson M.A., Newey S.E., Martin-Rendon E., Hawkes R., Blake D.J. Dysbindin, a novel coiled-coil-containing protein that interacts with the dystrobrevins in muscle and brain. *JBC* 2001;276: 24232-24241.
6. Blake DJ., Hawkes R., Benson M.A., Beesley P.W. Different dystrophin-like complexes are expressed in neurons and glia. *JCB* 1999; 147: 645-657.
7. Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV and et al. Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. *Am. J. Hum. Genet* 2002; 71: 337-348.
8. Ciciotte SL, Moriyama K, Gwynn B, Huizing MA, Gahl WA, Bonifacino JS, and et al. Cappuccino, a mouse model of Hermansky-Pudlak syndrome, encodes a novel protein that is part of the pallidin-muted complex (BLOC-1). *Blood* 2003; 101: 4402- 4407.
9. Numakawa T, Yagasaki Y, Ishimoto T, Okada T, Suzuki T, Iwata N, and et al. Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. *Hum. Mol. Genet* 2004; 13: 2699-2708.
10. Talbot K., Eidem W.L., Tinsley C.L., Benson M.A., Thompson E.W., Smith R.J. Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. *J. Clin. Invest* 2004;113: 1353-1363.
11. Derek W, Kevin M, Naiamh K. Dysbindin (DTNBP1) and the Biogenesis of lysosome-Related organelles complex1 (BLOC-1): Main and Epistatic Gene Effects Are Potential Contributors to schizophrenia susceptibility. *Biol Psychiatry* 2007;82: 124-129.
12. Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol Psychiatry* 2005; 10: 40-68.
13. Weickert CS, Straub RE, McClintock BW. Human

- dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. *Arch Gen Psychiatry* 2004; 61: 544-55.
14. Liao h, Chen C. Mutation analysis of the human dystrobrevin-binding protein1 gene in schizophrenic patients. *Schizophr Res* 2004; 71: 185-189.
15. D Morris, K McGhee, S Schwaiger, P Scully, J.Quinn, D.Meagher and et al. No evidence for association of the dysbindin gene [DTNBP1] with scizophrenia in an Irish population-based study. *Schizophr Res* 2003;60: 167-172.
16. Schwab SG, Knapp M, Mondabon S, Hallmayer J, Borrmann-Hassenbach M, Albus M. et al. Support for association of schizophrenia sample of triad families. *Am. J. Hum. Genet* 2003; 72: 185-190.
17. Tang JX, Zhou J, Fan JB, Li XW, Shi YY, Gu NF, and et al. Family-based association study of DTNBP1 in 6P22.3 and schizophrenia. *Mol Psychiatry* 2003; 8: 717-718.
18. Van den Oord E, Sullivan PF, Jiang Y P, Walsh D, O'Neill FA, Kendler KS et al. Identification of a high risk haplotype for the dystrobrevin binding protein 1 (DTNBP1) gene in the Irish study of high density schizophrenia families. *Mol Psychiatry* 2003; 8: 499-510.
19. Kirov G, Ivanov D, Williams NM, Preece A, Nikolov I, Milev R, and et al. Strong evidence for association between the dystrobrevin binding protein 1 gene (DTNBP1) and schizophrenia in 488 parent-offspring trios from Bulgaria. *Biol Psychiatry* 2004; 55: 971-975.
20. Williams NM, Preece A, Morris DW, Spurlock G, Bray NJ, Stephens M. and et al. Identification in 2 independent samples of a novel schizophrenia risk haplotype of the dystrobrevin binding protein gene (DTNBP1). *Arch Gen Psychiatry* 2004; 61: 336-344.
21. Li T, Zhang F, Liu X, Sun X, Sham PC, Crombie C and et al. Identifying potential risk haplotypes for schizophrenia at the DTNBP1 locus in Han Chinese and Scottish populations. *Mol Psychiatry* 2005; 10: 1037-1044.
22. Hollmann M., Heinemann S. Cloned glutamate receptors. *Annu Rev Neurosci* 1994; 17: 31-108.
23. Javitt DC, Zukin SR. Recent advances in the phenocyclidine model of schizophrenia. *Am J Psychiat* 1991; 148: 1301-1308.
24. Meador-Woodruff JH, Healy DJ. Glutamate Receptor Expression in Schizophrenic Brain. *Behav. Brain Res* 2000; 31:288-294.
25. Mohn AR, Gainetdinov A, Caron V, Koller BH. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell* 1999; 20: 427-436.
26. Dracheva S, Marras SAE, Elhakem V, Kramer FR, Davis KL. Haroutunian VN-methyle-D-aspartic acid receptor expression in dorsolateral prefrontal cortex of elderly patients with schizophrenia. *Am J Psychiat* 2001; 158: 1400-1410.
27. Karasawa J, Hashimoto K, Chali SH. D-Serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. *Behav. Brain Res* 2008; 186:78-83.
28. Galehdari H, Pooryasin A, Foroughmand AM, Daneshmand S, Saadat M. Association between the G1001C polymorphism in the GRIN1 gene promoter and schizophrenia in the Iranian population. *J Mol Neurosci*. 2009; 38: 178-181.
29. Bray NJ, Buckland PR, Owen MJ, O'Donovan MC. Cis-Acting variation in the expression of a high proportion of genes in human brain. *Hum Genet* 2003; 113: 149-153.
30. Joo JE, Lee K, Jeong S, Ahn YM, Koo YJ, Kim YS. The dysbindin gen (DTNBP1) and scizophrenia: NO support for an association in the Korean population. *Neurosci Lett* 2006; 407: 101-106.
31. Datta SR, McQuillin A, Puri V, Choudhury K, Thirumalai S, Lawrence J and et al. Failure to confirm allelic and haplotypic association between markers at the chromosome 6p22.3 dystrobrevin-binding protein 1 (DTNBP1) locus and schizophrenia. *Behav Brain Funct* 2007;3: 50.