A missense mutation in the ITGA8 gene, a cell adhesion molecule gene, is associated with schizophrenia in Japanese female patients

IRWAN SUPRIYANTO

(Degree)
博士（医学）

(Date of Degree)
2013-09-25

(Date of Publication)
2014-09-01

(Resource Type)
doctoral thesis

(Report Number)
甲第5950号

(URL)
https://hdl.handle.net/20.500.14094/D1005950

A missense mutation in the ITGA8 gene, a cell adhesion molecule gene, is associated with schizophrenia in Japanese female patients

細胞接着分子遺伝子である ITGA8 遺伝子のミスセンス変異と統合失調症の日本人女性患者との相関研究

Irwan Supriyanto、渡部 雄一郎、毛利 健太朗、白岩 恭一、Woraphat Ratta-Apha、
吉田 正邦、田宮 弦樹、笹田 徹、江口 典臣、岡崎 賢志、白川 治、
染矢 俊幸、菱本 明豊

神戸大学大学院医学研究科医科学専攻
精神医学
（指導教員：曽良 一郎 教授）

Irwan Supriyanto

Key words: Association study, cell adhesion molecule, integrin, neurofascin, schizophrenia

A missense mutation in the *ITGA8* gene, a cell adhesion molecule gene, is associated with schizophrenia in Japanese female patients

Irwan Supriyanto [a], Yuichiro Watanabe [b], Kentaro Mouri [a], Kyoichi Shiroiwa [a], Woraphat Ratta-Apha [a], Masakuni Yoshida [a], Genki Tamiya [a], Toru Sasada [a], Noriomi Eguchi [a], Kenji Okazaki [a], Osamu Shirakawa [c], Toshiyuki Someya [b], Akitoyo Hishimoto [a] *,

[a] Department of Psychiatry, Kobe University Graduate School of Medicine, Kobe, Japan

[b] Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

[c] Department of Neuropsychiatry, Kinki University School of Medicine, Osaka, Japan

*Corresponding author. Department of Psychiatry, Faculty of Medical Science, Kobe University Graduate School of Medicine,

7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Tel.: + 81 78 382 6065; fax: + 81 78 382 6079.

E-mail address: hishipon@med.kobe-u.ac.jp (A. Hishimoto)

Abstract

Background: Cell adhesion molecules (CAMs) play pivotal role in the development of the central nervous system (CNS) and have also been reported to play role in the pathophysiology of schizophrenia. Missense mutations in the CAMs genes might alter the binding of their ligands, increasing the vulnerability to develop schizophrenia.

Methods: We selected 15 missense single nucleotide polymorphisms (SNPs) in the CAMs genes of the CNS reported in the Kyoto Encyclopedia of Genes and Genomes (KEGG) and examined their association with schizophrenia in 278 patients and 284 control subjects (first batch). We genotyped the positive SNPs in 567 patients and 710 control subjects (second batch) and in 635 patients and 639 control subjects (replication samples).

Results: Genotypic and allelic distributions of rs2298033 in the ITGA8 gene between the schizophrenia and control groups were significantly different in both batches ($p = 0.005$, 0.007, respectively). Gender-based analysis revealed that the allelic and genotypic distributions of rs2298033 in the ITGA8 were significantly different between the schizophrenia and control groups among females in both batches ($p = 0.010$, 0.011 and 0.0086, 0.010, respectively) but not in males. Combine analysis revealed a more significant differences ($p = 0.0032$; 0.0035 and 0.0024; 0.0025, respectively), but not in the replication samples. The significant differences for rs2802808 of the NFASC gene were only observed in the female subgroups of the first batch.

Conclusion: These results suggest that both ITGA8 might have gender-specific roles in the development of schizophrenia. Further replication and functional studies are required to confirm these findings.

Keywords:

Cell adhesion molecules; schizophrenia; integrin; neurofascin; association study

Abbreviations

CAM, cell adhesion molecule; CNS, central nervous system; GWAS, genome wide association study; HWE, Hardy Weinberg equilibrium; LD, linkage disequilibrium; LTP, long term potentiation; SNP, single nucleotide polymorphism

1. Introduction

Schizophrenia is a common disorder caused by both genetic diathesis and environmental factors, but its etiology is still unclear. Thus, diagnosis and treatment of schizophrenia are based only on clinical assessment of symptoms and the course of the disorder. Modern treatments of schizophrenia are far from being able to cure the disorder and just relieve the symptoms. Therefore, understanding the pathophysiological processes underlying schizophrenia is considered to be essential for the development of a reliable treatment for schizophrenia (Gaur et al., 2008).

Changes in groups of molecules such as adhesion molecules, cytoskeletal proteins, neurotrophins, and cell signaling molecules have been observed in the brains of schizophrenia patients (Gaur et al., 2008; Maynard et al., 2001). Moreover, the genetic component of schizophrenia is reported to involve single nucleotide polymorphism (SNPs) that are not distributed randomly across the genome but are distributed across genes that share a common biological function or pathway (Lips et al., 2011). Therefore, rather than focusing on specific susceptibility loci, the study of schizophrenia should be broadened to collections of neuronal phenotypes (Costa et al., 2003), such as cell adhesion molecules (CAMs) in the central nervous system (CNS).

CAMs play an important role in the maintenance and modulation of synaptogenic activity within neuronal circuitries (Giagtzoglou et al., 2009; Kriebel et al., 2011) and have been reported to be important in the pathophysiology of schizophrenia (Lips et al., 2011). They also play an important role in axonal/dendritic growth, synapse formation and plasticity, and neurotransmission (Nakamoto et al., 2004; Moresco et al., 2005; Robertson et al., 2006; Goh et al., 2008; Corvin, 2010; Myers and Gomez, 2011; O'Dushlaine et al., 2011). Schizophrenia is a neurodevelopmental disorder that involves aberrant brain wiring or disconnectivity due to synaptogenic alterations (Maynard et al., 2001; Corvin, 2010; Jones

and Murray, 1991; Honer, 1999; Kirov et al., 2005; Hildebrandt et al., 2009; Chan et al., 2010a). The behavioral disturbances found in schizophrenia involve developmental disorders not only in neurons, but also in glial cells (Jones and Murray, 1991). Specific cell adhesion interactions between neurons, glial cell, and extra cellular matrices are critical for the appropriate migration and placement of cortical neurons (Stanco et al., 2009). Given their role in synaptogenesis, cortical placement, and neurotransmission, CAMs might play a role in the pathophysiology of schizophrenia.

The CAMs pathway reported in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Figure 1) has been reported to be associated with schizophrenia and bipolar disorders susceptibility in genome-wide association study (GWAS) populations (Corvin, 2010; O'Dushlaine et al., 2011). Amino acid changes in these CAMs might affect the protein function and contribute to the risk of developing psychiatric and neurologic disorders. In the present study, we examined the association between missense mutations in CAM genes in the CNS and schizophrenia.

2. Materials and methods

2.1. Subjects

This study was approved by the Ethical Committee for Genetic Studies of Kobe University Graduate School of Medicine and the Ethics Committee of Genetics at the Niigata University School of Medicine. Informed consent was obtained from all participants for this study. All participants were of Japanese descent and were recruited in the Kobe city area or Niigata area, Japan. Kobe sample is consisted of two batches of subjects. The first schizophrenia group consisted of 278 unrelated patients (124 males; mean age ± SD, 48.4 ± 10.5 years; 154 females: 51.7 ± 12.6 years). The first control group consisted of 284 unrelated healthy volunteers (123 males; mean age ± SD, 44.0 ± 1 5. 6 y ears; 161 females:

51.2 ± 15.5 years). There were no significant differences in the gender and age distributions between the schizophrenia and the control groups ($\chi 2 = 0.096$, $p = 0.757$ and $t = 1.793$, $df = 560$, $p = 0.074$; respectively). Two SNPs which showed significant association with schizophrenia in this pilot study were then genotyped in the second batch of subjects consisted of 567 schizophrenic patients (296 males; mean age ± SD, 53.4 ± 13.5 years; 271 females; mean age ± S D, 54.3 ± 15.0 y ears) and 710 controls (334 males; mean age ± SD, 52.5 ± 18.8 years; 376 females; mean age ± SD, 54.3 ± 15.0 years). There were no significant differences in the gender and age distribution between the schizophrenia and the control groups ($\chi 2 = 3.4$, $p = 0.067$ and $t = 0.163$, $df = 1246$, $p = 0. 871$; respectively).

Our two batches of sample are not completely independent. Some patients recruited in the first batch are also recruited in the second batch. Therefore we replicated our experiments in another independent group of samples recruited in Niigata area, Japan. The group consisted of 635 schizophrenic patients (345 males; mean age ± SD, 39.8 ± 13 .4 years; 290 females; mean age ± S D, 39.7 ± 14.5 years) and 639 controls (341 males; mean age ± SD, 36.7 ± 9.5 years; 298 females; mean age ± SD, 40.2 ± 11.8 years). The gender proportion was not significantly different ($\chi 2 = 0 .12$, $p = 0.730$), although the age distribution was slightly different ($t = 2.027$, $df = 1266$, $p = 0.043$).

Psychiatric assessment was conducted in each participant as previously described (Watanabe et al., 2006; Yoshida et al., 2012). In brief, the patients were diagnosed by at least two psychiatrists according to the DSM-IV criteria for schizophrenia on the basis of unstructured interviews and reviews of their medical records. None of the control subjects had present, past or family (up to first degree relatives) histories of psychiatric disorders or substance abuse (excluding nicotine dependence). All control subjects were interviewed and were screened for psychiatric disorders based on an unstructured interview by a psychiatrist.

2.2. SNPs selection and genotyping

First we identified neural adhesion molecule gene in KEGG and consulted NCBI dbSNP (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp) to identify any missense mutations they carried. Among 30 neural adhesion molecule genes identified in KEGG, 14 carried missense mutations according to NCBI dSNP (Supplementary Table 1). We then selected 15 missense mutations with minor allele frequencies of more than 3 % in the Japanese population (based on NCBI dbSNP database) and conducted an association study using our samples of schizophrenic patients and control subjects.

For genotype determination, peripheral blood was drawn from the subjects and the leukocyte DNA was extracted. We used TaqMan assays (Applied Biosystems, Foster City, CA, U.S.A.) for genotyping. We selected pre-designed Taqman SNP genotyping assays from the Applied Biosystems database (http://www.appliedbiosystem.com) for all 15 SNPs examined. Genotyping was performed according to the protocol recommended by manufacturer.

2.3. Data analysis

Genotype distributions were examined for Hardy-Weinberg equilibrium (HWE) and the SNPs were examined for linkage disequilibrium (LD) with Haploview v 4.2 software (Barret et al., 2005) (http://www.broad.mit.edu/mpg/haploview/). Haploview was also used to determine allelic/haplotypic frequencies, as well as an association between SNPs or haplotypes and schizophrenia. Permutation tests based on 10,000 replications were performed to calculate corrected P values of allelic or haplotypic analyses for multiple comparisons by the Haploview software, if necessary. Genotype-based association was tested with Cochran–Armitage test for trend. Odd ratios were calculated with the minor allele regarded as the risk

allele. Statistical significance was defined at $P < 0.05$. Power analysis was calculated with the program PS v2.1.31 (Dupont and Plummer, 1998).

3. Results

A nominally significant difference was observed for both genotypic and allelic distributions of rs2298033 in the ITGA8 gene between the schizophrenia and control groups (Cochran Armitage test for trend Z = 2.8, p = 0.005 and Chi square χ^2 = 7.32, p = 0.007, respectively) (Table 1). Gender based analysis revealed only female population showed a difference (Cochran Armitage test for trend Z = 2.6, p = 0.010 and Chi square χ^2 = 6.54, p = 0.011, respectively; OR = 0.500; 95% CI = 0.292 – 0.857). Gender-based analysis also revealed a significant difference in both genotypic and allelic distributions of rs2802808 in the NFASC gene in the female population (Cochran Armitage test for trend Z = 2.0, p = 0.044 and Chi square χ^2 = 4.5, p = 0.034, respectively) which was not observed in the analyses of all subjects (Table 2). However, the observed differences did not withstand correction for multiple comparisons. The genotypic and allelic distributions of the other 13 SNPs examined were not significantly different between the control and schizophrenia groups, although these negative results might be due to a lack of power in the pilot study. The distributions of all 15 SNPs examined were in HWE for both the schizophrenia and control groups.

In the second batch of subjects, the genotypic and allelic distributions of rs2298033 remained significantly different in both overall subjects (Cochran Armitage test for trend Z = 2.6, p = 0.0086 and Chi square χ^2 = 6.6, p = 0.010, respectively) and the female subgroup (Cochran Armitage test for trend Z = 2.5, p = 0.0115 and Chi square χ^2 = 6.4, p = 0.0114, respectively), but not the genotypic and allelic distributions of rs2802808. The differences withstood correction for multiple comparisons. Although we did not observed any significant

difference in the allelic nor genotypic distributions of the two SNPs in the Niigata sample, the combined analysis yielded in a more significant association between rs2298033 and schizophrenia in both overall subjects (Cochran Armitage test for trend Z = 2.9, p = 0.0032 and Chi square χ^2 = 8.5, p = 0.0035) and the female subgroup (Cochran Armitage test for trend Z = 3.0, p = 0.0024 and Chi square χ^2 = 9.17, p = 0.0025). The differences withstood correction for multiple comparisons. The distributions of these two SNPs were in HWE in both the schizophrenia and control groups (Table 3).

Six of the selected SNPs in the pilot study (rs2652098, rs2287926, rs309559, rs188703, rs160278, and rs160278) were located in the CSPG2 gene. Haplotypes analyses revealed that all but rs2652098 were in tight LD. No significant difference in the distributions of the haplotypes was observed between the schizophrenia and control groups (data not shown).

4. Discussion

Integrins are a superfamily of cell adhesion receptors that bind to extracellular matrix ligands, cell-surface ligands, and soluble ligands (Takada et al., 2007). Integrins have a heterodimeric structure consisting of non-covalently associated α and β subunit. ITGA8 encodes the integrin α8 subunit. The α8 subunit in the rat brain was found predominantly in neurons and was concentrated in the olfactory bulb, hippocampal formation, substantia nigra, ventral tegmental area, and superior olivary complex (Einheber et al., 1996). Its distribution in the human brain has not been investigated.

Integrins have been implicated in the extracellular signaling involved in the development of the nervous system (Bossy et al., 1991; Chan et al., 2007, 2010b; Benoit et al., 2009) and were shown to play pivotal roles in the placement of cortical neurons (Stanco et al., 2009). Integrins interact with a large number of signaling molecules and have been

implicated in the CNS physiology underlying synaptic and behavioral plasticity (Moresco et al., 2005; Chan et al., 2003, 2010b; Chun et al., 2001; Kramar et al., 2002). Several types of memory and long term potentiation (LTP), which are frequently reported to be impaired in psychiatric and cognitive disorders, are reported to be modulated by integrins (Chan et al., 2003, 2007). Amino acid changes due to missense mutations in ITGA8 might alter the binding of integrin-ligand complexes. Takada et al (2007) reported that small variations in the particular structure or charge of a ligand can strongly influence the binding affinity and the capacity of the integrins. These alterations might lead to the development of schizophrenia or protects individual against schizophrenia, depending on the site of the changes and the effect on the binding affinity.

Our results suggest that the amino acid change in rs2298033 of the ITGA8 gene might have a protective role against schizophrenia (Table 1), particularly in females (Tables 2 and 3). Rs2298033 (Ser577Phe) is an intolerant amino acid change that is reported to be deleterious (Ekwa-Ekoka et al., 2004). The change from serine (a hydrophilic amino acid) to phenylalanine (a hydrophobic amino acid) is a non conservative amino acid change, possibly resulting in a change of function of the protein, which appears to increase resistance to schizophrenia.

The NFASC gene encodes neurofascin, an immunoglobulin adhesion molecule localized in the nodes of Ranvier. Neurofascin functions in nerve conduction, particularly in GABAergic synapses. GABAergic innervation in the cortical layer, in which neurofascin might play role (Kriebel et al., 2011), is reduced in schizophrenia (Kriebel et al., 2011; Devaux and Gow, 2008; Pillai-Nair et al., 2005). In our pilot study, rs2802808 of the NFASC gene was associated with schizophrenia in females. However, the association was not significant in the second batch samples (Table 2 and 3).

Predisposition to schizophrenia is thought to be caused by multiple mutations in genes in neural development pathways (Kirov et al., 2005; Hildebrandt et al., 2009; Walsh et al., 2008). Therefore, it is very unlikely that amino acids changes in the ITGA8 and NFASC genes alone will suffice as predisposing factors towards schizophrenia. Mutations in these genes probably act in concert with other risk factors, both genetic and environmental factors.

There is some evidence of gender differences in the CAMs pathway. Ponthieux et al. (2003) reported a sex difference in the serum levels of CAMs. Bramachari and Pahan (2010) reported gender-specific expression of integrin in T cells which they thought contribute to gender difference in the prevalence of multiple sclerosis. Begic et al. (2010) reported that short-term changes in female sex hormone levels could modulate expression of soluble CAMs. However, to our knowledge no reports have shown gender-specific effects of the CAMs pathway in neurodevelopment. Nevertheless our findings suggest that integrins might possess a gender-specific property, particularly in schizophrenia patients. Although the prevalence of schizophrenia is relatively similar for males and females, the onset of symptoms is earlier in males than in females (Chu et al., 1988, Castle et al., 1998). Our findings suggest that although the types of CAMs in neurons are not different between males and females, mutations in these genes might contribute to the different time of onset of schizophrenia between males and females. Further studies are needed to determine whether the mutated protein functions differently in males and females.

We cannot fully rule out the possibility of a type I error due to our relatively small sample size. Because we did not examine SNPs in promoter region, we might have missed other functional SNPs possibly related to schizophrenia in CAMs genes. Due to a lack of power, our pilot study might not have been able to detect differences among the subjects and thus the SNPs were not examined in our second batch and replication samples. Nevertheless, CAMs might play significant roles in the development and pathophysiology of schizophrenia

and further studies are required to identify their roles. Functional mutations in these genes might uncover the true nature of the pathophysiology of schizophrenia.

In conclusion, we found an association between one missense mutation in the ITGA8 gene and schizophrenia in the Japanese population, particularly in females. The observed significant difference withstood correction for multiple. However, it would be interesting to do a further study with a larger sample size and a wider coverage of SNPs. Our results support that of O'Dushlaine et al. (2011) which reported that mechanisms involved in cell adhesion may contribute broadly to neurodevelopmental psychiatric phenotypes.

Acknowledgements

This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology in Japan. We thank you Ms. Y. Nagashima for the assistance during the experiments.

References

Barret JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 2: 263-5

Begic I, Cavka A, Mihalj M, Bacun T, Drenjancevic I. Influence of short-term changes in sex hormones on serum concentrations of cellular adhesion molecules in young healthy women. Med Glas 2012; 9(1): 32-36

Benoit YD, Lussier C, Ducharme PA, Sivret S, Schnapp LM, Basora N, et al. Integrin α8β1 regulates adhesion, migration and proliferation of human intestinal crypt cells via a predominant RhoA/ROCK-dependent mechanism. Biol Cell 2009; 101: 695-708

Bossy B, Bossy-Wetzel E, Reichardt LF. Characterization of the integrin α8 subunit: a new integrin β1-associated subunit, which is prominently expressed on axons and on cells in contact with basal laminae in chick embryos. EMBO J 1991; 10(9): 2375-85

Bramachari S, Pahan K. Gender-Specific Expression of β 1 Integrin of VLA-4 in Myelin Basic Protein-Primed T Cells: Implications for Gender Bias in Multiple Sclerosis. J Imunol 2010; 184: 6103-6113

Castle D, Sham P, Murray R. Differences in distribution of ages of onset in males and females with schizophrenia. Schizophr Res 1998; 33(3): 179-183

Chan CS, Weeber EJ, Kurup S, Sweatt JS, Davis RL. Integrin requirement for hippocampal synaptic plasticity and spatial memory. J Neurosci 2003; 23(18): 7107-16

Chan CS, Levenson JM, Mukhopadyay PS, Zong L, Bradley A, Sweatt JD, et al. α3-integrins are required for hippocampal long-term potentiation and working memory. Learn Mem 2007; 14: 606-15

Chan MK, Tsang TM, Harris LW, Guest P, Holmes E, Bahn S. Evidence for disease and antipsychotic medication effects in post-mortem brain from schizophrenia patients. Mol Psychiatry 2010a; 16(12): 1189-202

Chan CS, Chen H, Bradley A, Dragatsis I, Rosemund C, Davis RL. α8-integrins are required for hippocampal long-term potentiation but not for hippocampal-dependent learning, Genes Brain Behav 2010b; 9: 402-10

Chu CC, Abi-Dargam A, Ackerman B, Cetingok M, Klein HE, Sex difference in schizophrenia. Int J Soc Psychiatry 1988; 35: 237-44

Chun D, Gall CM, Bi X, Lynch G. Evidence that integrins contribute to multiple stages in the consolidation of long term potentiation in rat hippocampus. Neurosci 2001; 105: 815-29

Corvin AP. Neuronal cell adhesion genes. Key player in risk for schizophrenia, bipolar disorder, and other neurodevelopmental brain disorder? Cell Adh Migr 2010; 4(4): 511-4

Costa E, Grayson DR, Guidotti A. Epigenetic downregulation of GABAergic function in schizophrenia: Potential for pharmacological intervention? Mol Interv 2003; 3(4): 220-9

Devaux J, Gow A. Tight junctions potentiate the insulative properties of small CNS myelinated axons. J Cell Biol 2008; 183(5): 909-21

Dupont WD, Plummer Jr WD. Power and sample size calculations for studies involving linear regression. Control Clin Trials 1998; 19: 589–601

Einheber S, Schnapp LM, Salzer JL, Cappiello ZB, Milner TA. Regional and ultrastructural distribution of the alpha 8 integrin subunit in developing and adult rat brain suggests a role in synaptic function. J Comp Neurol 1996; 370(1): 105-34

Ekwa-Ekoka C, Diaz GA, Carlson C, Hasegawa T, Samudrala R, Lim K, et al. Genomic organization and sequence variation of the human integrin subunit α8 gene (*ITGA8*). Matrix Biol 2004; 23: 487-96

Gaur N, Gautam S, Gaur M, Sharma P, Dadheech G, Mishra S. The biochemical womb of schizophrenia: A review, Indian J CLin Biochem 2008; 23(4): 307-27

Giagtzoglou N, Ly CV, Bellen HJ. Cell adhesion, the backbone of the synapse: "Vertebrate" and "Invertebrate" Perspectives. Cold Spring Harb Perspect 2009; 1(aa003079): 1-26

Goh ELK, Young JK, Kuwako K, Tessier-Lavigne M, He Z, Griffin JW, et al. Beta I-integrin mediates myelin-associated glycoprotein signaling in neuronal growth cone. Mol Brain 2008: 1-14

Hildebrandt H, Muhlenhoff M, Oltmann-Norden I, Rockle I, Burkhardt H, Weinhold B, et al. Imbalance of neural cell adhesion molecule and polysialyltransferase alleles causes defective brain connectivity. Brain 2009; 132: 2831-8

Honer WG. Assessing the machinery of mind: Synapse in neuropsychiatric disorder. J Psychiatry Neurosci 1999; 24(2): 116-21

Jones P, Murray RM. The genetics of schizophrenia is the genetics of neurodevelopment. BJP 1991; 158: 615-23

Kirov G, O'Donovan MC, Owen MJ. Finding schizophrenia genes. J Clin Invest 2005; 115: 1440-8

Kramar EA, Bernard JA, Gall CM, Lynch G. Alpha3 integrin receptors contribute to the consolidation of long-term potentiation. Neurosci 2002; 110: 29–39

Kriebel M, Metzger J, Trinks S, Chugh D, Harvey RJ, Harvey K, et al. The cell adhesion molecule neurofascin stabilizes axo-axonic GABAergic terminal at the axon initial segment. J Biol Chem 2011; 286(27): 24385-93

Lips ES, Cornelisse LN, Toonen RF, Min JL, Hultman CM, the International Schizophrenia Consortium, et al. Functional gene group analysis identifies synaptic gene groups as risk factor for schizophrenia, Mol Psychiatry 2011: 1-11 (Epub ahead pints)

Maynard TM, Sikich L, Lieberman JA, LaMantia AS. Neural development, cell-cell signaling, and the "Two hit" hypothesis of schizophrenia, Schizophr Bull 2001; 27(3): 457-76

Moresco EM, Donaldson S, Williamson A, Koleske AJ. Integrin-mediated dendrite branch maintenance requires Abelson (Abl) family kinase. J Neurosci 2005; 25(26): 6105-18

Myers JP, Gomez TM. Focal adhesion kinase promotes integrin adhesion dynamics necessary for chemotropic turning of nerve growth cones, J Neurosci 2011; 31(38): 13585-95

Nakamoto T, Kain KH, Ginsberg MH. Neurobiology: New connections between integrins and axon guidance. Curr Biol 2004; 14 : 121-3

O'Dushlaine C, Kenny E, Heron E, Donohoe G, Gill M, The International Schizophrenia Consortium, et al. Molecular pathways involved in neuronal cell adhesion and membrane scaffolding contribute to schizophrenia and bipolar disorder susceptibility. Mol Psychiatry 2011; 16(3): 286-92

Pillai-Nair N, Panicker AK, Rodriguiz RM, Gilmore KL, Demyanenko GP, Huang JZ, et al. Neural cell adhesion molecule-secreting transgenic mice display abnormalities in GABAergic interneurons and alterations in behavior. J Neurosci 2005; 25(18): 4659-71

Ponthieux A, Herbeth B, Droesch S, Lambert D, Visvikis S. Age- and Sex-related Reference Values for Serum Adhesion Molecule Concentrations in Healthy Individuals: Intercellular Adhesion Molecule-1 and E-, P-, and L-Selectin. Clin Chem 2003; 49(9): 1544-1546

Robertson GS, Hori SE, Powell KJ. Schizophrenia: An integrative approach to modeling a complex disorder. J Psychiatry Neurosci 2006; 31(3): 157-67

Stanco A, Szekeres C, Patel N, Rao S, Campbell K, Kreidberg JA, et al. Netrin-1– αβ1 integrin interactions regulate the migration of interneurons through the cortical marginal zone, Proc Natl Acad Sci 2009; 106(18): 7595-600

Takada Y, Ye X, Simon S. The integrins. Genome Biol 2007; 8(5): 215

Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science 2008; 320: 539-43

Watanabe Y, Muratake T, Kaneko N, Nunokawa A, Someya T. No association between the brain-derived neurotrophic factor gene and schizophrenia in a Japanese population. Schizophr Res 2006;84(1):29–35.

Yoshida M, Shiroiwa K, Mouri K, Ishiguro H, Supriyanto I, Ratta-Apha W, et al. Haplotypes in the expression quantitative trait locus of interleukin-1 β gene are associated with schizophrenia. Schizophr Res 2012;140(1 –3):185–92.

Table 1 Associations of missense mutations in cell adhesion molecules with schizophrenia in the first batch of the sample (n control = 284, schizophrenia = 278)

Gene	Position	SNPs[a]	Amino acid changes	Phen[b]	Genotype[c]			Minor allele		p values			Odds ratio (95% CI)	Power
					MM	Mm	mm	Freq	allele	HWE[d]	gen[e]	allelic[f]		
PTPRF	chr 1	rs3748796	A/G	CON	0.936	0.060	0.004	0.034	G	0.537	0.586	0.570	0.819	0.056
	14028960		Tyr450Cys	SCZ	0.948	0.048	0.004	0.028		0.361			(0.412-1.630)	
NFASC	chr 1	rs2802808	C/G	CON	0.282	0.462	0.256	0.487	C	0.245	0.477	0.466	1.093	0.068
	56455070		Ile971Met	SCZ	0.246	0.488	0.265	0.510		0.785			(0.860-1.389)	
CNTN2	chr 1	rs2275697	G/A	CON	0.278	0.448	0.274	0.498	A	0.101	0.758	0.753	0.963	0.062
	56516379		Ala145Thr	SCZ	0.256	0.511	0.233	0.489		0.824			(0.759-1.220)	
ITGAV	chr 2	rs3738918	A/G	CON	0.922	0.071	0.007	0.042	G	0.160	0.172	0.168	1.458	0.115
	37720884		Ile359Val	SCZ	0.879	0.121	0.000	0.061		0.712			(0.850-2.501)	
CSPG2	chr 5	rs2652098	C/T	CON	0.774	0.194	0.032	0.129	T	0.053	0.516	0.504	0.885	0.050
	33402431		Ser300Leu	SCZ	0.779	0.210	0.011	0.116		1.000			(0.617-1.267)	
CSPG2	chr 5	rs2287926	G/A	CON	0.731	0.217	0.051	0.160	A	0.513	0.594	0.581	1.096	0.055
	33409767		Gly428Asp	SCZ	0.680	0.294	0.026	0.173		0.838			(0.792-1.517)	
CSPG2	chr 5	rs309559	A/G	CON	0.272	0.466	0.261	0.495	A	0.299	0.707	0.708	0.956	0.050
	33427728		Lys1516Arg	SCZ	0.246	0.540	0.213	0.483		0.229			(0.755-1.210)	
CSPG2	chr 5	rs188703	G/A	CON	0.300	0.473	0.226	0.463	G	0.475	0.959	0.960	0.994	0.058
	33428658		Arg1826His	SCZ	0.265	0.548	0.188	0.461		0.125			(0.785-1.259)	
CSPG2	chr 5	rs160278	T/A	CON	0.269	0.470	0.261	0.496	A	0.357	0.897	0.899	0.985	0.052
	33430083		Phe2301Tyr	SCZ	0.228	0.559	0.213	0.493		0.073			(0.778-1.246)	
CSPG2	chr 5	rs160277	G/T	CON	0.311	0.457	0.232	0.461	A	0.211	0.652	0.652	1.056	0.050
	33431990		Asp2937Tyr	SCZ	0.257	0.537	0.206	0.474		0.268			(0.834-1.338)	
NRCAM	chr 7	rs6958498	C/G	CON	0.746	0.226	0.029	0.142	G	0.328	0.404	0.383	1.159	0.082
	45867456		Pro545Ala	SCZ	0.720	0.239	0.041	0.160		0.105			(0.832-1.614)	
	15689716		Ser577Phe	SCZ	0.850	0.150	0.000	0.075		0.410			**(0.380-0.860)**	

PVRL1	chr 11	rs7940667	A/C	CON	0.869	0.127	0.004	0.067	A	1.000	0.754	0.753	1.077	0.051
	23073060		Val361Gly	SCZ	0.863	0.129	0.007	0.072		0.813			(0.678-1.712)	
CDH2	chr 18	rs2289664	C/T	CON	0.971	0.029	0.000	0.015	C	1.000	0.349	0.354	1.528	0.141
	7021406		Asn845Ser	SCZ	0.956	0.044	0.000	0.022		1.000			(0.620-3.769)	
CDH2	chr 18	rs17445840	G/A	CON	0.849	0.151	0.000	0.076	A	0.375	0.792	0.795	0.941	0.140
	7082796		Ala118Thr	SCZ	0.865	0.128	0.008	0.071		0.778			(0.597-1.485)	

[a] SNP single nucleotide polymorphisms, SNP identification number and position are determined by NCBI dbSNP (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp gene build assembly GRCh37 37.1); [b] Phenotype, CON control, SCZ schizophrenia; [c] M Major allele, m minor allele; [d] HWE Hardy Weinberg equilibrium; [e] Genotypic p value, determined with Cochran Armitage test for trend; [f] allelic p value, determined with Chi square

Table 2 Gender-based analysis of missense mutations in NFASC and ITGA8 in the first batch of the sample

Gene	SNPs [a]	Sex	Phen [b]	Genotype [c]			Allele [c]		p values			Odds ratio (95% CI)
				MM	Mm	mm	M	m	HWE [d]	gen [e]	allelic [f]	
NFASC	rs2802808	male	CON	0.225	0.483	0.292	0.467	0.533	0.857	0.197	0.201	0.792
			SCZ	0.258	0.533	0.208	0.525	0.475	0.595			(0.553-1.133)
		female	CON	0.325	0.446	0.229	0.548	0.452	0.257	**0.044**	**0.034**	1.417
			SCZ	0.236	0.450	0.314	0.461	0.539	0.322			(1.026-1.959)
ITGA8	rs2298033	male	CON	0.793	0.207	0.000	0.897	0.103	0.502	0.239	0.263	0.698
			SCZ	0.851	0.149	0.000	0.926	0.074	1.000			(0.370-1.315)
		female	CON	0.737	0.244	0.019	0.859	0.141	1.000	**0.010**	**0.011**	**0.500**
			SCZ	0.848	0.152	0.000	0.924	0.076	0.845			**(0.292-0.857)**

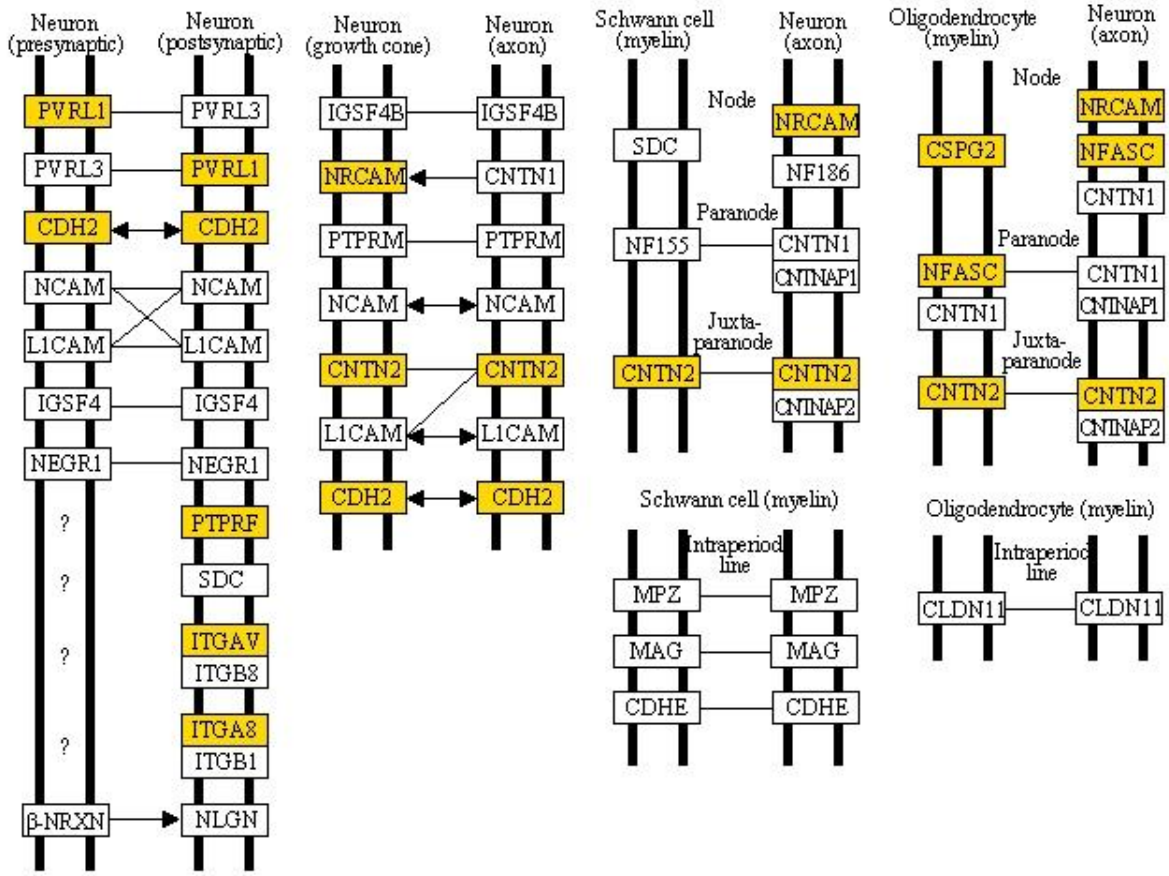
[a] SNP single nucleotide polymorphisms, SNP identification number and position are determined by NCBI dbSNP (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp); [b] Phenotype, CON control, SCZ schizophrenia; [c] M Major allele, m minor allele; [d] HWE Hardy Weinberg equilibrium; [e] Genotypic p value, determined with Cochran Armitage test for trend; [f] allelic p value, determined with Chi square

Table 3 Associations of missense mutations in NFASC and ITGA8 genes with schizophrenia in the second batch of the sample (n control 710, schizophrenia 567) and the replication sample (n control = 639, schizophrenia = 635)

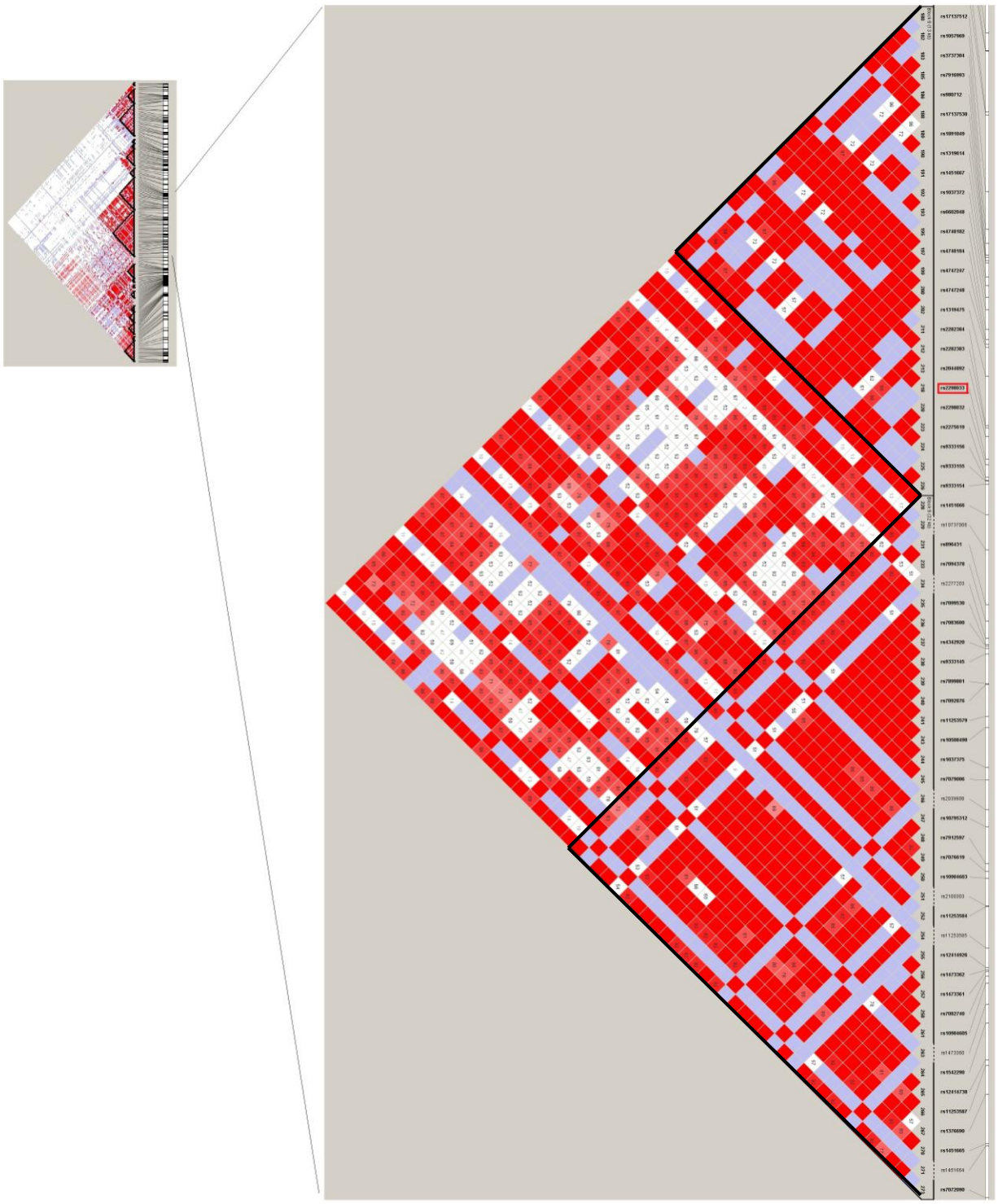
Gene	SNPs [a]	Phen [b]	Genotype [c]			Allele [c]		p values			Odds ratio (95% CI)	Power
			MM	Mm	mm	M	m	HWE [d]	gen [e]	allelic [f]		
Kobe samples												
NFASC	rs2802808	CON	0.278	0.486	0.236	0.521	0.479	0.535	0.694	0.691	0.968	0.059
		SCZ	0.285	0.488	0.227	0.529	0.471	0.673			(0.813-1.154)	
	male	CON	0.267	0.494	0.239	0.514	0.486	0.901	0.177	0.180	0.855	
		SCZ	0.297	0.511	0.192	0.553	0.447	0.690			(0.717-1.019)	
	female	CON	0.288	0.479	0.233	0.527	0.473	0.515	0.426	0.414	1.096	
		SCZ	0.272	0.463	0.265	0.504	0.496	0.275			(0.920-1.307)	
ITGA8	rs2298033	CON	0.754	0.235	0.012	0.871	0.129	0.311	**0.0086**	**0.010**	**0.717**	0.440
		SCZ	0.812	0.185	0.004	0.904	0.096	0.202		**(0.021)**	**(0.556-0.926)**	
	male	CON	0.765	0.232	0.003	0.881	0.119	0.077	0.274	0.302	0.823	
		SCZ	0.799	0.201	0.000	0.900	0.100	0.088			(0.621-1.090)	
	female	CON	0.743	0.238	0.019	0.862	0.138	1.000	**0.0115**	**0.0114**	**0.625**	
		SCZ	0.825	0.167	0.008	0.909	0.091	1.000		**(0.0287)**	**(0.472-0.828)**	
Niigata samples												
NFASC	rs2802808	CON	0.255	0.519	0.225	0.515	0.485	0.532	0.772	0.778	1.022	0.055
		SCZ	0.243	0.532	0.224	0.509	0.491	0.121			0.877-1.192)	
	male	CON	0.254	0.516	0.230	0.512	0.488	0.637	0.613	0.624	1.054	
		SCZ	0.227	0.544	0.230	0.499	0.501	0.109			(0.853-1.304)	
	female	CON	0.257	0.523	0.221	0.518	0.482	0.790	0.884	0.886	0.984	
		SCZ	0.263	0.519	0.218	0.522	0.478	0.678			(0.788-1.229)	
ITGA8	rs2298033	CON	0.779	0.206	0.016	0.881	0.119	0.791	0.131	0.128	0.823	0.191
		SCZ	0.812	0.177	0.011	0.900	0.100	0.869			(0.641-1.058	
	male	CON	0.792	0.196	0.012	0.890	0.110	1.000	0.640	0.634	0.920	
		SCZ	0.813	0.169	0.017	0.898	0.102	0.247			(0.652-1.298)	

		female	CON	0.764	0.216	0.020	0.872	0.128	0.692	0.087	0.089	0.728	
			SCZ	0.810	0.187	0.003	0.903	0.097	0.448			(0.505-1.050)	
Combined													
NFASC	rs2802808		CON	0.266	0.499	0.235	0.515	0.485	1.000	0.666	0.668	1.028	0.058
			SCZ	0.250	0.517	0.233	0.509	0.491	0.425			(0.906-1.166)	
		male	CON	0.268	0.503	0.229	0.519	0.481	0.848	0.955	0.956	1.005	
			SCZ	0.244	0.547	0.208	0.518	0.482	0.136			(0.844-1.197)	
		female	CON	0.264	0.495	0.241	0.511	0.489	0.829	0.565	0.561	1.056	
			SCZ	0.257	0.482	0.261	0.498	0.502	0.726			(0.880-1.266)	
ITGA8	rs2298033		CON	0.766	0.221	0.014	0.876	0.124	0.645	**0.0032**	**0.0035**	**0.767**	0.541
			SCZ	0.812	0.181	0.008	0.902	0.098	0.575		**(0.0069)**	**(0.642-0.917)**	
		male	CON	0.779	0.214	0.007	0.886	0.114	0.212	0.276	0.284	0.873	
			SCZ	0.807	0.183	0.010	0.899	0.101	1.000			(0.680-1.120)	
		female	CON	0.752	0.228	0.020	0.866	0.134	0.785	**0.0024**	**0.0025**	**0.674**	
			SCZ	0.817	0.178	0.005	0.906	0.094	0.518		**(0.0052)**	**(0.521-0.871)**	

[a] SNP single nucleotide polymorphisms, SNP identification number and position are determined by NCBI dbSNP (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp); [b] Phenotype, CON control, SCZ schizophrenia; [c] M Major allele, m minor allele; [d] HWE Hardy Weinberg equilibrium; [e] Genotypic p value, determined with Cochran Armitage test for trend; [f] allelic p value, determined with Chi square, correction for multiple comparisons in parentheses



Supplementary figure 1. Experimentally derived cell adhesion molecules (CAMs) pathways map in the neural system reported in the Kyoto Encyclopedia of Genes and Genomes. Genes examined in this study are highlighted (http://www.kegg.jp/kegg/pathway/hsa/hsa04514.html).



Supplementary figure 2. Haplotype block containing rs2298033 of the *ITGA8* gene constructed based on genotype data of the JPT population in Hapmap (release #27) (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap27_B36/)

Supplementary Table 1. Missense mutations in cell adhesion molecules genes based on the KEGG pathway map analysis in the Japanese population

Gene name	Strand	Chr	Gen build Assembly	rs number	Function	Chr Position	Cont Position	mRNA allele change	Protein change	(fwd) Genotype freqency	allele freqency
CADM1 (IGSF4)	-	11	37.1 GRCh37	rs74750431	**missense**	115049423	18611839	GTG→GGG	Val→Gly 356		A C 0.852 0.148
				rs74751890	cds-synon	115085422	18647838	ATC→ATA	Ile→Ile 300		G T 0.989 0.011
				rs45525440	**missense**	115085467	18647883	GAT→GAG	Asp→Glu 285		A C 0.989 0.011
CADM3 (IGSF4B)	+	1	37.1 GRCh37	rs862999	cds-synon	159169641	10658283	CTT→CTC	Leu→Leu 351	C/C C/T 0.841 0.159	C T 0.920 0.080
NRCAM	–	7	37.1 GRCh37	rs401433	cds-synon	107824889	45857732	GCG→GCC	Ala→Ala 735	C/C C/G 0.795 0.205	C G 0.898 0.102
				rs6958498	**missense**	107834613	45867456	CCT→GCT	Pro→Ala 545	C/C C/G 0.727 0.273	C G 0.864 0.136
				rs404287	cds-synon	107834734	45867577	GCG→GCA	Ala→Ala 534	C/T T/T 0.267 0.733	C T 0.133 0.867
				rs381318	cds-synon	107838464	45871307	GTC→GTA	Val→Val 429	A/A A/C 0.721 0.279	A C 0.860 0.140
				rs1269621	cds-synon	107849908	45882751	AAC→AAT	Asn→Asn 344	A/A A/G G/G 0.455 0.500 0.045	A G 0.705 0.295
				rs2072546	cds-synon	107872816	45905659	AAC→AAT	Asn→Asn	A/A A/G G/G	A G

									127	0.267 0.444 0.289	0.489 0.511
NCAM	+	11	37.1 GRCh37	rs584427	cds-synon	113103996	1666412	GTT→GTG	Val→Val 540	A/A A/C C/C 0.114 0.341 0.545	A C 0.284 0.76
L1CAM	-	X	37.1 GRCh37	rs2071643	cds-synon	153132261	4050199	GGG→GGA	Gly→Gly 758	A/A A/G G/G 0.068 0.023 0.909	A G 0.080 0.920
CNTN1	+	12	37.1 GRCh37	rs935105	cds-synon	41330611	3473917	AAT→AAC	Asn→Asn 338	A/A A/G G/G 0.933 0.044 0.022	A G 0.956 0.044
				rs1056019	cds-synon	41337435	3480741	AAC→AAT	Asn→Asn 472	C/C C/T T/T 0.311 0.467 0.222	C T 0.544 0.456
				rs11553341	**missense**	41414190	3557496	GAG→GGG	Glu→Gly 824	A/A A/G 0.977 0.023	A G 0.989 0.011
CNTN2 (axonal)	+	1	37.1 GRCh37	rs75898472	**missense**	205022315	56510957	ATG→AGG	Met→Arg 1		G T 0.023 0.977
				rs9787172	cds-synon	205027390	56516032	AAC→AAT	Asn→Asn 99	C/C C/T T/T 0.933 0.044 0.022	C T 0.956 0.044
				rs2275697	**missense**	205027737	56516379	GCT→ACT	Ala→Thr 145	C/C C/T T/T 0.256 0.488 0.256	C T 0.500 0.500
				rs2305276	**missense**	205035721	5624363	CGG→TGG	Arg→Trp 657	A/G G/G 0.027 0.973	A G 0.014 0.986
				rs2229868	cds-synon	205041158	56529800	AGC→AGT	Ser→Ser 876	C/C C/T T/T 0.111 0.556 0.333	C T 0.389 0.611
				rs17416074	**missense**	205042840	56531482	GTC→ATC	Val→Ile 1024	A/G G/G 0.022 0.978	A G 0.011 0.989
NRXN1	–	2	37.1 GRCh37	rs75575150	cds-synon	50765701	29587588	GAT→GAC	Asp→Asp 651		A G 0.977 0.023

				rs2303298	cds-synon	50850686	29672573	CC**C**→CC**T**	Pro→Pro 333	A/A 0.022	A/G 0.333 G/G 0.644 A 0.189 G 0.811
NRXN2	−	11	37.1 GRCh37	rs3825074	cds-synon	64415767	9721562	GG**C**→GG**T**	Gly→Gly 1109	C/C 0.556	C/T 0.378 T/T 0.067 C 0.744 T 0.256
				rs526338	cds-synon	64418900	9724695	AT**C**→AT**T**	Ile→Ile 915		A 0.239 G 0.761
				rs2285341	cds-synon	64453268	9759063	GC**C**→GC**T**	Ala→Ala 334	A/G 0.091	G/G 0.909 A 0.045 G 0.955
NRXN3	+	14	37.1 GRCh37	none							
CNTNAP1 (NRXN4)	+	17	37.1 GRCh37	rs2271029	cds-synon	40835922	6110074	**A**GA→**C**GA	Arg→Arg 51	A/A 0.310	A/C 0.500 C/C 0.190 A 0.560 C 0.440
				rs77725092	cds-synon	40849573	6123725	GA**G**→GA**A**	Glu→Glu 1190		A 0.034 G 0.966
CNTNAP2 (NRXN4)	+	7	37.1 GRCh37	rs2286128	cds-synon	147183066	7778689	TC**G**→TC**A**	Ser→Ser 570	C/C 0.889	C/T 0.111 C 0.944 T 0.056
				rs74354654	cds-synon	147600748	8196371	TG**C**→TG**T**	Cys→Cys 730		C 0.977 T 0.023
				rs10240503	cds-synon	147674978	8270610	TC**A**→TC**G**	Ser→Ser 760	A/A 0.489	A/G 0.467 G/G 0.044 A 0.722 G 0.278
				rs75688908	cds-synon	147869455	8465078	GG**C**→GG**A**	Gly→Gly 965		A 0.023 C 0.977
				rs9648691	cds-synon	148106490	8702113	GC**C**→GC**A**	Ala→Ala	A/A	A/G G/G A G

									1241	0.136 0.364 0.500	0.318 0.682
NLGN1	+	3	37.1 GRCh37	rs74718952	cds-synon	173993151	80488297	CTC→CTT	Leu→Leu 231		C T 0.989 0.011
				rs7646919	cds-synon	173997153	80492299	AAG→AAA	Lys→Lys 454	A/G G/G 0.159 0.841	A G 0.080 0.920
				rs16858840	cds-synon	173998955	80494101	CCC→CCT	Pro→Pro 778		C T 0.977 0.023
NLGN2	+	17	37.1 GRCh37	rs74879880	**missense**	7318307	6921681	ACC→CCC	Thr→Pro 293		A C 0.818 0.182
				rs224123	cds-synon	7318396	6921770	AGC→AGT	Ser→Ser 322	A/A A/G G/G 0.889 0.089 0.022	A G 0.933 0.067
				rs12947017	cds-synon	7318935	6922309	GGC→GGT	Gly→Gly 381		C T 0.932 0.068
NLGN3	+	X	37.1 GRCh37	none							
NLGN4X	-	X	37.1 GRCh37	rs7049300	cds-synon	5821786	3703548	ACC→ACT	Thr→Thr 311	A/A A/G G/G 0.111 0.089 0.800	A G 0.156 0.844
NLGN4Y	+	Y	37.1 GRCh37	none							
NEGR1	-	1	37.1 GRCh37	rs3795696	cds-synon	72058543	42030461	GCT→GCA	Ala→Ala 299	A/A A/T T/T 0.689 0.289 0.022	A T 0.833 0.167
				rs1413368	cds-synon	72058552	42030470	ACC→ACT	Thr→Thr 296	A/A A/G G/G 0.578 0.400 0.022	A G 0.778 0.222
NFASC	+	1	37.1	rs3795564	**missense**	204924020	56412662	ACG→ATG	Thr→Met	A/G G/G	A G

			GRCh37						159	0.023 0.977	0.011 0.989
				rs2246662	cds-synon	204943947	56432589	GTC→GTA	Val→Val 518	G/G G/T T/T 0.044 0.356 0.600	G T 0.222 0.778
				rs6667532	cds-synon	204948659	56437301	CCA→CCG	Pro→Pro 716	A/A A/G 0.978 0.022	A G 0.989 0.011
				rs2802808	**missense**	204966428	56455070	ATC→ATG	Ile→Met 971	C/C C/G G/G 0.205 0.500 0.295	C G 0.455 0.545
				rs4951151	cds-synon	204970302	56458944	CCT→CCC	Pro→Pro 1008	C/C C/T T/T 0.023 0.273 0.705	C T 0.159 0.841
CDH2	-	18	37.1 GRCh37	rs2289664	**missense**	25532304	7021406	AAT→AGT	Asn→Ser 845	C/T T/T 0.067 0.933	C T 0.033 0.967
				rs1041985	cds-synon	25543387	7032489	GCC→GCT	Ala→Ala 816	C/C C/T T/T 0.372 0.442 0.186	C T 0.593 0.407
				rs17857112	**missense**	25570299	7059401	ACA→GCA	Thr→Ala 454	C/C T/T 0.023 0.977	C T 0.023 0.977
				rs1041970	**missense**	25589796	7078898	AGT→ACT	Ser→Thr 196	C/G G/G 0.023 0.977	C G 0.012 0.988
				rs17445840	**missense**	25593694	7082796	GCA→ACA	Ala→Thr 118	C/C C/T 0.756 0.244	C T 0.878 0.122
CSPG2 (VCAN)	+	5	37.1 GRCh37	rs12332199	cds-synon	82786194	33380553	ACT→ACC	Thr→Thr 116	C/C C/T T/T 0.067 0.311 0.622	C T 0.222 0.778
				rs35042106	cds-synon	82786239	33380598	GAC→GAT	Asp→Asp 131		C T 0.852 0.148
				rs4470745	cds-synon	82789647	33384006	GTA→GTG	Val→Val 215	A/A A/G 0.867 0.133	A G 0.933 0.067

				rs2652098	**missense**	82808072	33402431	TCG→TTG	Ser→Leu 300	A/G 0.341	G/G 0.659	A 0.170	G 0.830
				rs2287926	**missense**	82815408	33409767	GGC→GAC	Gly→Asp 428	A/A 0.022	A/G 0.289	G/G 0.689	A 0.167	G 0.833
				rs2548541	cds-synon	82833145	33427504	CAG→CAA	Gln→Gln 1441	A/A 0.467	A/G 0.422	G/G 0.111	A 0.678	G 0.322
				rs309559	**missense**	82833369	33427728	AAA→AGA	Lys→Arg 1516	C/C 0.159	C/T 0.614	T/T 0.227	C 0.466	T 0.534
				rs188703	**missense**	82834299	33428658	CGT→CAT	Arg→His 1826	C/C 0.341	C/T 0.545	T/T 0.114	C 0.614	T 0.386
				rs309557	cds-synon	82834630	33428989	GGT→GGC	Gly→Gly 1936	A/A 0.227	A/G 0.614	G/G 0.159	A 0.534	G 0.466
				rs160279	cds-synon	82835545	33429904	AGA→AGG	Arg→Arg 2241				C 0.450	T 0.550
				rs160278	**missense**	82835724	33430083	TTT→TAT	Phe→Tyr 2301	A/A 0.227	A/T 0.614	T/T 0.159	A 0.534	T 0.466
				rs3734094	cds-synon	82835765	33430124	GTA→CTA	Val→Leu 2315	C/C 0.844	C/G 0.156		C 0.922	G 0.078
				rs76091728	**nonsense**	82836342	33430701	TCA→TAA	Ser→OCH 2507				A 0.011	C 0.989
				rs75771891	cds-synon	82836565	33430924	GAT→GAC	Asp→Asp 2581				C 0.114	T 0.886
				rs160277	**missense**	82837631	33431990	GAT→TAT	Asp→Tyr 2937	A/A 0.116	A/C 0.535	C/C 0.349	A 0.384	C 0.616
SDC1 (SDC)	-	2	37.1 GRCh37	rs2230924	cds-synon	20403949	4074225	GAG→GAA	Glu→Glu 84				A 0.239	G 0.761

PVRL1	-	11	37.1 GRCh37	rs76403536	cds-synon	119508832	23071248	ACC→ACT	Thr→Thr 451		A G 0.114 0.886
				rs7940667	**missense**	119510644	23073060	GTT→GGT	Val→Gly 361	A/C C/C 0.156 0.844	A C 0.078 0.922
PVRL3	+	3	37.1 GRCh37	none							
PTPRF	+	1	37.1 GRCh37	rs1065771	cds-synon	44035352	14007270	GCC→GCT	Ala→Ala 157	C/C C/T T/T 0.682 0.295 0.023	C T 0.830 0.170
				rs3748796	**missense**	44057042	14028960	TAC→TGC	Tyr→Cys 450	A/A A/G 0.930 0.070	A G 0.965 0.035
				rs2304354	cds-synon	44057535	14029453	ATC→ATT	Ile→Ile 528	C/C C/T 0.956 0.044	C T 0.978 0.022
				rs3748800	**missense**	44058143	14030061	GAC→AAC	Asp→Asn 562	A/G G/G 0.044 0.956	A G 0.022 0.978
				rs3828151	cds-synon	44058265	14030183	GCC→GCA	Ala→Ala 602	A/A A/C C/C 0.045 0.386 0.568	A C 0.239 0.761
				rs631248	cds-synon	44071221	14043139	GTG→GTA	Val→Val 1137	A/A A/G G/G 0.289 0.578 0.133	A G 0.578 0.422
				rs1065772	cds-synon	44072018	14043936	ACC→ACT	Thr→The 1197	C/C C/T T/T 0.622 0.333 0.044	C T 0.789 0.211
				rs10890266	cds-synon	44072066	14043984	CCC→CCT	Pro→Pro 1213	C/C C/T T/T 0.622 0.333 0.044	C T 0.789 0.211
				rs641365	cds-synon	44083507	14055425	GGT→GGC	Gly→Gly 1432	C/C C/T T/T 0.267 0.578 0.156	C T 0.556 0.444
				rs1143702	cds-	44086831	14058749	TAC→TAT	Tyr→Tyr	C/C C/T T/T	C T

					synon				1861	0.227 0.523 0.250	0.489 0.511
PTPRM	+	18	37.1 GRCh37	rs2230601	cds-synon	8069868	8059868	AA**C**→AA**T**	Asn→Asn 439	C/C C/T T/T 0.465 0.488 0.047	C T 0.709 0.291
				rs593978	cds-synon	8387195	8377195	GA**G**→GA**A**	Glu→Glu 1390	C/C C/T T/T 0.035 0.465 0.500	C T 0.267 0.733
				rs593950	cds-synon	8387219	8377219	AC**G**→AC**C**	Thr→Thr 1398	C/C C/G G/G 0.068 0.295 0.636	C G 0.216 0.784
ITGAV	+	2	37.1 GRCh37	rs3738918	**missense**	187511466	37720884	**A**TC→**G**TC	Ile→Val 359	A/A A/G 0.907 0.093	A G 0.953 0.047
				rs2230616	**missense**	187532417	37741835	**A**TC→**G**TC	Ile→Val 737	A/A A/G 0.911 0.089	A G 0.956 0.044
ITGA8	-	10	37.1 GRCh37	rs1041135	**missense**	15573050	15513050	G**C**A→G**T**A	Ala→Val 994	C/C C/T T/T 0.600 0.378 0.022	C T 0.789 0.211
				rs2298033	**missense**	15649710	15589710	T**C**C→T**T**C	Ser→Phe 577	C/C C/T T/T 0.800 0.178 0.022	C T 0.889 0.111
ITGB1	-	10	37.1 GRCh37	rs76211561	**missense**	33200525	33140525	TT**G**→TT**T**	Leu→Phe 594		A C 0.034 0.966
				rs2230396	cds-synon	33209266	33149266	GG**C**→GG**A**	Gly→Gly 392	A/A A/C C/C 0.477 0.318 0.364	A C 0.636 0.364
				rs2230395	cds-synon	33211227	33151227	TG**T**→TG**C**	Cys→Cys 261	A/A A/G G/G 0.591 0.318 0.091	A G 0.750 0.250
				rs2230394	cds-synon	33217110	33157110	TA**C**→TA**T**	Tyr→Tyr	C/C C/T T/T	C T

									153	0.489 0.311 0.200	0.644 0.356
ITGB8	+	7	37.1 GRCh37	rs3735619	cds-synon	20418678	20408678	GC**C**→GC**T**	Ala→Ala 131	C/C C/T T/T 0.400 0.400 0.200	C T 0.600 0.400
				rs6968952	cds-synon	20421490	20411490	TA**T**→TA**C**	Tyr→Tyr 314	C/C C/T T/T 0.140 0.419 0.442	C T 0.349 0.651
				rs80015015	**missense**	20441504	20431504	T**G**T→T**A**T	Cys→Tyr 481		A G 0.057 0.943

* The genes were selected based on the cell adhesion molecule reported in the KEGG (http://www.kegg.jp/kegg/pathway/hsa/hsa04514.html)
** SNP identification number, position, and frequencies are based on the reported data in the NCBI dbSNP (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp)
*** Highlighteds are the selected SNPs for genotypings

Supplementary table 2. List of the SNPs in the haplotype block containing rs2298033 of *ITGA8* gene

No.	SNP	position	No.	SNP	position	No.	SNP	position
1.	rs17137512	15676972	22.	rs2275619	15690591	43.	rs7912597	15709063
2.	rs1057969	15678757	23.	rs9333156	15690749	44.	rs7076619	15709436
3.	rs3737304	15679417	24.	rs9333155	15690790	45.	rs10904603	15709749
4.	rs7916993	15679968	25.	rs9333154	15690923	46.	rs2100303	15711057
5.	rs980712	15680110	26.	rs1451666	15692374	47.	rs11253584	15711117
6.	rs17137530	15680226	27.	rs10737006	15694064	48.	rs11253585	15713068
7.	rs1891049	15680366	28.	rs896431	15696680	49.	rs12414926	15714011
8.	rs1319614	15681034	29.	rs7094378	15697459	50.	rs1473362	15714141
9.	rs1451667	15681444	30.	rs2277203	15698303	51.	rs1473361	15714213
10.	rs1037372	15682000	31.	rs7099530	15698602	52.	rs7082740	15714422
11.	rs6602048	15682615	32.	rs7083600	15698665	53.	rs10904605	15714770
12.	rs4748182	15683522	33.	rs4342920	15698781	54.	rs1473360	15715786
13.	rs4748184	15684004	34.	rs9333145	15699017	55.	rs1542290	15716686
14.	rs4747247	15684236	35.	rs7899801	15700439	56.	rs12414738	15718422
15.	rs4747248	15684396	36.	rs7092876	15700508	57.	rs11253587	15718696
16.	rs1319475	15685759	37.	rs11253579	15702014	58.	rs1376690	15720079
17.	rs2282384	15688110	38.	rs10508490	15702543	59.	rs1451665	15722433
18.	rs2282383	15688249	39.	rs1037375	15704458	60.	rs1451664	15722545
19.	rs2044892	15688654	40.	rs7079006	15705060	61.	rs7072090	15725019
20.	**rs2298033**	**15689716**	41.	rs2039908	15706485			
21.	rs2298032	15690005	42.	rs10795312	15707285			