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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

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

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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 \pm SD, 48.4 \pm 10.5 years; 154 females: 51.7 \pm 12.6 years). The first control group consisted of 284 unrelated healthy volunteers (123 males; mean age \pm SD, 44.0 \pm 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 \pm SD, 39.8 \pm 13 .4 years; 290 females; mean age \pm S D, 39.7 \pm 14.5 years) and 639 controls (341 males; mean age \pm SD, 36.7 \pm 9.5 years; 298 females; mean age \pm SD, 40.2 \pm 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 $\chi^2 = 7.32$, 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 $\chi^2 = 6.54$, 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 $\chi^2 = 4.5$, 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 $\chi^2 = 6.6$, p = 0.010, respectively) and the female subgroup (Cochran Armitage test for trend Z = 2.5, p = 0.0115 and Chi square $\chi^2 = 6.4$, 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 $\chi^2 = 8.5$, p = 0.0035) and the female subgroup (Cochran Armitage test for trend Z = 3.0, p = 0.0024 and Chi square $\chi^2 = 9.17$, 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 α 8 subunit. The α 8 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.

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| | | | Amino soid | Dhon | G | enotype | , c | Minor | r allele | | p values | 5 | Odds ratio | |
|-------|----------|--------------------------|------------|------|-------|---------|-------|-------|----------|-------|----------|---------|---------------|-------|
| Gene | Position | SNPs ^a | changes | b b | MM | Mm | mm | Freq | allele | HWE | gen e | allelic | (95% CI) | Power |
| 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 | С | 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 | А | 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 | Т | 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 | А | 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 | А | 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 | А | 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 | А | 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) | |
| 18 | 15689716 | | Ser577Phe | SCZ | 0.850 | 0.150 | 0.000 | 0.075 | | 0.410 | | | (0.380-0.860) | |

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

| 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 | С | 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 | А | 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 (<u>http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp</u> 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

| Gene | SND _a a | Sow | Dhon ^b | Ge | notype ' | 2 | Alle | ele ^c | | p values | | Odds ratio |
|-------|--------------------|--------|-------------------|-------|----------|-------|-------|------------------|------------------|------------------|----------------------|---------------|
| Gene | SINFS | Sex | Flien – | MM | Mm | mm | Μ | m | HWE ^d | gen ^e | allelic ^f | (95% CI) |
| 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) |

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

^a SNP single nucleotide polymorphisms, SNP identification number and position are determined by NCBI dbSNP (<u>http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp</u>); ^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

| C | | D L b | (| Genotype | c | Alle | ele ^c | | p values | | Odds ratio | D |
|-------------|-----------|-------------------|-------|----------|-------|-------|------------------|------------------|------------------|----------------------|-----------------|-------|
| Gene | SNPs " | Phen [®] | MM | Mm | mm | Μ | m | HWE ^d | gen ^e | allelic ^f | (95% CI) | Power |
| Kobe samp | oles | | | | | | | | | | | |
| 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 san | nples | | | | | | | | | | | |
| 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) | |

Table 3 Associations of missense mutations in NFASC and ITGA8 genes with schizophrenia in the second batch of the sample (n control710, schizophrenia 567) and the replication sample (n control = 639, schizophrenia = 635)

| | 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 (<u>http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp</u>); ^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/)

| Gene name | Stran d | Chr | Gen build Assembly | rs number | Function | Chr Position | Cont Position | mRNA allele change | Protein change | (fwd) Genotype freqency | allele freqen | су |
|-----------|------------|-----|-----------------------|------------|---------------|-----------------|------------------|--------------------------------------|-------------------|----------------------------|------------------|-------|
| CADM1 | - | 11 | 37.1 | rs74750431 | missense | 115049423 | 18611839 | G T G→G <mark>G</mark> G | Val→Gly | | А | С |
| (IGSF4) | | | GRCh37 | | | | | | 356 | | 0.852 | 0.148 |
| | | | | rs74751890 | cds- synon | 115085422 | 18647838 | AT <mark>C</mark> →AT <mark>A</mark> | Ile→Ile | | G | Т |
| | | | | | | | | | 300 | | 0.989 | 0.011 |
| | | | | rs45525440 | missense | 115085467 | 18647883 | GA T →GA G | Asp→Glu | | А | С |
| | | | | | | | | | 285 | | 0.989 | 0.011 |
| CADM3 | + | 1 | 37.1 | rs862999 | cds- synon | 159169641 | 10658283 | CT T →CT C | Leu→Leu | C/C C/T | С | Т |
| (IGSF4B) | | | GRCh37 | | - | | | | 351 | 0.841 0.159 | 0.920 | 0.080 |
| NRCAM | - | 7 | 37.1 | rs401433 | cds- synon | 107824889 | 45857732 | GC G →GCC | Ala→Ala | C/C C/G | С | G |
| | | | GRCh37 | | - | | | | 735 | 0.795 0.205 | 0.898 | 0.102 |
| | | | | rs6958498 | missense | 107834613 | 45867456 | CCT→GCT | Pro→Ala | C/C C/G | С | G |
| | | | | | | | | | 545 | 0.727 0.273 | 0.864 | 0.136 |
| | | | | rs404287 | cds- synon | 107834734 | 45867577 | GC G →GCA | Ala→Ala | C/T T/T | С | Т |
| | | | | | - | | | | 534 | 0.267 0.733 | 0.133 | 0.867 |
| | | | | rs381318 | cds- synon | 107838464 | 45871307 | GT C →GT A | Val→Val | A/A A/C | А | С |
| | | | | | | | | | 429 | 0.721 0.279 | 0.860 | 0.140 |
| | | | | rs1269621 | cds- synon | 107849908 | 45882751 | AA <mark>C</mark> →AA T | Asn→Asn | A/A A/G G/G | А | G |
| | | | | | | | | | 344 | 0.455 0.500 0.045 | 0.705 | 0.295 |
| | | | | rs2072546 | cds- synon | 107872816 | 45905659 | AA C →AA T | Asn→Asn | A/A A/G G/G | A | G |

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

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

| | | | | | cds- | | | | | | |
|---------|---|----|--------|------------|---------------|-----------|----------|--------------------------------------|---------|-------------------|-------------|
| | | | | rs2303298 | synon | 50850686 | 29672573 | CCC→CCT | Pro→Pro | A/A A/G G/G | A G |
| | | | | | | | | | 333 | 0.022 0.333 0.644 | 0.189 0.811 |
| NRXN2 | - | 11 | 37.1 | rs3825074 | cds- synon | 64415767 | 9721562 | GG <mark>C</mark> →GGT | Gly→Gly | C/C C/T T/T | С Т |
| | | | GRCh37 | | 5 | | | | 1109 | 0.556 0.378 0.067 | 0.744 0.256 |
| | | | | rs526338 | cds- svnon | 64418900 | 9724695 | AT C →AT T | Ile→Ile | | A G |
| | | | | | 5 | | | | 915 | | 0.239 0.761 |
| | | | | rs2285341 | cds- synon | 64453268 | 9759063 | GC C →GC T | Ala→Ala | A/G G/G | A G |
| | | | | | 5 | | | | 334 | 0.091 0.909 | 0.045 0.955 |
| NRXN3 | + | 14 | 37.1 | none | | | | | | | |
| | | | GRCh37 | | | | | | | | |
| CNTNAP1 | + | 17 | 37.1 | rs2271029 | cds- synon | 40835922 | 6110074 | A GA→ C GA | Arg→Arg | A/A A/C C/C | A C |
| (NRXN4) | | | GRCh37 | | 5 | | | | 51 | 0.310 0.500 0.190 | 0.560 0.440 |
| | | | | rs77725092 | cds- synon | 40849573 | 6123725 | GA G →GAA | Glu→Glu | | A G |
| | | | | | 5 | | | | 1190 | | 0.034 0.966 |
| CNTNAP2 | + | 7 | 37.1 | rs2286128 | cds- synon | 147183066 | 7778689 | TC G →TCA | Ser→Ser | C/C C/T | С Т |
| (NRXN4) | | | GRCh37 | | -) | | | | 570 | 0.889 0.111 | 0.944 0.056 |
| | | | | rs74354654 | cds- synon | 147600748 | 8196371 | TG <mark>C</mark> →TG T | Cys→Cys | | С Т |
| | | | | | Synon | | | | 730 | | 0.977 0.023 |
| | | | | rs10240503 | cds- synon | 147674978 | 8270610 | TC A →TC G | Ser→Ser | A/A A/G G/G | A G |
| | | | | | Synon | | | | 760 | 0.489 0.467 0.044 | 0.722 0.278 |
| | | | | rs75688908 | cds- synon | 147869455 | 8465078 | GG <mark>C</mark> →GG <mark>A</mark> | Gly→Gly | | A C |
| | | | | | | | | | 965 | | 0.023 0.977 |
| | | | | rs9648691 | cds- synon | 148106490 | 8702113 | GC C →GCA | 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 | rs74718952 | cds- synon | 173993151 | 80488297 | C T C→CT T | Leu→Leu | | СТ |
| | | | GRCh37 | | | | | | 231 | | 0.989 0.011 |
| | | | | rs7646919 | cds- synon | 173997153 | 80492299 | AA G →AA A | Lys→Lys | A/G G/G | A G |
| | | | | | 5 | | | | 454 | 0.159 0.841 | 0.080 0.920 |
| | | | | rs16858840 | cds- synon | 173998955 | 80494101 | CC C →CC T | Pro→Pro | | С Т |
| | | | | | | | | | 778 | | 0.977 0.023 |
| NLGN2 | + | 17 | 37.1 | rs74879880 | missense | 7318307 | 6921681 | ACC→CCC | Thr→Pro | | A C |
| | | | GRCh37 | | | | | | 293 | | 0.818 0.182 |
| | | | | rs224123 | cds- synon | 7318396 | 6921770 | AG C →AG T | Ser→Ser | A/A A/G G/G | A G |
| | | | | | - | | | | 322 | 0.889 0.089 0.022 | 0.933 0.067 |
| | | | | rs12947017 | cds- synon | 7318935 | 6922309 | GG <mark>C</mark> →GG T | Gly→Gly | | С Т |
| | | | | | | | | | 381 | | 0.932 0.068 |
| NLGN3 | + | X | 37.1 | none | | | | | | | |
| | | | GRCh37 | | | | | | | | |
| NLGN4X | - | Х | 37.1 | rs7049300 | cds- synon | 5821786 | 3703548 | AC C →AC T | Thr→Thr | A/A A/G G/G | A G |
| | | | GRCh37 | | | | | | 311 | 0.111 0.089 0.800 | 0.156 0.844 |
| NLGN4Y | + | Y | 37.1 | none | | | | | | | |
| | | | GRCh37 | | | | | | | | |
| NEGR1 | - | 1 | 37.1 | rs3795696 | cds- synon | 72058543 | 42030461 | GC T →GCA | Ala→Ala | A/A A/T T/T | A T |
| | | | GRCh37 | | | | | | 299 | 0.689 0.289 0.022 | 0.833 0.167 |
| | | | | rs1413368 | cds- synon | 72058552 | 42030470 | AC <mark>C</mark> →AC T | Thr→Thr | A/A A/G G/G | A G |
| | | | | | - | | | | 296 | 0.578 0.400 0.022 | 0.778 0.222 |
| NFASC | + | 1 | 37.1 | rs3795564 | missense | 204924020 | 56412662 | A <mark>C</mark> G→A T G | Thr→Met | A/G G/G | A G |

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

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

| PVRL1 | - | 11 | 37.1 | rs76403536 | cds- synon | 119508832 | 23071248 | AC C →AC T | Thr→Thr | | A G |
|-------|---|----|--------|------------|---------------------------------------|-----------|----------|---------------------------|---------|-------------------|-------------|
| | | | GRCh37 | | , , , , , , , , , , , , , , , , , , , | | | | 451 | | 0.114 0.886 |
| | | | | rs7940667 | missense | 119510644 | 23073060 | G T T→G G T | Val→Gly | A/C C/C | A C |
| | | | | | | | | | 361 | 0.156 0.844 | 0.078 0.922 |
| PVRL3 | + | 3 | 37.1 | none | | | | | | | |
| | | | GRCh37 | | | | | | | | |
| PTPRF | + | 1 | 37.1 | rs1065771 | cds- synon | 44035352 | 14007270 | GC C →GC T | Ala→Ala | C/C C/T T/T | С Т |
| | | | GRCh37 | | ~, | | | | 157 | 0.682 0.295 0.023 | 0.830 0.170 |
| | | | | rs3748796 | missense | 44057042 | 14028960 | TAC→TGC | Tyr→Cys | A/A A/G | A G |
| | | | | | | | | | 450 | 0.930 0.070 | 0.965 0.035 |
| | | | | rs2304354 | cds- synon | 44057535 | 14029453 | AT C →AT T | Ile→Ile | C/C C/T | С Т |
| | | | | | ~, | | | | 528 | 0.956 0.044 | 0.978 0.022 |
| | | | | rs3748800 | missense | 44058143 | 14030061 | GAC→AAC | Asp→Asn | A/G G/G | A G |
| | | | | | | | | | 562 | 0.044 0.956 | 0.022 0.978 |
| | | | | rs3828151 | cds- synon | 44058265 | 14030183 | GC C →GCA | Ala→Ala | A/A A/C C/C | A C |
| | | | | | 2 | | | | 602 | 0.045 0.386 0.568 | 0.239 0.761 |
| | | | | rs631248 | cds- synon | 44071221 | 14043139 | GT G →GT A | Val→Val | A/A A/G G/G | A G |
| | | | | | ~, | | | | 1137 | 0.289 0.578 0.133 | 0.578 0.422 |
| | | | | rs1065772 | cds- synon | 44072018 | 14043936 | AC C →AC T | Thr→The | C/C C/T T/T | С Т |
| | | | | | ~, | | | | 1197 | 0.622 0.333 0.044 | 0.789 0.211 |
| | | | | rs10890266 | cds- synon | 44072066 | 14043984 | CC C →CC T | Pro→Pro | C/C C/T T/T | С Т |
| | | | | | | | | | 1213 | 0.622 0.333 0.044 | 0.789 0.211 |
| | | | | rs641365 | cds- synon | 44083507 | 14055425 | GG T →GG C | Gly→Gly | C/C C/T T/T | СТ |
| • | | | | | | | | | 1432 | 0.267 0.578 0.156 | 0.556 0.444 |
| | | | | rs1143702 | cds- | 44086831 | 14058749 | TA C →TA T | Tyr→Tyr | C/C C/T T/T | С Т |

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

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

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

| No. SNP position No. SNP position No. | SNP position |
|---|------------------|
| 1. rs17137512 15676972 22. rs2275619 15690591 43. rs7 | 912597 15709063 |
| 2. rs1057969 15678757 23. rs9333156 15690749 44. rs76 | 076619 15709436 |
| 3. rs3737304 15679417 24. rs9333155 15690790 45. rs10 | 0904603 15709749 |
| 4. rs7916993 15679968 25. rs9333154 15690923 46. rs2 | 100303 15711057 |
| 5. rs980712 15680110 26. rs1451666 15692374 47. rs1 | 1253584 15711117 |
| 6. rs17137530 15680226 27. rs10737006 15694064 48. rs1 | 1253585 15713068 |
| 7. rs1891049 15680366 28. rs896431 15696680 49. rs12 | 2414926 15714011 |
| 8. rs1319614 15681034 29. rs7094378 15697459 50. rs14 | 473362 15714141 |
| 9. rs1451667 15681444 30. rs2277203 15698303 51. rs14 | 473361 15714213 |
| 10. rs1037372 15682000 31. rs7099530 15698602 52. rs70 | 082740 15714422 |
| 11. rs6602048 15682615 32. rs7083600 15698665 53. rs10 | 0904605 15714770 |
| 12. rs4748182 15683522 33. rs4342920 15698781 54. rs14 | 473360 15715786 |
| 13. rs4748184 15684004 34. rs9333145 15699017 55. rs1: | 542290 15716686 |
| 14. rs4747247 15684236 35. rs7899801 15700439 56. rs12 | 2414738 15718422 |
| 15. rs4747248 15684396 36. rs7092876 15700508 57. rs1 | 1253587 15718696 |
| 16. rs1319475 15685759 37. rs11253579 15702014 58. rs12 | 376690 15720079 |
| 17. rs2282384 15688110 38. rs10508490 15702543 59. rs1 | 451665 15722433 |
| 18. rs2282383 15688249 39. rs1037375 15704458 60. rs1 | 451664 15722545 |
| 19. <u>rs2044892</u> 15688654 40. rs7079006 15705060 61. rs70 | 072090 15725019 |
| 20. rs2298033 15689716 41. rs2039908 15706485 | |
| 21. rs2298032 15690005 42. rs10795312 15707285 | |

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