Human Rho guanine nucleotide exchange factor 11 gene is associated with schizophrenia in a Japanese population

Yutaka Mizuki, Manabu Takaki*, Yuko Okahisa, Shinji Sakamoto,

Masafumi Kodama, Hiroshi Ujike, Yosuke Uchitomi

Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan

*Corresponding author: Manabu Takaki M.D., Ph.D.

Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan

E-mail address: manabuta@cc.okayama-u.ac.jp

Tel.: +81-86-235-7242

Fax: +81-86-235-7246

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Running head: Association of *ARHGEF11* with schizophrenia

Abstract

Objective: The human Rho guanine nucleotide exchange factor 11 (*ARHGEF11*) gene is one of the candidate genes for type 2 diabetes mellitus (T2DM). *ARHGEF11* is mapped to chromosome 1q21, which has susceptible risk loci for T2DM and schizophrenia. We hypothesized that *ARHGEF11* contributes to the pathogenesis of schizophrenia.

Method: We selected eight single nucleotide polymorphisms of *ARHGEF11* that had significant associations with T2DM for a case-control association study of 490 patients with schizophrenia and 500 age- and sex- matched controls.

Results: We did not find any differences in allelic, genotypic associations, or minor allele frequencies with schizophrenia. Analysis of the rs6427340-rs6427339 haplotype and the rs822585-rs6427340-rs6427339 haplotype combination provided significant evidence of an association with schizophrenia (global permutations *P*=0.00047 and 0.0032, respectively). **C-C of the rs6427340-rs6427339 haplotype and A-C-C of the rs822585-rs6427340-rs6427339 haplotype carried higher risk factors for schizophrenia (permutation *P*=0.0010 and 0.0018, respectively). A-C-T of the rs822585-rs6427340-rs6427339 haplotype had a possible protective effect (permutation *P*=0.031).**

Conclusion: These results provide new evidence that *ARHGEF11* may constitute a risk factor for schizophrenia.

Keywords

ARHGEF11, Case-control study, Schizophrenia, Type 2 diabetes mellitus

1. Introduction

It is well established that patients with schizophrenia are at increased risk for the development of type 2 diabetes mellitus (T2DM) (Bushe and Holt, 2004; Suvisaari *et al.*, 2008). Epidemiological surveys show that the prevalence of T2DM in patients with schizophrenia is approximately 6% to 21%, two to three times higher than in the general population (Bushe and Holt, 2004; Suvisaari *et al.*, 2008). Some studies have suggested that the medication used to treat schizophrenia may contribute to the risk of diabetes (Scheen and De Hert, 2007; Tschoner *et al.*, 2007). However, apart from adverse effects related to medication, recent studies show an elevated risk of T2DM among drug-naïve or first-episode patients with schizophrenia and their relatives (Ryan et al., 2003, Spelman et al., 2007; Guest et al., 2010). Thus, T2DM and schizophrenia are common diseases that seem to share a complex mode of inheritance that includes both genetic factors and environmental determinants (Gough and O'Donovan, 2005).

Previous genetic studies show that T2DM and schizophrenia have a number of overlapping risk loci, including chromosomes 1p13, 1p36, 1q21-24, 1q25, 2q14, 2q33, 2q36, 3p22, 3q29, 4q25, 5q13, 6p21, 6q25, 7p15, 7p21, 7q21, 7q31, and 9p24 (Lin and Shuldiner, 2010). Chromosome 1q21 was reported to have a linkage to T2DM by several previous studies (Vionnet *et al.*, 2000; Das and Elbein, 2007). This location has also been implicated

as a schizophrenia susceptibility locus (Brzustowicz *et al.*, 2000; Craddock *et al.*, 2005). The human Rho guanine nucleotide exchange factor 11 (*ARHGEF11*) gene is located in chromosome 1q21, and *ARHGEF11* variants have been associated with T2DM in multiple ethnic populations (Fu *et al.*, 2007; Ma *et al.*, 2007; Böttcher *et al.*, 2008; Jin *et al.*, 2010; Liu *et al.*, 2011).

ARHGEF11 is expressed in the pancreas, liver, adipose tissue (Fukuhara *et al.*, 1999), and multiple brain regions (Huerta *et al.*, 2006). It mediates interaction with the actin cytoskeleton (Banerjee and Wedegaertner; 2004) and activates RhoA GTPases that belong to small GTP-binding proteins of Rho families (Fukuhara *et al.*, 1999; Rümenapp *et al.*, 1999). Rho GTPases play fundamental roles in the regulation of G protein signaling and a number of cellular processes, including insulin secretion (Hirosumi *et al.*, 2002), insulin signaling (Kowluru and Veluthakal, 2005), and lipid metabolism (Houssa *et al.*, 1999). Rho GTPases are also implicated in the regulation of neuronal morphogenesis, including migration, polarity, axon growth, axon guidance, dendrite elaboration, dendrite plasticity, and synapse formation (Luo, 2000).

In a yeast two-hybrid screen, *ARHGEF11* directly interacted with disrupted-in-schizophrenia 1 (*DISC1*), which is one of the candidate genes for schizophrenia (Millar *et al.*, 2003). DISC1 modulates the structure and function of the spines of the glutamate synapse via another member of the RhoGEF family, KALRN (Hayashi-Takagi *et*

al., 2010).

We hypothesized that ARHGEF11 contributes to the pathogenesis of schizophrenia. Therefore, we examined the association between patients with schizophrenia and *ARHGEF11*.

2. Methods

2.1. Subjects

The subjects of this association study comprised 490 unrelated patients fulfilling the ICD-10 (International Classification of Disease, Version 10, WHO 1992) diagnostic criteria for schizophrenia (250 males and 240 females, average age ± standard deviation (S.D.), 50.5 ± 12.8 years; 223 were diagnosed with the paranoid subtype, 241 with the hebephrenic subtype, 18 with the catatonic subtype, and 8 with the undifferentiated subtype), and 500 age-, sex-, and geographical origin-matched control subjects (250 males and 250 females, average age ± S.D., 51.0 ± 14.5 years). Diagnosis of schizophrenia and determination of subtype were performed by two trained psychiatrists on the basis of all available information and unstructured interviews. We excluded the catatonic subtype due to the low sample size and the undifferentiated subtype due to its heterogeneity. Controls were recruited primarily from medical staff members. Psychiatrically, medically, and neurologically healthy controls were

evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services. All subjects were Japanese. This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. Written informed consent was obtained from all participants.

2.2. Genotyping

Peripheral blood was obtained from the subjects, and genomic DNA was extracted from peripheral leukocytes using a standard procedure. We selected eight single nucleotide polymorphisms (SNPs), rs822585, rs6427340, rs6427339, rs1006168, rs12136088, rs2275199 (N1207N), rs868188 (S1456G), and rs945508 (R1467H), of *ARHGEF11* for genetic association analyses **(see Fig. 1).** These eight SNPs have shown significant associations with T2DM (Fu *et al.,* 2007; Ma *et al.,* 2007; Böttcher *et al.,* 2008; Jin *et al.,* 2010; Liu *et al.,* 2011). The concentration and purity of DNA samples was assessed using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). The concentration of DNA samples was adjusted to 20 ng/μl. The purity was estimated from the ratio of the absorbance values measured at wavelengths of 260 nm and 280 nm (defined as a 260/280 ratio between 1.8 and 2.0). Eight SNPs (rs822585, rs6427340, rs6427339, rs1006168, rs12136088, rs2275199, rs868188, and rs945508) were genotyped with a TaqMan assay (Assay ID:

C___1818078_10, C___1818022_20, C___1818021_20, C___1818013_10, C__31432570_10, C__16183901_10, C___1468852_10, and C___1468853_10, respectively, Applied Biosystems, USA) with a Real Time QPCR System Mx3000P (Agilent Technologies, USA). PCR reaction was carried out on 16 μl of a solution containing approximately 0.2 μl TE buffer, 0.2 μl ready-to-use PCR probe, 2 μl genomic DNA, 8 μl Brilliant II FAST QPCR Master Mix (Agilent Technologies), and 5.6 μl DNase-free water. PCR cycling conditions consisted of an initial incubation at 95°C for 2 min, followed by 40 cycles of 92°C for 30 sec and 60°C for 1 min, for the annealing/elongation steps. An experienced researcher conducted the genotyping and read out the data, and the genetic variants were verified by repeat PCR.

2.3. Statistical analyses

Statistical analyses were performed using Statcel software for Windows version 2.0 (OMS Ltd., Japan). Associations between categorical variables were analyzed using χ^2 test and continuous variables were analyzed using Mann-Whitney's U test. Deviation from Hardy-Weinberg equilibrium and case-control association were examined by χ^2 test. SNPs with Hardy-Weinberg equilibrium below 0.05 in either patients or controls were excluded from further analyses. We evaluated pairwise linkage disequilibrium (LD) among the SNPs

by χ^2 test, and *D'* and r^2 values. In haplotype analyses, we calculated the permutation *P* value by using 100,000 simulations to avoid the possibility that a large error could occur in the χ^2 test when the haplotype frequency was extremely small. To avoid misleading global haplotype results caused by rare haplotypes, we limited haplotypes to those having frequencies of at least 1% in either patients or controls. These statistical analyses were performed by using the software SNPAlyze (version 4.2; Dynacom Co., Japan). The statistical significance was set at $P < 0.05$. **Comparisons between schizophrenia patients of paranoid and hebephrenic subtypes and normal controls reveal *P* values <0.05/3=0.016.** Furthermore, we performed a post hoc power calculation using a G* power program (Faul *et al*., 2007). We found that the sample size had >0.90 power for detecting a significant association (alpha level <0.05) when an effect size index of 0.2 was used.

2.4. In silico analysis of SNP functions

To investigate the functions of non-synonymous SNPs of *ARHGEF11*, we used the in silico analysis program Polyphen on the SNPinfo Web Server **(http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm).**

3. Results

3.1. Genetic association analyses

The genotype and allele frequencies for each SNP of patients with schizophrenia and controls are shown in Table 1. The eight SNPs did not deviate from Hardy-Weinberg equilibrium. Therefore, we performed the following analyses. **We did not find significant differences in genotype, allele frequencies, or minor allele frequencies between patients with schizophrenia and controls. After stratification by subtype of schizophrenia, we did not find significant differences in genotype, allele frequencies, or minor allele carrier frequencies.**

3.2. Haplotype association analyses

Table 2 shows the pairwise LD among eight SNPs of *ARHGEF11* using *D'* and r^2 values as an index. According to these results, we found two LD blocks, block 1 containing rs822585, rs6427340, and rs6427339 (*D'* ranging between 0.93 and 0.98, and r^2 ranging between 0.85 and 0.92), and block 2 containing rs1006168 and rs12136088 (*D'* of 0.98, and r^2 of 0.97). We then analyzed two- and three-loci haplotypes in block 1, and two-loci haplotypes in block 2 of patients with schizophrenia and controls. We found significant

differences in the frequencies of rs6427340-rs6427339 and rs822585-rs6427340-rs6427339 haplotypes of *ARHGEF11* in block 1 (global permutation *P*=0.00047 and 0.0032, respectively). The frequencies of C-C of rs6427340-rs6427339 and A-C-C of rs822585-rs6427340-rs6427339 in schizophrenia were higher (permutation *P*=0.0010 and 0.0018, respectively) than in controls, indicating a higher risk for schizophrenia. The frequencies of A-C-T of rs822585-rs6427340-rs6427339 in schizophrenia were lower (permutation *P*=0.031) than in controls, indicating a possible protective effect (see Table 3).

After stratification by subtype of schizophrenia, we found significant differences in the frequencies of the rs6427340-rs6427339 and rs822585-rs6427340-rs6427339 haplotypes between the paranoid subtype and controls (global permutation *P*=0.0018 and 0.0077, respectively) and between the hebephrenic subtype and controls (global permutation *P*=0.00007 and 0.00025, respectively). The frequencies of C-C of rs6427340-rs6427339 and A-C-C of rs822585-rs6427340-rs6427339 in the hebephrenic subtype (permutation *P*=0.00005 and 0.000083, respectively) were higher than in controls, indicating a higher risk for schizophrenia. The frequency of C-T of rs6427340-rs6427339 in the paranoid subtype (permutation *P*=0.016) was lower than in controls, indicating a possible protective effect (see Table 3).

3.3. In silico analysis of SNP functions

Rs868188 (S1456G) had a predicted sequence conservation score of 0.012 and was predicted to be “benign”. Rs945508 (R1467H) had a predicted sequence conservation score of 0.168 and was predicted to be “possibly damaging”.

4. Discussion

To the best of our knowledge, this is the first genetic association study of *ARHGEF11* in patients with schizophrenia. Though we did not find allelic and genotypic associations with schizophrenia, analysis of the haplotype combinations provided significant evidence for an association with schizophrenia. It was reported that having the A allele of rs822585, C allele of rs6427340, or T allele of rs6427339 was associated with T2DM in an Amish population (Fu *et al.,* 2007). **In our study, the frequency of A-C-T of rs822585-rs6427340-rs6427339 was significantly higher in controls than in patients with schizophrenia. This result contradicts the view that liability to T2DM is associated with vulnerability to schizophrenia. In the dbSNP database of the NCBI Web Server (http://www.ncbi.nlm.nih.gov/projects/SNP), the T allele of rs822585, T allele of rs6427340, and C allele of rs6427339 were estimated to be minor alleles in a Japanese population (each allele frequency was 20%, 24%, and 24%, respectively). This data is**

consistent with our results, and different from that reported for an Amish population. A possible explanation for this incongruity may be the difference in ethnic backgrounds. In several other individual haplotypes, the frequencies of C-C of rs6427340-rs6427339 and A-C-C of rs822585-rs6427340-rs6427339 were associated with higher risks for schizophrenia. These haplotypes were also strongly associated with the hebephrenic subtype after stratification by subtype of schizophrenia. However, these differences may be mainly due to the low frequencies of rare haplotypes in the controls (haplotype frequency was 0.004).

Minor A allele carriers (GA + AA genotype) of rs945508 (R1467H) were reported to have markedly higher risk of T2DM, higher body mass index, higher serum fasting plasma glucose, higher fasting insulin, and higher homeostasis model assessment of insulin resistance values (Liu *et al*., 2011). G allele carriers (GA＋GG genotype) of rs868188 (S1456G) were reported to have a higher incidence of T2DM (Ma *et al.,* 2007). Rs868188 (S1456G) and rs945508 (R1467H) are non-synonymous SNPs that result in an amino acid substitution. In silico analysis predicted rs868188 (S1456G) to be “benign”, most likely having no phenotypic effect. Rs945508 (R1467H) was predicted to be “possibly damaging”, in that it may affect the protein function and/or structure of ARHGEF11. **Although we did not find significant allelic or genotypic associations or minor allele carrier frequencies, the allele frequencies of rs822585, rs6427340, rs6427339, and rs868188 (S1456G) (*P*=0.031, 0.036, 0.023, and**

0.020, respectively) and minor allele carrier frequencies of rs945508 (R1467H) (*P*=0.046) of ARHGEF11 showed marginally significant associations (P<0.05) between the paranoid subtype of schizophrenia and controls. Because our sample size was small after stratification by subtype of schizophrenia, we may need to confirm whether these results are only marginal or not using a larger sample.

It is reported that the expression levels of ARHGEF11 mRNA were higher in the postmortem thalamus of patients with schizophrenia than of controls (Davidkova *et al.*, 2003). Although the function of ARHGEF11 mRNA overexpressed in the thalamus is unclear, the thalamus provides a nodal link for multiple functional circuits, and schizophrenia-associated abnormalities in thalamic expression of genes could reflect aberrations in extrathalamic components of these circuits (Byne *et al.*, 2009). Furthermore, it is reported that abnormalities in the thalamus may relate to hallucinations in patients with schizophrenia (Martínez-Granados *et al.*, 2008). *ARHGEF11* may be associated with the function of the thalamus and related to positive symptoms in patients with schizophrenia.

Several points should be considered in the present study. The first is that the selection of the eight SNPs not based on a Tagger selection program such as Haploview. Therefore, further analysis of the remaning SNPs is needed to confirm that *ARHGEF11* is associated with schizophrenia. The second is that we subcategorized the subjects with schizophrenia according to ICD-10 criteria because the schizophrenia subtypes appear to be

more homogeneous than the broader disorder (Mirnics and Lewis, 2001). However, the subtype categories of schizophrenia were eliminated from the Diagnostic and Statistical Manual of Mental Disorders-5 due to their limited diagnostic stability, low reliability, and poor validity. Therefore, it might be useless to dichotomize the paranoid and hebephrenic subtypes. **The third is that we considered *P* values <0.05 or 0.016 as statistically significant, and therefore found significant associations. The *P* value for a significance threshold may be 0.0062 (0.05/8) or 0.0020 (0.05/8X3) instead of 0.05 or 0.016 if multiple allelic frequencies and genotypic distribution (8SNPs) are considered.** Although significant differences might be eliminated after correction for multiple testing, we found significant associations with schizophrenia in several haplotype combinations.

In conclusion, these findings indicated that *ARHGEF11* may affect the susceptibility to schizophrenia in a Japanese population. Further replication studies in distinct populations and larger sample are necessary to confirm the association between *ARHGEF11* and schizophrenia.

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References

Banerjee J, Wedegaertner PB. 2004. Identification of a novel sequence in PDZ-RhoGEF that mediates interaction with the actin cytoskeleton. *Mol Biol Cell* **15**: 1760-1775.

Böttcher Y, Schleinitz D, Tönjes A, Blüher M, Stumvoll M, Kovacs P. 2008. R1467H variant in the rho guanine nucleotide exchange factor 11 (ARHGEF11) is associated with impaired glucose tolerance and type 2 diabetes in German Caucasians. *J Hum Genet* **53**: 365-367.

Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS. 2000. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. *Science* **288**: 678-682.

Bushe C, Holt R. 2004. Prevalence of diabetes and impaired glucose tolerance in patients with schizophrenia. *Br J Psychiatry Suppl.* **47**: S67-71.

Byne W, Hazlett EA, Buchsbaum MS, Kemether E. 2009. The thalamus and schizophrenia: current status of research. *Acta Neuropathol* **117**: 347-368.

Craddock N, O'Donovan MC, Owen MJ. 2005. The genetics of schizophrenia and bipolar

disorder: dissecting psychosis. *J Med Genet* **42**: 193-204.

Das SK, Elbein SC. 2007. The search for type 2 diabetes susceptibility loci: the chromosome 1q story. *Curr Diab Rep* **7**: 154-164.

Davidkova G, McCullumsmith RE, Meador-Woodruff JH. 2003. Expression of ARHGEF11 mRNA in schizophrenic thalamus. *Ann NY Acad Sci* **1003**: 375-377.

Faul F, Erdfelder E, Lang AG, Buchner A. 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods* **39**: 175-191.

Fu M, Sabra MM, Damcott C, Pollin TI, Ma L, Ott S, Shelton JC, Shi X, Reinhart L, O'Connell J, Mitchell BD, Baier LJ, Shuldiner AR. 2007. Evidence that Rho guanine nucleotide exchange factor 11 (ARHGEF11) on 1q21 is a type 2 diabetes susceptibility gene in the Old Order Amish. *Diabetes* **56**: 1363-1368.

Fukuhara S, Murga C, Zohar M, Igishi T, Gutkind JS. 1999. A novel PDZ domain containing guanine nucleotide exchange factor links heterotrimeric G proteins to Rho. *J Biol Chem* **274**:

5868-5879.

Gough SC, O'Donovan MC. 2005. Clustering of metabolic comorbidity in schizophrenia: a genetic contribution? *J Psychopharmacol* **19**: 47-55.

Guest PC, Wang L, Harris LW, Burling K, Levin Y, Ernst A, Wayland MT, Umrania Y, Herberth M, Koethe D, van Beveren JM, Rothermundt M, McAllister G, Leweke FM, Steiner J, Bahn S. 2010. Increased levels of circulating insulin-related peptides in first-onset, antipsychotic naive schizophrenia patients. *Mol Psychiatry* **15**: 118-119.

Hayashi-Takagi A, Takaki M, Graziane N, Seshadri S, Murdoch H, Dunlop AJ, Makino Y, Seshadri AJ, Ishizuka K, Srivastava DP, Xie Z, Baraban JM, Houslay MD, Tomoda T, Brandon NJ, Kamiya A, Yan Z, Penzes P, Sawa A. 2010. Disrupted-in-Schizophrenia 1 (DISC1) regulates spines of the glutamate synapse via Rac1. *Nat Neurosci* **13**: 327-332

Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. 2002. A central role for JNK in obesity and insulin resistance. *Nature* **420**: 333-336.

Houssa B, de Widt J, Kranenburg O, Moolenaar WH, van Blitterswijk WJ. 1999.

Diacylglycerol kinase theta binds to and is negatively regulated by active RhoA. *J Biol Chem* **274**: 6820–6822.

Huerta I, McCullumsmith RE, Haroutunian V, Giménez-Amaya JM, Meador-Woodruff JH. 2006. Expression of excitatory amino acid transporter interacting protein transcripts in the thalamus in schizophrenia. *Synapse* **59**: 394-402.

Jin QS, Kim SH, Piao SJ, Lim HA, Lee SY, Hong SB, Kim YS, Lee HJ, Nam M. 2010. R1467H Variants of Rho guanine nucleotide exchange factor 11 (ARHGEF11) are associated with type 2 diabetes mellitus in Koreans. *Korean Diabetes J* **34**: 368-373.

Kowluru A, Veluthakal R. 2005. Rho guanosine diphosphate-dissociation inhibitor plays a negative modulatory role in glucose-stimulated insulin secretion. *Diabetes* **54**: 3523-3529.

Lin PI, Shuldiner AR. 2010. Rethinking the genetic basis for comorbidity of schizophrenia and type 2 diabetes. *Schizophr Res* **123**: 234-243.

Liu J, Chen X, Guo Q, Ma X, Zhang J, Huang X, Zhang X, Zhang S. 2011. Association of ARHGEF11 R1467H polymorphism with risk for type 2 diabetes mellitus and insulin

resistance in Chinese population. *Mol Biol Rep* **38**: 2499-2505.

Luo L. 2000. Rho GTPases in neuronal morphogenesis. *Nat Rev Neurosci* **1**: 173-180.

Ma L, Hanson RL, Que LN, Cali AM, Fu M, Mack JL, Infante AM, Kobes S; International Type 2 Diabetes 1q Consortium, Bogardus C, Shuldiner AR, Baier LJ. 2007. Variants in ARHGEF11, a candidate gene for the linkage to type 2 diabetes on chromosome 1q, are nominally associated with insulin resistance and type 2 diabetes in Pima Indians. *Diabetes* **56**: 1454-1459.

Martínez-Granados B, Brotons O, Martínez-Bisbal MC, Celda B, Martí-Bonmati L, Aguilar EJ, González JC, Sanjuán J. 2008. Spectroscopic metabolomic abnormalities in the thalamus related to auditory hallucinations in patients with schizophrenia. *Schizophr Res* **104**: 13-22.

Millar JK, Christie S, Porteous DJ. 2003. Yeast two-hybrid screens implicate DISC1 in brain development and function. *Biochem Biophys Res Commun* **311**: 1019-1025.

Mirnics K, Lewis DA. 2001. Genes and subtypes of schizophrenia. *Trends Mol Med* **7**: 281-283.

Rümenapp U, Blomquist A, Schwörer G, Schablowski H, Psoma A, Jakobs KH. 1999. Rho-specific binding and guanine nucleotide exchange catalysis by KIAA0380, a dbl family member. *FEBS Lett* **459**: 313-318.

Ryan MC, Collins P, Thakore JH. 2003. Impaired fasting glucose tolerance in first-episode, drug-naive patients with schizophrenia. *Am J Psychiatry* **160**: 284-289.

Scheen AJ, De Hert MA. 2007. Abnormal glucose metabolism in patients treated with antipsychotics. *Diabetes Metab* **33**: 169-175.

Spelman LM, Walsh PI, Sharifi N, Collins P, Thakore JH. 2007. Impaired glucose tolerance in first-episode drug-naive patients with schizophrenia. *Diabet Med* **24**: 481-485.

Suvisaari J, Perälä J, Saarni SI, Härkänen T, Pirkola S, Joukamaa M, Koskinen S, Lönnqvist J, Reunanen A. 2008. Type 2 diabetes among persons with schizophrenia and other psychotic disorders in a general population survey. *Eur Arch Psychiatry Clin Neurosci* **258**: 129-136.

Tschoner A, Engl J, Laimer M, Kaser S, Rettenbacher M, Fleischhacker WW, Patsch JR,

Ebenbichler CF. 2007. Metabolic side effects of antipsychotic medication. *Int J Clin Pract* **61**: 1356-1370.

Vionnet N, Hani EH, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Leprêtre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P. 2000. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. *Am J Hum Genet* **67**: 1470-1480.

Table 1.

Genotype and allele frequencies of eight single nucleotide polymorphisms of *ARHGEF11* in controls and patients with schizophrenia.

	Location	*n*	Genotype			*P* value	Minor allele carrier	*P* value	Allele		*P* value
rs822585	5' flanking		A/A (%)	A/T (%)	T/T (%)		A/T+T/T (%)		A (%)	T (%)	
Control		488	318 (65.2)	143 (29.3)	27 (5.5)		170 (34.8)		779 (79.8)	197 (20.2)	
Schizophrenia		462	280 (60.6)	153 (33.1)	29 (6.3)	0.34	182 (39.4)	0.15	713 (77.2)	211 (22.8)	0.15
Paranoid subtype		209	118 (56.4)	76 (36.4)	15 (7.2)	0.093	91 (43.6)	0.030	312 (74.6)	106 (25.4)	0.031
Hebephrenic subtype		228	145 (63.6)	70 (30.7)	13 (5.7)	0.91	83 (36.4)	0.68	360 (78.9)	96 (21.1)	0.70
rs6427340	Intron 2		C/C (%)	C/T (%)	T/T (%)		C/T+T/T (%)		C (%)	T (%)	
Control		490	323 (65.9)	141 (28.8)	26 (5.3)		167 (34.1)		787 (80.3)	193 (19.7)	
Schizophrenia		475	298 (62.7)	149 (31.4)	28 (5.9)	0.58	177 (37.3)	0.30	745 (78.4)	205 (21.6)	0.30
Paranoid subtype		215	124 (57.8)	76 (35.3)	15 (6.9)	0.10	91 (42.2)	0.036	324 (75.3)	106 (24.7)	0.036
Hebephrenic subtype		234	154 (65.8)	67 (28.6)	13 (5.6)	0.99	80 (34.2)	0.98	375 (80.1)	93 (19.9)	0.93
rs6427339	Intron 2		T/T (%)	T/C (%)	C/C (%)		T/C+C/C (%)		T (%)	C (%)	
Control		489	319 (65.2)	143 (29.3)	27 (5.5)		170 (34.8)		781 (79.9)	197 (20.1)	
Schizophrenia		475	296 (62.3)	139 (29.3)	40 (8.4)	0.19	179 (37.7)	0.35	731 (76.9)	219 (23.1)	0.12
Paranoid subtype		213	122 (57.3)	73 (34.3)	18 (8.4)	0.095	91 (42.7)	0.045	317 (74.4)	109 (25.6)	0.023
Hebephrenic subtype		235	153 (65.1)	60 (25.5)	22 (9.4)	0.12	82 (34.9)	0.97	366 (77.9)	104 (22.1)	0.38
rs1006168	Intron 6		A/A (%)	A/C (%)	C/C (%)		A/C+C/C (%)		A (%)	C (%)	
Control		493	194 (39.4)	232 (47.0)	67 (13.6)		299 (60.6)		620 (62.9)	366 (37.1)	
Schizophrenia		467	186 (39.8)	218 (46.7)	63 (13.5)	0.98	281 (60.2)	0.88	590 (63.2)	344 (36.8)	0.89
Paranoid subtype		212	78 (36.8)	104 (49.1)	30 (14.1)	0.81	134 (63.2)	0.52	260 (61.3)	164 (38.7)	0.57
Hebephrenic subtype		229	97 (42.4)	102 (44.5)	30 (13.1)	0.74	132 (57.6)	0.44	296 (64.6)	162 (35.4)	0.52
rs12136088	Intron 8		T/T (%)	T/G (%)	G/G (%)		T/G+G/G (%)		T (%)	G (%)	
Control		494	194 (39.3)	231 (46.8)	69 (13.9)		300 (60.7)		619 (62.7)	369 (37.3)	
Schizophrenia		474	192 (40.5)	221 (46.6)	61 (12.9)	0.85	282 (59.5)	0.69	605 (63.8)	343 (36.2)	0.59
Paranoid subtype		215	81 (37.7)	104 (48.4)	30 (13.9)	0.91	134 (62.3)	0.69	266 (61.9)	164 (38.1)	0.77
Hebephrenic subtype		233	99 (42.5)	106 (45.5)	28 (12.0)	0.63	134 (57.5)	0.41	304 (65.2)	162 (34.8)	0.33
rs2275199 (N1207N)	Exon 37		C/C (%)	C/T (%)	T/T (%)		C/T+T/T (%)		C (%)	T (%)	
Control		492	203 (41.3)	229 (46.5)	60 (12.2)		289 (58.7)		635 (64.5)	349 (35.5)	
Schizophrenia		480	198 (41.3)	217 (45.2)	65 (13.5)	0.80	282 (58.7)	1.00	613 (63.9)	347 (36.1)	0.75
Paranoid subtype		217	78 (35.9)	105 (48.4)	34 (15.7)	0.27	139 (64.1)	0.18	261 (60.1)	173 (39.9)	0.11
Hebephrenic subtype		236	107 (45.3)	101 (42.8)	28 (11.9)	0.56	129 (54.7)	0.30	315 (66.7)	157 (33.3)	0.40
rs868188 (S1456G)	Exon 39		A/A (%)	A/G (%)	G/G (%)		A/G+G/G (%)		A (%)	G (%)	
Control		496	168 (33.9)	223 (44.9)	105 (21.2)		328 (66.1)		559 (56.4)	433 (43.6)	
Schizophrenia		480	174 (36.3)	208 (43.3)	98 (20.4)	0.73	306 (63.7)	0.44	556 (57.9)	404 (42.1)	0.48
Paranoid subtype		216	86 (39.8)	100 (46.3)	30 (13.9)	0.056	130 (60.2)	0.13	272 (62.9)	160 (37.1)	0.020
Hebephrenic subtype		236	78 (33.1)	98 (41.5)	60 (25.4)	0.41	148 (66.9)	0.83	254 (53.8)	218 (46.2)	0.36
rs945508 (R1467H)	Exon 39		G/G (%)	G/A (%)	A/A (%)		G/A+A/A (%)		G (%)	A (%)	
Control		488	363 (74.4)	109 (22.3)	16 (3.3)		125 (25.6)		835 (85.6)	141 (14.4)	
Schizophrenia		484	348 (71.9)	115 (23.8)	21 (4.3)	0.56	136 (28.1)	0.38	811 (83.8)	157 (16.2)	0.27
Paranoid subtype		219	147 (67.1)	63 (28.8)	9 (4.1)	0.13	72 (32.9)	0.046	357 (81.5)	81 (18.5)	0.053
Hebephrenic subtype		238	180 (75.6)	46 (19.3)	12 (5.1)	0.36	58 (24.4)	0.72	406 (85.3)	70 (14.7)	0.89

Table 2.

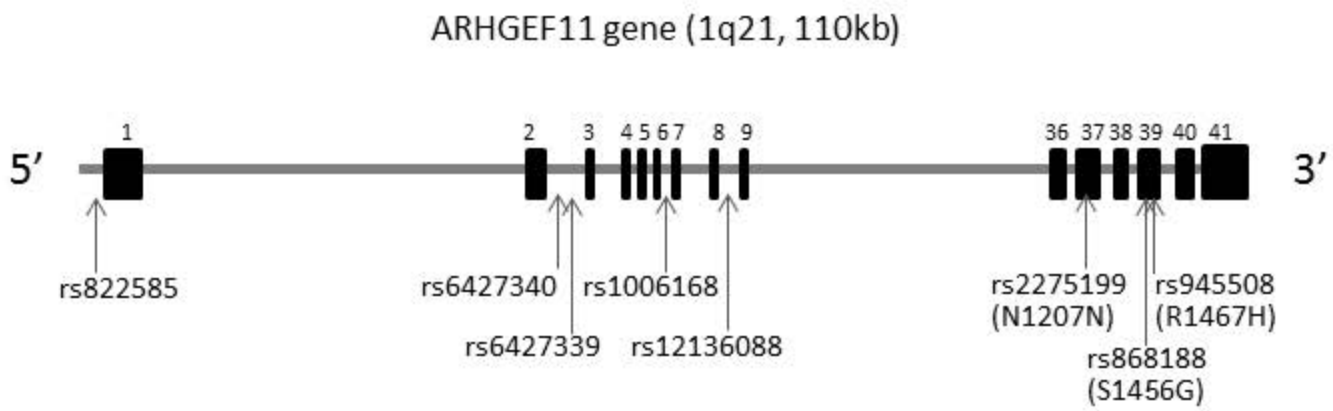
Pairwise linkage disequilibrium between eight single nucleotide polymorphisms of *ARHGEF11* .

	rs822585	rs6427340	rs6427339	rs1006168	rs12136088	rs2275199	rs868188	rs945508
rs822585		**0.92**	**0.85**	0.13	0.13	0.069	0.057	0.56
rs6427340	**0.98**		**0.90**	0.14	0.14	0.077	0.055	0.61
rs6427339	**0.93**	**0.97**		0.12	0.13	0.065	0.057	0.56
rs1006168	**0.89**	**0.97**	**0.86**		**0.97**	0.78	0.36	0.08
rs12136088	**0.91**	**0.98**	**0.90**	**0.98**		0.79	0.36	0.081
rs2275199	0.67	0.72	0.65	**0.90**	**0.91**		0.39	0.071
rs868188	0.53	0.53	0.52	**0.91**	**0.91**	**0.96**		0.12
rs945508	**0.92**	**0.93**	**0.92**	**0.86**	**0.87**	**0.83**	**0.97**	

Lower left and upper right diagonals show D' and r^2 values, respectively. D' values >0.8 or r^2 values >0.8 are shown in bold.

We define significant LD if D' and r^2 are greater than 0.8.

Rs822585, rs6427340, and rs6427339 constitute one block (block 1), and rs1006168 and rs12136088 constitute another block (block 2).


ARHGEF11 gene (1q21, 110kb)
5’
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rs822585
rs6427340
rs6427339
rs1006168
rs12136088
rs2275199
(N1207N)
rs868188
(S1456G)
rs945508
(R1467H)

Table 3.
Estimated haplotype frequencies and association significance for *ARHGEF11* .

	Haplotype	Haplotype frequency		Permutation *P* value	
Schizophrenia		Control	Schizophrenia	Global	Individual
Block1					
rs822585-rs6427340	A-C	0.80	0.77	0.27	0.13
	T-T	0.19	0.21		0.25
	T-C	0.007	0.01		0.2
rs6427340-rs6427339	C-T	0.80	0.76	**0.00047**	0.071
	T-C	0.19	0.21		0.55
	C-C	0.004	0.02		**0.0010**
rs822585-rs6427340-rs6427339	A-C-T	0.79	0.75	**0.0032**	**0.031**
	T-T-C	0.19	0.21		0.35
	A-C-C	0.004	0.02		**0.0018**
	T-C-T	0.007	0.01		0.21
Block2					
rs1006168-rs12136088	T-T	0.62	0.63	0.16	0.75
	G-G	0.37	0.36		0.63

	Haplotype	Haplotype frequency		Permutation *P* value	
Paranoid subtype		Control	Paranoid subtype	Global	Individual
Block 1					
rs822585-rs6427340	A-C	0.80	0.75	0.077	0.043
	T-T	0.19	0.24		0.025
rs6427340-rs6427339	C-T	0.80	0.74	**0.0018**	**0.016**
	T-C	0.19	0.24		0.080
	C-C	0.004	0.01		0.063
rs822585-rs6427340-rs6427339	A-C-T	0.79	0.74	**0.0077**	0.019
	T-T-C	0.19	0.24		0.049
	A-C-C	0.004	0.01		0.064
Block 2					
rs1006168-rs12136088	T-T	0.62	0.61	0.69	0.74
	G-G	0.37	0.38		0.67

	Haplotype	Haplotype frequency		Permutation *P* value	
Hebephrenic subtype		Control	Hebephrenic subtype	Global	Individual
Block 1					
rs822585-rs6427340	A-C	0.80	0.78	0.17	0.50
	T-T	0.19	0.19		0.94
	T-C	0.007	0.02		0.051
rs6427340-rs6427339	C-T	0.80	0.77	**0.000070**	0.26
	T-C	0.19	0.19		0.78
	C-C	0.004	0.03		**0.000050**
rs822585-rs6427340-rs6427339	A-C-T	0.79	0.76	**0.00025**	0.11
	T-T-C	0.19	0.19		0.89
	A-C-C	0.004	0.03		**0.000083**
	T-C-T	0.007	0.02		0.050
Block 2					
rs1006168-rs12136088	T-T	0.62	0.64	0.073	0.50
	G-G	0.37	0.34		0.32
	G-T	0.001	0.01		0.0077

Rs822585, rs6427340, and rs6427339 constitute block 1. Rs1006168 and rs12136088 constitute block 2.
Global permutation *P* values and individual permutation *P* values are indicated. Significant *P* values are in bold.
Statistical significance was $P < 0.05$. Comparisons among schizophrenia subtypes and controls reveal $P < 0.05/3=0.016$.
We limited haplotypes to those having frequencies of at least 1% either patients or controls.

Fig. 1.

Schematic diagram of the ARHGEF11 gene with introns and exons drawn to scale. The positions of the eight single nucleotide polymorphisms examined in this study are indicated.