incidence of gastrointestinal side effects but not for the incidence of all side effects. Furthermore, the A-1438G polymorphism had no significant effect on discontinuation in this study. Our results may indicate that the A-1438G polymorphism is only strongly related to the gastrointestinal side effects induced by fluvoxamine. While the subjects in the former study were 65 years of age or older, the mean age of the subjects in this study was 40.2±15.7 years. Since elderly patients are thought to have different pharmacodynamic and pharmacokinetic profiles from those of younger patients, the difference in age may explain the discrepancy between the two studies. In addition, the difference in medication, paroxetine vs. fluvoxamine, may cause disagreement of the results, since the two SSRIs have been reported to have different pharmacodynamic and pharmacokinetic profiles (Bourin et al, 2001). On the contrary, Yoshida et al (2003) reported that the A-1438G polymorphism of the 5-HT2A gene had no significant effect on the incidence of nausea. Their results disagree with those presented in this study. This may result from differences between the two studies in the numbers of subjects and other methodological points such as the dosage schedules and periods of observation.

A postmortem brain study found that the C allele of T102C (in complete linkage disequilibrium with the G allele of A-1438G) was associated with lower messenger ribonucleic acid (mRNA) and lower protein expression than the T allele (Polesskaya and Sokolov, 2002). Parsons et al (2004) reported that the presence of the A allele significantly increased promoter activity compared to the G allele. However, a study by Bray et al (2004) failed to replicate the differences in mRNA expression. Since the possible role of A-1438G in promoter function remains unclear, further studies are needed to clarify why this polymorphism affects the incidence of gastrointestinal side effects induced by fluvoxamine. Moreover, sleep disturbances (Landolt et al, 1999) and sexual dysfunction (Sargent et al, 1998) are preferentially associated with the 5-HT2A receptor and it has been reported that SSRI-induced gastrointestinal side effects are mediated by the 5-HT3 receptor (Bergeron and Blier, 1994). To our knowledge, there have been no previous studies investigating the relationship between polymorphisms of the 5-HT3A and 3B genes and the gastrointestinal side effects induced by SSRI. In the current study, the polymorphisms of the 5-HT3A and 3B genes had no significant effects on the onset of fluvoxamine-induced gastrointestinal side

effects. Tremblay et al (2003) reported that an insertion/deletion polymorphism in the promoter region of the 5-HT3B gene had a significant effect on the incidence of nausea and vomiting induced by cancer chemotherapy and that the Tyr129Ser polymorphism of the 5-HT3B gene, detected in this study, did not affect these side effects. On the other hand, Kaiser et al (2004) reported that polymorphisms of the 5-HT3A receptor gene may not serve as pharmacogenetic predictors of antiemetic treatment with 5-HT3 receptor antagonists in cancer patients. Since it is possible that polymorphisms of the 5-HT3A and 3B genes other than those detected in this study have significant effects on the gastrointestinal side effects, further studies are needed to clarify the impact of polymorphisms of the 5-HT3 gene on SSRI-induced gastrointestinal side effects.

Similar to A-1438G of the 5-HT2A receptor gene, the CYP2D6 polymorphism also showed a significant effect on the incidence of gastrointestinal side effects. Cox regression analysis showed that the combination of the A-1438G genotype and the CYP2D6 genotype could strongly predict the incidence of fluvoxamine-induced gastrointestinal side effects (Table 5). Indeed, there were six LMs of CYP2D6 who had the G/G genotype of the 5-HT2A receptor

gene, and all of them suffered from gastrointestinal side effects. Among 11 LMs of CYP2D6 who had the A/G genotype, nine (81.8%) suffered from gastrointestinal side effects. In clinical situations, taking account of these results, tailor-made pharmacotherapy for fluvoxamine based on genetic factors may be possible. For example, LMs with the G/G or A/G genotype should be treated by antidepressants other than SSRIs or should be treated at lower starting doses of fluvoxamine than the other patients. Kasper et al (1992) reported that an increased incidence of nausea is associated with higher plasma concentrations of fluvoxamine. Since it has been shown that the plasma concentrations of fluvoxamine depend on the CYP2D6 polymorphism, our results support the preceding study. However, Murphy et al (2003) reported that the CYP2D6 genotype did not influence the side effects from paroxetine. Gerstenberg et al (2003) also reported that the number of mutated CYP2D6 alleles was not related to the development of nausea induced by fluvoxamine. CYP2D6 gene polymorphisms are known to have ethnic differences, for example, the CYP2D6*10 allele, causing decreased enzyme activity, had a higher frequency in an Asian population (51%) (Johansson et al, 1994) than in a white population (2.8%) (Bertilsson and

Dahl, 1996). These ethnic differences in the genetic polymorphisms may produce the discrepancy between the results in Murphy et al (2003) and those in the present study. However, similar to this study, the subjects in the study by Gerstenberg et al were all Japanese patients. In the former study, the patients were divided into three genotype groups by the number of CYP2D6 mutated alleles: *1/*1, *1/*5 or *1/*10 and *5/*10 or *10/*10, whereas in this study, the patients were divided into two genotype groups by the degree of enzyme activity: *1/*1 or *1/*10 and *10/*10, *1/*5 or *5/*10. We previously reported that one *5 allele had a greater impact on the metabolism of haloperidol, a substrate of CYP2D6, than one *10 allele (Someya et al, 2003). Since *1/*10 has only one mutation causing decreased enzyme activity, it was supposed that the enzyme activity of *1/*10 was almost equal to that of the *1/*1 genotype in this study. These differences in analysis may explain the inconsistency between the two studies.

It was demonstrated that a pharmacodynamic factor such as the A-1438G polymorphism of the 5-HT2A receptor gene and a pharmacokinetic factor such as the CYP2D6 gene

polymorphism had synergistic effects on the prediction of the gastrointestinal side effects induced by fluvoxamine in Japanese depressed patients. However, since there have been several previous studies that were inconsistent with our results, much research remains to be done to explain the discrepancies.

Acknowledgements

This research was supported by Grants-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Research (JSPS) (#13670991 and #13671000).

References

Banes A, Florian JA, Watts SW. Mechanisms of 5-hydroxytryptamine(2A) receptor activation of the mitogen-activated protein kinase pathway in vascular smooth muscle. J Pharmacol Exp Ther. 1999 Dec;291(3):1179-87.

Bergeron R, Blier P. Cisapride for the treatment of nausea produced by selective serotonin reuptake inhibitors. Am J Psychiatry. 1994 Jul;151(7):1084-6.

Bertilsson, L & Dahl ML: Polymorphic drug oxidation. Relevance to the treatment of psychiatric disorders. (1996): *CNS drugs*, 5: 200-223.

Bourin M, Chue P, Guillon Y. Paroxetine: a review. CNS Drug Rev. 2001 Spring;7(1):25-47.

Bray NJ, Buckland PR, Hall H, Owen MJ, O'Donovan MC. The serotonin-2A receptor gene locus does not contain common polymorphism affecting mRNA levels in adult brain. Mol Psychiatry. 2004 Jan;9(1):109-14.

Erdmann J, Shimron-Abarbanell D, Rietschel M, Albus M, Maier W, Korner J, Bondy B, Chen K, Shih JC, Knapp M, Propping P, Nothen MM. Systematic screening for mutations in the human serotonin-2A (5-HT2A) receptor gene: identification of two

naturally occurring receptor variants and association analysis in schizophrenia. Hum Genet. 1996 May;97(5):614-9.

Gerstenberg G, Aoshima T, Fukasawa T, Yoshida K, Takahashi H, Higuchi H, Murata Y, Shimoyama R, Ohkubo T, Shimizu T, Otani K. Relationship between clinical effects of fluvoxamine and the steady-state plasma concentrations of fluvoxamine and its major metabolite fluvoxamino acid in Japanese depressed patients. Psychopharmacology (Berl). 2003 Jun;167(4):443-8.

Greenblatt DJ, von Moltke LL, Harmatz JS, et al. Drug interactions with newer antidepressants: Role of human cytochromesP450. *J Clin Psychiatry* 1998; 59[suppl 15]: 19-27.

Hartter S, Wetzel H, Hammes E, Torkzadeh M, Hiemke C. Serum concentrations of fluvoxamine and clinical effects. A prospective open clinical trial. Pharmacopsychiatry. 1998 Sep;31(5):199-200.

Janssen P, Prins NH, Meulemans AL, Lefebvre RA. Pharmacological characterization of the 5-HT receptors mediating contraction and relaxation of canine isolated proximal

stomach smooth muscle. Br J Pharmacol. 2002 May;136(2):321-9.

Johansson, I, Oscarson M, Yue Q, Bertilsson L, Sjöqvist F & Ingelman-Sundberg M: Genetic analysis of the Chinese cytochrome P4502D6 locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for deberisoquine hydroxylation. (1994): *Mol Pharmacol*, 46: 452-459.

Kaiser R, Tremblay PB, Sezer O, Possinger K, Roots I, Brockmoller J. Investigation of the association between 5-HT3A receptor gene polymorphisms and efficiency of antiemetic treatment with 5-HT3 receptor antagonists. Pharmacogenetics. 2004 May;14(5):271-8.

Kasper S, Fuger J, Moller HJ. Comparative efficacy of antidepressants. Drugs. 1992;43 Suppl 2:11-22; discussion 22-3.

Landolt HP, Meier V, Burgess HJ, Finelli LA, Cattelin F, Achermann P, Borbely AA. Serotonin-2 receptors and human sleep: effect of a selective antagonist on EEG power spectra. Neuropsychopharmacology. 1999 Sep;21(3):455-66

Murphy GM Jr, Kremer C, Rodrigues HE, Schatzberg AF. Pharmacogenetics of antidepressant medication intolerance. Am J Psychiatry. 2003 Oct;160(10):1830-5.

Niesler B, Flohr T, Nothen MM, Fischer C, Rietschel M, Franzek E, Albus M, Propping P, Rappold GA. Association between the 5' UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. Pharmacogenetics. 2001 Aug;11(6):471-5.

Parsons MJ, D'Souza UM, Arranz MJ, Kerwin RW, Makoff AJ. The -1438A/G polymorphism in the 5-hydroxytryptamine type 2A receptor gene affects promoter activity. Biol Psychiatry. 2004 Sep 15;56(6):406-10.

Polesskaya OO, Sokolov BP. Differential expression of the "C" and "T" alleles of the 5-HT2A receptor gene in the temporal cortex of normal individuals and schizophrenics. J Neurosci Res. 2002 Mar 15;67(6):812-22.

Sargent PA, Williamson DJ, Cowen PJ.Brain 5-HT neurotransmission during paroxetine treatment. Br J Psychiatry. 1998 Jan;172:49-52.

Someya T, Shimoda K, Suzuki Y, Sato S, Kawashima Y, Hirokane G, Morita S, Yokono A, Takahashi S. Effect of CYP2D6 genotypes on the metabolism of haloperidol in a Japanese psychiatric population. Neuropsychopharmacology. 2003 Aug;28(8):1501-5.

Steen, VM, Andreassen OA, Daly AK, Tefre T, Borresen A-L, Idle JR & Gulbrandsen A-K:

Detection of the poor metabolizer-associated CYP2D6(D) gene deletion allele by long-PCR technology. (1995): *Pharmacogenetics*, 5: 215-223.

Takahashi H, Yoshida K, Ito K, Sato K, Kamata M, Higuchi H, Shimizu T, Ito K, Inoue K, Tezuka T, Suzuki T, Ohkubo T, Sugawara K. No association between the serotonergic polymorphisms and incidence of nausea induced by fluvoxamine treatment. Eur Neuropsychopharmacol. 2002 Oct;12(5):477-81.

Tremblay PB, Kaiser R, Sezer O, Rosler N, Schelenz C, Possinger K, Roots I, Brockmoller J. Variations in the 5-hydroxytryptamine type 3B receptor gene as predictors of the efficacy of antiemetic treatment in cancer patients. J Clin Oncol. 2003 Jun 1;21(11):2147-55.

Trindade E, Menon D, Topfer LA, Coloma C. Adverse effects associated with selective serotonin reuptake inhibitors and tricyclic antidepressants: a meta-analysis. CMAJ. 1998 Nov 17;159(10):1245-52.

Trindade E, Menon D, Topfer LA, Coloma C.Adverse effects associated with selective serotonin reuptake inhibitors and tricyclic antidepressants: a meta-analysis. CMAJ. 1998

Nov 17;159(10):1245-52.

Yoshida K, Naito S, Takahashi H, Sato K, Ito K, Kamata M, Higuchi H, Shimizu T, Itoh K, Inoue K, Suzuki T, Ohkubo T. Monoamine oxidase A gene polymorphism, 5-HT 2A receptor gene polymorphism and incidence of nausea induced by fluvoxamine. Neuropsychobiology. 2003;48(1):10-3.

http://www.imm.ki.se/CYPalleles/

Figure legends

Figure 1. Effect of the A-1438G polymorphism of the 5-HT2A receptor gene on the cumulative 12 week incidence of gastrointestinal side effects induced by fluvoxamine.

Figure 2. Effect of the CYP2D6 genotype on the cumulative 12 week incidence of gastrointestinal side effects induced by fluvoxamine.

Figure 3. Combination effect of the A-1438G polymorphism of the 5-HT2A receptor gene and the CYP2D6 gene polymorphism on the cumulative 12 week incidence of gastrointestinal side effects induced by fluvoxamine.

TABLE 1. Characteristics of the demographic data and fluvoxamine-induced side effects by comparison of the A-1438G genotypes of the 5-HT2A receptor

	5-HT2A gene A-1438G polymorphism		
	A/A N=28	A/G N=41	G/G N=27
Sex (M/F)	12/16	24/17	11/16
Age	40.8 (17.6)	41.3 (15.4)	38.2 (14.7)
Baseline HAM-D-17 score	21.6 (5.2)	20.2 (5.4)	20.0 (4.9)
Discontinuation	7	11	7
P		0.986	
Gastrointestinal side effects			
Number of patients	10	25	18
Onset week	3.4 (2.9)	3.9 (2.7)	2.1 (2.0)
P		0.073	
Onset dose (mg)	60.0 (42.8)	75.0 (53.5)	45.8 (43.9)
P		0.16	
Onset concentration (ng/ml)	46.5 (70.3)	25.0 (27.8)	24.6 (32.9)
P		0.366	
Cumulative number of side effects	0.7 (1.2)	1.7 (2.0)	2.0 (2.7)
P		0.05	
All side effects			
Number of patients	21	34	22
Onset week	3.2 (2.9)	3.1 (2.8)	1.9 (1.7)
P		0.139	
Onset dose (mg)	56.0 (31.5)	62.5 (47.8)	39.8 (24.0)
P		0.097	
Cumulative number of side effects	2.8 (3.3)	5.3 (6.7)	5.0 (4.6)
P		0.147	

TABLE 2. Characteristics of the demographic data and fluvoxamine-induced side effects by comparison of the 5-HT3A receptor gene polymorphisms

	5-HT3A gene					
	Pro16Ser			C195T		
	Pro/Pro N=76	Pro/Ser N=18	Ser/Ser N=2	C/C N=8	C/T N=35	T/T N=50
Sex (M/F)	38/38	9/9	0/2	2/6	18/17	25/25
Age	41.2 (15.5)	39.1 (16.2)	17.0 (5.7)	29.3 (15.5)	38.5 (13.3)	43.5 (16.6)
Baseline HAM-D-17 score	20.1 (5.2)	21.8 (5.2)	24.5 (0.7)	22.3 (5.7)	19.9 (5.1)	20.6 (5.3)
Gastrointestinal side effects						
Number of patients	44	7	2	5	19	26
All side effects						
Number of patients	63	12	2	6	29	39

TABLE 3. Characteristics of the demographic data and fluvoxamine-induced side effects by comparison of the 5-HT3B receptor gene polymorphisms

	5-HT3B gene		
	Tyr129Ser		
	Tyr/Tyr N=54	Tyr/Ser N=37	Ser/Ser N=5
Sex (M/F)	29/25	17/20	1/4
Age	40.2 (16.6)	39.8 (15.4)	44.4 (9.9)
Baseline HAM-D-17 score	21.2 (5.2)	20.1 (5.3)	17.2 (4.3)
Gastrointestinal side effects			
Number of patients	28	22	3
All side effects			
Number of patients	42	30	5

TABLE 4. Characteristics of the demographic data and fluvoxamine-induced side effects by comparison of the CYP2D6 genotype groups

	CYP2D6 genotype (phenotype)		
	*1/*1, *1/*10 (NMs) N=75		*1/*5, *10/*10, *5/*10 (LMs) N=22
Sex (M/F)	35/40		12/10
Age	39.1 (16.1)		43.8 (14.2)
Baseline HAM-D-17 score	20.3 (5.3)		21.4 (5.0)
Discontinuation	17		8
P		0.267	
Gastrointestinal side effects			
Number of patients	37		17
Onset week	3.1 (2.7)		3.4 (2.6)
P		0.723	
Onset dose (mg)	64.9 (50.5)		58.8 (47.6)
P		0.679	
Onset concentration (ng/ml)	29.7 (44.8)		27.4 (30.3)
P		0.855	
Cumulative number of side effects	2.7 (2.3)		2.8 (1.9)
P		0.849	
All side effects			
Number of patients	58		20
Onset week	2.7 (2.4)		3.3 (3.2)
P		0.357	
Onset dose (mg)	56.0 (37.3)		51.3 (44.0)
P		0.638	
Cumulative number of side effects	5.3 (4.5)		6.8 (7.7)
P		0.285	

TABLE 5. Combination effect of the A-1438G polymorphism of the 5-HT2A receptor and the CYP2D6 phenotype

	Hazard ratio	95% CI	*P*
Sex	0.65	0.368-1.146	0.136
Age	0.986	0.968-1.005	0.148
Baseline HAM-D-17 score	1.045	0.988-1.106	0.124
Combination of 5-HT2A and CYP2D6 polymorphisms			
LMs with A/A	0.859	0.179-4.122	0.849
NMs with A/G	1.681	0.717-3.939	0.232
LMs with A/G	4.147	1.558-11.038	0.004
NMs with G/G	2.491	0.997-6.223	0.051
LMs with G/G	4.242	1.444-12.459	0.009

Figure 1

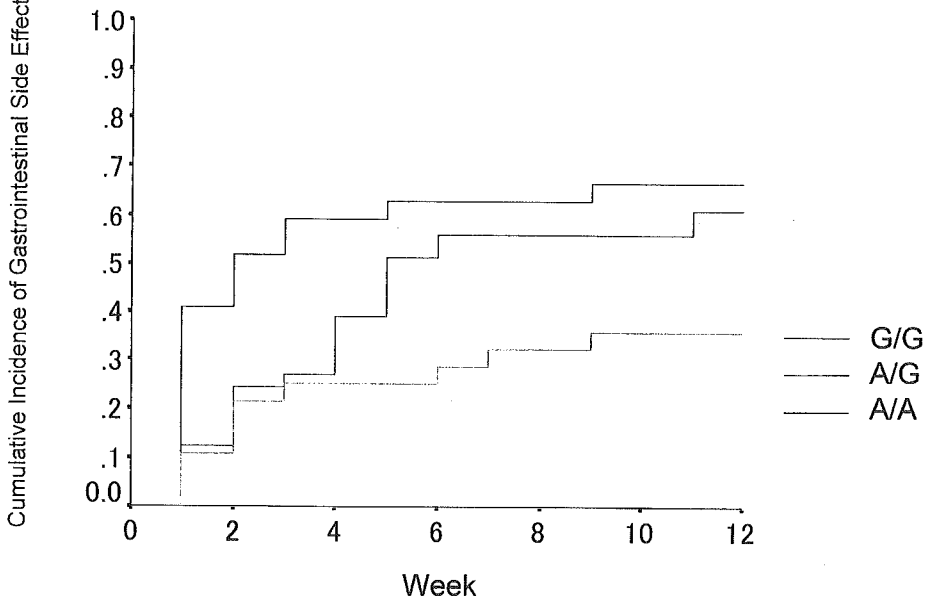


Figure 2

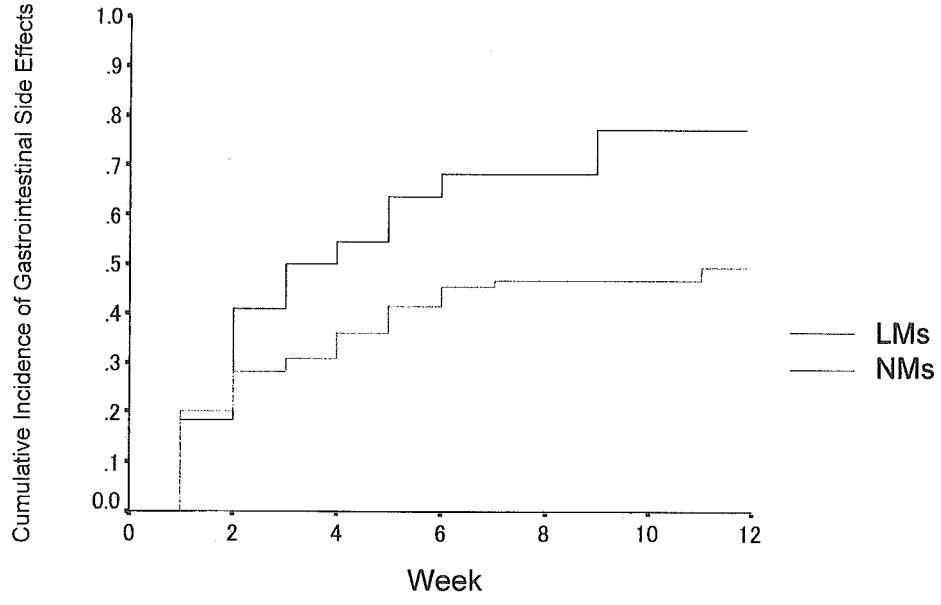
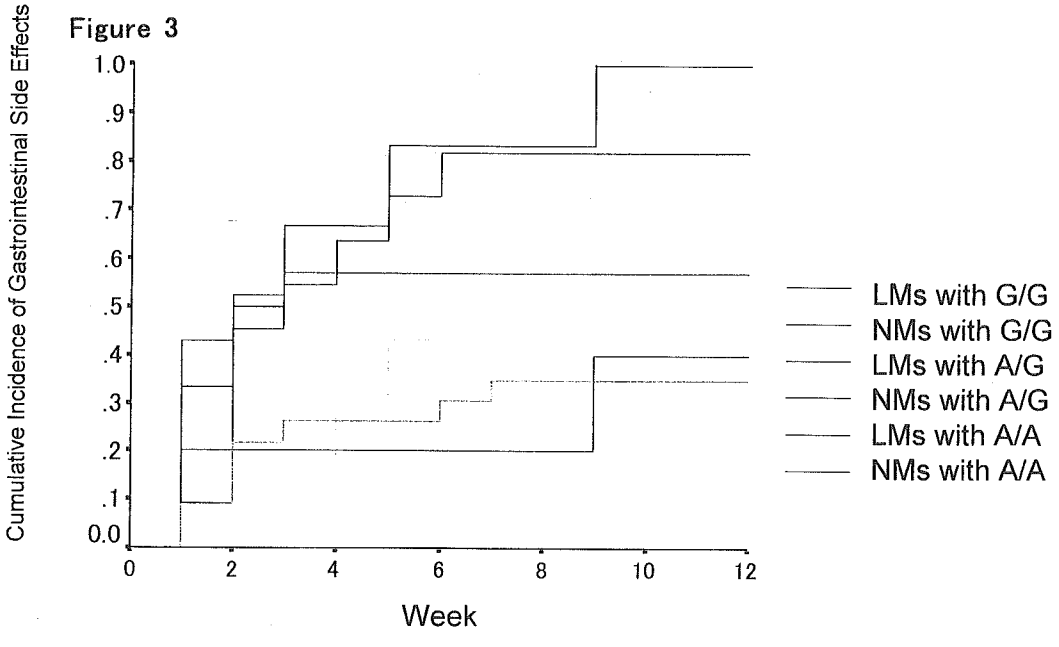


Figure 3



Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 396 (2006) 117–120
Neuroscience Letters
www.elsevier.com/locate/neulet

Supportive evidence for *neuregulin* 1 as a susceptibility gene for schizophrenia in a Japanese population

Naoki Fukui, Tatsuyuki Muratake, Naoshi Kaneko, Hideki Amagane, Toshiyuki Someya *

Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, 757 Asahimachi-ichibancho, Niigata 951-8510, Japan

Received 26 July 2005; received in revised form 18 October 2005; accepted 8 November 2005

Abstract

Schizophrenia is a complex genetic disorder and affects approximately 1% of the population worldwide. Recently, Stefansson et al. identified *neuregulin* 1 (*NRG*1) on 8p12 as a susceptibility gene for schizophrenia in the Icelandic population. It was reported that the at-risk haplotype ("HapICE") constructed from five SNPs and two microsatellite markers was found to be over-represented in patients with schizophrenia compared to controls. Since then several independent studies have supported the association of *NRG*1 with schizophrenia. We performed a case–control association study using the four SNPs in a Japanese sample. We genotyped three SNPs (SNP8NRG221533, SNP8NRG241930, and SNP8NRG243177) from Stefansson et al. and one SNP (rs1081062) located in intron 1 of *NRG*1. There were no significant differences in allele frequencies for each SNP between cases and controls, however, homozygotes of minor alleles in SNP8NRG241930, SNP8NRG243177, and rs1081062 were associated with an increased risk of schizophrenia ($P=0.025$, OR = 4.14; $P=0.041$, OR = 1.43; and $P=0.0023$, OR = 3.06, respectively). Furthermore, the haplotype constructed from four SNPs shows a significant association with schizophrenia (permutation $P=0.026$). Our data support the hypothesis that *NRG*1 gene is a susceptibility gene for schizophrenia.

Keywords: Schizophrenia; *Neuregulin* 1; Case–control study; Single nucleotide polymorphism; Haplotype

Schizophrenia is a common mental disorder, which affects ~1% of the general population worldwide. Although the genetic factors of schizophrenia still remain to be elucidated, several genome scan studies have identified a number of sites on the human genome associated with risk of schizophrenia. A genome-wide scan, which took advantage of the extensive pedigree information and the relative genetic isolation of the Icelandic population, identified *neuregulin* 1 (*NRG*1) on 8p12 as a susceptibility locus for schizophrenia [7]. The Icelandic study identified an at-risk haplotype (HapICE) consisting of five SNPs (SNP8NRG221132, SNP8NRG221533, SNP8NRG241930, SNP8NRG243177, and SNP8NRG433E1006), and two microsatellite markers at the 5′ end of the *NRG*1 gene. HapICE is present at a frequency of 15.4% in affected individuals, compared to 7.5% in controls (relative risk ratio, 2.1). In a follow-up study in Scottish patients, an estimated risk ratio of 1.8 was observed with HapICE [6].

Subsequently, several replication studies have reported an association between the 5′ end of the *NRG*1 gene and schizophrenia, an association not only with HapICE but also other haplotypes incorporated in further polymorphisms. Williams et al. [9] replicated these findings using one SNP (SNP8NRG221533) and two microsatellite markers of HapICE with British and Irish samples (relative risk ratio, 1.2). Yang et al. [10] reported a positive association in the Han Chinese population using one SNP from HapICE and two other SNPs located in the middle of *NRG*1 gene. Another independent analysis using 13 microsatellite markers located at the 5′ end of the *NRG*1 gene found two groups of haplotypes, which were significantly associated with schizophrenia in the Han Chinese population [8]. Li et al. [4] identified an associated haplotype at the same position as HapICE, albeit with a different set of markers and alleles, in the Han Chinese population. In another Chinese study, Zhao et al. [11] genotyped three of the five SNPs and the two microsatellite markers of HapICE, and found an associated haplotype constructed from different alleles of HapICE. Furthermore, in an Irish study, Corvin et al. [2] found an association with a 2-marker-haplotype, which shared common alleles with 1 of HapICE.

* Corresponding author. Tel.: +81 25 227 2209; fax: +81 25 227 0777.
E-mail address: someya@med.niigata-u.ac.jp (T. Someya).

0304-3940/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2005.11.015

On the other hand, Iwata et al. [3] failed to find an association between HapICE in the *NRG*1 gene and schizophrenia using large Japanese samples. In the Japanese study, HapICE is present at a frequency of 4.5% in affected individuals and 4.3% in controls; therefore, HapICE was reported not to be a risk for schizophrenia in a Japanese population. There is a possibility, however, that another haplotype besides HapICE is associated with schizophrenia in a Japanese population from the results of the previous studies mentioned above. Here, we performed a case–control association study to find a different haplotype associated with the risk of schizophrenia using SNPs at the 5′ end of the *NRG*1 gene.

The subjects in this study were 349 schizophrenic patients (161 females and 188 males) and 424 control subjects (207 females and 217 males). All of the subjects were Japanese and the controls were healthy volunteers with no history of psychiatric disorders. The mean ages and S.D. of the cases and the controls were 41.9 ± 14.9 and 38.3 ± 8.9 years, respectively. All patients were diagnosed as having schizophrenia according to the DSM-IV criteria [1]. The mean ages and S.D. of onset is 23.7 ± 7.9 and 35% of the patients had family history of schizophrenia in their first relatives. None of the patients or the controls was related. All subjects were residents of the Niigata area in northern central Japan. Written informed consent was obtained from all patients prior to their participation in this study. This study was approved by the Ethics Committee on Genetics of Niigata University School of Medicine.

We selected three SNPs (SNP8NRG221533, SNP8NRG-241930, and SNP8NRG243177) from an Icelandic study (http://www.decode.com/nrg1/markers/). These three SNPs were located upstream from exon 1. Because two microsatellite markers of HapICE located in intron 1 were not genotyped in this study, to cover exon 1, we selected one SNP (rs1081062) from dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/). This SNP (rs1081062) was located in intron 1 and was located closer to exon 1 than two microsatellite markers of HapICE. We did not examine SNP8NRG221132 and SNP8NRG433E1006, which construct HapICE, because the minor allele frequencies of the SNPs were very low (0.000 and 0.011, respectively) in the Japanese population [3]. These four SNPs were genotyped by TaqMan assay. The probe sets were designed and synthesized by Applied Biosystems, USA. We carried out PCR using TaqMan Universal Master Mix, No AmpErase UNG (Applied Biosystems), 5 ng of DNA from leukocytes of the subjects, 0.9 μM of each primer and 200 nM of each probe in a total volume of 5 μl. Each 96-well plate contained 94 samples and two no-DNA template controls. Thermal cycler conditions were 95 °C for 10 min, 40 cycles of 92 °C for 15 s, and 60 °C for 1 min. Fluorescence and allelic discrimination were measured with an ABI PRISM 7900HT Sequence Detection System using SDS 2.0 software (Applied Biosystems).

We analyzed association and Hardy–Weinberg equilibrium using the χ^2 test or Fisher exact test. Haplotype frequencies were estimated using the expectation maximization algorithm with the SNPAlyse program (Dynacom, Japan). Pair-wise linkage disequilibrium (LD) indices, D' and r^2, were calculated for all subjects. Because we identified modest LD among four SNPs, we assessed haplotype association between cases and controls. The level of significance was set at 0.05.

The genotype frequencies of all SNPs in the control group were not significantly different from the values expected from the Hardy–Weinberg equilibrium (all of the $P > 0.05$, d.f. = 2). There were no significant differences in allele frequencies for each SNP between cases and controls. On the contrary, homozygotes of minor allele at SNP8NRG241930, SNP8NRG243177, and rs1081062 have been associated with an increased risk of schizophrenia (Table 1). This result may indicate that these homozygotes, rather than heterozygotes, confer susceptibility to schizophrenia by altering expression of *NRG*1 gene. Furthermore, the haplotype constructed from SNP8NRG221533, SNP8NRG241930, SNP8NRG243177, and rs1081062 showed a significant association with schizophrenia (Table 2A). Haplotype0000, which was constructed from major alleles of all SNPs, occurred more frequently in controls than in cases. On the other hand, haplotype0001 and haplotype0110, which contain minor alleles of rs1081062 or SNP8NRG241930 and SNP8NRG243177 occurred more frequently in cases than in controls (Table 2B). These results suggest that a combination of major alleles could be associated with a decreased risk of schizophrenia and minor alleles of SNP8NRG241930, SNP8NRG243177, and rs1081062 associated with an increased risk of schizophrenia in a Japanese population.

This study indicates that the *NRG*1 gene is associated with an increased risk of schizophrenia in a Japanese population. However, Iwata et al. failed to replicate the results reported by Stefannsson et al. using a larger Japanese sample. One of the discrepancies between our results and those of Iwata et al. is that, in our analysis, genotypes in SNP8NRG241930 and SNP8NRG243177, showed a significant association with schizophrenia. There may be differences in the clinical backgrounds that influence the results of a genotype analysis between our samples and those of Iwata et al. For example, Iwata et al.'s paper showed 11.5% of the patients had family history of schizophrenia, on the other hand, 35% of our patients had family history of schizophrenia in their first relatives. This difference may influence the results of analyses between two studies.

In our haplotype analysis, the frequency of 3-SNP-haplotype, 000 (SNP8NRG221533–SNP8NRG241930–SNP8NRG2431-77), which is constructed from the same SNP alleles of HapICE, were 48.0% in the controls and 46.0% in the cases (permutation $P = 0.428$, detail data not shown). We did not find an association between the haplotype constructed from same SNP alleles of HapICE and schizophrenia, so this result does not contradict the results of Iwata et al. Only 4-SNP-haplotype, 0110 and 0001 constructed from SNP8NRG221533, SNP8NRG241930, SNP8NRG243177, and rs1081062, shows a significant association with schizophrenia. This may indicate that this 4-SNP-haplotype is the risk haplotype of the *NRG*1 gene in a Japanese population.

In comparison with the results reported by the Icelandic study, we found differences in allele frequencies between Icelandic and Japanese schizophrenia groups (this study versus Icelandic study: SNP8NRG221533(C); 53.1% versus 36.4%, SNP8NRG241930(G); 89.4% versus 68.3%,

Table 1
Allelic and genotypic analysis

Marker name, intermarker distance (kb)	LD[a]	Subject group (number)	Minor allele (frequency)	Genotype (frequency)			Allele 1 vs. allele 0		Genotype 1.1 vs. 1.0+0.0		
				1.1	1.0	0.0	χ^2	P	Odd ratio (95% CI)	χ^2	P
SNP8NRG221533, 20.2		Case (349)	326 (0.467)	76 (0.218)	174 (0.499)	99 (0.284)	0.035	0.85	1.16 (0.818–1.65)	0.70	0.42
	$D'=0.17$, $r^2=0.004$	Control (424)	392 (0.462)	82 (0.193)	228 (0.537)	114 (0.269)					
SNP8NRG241930, 1.3		Case (349)	90 (0.129)	10 (0.0287)	70 (0.201)	269 (0.771)	1.9	0.16	4.14 (1.13–15.2)	–	0.025[b]
	$D'=0.984$, $r^2=0.143$	Control (424)	90 (0.106)	3 (0.0071)	84 (0.198)	337 (0.795)					
SNP8NRG243177, 4.7		Case (349)	346 (0.496)	88 (0.252)	170 (0.487)	91 (0.261)	3.1	0.076	1.43 (1.01–2.01)	4.2	0.041
	$D'=0.260$, $r^2=0.009$	Control (424)	382 (0.451)	81 (0.191)	220 (0.519)	123 (0.290)					
rs1081062		Case (349)	104 (0.149)	24 (0.0688)	56 (0.161)	269 (0.771)	2.7	0.098	3.06 (1.44–6.48)	9.3	0.0023
		Control (424)	102 (0.120)	10 (0.0236)	82 (0.193)	332 (0.783)					

SNP8NRG221533; 0 = C*, 1 = T, SNP8NRG241930; 0 = G*, 1 = T, SNP8NRG243177; 0 = T*, 1 = C, rs1081062; 0 = T, 1 = C; (*) same alleles constructed HapICE.
[a] Linkage disequilibrium of adjacent SNPs is shown by D' and r^2.
[b] Calculated by Fisher exact test.

Table 2A
Haplotype analysis

Marker name	Permutation P		
	Two-SNP	Three-SNP	Four-SNP
SNP8NRG221533			
	0.176		
SNP8NRG241930		0.054	
	0.117		0.026
SNP8NRG243177		0.088	
	0.077		
rs1081062			

Table 2B
Frequency of individual haplotypes constructed from four SNPs

Haplotype	Frequency in subjects		Permutation P
	Case	Control	
0000	0.365	0.419	0.031
1010	0.322	0.302	0.43
0001	0.094	0.061	0.035
0110	0.061	0.030	0.0044
1110	0.060	0.063	0.77
1011	0.038	0.026	0.34
1000	0.037	0.056	0.091

0 = major allele; 1 = minor allele; the marker order, from left to right, is as follows: SNP8NRG221533, SNP8NRG241930, SNP8NRG243177 and rs1081062.

SNP8NRG243177(T); 54.4% versus 38.9%). These differences indicate that the same functional variant, which has not been identified, may be carried on different alleles or haplotypes in different populations.

Previously, several reports on the association between schizophrenia and *NRG1* gene, including our study, identified associated SNPs and haplotypes at 5′ region of *NRG1* gene. Interestingly, in the latest study, Petryshen et al. [5] identified associated SNPs and haplotypes at 3′ region of the *NRG1* gene in the Portuguese population. One of the associated haplotypes spans an exon encoding a domain only used by the sensory and motor neuron-derived factor (SMDF) variant of the *NRG1* gene. Furthermore, this Portuguese study reported that SMDF expression in peripheral leukocytes is significantly higher in schizophrenic patients than in their unaffected siblings.

HapICE and our risk haplotype are located around exon 1, which encodes the GGF2 glial growth factor 2 (GGF2) variant of *NRG1* gene. Unfortunately, we do not have an answer as to whether our risk haplotypes alter the expression of the GCF2 variant, or whether these haplotypes are related indirectly to SMDF expression in a linkage disequilibrium manner with its regulatory region. These issues should be resolved to understand the pathogenesis of schizophrenia.

Acknowledgements

We would like to express our deep gratitude to all of the patients. We thank Hiroshi Kusano, Miki Inomata and Hiromi

Tanabe for excellent technical assistance and Yuuichiro Watanabe for reviewing the manuscript.

References

[1] American Psychiatric Association, The Diagnostic and Statistical Manual for the DSM-IV, American Psychiatric Association, 1994.

[2] A.P. Corvin, D.W. Morris, K. McGhee, S. Schwaiger, P. Scully, J. Quinn, D. Meagher, D.S. Clair, J.L. Waddington, M. Gill, Confirmation and refinement of an 'at-risk' haplotype for schizophrenia suggests the EST cluster, Hs.97362, as a potential susceptibility gene at the *neuregulin*-1 locus, Mol. Psychiatry 9 (2004) 208–212.

[3] N. Iwata, T. Suzuki, M. Ikeda, T. Kitajima, Y. Yamanouchi, T. Inada, N. Ozaki, No association with the *neuregulin* 1 haplotype to Japanese schizophrenia, Mol. Psychiatry 9 (2004) 126–127.

[4] T. Li, H. Stefansson, E. Gudfinnsson, G. Cai, X. Liu, R.M. Murray, V. Steinthorsdottir, D. Januel, V.G. Gudnadottir, H. Petursson, A. Ingason, J.R. Gulcher, K. Stefansson, D.A. Collier, Identification of a novel *neuregulin* 1 at-risk haplotype in Han schizophrenia Chinese patients, but no association with the Icelandic/Scottish risk haplotype, Mol. Psychiatry 9 (2004) 698–704.

[5] T.L. Petryshen, F.A. Middleton, A. Kirby, K.A. Aldinger, S. Purcell, A.R. Tahl, C.P. Morley, L. McGann, K.L. Gentile, G.N. Rockwell, H.M. Medeiros, C. Carvalho, A. Macedo, A. Dourado, J. Valente, C.P. Ferreira, N.J. Patterson, M.H. Azevedo, M.J. Daly, C.N. Pato, M.T. Pato, P. Sklar, Support for involvement of *neuregulin* 1 in schizophrenia pathophysiology, Mol. Psychiatry 10 (2005) 366–374.

[6] H. Stefansson, J. Sarginson, A. Kong, P. Yates, V. Steinthorsdottir, E. Gudfinnsson, S. Gunnarsdottir, N. Walker, H. Petursson, C. Crombie, A. Ingason, J.R. Gulcher, K. Stefansson, D. St Clair, Association of *neuregulin* 1 with schizophrenia confirmed in a Scottish population, Am. J. Hum. Genet. 72 (2003) 83–87.

[7] H. Stefansson, E. Sigurdsson, V. Steinthorsdottir, S. Bjornsdottir, T. Sigmundsson, S. Ghosh, J. Brynjolfsson, S. Gunnarsdottir, O. Ivarsson, T.T. Chou, O. Hjaltason, B. Birgisdottir, H. Jonsson, V.G. Gudnadottir, E. Gudmundsdottir, A. Bjornsson, B. Ingvarsson, A. Ingason, S. Sigfusson, H. Hardardottir, R.P. Harvey, D. Lai, M. Zhou, D. Brunner, V. Mutel, A. Gonzalo, G. Lemke, J. Sainz, G. Johannesson, T. Andresson, D. Gudbjartsson, A. Manolescu, M.L. Frigge, M.E. Gurney, A. Kong, J.R. Gulcher, H. Petursson, K. Stefansson, *Neuregulin* 1 and susceptibility to schizophrenia, Am. J. Hum. Genet. 71 (2002) 877–892.

[8] J.X. Tang, W.Y. Chen, G. He, J. Zhou, N.F. Gu, G.Y. Feng, L. He, Polymorphisms within 5′ end of the *neuregulin* 1 gene are genetically associated with schizophrenia in the Chinese population, Mol. Psychiatry 9 (2004) 11–12.

[9] N.M. Williams, A. Preece, G. Spurlock, N. Norton, H.J. Williams, S. Zammit, M.C. O'Donovan, M.J. Owen, Support for genetic variation in *neuregulin* 1 and susceptibility to schizophrenia, Mol. Psychiatry 8 (2003) 485–487.

[10] J.Z. Yang, T.M. Si, Y. Ruan, Y.S. Ling, Y.H. Han, X.L. Wang, M. Zhou, H.Y. Zhang, Q.M. Kong, C. Liu, D.R. Zhang, Y.Q. Yu, S.Z. Liu, G.Z. Ju, L. Shu, D.L. Ma, D. Zhang, Association study of *neuregulin* 1 gene with schizophrenia, Mol. Psychiatry 8 (2003) 706–709.

[11] X. Zhao, Y. Shi, J. Tang, R. Tang, L. Yu, N. Gu, G. Feng, S. Zhu, H. Liu, Y. Xing, S. Zhao, H. Sang, Y. Guan, D. St Clair, L. He, A case control and family based association study of the *neuregulin* 1 gene and schizophrenia, J. Med. Genet. 41 (2004) 31–34.