Abstract

Background: Several lines of evidence have supported possible roles of the sigma receptors in the etiology of schizophrenia and mechanisms of antipsychotic efficacy. An association study provided genetic evidence that the sigma receptor type 1 gene ($\text{SIGMAR1}$) was a possible susceptibility factor for schizophrenia, however, it was not replicated by a subsequent study. It is necessary to evaluate further the possibility that the $\text{SIGMAR1}$ gene is associated with susceptibility to schizophrenia.

Methods: A case-control association study between two polymorphisms of the $\text{SIGMAR1}$ gene, G-241T/C-240T and Gln2Pro, and schizophrenia in Japanese population, and meta-analysis including present and previous studies.

Results: There was no significant association of any allele or genotype of the polymorphisms with schizophrenia. Neither significant association was observed with hebephrenic or paranoid subtype of schizophrenia. Furthermore, a meta-analysis including the present and previous studies comprising 779 controls and 636 schizophrenics also revealed no significant association between the $\text{SIGMAR1}$ gene and schizophrenia.

Conclusion: In view of this evidence, it is likely that the $\text{SIGMAR1}$ gene does not confer susceptibility to schizophrenia.
various tissues and supposed to be involved in diverse physiological functions. Especially, substantial roles of sigma receptors in pathogenesis of psychoses including schizophrenia have been suggested by many preclinical and clinical studies. In preclinical studies, putative sigma receptor antagonists including BMY-14802 and NE-100 improved two classes of animal models of schizophrenia, behavioral sensitization to psychostimulants [3-5] and phencyclidine-induced cognitive dysfunction [6]. Binding studies revealed that sigma receptors are expressed densely in mesolimbic and mesocortical areas including cortex and hippocampus [7,8], where structural abnormalities were reported in schizophrenic brains. In addition, postmortem studies showed a reduced sigma receptor density in the brain of schizophrenia compared to that of controls [9,10]. In clinical trials, putative selective antagonists of sigma receptors, such as SL 82.0715 and panamesine, showed successful therapeutic effects for schizophrenia [11-13]. Most conventional neuroleptics showed a significant affinity to sigma receptors. Among them, haloperidol, which has a potent antipsychotic effects in treatment of schizophrenia, showed a most potent affinity to sigma receptors [7].

Based on these rationales, Ishiguro et al. [14] screened promoter and exonic regions of sigma receptor type 1 gene (SIGMAR1 or SR-BP1, OMIM No. *601978), and found two polymorphisms, G-241T/C-240T (rs1799729) and Gln2Pro (A61C, rs1800866). They showed that the two polymorphisms were in complete linkage disequilibrium with each other, and a significant association between the TT-241-240/Pro2 haplotype and schizophrenia in a Japanese population (odds ratio = 1.27, P = 0.04). However, this result was not replicated by a subsequent study. Thus, Ohmori et al. [15] examined an association study using the same polymorphisms of the SIGMAR1 gene and found no significant difference in the allelic or genotypic distribution between schizophrenic patients and controls. They found slight increased homozygosity for TT-241-240 and Pro2 in schizophrenics compared with controls (P = 0.045), but the significance did not remain after a Bonferoni correction was applied to avoid false results. Therefore, it should be necessary to clarify further whether TT-241-240/Pro2 haplotype of the SIGMAR1 gene is a risk factor for susceptibility of schizophrenia. To this end, we examined association study using these polymorphisms of the SIGMAR1 gene in schizophrenic in Japanese population and meta-analysis of the present and previous results.

**Methods**

**Subjects**

Patient with schizophrenia and control subjects were recruited from the middle western area of Japan. They were provided with information about this study and gave their written informed consent to participate. A total of 198 schizophrenia patients (106 men and 92 women; mean age, 45.1 ± 12.5 y) fulfilling the international statistical classification of disease, revision 10 (ICD-10) diagnostic criteria for schizophrenia were registered in five hospitals: Okayama university graduate school of medicine and dentistry, Zikei hospital, Kibougaoka hospital, Aoyama hospital, Yui hospital. Assessment for diagnosis and subtypes of schizophrenia was carried out by two trained psychiatrists on the basis of all available information. Schizophrenia subtypes included 90 participants of the paranoid type, 107 participants of the hebephrenic type and 2 of the catatonic type. A total of 206 healthy volunteers recruited primarily from medical staff were examined as age- and gender-matched controls (106 men and 100 women; mean age, 42.1 ± 13.3 y). Only unaffected subjects who had no known past and family history of major psychiatric disorders were included in this study. This study was approved by the Ethics Committee of all five hospitals.

**Mutation screening and genotyping**

The genomic DNA was extracted from peripheral leukocytes using standard procedures. 5' flanking region (up to -585 bp) and each of the four exons with exon-intron boundary of the SIGMAR1 gene was amplified by polymerase chain reaction (PCR) in a total volume of 15 µl with 10% dimethyl sulfoxide and 0.75 units superTaq DNA polymerase (Sawady Technology, Japan) in the reaction mixture using the corresponding primer pairs (for 5' flanking to exon3; 5'-TGGTGGAAAGTCTCCACAGAG ATGA-3' (position of NT 8413 is 34629678-99), 5'-GTCACCCTCCACACGACTCCACAGTCC-3' (34628383-60), for exon 4; 5'-GAGACGGTAGTAACACGGCGCTTGCTGTG-3' (34625854-30), 5'-GTTAGTGAGTCAACAGTGAGTGAAGTG-3' (34625318-42). The PCR products from 25 subjects (15 schizophrenics and 10 controls) were directly sequenced to screen novel polymorphism (sequence primer for 5' flanking to exon3, 5'-GCCCTCCACACTGCCAGATCTCGT-3' (34628379-56), 5'-TGACATGCTGGGCGCTGTGAGT-3' (34627903-880), 5'-CGAAGGCGCCTCCGGACCTAGG-3' (34627428-405), and for exon 4, 5'-GCTAAGCCTGATGCTGTGCTGCT-3' (34625326-49), 5'-GTTGAGTACACGGCGCTTGAGGC-3' (34625849-26). For genotype of G-241T/C-240T and Gln2Pro polymorphism, PCR amplifications were carried out using each pair of the primers for the targeted regions of the SIGMAR1 gene according to Ishiguro et al. [14], and PCR products were digested by restriction enzyme of PsI I and Hha I for G-241T/C-240T and Gln2Pro, and were separated on 2% and 4.5% of NuSieve GTG agarose gel, respectively.

**Statistical analysis**

Deviation of the genotype counts from the Hardy-Weinberg equilibrium was tested using a chi-square goodness-
of-fit test. The statistical significance of differences in the genotype and allele frequencies between patients and controls were tested by Chi square test at a significance level of .05 two-tailed. For meta-analysis, numbers of each genotype or each allele of three studies by Ishiguro et al. [14], Ohmori et al. [15] and us were added, and were analysed by by Chi square test. Statistical power analysis was examined using software G*Power (version 2.1.2) [16].

**Results and Discussion**

Screening of 5' promoter region and four exons of the SIGMAR1 gene identified the two previously reported polymorphisms, G-241T/C-240T and Gln2Pro, but not any novel one. The genotypic distributions for patient and control groups did not deviate significantly from the Hardy-Weinberg equilibrium at the two polymorphisms of the SIGMAR1 gene. Frequencies of each genotype and allele of the polymorphisms were similar to those of previous studies. In consistent with the previous studies, G-241T/C-240T and Gln2Pro polymorphisms showed complete linkage disequilibrium with each other, and resulted in two haplotypes, GC-241-240/Gln2 and TT-241-240/Pro2. Subsequently, results of only Gln2 Pro was shown in table 1. The present study revealed that there was no significant difference in the genotype frequencies ($x^2 = 0.05$, d.f. = 2, $P = 0.97$) or allele frequencies ($x^2 = 0.02$, d.f. = 1, $P = 0.88$) of Gln2Pro polymorphism between schizophrenia patients and controls. No association was observed between the polymorphism and any of the diagnostic subtypes, hebephrenic type (genotype, $x^2 = 0.01$, d.f. = 2, $P = 0.99$, allele, $x^2 = 0.00$, d.f. = 1, $P = 1.00$) and paranoid type (genotype, $x^2 = 0.06$, d.f. = 2, $P = 0.97$, allele, $x^2 = 0.00$, d.f. = 1, $P = 1.00$).

Table 2 showed comparison among the present study and the two previous studies of case-control association with the SIGMAR1 gene. Values of allelic and genotypic distribution of Gln2Pro polymorphism were almost similar among the three studies. However, a difference of allelic frequencies between controls and schizophrenics in Ishiguro's and Ohmori's study was 4.8 and 4.6 point, respectively, and that of the present study was only 0.6 point. Ishiguro's study revealed a marginal significant difference between the groups, but Ohmori's one failed, suggesting a negative result by the latter study may result from lack of power to detect significance difference. However, it is possible that Ishiguro's result may be a false positive due to population stratification, which is considered as unavoidable in such case-control association design studies.

To reduce possible population stratification and to verify the negative results of association between schizophrenia...
and the SIGMAR1 gene found by the present study, meta-analysis was examined by getting data of the present and the two previous studies [14,15] together, which comprised 779 controls and 636 schizophrenics in total (Table 2). Meta-analysis revealed that there was no significant association in the genotype frequencies ($x^2 = 3.57, \text{d.f.} = 2, P = 0.17$) or allele frequencies ($x^2 = 3.57, \text{d.f.} = 1, P = 0.06$) of the SIGMAR1 gene with schizophrenia. The power analysis showed that the present sample size had a power of $>0.9999$ to detect significant allelic associations and a power of 0.93 to detect genotypic associations, given that the gene effect is small (the effective sample size set at 0.1). Therefore, it is likely that the SIGMAR1 gene is not associated with susceptibility to schizophrenia, and genetic studies did not provide any additional support for sigma receptor hypothesis for pathogenesis of schizophrenia. However, pharmacological studies suggested that there are at least two subtypes of sigma receptors, type 1 and type 2 [17]. Although a gene for sigma receptor type 2 has not been cloned yet, the possibility remains that genetic variance of sigma receptor type 2 or unknown subtypes could precipitate development of schizophrenia.

Conclusions The present study and meta-analysis including the previous two studies have suggested that the SIGMAR1 gene does not confer susceptibility to schizophrenia.

Competing interests None declared.

Authors’ contributions NU carried out genotyping and mutation screening, data collection, data analysis, and drafted the manuscript. HU conceived of the study, its design and coordination, participated in data analysis, and edited the manuscript. KN and YT assisted genotyping and sequencing, and data analyses. All authors recruited subjects and made a diagnosis. SK provided financial support and edit the manuscript. All authors read and approved the final manuscript.

Acknowledgements The present study was supported in part by a grant from the Zikei Institute of Psychiatry (Okayama, Japan).

References
1. Martin WR, Eades CE, Thompson JA and Huppler RE: The effects of morphine- and nalorphin-like drugs in the nondependent and morphine-dependent chronic spinal dog. J Pharmacol Exp Ther 1976, 197:517-522.
2. Walker JM, Bowen WD, Walker FO, Matsumoto RR, De CB and Rice KC: Sigma receptors: biology and function. Pharmacol Rev 1990, 42:355-402.
3. Ujike H, Kanzaki A, Okumura K, Akiyama K and Otsuki S: Sigma (2) antagonist BMY 14802 prevents methamphetamine-induced sensitization. Life Sci 1992, 50(16):PL-129-PL-134.
4. Ujike H, Okumura K, Zushi Y, Akiyama K and Otsuki S: Persistent supersensitivity of $\sigma$ – receptors develops during repeated methamphetamine treatment. Eur J Pharmacol 1992, 211:323-328.
5. Ujike H, Kuroda S and Otsuki S: $\sigma$ Receptor antagonists block the development of sensitization to cocaine. Eur J Pharmacol 1999, 396:123-128.
6. Okuyama S, Ogawa S, Nakazato A and Tomizawa K: Effect of NE-100, a novel sigma receptor ligand, on phenycyclidine-induced delayed cognitive dysfunction in rats. Neurosci Lett 1995, 189:60-62.
7. Tam SW: Naloxone-inaccessible sigma receptor in rat central nervous system. Proc Natl Acad Sci U S A 1983, 80:6703-6707.
8. Ujike H, Akiyama K and Kuroda S: [H]JYM-09151-2 (nemonapride), a potent radioligand for both sigma 1 and sigma 2 receptor subtypes. Neuropept. 1996, 7:1057-1061.
9. Weissman AD, Casanova MF, Kleinman JE, London ED and De SE: Selective loss of cerebral cortical sigma, but not PCP binding sites in schizophrenia. Biol Psychiatry 1991, 29:41-54.
10. Feldmeier DM, Tang, SW, Bunney WJ, Potkin SG and Jones EG: Decrease in sigma but no increase in striatal dopamine D4 sites in schizophrenic brains. Eur J Pharmacol 1996, 314:R3-5.
11. Modell S, Naber D and Holzbach R: Efficacy and safety of an opiate sigma-receptor antagonist (SL 82.0715) in schizophrenic patients with negative symptoms: an open dose-range study. Pharmacopsychiatry 1996, 29:63-66.
12. Muller MJ, Grunder G, Wetzel H, Muller-Siecheneder F, Marx-Danigkeit P and Benkert O: Antipsychotic effects and tolerability of the sigma ligand EMD 57445 (panemone) and its metabolites in acute schizophrenia: an open clinical trial. Psychiatry Res 1999, 89:275-280.
13. Huber MT, Gortthardt U, Schreiber W and Krieg JC: Efficacy and safety of the sigma receptor ligand EMD 57445 (panemone) in patients with schizophrenia: an open clinical trial. Pharmacopsychiatry 1999, 32:68-72.
14. Ishiguro H, Ohtsuki T, Toru M, Itoaka M, Aoki J, Shibuya H, Kuru-maj A, Okubo Y, Iwawaki A and Ota K et al.: Association between polymorphisms in the type 1 sigma receptor gene and schizophrenia. Neurosci Lett 1998, 257:45-48.
15. Ohmori O, Shinkai T, Suzuki T, Ohto C, Kojima H, Terao T and Nakamura J: Polymorphisms of the sigma(1) receptor gene in schizophrenia: An association study. Am J Med Genet 2000, 96:118-122.
16. Erdelieber E, Faul F and Buchner A: G POWER: A general power analysis program. Behavior Research Methods, Instruments, and Computers 1996, 28:1-11.
17. Quirion R, Bowen WD, Itzhak Y, Junier JL, Musacchio JM, Rothman RB, Su TP, Tam SW and Taylor DP: A proposal for the classification of sigma binding sites. Trends Pharmacol Sci 1992, 13:85-86.

Pre-publication history The pre-publication history for this paper can be accessed here: http://www.biomedcentral.com/1471-244X/3/13/prepub

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