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Genetic association between the dopamine D3 gene polymorphism (Ser9Gly) and schizophrenia in Japanese populations: Evidence from a case–control study and meta-analysis

Kensuke Utsunomiya[a,b,*], Takahiro Shinkai[a], Vincenzo De Luca[c], Rudi Hwang[c], Shinichi Sakata[a], Yuko Fukunaka[a], Hsin-I. Chen[a,d], Osamu Ohmori[a,e], Jun Nakamura[a]

[a] Department of Psychiatry, School of Medicine, University of Occupational and Environmental Health, Yahatanishi-ku, Kitakyushu 807-8555, Japan
[b] Health Management Department, Shimonoseki Shipyard & Machinery Works, Mitsubishi Heavy Industries, Ltd., Shimonoseki 750–8505, Japan
[c] Neurogenetics Section, Centre for Addiction and Mental Health, Clarke Division, Department of Psychiatry, University of Toronto, Toronto, Ontario M5T 1R8, Canada
[d] Department of Public Health, Kaohsiung Medical University, Kaohsiung 807, Taiwan
[e] Wakato Hospital, Wakamatsu-ku, Kitakyushu 808-0132, Japan

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ABSTRACT

Dysregulation in the dopaminergic system has been implicated in the pathophysiology of schizophrenia (SCZ). Dopamine D3 receptors (DRD3) concentrated in limbic regions of the brain (important for cognitive, emotional and endocrine function) may be particularly relevant to SCZ. A recent meta-analysis with mixed ethnicities reported a marginal significant association between the Ser9Gly homozygosity in the first exon of the *DRD3* gene and SCZ. To further evaluate the controversial association between this polymorphism and SCZ, a case–control study and meta-analysis was conducted using the homogeneous Japanese population. In our Japanese case–control sample (246 cases/198 controls), we found an association between the *DRD3* Ser9Gly polymorphism and SCZ (genotype: $\chi^2 = 9.76$, d.f. = 2, $p = 0.008$; Ser allele versus Gly allele: $\chi^2 = 7.96$, d.f. = 1, $p = 0.0048$; OR = 0.65; 95% CI = 0.48–0.88). However in a meta-analysis of nine Japanese case–control studies comprising 2056 subjects the association between *DRD3* Ser9Gly polymorphism and SCZ did not persisted. The Mantel–Haenszel pooled OR for SCZ among carriers of the *DRD3* Ser9Gly homozygosity (Ser/Ser homozygotes and Gly/Gly homozygotes) of the nine Japanese studies was 1.16 (95% CI 0.97–1.39), pointing to a non-significant effect of the *DRD3* Ser9Gly homozygosity as a risk factor for SCZ. Overall, our results suggest that the *DRD3* Ser9Gly polymorphism may not confer susceptibility to SCZ in the Japanese population. Given that the Ser9Gly variant may play a putative role in DRD3 function, further studies on the *DRD3* with linked variants are warranted.
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The dopamine D3 receptor (DRD3) has been suggested to play an important role in the pathophysiology of schizophrenia (SCZ). Postmortem studies have shown a selective loss of *DRD3* gene mRNA in the parietal and motor cortical regions of patients with chronic SCZ [21]. *DRD3* also has a restricted pattern of expression in limbic areas of the brain, areas which are involved in cognitive, emotional, endocrine functions, etc. [24]. Since typical and atypical antipsychotics (APs) have a relatively high affinity for DRD3 [22], it has been postulated that DRD3 may mediate the therapeutic mechanisms of APs. Recently, DRD3-selective antagonists have been proposed as novel APs (reviewed in [23]).

DRD3 has a polymorphic site in the first exon that leads to a serine to glycine amino acid substitution at position 9 (Ser9Gly) in the extracellular N-terminal domain of the receptor [13]. This polymorphism has been associated with altered dopamine binding affinity [16], indicating a possible functional effect. In human embryonic kidney transfected cells, although the Gly9 variant did not differ from the Ser9 variant with respect to glycosylation and to anterograde and retrograde trafficking, dopamine had an affinity that was four to five times higher. With the Gly9 variant, the dopamine-mediated cyclic adenosine monophosphate (cAMP) response was increased, and the mitogen-associated protein kinase signal was prolonged, as compared with the Ser9 variant [10].

Several association studies between SCZ and the *DRD3* Ser9Gly polymorphism have been previously conducted. Initial case–control studies reported an association between homozygosity of this polymorphism and SCZ in two independent samples [3]. Although subsequent studies did not confirm these findings

* Corresponding author at: Department of Psychiatry, School of Medicine, University of Occupational and Environmental Health Yahatanishi-ku, Kitakyushu 807-8555, Japan. Tel.: +81 93 691 7253; fax: +81 93 692 4894.
E-mail address: k-utsu@med.uoeh-u.ac.jp (K. Utsunomiya).

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[14,19] a recent large meta-analysis also found homozygosity to be associated with SCZ (4140 patients and 4408 controls) [11]. However, when four additional samples were added to the original meta-analysis [11], the initial association observed between *DRD*3 Ser9Gly homozygosity and SCZ became non-significant [12]. Despite conducting a meta-analysis with over 11,000 individuals [12], the relationship between *DRD*3 Ser9Gly polymorphism and SCZ remains inconclusive.

One possible explanation for the inconsistencies in previous studies may be due to ethnic heterogeneity within the samples studied. When an association was investigated in a more ethnically homogeneous northern or middle European Caucasian sample, indeed a positive result was detected (odds ratio (OR) = 1.16, 95% confidence interval (CI) = 1.01–1.33) [5]. To further evaluate the controversial association between this polymorphism and SCZ using a homogeneous population, in the present study we conducted a Japanese case–control study and meta-analysis.

Our sample included 246 unrelated chronic in-patients with SCZ (128 male/118 female, mean age ± standard deviation (S.D.): 51.1 ± 11.2 years) and 198 normal controls (102 male/94 female, mean age ± S.D.: 55.0 ± 6.3 years). Most of patients used in this study were chronic schizophrenia in-patients (mean duration of illness, 26.4 ± 11.1 years). All subjects in this study were unrelated Japanese recruited from Kitakyushu district, Japan. Subjects were assessed for the diagnosis of SCZ using the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria [1]. This was performed by four psychiatrists with consensus based on clinical interviews and case records. None of the subjects had significant neurological comorbidity, epilepsy, mental retardation, or history of substance abuse. All patients had been admitted to one of five hospitals within a 30-km radius of the University of Occupational and Environmental Health (UOEH); the controls also lived within this area. Control subjects were recruited mostly from medical staff and hospital/university employees at above-mentioned five hospitals or our institution. Although no structured interviews performed with the control subjects, at least two experienced psychiatrists interviewed every subject directly and confirmed that they lacked a family history of SCZ or other psychiatric disorders in their first- and second degree relatives. In addition we limited the controls to those who were older than 45 years of age as SCZ often develops during the late teen and young adult years.

This study was approved by the Ethics Committee of UOEH. All subjects for this study were recruited with fully informed written consent.

Two 7 ml EDTA tubes of blood were drawn from patients and their parents, and genomic DNA was extracted using a standard method [2]. Genotypes were assessed by the TaqMan allele specific assay method (Applied Biosystems, Foster City, CA) according to the manufacturer's protocols at the Bioinformation Research Center, UOEH. The Ser9Gly polymorphism (dbSNP ID: rs6280; ABI assay ID: C_949770_10) site was amplified by polymerase chain reaction (PCR) using the following primers: 5′-GCCCCACAGGTGTAGTTCAGGTGGC-3′ (forward) and 5′-ACTCAGCTGGCTCAGAGATGCCATA-3′ (reverse). Two dual labeled probes centered on the SNP and differing in sequence by the 1-bp polymorphism of the SNP site itself were designed by Applied Biosystems Inc. The probes were labeled with 5′ reporter fluors VIC or 6-FAM, and 3′ quencher. The probe sequences were: VIC-ACAGCTGGGCCCTT and 6-FAM-ACAGCTGAGCCCTT. PCR amplifications were performed on an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems) with the reaction mixture in a total volume of 25 μl, consisting of 40 ng of genomic DNA, 2× TaqMan Universal Master Mix (Applied Biosystems), 40× Assay-By-Design SNP Genotyping Assay Mix (Applied Biosystems), which includes the primers and labeled probes above, and deionized H_2O. After denaturing at 95 °C for 10 min, 45 cycles of PCR were performed under the following conditions: 92 °C for 15 s and 60 °C for 1 min. All genotypes were reported with the allelic discrimination program using the ABI software and confirmed by two experienced researchers. Approximately 0.2% of samples yielding ambiguous genotype calls were excluded from the study. Additionally, a random sample of approximately 5% of subjects was genotyped twice to assess the genotyping error rate, and this showed 100% concordance between genotyping runs.

The fitness of genotype frequency distribution to the Hardy–Weinberg equilibrium was calculated by the χ^2 goodness-of-fit test. Allele frequency differences between patients and controls were analyzed using the χ^2 test. Statistical analyses were performed using the *Statistical Package for the Social Sciences*, *version* 10.0 (SPSS, Tokyo, Japan). Power calculation was performed with the *SPSS Sample Power*. For meta-analysis, from nine published association studies between the *DRD*3 Ser9Gly polymorphism and schizophrenia using Japanese samples detected by PubMed search, we used eight sets of data [7–9,17,18,20,27], excluding one very small study ($n = 18$) [6]. The Mantel–Haenszel pooled OR was used to combine results from different studies. The *STATA* 8.0 program (Stata Corporation, College Station, TX, USA), a general package for statistics and genetic epidemiology, was employed for the meta-analysis.

Distribution of *DRD*3 Ser9Gly genotype and allele frequencies in the present case–control sample are shown in Table 1. The genotype distribution of the polymorphism did not deviate significantly from the Hardy–Weinberg equilibrium for both cases and controls. The allele frequency of Gly allele was 0.315 in the patient group and 0.23 in the control group. A significant association between the polymorphism and SCZ was observed for both genotype and allele frequencies (genotype: $\chi^2 = 9.76$, d.f. = 2, $p = 0.008$; Ser allele versus Gly allele: $\chi^2 = 7.96$, d.f. = 1, $p = 0.0048$; OR = 0.65; 95% CI = 0.48–0.88). There was a trend for an excess of Gly-containing genotypes (Ser/Gly + Gly/Gly) among patients in comparison to controls (OR = 0.69; 95% CI = 0.47–1.00; $p = 0.051$). However, no significant association between the Ser9Gly homozygosity and SCZ was found (OR = 0.94; 95% CI = 0.64–1.38).

All Japanese populations included in the present meta-analysis were in Hardy–Weinberg equilibrium for both cases and controls. The Mantel–Haenszel pooled OR for SCZ among carriers of the *DRD*3 Ser9Gly homozygosity (Ser/Ser homozygotes and Gly/Gly homozygotes) of the nine Japanese studies was 1.16 (95% CI 0.97–1.39), pointing to a non-significant effect of the *DRD*3 Ser9Gly homozygosity as a risk factor for SCZ (Fig. 1). There was no evidence for the Ser/Ser genotype (versus Ser/Gly and Gly/Gly genotypes; the Mantel–Haenszel pooled OR = 1.08, 95% CI: 0.90–1.29) as a risk factor for the disease. The meta-analysis was also not significant with the Ser allele (versus Gly allele; the DerSimonian-Laird pooled OR = 1.03, 95% CI: 0.82–1.29), analyzed using a random model since there was evidence for heterogeneity ($\chi^2 = 19.11$, d.f. = 8, $p = 0.014$).

In our Japanese sample (246 cases/198 controls), a significant difference in both genotype distribution and allele frequency between patients and healthy subjects was observed for the *DRD*3 Ser9Gly polymorphism. However, a subsequent meta-analysis using nine Japanese studies (total 1051 cases/1005 controls) revealed no significant effect of this polymorphism on the risk of SCZ. Overall, our findings suggest that this polymorphism may not confer susceptibility to SCZ in Japanese population.

In a recent meta-analysis of published case–control studies that included 8761 individuals [11] an association between homozygosity and SCZ was found. This association was most marked among European Caucasian samples. The lack of association among other studies may be explained as being due to genetic

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