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The *dopamine D3 receptor* (*DRD3*) gene and risk of schizophrenia: Case–control studies and an updated meta-analysis

Ayako Nunokawa [a], Yuichiro Watanabe [a,b,*], Naoshi Kaneko [a], Takuro Sugai [a], Saori Yazaki [c], Tadao Arinami [c], Hiroshi Ujike [d], Toshiya Inada [e], Nakao Iwata [f], Hiroshi Kunugi [g], Tsukasa Sasaki [h], Masanari Itokawa [i], Norio Ozaki [j], Ryota Hashimoto [k], Toshiyuki Someya [a]

[a] *Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, 757 Asahimachidori-ichibancho, Chuo-ku, Niigata 951-8510, Japan*
[b] *Health Administration Center, Niigata University, 8050 Ikarashi-ninocho, Nishi-ku, Niigata 950-2181, Japan*
[c] *Department of Medical Genetics, Doctoral Program in Social and Environmental Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan*
[d] *Department of Neuropsychiatry, Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan*
[e] *Seiwa Hospital, Institute of Neuropsychiatry, 91 Bentencho, Shinjuku-ku, Tokyo 162-0851, Japan*
[f] *Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan*
[g] *Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan*
[h] *Health Service Center, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan*
[i] *Schizophrenia Research Project, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, Japan*
[j] *Department of Psychiatry, School of Medicine, Nagoya University, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan*
[k] *The Osaka-Hamamatsu Joint Research Center for Child Mental Development, Osaka University Graduate School of Medicine, D3, 2-2, Yamadaoka, Suita, Osaka, 5650871, Japan*

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ABSTRACT

The dopamine D3 receptor (DRD3) has been suggested to be involved in the pathophysiology of schizophrenia. *DRD3* has been tested for an association with schizophrenia, but with conflicting results. A recent meta-analysis suggested that the haplotype T–T–T–G for the SNPs rs7631540–rs1486012–rs2134655–rs963468 may confer protection against schizophrenia. However, almost all previous studies of the association between *DRD3* and schizophrenia have been performed using a relatively small sample size and a limited number of markers. To assess whether *DRD3* is implicated in vulnerability to schizophrenia, we conducted case–control association studies and performed an updated meta-analysis. In the first population (595 patients and 598 controls), we examined 16 genotyped single nucleotide polymorphisms (SNPs), including tagging SNPs selected from the HapMap database and SNPs detected through resequencing, as well as 58 imputed SNPs that are not directly genotyped. To confirm the results obtained, we genotyped the SNPs rs7631540–rs1486012–rs2134655–rs963468 in a second, independent population (2126 patients and 2228 controls). We also performed an updated meta-analysis of the haplotype, combining the results obtained in five populations, with a total sample size of 7551. No supportive evidence was obtained for an association between *DRD3* and schizophrenia in our Japanese subjects. Our updated meta-analysis also failed to confirm the existence of a protective haplotype. To draw a definitive conclusion, further studies using larger samples and sufficient markers should be carried out in various ethnic populations.

1. Introduction

The dopamine D3 receptor (DRD3) has been suggested to be involved in the pathophysiology of schizophrenia (for a

* Corresponding author. Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, 757 Asahimachidori-ichibancho, Chuo-ku, Niigata 951-8510, Japan. Tel.: +81 25 227 2213; fax: +81 25 227 0777.
E-mail address: yuichiro@med.niigata-u.ac.jp (Y. Watanabe).

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review, Schwartz et al., 2000). DRD3 has relatively strong affinity for both first- and second-generation antipsychotics (Sokoloff et al., 1990). Postmortem studies have revealed changes in the mRNA and protein levels of DRD3 in the brains of patients with schizophrenia (Gurevich et al., 1997; Meador-Woodruff et al., 1997; Schmauss et al., 1993). Altered levels of *DRD3* mRNA in blood lymphocytes of patients with schizophrenia have also been reported (Ilani et al., 2001; Vogel et al., 2004). *DRD3* is located on 3q13.3 where some linkage analyses have suggested a region of susceptibility to schizophrenia (Brzustowicz et al., 2000; Kaneko et al., 2007). Therefore, *DRD3* is a promising functional and positional candidate gene for schizophrenia.

More than 60 studies have tested an association between *DRD3* and schizophrenia (Allen et al., 2008). The most extensively investigated *DRD3* polymorphism is Ser9Gly (rs6280) in exon 2 resulting in a serine to glycine substitution at codon 9. This polymorphism has been reported to be associated with altered dopamine binding affinity, suggesting that the Ser9Gly polymorphism may be functional (Lundstrom and Turpin, 1996). An initial study reported an association between homozygosity of this polymorphism and schizophrenia (Crocq et al., 1992). Some studies showed an association of the Ser allele with schizophrenia (Ishiguro et al., 2000; Shaikh et al., 1996), whereas others reported that the Gly allele was over-represented in patients with schizophrenia (Kennedy et al., 1995; Utsunomiya et al., 2008). However, two recent large meta-analyses did not provide evidence for an association between the Ser9Gly polymorphism and schizophrenia (Allen et al., 2008; Ma et al., 2008). Therefore, if *DRD3* is implicated in genetic susceptibility to schizophrenia, this cannot be wholly accounted for by the Ser9Gly polymorphism. This view has been supported by two studies using tagging single nucleotide polymorphisms (SNPs) based on linkage disequilibrium (LD) (Domínguez et al., 2007; Talkowski et al., 2006). A recent meta-analysis showed that the second most common haplotype (T–T–T–G) for the SNPs rs7631540–rs1486012–rs2134655–rs963468 was less frequent in patients with schizophrenia than in control subjects, suggesting that this haplotype may confer protection against schizophrenia (Costas et al., 2009).

Almost all previous studies on the association between *DRD3* and schizophrenia have been performed using a relatively small sample size and a limited number of markers. Here, we tried to increase the power by increasing the sample size and testing more markers, including tagging SNPs selected from the HapMap database and SNPs detected through resequencing of whole exon regions of *DRD3*. First, we conducted a moderate-scale case–control association study (595 patients and 598 controls) using 16 genotyped SNPs and 58 imputed SNPs that have not been directly genotyped. Second, we carried out an independent large-scale case–control association study (2126 patients and 2228 controls) to confirm the results of the first study, specifically to test the association of the haplotype T–T–T–G for the SNPs rs7631540–rs1486012–rs2134655–rs963468 with schizophrenia. Third, we performed an updated meta-analysis of this haplotype to assess the collective evidence across individual studies.

2. Materials and methods

The present study was approved by the Ethics Committee of each participating institute, and written informed consent was obtained from all participants. All participants were unrelated Japanese subjects.

2.1. Subjects

The first population consisted of 595 patients with schizophrenia (313 men and 282 women; mean age, 40.2 [SD 14.1] years) and 598 control subjects (311 men and 287 women; mean age, 38.1 [SD 10.5] years). These subjects partially overlapped with those in the report of Tanaka et al. (1996). Case and control groups were matched for sex ($p = 0.836$). Although the mean age of the patients was significantly higher than that of the control subjects ($p = 0.004$), the difference in mean age between the groups was relatively small (2.1 years). The second population consisted of 2126 patients with schizophrenia (1137 men and 989 women; mean age, 47.3 [SD 14.3] years) and 2228 control subjects (1189 men and 1039 women; mean age, 46.6 [SD 13.9] years). Case and control groups were matched for sex ($p = 0.940$) and age ($p = 0.083$).

We conducted a psychiatric assessment of every participant, as described previously (Nunokawa et al., 2007). In brief, the patients were diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* (DSM-IV) criteria by at least two experienced psychiatrists, on the basis of all available sources of information, including unstructured interviews, clinical observations and medical records. The control subjects were mentally healthy subjects with no self-reported history of psychiatric disorders; they showed good social and occupational skills, but were not assessed using a structured psychiatric interview.

The subjects for resequencing of exon regions were six patients with schizophrenia from a Japanese single multiplex schizophrenia pedigree. In this pedigree, our previous linkage analysis revealed that 3q is one of the candidate regions for schizophrenia (Kaneko et al., 2007). These patients were diagnosed according to the DSM-IV criteria by two experienced psychiatrists, on the basis of all available sources of information, including direct interviews using the Structured Clinical Interview for DSM-IV Axis I disorders and Axis II disorders, medical records, and information from reliable relatives and psychiatric professionals.

2.2. Tagging SNP selection

Tagging SNPs for *DRD3*, covering gene region and the 5′ and 3′ flanking regions (chr3:115307882..115402406), were selected from the HapMap database (release#22, population: Japanese in Tokyo [JPT], minor allele frequency [MAF]: more than 0.05). We applied the criterion of an r^2 threshold greater than 0.8 in the '*aggressive tagging: use 2- and 3-marker haplotype*' mode using the 'Tagger' program (de Bakker et al., 2005), as implemented in Haploview v4.0 (Barrett et al., 2005); rs6280 (Ser9Gly) was forced to be selected as a tagging SNP. To confirm the existence of a common protective haplotype (Costas et al., 2009), we also included rs963468.

2.3. Resequencing of exon regions

All seven exons of *DRD3* were screened for polymorphisms using direct sequencing of PCR products. The sequences