Association of polymorphisms in the haplotype block spanning the alternatively spliced exons of the NTNG1 gene at 1p13.3 with schizophrenia in Japanese populations

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Abstract

Chromosome 1p13 is linked with schizophrenia in Japanese families, and one of the candidate genes in this region is the *netrin G1* (*NTNG1*) gene at 1p13.3. Associations of 56 tag single nucleotide polymorphisms (SNPs) with schizophrenia were explored by transmission disequilibrium analysis in 160 Japanese trios and by case-control analysis in 2174 Japanese cases and 2054 Japanese controls. An association between SNP rs628117 and schizophrenia was identified by case-control comparison (nominal allelic $p = 0.0009$, corrected $p = 0.006$). The associated polymorphism is located in intron 9 and in the haplotype block encompassing the alternatively spliced exons of the gene. Allelic association of a different SNP in the same haplotype block in Japanese families was previously reported. These findings support that the *NTNG1* gene is associated with schizophrenia in the Japanese.

Keywords: netrin; schizophrenia; alternative splicing; association;

Schizophrenia is a common disorder with a lifetime morbidity risk of 1%. A large number of family, twin, and adoption studies have revealed that individual differences in susceptibility are predominantly genetic, with a heritability of 0.70-0.85 and a 10-fold increased risk in siblings of probands [5]. Genome-wide linkage analysis of Japanese Schizophrenia Sib-pair Linkage Group (JSSLG) samples comprising 236 Japanese families with 268 non-independent affected sib pairs with schizophrenia revealed significant evidence for linkage of schizophrenia to chromosome 1p21.2-1p13.2 (LOD = 3.39)[3]. One of the candidate genes in the chromosome 1p linkage region is *NTNG1*, which is located at 1p13.3. A potential association between a polymorphism and haplotypes with schizophrenia was reported in Japanese families [1]. This study also reported differential expression of NTNG1 isoforms between in Brodmann area 46 of postmortem brains between patients with schizophrenia and controls. The *NTNG1* gene encodes netrin G1, which is involved in the formation and/or maintenance of glutamatergic neuronal circuitry [1, 6]. A case of Rett syndrome and a *de novo* translocation causing disruption of the *NTNG1* gene has been reported [2]. In the present study, we examined *NTNG1* as a candidate gene in the 1p linkage region that we previously reported.

Subjects were of Japanese descent and were recruited from the main island of Japan. Transmission disequilibrium test (TDT) was performed for 160 trios consisting of probands and their parents. The probands were 86 men and 74 women and the mean age ± SD were 29.3± 9.7 years. Among them, 26 trios had been included in the JSSLG linkage study. Independent case-control subjects were a total of 2174 unrelated patients with schizophrenia (mean age ± SD: 48.8 ± 14.5 years, 1187 men and 987 women) and 2056 mentally healthy unrelated subjects (mean age ± SD: 49.1 ± 14.3 years, 1109 men and 947 women) without self-reported family histories of mental illness within second-degree relatives. Consensual diagnosis was made according to DSM-IV criteria by at least two experienced psychiatrists on the basis of direct interviews, available medical records, and information provided by hospital staff and relatives. None of the patients had

additional Axis I disorders as defined by DSM-IV. The present study was approved by the Ethics Committees of the University of Tsukuba, Niigata University, Fujita Health University, Nagoya University, Okayama University, and Teikyo University, and all participants provided written informed consent.

Single nucleotide polymorphisms (SNPs) were typed with the TaqMan system (Applied Biosystems, Foster City, California). Polymerase chain reaction was performed in an ABI 9700 thermocycler, and fluorescence was determined with an ABI 7900 sequence detector with single-point measurement and SDS v2.2 software (Applied Biosystems). A total of 55 tag SNPs in the *NTNG1* gene region were selected according to the Japanese genotype data of the HapMap database (http://www.hapmap.org/) and genotyped in 160 TDT trios. These tag SNPs were selected to distinguish haplotypes with minimal haplotype frequency > 0.1 represented for each block. These 160 trios had approximately 80% power to detect SNP association with the use of a 5% significance given the genetic model of risk allele frequency > 0.2, additive model with genotype relative risk > 1.9, and r^2 capture level of the selected tag SNPs > 0.8. SNPs that showed nominally significant association with schizophrenia in the TDT trios were genotyped, along with two additional SNPs, in the case-control subjects.

The two additional SNPs were genotyped to construct the haplotypes previously reported by Aoki-Suzuki et al. [1] to be associated with schizophrenia. Hardy-Weinberg equilibrium, linkage disequilibrium, and allele/haplotype frequencies, as well as association between a SNP or haplotype and schizophrenia, were analyzed with the Haploview software program (http://www.broad.mit.edu/mpg/haploview/). Permutation tests were also performed to calculate corrected p values for multiple testing by the Haploview software. Genotype-based associations were tested with the Cochran-Armitage test for trend. Statistical significance was accepted at $p < 0.05$.

SNPs screened for association with schizophrenia are listed in Table 1. Six SNPs showed nominal allelic p values < 0.05, however, no SNP was significantly associated with schizophrenia after permutation tests. Among these five SNPs, rs4307594, rs7522583, and

rs4364907 were found in linkage disequilibrium in each other in the TDT sample ($r^2 > 0.9$). We then examined eight SNPs in case-control subjects (Table 2). Among these, four (rs1335059, rs4364907, rs4132604, and rs628117) showed nominally significant association in TDT screening, and one (rs96501) was previously reported to be associated with schizophrenia (Aoki-Suzuki et al., 2005). Three (rs2218404, 1373336, and rs1444042) were used for constructing haplotypes previously reported to be associated with schizophrenia (Aoki-Suzuki et al., 2005). SNPs rs1335059, rs4364907, and rs4132604 were examined in a part of the case-control subjects consisted of 294 men and 282 women (mean age ± SD: 49.6± 14.8 years) in the patient group and 289 men and 287 women (mean age ± SD: 48.9± 12.5 years) in the control group. Among the eight SNPs, rs1444042 (nominal allelic $p = 0.006$; permutated $p = 0.04$) and rs628117 (nominal allelic $p = 0.0009$; permutated $p = 0.006$) showed significant association even after the permutation procedure. These two SNPs were in almost complete linkage disequilibrium with each other ($r^2 = 0.96$). These permutated p values were almost the same as those after Bonferroni correction for 7 tests. The odds ratio for homozygous AA carriers of rs628117 against the other genotypes carrier was 1.17 (95% confidence interval, 1.04-1.32) and the odds ratio for the A allele against the G allele was 1.17 (95% confidence interval, 1.07 – 1.29). No apparent gender difference was observed for the association: the odds ratio for the Allele against the G allele was 1.14 ($p = 0.06$) in the male subjects and 1.20 ($p = 0.02$) in the female subjects. Aoki-Suzuki et al. [1] reported that the minor allele frequency of rs144042 in 186 Japanese unrelated subjects (0.32) was close to that in the schizophrenia group in the present study (0.31). Although information of rs144042 is not uploaded in the JSNP database (http://snp.ims.u-tokyo.ac.jp/map/cgi-bin/searchTypingData.cgi), the minor allele frequency of rs628117, which is in linkage disequilibrium with rs144042, in 933 Japanese unrelated volunteers in the JSNP database is 0.27, similar with that in the control group in the present study.

Because rs2218404 (G1-14), rs1373336 (G1-17), and rs1444042 (G1-18) were in linkage disequilibrium (Figure 1), and haplotype association was reported previously, we

constructed haplotypes with these three SNPs. These three SNPs distinguished the common haplotypes with a frequency > 0.1 in this haplotype block, according to the Japanese genotype data of the HapMap database. The global *p* value for the haplotype association was 0.24, and no single haplotype association was found (Table 3).

Aoki-Suzuki et al. (2005) reported association of specific haplotypes encompassing alternatively spliced exons of *NTNG1* with schizophrenia and that mRNA isoform expression differed significantly between schizophrenic and control brains [1]. In the present study, we found significant associations of two SNPs in linkage disequilibrium with schizophrenia. These SNPs are located in the haplotype block encompassing the alternatively spliced exons reported by Aoki-Suzuki et al. [1]. However, the SNPs associated with schizophrenia identified in the present study differed from that of Aoki-Suzuki et al. [1]. They reported nominal association of SNP G1-19 and association of haplotypes constructed of SNPs G1-14, G1-17, and G1-18 with schizophrenia. The associated haplotype was the fourth most common. In the present study, allelic association was observed for SNP G1-18 but not SNP G1-19. SNP G1-18 and G1-19 were not in linkage disequilibrium with each other (D' = 0.38, r-square = 0.002). The haplotype association was not confirmed in the present study; the most significant associated haplotype was the second most common one (nominal $p = 0.05$), not the fourth most common one. Further studies are necessary to reconcile these differences.

We did not search for nonsynonymous variations because Aoki-Suzuki et al. (2005) did not any. They evaluated isoform-specific expression of the *NTNG1* gene and found decreased expression of exon 8- and exon 9- skipping isoforms in Brodmann area 46 of postmortem brains in schizophrenia compared to that in controls. The SNPs associated with schizophrenia in the present study and the SNPs and haplotype associations reported by Aoki-Suzuki et al. [1] are located in the same haplotype block spanning alternatively spliced exons.

There were some inconsistencies between our own two samples of trio and case-control samples. The discrepancy is probably due to relatively low detection power of

the 160 trio sample in which *NTNG1* was most comprehensively screened for association with schizophrenia. The 4 tag SNPs that reached < 0.05 probability in the trios are roughly the number that would be expected by chance and these associations were not replicated in the case-control sample. Therefore, *NTNG1* is unlikely to contain common polymorphisms that exert a strong effect on schizophrenia susceptibility in Japanese samples.

Many issues remain to be addressed. Discordant findings must be reconciled by further studies with large samples. In addition, the functional significance of each mRNA isoform should be assessed, and the relation between the genetic association with schizophrenia and mRNA or protein regulation should be elucidated. Eastwood and Harrison [4] reported that expression of the *NTNG1* c isoform was reduced in schizophrenia and bipolar disorder but that the SNP rs1373336 did not affect *NTNG1* mRNA expression. The SNP rs1373336 was not found to be associated with schizophrenia in the present study.

In conclusion, results of the present study support genetic associations of polymorphisms in the haplotype block spanning the alternatively spliced exons of the *NTNG1* gene with schizophrenia.

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Figure legend

Figure 1

Genomic structure of the *NTNG1* gene spanning 341,731 kb on chromosome 1p13.3 and linkage disequilibrium between SNPs genotyped. The SNP numbers are shown in Table 1. Linkage disequilibrium as expressed by D prime was determined with the use of Haploview software version 3.32 and haplotype blocks were determined by Gabriel's block definition.