Association study of the frizzled-3 (FZD3) gene with schizophrenia and mood disorders

Short Communication

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Summary. Two research groups have recently reported a significant association between schizophrenia and genetic variants of Frizzled-3 (FZD3) gene. We examined a possible association in a Japanese sample of schizophrenia, bipolar disorder, unipolar depression and controls with four single nucleotide polymorphisms (SNPs), tested in previous reports. We failed to find significant association in the four SNPs or haplotype analysis. The FZD3 gene might not play a role in conferring susceptibility to major psychosis in our sample.

Keywords: FZD3, schizophrenia, mood disorder, association study, single nucleotide polymorphism (SNP).

Introduction

Schizophrenia is a complex genetic disorder characterized by disturbances of cognition, emotion and social functioning. This disease is believed to involve genetic abnormalities in developmental/plasticity related processes during a critical period in neuronal growth (Weinberger et al., 2001). Wnt signal transduction cascades have been implicated in a variety of neurodevelopmental processes, e.g. segmentation, central nervous system patterning, and cell divisions (Wodarz and Nusse, 1998). Wnt proteins signal via cell surface transmembrane receptors, termed frizzleds, which display many properties.
characteristic of members of the superfamily of G-protein-coupled receptors (Wang and Malbon, 2004). The frizzled-3 (FZD3) gene, a member of frizzles, is located on chromosome 8p21, repeatedly suggested as a positive linkage locus for schizophrenia (Lewis et al., 2003; McGuffin et al., 2003). The FZD3 gene consists of 8 exons and 7 introns, spanning approximately 70 Kb (Kirikoshi et al., 2000). In accordance with this, two research groups have recently reported a significant association between schizophrenia and the FZD3 gene in Japanese and Chinese samples (Katsu et al., 2003; Yang et al., 2003). We tried to replicate these findings in an independent Asian sample. Furthermore, we also examined the possible association between the FZD3 gene with mood disorders, since schizophrenia and mood disorders might share the genetic vulnerability (Berrettini, 2003).

**Methods and materials**

**Subjects**

Subjects were 427 patients with schizophrenia (221 males and 206 females with mean age of 44.2 years [SD 14.5]), 91 with bipolar disorder (40 and 51; 53.6 years [SD 14.8]), and 396 with major depression (155 and 241; 53.4 years [SD 16.1]) and 473 healthy controls (228 and 245; 36.1 years [SD 12.5]). All the subjects were biologically unrelated Japanese. Consensus diagnosis was made for each patient by at least two trained psychiatrists according to the DSM-IV criteria. Controls were healthy volunteers who had no current or past contact to psychiatric services. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethical committees.

**SNP genotyping**

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. We genotyped four SNPs (single nucleotide polymorphisms; dbSNP accession: rs960914 in intron3, rs2241802 in exon5: A435G, L145L, rs2323019 in intron5 and rs352203 in intron5) in the FZD3 gene, which were examined in the previous two studies (Katsu et al., 2003; Yang et al., 2003). Genotyping was performed with the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Hashimoto et al., 2004a, b). Briefly, primers and probes for detection of the SNPs are: rs960914: forward primer 5′-CTTTTATAAAGAAATTTGAAACATCAGAACATGGGA-3′, reverse primer 5′-ACTTTTTTCACGCTTGGGAGATATTTCT-3′, probe 1 5′-VIC-CTGAATGGCTGCTATC-MGB-3′, and probe 2 5′-FAM-TCTGAATGGCTACTATC-MGB-3′; rs2241802: forward primer 5′-ATGAGCCATATCCTCGACTTGTG-3′, reverse primer 5′-GGACACAAAAACCATAGTCTCTCT-3′, probe 1 5′-VIC-TCCAGCTAAATTCAG-MGB-3′, and probe 2 5′-FAM-TCTGAATGGCTACTATC-MGB-3′; rs2323019: forward primer 5′-GAATATTACTTTGTTTTCTAGATCTTGAATGAAAC-3′, reverse primer 5′-CCAACCTGTTAA TAATGCTTTTTG-3′, probe 1 5′-VIC-TCTATTTATGCTCAATATTAA-TGGTCTTTTGG-3′, and probe 2 5′-TCATTTATGCTCAATATTAA-MGB-3′; rs352203: forward primer 5′-CTCAGAAAATATTCTATACGATAAGC-3′, reverse primer 5′-CAACCAGACATAACAGATTTACAGTTTCTAT-3′, probe 1 5′-VIC-TCTGAGTGTATCTGCTATGCTATTC-MGB-3′, and probe 2 5′-FAM-TTTCCTTCATATCCTATGCTATTC-MGB-3′. PCR cycling conditions were: at 95°C for 10 minutes, 45 cycles of 92°C for 15 seconds and 60°C for 1 minute.

**Statistical analysis**

Statistical analysis of association studies was performed using SNPAllyse software (DYNACOM, Yokohama, Japan). The presence of Hardy-Weinberg equilibrium was examined using the $\chi^2$ test for goodness of fit. Allele distributions between patients and controls were analyzed by the