DISCUSSION

A total of nine novel polymorphisms were found in the present study. They were two nucleotide sequence variants (201A→G, 246G→A) in the promoter region, two variants (1260T→A, 1266T→C) in intron 1, and a silent mutation (Leu306Leu) in exon 5 of the CCK-AR gene, one nucleotide sequence variant (-215C→A) in the promoter region, two missense mutations in exon 1 (Leu37Phe) as well as in exon 5 (Arg319Glu) of the CCK-BR gene, and one nucleotide sequence variant (-196A→G) in the promoter region of the CCK gene.

The sequence AAGACTGG (201-208) including the 201A→G nucleotide substitution in the promoter region of the CCK-AR gene may correspond to the cap signal (TCAGTCTT) reported by Bucher (1990). Moreover, the 246G→A nucleotide substitution modifies the sequence from GAAGGTGGAG to GAAGATGGAG (242-251) within a region corresponding to the GATA-1 binding site (Merika and Orkin, 1993). These polymorphisms may therefore affect the regulation of gene transcription, and may have functional significance in the disease. Note however, that we have not performed a transcriptional assay to assess the impact of these mutations. The 1260T→A, and the 1266T→C nucleotide substitutions in intron 1 of the CCK-AR gene displayed a high degree of polymorphism and were apparently linked with each other. The two polymorphic sites precede exon 2 and lie within the 3' mRNA splicing acceptor site of the consensus sequence, as described by Shapiro and Senapathy (1987). The transversion found at the 1260T→A position is located where the nucleotide is usually C or T. A similar phenomenon has been observed for the 1270C→G transversion of the CCK gene in reports by Bowen et al. (1998). This polymorphism may therefore affect the splicing of exon 2 of the CCK-AR gene.

CCK-BR is a member of the G-protein coupled, heptahelical receptor superfamily; possessing three extracellular and intracellular loops. The Leu37Phe mutation site is located immediately downstream from the potential site for *N*-linked glycosylation in the putative extracellular amino terminus. The Arg319Glu mutation site is located immediately upstream from the potential site for threonine phosphorylation in the terminus part of third intracellular loop; it activates G-protein, and regulates the receptor effects (Song et al., 1993; Lee et al., 1993; Pisegna et al., 1992). Thus, these mutations of the CCK-BR gene might influence the receptor function in a G-protein mediated signal transduction manner. However, no significant correlation was found between genotype frequencies of variants of the CCK-BR gene and schizophrenic patients; including their clinical details. The data suggest that CCK-BR gene polymorphisms have no association with schizophrenia or its clinical heterogeneity. However, it is necessary to determine if the -215C→A in the promoter of the CCK-BR gene is related to transcription regulation or is of any functional significance.

The promoter region of the CCK gene contains several cis-elements binding with trans-acting factors, such as bHLH-ZIP, CRE/TRE, Sp 1, and TFIID that regulate transcription of the gene (Nielsen et al., 1996). A nucleotide substitution (-196A→G) in the promoter of the CCK gene changes a core sequence of GGGAGGG to GAGAGGG (from -199 to -193). The former sequence corresponds to the putative MAZ consensus cis-element sequence (GGGAGGG) (Ashfield et al., 1994). Therefore, it is speculated that this variant may disrupt a putative transcription factor binding site, which affects dopaminergic function. However, a transcriptional assay to assess the impact of these mutations has not been performed. Further experiments are required in order to confirm this hypothesis. Comparison of the genotype distributions and allele frequencies of the polymorphisms in the CCK gene indicated no

significant association between patients and controls. Moreover, no significant correlation was found between genotype frequencies of variants of the CCK gene and clinical details of the patients. Recently, a positive association has been reported between both panic disorder (Wang et al., 1998) and alcoholism (Harada et al., 1998), and polymorphism of the Sp 1 binding cis-element (-45C→T) in the promoter region of the CCK gene. On the other hand, an association analysis of the CCK gene in schizophrenia yielded negative results (Bowen et al., 1998). Our data have confirmed that the polymorphisms of the CCK gene have no association with schizophrenia and its clinical heterogeneity.

The frequency of the 201A allele in the promoter region of the CCK-AR gene was significantly higher in the schizophrenic group, especially in the paranoid type, than in the control group. Since dysfunction of dopaminergic neurotransmission might relate to the pathogenesis of schizophrenia, especially for the paranoid type (Snyder, 1975; Seeman, 1974), this polymorphism may affect the regulation of gene transcription of the CCK-AR, which may in turn relate to functional significance in the paranoid schizophrenia through interaction with dopaminergic neurotransmission. However, these differences were not significant after the Bonferroni correction suggesting that the significance was involved in type I error. In addition, the sample size in the present study may have been too small to reach a final conclusion, since the power estimated from the 201A allele frequencies of the patients and the controls was less than 60% ($W = 0.127$, $P = 0.05$, $df = 1$) using the methods described by Cohen (1977). Further study using a larger sample as well as other ethnic population groups will be necessary to determine the involvement of the CCK-AR gene in susceptibility for schizophrenia.

CONCLUSION

Genetic polymorphisms in the promoter and coding regions of the CCK-AR, the CCK-BR, and the CCK gene were analyzed in schizophrenic patients and in healthy controls. Nine novel polymorphisms were identified in the CCK-AR, the CCK-BR, and the CCK genes in addition to polymorphic alleles reported previously. The genotype distributions and allele frequencies of the polymorphisms in the CCK-BR and the CCK genes have shown no significant differences between patients and controls. Statistical analysis suggested that the 201A allele frequency in the promoter region of the CCK-AR gene was higher in the schizophrenic group, especially in paranoid type, than the control group at a rate that was not quite significant after Bonferroni correction.

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