REVIEW

Genetic polymorphisms of serotonin and dopamine transporters in mental disorders

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Abstract : Transporter-assisted uptake of serotonin (5-HT) and dopamine (DA) has been accounted for activities in human behavior or mental status, because they are the sites of action of widely used antidepressant and psychoactive drugs. Both the human serotonin transporter (5-HTT) and human dopamine transporter (DAT1) genes are good candidates for etiological involvement in various psychiatric conditions. The serotonin transporter gene has two types of functional polymorphisms. One is serotonin transporter linked polymorphic region (5-HTTLPR) consisting of length variation of the repetitive sequence containing 20〜23-bp-long repeat elements in the 5'-upstream region of the gene. Another polymorphism is that serotonin transporter variable number of tandem repeats (5-HTTVNTR) in its second intron. Both polymorphisms affect the transcription ratio of 5-HTT gene and may modify neuronal transmission by changing its protein expression. On the other hand, DAT1 gene has a variable number of tandem repeats type polymorphism (DAT1VNTR) in the 3'-untranslated region of the mRNA, which was also reported to change its gene expression. So polymorphic variations of transporters would change the behavioral and neuropathological tendency. Here, the feature of those two transporters and their relations to psychiatric disorders are described.
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Keywords **:** *human serotonin transporter (5-HTT), human dopamine transporter (DAT1), functional polymorphism, association study, psychiatric disorders*

INTRODUCTION

Monoamines play important roles in the control of many basic functions, including movement, emotional behavior, cognition and neuroendocrine regulation. Psychiatric disorders such as schizophrenia, mood disorder, substance abuse and developmental disorders are widely accepted to have a basis in a dysfunction of the brain monoamine systems. Neurological disorders such as Parkinson disease and Huntington disease are also related to monoamine transmission. The concentration of monoamines in a synaptic cleft is the primary determinant of the intensity of neuronal signaling, while the functional state of monoamine neurons is determined by a delicate balance between the amount of synthesized, stored, released, re-uptaken and metabolized monoamines. The monoamine transporters are functional proteins that act to take released monoamines back up into presynaptic terminals, and their gene expressions seem to control the transmission of neurons (Figure 1). Dopamine, serotonin and noradrenaline has its specific transporter. Those three types of transporters are thought to be derived from the same origin, the structure of which includes twelve putative transmembrane domains with both N-and C-terminal domains located in the cytoplasm. The mechanism of the

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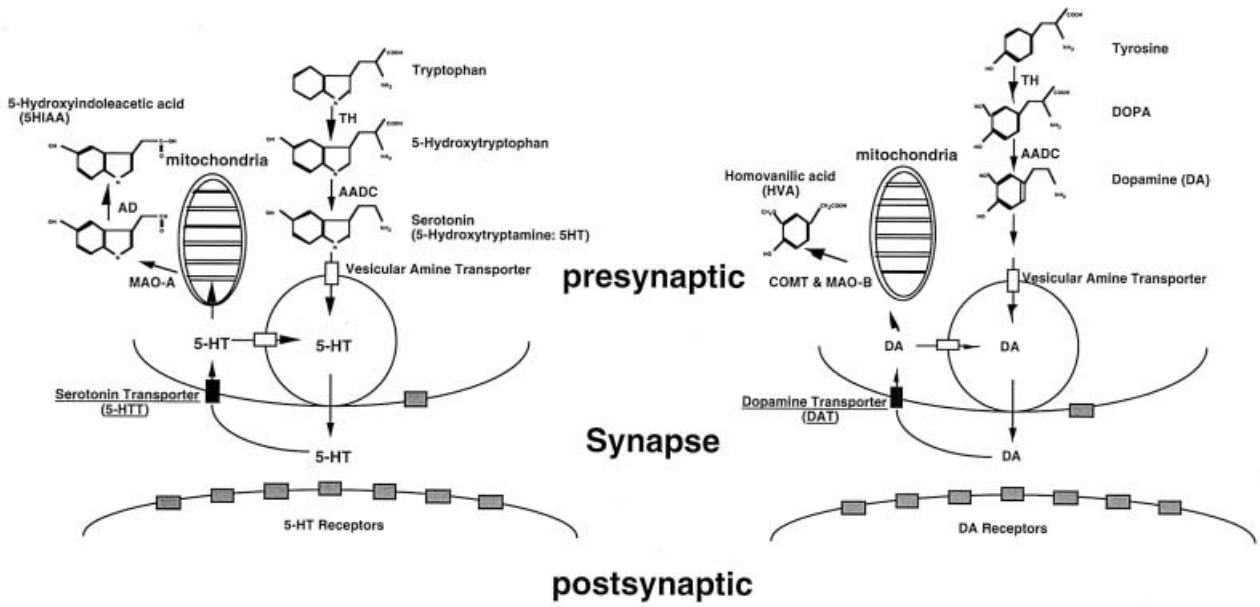

Figure 1. **The mechanism of neurotransmission in serotonin and dopamine synapses. The monoamine transporters (5-HTT and DAT) are functional proteins that act to take released monoamines back up into presynaptic terminals and regulate the amount of monoamines in the synaptic cleft. The up-taken monoamines are reused or degraded by their own mechanisms. So the expression of the monoamine transporter genes seems to control the neuronal transmission. TH, tryptophan or tyrosine hydroxylase ; AADC, aromatic amino acid decarboxylase ; MAO, monoamine oxidase ; AD, aldehyde dehydrogenase ; DOPA, dihydroxyphenylalanine ; COMT, catecholamine O-methyltransferase.**

transporter-mediated uptake of monoamines seems to be related with an electronic transport of sodium and chloride ions. In this review, the functional polymorphisms and relation to mental functions of both serotonin and dopamine transporters are discussed.

Serotonin transporter (5-HTT)

In the serotonin neuron, tryptophan is transferred to 5-hydroxytryptophan, and successively to 5-hydroxytryptamine (serotonin, 5-HT) by the tryptophan hydroxylase and the aromatic amino acid dehydroxylase, respectively. The final product, 5-HT is taken into synaptic vesicles by vesicular amino acid transporters, released by stimulus to synaptic clefts, and works as a neurotransmitter. The released 5-HT is taken up by serotonin transporter (5-HTT) and the neurotransmission is finished. 5-HT recovered in presynaptic neurons is degraded by monoamine oxidase A to 5-hydroxyindole acetic acid (5-HIAA) or re-used following to entering synaptic vesicles again. Since 5-HTT is a functional protein that regulates the amount of 5-HT in a synaptic gap, the agents that bind to 5-HTT, for example, serotonin reuptake inhibiting antidepressants, cocaine and 3, 4-methylenedioxymethanphetamine (MDMA ; known as "ecstasy") will modify the serotonergic transmission. These agents elevate mood in depressed or normal subjects. Therefore, 5-HTT is one of the targets in the etiological study of psychiatric disorders including mood and anxiety disorders.

The sequence analysis of 5-HTT revealed a length of 630 amino acid residues with about 2.5 kbp mRNA in chromosome 17q11.1-q12 (1, 2). The 5-HTT gene spans about 38 kbp and consists of 15 exons. One well-known polymorphism is a functional polymorphism in its 5'-flanking region, which is known as a serotonin transporter linked polymorphic region (5-HTTLPR). This polymorphism consists of length variation of the repetitive sequence containing GC-rich, 20～23-bp-long repeat elements (3). Two types, 16 repeats (long) or 14 repeats (short), were reported in 5-HTTLPR and a transfection study of serotonergic cells *in vitro* showed transcriptional efficiency in long repeats were higher than that in short repeats. With NEO five factor personality traits questionnaire, Lesch *et al.* reported that the 5-HTTLPR was associated with anxiety-related personality traits (4). This genetic variation of non-human primates influenced the CNS function, too (5). However, in addition to those two types of repeat polymorphism, two other repeat forms of alleles (19- and 20- repeat allele) were found in Japanese subjects (6). Our group found more than 14 types of alleles in the 5-HTTLPR in Japanese and Cauca-

sians (7, Figure 2). Four variants in 14-repeart (s) type allele and six variants in 16-repeat (l) type allele were detected as a single band by a usual agarose gel, respectively. But the functional difference of the silencer activity was significant among each variant in the serotonergic culture cells (8). Another polymorphism is a variable number of tandem repeat (VNTR) elements of 17 bp in the second intron of the 5-HTT gene (9, 10). This VNTR polymorphism may be also of functional significance because the amount of 5-HTT gene products is significantly higher in 12-repeat allele of 5-HTTVNTR than in 10-repeat allele if activated with morphogens (11).

Many studies have been performed to find a relation between 5-HTTLPR and mental disorders, including anxiety-related traits (4), depression (10, 12), seasonal affective disorder (13), autism (14), alcoholism (15), and schizophrenia (16). The association studies analyzing both 5-HTTLPR and 5 HTTVNTR polymorphisms were also reported (17, 18). However, the relation between 5-HTT gene polymorphisms and mental disorders is not clear yet. Ambiguous associations between 5-HTTLPR and psychiatric disorders is because the 5-HTTLPR polymorphism with diseases has been studied for only the s and l variants under the size of repeat length to determine genotypes. It should be taken into account that there are over fourteen variants and the functional difference among the alleles of 5-HTTLPR (7, 8). The more refined experiments are necessary to clarify whether the 5-HTT gene polymorphisms is associated with mental disorders or not.

The efficacy of selective serotonin re-uptake inhibitors (SSRI) was reported to be lower in s type allele than in l type allele of 5-HTTLPR (19, 20) but the results were controversial (21, 22). A preliminary report showed that short variant homozygotes had higher probability to get an antidepressant-induced mania in bipolar disorder than long variant homozygotes (23). These results suggesting a difference in therapeutic response between s and l variants of 5-HTTLPR should be examined with reference to the functio-

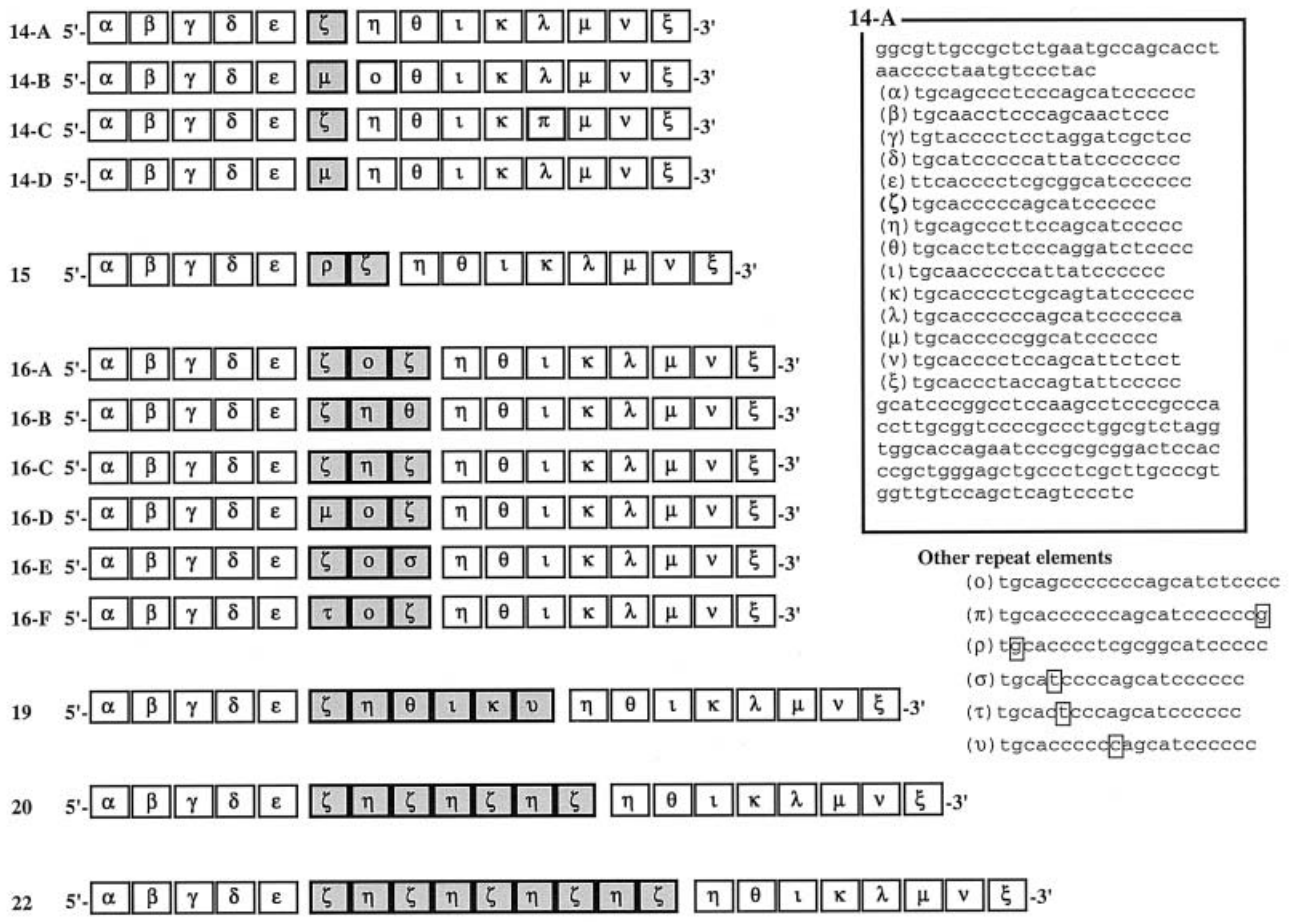

Figure 2. Nucleotide sequences and repeat architectures of 5-HTTLPR alleles. The sequence of the 14-A allele is shown in the upper right corner in the inset. The repeat elements of 14-A were slightly different from each other, and they were named in order from the 5'-end as α, β, ..., and ξ. The elements shown in dark boxes are inserted in the place of ζ element of the 14-A allele. In the lower right corner, the repetitive elements other than those in the 14-A are shown. The nucleotides in boxes are different from the nucleotides in similar repeat elements. (modified from reference 7)

nal expressional difference of the 5-HTTLPR.

Dopamine transporter (DAT)

Human dopamine transporter (DAT 1) is a monoamine transporter that has high selectivity to dopamine, and has 620 amino acid residues, M.W. 68,517 (24). The DAT1 gene is in chromosome 5p15.3, with fifteen exons, and its transcript is about 3.5 kbp long (25). Psychotropic agents such as cocaine, amphetamine, phencyclidine and 1-methyl-4-phenylpyridinium (MPP) bind to DAT. The disruption of the mouse DAT gene results in spontaneous hyperlocomotion, demonstrating its critical role in behavioral regulation (26). The dopamine transporter is an obligatory target of cocaine and amphetamine because these psychostimulants had no effect on locomotor activity or dopamine release and uptake in mice lacking the transporter. It was also demonstrated that DAT knockout mice have elevated dopaminergic tone and were about 12 times more active than normal mice when placed in new surroundings. Additionally, these mice were impaired in spatial cognitive function, and they showed a decrease in locomotion in response to methylphenidate (27). It is suggested that the DAT knockout mice seem to be a model animal of attention deficit hyperactive disorder (ADHD) in children. Although these DAT knockout mice exhibited high levels of extracellular dopamine, they paradoxically still self-administered cocaine (28). These studies suggested that DAT gene would be one of the targets for the study of substance abuse. Thus, the dopamine transporter is a very important regulatory factor in dopamine neurotransmission. Since there is a functional VNTR polymorphism in the 3'-untranslated region of the DAT1 gene (29), this polymorphism may affect the mental conditions. The association studies between the DAT1 gene and mental disorders are shown below.

DAT1 gene and ADHD

Attention deficit-hyperactivity disorder (ADHD) is one of the common psychiatric disorders of childhood, affecting 3-5% of school age children and often continuing into adolescence and adulthood (30). The etiology of ADHD is unknown, however, family, adoption and twin studies support the influence of strong genetic factors. DAT1 gene is one of the good candidates for the responsible gene for ADHD because the medicines prescribed in psychiatric practice used for ADHD, methylphenidate, pemoline and bupropion seems to have their action through the inhibition of the dopamine transporter. Cook *et al.* reported that the VNTR polymorphism of the DAT1 gene was associated with ADHD using the haplotype-based haplotype relative risk (HHRR) method (31). Several association studies support the DAT1 gene contributes some effect on ADHD. Especially, 480-bp allele (10-repeat allele) will be as the high-risk allele and the relationship between the DAT1 gene and the combined type of ADHD is strong (32). The patients who have homozygosity for 10-allele at the DAT1 gene did not respond well to methylphenidate in two pharmacogenetic studies but this mechanism was still unknown (33, 34).

DAT1 and Alcoholism

Animal studies suggest that the development of substance abuse is associated with dopaminergic activity in striatum and the limbic system (28). The single emission computed tomography (SPECT) study showed the striatal DAT density was different between alcoholics and controls (35). The association between alcoholics and the DAT1 gene was ambiguous because of populational difference, stratification (36). Our group showed the relationship between them using haplotype analysis with 10-repeat allele and a new single nucleotide polymorphism (SNP)(37, Figure 3). We also indicated a gene dose effect of 10-repeat allele with 2319GtoA SNP on the risk for alcoholism. The reporter assay showed the DAT expression of 10-repeat allele was functionally lower than that of 9-repeat allele (29) and it is suggested that the lower expression of the DAT1 gene results in higher tendency of substance abuse.

DAT1 and Smoking

The behavioral and neurobiological effects of nicotine are similar to addictive drugs and nicotine made the release of dopamine in basal ganglia in animal study (38). So DAT1 gene is one of candidates for heritable influences on cigarette smoking. 9-repeat allele of the DAT1VNTR was reported to be associated with lack of smoking, late initiation of smoking, and length of quitting attempts (39). The study by examining both smoking behavior

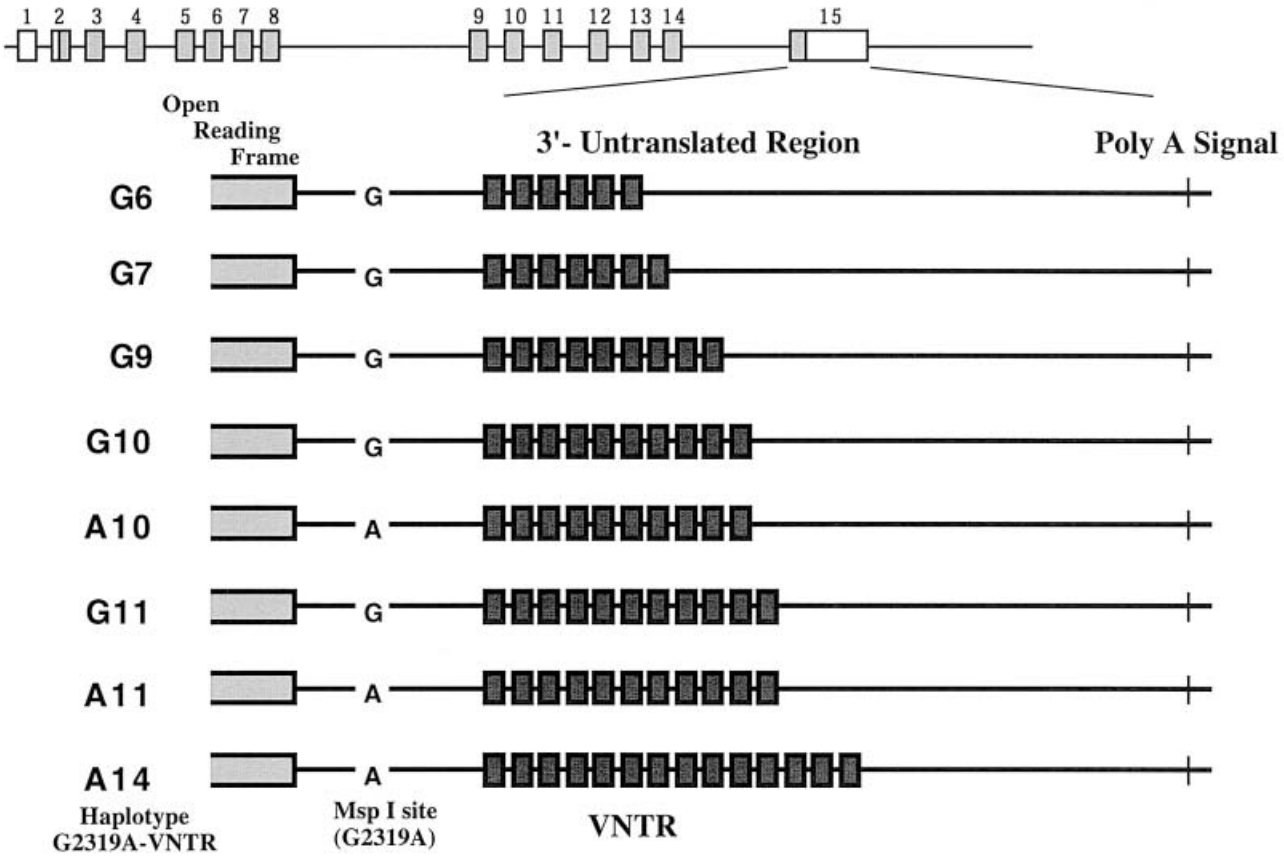

Figure 3. The haplotypes of the DAT1 gene. The open space square means the 5'- or 3'-untranslated regions and the dotted space square means the open reading frame in the genomic structure on the top of this figure. The exon 15 including the G2319A polymorphism is shown below. There were eight haplotypes ; the G2319A polymorphism and the repeat number of the VNTR is shown on the left of this figure. G2319A polymorphism is distinguished with *Msp* I restriction enzyme. VNTR, Variable number of tandem repeats. (modified from reference 37)

and personality traits of more than thousand individuals in a diverse population of nonsmokers, current smokers, and former smokers, confirmed the significant association between 9-repeat allele of DAT1VNTR and smoking status (40). The 9-repeat was also associated with low scores for a personality trait of novelty seeking in this study. But Jorm *et al.* did not replicate this association in 861 Caucasian subjects (41). Further study should be performed.

CONCLUSIONS

Monoamines are important neurotransmitters for human behavior and cognition. The function of monoamine systems is regulated by many factors including receptors, transporters and their metabolizing enzymes. Monoamine transporters functionally uptake monoamines into presynaptic neurons and cease their transmission. The genetic polymorphisms of the transporters change their expression and should affect the personality difference and the susceptibility to mental disorders. The more sophisticated genetic studies should be done with their polymorphic expressional differences. To clarify the mechanism of the transporter gene expression will contribute to the better understanding of mental functions and disorders.

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