REVIEW ARTICLE

Genetic polymorphism of serotonin transporter 5-HTTLPR: involvement in smoking behaviour

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Abstract

Data suggest that the serotonin (5-hydroxytryptamine, 5-HT) system is implicated in the pathogenesis of multiple neuropsychiatric disorders and may also be involved in smoking behaviour since nicotine increases brain serotonin secretion. It is known that smoking behaviour is influenced by both genetic and environmental factors. The present review examines the role of the serotonin transporter gene (5-HTT) in smoking behaviour and investigating studies that showed association of 5-HTT gene with smoking. This study discusses a polymorphism which has been investigated by many researchers, as the bi-allelic insertion/deletion polymorphism in the 5′- flanking promoter region (5-HTTLPR). This gene has received considerable attention in attempts to understand the molecular determinants of smoking. Therefore, in the present study, the relationship between genetic polymorphism of serotonin transporter in smoking behaviour is reviewed considering the interactive effect of genetic factors.

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Introduction

Tobacco smoking is one of the most potent and prevalent addictive habits influencing human behaviour and continues to be the second major cause of death in the world (WHO 2002) and it is associated with economic losses to society (Centers for Disease Control and Prevention 2008). More than 98% of tobacco users smoke cigarettes. Most of them are physically dependent on nicotine, which is the primary component present in tobacco and responsible for the smoking addiction (Li 2006). It is well documented that nicotine dependence results from an interplay of neurobiological, environmental and genetic factors (Amos *et al*. 2010).

There are a number of candidate genes to link with the smoking habit (Li 2006), since genetic variability (polymorphic alleles) probably plays an important role in many human traits including nicotine dependence (Li *et al*. 2007). Batra *et al*. (2003) have reviewed the scientific evidence that support a role for genetic influences on smoking. A better understanding of the genetic and environmental influences and their interactions should reinforce the concept of tobacco smoking as a chronic addictive disease that needs to be addressed. Furthermore, insights into the genetic contributions to smoking can potentially lead to more effective strategies to reduce smoking. It is increasingly recognized that smokers are not a homogeneous group; moreover, genetic influences on different stages of smoking such as initiation, maintenance, and cessation may not be identical.

The role of the serotonin transporter gene (5-HTT) effects in nicotine dependence has been investigated, as 5-HTT is a neuromodulator with widespread influences in the central nervous system. Despite detailed knowledge about the molecular biology of cellular signalling, it is not possible to anticipate the responses of neuronal networks to a global action of 5-HT (Manzke *et al*. 2009).

The 5-HTT is a polymorphic gene in the human population and such genetic variation may influence human disturbances of the mood and, consequently, smoking behaviour. The aim of the current review is to provide some information about one genetic polymorphism of the serotonin transporter (5-HTTLPR) and its involvement in smoking behaviour.

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The serotonergic neurotransmission

A large number of evidences suggests that personality and behavioural traits may be related to brain monoamine changes (Blum *et al.* 2000; Gerra *et al.* 2000; Young *et al.* 2002), and particularly with a dysfunction of serotonin (5-HT) transmission (Virkkunen and Linnoila 1990; Gerra *et al.* 1995; Coccaro *et al.* 1996; Tiihonen *et al.* 1997).

Evidence also indicates that nicotine increases serotonin release in the brain and that symptoms of nicotine withdrawal may be modulated by diminished serotonergic neurotransmission (Ribeiro *et al.* 1993).

The serotonin system is implicated in the pathogenesis of multiple neuropsychiatric disorders and has received considerable attention in attempts to understand the molecular determinants of smoking (Veenstra-Vander *et al.* 2000). It is known that serotonergic neurotransmission in brain is involved in the inhibitory control of behaviour (Erritzoe *et al*. 2009).

Serotonergic transmission is regulated by coordinated actions of several 5-HT-related proteins: 5-HT autoreceptors, enzymes that synthesize (tryptophan hydroxylase, TPH) or degrade (monoamine oxidase, MAO) serotonin and 5-HTT that cleans the synaptic cleft by taking released 5-HT back into nerve terminals. It was suggested that genetically heritable variations in function of these 5-HT-related proteins affects 5-HT levels in the synaptic cleft, and consequently its role in neurotransmission (Lesch 2005).

The serotonin transporter (5-HTT)

5-HTT is a member of the sodium-and-chloride-dependent neurotransmitter transporter (SLC6) family (Saier 1999). It is a 630-amino-acid protein and has 12 transmembrane domains. Its C and N terminal regions lie in the cytoplasm (Amara and Kuhar 1993; Rudnick and Clark 1993; Tate and Blakely 1994; Qian *et al.* 1995) and it is responsible for regulating the magnitude and duration of serotonergic neurotransmission (figure 1).

This protein selectively transports serotonin together with Na^+ and Cl^- into cells and in the same reaction transports K^+ out of the cell modulating serotonergic signalling and neurotransmission (Blakely *et al.* 1994). It has been shown

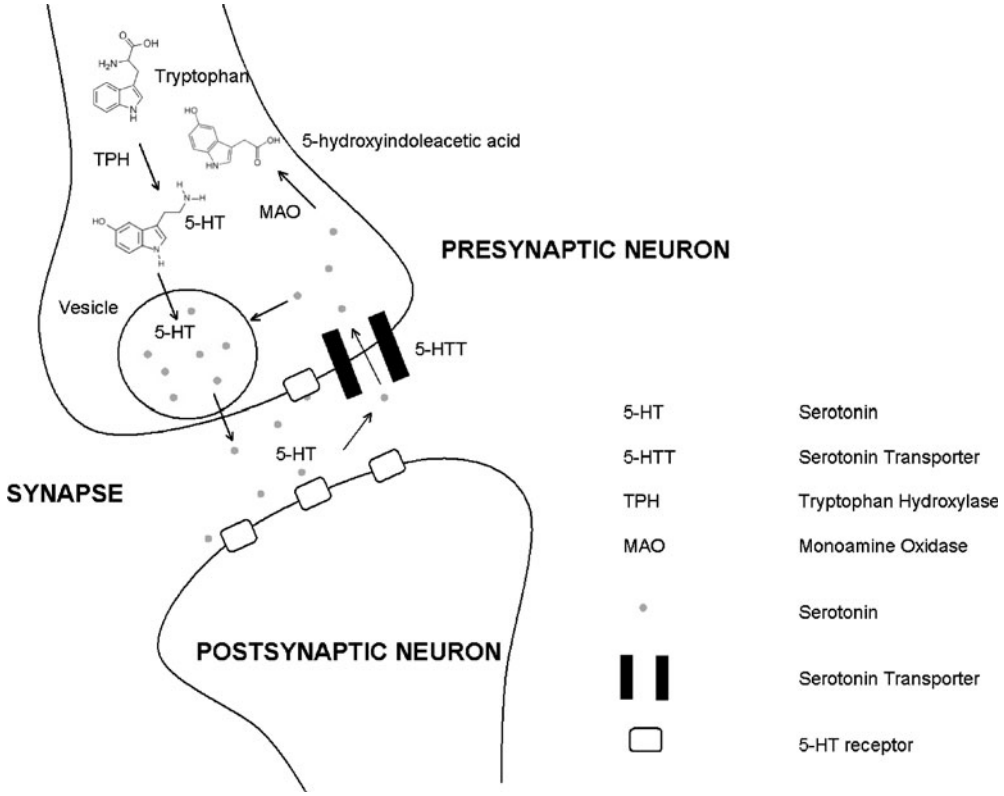

Figure 1. Schematic view of the serotonergic neurotransmission. Inside the neuron, tryptophan can be converted into serotonin (5-HT) through the action of the enzyme tryptophan hydroxylase (TPH). The serotonin is then being stored in vesicles. After received a neuron stimulation, the 5-HT contained vesicle fuses with the neuronal membrane, so that neurotransmitter is released in the synaptic cleft. In the synaptic cleft, serotonin can bind to receptors on neurons, both postsynaptic and presynaptic and trigger the effector functions of serotonergic neurotransmission. The concentration of serotonin in the cleft can be modulated by the serotonin transporter (5-HTT) that activates neuronal reuptake of serotonin. Back in the presynaptic neuron, serotonin can return the vesicles and/or be degraded by monoamine oxidase (MAO) in an inactive metabolite, the 5-hydroxyindoleacetic acid.

that 5-HTT is also a receptor for antidepressant drugs (fluoxetine, sertraline and paroxetine) and psychostimulant drugs of abuse such as 3,4–methylenedioxymethamphetamine (MDMA; known as ecstasy), amphetamine, and cocaine, suggesting that these drugs are able to modulate the transport of serotonin into the cell (Tatsumi *et al.* 1997).

Heterogeneous expression of various subtypes of serotonin receptors (5-HTR) in a variety of neurons differently equipped with cell-specific transmitter receptors resulting in various forms of network adjustment and, hence, motor behaviour (Manzke *et al.* 2009).

The human 5-HTT protein is one of the candidates for nicotine dependence and the gene coding for it, named *SLC6A4*, has been mapped to chromosome 17, q11.1–q12. (Lesch *et al*. 1994; Gelernter *et al*. 1995). This gene is a candidate for smoking predisposition because a polymorphism in its 5′-flanking region is associated with its transcriptional efficacy (Heils *et al.* 1996; Lesch *et al.* 1996).

Serotonin transporter polymorphisms

Three common polymorphisms associated with the serotonin transporter have been described: an insertion/deletion in the promoter region, called 5-HTTLPR (Heils *et al.* 1996), a variable number of tandem repeat (VNTR) in intron 2 (Lesch *et al.* 1994; Ogilvie *et al.* 1996) (5-HTTVNTR2) and a 3′ untranslated region (UTR) G/T single nucleotide polymorphism (SNP - I425V) (Battersby *et al.* 1999).

The most studied genetic variant of the human 5-HTT gene is a polymorphism known as the serotonin transporter gene-linked polymorphic region (5-HTTLPR). This common polymorphism is due to a 44–bp deletion/insertion of the repetitive sequence containing GC-rich, 20∼23-bp-long repeat elements (figure 2), in the 5′ flanking region located ∼1.4–kb upstream of the transcription start site (Heils *et al.* 1996) that results in differential expression of 5-HTT binding sites in cell lines (Lesch *et al.* 1996). It occurs in populations as two prevalent alleles, one consisting of 14 repeats (the short allele S) and another of 16 repeats (the long allele L) (Caspi *et al.* 2003).

The 5-HTTLPR is also known to be associated with altered serotonin transporter activity. The short (S) form of this polymorphism is associated with lower basal transcriptional efficiency of the 5-HTT gene promoter, resulting in lower serotonin uptake activity, when compared with the long form (L) (Lesch *et al.* 1994; Heils *et al.* 1996). Hranilovic *et al.* (2004) found that the relative expression of 5-HTT mRNA in lymphoblastoid cell lines with L/L homozygotes alleles was about 30% higher than in cells containing at least one S allele. Lesch *et al.* (1996) analysed homozygous cells for the L allele and observed mRNA levels that were 1.4 to 1.7 times higher than in cells containing copies of the S allele. These results confirm that the genotype has an influence on the expression levels.

Heils *et al.* (1997) showed the influence of the 5-HTT gene promoter polymorphism at the protein level. They analysed the binding and uptake of serotonin in lymphoblasts by two kinds of isotypes. The genotype L/L presented 30%–40% more serotonin transporter in the membrane and 1.9 to 2.2 times greater serotonin uptake than the genotype carrying one or two endogenous copies of the short form.

It is known that different alleles of the 5-HTTLPR variant, which interfere with the expression of 5-HTT, could also alter serotonergic neurotransmission, such as the 5-HT1A receptor. In an association study between 5-HTTLPR genotypes and 5-HT1A receptors expression, David *et al*. (2005) explained that, mechanistically, the lower transcriptional efficiency associated with the S allele of the 5-HTTLPR may lead to decreased 5-HTT function, which in turn may lead to a lifelong increase in 5-HT, which may in turn desensitize and downregulate 5-HT1A receptors.

5-HTTLPR polymorphism and smoking behaviour

It was demonstrated that smoking habit may be associated with diminished serotonin neurotransmission determined

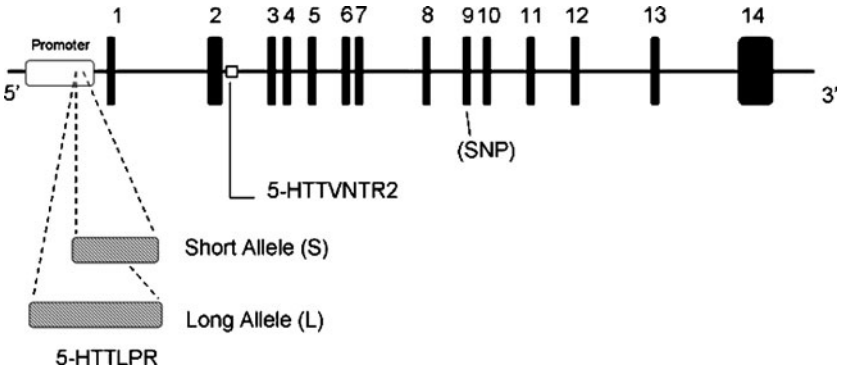

Figure 2. Schematic view of the polymorphisms associated with the serotonin transporter gene. Three common polymorphisms associated with the serotonin transporter have been described. The insertion/deletion in the promoter region, called 5-HTTLPR, a variable number tandem repeat (VNTR) in the intron 2 and a 3′ untranslated region (UTR) G/T single nucleotide polymorphism (SNP - I425V). The HTTLPR polymorphism is due to a 44-bp deletion (S)/insertion (L) of the repetitive sequence containing GC-rich, 20∼23-bp-long repeat elements.

by genetic polymorphisms and the 5-HTTLPR variant has demonstrated associations with smoking-related phenotypes (Ishikawa *et al.* 1999; Lerman *et al.* 2000; Chu *et al.* 2009). According to this, the 5-HTTLPR may be a plausible candidate gene for smoking predisposition because its role in psychological traits is relevant to smoking behaviour, such as anxiety-related-personality traits (Lesch *et al.* 1996) and depression (Collier *et al.* 1996). Thus, a selective review with a systematic procedure was performed to investigate studies showing association between the 5-HTTLPR polymorphism and smoking behaviour (table 1).

Further, most of the demonstrated data have been obtained from research designs that have limitations in the study of complex behaviours. The inconsistency in results is apparently due to the heterogeneity of the samples. Lerman *et al.* (1998) found significant racial differences in the distribution of 5-HTT genotypes. Their results showed that Caucasians were significantly more likely to carry the short variant of the gene than were African Americans. Moreover, studies involving variable ethnic groups may contribute to different genotypes. In this context, it is known that the L allele frequency is lower in the Japanese (0.16) than Europeans, Americans (0.6) and African Americans (0.7) (Gelernter *et al*. 1997).

According to Tyndale (2003), the release of serotonin may increase due to nicotine, suggesting that variations in the serotonergic system may influence some aspects of smoking, such as mood variations during nicotine withdrawal. Ribeiro *et al.* (1993) has also verified that the serotonin released is increased in the brain cortical region of rats treated with nicotine, and nicotine withdrawal seems to be related to the subsequent serotonin decrease.

Fletcher *et al.* (1999) reported that the depletion of the monoamine neurotransmitter serotonin in animals consistently leads to an impulsive behavioural pattern with increased response to conditioned reinforcers and manipulations that decrease brain 5-HT neurotransmission that have been shown to elevate selfadministration of food (Waldbillig *et al.* 1981) and alcohol (Lyness and Smith 1992; Ciccocioppo *et al.* 1999) as well as tobacco smoking (Hitsman *et al.* 2007). On the other hand, manipulations that increase 5-HT levels inhibit intake of food (Halford *et al.* 2005), alcohol (Johnson 2008), and nicotine in animals and humans (Olausson *et al.* 2002).

A highly significant association has been reported between the serotonin transporter gene and the smoking habit, suggesting that it influences smoking. The 5-HTTLPR alleles have been associated with altered transcriptional activity and individuals with different alleles might be at risk for nicotine dependence or may stop smoking more easily. Further, many studies have failed to substantiate this hypothesis and a growing number of studies in the literature have examined the role of the 5-HTT gene in smokers, but the results have been contradictory.

Ishikawa *et al.* (1999) suggested a neurochemical hypothesis that postulated how the long allele could be a risk factor for smoking behaviour, based on some results. Nicotine increases brain serotonin secretion, and nicotine withdrawal has the opposite effect (Ribeiro *et al.* 1993; Mihailescu *et al.* 1998), leading to the hypothesis that the appetite and mood disturbances associated with nicotine withdrawal may be mediated by diminished serotoninergic transmission. Fluoxetine treatment effectively prevents the increased food intake and weight gain in smokers who reduce their nicotine intake (Pomerleau *et al.* 1991). Fluoxetine antagonizes the response to nicotine that evokes hyppocampal noradrenaline release in the rat (Hennings *et al.* 1997). The 5-HTTLPR short allele is associated with lower transcriptional activity.

Gerra *et al.* (2005) suggested that the dysfunction of the serotonin reuptake mechanism maybe provoked by the short

Table 1. Association studies of 5-HTTLPR polymorphism on smoking behaviour.

Region	Samples (*n*)	Association with smoking behaviour	Reference
USA	268 smokers and 230 nonsmokers	No	Lerman *et al*. (1998)
Japan	82 non smokers, 103 ex-smokers and 202 smokers	Long allele	Ishikawa *et al*. (1999)
USA	458 nonsmokers, 124 ex-smokers and 177 smokers	Short allele	Hu *et al.* (2000)
USA	185 smokers	Short allele	Lerman *et al*. (2000)
Israel	54 smoked in the past and 190 smokers	Long allele	Kremer *et al*. (2005)
Italy	103 nonsmokers and 107 smokers	Short allele	Gerra *et al*. (2005)
Austria	470 smokers and 419 nonsmokers	No	Trummer *et al*. (2006)
Poland	149 smokers and 158 nonsmokers	No	Sieminska *et al*. (2008)
Denmark	642 non-smokers, 396 ex-smokers and 327 smokers	No	Rasmussen *et al*. (2009)
China	144 smokers and 135 nonsmokers	Long allele	Chu *et al*. (2009)
Sweden	80 smokers and 120 nonsmokers	Long allele	Nilsson *et al*. (2009)
Greece	172 smokers and 254 nonsmokers	No	Iordanidou *et al*. (2010)

allele, and this may contribute to induce novelty seeking and aggressive behaviour. According to Cadoret *et al.* (2003) and Lakatos *et al.* (2003) this aggressive behaviour might also contribute to nicotine addiction vulnerability.

Nicotine dependence, like many other drug dependencies, is a complex behaviour with both genetic and environmental components. In this review, it was demonstrated that the serotonergic system is closely linked to personality traits, mood disorders and consequently to smoking behaviour. The characteristics of the serotonin transporter (genetic and structural) and its central role in regulating neurotransmission were reviewed. It was demonstrated that a polymorphism in the promoter region of this gene, the *5-HTTLPR*, modulates the mRNA and protein levels and that such allelic variants may influence nicotine dependence. In terms of transcription regulation, the presence of the short or long alleles appears to have a great influence. It is known that among multiple levels of gene regulation, there is the possibility of a dynamic control of transcription by sequences within introns, promoters and unstranslated regions. Some genetic alterations in these sequences could allow increased or decreased transcription compared with the wild–type sequence. Data from the 5-HTTLPR genetic polymorphism suggest that this regulatory promoter region is subject to alterations. Hence, we believe that the presence of the long allele must facilitate the assembly of some transcriptional factors and may contribute to overexpression, highlighting the importance of noncoding sequences in regulating this gene, and consequently in its phenotypic expression.

The 5-HTT gene has shown evidence in association with smoking cessation, in a comparison of current smokers with ex-smokers. Studies from Gilbert *et al*. (2009), Bloch *et al.* (2010) and Carlson *et al.* (2009) reported a significant association of the short allele in nicotine replacement therapy. Munafò *et al*. (2006) predicted that possession of one or more copies of the short carriers (S allele) of the 5-HTTLPR polymorphism would be associated with reduced likelihood of successful cessation. Data from David *et al*. (2007, 2008) do not support a statistically or clinically significant moderating effect of these specific 5-HT pathway genetic variants on smoking cessation. The results from Gilbert *et al.* (2009) suggested that the effects of genotype and treatment may vary across different durations of abstinence, treatment doses, and genotypes. Individuals with one or two short 5-HTTLPR alleles experienced larger increases in negative affect symptoms than those without a short allele. It is possible that the S allele influences smoking cessation via increased anxiety-related withdrawal symptomatology, given evidence of an association of this polymorphism with anxiety-related traits (Munafò *et al.* 2003).

In this review, studies reported that nicotine dependence, like many other drug dependencies, is a complex behaviour with both genetic and environmental factors contributing to the variance. Most of the genetic data have been obtained from research designs that have limitations in the study of complex behaviours. The results obtained through this line of research will eventually aid clinicians to recognize that smokers are not a homogeneous group. In future, there is a need for prospective studies to show that 5-HTTLPR is not a major factor determining smoking behaviour and there are interactions between the 5-HTTLPR polymorphism and psychological traits in smoking behaviour.

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