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ORIGINAL RESEARCH ARTICLE

Allelic variation of serotonin transporter expression is associated with depression in Parkinson's disease

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Idiopathic Parkinson's disease (PD) is a common neurodegenerative disorder with prominent motor symptoms. However, depression is common in PD, affecting about 40% of PD patients. Since there is extensive evidence of degeneration of serotonin (5HT) neurons and loss of the 5HT transporter (5HTT) in PD, we assessed whether a functional polymorphism in the promoter of the 5HTT gene (5HTT gene-linked polymorphic region, 5HTTLPR), which determines high or low 5HT uptake, is associated with depressive symptomatology in PD patients. We found that patients with the short allele of the 5HTTLPR had significantly higher scores on the Hamilton Depression Scale. A functional promoter polymorphism of the monoamine oxidase A (MAOA) gene showed no association. Thus, the 5HTTLPR but not the MAOA gene promoter-associated polymorphism may be a risk factor for depression in PD patients, while neither polymorphism increases the risk for development of Parkinson's disease itself. *Molecular Psychiatry* (2001) **6,** 350–352.

PD is the second most common neurodegenerative disease and affects about 1% of the Western population older than 50 years. Rare forms of PD are transmitted in an autosomal dominant or autosomal recessive mode of inheritance: for these, mutations in the Park 1 locus (α-synuclein) or the Park 2 locus have been demonstrated, while the underlying mutation in a third locus, Park 3, has not yet been identified.[1] The vast majority of PD cases, however, are sporadic and are probably caused by the interaction of several genes with multiple environmental factors. One of these genes may be the ϵ4 allele of the apolipoprotein E gene, which in conjunction with a Park 1 promoter polymorphism has very recently been shown to increase the risk for developing sporadic PD.[1]

The most striking symptoms of PD relate to the motor system, with rigidity, tremor, and akinesia. However, psychiatric manifestations including depression and dementia syndrome are common in PD patients. Depression is a frequent and often presymptomatic feature and has been shown to affect about 40% of PD patients.[2–4] This may relate to the fact that neurodegeneration in PD is not restricted to the dopaminergic neurons of the substantia nigra but also affects serotonergic neurons. The cell bodies of serotonergic neurons are located in the raphe nuclei of the brainstem. The axons of these serotonergic neurons project to almost all regions of the brain. The neurotransmitter they produce, serotonin (5HT), influences many physiologic functions including motor activity, food intake, reproductive activity, sleep, and neuroendocrine rhythms, as well as cognition and emotional states, including mood and anxiety. This diversity of effects is due to the fact that 5HT influences the activity and interaction of several other neurotransmitters. 5HT exerts its effects via at least 14 known pre- and postsynaptic 5HT receptor subtypes. The reuptake of 5HT released into the synaptic cleft, on the other hand, is mediated by a single protein, the 5HT transporter (5HTT). The 5HTT thus plays a critical role in the fine-tuning of serotonergic neurotransmission by determining the duration and the amount of 5HT present in the synaptic cleft.

In PD, there is an extensive loss of serotonergic raphe neurons, which may be more marked in the median raphe nucleus than in the dorsal raphe nucleus. Moreover, PD brains with Lewy bodies in the substantia nigra also had Lewy bodies in the raphe nuclei.[5–7] The loss of serotonergic neurons in the raphe nuclei is paralleled by a loss of serotonergic terminals as indicated by a reduction of 5HTT of up to 40–50% in various serotonergic projection areas including frontal cortex, temporal cortex, and putamen.[5,8,9] Given this loss of serotonergic neurons and 5HTT in PD, it is interesting to note that in humans there is a genetically driven variation in 5HTT expression. Analysis of the 5′-flanking regulatory region of the human 5HTT gene revealed a polymorphism that results in allelic variation in functional 5HTT expression. This polymorphic repetitive element (5HTT gene-linked polymorphic region, 5HTTLPR) is located approximately 1.4 kb upstream of the transcription initiation site and consists predominantly of either 14 or 16 repeat elements. The long (l) allele with 16 repeat elements leads to more 5HTT mRNA, 5HTT protein, and 5HT uptake, than the short (s) allele with 14 repeat elements.[10] This relationship between 5HTTLPR genotype, 5HTT gene transcription, and 5HT uptake activity has also been shown for 5HTT mRNA concentrations in the raphe complex of human postmortem brain[11] and for 5HTT binding sites *in vivo*.[12] 5HT uptake is dysregulated in affective dis-

Table 1 Depression by genotype. Depressed = 18 or higher on HAM-D

Genotype	*Depressed*	*Nondepressed*
l/l	0	25
l/s	2	33
s/s	2	10

Indicated are number of patients. Fisher's exact: $P = 0.11$.

orders such as depression and bipolar affective disorder and remains decreased after recovery. Moreover, the s allele of the 5HTTLPR (which results in less 5HT uptake) has been shown in some studies but not in others to be associated with an increased risk for these disorders (for review see Lesch).[13] We therefore queried whether the s allele of the 5HTTLPR represents a risk factor for the development of depression in PD patients. For comparison, we investigated a polymorphism in the 5′-flanking regulatory region of another gene, monoamine oxidase A (MAOA), in which the low-activity, short alleles (2 and 3) but not the long alleles (3a, 4, and 5) have been shown in some studies[14,15] but not in others[16] to be associated with panic disorder, depression or bipolar affective disorder.

We have now investigated a sample of 72 patients with idiopathic PD. In this sample, the frequency of the 5HTTLPR genotypes and alleles was similar compared to 301 control subjects. The severity of PD symptoms (UPDRS score) in the PD patients did not differ significantly between the 5HTTLPR genotypes. The HAM-D score was significantly higher in PD patients of the s/s genotype than in PD patients of the l/l genotype (8.4 *vs* 3.8, $P = 0.02$). Heterozygotes showed an intermediate value (4.7), which was not statistically different from the l/l or s/s genotype. Cut-off scores may be applied to the HAM-D scale to define typical depressed states. When a cut-off score of 18 is applied to the HAM-D scale,[17] two of 12 (17%) of the s/s genotype and two of 35 (6%) of the l/s genotype were depressed, while none in the l/l group were (Fisher's exact, $P = 0.11$) (Table 1). If a less severe cut-off score of 10 is used, 42% of the s/s genotype and 29% of the l/s genotype fall in the 'depressed' group, but only 8% of the l/l patients (Fisher's exact, $P = 0.03$) (Table 2).

For the MAOA gene promoter polymorphism, the frequency of the low-activity, short alleles (2 and 3) was slightly but non-significantly lower in the PD patients, compared to controls (34% *vs* 35%). Correspondingly, the high-activity, long alleles (3a, 4, and 5) were slightly more prevalent in the PD patients, compared to controls (66% *vs* 65%). Regarding depression in PD patients, the HAM-D score was not associated with genotype groups or alleles of the MAOA gene promoter polymorphism.

Table 2 Depression by genotype. Depressed = 10 or higher on HAM-D

Genotype	*Depressed*	*Nondepressed*
l/l	2	23
l/l	10	25
s/s	5	7

Indicated are number of patients. Fisher's exact: $P = 0.03$.

One previous study[18] has investigated the association between 5HTTLPR genotype and PD, but no association between the 5HTTLPR and PD was found in 47 patients. We have now extended this finding to a further 72 patients and shown that the 5HTTLPR does not represent a risk factor for PD. In addition, the MAOA promoter polymorphism does not represent a risk factor for PD, despite the fact that MAOA is involved in the metabolism of dopamine.

Our results show that the 5HTTLPR is associated with measures of depression in PD. This finding supports the results of a pilot study of 32 patients with PD, which also found an association of the short allele with depressive symptoms in PD.[17] Given the low depression scores among PD patients of the l/l genotype in both our study and the study of Menza and coworkers,[17] we suggest that it may be helpful to screen the 5HTTLPR genotype in PD patients. Together with other genetic markers yet to be described, it could be possible in the future to pinpoint those PD patients most at risk for developing depression. This is all the more important since in a study of patients with delusional depression, those with the s/s genotype responded poorly to selective 5HT reuptake inhibitors such as fluvoxamine, compared to those of the l/l or l/s genotypes.[19,20] Moreover, depression may be associated with a more rapid cognitive and motor decline of PD patients.[21] Thus, the 5HTTLPR might in the future be of diagnostic and potentially also of therapeutic and prognostic value for depression in PD patients.

The pathophysiology of the association of allelic variation of 5HTT expression with depression in PD is currently not known, and it remains to be investigated whether 5HT neuron degeneration is more pronounced in depressed than in non-depressed PD patients. Given the recent advances in *in vivo* imaging of the 5HTT,[12] this can now be investigated with PET or SPECT studies without having to resort to neuropathological investigations. Such studies may yield further insight into the mechanism of 5HTT-associated neurodegeneration.

Methods

Subjects

The diagnosis of 72 patients with idiopathic PD who were admitted to the Hospital for Parkinson's Disease, Bad Nauheim, Germany, was based on the clinical examination and history. The type of PD was hypokinetic-rigid type in 30 patients, tremor-dominant type in six patients and equivalence type in 36 patients. There were 39 males and 33 females. Mean disease duration was 9.9 years. PD symptoms were scored on the Unified Parkinson's Disease Rating Scale (UPDRS),[22] which was administered on admission.

Concomitantly, the 21-item Hamilton Rating Scale for Depression (HAM-D) was administered by clinicians who were blinded to the genotypes. The control subjects were blood donors (n = 301). All subjects were unrelated and of German Caucasian descent.

DNA analysis

DNA was extracted from peripheral venous blood after informed consent with approval of the local ethics committee, and the 5HTTLPR and the MAOA gene-associated promoter polymorphism were analysed by polymerase chain reaction (PCR) according to the methods described previously.[10,14]

Statistical procedures

Allele and genotype frequencies (PD patients *vs* controls) were compared by Fisher's exact test. Moreover, HAM-D scores in PD patients with different genotypes were compared by Student's *t*-test, while genotype frequencies in depressed *vs* non-depressed PD patients were compared by Fisher's exact test.

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