

Association between serotonin transporter gene polymorphism and chronic fatigue syndrome

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Abstract

Interaction between the hypothalamo–pituitary–adrenal axis and the serotonergic system is thought to be disrupted in chronic fatigue syndrome (CFS) patients. We examined a serotonin transporter (5-HTT) gene promoter polymorphism, which affects the transcriptional efficiency of 5-HTT, in 78 CFS patients using PCR amplification of the blood genomic DNA. A significant increase of longer (L and XL) allelic variants was found in the CFS patients compared to the controls both by the genotype-wise and the allele-wise analyses (both $p < 0.05$, by χ^2 test and Fisher's exact test). Attenuated concentration of extracellular serotonin due to longer variants may cause higher susceptibility to CFS.

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Chronic fatigue syndrome (CFS) is defined as a sensation of abnormally prolonged fatigue, according to the guidelines of the US Centers for Disease Control and Prevention [1]. Yet the precise pathophysiology is unknown to date; cytokines, neuropeptides, or neurotransmitters are considered to be responsible for the abnormal immune response and disrupted hypothalamo–pituitary–adrenal (HPA) axis found in the patients [2–5]. Genetic backgrounds have been searched widely, although they failed to find any candidate genes.

Serotonin (5-hydroxytryptamine, 5-HT) modulates diverse brain functions through interactions with 14 different 5-HT receptor subtypes and is known to regulate sleep, appetite, pain, and inflammation, all of which are related to the symptoms of CFS. Several 5-HT agonist challenge tests showed a disturbance of the

5-HT–HPA axis interaction in the CFS patients, suggesting the 5-HT involvement in the etiology of CFS [2,6]. The complex 5-HT neuronal system is under a bottleneck control by a single protein, 5-HT transporter (5-HTT). By controlling reuptake of 5-HT from the extracellular space, 5-HTT regulates the duration and strength of the interactions between 5-HT and its receptors.

A polymorphism within the 5-HTT 5' upstream region (5-HTTLPR) has been reported, of which the majority is composed of either 14 (S) or 16 (L) repetitive elements. In humans, although infrequent, 18 and 20 repetitive elements (XL) are also present [7–10]. An in vitro transcriptional assay indicated that the activity of the human 5-HTT promoter is regulated by these polymorphic repetitive elements, resulting in differences in the efficacy of 5-HTT reuptake among the allelic variants [11].

In this study, we have investigated the 5-HTTLPR in the CFS patients and the controls.

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Table 1
Comparison of genotype distribution and allele frequencies between the CFS patients and the control subjects

	CFS patients	Controls	χ^2 test	Fisher's exact test
<i>Genotype</i>	<i>n</i> = 78	<i>n</i> = 50	$\chi^2 = 7.887, p = 0.031$	$p = 0.026$
S/S	42	39		
L/S	32	10		
L/L	3	1		
XL/L	1	0		
<i>Allele</i>	<i>n</i> = 156	<i>n</i> = 100	$\chi^2 = 7.233, p = 0.016$	$p = 0.012$
S	116	88		
L	39	12		
XL	1	0		

Subjects and methods

Seventy-eight patients (35.67 ± 8.2 years old), who were treated in the Osaka University Medical Hospital, were enrolled in this study. A diagnosis of CFS was made based on the clinical criteria proposed by Fukuda et al. [1]. Written informed consent was taken from each patient prior to the study, which was approved by the Ethical Committees of both Osaka University and Tsukuba University. For the age-matched controls, 50 healthy volunteers (28.0 ± 8.4 years old) were subjected to the study.

Genomic DNA was extracted from the whole blood using the Genomic DNA Isolation Reagent (Prepman Ultra, Applied Biosystems, Foster City, CA, USA). The extracted DNA was amplified by a polymerase chain reaction (PCR) as described previously [10]. Oligonucleotide primers flanking the 5-HTTLPR and corresponding to the nucleotide positions -1416 to -1397 ($5'$ -GGCGTTGCCGCTCTGAA TGC) and -910 to -888 ($5'$ -GAGGGACTGAGCTGGACAACCAC) of the 5-HTT gene 5'-flanking regulatory region were used to generate 484, 528, or 613 bp fragments, corresponding to the S, L, and XL alleles, respectively. The resultant PCR products were visualized by a 2% agarose gel electrophoresis followed by an ethidium bromide staining. The genotype distribution and the allele frequencies of the 5-HTTLPR were statistically analyzed between the CFS patients and the controls by Pearson's χ^2 test and Fisher's exact test.

Results and discussion

As shown in Table 1, we have found significant differences between the CFS patients and the controls both by the genotype distribution ($p < 0.05$ by Pearson's χ^2 test and Fisher's exact test), and the allele frequencies ($p < 0.05$ by Pearson's χ^2 test and Fisher's exact test). Since two other 5-HT related polymorphisms we have studied, the 5-HT 2A receptor promoter polymorphism and the 5-HTT intron 2 VNTR polymorphism, showed no significant difference between the CFS patients and the controls (data not shown), we speculate that 5-HTTLPR is closely linked to the pathophysiology of CFS.

The interaction between the HPA axis and the 5-HT system is probably mediated partially by the 5-HT_{1A} receptors in the hippocampus and partially by the neuroendocrine activation by the serotonergic neurons in the paraventricular nucleus or the hypothalamus. To date, some replicated results of challenge tests with CFS patients are reported, including enhanced prolactin response to a selective 5-HT agonist, D-fenfluramine.

This indicates that a hypofunction of the 5-HT system and/or a hypersensitivity of the serotonin receptors in the brain may result in the irregular reactions in the primary endocrine stress system in the pathophysiology of CFS [2,6,12,13].

The L allele, which is more frequently observed in the CFS patients, is supposed to retain higher transcriptional activity compared to the S allele [11,14]. This may result in the lower concentration of 5-HT in the extracellular space, namely, active 5-HT. To date, no functional analysis of XL allele has been reported. However, from our recent findings describing a strict association between the longer variants and sudden infant death syndrome [10], the functional alteration of the XL variant as similar to the L variant is strongly suspected.

To our knowledge, this is the first reported genetic linkage to CFS and it emphasizes the '5-HT system dysfunction hypothesis' when considering the etiology of the disease. We postulate that the individuals carrying the longer alleles are primarily susceptible to CFS, because of the relative hypofunction of the 5-HT system due to the lower 5-HT concentration in the extracellular space, compared to the ones with the short allele.

This hypothesis is also supported by our preliminary observation of the trial of Selective Serotonin Reuptake Inhibitor, fluvoxamine, for the treatment of 39 Japanese patients with CFS. Twenty-eight out of the 39 patients were free from side effects and were given fluvoxamine for more than 2 months. Two patients were cured of CFS after the treatment and 8 of them recovered enough to return to work, suggesting that the serotonergic hypofunction might be involved in at least a part of the pathogenesis of CFS. As CFS is a relatively new and still obscure syndrome, our present study is expected to be applied to find a reliable biochemical marker, which has not been established until now, and an effective treatment for the disease.

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References

- [1] K. Fukuda, S.E. Straus, I. Hickie, M.C. Sharpe, J.G. Dobbins, A. Komaroff, The chronic fatigue syndrome: a comprehensive approach to its definition and study, International Chronic Fatigue Syndrome Study Group, *Ann. Intern. Med.* 121 (1994) 953–959.
- [2] A.J. Parker, S. Wessely, A.J. Cleare, The neuroendocrinology of chronic fatigue syndrome and fibromyalgia, *Psychol. Med.* 31 (2001) 1331–1345.
- [3] A.L. Komaroff, The biology of chronic fatigue syndrome, *Am. J. Med.* 108 (2000) 169–171.
- [4] J. Bearn, S. Wessely, Neurobiological aspects of the chronic fatigue syndrome, *Eur. J. Clin. Invest.* 24 (1994) 79–90.
- [5] H. Kuratsune, K. Yamaguti, G. Lindh, B. Evengard, G. Hagberg, K. Matsumura, M. Iwase, H. Onoe, M. Takahashi, T. Machii, Y. Kanakura, T. Kitani, B. Langstrom, Y. Watanabe, Brain regions involved in fatigue sensation: reduced acetylcarnitine uptake into the brain, *NeuroImage* 17 (2002) 1256–1265.
- [6] M. Sharpe, K. Hawton, A. Clements, P.J. Cowen, Increased brain serotonin function in men with chronic fatigue syndrome, *BMJ (Clinical Research Ed.)* 315 (1997) 164–165.
- [7] A. Heils, A. Teufel, S. Petri, G. Stober, P. Riederer, D. Bengel, K.P. Lesch, Allelic variation of human serotonin transporter gene expression, *J. Neurochem.* 66 (1996) 2621–2624.
- [8] S.J. Delbruck, B. Wendel, I. Grunewald, T. Sander, D. Morris-Rosendahl, M.A. Crocq, W.H. Berrettini, M.R. Hoehe, A novel allelic variant of the human serotonin transporter gene regulatory polymorphism, *Cytogenet. Cell Genet.* 79 (1997) 214–220.
- [9] F. Murakami, T. Shimomura, K. Kotani, S. Ikawa, E. Nanba, K. Adachi, Anxiety traits associated with a polymorphism in the serotonin transporter gene regulatory region in the Japanese, *J. Human Genet.* 44 (1999) 15–17.
- [10] N. Narita, M. Narita, S. Takashima, M. Nakayama, T. Nagai, N. Okado, Serotonin transporter gene variation is a risk factor for sudden infant death syndrome in the Japanese population, *Pediatrics* 107 (2001) 690–692.
- [11] K.P. Lesch, D. Bengel, A. Heils, S.Z. Sabol, B.D. Greenberg, S. Petri, J. Benjamin, C.R. Muller, D.H. Hamer, D.L. Murphy, Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region, *Science* 274 (1996) 1527–1531.
- [12] J. Bearn, S. Wessely, Neurobiological aspects of the chronic fatigue syndrome, *Eur. J. Clin. Invest.* 24 (1994) 79–90.
- [13] J. Bearn, T. Allain, P. Coskeran, N. Munro, J. Butler, A. McGregor, S. Wessely, Neuroendocrine responses to *D*-fenfluramine and insulin-induced hypoglycemia in chronic fatigue syndrome, *Biol. Psychiatry* 37 (1995) 245–252.
- [14] S. Eddahibi, N. Hanoun, L. Lanfumey, K.P. Lesch, B. Raffestin, M. Hamon, S. Adnot, Attenuated hypoxic pulmonary hypertension in mice lacking the 5-hydroxytryptamine transporter gene, *J. Clin. Invest.* 105 (2000) 1555–1562.