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Efficacy of tricyclic antidepressant is associated with beta2-adrenoceptor genotype in patients with interstitial cystitis

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ABSTRACT

We examined the relationship between the Arg16Gly polymorphism of β2-adrenoceptor (ADRB2) and the efficacy of tricyclic antidepressant therapy in patients with interstitial cystitis (IC). We studied 55 IC patients and 113 controls. The IC patients were treated with imipramine hydrochloride, and the efficacy of treatment was categorized by patients' satisfaction (no change, fair, or good). Genomic DNA was extracted from the controls and IC patients, and the Arg16Gly polymorphism of ADRB2 was analyzed. The Arg16Gly polymorphism showed a significant difference in prevalence between IC patients and controls, and Arg/Arg was associated with increase in the risk of IC than Arg/Gly or Gly/Gly. Regarding the tricyclic antidepressant therapy, there was a significant difference in the prevalence of this polymorphism between IC patients with no change or a fair response to treatment and controls, and Arg/Arg was associated with decrease in the response rate to tricyclic antidepressant therapy than Arg/Gly or Gly/Gly. Therefore, these results suggest that the Arg16Gly polymorphism of ADRB2 is related to down-regulation of ADRB2 expression in the detrusor muscle, so that the response of IC to tricyclic antidepressant therapy depends on the Arg16Gly polymorphism.

Interstitial cystitis (IC) is a chronic disorder with symptoms that include urinary urgency, frequency, nocturia, and bladder pain (10, 12). On cystoscopy, petechial hemorrhages and/or Hunner's ulcers are found after distension of the bladder (9). Several possible causative factors have been proposed, but the etiology and pathophysiology of IC are still unknown. Epidemiological studies have shown that patients with IC are 100 times more likely to have inflammatory bowel disease than healthy persons (1). IC is often associated with allergic diseases, and 40 to 80% of these patients have allergies (16). These findings suggest that IC is an immunological disease of the bladder.

Regarding the treatment of IC, a randomized trial showed the marked improvement of symptoms after 4 months of tricyclic antidepressant therapy (17). Tricyclic antidepressant blocks the active transport system that is involved in the re-uptake of serotonin and noradrenaline (4). The detrusor muscle of the bladder shows abundant expression of the β2-adrenoceptor (ADRB2), and polymorphisms of the ADRB2 gene have been suggested to show a close association with various allergic diseases. For example, an arginine-to-glycine substitution at codon 16 (Arg16Gly) is related to down-regulation of ADRB2 (6), which is thought to be one of the pathogenic factors in asthma (7). Therefore, the effect of tricyclic antidepressant on CI may be related to polymorphism of the ADRB2 (Arg16Gly) gene and to

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down-regulation of ADRB2 expression by the detrusor muscle. In the present study, we examined the relationship between the response of IC to tricyclic antidepressant therapy and polymorphism of the ADRB2 (Arg16Gly) gene.

MATERIALS AND METHODS

Subjects. A total of 168 subjects were enrolled in this study, including 55 IC patients (8 men and 47 women) and 113 normal controls (39 men and 74 women) with no history of allergic or autoimmune diseases. The mean (± standard deviation) age of the IC patients and controls was 58.1 ± 15.2 years and 36.5 ± 10.2 years, respectively. IC was diagnosed in accordance with the criteria of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (5). Patients who met the diagnostic criteria for IC were over 18 years old, had suffered from pelvic pain and/or urgency for at least 12 months, had no urinary tract infection within the previous 3 months, voided at least 8 times daily with nocturia, and had a bladder capacity of 350 mL or less. Patients with benign or malignant bladder tumors were excluded. In addition to the above criteria, a diagnosis of IC was made by a qualified urologist in all of the IC patients. All patients had already received conservative treatment, including hydrostatic bladder distension or oral medications (antiallergy or anticholinergic agents), but satisfactory results had not been obtained with these treatments. Imipramine hydrochloride (a tricyclic antidepressant) was administered to the IC patients at a dose of 75 mg/day. After 12 weeks, a qualified urologist comprehensively evaluated the efficacy of tricyclic antidepressant therapy by determining each patient's satisfaction with the treatment and assigning them to 1 of 3 categories (no change, fair, or good). All subjects gave informed consent to the study and the protocol was approved by the University of the Ryukyus.

Polymorphism of the ADRB2 (Arg16Gly) gene. Analysis of ADRB2 gene polymorphism (Arg16Gly) was done according to the method reported previously (15). Briefly, genomic DNA was extracted from whole blood samples collected from each subject, and the Arg16Gly polymorphism of ADRB2 was examined by direct DNA sequencing. Genomic DNA was used as the template and PCR was performed with following pair of primer: 5'-ccttcttgctg gcaccccat (forward) and 5'-ggaagtccaaaactgacca (reverse). Then, sequencing of the PCR products was performed with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Japan), and the sequences thus obtained were analyzed with an ABI PRISM^{R} 310 Genetic analyzer (Applied Biosystems, Japan)(15).

Statistical analysis. The relationship between the Arg16Gly polymorphism of ADRB2 and IC was assessed by calculating odds ratios (OR). Comparison of the distribution of Arg16Gly polymorphism was done by the χ^2 test. For all analyses, a probability of less than 0.05 was defined as indicating statistical significance.

RESULTS

The frequencies of the Arg16Gly polymorphism in the IC patients and controls are shown in Table 1. Statistical analysis of ADRB2 gene showed that there was a significant difference ($p = 0.018$) in the prevalence of the Arg16Gly polymorphism between IC patients and controls. The ratio of Arg/Arg was higher in IC patients than control, and it was associated with a 3.66-fold increase in the risk of IC than those with Arg/Gly or Gly/Gly. Some of the IC patients complained of a dry mouth while taking imipramine, but there were no severe side effects.

We classified the IC patients according to their subjective response to treatment (no change, fair, or good) in order to examine the relationship between the Arg16Gly polymorphism and the efficacy of tricyclic antidepressant therapy. As the results, 30 patients (55%) had no change, 16 patients (29%) had fair response, and 9 patients (16%) had good response for therapy. Statistical analysis of ADRB2 gene showed that there was a significant difference ($p = 0.002$) in the prevalence of Arg16Gly polymorphism between IC patients with no change or a fair response to tricyclic antidepressant therapy and controls (Table 2). However, significant difference ($p = 0.647$) in the prevalence of the Arg16Gly polymorphism was not observed between IC patients with a good response to tricyclic antidepressant therapy and controls. In IC patients, we also compared the prevalence of Arg16Gly polymorphism between no change or fair response groups and good response group to tricyclic antidepressant therapy. The ratio of Arg/Arg was higher in IC patients with no change or fair response to the tricyclic antidepressant, and it was a 2.97-fold decrease of the response rate to tricyclic antidepressant therapy than those with Arg/Gly or Gly/Gly.

Table 1 *The frequencies of the Arg16Gly polymorphism in the IC patients and controls*

	Number of patients with IC (%)	Number of Controls (%)
ADRB2 (Arg16Gly)		
Arg/Arg	12 (21.8)	8 (7.0)
Arg/Gly	34 (61.8)	78 (69.0)
Gly/Gly	9 (16.4)	27 (24.0)

・Significant difference ($p = 0.018$) in the prevalence of the Arg16Gly polymorphism between IC patients and controls.
・Arg/Arg was associated with a 3.66-fold increase in the risk of IC than those with Arg/Gly or Gly/Gly.

Table 2 *Relatiohship between efficacy of tricyclic antidepressant therapy and Arg16Gly polymorphism*

	Number of IC patients (%)			Number of Controls (%)
	Evaluation of tricyclic antidepressant therapy			
	no change	fair	good	
ADRB2 (Arg16Gly)				
Arg/Arg	7 (23.3)	4 (25.0)	1 (11.1)	8 (7.0)
Arg/Gly	19 (63.4)	8 (50.0)	7 (77.8)	78 (69.0)
Gly/Gly	4 (13.3)	4 (25.0)	1 (11.1)	27 (24.0)

・Significant difference ($p = 0.002$) in the prevalence of Arg16Gly polymorphism between IC patients with no change or a fair response to tricyclic antidepressant therapy and controls.
・Arg/Arg was associated with a 2.97-fold decrease of the response rate to tricyclic antidepressant therapy than those with Arg/Gly or Gly/Gly.

DISCUSSION

IC is a chronic inflammatory disease that is characterized by pain of the bladder, urinary urgency and frequency associated with a small bladder capacity (10, 12). On cystoscopy, the mucosa of the bladder is thin, and petechial hemorrhages (glomerulations) and/or Hunner's ulcers are found after distension of the bladder (9). Several possible causes of IC have been proposed, including mast cell activation, neuropathy, urinary toxins, and altered urothelial permeability. However, the etiology and pathophysiology of IC are still unknown.

Clinically, IC patients show many features of immunological disease, and these patients are 100 times more likely to have inflammatory bowel disease and 30 times more likely to have systemic lupus erythematosus compared with healthy controls (1). Some studies have shown that 40 to 80% of IC patients have allergies (6). In our previous study, we examined the role of some gene polymorphisms (α1d-adrenoceptor, β2-adrenoceptor, and β3-adrenoceptor, interleukin-4, and interleukin-4 receptor) in patients with IC. We found that about 35% of patients had allergic diseases or autoimmune conditions, and that Arg16Gly polymorphism of the ADRB2 gene showed the most significant relation with a predisposition to IC (15). Therefore, it is reasonable to suspect that IC may be a specific immunological disease or chronic inflammatory condition of the bladder, and there seems to be a relationship between IC and ADRB2 gene polymorphism (Arg16Gly).

Regarding the treatment of IC, hydrostatic distension to expand the bladder, dimethyl sulfoxide (11), muscarinic antagonists (2), and anti-inflammatory agents (prednisone, pentosanpolysulfate, and nalmefene) have been used (3, 14, 19). However, it is difficult to obtain satisfactory results with these treatments, and patients often complain of pain or side effects. Tricyclic antidepressant is also used for the treatment of IC, and long-term administration of such drugs is reported to be a feasible, safe, and effective management strategy (16, 17, 18). It is thought that tricyclic antidepressant may improve IC by raising the pain threshold of the bladder, but the fundamental mechanism of action is unclear. Tricyclic antidepressants also block the active transport system responsible for re-uptake of serotonin and noradrenaline (4). The bladder detrusor muscle shows high ADRB2 expression and some polymorphisms of this gene on chromosome 5q31-q33 are suggested to be closely associated with allergic diseases. In particular, Arg16Gly polymorphism of ADRB2 involving the substitution of an amino acid is reported to be related to the down-regulation of

ADRB2 (6), and low activity of this receptor may be a pathologic factor in asthma (7). Therefore, we considered the possibility that IC represents an immunological response to the expression of defective ADRB2 in detrusor muscle, so that tricyclic antidepressants may act by down-regulation of ADRB2 expression. If the ADRB2 in the bladder provokes an immune response by acting as an antigen in patients with IC, symptoms may be improved by down-regulation of ADRB2 expression by tricyclic antidepressant therapy. However, Arg16Gly polymorphism of ADRB2 is related to its down-regulation (6), so the effect of tricyclic therapy may also depend on the ADRB2 genotype. Indeed, the present study showed that Arg/Arg was associated with an increased risk of IC and also influenced the response to tricyclic antidepressant therapy compared with Arg/Gly or Gly/Gly. It has also been reported that Arg16Gly polymorphism of ADRB2 is associated with a greater decrease of the diastolic blood pressure in response to benazepril treatment (8), while the Gly16 allele of the ADBR2 gene confers resistance to parasitic infections (13). Cellular immunity and humoral immunity usually maintain a balance, but infection sometimes induces allergy or autoimmune disease by upsetting this balance. Therefore, Arg16Gly polymorphism of ADRB2 may be related to the occurrence of certain infections and allergic diseases in IC patients.

IC is a complex clinical syndrome that probably arises from an interaction between environmental and genetic influences, but analysis of ADRB2 gene polymorphism may be useful for selecting the most effective medical therapy for these patients.

Our analysis of ADRB2 genotypes showed a significant difference in the prevalence of the Arg16Gly polymorphism between IC patients and controls, and Arg/Arg was associated with a 3.66-fold increase in the risk of IC than those with Arg/Gly or Gly/Gly. Regarding the relationship between the Arg16Gly polymorphism and the efficacy of tricyclic antidepressant therapy, a significant difference of Arg16Gly polymorphism prevalence was observed between IC patients with no change or a fair response to tricyclic treatment and the controls, while there was no such difference between IC patients with a good response to tricyclic treatment and the controls. In patients with IC, Arg/Arg was associated with a 2.97-fold decrease of the response rate to tricyclic antidepressant therapy than those with Arg/Gly or Gly/Gly. Therefore, it is suggested that Arg16Gly polymorphism of ADRB2 is related to the down-regulation of ADRB2 expression by the detrusor muscle of the bladder, and that the effect of tricyclic antidepressant therapy on IC depends on the ADRB2 genotype.

REFERENCES

1. Alagiri M, Chottiner S, Ratner V, Slade D and Hanno PM (1997) Interstitial cystitis: unexplained associations with other chronic disease and pain syndromes. *Urology* **49**, 52–57.
2. Barbalias GA, Liatsikos EN, Athanasopoulos A and Nikiforidis G (2000) Interstitial cystitis: bladder training with intravesical oxybutynin. *J Urol* **163**, 1818–1822.
3. Chiang G, Patra P, Letourneau R, Jeudy S, Boucher W, Green M, Sant GR and Theoharides TC (2000) Pentosan-polysulfate inhibits mast cell histamine secretion and intracellular calcium ion levels: an alternative explanation of its beneficial effect in interstitial cystitis. *J Urol* **164**, 2119–2125.
4. Coppen A and Wood K (1979) Adrenergic and serotonergic mechanisms in depression and their response to amitriptyline. *Ciba Found Symp* **74**, 157–166.
5. Gillenwater JY and Wein AJ (1988) Summary of the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases Workshop on Interstitial Cystitis. National Institute of Health, Bethesda, Maryland, August 28-29, 1987. *J Urol* **140**, 203–206.
6. Green SA, Turki J, Innis M and Liggett SB (1994) Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry* **33**, 9414–9419.
7. Holloway JW, Dunbar PR, Riley GA, Sawyer GM, Fitzharris PF, Pearce N, Le Gros GS and Beasley R (2000) Association of beta2-adrenergic receptor polymorphisms with severe asthma. *Clin Exp Allergy* **30**, 1097–1103.
8. Huang G, Xing H, Hao K, Peng S, Wu D, Guang W, Huang A, Hong X, Wang Y, Feng Y, Zhang Y, Li J, Chen C, Wang B, Zhang X, Li D, Yu Y, Liu J, Zhu G, Huo Y, Chen D, Hou Y, Wang X, Xu X, Niu T and Xu X (2004) Beta2 adrenergic receptor gene Arg16Gly polymorphism is associated with therapeutic efficacy of benazepril on essential hypertension in Chinese. *Clin Exp Hypertens* **26**, 581–592.
9. Johansson SL and Fall M (1990) Clinical features and spectrum of light microscopic changes in interstitial cystitis. *J Urol* **1143**, 1118–1124.
10. Lilly JD and Parsons CL (1990) Bladder surface glycosaminoglycans is a human epithelial permeability barrier. *Surg Gynecol Obstet* **171**, 493–496.
11. Melchior D, Packer CS, Johnson TC and Kaefer M (2003) Dimethyl sulfoxide: does it change the functional properties of the bladder wall? *J Urol* **170**, 253–258.
12. Parsons CL, Stein PC, Bidair M and Lebow D (1991) Epithelial dysfunction in non-bacterial cystitis (interstitial cystitis). *J Urol* **145**, 732–735.
13. Ramsay CE, Hayden CM, Tiller KJ, Burton PR, Hagel I, Palenque M, Lynch NR, Goldblatt J and LeSouef PN (1999) Association of polymorphisms in the beta2-adrenoreceptor gene with higher levels of parasitic infection. *Hum Genet* **104**, 269–274.
14. Soucy F and Gregoire M (2005) Efficacy of prednisone for severe refractory ulcerative interstitial cystitis. *J Urol* **173**, 841–843.
15. Sugaya K, Nishijima S, Yamada T, Miyazato M, Hatano T and Ogawa Y (2002) Molecular analysis of adrenergic recep-

tor genes and interleukin-4/interleukin-4 receptor genes in patients with interstitial cystitis. *J Urol* **168**, 2668–2671.
16. van de Merwe JP, Yamada T and Sakamoto Y (2003) Systemic aspects of interstitial cystitis, immunology and linkage with autoimmune disorders. *Int J Urol* **10 Suppl**, S35–38.
17. van Ophoven A, Pokupic S, Heinecke A and Hertle L (2004) A prospective, randomized, placebo controlled, double-blind study of amitriptyline for the treatment of interstitial cystitis. *J Urol* **172**, 533–536.
18. van Ophoven A and Hertle L (2005) Long-term results of amitriptyline treatment for interstitial cystitis. *J Urol* **174**, 1837–1840.
19. Wang DS, Sternbach G and Varon J (1998) a long-acting opioid antagonist. Clinical applications in emergency medicine. *J Emerg Med* **16**, 471–475.