Analysis of Correlation between Prescription Drugs for Climacteric Disorders and CA Repeat Polymorphism of Estrogen Receptor β Gene

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To establish drug therapy for climacteric disorders based on CA repeat polymorphism in the estrogen receptor β gene, the present study investigated the issuance of prescriptions to patients with climacteric disorders in relation to CA repeat polymorphism. Prescription data used in this study included that for drugs that were prescribed for more than 3 months to 63 patients undergoing treatment for climacteric disorders. The 195 prescriptions analyzed included traditional Kampo medicines (49.7%), central nervous system medicines (26.7%) and Hormones (21.5%). The scale of combination therapy with Kampo to monotherapy with Kampo was 2.14 for SS genotype subjects, 1.43 for SL genotype subjects and 0.86 for LL genotype subjects. Thus the usage of combination therapy in SS subjects was 2.5 times greater than that in LL subjects. The percentages of the medicines predominantly prescribed for treatment of climacteric disorders-Kamishoyosan, Keishibukuryogan and Tokishakuyakusan-for each genotype were 72.7% for SS, 47.1% for SL and 38.5% for LL, and the percentage for the SS genotype was significantly higher than that for the LL genotype. The prescription rate for Keishibukuryogan for patients with the SS genotype was 31.8% versus 10.0% for the other 2 genotypes, the rate for SS being significantly higher.

In conclusion, the use of CA repeat polymorphism in addition to symptoms in the selection of drugs for climacteric disorders may allow therapy to be personalized.

Key words —— climacteric disorders, prescription data, estrogen receptor β gene polymorphism, Kampo medicine, Keishibukuryogan

Introduction

The term “climacteric disorders” refers to the various symptoms that appear in women during climacterium. Unidentified complaints primarily involving autonomic dysregulation syndromes, which are caused by rapid reductions in the secretion of estrogen, and the lack of correspondence to organic changes are the main subjective symptoms. The main clinical symptoms extensively include vasomotor symptoms, such as hot flashes, psychoneurotic symptoms, such as depression, frustration and insomnia, and muscular and algetic symptoms, such as headaches, stiff shoulders, cold sensations and lower back pain. It was reported that such disorders occurred in 50–80% of women between the ages of 40 and 70 years, and these symptoms vary among individuals. However, it has not been clarified what causes such variations in patient symptoms in patients. Based on CA repeat polymorphism analysis of estrogen receptor (ER) β in women during climacterium, we identified a correlation between this polymorphism and the symptoms associated with climacteric disorders. ER is a member of the nuclear receptor superfamily, having 2 isoforms, α and β. Both ERs have polymorphisms, and numerous studies have been conducted on the relation between these polymorphisms and diseases. In particular, the cytosine-adenine (CA) dinucleotide repeat polymorphism (microsatellite polymorphism), which we identified as being correlated with the symptoms associated with climacteric disorders, is reportedly correlated with low-bone density in postmenopausal women and Alzheimer’s Disease.

Because supplementation of estrogen, which is low in
women with climacteric disorders, has been considered appropriate treatment for climacteric disorders, hormone replacement therapy (HRT) is the primary treatment in Western countries. In Japan, however, climacteric disorders have not been widely recognized, and thus achievements with established therapies are scarce. Currently, in addition to the estrogen drugs used in HRT, Kampo medicines and central nervous system (CNS) agents have been utilized in the treatment of patients with climacteric disorders in Japan. However, due to a lack of evidence, enforcement of drug treatments for climacteric disorders depends on doctor, experience. In addition, it has been reported that the risks exceeded the benefits of HRT in America. Therefore, further clinical studies for appropriate utilization of HRT, and the establishment of evidence regarding alternatives to HRT is required.

The relation between CA repeat polymorphism and the symptoms associated with climacteric disorders has been verified, and thus drug efficacy for individuals with these disorders may vary depending on this polymorphism. To establish evidence of drug treatments for climacteric disorders based on polymorphism analysis, the present study analyzed the prescription data of patients with climacteric disorders in terms of correlations with CA repeat polymorphism.

**Materials and Methods**

Precautions were taken to ensure that all genetic information used in this study remained confidential. This study was conducted with the approval of the Ethics Committee of the Graduate School of Pharmaceutical Sciences of Chiba University and Chiba Prefectural Togane Hospital. A survey of prescription drugs was also obtained with epidemiological investigation approval by these Ethics Committees.

Subjects: Subjects comprised 63 patients (average age: 52.6±4.1) diagnosed with climacteric disorders at the Women's Outpatient Ward at Chiba Prefectural Togane Hospital, and in whom drug treatment was performed. All subjects consented to participate in the study.

Prescription drug analysis: The consultation period was made from January, 2002 into December, 2004. Drugs that were prescribed for various symptoms associated with climacteric disorders for more than 3 months were identified in the ordering system from among the drugs prescribed to the subjects. Extracted data were classified according to the drug efficacy classification codes. For each Kampo medicine, CNS agents and Hormones medication classified, prescription rates were analyzed based on CA repeat polymorphism in the ER β gene. The relation between the prescription and patient genotype was then investigated. In addition, the follow-up period of prescription data was from 1 year and 3 months to 2 year, that was one and 10 months on an average per one patient. Doctors who wrote prescription of the extracted medicine were two persons.

Genetic Analysis: Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Mini Kit (QIAGEN, Hilden) according to the standard protocol. Polymerase chain reaction (PCR) was performed with oligonucleotide primers designed to amplify a polymorphic (CA) repeat in intron 6 of the human ER β gene. PCR was performed in a 75-μL reaction mixture with the following components: 150 ng of human genomic DNA, 2.5 pmol of each primer (5'-CAA TTC CCA ATT CTA AGC CT-3' and 5'-ATT CTT CTT TAG GCC AGG CA-3'), each dNTP at 200 μM, 7.5 μL of 10× reaction buffer (Transgenomic Inc. Omaha) and 1 U of Optimase polymerase (Transgenomic Inc.). Cycling consisted of 30 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 30 s. A volume of 15 μL of each PCR product was analyzed using the WAVE DNA Fragment Analysis System (Transgenomic Inc.), which separates amplified DNA fragments on a DNasep column containing hydrophobic nonporous particles. Based on previous studies, we divided all alleles into two groups of approximately equal size; those with short alleles and those with long alleles. Splitting of the groups was performed with the median as the cut-off. The cut-off limit was 21 repeats (alleles containing≤21 repeats = short (S) and ≥22 repeats =long (L)). All subjects were separated into subgroups comprising those with two short alleles (SS), those with one short and one long allele (SL) and those with two long alleles (LL).

Statistical analysis: Statistical analysis was performed by the χ²-test. Moreover, levels of significance were defined as less than 0.05.

**Results**

The number of extracted prescriptions was 195. These prescriptions included 97 for Kampo medicines (49.7%), 52 for CNS agents (26.7%) and 42 for Hormones (21.5%) (Fig. 1).

Among the 23 types of Kampo medicines prescribed, the
most prescribed medicine was Kamishoyosan (KSS) (18 cases; 18.6%) followed by Hangekouboku (11 cases; 11.3%) and Keishibukuryogan (KBG) (10 cases; 10.2%). Among the CNS agents prescribed, the most prescribed were the psychotherapeutic drugs (28 cases; 53.8%); selective serotonin reuptake inhibitors (SSRIs) were prescribed in 16 cases (57.1%), while hypnotic-sedative and antianxiety drugs were prescribed in 21 cases (40.4%), among which benzodiazepine drugs accounted for 17 cases (81.0%). All Hormones prescribed were drugs typically used in HRT.

CA repeat genotype was SS in 29 subjects, followed by SL in 19 subjects and LL in 15 subjects.

Prescription frequency for Kampo medicines, CNS agents and Hormones by genotype are shown in Fig. 2.

The prescription rates of Kampo medicines were 75.9% for SS subjects, 89.5% for SL subjects and 86.7% for LL subjects; high rates of Kampo medication prescription to all genotypes were thus confirmed. Prescription rates of CNS agents and Hormones were lower than those of Kampo medicines; however, the rates also decreased in order for the SS, SL and LL genotypes. And the scale of the combination therapy with Kampo medicines and other medicines against the monotherapy with Kampo medicines was 2.14 for SS subjects, 1.43 for SL subjects and 0.86 for LL subjects. The ratio of the combination therapy in SS subjects was 2.5 times greater than that in LL subjects (Table 1).

Analysis by group for the 3 Kampo medicines frequently prescribed for the treatment of climacteric disorders (KSS, KBG and tokishakuyakusan), as well as for other Kampo medicines, was performed. The results showed the prescription rates for the 3 main Kampo medicines were 72.7% for SS subjects, 47.1% for SL subjects and 38.5% for LL subjects; the rates were again observed to decrease in order for the SS, SL and LL genotypes. In addition, the prescription rate for the SS genotype was significantly higher than that for the LL genotype (Fig. 3).

The KBG prescription rate for SS subjects was 31.8%, while that for the other genotypes was 10.0%, and this represented a significant difference (Fig. 4). The same tendency was observed for KSS prescription rates.

Discussion

In the present study, because the drugs prescribed for more than 3 months were extracted for analysis, the prescription drug data was considered to indicate constant effects on the symptoms associated with climacteric disorders.

On analysis of the number of prescriptions, approximately half of the extracted drugs were Kampo medicines, thus suggesting that HRT is not widely utilized in Chiba Prefectural Togane Hospital. A range of Kampo medicines, as many as 23 types, were extracted. This variety may be due to the Kampo-specific concept of "Sho", which includes symptoms, signs and evidence upon treatment, and the diverse symptoms observed in climacteric disorders. The CNS agents extracted mostly comprised SSRIs and benzodiazepine drugs. It has been reported that SSRIs were effective against hot flashes in addition to depressive symptoms8, which suggests that such drugs may also be effective against the climacteric symptoms observed in Japanese women. However, appropriate dosage and duration of administration for various climacteric symptoms have not been established. It may therefore be necessary to establish further evidence for appropriate usage of these drugs.

Investigation of prescription rates based on the CA repeat polymorphism genotype and drug efficacy classification indicated that patients with SS genotype may require CNS agents and Hormone medicines at a higher level than those with other genotypes. Moreover, since the combination therapy with Kampo medicines and others in SS subjects was...
Table 1. Number of Mono and Combination Therapy of Kampo Medicines, and the Combination Scale According to CA Repeat Genotype of ER β gene.

<table>
<thead>
<tr>
<th>CA repeat genotype</th>
<th>Monotherapy of Kampo ( \alpha )</th>
<th>Kampo + HRT</th>
<th>Kampo + CNS</th>
<th>Kampo + HRT + CNS</th>
<th>Combination scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>7</td>
<td>15</td>
<td>4</td>
<td>5</td>
<td>2.14</td>
</tr>
<tr>
<td>SL</td>
<td>7</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>1.43</td>
</tr>
<tr>
<td>LL</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Combination scale = \( b / a \), Kampo: Kampo medicines, CNS: CNS agents, HRT: Hormones

Fig. 3. Prescription frequency for 3 main Kampo medicines for climacteric disorders according to CA repeat genotype of ER \( \beta \) gene.

Fig. 4. Prescription frequency for Keishibukuryogan (KBG) for climacteric disorders according to CA repeat genotype of ER \( \beta \) gene.

2.5 times higher than that in LL subjects, it was suggested that the medication for the SS genotype was more complicated compared then with other genotypes. Westberg et al. reported that the level of sex steroid hormone-binding globulin in the patients who had long CA repeat was lower than that in patients who had short CA repeat\(^{17}\). In a previous study, we found that among 51 Japanese subjects, those with the SS genotype tended to have symptoms including hot flashes and depression\(^{16}\). It is presumed that the climacteric disorders change with CA repeat polymorphism. Thus, the CA repeat polymorphism genotype may be utilized upon selecting drug treatments.

No relation between the CA repeat genotype and prescription rates for Kampo medicines overall was identified, however, analysis of the 3 main Kampo medicines showed that the prescription rates decreased in order for the SS, SL and LL genotypes. The 3 main Kampo medicines have been used in Japanese traditional medicine for improving the “oketsu” syndrome, which refers to blood stasis. It has been reported that the 3 main Kampo medicines show similar effects to HRT\(^{16,18}\). From the evaluation of the validity of Kampo medicines for climacteric disorders, Kita reported that KBG was the most effective in hot flash and insomnia, and KSS was the most effective in depression and insomnia\(^{20}\). The efficacy of KBG against hot flashes was reported in a preliminary study\(^{21}\). Therefore, there may be a correlation between the CA repeat genotype and prescription of the 3 main Kampo medicines in climacteric disorder treatments. It was also indicated that the application of Kampo medicines, particularly KBG when hot flashes were intense, was effective in patients with the SS genotype.

The progress report of the Women’s Health Initiative (WHI) Randomized Controlled Trial in July 2002 revealed that, in comparison with the placebo group, the incidences of colon cancer and femoral neck fractures were reduced by 37% and 33%, respectively, with estrogen and progesterin co-administration. However, the incidences of breast cancer and cardiovascular incidents, such as myocardial infarction, were increased by 26% and 29%, respectively. Thus, as mentioned earlier, the health risks of HRT apparently exceed the benefits in women as a whole\(^{22}\). It is reported that the risk of stroke is high, also in an the WHI estrogen-alone trial\(^{23}\). Kampo medicines may be used as alternative drugs to HRT, and with the application of genetic information, such as CA
repeat polymorphism, in addition to subjective symptoms and the experience of doctors, personalization of drug treatment for climacteric disorders may be possible. Especially, it was thought that the better medical treatment was possible for the person of SS genotype by using the 3 main Kampo medicines.

The present study identified a new approach, genetic analysis, for drug treatment of climacteric disorders, which has previously been largely dependent on doctor experience. With further large-scale investigations using a larger number of cases, it is expected that the results can be practically applied to the selection of drugs for the treatment of climacteric disorders in the future.

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