Original Article
Correlation between the anxiety state of patients with psoriasis and the REL polymorphism

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Abstract: Objective: This study explored the correlation between the anxiety state of patients with psoriasis and the REL polymorphism. Methods: The Hamilton Depression Scale (HAMA), Self-Rating Anxiety Scale (SAS), Hospital Anxiety and Depression Scale (HAD), and State and Trait Anxiety Inventory (SAI and TAI) were used to evaluate the anxiety of the subjects. Whole-blood DNA specimens were collected from the subjects, and the polymerase chain reaction-restriction fragment length polymorphism was used to perform the rs702873-locus SNP genotyping of REL in 96 psoriasis patients demonstrating anxiety, 118 psoriasis patients not showing anxiety, and 212 healthy controls. Results: The REL gene-locus SNP genotyping results of the group with psoriasis at the locus of rs702873 conformed to the Hardy-Weinberg equilibrium. Compared with the control group, the psoriasis group achieved markedly higher HAMA, SAS, STAI, and TAI scores with statistically significant differences (HAMA: t = 2.204, P < 0.05; SAS: t = 2.755, P < 0.05; SAI: t = 6.560, P < 0.05; TAI: t = 2.558, P < 0.05). The GG, GA, and AA genotype frequencies of the group with psoriasis at the locus of rs702873 in the REL gene were 21.56%, 53.64% and 24.8%, respectively, whereas those of the control group were 36.67%, 44.85% and 18.48%, correspondingly, with statistically significant difference in the genotype distribution (P < 0.05). The G-allele frequency of the psoriasis group significantly decreased compared with that of the control group (P < 0.05, OR = 0.793, 95% CI: 0.706-0.89). Stratified analysis of the age of onset and family history was conducted in the psoriasis group, and insignificant differences were detected among different phenotype distributions in the genotype at the locus of rs702873 (P > 0.05). The genotype GA distribution of the psoriasis group with anxiety was apparently higher than that of the psoriasis group without anxiety, and a statistically significant difference (P < 0.05) was found. However, no statistically significant difference existed between the two groups in the GG or AA genotype distribution (P > 0.05). The G-allele frequency of the psoriasis group with anxiety was higher than that of the psoriasis group without anxiety, and a statistically significant difference (P < 0.05) was detected. Conclusions: Patients with psoriasis often demonstrate anxiety, and the locus of rs702873 in the REL gene G > A is related to the susceptibility to anxiety.

Keywords: Psoriasis, anxiety, REL gene, genotyping

Introduction

Psoriasis is a common and recurrent chronic inflammatory skin disease, involving about 1% to 3% of the global population. The incidence of psoriasis in China shows a significantly increasing trend. In 2010, an epidemiological investigation on psoriasis showed that the incidence of the psoriasis in China was 0.47%, and over 3 million patients suffered from psoriasis throughout the country [1-3]. Previous studies have shown that psoriasis patients usually manifest abnormal psychological symptoms, and their psychological disorders closely related to extensive skin lesions, which are difficult to cure and yielding high recurrence rate. However, the exact pathogenesis of psoriasis remains unclear. At present, psoriasis considered as a complex disease induced under the cross-coupling effects of genetic and environmental factors [4, 5]. Recent observation by Genome Wide Association Study (GWAS) showed that the polymorphism locus of rs702873 in the REL gene was associated with the susceptibility of the Chinese Han population to psoriasis. Independently, psoriasis increases the risk for anxiety, which closely related to the REL polymorphism. Hence, the
REL polymorphism related to the mental state of a patient with psoriasis.

In the present study, the anxiety of each subject was measured by the Hamilton Depression Scale (HAMA), Self-Rating Anxiety Scale (SAS), Hospital Anxiety and Depression Scale (HAD), and State-Trait Anxiety Inventory (STAI). Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was used to exploring the relationship between the anxiety state of patients with psoriasis and the genetic susceptibility of the REL rs702873 polymorphism.

Materials and methods

Subjects investigated

A total of 214 psoriasis patients admitted to our inpatient and outpatient departments from May 2012 until December 2014 were enrolled in this study. The subjects consisted of 114 males and 100 females. The subjects aged 39-76 years old, and the average age was 58.12±9.57 years. Inclusion criteria: Pursuant to the diagnostic criteria of the Guidelines for Clinical Diagnosis and Treatment: Volume of Dermatology and Venereology promulgated by the Chinese Medical Association [6]. Among them, 176 patients presented with psoriasis vulgaris (accounting for 82.24%), 17 patients presented with erythrodermic psoriasis (7.94%), 14 patients presented with pustular psoriasis (6.54%), and 7 patients presented with arthropathic psoriasis (3.27%). Exclusion criteria: Patients with autoimmune diseases and/or systematic disorders were excluded from this study.

Grouping

The patients with psoriasis were divided into two groups according to the diagnostic criterion for anxiety (HAMA ≥ 14 points or SAS ≥ 50 points). First group is 96 psoriasis patients with anxiety, comprising 52 males aged 38-76 years old (average: 59.27±9.46 years) and 44 females aged 39-74 years old (average: 60.87±10.35 years). The second group is 118 psoriasis patients without anxiety, consisting of 62 males aged 36-78 years old (average: 59.51±11.41 years) and 56 females aged 41-76 years old (average: 62.51±11.69 years). The control group comprised 212 persons aged 37-77 years old (average: 59.45±10.24 years), who underwent physical examination at our hospital. The control group consisted of 112 males and 100 females. The present study has been reviewed by the academy ethics committee and has therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki. All persons gave their informed consent prior to their inclusion in the study.

Research tools

The Hamilton Depression Scale (HAMA) [7] was used to measuring the severity of anxiety of patients with neurosis or other mental illness. High HAMA score indicates high severity. Cutoff values: A total score of > 29 points may indicate severe anxiety, over 21 points may indicate apparent anxiety, over 14 points may indicate anxiety, over 7 points may indicate presence of some anxiety symptoms, and below 7 points may indicate absence of anxiety symptoms. The cutoff value used in the present study was 14 points for HAMA-14.

The Self-Rating Anxiety Scale (SAS) [8] was used to measuring the subjective feelings of the patients with anxiety. A total SAS score of ≥ 50 points indicates presence of anxiety symptoms.

The State-Trait Anxiety Inventory (STAI) [9] was used to measuring the anxiety symptoms of adults, and this method provides wide applicability. STAI used to evaluate situational anxiety, trait anxiety and mental health survey of patients with anxiety. The use of self-rating questionnaires is a simple and highly effective analytic technique.

The Hospital Anxiety and Depression Scale (HAD) [10] was used to evaluate the anxiety and depression of patients in a general hospital; HAD is a reliable diagnostic tool for emotional disorders diagnosed at general hospitals and is widely used in studies of psychosomatic diseases because of its high reliability and high efficiency.

Research methods

Investigation of general clinical data: The general clinical data included the following: population statistics; socio-economic and cultural characteristics; history of hypertension, hyperlipidemia, and diabetes mellitus; family history
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Table 1. Comparison of the general clinical data of the two groups (X ±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Gender (M/F)</th>
<th>Average age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group with Psoriasis</td>
<td>114/100</td>
<td>58.12±9.57</td>
</tr>
<tr>
<td>Control Group</td>
<td>112/100</td>
<td>59.45±10.51</td>
</tr>
</tbody>
</table>

P 0.891 0.642

Statistical validity analysis and Hardy-Weinberg genetic equilibrium test.

Table 2. Genotype Distributions of the Two Groups at the Locus of rs702873 in the REL Gene: G > A in the Hardy-Weinberg Equilibrium

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotyping at rs702873</th>
<th>χ² (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoriasis</td>
<td>GG 46 GA 115 AA 53</td>
<td>1.022 0.601</td>
</tr>
<tr>
<td>Control</td>
<td>GG 51 GA 107 AA 56</td>
<td></td>
</tr>
<tr>
<td>Observed</td>
<td>GG 78 GA 95 AA 39</td>
<td>0.930 0.628</td>
</tr>
<tr>
<td>Expected</td>
<td>GG 74 GA 103 AA 35</td>
<td></td>
</tr>
</tbody>
</table>

Comparison between the psoriasis group and the control group in the anxiety scale.

Table 3. Comparison between the psoriasis group and the control group in the anxiety scale (X ±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>HAMA</th>
<th>SAS</th>
<th>SAI</th>
<th>TAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoriasis</td>
<td>96</td>
<td>9.3±6.3</td>
<td>40.4±8.8</td>
<td>43.8±9.4</td>
<td>36.7±8.6</td>
</tr>
<tr>
<td>Control</td>
<td>212</td>
<td>7.3±5.1</td>
<td>36.5±7.9</td>
<td>33.7±8.6</td>
<td>33.1±7.6</td>
</tr>
</tbody>
</table>

Note: *P < 0.05, compared with the control group.

Experimental method

Reagents and instruments: Restriction enzyme NHe I (US Fermentas, Inc.), DNA Marker I, Taq DNA polymerase (Sangon), gel imaging analysis system (US Eastman Kodak Corp.), J-251 high-speed centrifuge (Beckman Coulter), 3720 gradient PCR instrument (ABI), and RT-6100 microplate reader (Rayto) used in this study.

DNA extraction and PCR reaction: Up to 3 ml of fasting peripheral blood sampled venously from each subject, who has not taken any lipid-reducing or anticoagulant drugs. A blood genomic DNA extraction kit (Sangon) used for DNA extraction from the blood. The extracted DNA samples placed and stored at -20°C.

Primer 5.0 software was used to designing PCR and genotyping primers. The primer sequences at the polymorphism locus of rs702873 in the REL gene were 5’-GTATGCACTGCCCAGTT-3’ upstream and 5’-TAAACACGGGATCTCAGT-3’ downstream. The primers synthesized by Invitrogen kit and purified by PAGE. The PCR system (15 µl) consisted of 10 × buffer (2 µl); dNTPs (2.5 mol/L); 0.3 µl; Taq enzyme (2.5 U/µl); 0.2 µl; upstream and downstream primers (10 µM), 0.1 µl each; genomic DNA: 2.0 µl; and double-distilled water (DDW): 10.3 µl. The amplification conditions were 5 min initial denaturation at 94°C; 45 s deformation at 94°C; 45 s annealing at 60°C and 45 s elongation at 72°C; a total of 38 cycles; and 10 min elongation at 72°C.

The PCR product at the polymorphism locus of rs702873 in the REL gene was digested with

of cardiovascular diseases; smoking, alcohol, and dietary habits; height; weight; blood pressure; blood lipid level; age; course of disease; and adverse life events.

Questionnaires: Under a unified instruction, the subjects completed the questionnaires for HAMA, SAS, STAI, and HAD, as guided by qualified physicians. The rating scale was completed by means of conversation and observation, and the rest were done by the subjects themselves. The presence of anxiety was determined as follows: HAMA ≥ 14 points or SAS ≥ 50 points (total points).
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Table 4. Comparison between the psoriasis group and the control group in the genotype distribution at the locus of rs702873 in the REL gene (G > A)

<table>
<thead>
<tr>
<th>Group</th>
<th>rs702873 (N %)</th>
<th>G-allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td>Psoriasis group</td>
<td>46 (21.50)</td>
<td>115 (53.74)</td>
</tr>
<tr>
<td>Control group</td>
<td>78 (36.79)</td>
<td>95 (44.81)</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Corrected OR value (95% CI)</td>
<td>1</td>
<td>2.044 (1.882-2.219)</td>
</tr>
</tbody>
</table>

Figure 2. Amplification results of 11 normal primers and mutant primers in genotype samples of GG, GA, AA, the mutant site of rs702873 respectively. M is 1200bp DNA Marker.

All the measurement data were assessed by mean and standard deviation. SPSS 13.0 was used to statistical analysis. The REL gene genotype distributions and allele frequencies at the polymorphism locus of rs702873 in different populations compared by χ² test, and the between-group comparison of the mean values was based on t-test. To determine whether the genotype distribution is consistent, the Hardy-Weinberg equilibrium (HWE) was evaluated by chi-square goodness-of-fit test with criterion of α = 0.05. A value of P < 0.05 considered statistically significant.

Results

Analysis of the general clinical data of the two groups

No statistical significance existed between the two populations in terms of age or gender (P > 0.05), but the results were comparable, as presented in Table 1.

The statistical effect of sample size was analyzed with G power (effect size = 0.1). The statistical effect of this study sample was 78.4%. This suggests that the sample size for the study is sufficient, to detect the difference of gene locus rs702873 of REL gene between psoriasis group and control group.

To investigate the reliability and representativeness of the experimental data, the genotype of the group with psoriasis at the locus of rs702873 in the REL gene was tested by using the HWE. The results showed that the expected value of the gene locus agreed well with the observed value, and the gene frequencies reached the genetic equilibrium, indicating that the subjects investigated were representative as presented in Table 2.

The scores of HAMA, SAS, SAI, and TAI in the psoriasis group were significantly higher than the control group, and statistically significant differences were observed (HAMA: t = 2.204, P < 0.05; SAS: t = 2.755, P < 0.05; SAI: t = 6.560, P < 0.05; TAI: t = 2.558, P < 0.05), as presented in Table 3; Figure 1.

Genotype distributions of the psoriasis group and the control group at the locus of rs702873 in the REL gene: G > A.

Case-control association study showed that the GG, GA, and AA genotype frequencies of the group with psoriasis at the locus of rs702873 in the REL gene and those of the control group were 21.56%, 53.64%, and 24.8%; 36.67%, 44.85%, and 18.48%, correspondingly. Statistically significant differences were found.
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between the two groups in terms of genotype distribution \( (P < 0.05) \), and the G-allele frequency of the psoriasis group decreased sharply compared with that of the control group \( (P < 0.05, \ OR = 0.793; \ 95\% \ CI: \ 0.706-0.89) \), as presented in Table 4. Gene typing electrophoresis was showed in Figure 2.

Psoriasis group and control group at the locus of rs702873 in the REL gene: analysis of the correlation between G > A and psoriasis vulgaris phenotype.

Stratified analysis was applied in the psoriasis group in accordance with the age of onset and family history. No significant differences were detected among various phenotype distributions in the genotype at the locus of rs702873 \( (P > 0.05) \), as presented in Table 5.

Genotype and allele frequency distribution in patients with psoriasis at the locus of rs702873 in the REL gene: presence or absence of anxiety.

The genotype GA distribution of the psoriasis group with anxiety was apparently higher than that of the psoriasis group without anxiety, and a statistically significant difference was found \( (P < 0.05) \). However, no statistically significant difference existed between the two groups in terms of GG or AA genotype distribution \( (P > 0.05) \). The G-allele frequency of the psoriasis group with anxiety was higher than that of the psoriasis group without anxiety, and a statistically significant difference was detected \( (P < 0.05) \), as presented in Table 6.

Discussion

Psoriasis is a common chronic inflammatory skin disease clinically manifested with red spots, pimples, and scales. This disease was divided into four types according to clinical manifestations: psoriasis vulgaris, arthropathic psoriasis, pustular psoriasis, and erythrodermic psoriasis, of which psoriasis vulgaris is the most common clinically, accounting for 90% of all cases. Large-scale clinical epidemiological surveys showed the incidence of this disease among adults in Western and Northern Europe was 1.5% to 2.0%, and that in Asia was 0.2% to 1%, with about 0.123% in China [11-13]. Psoriasis is prevalent among young individuals, and the age of onset is about 20 years old. Psoriasis characterized by high incidence, high recurrence rate, and long course of disease, which significantly affect the patients' mental state and physical health [14, 15]. At present, psoriasis is a research focus in the field of dermatology.

Psoriasis and anxiety

Psoriasis is difficult to cure. This disease affects the patients' image and reduces their quality of

Table 5. Phenotype distributions of patients with psoriasis at the locus of rs702873 in the REL gene \( (G > A) \)

<table>
<thead>
<tr>
<th>Item</th>
<th>n</th>
<th>Genotype</th>
<th>P</th>
<th>Corrected OR value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GG (%)</td>
<td>GA (%)</td>
<td>AA (%)</td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 40</td>
<td>148</td>
<td>33 (22.30)</td>
<td>76 (51.35)</td>
<td>39 (26.35)</td>
</tr>
<tr>
<td>≥ 40</td>
<td>66</td>
<td>15 (22.73)</td>
<td>36 (53.55)</td>
<td>15 (22.73)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25</td>
<td>6 (24.0)</td>
<td>13 (52.00)</td>
<td>6 (24.0)</td>
</tr>
<tr>
<td>None</td>
<td>189</td>
<td>43 (22.75)</td>
<td>98 (51.85)</td>
<td>48 (25.40)</td>
</tr>
</tbody>
</table>

Table 6. Genotype and allele frequency distribution in patients with psoriasis at the locus of rs702873 in the REL gene (with or without anxiety)

<table>
<thead>
<tr>
<th>Group</th>
<th>rs702873 (N %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
</tr>
<tr>
<td>Psoriasis group with anxiety</td>
<td>17 (17.71)</td>
</tr>
<tr>
<td>Psoriasis group without anxiety</td>
<td>29 (24.58)</td>
</tr>
<tr>
<td>Control group</td>
<td>78  (36.79)</td>
</tr>
</tbody>
</table>

Note: \(^aP < 0.05\), compared with the control group; \(^bP < 0.05\), compared with the psoriasis group without anxiety.
life. Compared with normal individuals, patients with psoriasis exhibit poor mental health and may suffer from severe anxiety and depression, as well as other psychological disorders. Many scholars have used SAS, SDS, SCL-90, and DLQs and found that patients with psoriasis suffer from anxiety, depression, and psychological disorders related to sexual intercourse and social life [15]. Researches have also found that the occurrence, development, and recurrence of psoriasis closely related to the patients' emotional personality, stress, anxiety, and other psychological factors [16], and the patients' mental pressure positively correlated with the severity of psoriasis [17]. Moreover, about one-third of the patients with psoriasis are more susceptible to symptoms of anxiety related to skin lesions; tension, anxiety, depression, and other psychological factors may promote the release of substance P, thus stimulating cell proliferation and keratinocyte formation, causing immune system abnormalities, and aggravating and inducing psoriasis [18]. In the present study, the anxiety-testing results of the subjects showed that the HAMA, SAS, SAI, and TAI scores of the group with psoriasis were apparently higher than the control group. This finding indicates that the progress of psoriasis closely related to anxiety. Psoriasis patients may suffer from tension, anxiety, depression, and other aggravating psychological factors and autoimmune disorders, which can induce skin lesions, thereby reversely stimulating the onset of psoriasis. This result is consistent with the above literature. Studies also showed that the life quality of patients with psoriasis can be improved through health education [19]. Thus, psoriasis is a typical physical and mental disease.

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Psoriasis and genotype distribution at the locus of rs702873 in the REL gene (G > A)

The pathogenesis of psoriasis is complicated. To date, psoriasis considered as a complex disease induced by immunological and environmental factors under certain genetic background [20]. Epidemiological studies and clinical practices have shown that psoriasis often occurs along a family history and a genetic tendency. Surveys have indicated that the incidence of psoriasis among individuals with family history of the disease is higher than normal. Among the patients with early psoriasis (age of onset: < 40 years), the incidence among the first-degree relatives of probands is 10 times that of normal, and the incidence among monozygotic twins is significantly higher than that among dizygotic twins, with similarity in the age of onset, genotype and distribution, severity, and course of disease [21, 22]. All these phenomena reveal that hereditary factors closely related to the incidence of psoriasis.

The NF-κB/Rel protein consists of five family members in mammalian cells: p50 (NFκB1), p52 (NFκB2), Rel A (p65), Rel B, and c Rel, which paired into dipolymers or heterodimers in a certain way, constituting aggregates of heterogeneity. The amino termini of all family members correspond to a conserved Rel homology domain (RHD) consisting of about 300 amino acids, including dimerization domain, κB-binding site, nuclear localization signal (NLS), and DNA-binding site. Among them, the heterodimer consisting of p50/p65 (Rel-A) is the most typical NF-κB dipolymer with the highest content in cells and the highest affinity with κB, far above those of other dimers, and this dipolymer plays an important role in the transcriptional regulation. Thus, NF-κB refers to p50/p65 (Rel-A) customarily. p50 contains NLS, whereas Rel-A contains transcriptional activation domains. In quiescent cells, NF-κB and IκB combined into trimers, which are present in the cytoplasm. When cells are stimulated, protein kinase (IKKs) are activated; IκB is phosphorylated, cleaved, and dissociated from trimers; and NF-κB is quickly shifted from the cytoplasm to the nucleus, showing specific binding with the κBlocus, thereby promoting the gene transcription [23-26].

Preliminary sequencing results showed that the REL gene was susceptible to psoriasis. Hence, in the present study, the REL gene considered as a candidate gene to explore the genetic correlation with susceptibility to psoriasis. The prerequisite for exploring the correlation between the genetic background and the disease is to ensure that all the samples are representative, thereby reaching the genetic equilibrium. The HWE is the most important rule of population genetics and also a prerequisite and foundation for studying population gene polymorphisms. According to the test results, the measured value may not match with the theoretical value, probably because of the very small sample size without representativeness or at least
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not meeting a condition for the equilibrium, that is, the subjects did not meet the genetic equilibrium as a whole. According to the HWE results, the expected values of the subjects investigated in the present experiment at the locus of rs702873 in the REL gene agreed well with the observed values, indicating that the subjects were representative. Up to 214 patients with psoriasis and 212 healthy control subjects compared in the genotyping of the REL gene at the polymorphic locus of rs702873 in the peripheral blood DNA, and statistically significant differences was found between the two groups in the genotyping. The results showed that the polymorphic locus of rs702873 related to the susceptibility of the northern Chinese Han population to psoriasis, while the G-allele was associated with the risk of psoriasis. Furthermore, this study displayed the correlation between different genotypes at the locus and the part of the clinical phenotypes of the southern Chinese Han population with psoriasis vulgaris, such as age of onset and family history, and no marked correlation between the polymorphic locus of rs702873 and that of psoriasis vulgaris.

Psoriasis group with anxiety and genotype distribution at the locus of rs702873 in the REL gene (G > A)

In the present study, 214 patients with psoriasis vulgaris completed the questionnaire-based anxiety survey. On the basis of the results, the patients were classified into two groups: 96 psoriasis patients with anxiety and 118 psoriasis patients without anxiety. The genotype GA distribution in the psoriasis group with anxiety was apparently higher than that in the psoriasis group without anxiety. However, no statistically significant difference existed between the two groups in terms of GG or AA genotype distribution. The G-allele frequency of the psoriasis group with anxiety was higher than that of the psoriasis group without anxiety. By retrieving the public database dbSNP, the G-allele frequency distributions at rs702873 in Europeans, Asians, and Africans were 0.751, 0.523 and 0.518, respectively. This probably because the Chinese Han population can be classified into three populations according to genetic background, migration history, and living environment; for instance, the northern, middle, and southern populations, as well as the race and population differences are attributed to the different prevalence ratios and incidence [27].

In summary, the present study confirmed that patients with psoriasis vulgar were more susceptible to anxiety compared with the normal population, and the locus of rs702873 in the REL gene (G > A) of psoriasis patients with anxiety was related to the presence of anxiety, which was of great significance to guide the evaluation of the severity of psoriasis.

Disclosure of conflict of interest

None.

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References


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