Relationship between gene expressions of aquaporin 3 and 4 and various degrees of spleen-stomach dampness-heat syndrome in chronic superficial gastritis

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Objective: To explore the relationship between gene expressions of aquaporin (AQP) 3 and 4 and various degrees of spleen-stomach dampness-heat syndrome in chronic superficial gastritis (CSG).

Methods: Twenty-four CSG patients were divided into three groups according to the degrees of spleen-stomach dampness-heat syndrome: mild, moderate and severe groups. There were 8 patients in each group, and another 8 healthy persons were selected as normal control. Samples of mucosa of upper stomach in the included patients and normal persons were collected by gastroscopy, and then were stored in liquid nitrogen. The gene expressions of AQP3 and AQP4 in gastric tissue were examined by fluorescence quantitative polymerase chain reaction.

Results: The gene expressions of AQP3 and AQP4 in the moderate and severe groups were higher than that in the mild group and normal group (P <0.05, P <0.01). The gene expression of AQP3 in the severe group was higher than that in the moderate group (P <0.05).

Conclusion: The gene expressions of AQP3 and AQP4 in gastric mucosa are different in patients with various degrees of spleen-stomach dampness-heat syndrome of CSG. There is a relationship between expressions of AQP3 and AQP4 genes and the degrees of spleen-stomach dampness-heat syndrome, and the gene expressions will increase with the aggravation of the dampness-heat syndrome.

Keywords: gastritis; aquaporin; gene expression; dampness-heat syndrome; fluorescence quantitative polymerase chain reaction

慢性浅表性胃炎不同程度脾胃湿热证与胃黏膜水通道蛋白3、4基因表达的相关性

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目的：探讨慢性浅表性胃炎(chronic superficial gastritis, CSG)不同程度脾胃湿热证与胃黏膜水通道蛋白(aquaporin, AQP)3、4基因表达的相关性。

方法：共纳入24例CSG患者，按脾胃湿热证不同程度分为轻、中、重度组，另选择8名健康人作为正常对照。

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Spleen-stomach dampness-heat syndrome is one of the common syndrome types in cases of spleen and stomach diseases, which plays an important role in digestive diseases. It was previously demonstrated that there was a significant difference in positive expression of aquaporin 3 (AQP3) between the patients with chronic superficial gastritis (CSG) and normal population, and the AQP3 expression in CSG patients with spleen-stomach dampness-heat syndrome was clearly different from the normal (P < 0.05), while there was no remarkable difference in the AQP3 expression between the spleen-stomach dampness-heat syndrome and spleen deficiency syndrome[1]. In the CSG cases of spleen-stomach dampness-heat syndrome, the proportion of dampness over heat in the patients with positive expression of AQP3 was obviously higher than that in the negatives. AQP3 has a close relationship with water metabolism, and has some function to spleen-stomach dampness-heat syndrome[2]. Maybe the high expression of AQP3 is induced by osmolality change which results from the internal dampness. At the same time, it may make a contribution to flowing of body fluids and getting rid of internal dampness[3]. The protein and gene expressions in the patients with spleen-stomach dampness-heat syndrome were higher than those in the patients with spleen deficiency syndrome and normal population, while protein and gene expressions in the patients with spleen deficiency syndrome were lower than those in normal population[4]. The comparison between subtypes of spleen-stomach dampness-heat syndrome showed a tendency as dampness over heat was more common than dampness equal to heat, and dampness equal to heat was more common than heat over dampness. So we use quantified classification standard of CSG and spleen-stomach dampness-heat syndrome to investigate the relationship between degree of dampness-heat syndrome and gastric mucosa aquaporin[5,6].

In this research, the correlativity between degree of dampness-heat syndrome and gastric mucosa AQP3 and AQP4 was discussed by considering water metabolism as a pointcut based on quantitative diagnosis standard of spleen-stomach dampness-heat syndrome.

1 Clinical data and methods
1.1 Clinical Data
1.1.1 Study objects Twenty-four CSG adult re-
0.5 cm, were put into liquid nitrogen immediately, and preserved in a cold storage freezer at −70°C to be tested. Then, the gene expressions of AQP3 and 4 were tested with fluorescent quantitative polymerase chain reaction (FQ-PCR).

With reference to DNA and mRNA gene sequences generated by NCBI Genebank, the sequences of sense and antisense primers and fluorescence probes of AQP3 and 4 were designed by Da’an Gene Test Center at Sun Yat-sen University, which was generated into undercounter high-throughput DNA synthesizer. The details were as follows:

**Sequence name:** AQP3
**F:** 5'-ACC TTC ATG GGC TTC AAT TCT-3'
**R:** 5'-AGT GAA AAG GGC AGG TCC AA-3'
**Probe:** 5'-FAM-CTA TGC CGT CAA CCC AGC TCG TGA-TAMRA-3'

**Sequence name:** AQP4
**F:** 5'-CGG AGC CAG CAT GAA TCC-3'
**R:** 5'-TAT CCA GTG GTT TTC CCA GTT TC-3'
**Probe:** 5'-FAM-CTC GAT CCT TTG GCC CTG CAG TTA TCA-TAMRA-3'

After extraction and identification by RNA organization, PE9600 PCR was used to make reverse transcription reaction. Then its products were reacted by FQ-PCR. The reaction of positive and quantitative standard products followed the system: 5 quantitative PCR buffer (American ABI Company) 10 μL, sense primer F (25 μmol/L) 1 μL, antisense primer R (25 μmol/L) 1 μL, dNTPs (10 mmol/L) (Sigma Company) 0.5 μL, fluorescent probe (20 μmol/L) (Shanghai Sangon Biologcal Engineering Technology and Services Co., Ltd) 1 μL, Taq Enzyme (American ABI Company) 1.5 μL, cDNA 5 μL, ddH2O 30 μL.

The reaction conditions were as follows: 93°C 2 minutes, then 93°C 45 seconds, 55°C 1 minute, this cycle was repeated for 55 times in all. After reaction, the amplifying and quantitative curve of positive standard product was automatically analyzed and drawn out by computer.

The original copy numbers of gene expressions of AQP3 and AQP4 in each group were made out and then transformed logarithmically with further analysis and calculated by computer according to the standard curve.

### 1.3 Statistical analysis
The data were expressed in $\overline{x} \pm s$. The comparisons among independent multiple groups were analyzed in variance. The $q$ test was used to compare between any two groups. The chi-square test was used to count data. The significant level was set as $a = 0.05$ calculating with SPSS for Windows 11.5.

### 2 Results

#### 2.1 Baseline data
There were 3 males and 5 females in the mild group. Their mean age was (32.5 ± 7.05) years with a range of 25 to 51 years. And their average course of disease varied from (1.35 ± 0.38) years with a range of 3 months to 3 years. There were 4 males and 4 females in the moderate group. Their mean age was (36.38 ± 8.01) years with a range of 28 to 49 years. And their average course of disease was varied from (1.45 ± 0.56) years with a range of 2 months to 3.5 years. There were 5 males and 3 females in the severe group. Their mean age was (33.75 ± 8.01) years with a range of 23 to 53 years. And their average course of disease was varied from (1.52 ± 0.75) years with a range of 4 months to 3.5 years. There were 4 males and 4 females in the normal group with average age (25.88 ± 5.22) years. There were no significant differences among the four groups.

#### 2.2 Gene expressions of AQP3 and AQP4
The AQP3 expression in the moderate and severe groups was higher than that in the normal and mild groups ($P < 0.05$, $P < 0.01$). The gene expression of AQP3 in the severe group was higher than that in the moderate group ($P < 0.05$). The AQP4 expression in the moderate and severe groups was higher than that in the normal and mild groups ($P < 0.05$, $P < 0.01$), while the comparison between the moderate and severe groups had no statistical difference. Details were shown in Table 1.

### Table 1 Comparison of AQP3 and AQP4 gene expressions among the four groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>AQP3 Copy/μg RNA (×10^4)</th>
<th>Logarithm of copy number</th>
<th>AQP4 Copy/μg RNA (×10^4)</th>
<th>Logarithm of copy number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8</td>
<td>1.263 ± 1.642 0</td>
<td>3.849 5 ± 0.507 2</td>
<td>2.802 ± 3 ± 7.877 7</td>
<td>7.038 4 ± 0.670 6</td>
</tr>
<tr>
<td>Mild</td>
<td>8</td>
<td>0.711 9 ± 0.456 4</td>
<td>3.884 8 ± 0.442 2</td>
<td>1.827 6 ± 1.954 5</td>
<td>7.045 4 ± 0.501 9</td>
</tr>
<tr>
<td>Moderate</td>
<td>8</td>
<td>5.139 0 ± 4.820 1</td>
<td>4.524 5 ± 0.461 7</td>
<td>9.124 8 ± 5.365 2</td>
<td>7.830 7 ± 0.443 8</td>
</tr>
<tr>
<td>Severe</td>
<td>8</td>
<td>75.843 9 ± 123.523 7</td>
<td>5.384 9 ± 0.579 4</td>
<td>28.456 3 ± 31.245 3</td>
<td>8.242 6 ± 0.501 3</td>
</tr>
</tbody>
</table>

* $P < 0.05$, ** $P < 0.01$ vs normal group; $P < 0.05$, $\triangle P < 0.01$, vs mild group; $P < 0.05$, vs moderate group.

### 3 Discussion

AQP3s are basis of molecular biology to keep balance of internal water metabolism and important channels that mediate the efficient movement of water across the membrane. If expressions of AQP3s on different tissue cell membrane were higher or lower than normal level, its effects on discharge and absorption of water would break water balance in general and local[11-20].
Now, 11 kinds of subtypes have been found in human body. AQP3 is mainly expressed at basolateral membrane of cardiac glandular cell and rats’ non-gland area, so it is presumed that AQP3 is likely to associate with alternative transportation of water when the stomach is discharging gastric acid[9]. Differential AQP4 expressions appear into basement of gastric parietal cell in rats, mice, and gastric chief cell and parietal cell of human. AQP4 is expressed differently at different gastric gland zone. There are low or no AQP4 expressions on the neck of gastric gland or the upper side of parietal cell. Only at the bottom of parietal cell are there lots of AQP4 expressions. However, as the gastric acid is mainly secreted by the parietal cell at the bottom of gastric gland, there is a possibility of AQP4 taking part in the secretion of gastric acid and/or maintaining the normal volume[5].

According to TCM, the spleen governing transportation and transformation provides nutrition to the whole body by converting water and food into nutrients; the nutrient water was differentiated into physiologic juice as essence blood distributing to the whole body. In addition, the spare juice was excreted by transforming into saliva, sweat, and urine and transporting to lung and kidney. If the function of the spleen governing transportation and transformation is strong, it can prevent the abnormal retention of internal water, whereas, spleen-stomach dampness-heat syndrome will be caused by heat that was transformed by gathering of endogenous turbid dampness because of disordered function of the spleen governing transportation and transformation. From this, it can be seen that spleen-stomach dampness-heat syndrome is the outcome of disordered function and the disturbance of water metabolism.

Spleen-stomach dampness-heat syndrome is one of the common syndrome types in spleen-stomach diseases. The syndrome standardization is established initially by using combination of pharmacological components, modern epidemiology and statistic method through the procedures such as selection of the correlated factors, handling grade points to the correlated factors, confirmation of diagnosing cut-point and so on. And it is proved to have a good diagnosing effect through retrospective analysis[10]. The further study is based on syndrome standardization.

The gene expressions of AQP3 and AQP4 in gastric mucosa vary with the changes of “dampness”[11]. We adopted the quantitation and classifying standard of spleen-stomach dampness-heat syndrome to explore correlation between the degree of dampness-heat and AQP3 and AQP4 of gastric mucosa in order to further explore the relationship between “dampness-heat” and aquaporin. It was found that the gene expressions of AQP3 and AQP4 in gastric mucosa increased with the severity of spleen-stomach dampness-heat. We speculate that AQP3 and AQP4 expressions were the result of the local reaction of water disturbance of spleen-stomach dampness-heat syndrome at gastric mucosa, and meanwhile the level of gene expressions of AQP3 and AQP4 could reflect the severity of dampness-heat and the internal relations between AQP3 and AQP4 of gastric mucosa and “dampness-heat”.

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