Overexpression of AQP5 Was Detected in Axillary Sweat Glands of Primary Focal Hyperhidrosis Patients

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Introduction

Primary focal hyperhidrosis (PFH) is a disorder characterized by excessive sweating in specific body areas such as the palms, axillae, feet, or forehead where the eccrine sweat glands are concentrated [1]. Patients sweat in response to thermal and emotional stimuli but also spontaneously without any apparent trigger. Sweating, however, should be severe enough to significantly interfere with the patient’s occupation or enjoyment of life [2]. A US national survey estimated that 1.4% of the US population (4.0 million individuals) suffer from axillary hyperhidrosis [3]. One third of these axillary hyperhidrosis pa-

Key Words
Primary focal hyperhidrosis · Axillary sweat glands · Aquaporin 5

Abstract

Background: The expression of aquaporin 5 (AQP5) in human axillary sweat glands has never been studied so far. Objective: To detect the expression of AQP5 in axillary sweat glands of patients with primary focal hyperhidrosis (PFH) relative to control subjects. Methods: The morphological characteristics and the number of sweat coils in axillary sweat glands were compared between two groups by using transmission electron microscopy. The expression of AQP5 was detected by immunohistochemistry, Western blot analysis, and real-time transcription polymerase chain reaction. Results: There were no significant differences between the two groups in terms of morphological characteristics and the number of sweat coils in axillary sweat glands. The expressions of AQP5 protein and AQP5 mRNA were significantly higher in the patient group than in the control group. Conclusion: AQP5 is involved in the secretion of human axillary sweat glands. The overexpression of AQP5 in sweat glands is probably one pathogenetic mechanism underlying PFH.

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Patients (1.3 million individuals) stated that their sweating was intolerable and interfered with their daily life.

As sweating is mediated through the sympathetic nervous system, selective sympathectomy appears to be the only effective and sustainable surgical treatment for PFH [4]. Although the cause of PFH remains unclear, it is postulated that the overactivity of the sympathetic cholinergic fibers passing through the upper dorsal sympathetic ganglia causes abnormal innervation of the eccrine glands responsible for sweat secretion resulting in subsequent vasoconstriction and cooling of skin [5].

Aquaporins (AQPs) are water-selective channels that enhance the water permeability through the plasma membrane of the cells and were first discovered in 1991 [6]. Based on previous reports [7–9] we speculated that aquaporin 5 (AQP5) might play an important role in the excessive secretion of sweat glands of PFH patients. So the aim of this study was to measure the expression of AQP5 protein and AQP5 mRNA in axillary sweat glands of patients with PFH (P group) relative to control subjects (C group).

**Subjects and Methods**

For further details, see the supplementary materials (for all online suppl. material, see www.karger.com/doi/10.1159/000444081) [10] (tables 1, 2; fig. 1).

**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>P group (n = 35)</th>
<th>C group (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>21.2 ± 2.4</td>
<td>20.7 ± 2.1</td>
</tr>
<tr>
<td>(range 14 – 42)</td>
<td>(range 18 – 33)</td>
<td></td>
</tr>
<tr>
<td>Sex, male:female</td>
<td>17:18</td>
<td>15:13</td>
</tr>
<tr>
<td>Laterality, left:right</td>
<td>16:19</td>
<td>16:12</td>
</tr>
</tbody>
</table>

**Table 2. List of primers for AQP5 and β-actin amplification**

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
<th>Product size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target gene AQP5</td>
<td>Forward primer</td>
<td>5'-GCTGCCATCCTTTACTT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>5'-GTTCCCTTCCGTCCTC-3'</td>
</tr>
<tr>
<td>Housekeeping gene β-actin</td>
<td>Forward primer</td>
<td>5'-ATCATGTTTTTGAACCTTCAACA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>5'-CATCTCTTGCTGGAGTCCA-3'</td>
</tr>
</tbody>
</table>

**Results**

**Morphological Characteristics of Sweat Glands**

Using hematoxylin-eosin-stained histological sections and ultrastructural examination of axillary sweat glands, we found there were no significant differences between the two groups in terms of morphological characteristics (fig. 2a–d). There is no significant difference between the P group (34.95 ± 8.36) and C group (32.43 ± 7.51; p > 0.05) in terms of the number of sweat coils in axillary sweat glands. However, the number of secretory granules in the P group was significantly higher than in the C group (fig. 2e, f).

**Expression of AQP5 in Axillary Sweat Glands**

The immunohistochemical results showed that AQP5 was expressed predominantly on the basolateral plasma membrane and luminal membrane of epithelial cells in
sweat gland tubules. By using Image Pro-plus 6.0 software compared with the integrated optical density of the two groups, the expression of AQP5 increased significantly in the P group (8.15 ± 1.71) compared to the C group (3.50 ± 1.02; p < 0.01; fig. 3a, b). The results of immunofluorescence histochemistry showed that the average absorbance of AQP5 increased significantly in the P group (1.27 ± 0.11) compared to the C group (1.11 ± 0.12; p < 0.01; fig. 3c, d). By using the Western blot analysis, the level of skin AQP5 in the P group (1.43 ± 0.35) was significantly higher than that in the C group (0.75 ± 0.26; p < 0.01, fig. 4).

**Relative Expression of AQP5 mRNA by Real-Time PCR**

Real-time PCR was performed on total RNA from axillary skin tissues (fig. 5) by using specific primers. By using the $2^{-\Delta \Delta C_t}$ method, to compare the relative quantification of AQP5 mRNA (normalized AQP5 mRNA amount relative to the C group) between the C and P groups, we found the relative expression of AQP5 mRNA of the C group (1.02 ± 0.4) to be significantly lower than that of the P group (3.39 ± 0.84; p < 0.01).

**Discussion**

The primary cause of PFH is still unknown. There appears to be an overactive response to both heat and emotional stimuli, mediated through the sympathetic nervous system, and more research is focused on the sympathetic nervous system [11, 12]. However, studies for sweat
**Fig. 3.** The black arrows show that immunohistochemical staining revealed anti-AQP5 labeling of basolateral plasma membrane and luminal membrane domains of the secretory parts of axillary sweat glands in the C group (a) and P group (b). Original magnification ×200. Immunofluorescence staining shows the expression of AQP5 in the C group (c) was lower than that in the P group (d). White arrows point to sweat coils. Original magnification ×200.

**Fig. 4.** Expression of AQP5 protein was investigated in lysates of human axillary sweat glands in two groups. Immunoblotting analysis of AQP5 (32 kDa) and β-actin (43 kDa) was performed in lysates of human axillary sweat glands.

**Fig. 5.** Products of the predicted sizes were generated by RT-PCR of AQP5 (307 bp) and β-actin (318 bp) in human axillary sweat glands of the C and P groups.
glands with hypersecretion, especially those closely related to water, electrical and ion channels, have rarely been reported. In mammalian cells, AQP5 belongs to AQP subtypes which allow water to pass through the plasma membrane by osmosis [13, 14]. AQP5 has previously been detected in acinar cells of adult humans, mice, and rats. In addition, AQP5 has also been found to be expressed in several secretory glands including salivary glands, airway submucosal glands, lacrimal glands, sweat glands, as well as in alveolar type I epithelial cells of rats [15–20]. However, the expression of AQP5 protein and AQP5 mRNA in the sweat gland has, so far, only been studied in rats and mice.

To our knowledge, it was the first time that the expressions of AQP5 protein and AQP5 mRNA were detected in human axillary sweat glands. Accordingly, we harvested some important results as follows. To begin with, we verified that there was no significant difference in morphological characteristics and numbers of sweat glands between the two groups. Next, the number of secretory granules in the C group was significantly lower than in the P group, which illustrated the hypersecretion of axillary sweat glands in axillary hyperhidrosis patients. Finally, previous studies indicated that there are still controversies on whether AQP5 plays a significant role in sweat secretion [21, 22]. However, in our study we found that the expression of AQP5 protein and mRNA were markedly increased in the P group, which may explain the hypersecretion of sweat glands in PFH patients. In summary, our study supported the hypothesis that, in humans, AQP5 may be involved in sweat secretion, and the overexpression of AQP5 in sweat glands may contribute to the pathogenesis of PFH. Endoscopic thoracic sympathectomy is the main method of a radical cure for PFH; however, its side effects like compensatory hyperhidrosis, bradycardia and Horner’s syndrome are impossible to be avoided completely [23]. Ma et al. [24] reported that topiramate could reduce sweat secretion along with a decreased AQP5 expression by sweat glands in mice, suggesting that AQP5 may be involved in topiramate-induced hypohidrosis. So our study raises the possibility that the specific inhibition of AQP5 may provide a new nonsurgical treatment for PFH patients without the side effect and may avoid the damages of endoscopic thoracic sympathectomy.

Although we have found the presence of AQP5 in human axillary sweat glands and detected the overexpression of AQP5 in axillary sweat glands of PFH patients, the main roles of AQP5 in the human sweat glands remain to be clarified. Further investigations on the identification of the relationship between AQP5 and PFH will increase our understanding of the pathogenic mechanisms of PFH.

Acknowledgments

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Statement of Ethics

This study was approved by the Ethics Committee of Fujian Medical University (No. 2011-006).

Disclosure Statement

The authors have no conflicts of interest to declare.

References

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