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论文题目：A Systematic Analysis on ST36 Acupuncture Stimulation for Rhinitis Treatment

A Systematic Analysis on ST36 Acupuncture Stimulation for Rhinitis Treatment

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Abstract

Acupuncture has been shown to be beneficial in the physical therapy of pain and inflammatory diseases, while its underlying mechanisms still remain elusive. This limits the utility of this traditional medicine tool for targeted disease treatment by stimulating specific acupuncture points. A number of recent studies have suggested that electroacupuncture stimulation in ST36 (足三里穴) can treat systemic inflammation via the ProkR2+ neurons in the vagal-adrenal axis. I reasoned that such studies provide useful resources to track the same types of neurological pathways for finding new treatment targets (certain acupuncture points for treating specific inflammatory diseases). Therefore, this study aims to demonstrate other ProkR2 associated neurons/neural circuits and reveal translational insights by stimulating ST36. Through comprehensive data mining from Allen Brain Atlas, I found that Prokr2+ neurons are also highly enriched in the olfactory cortices of the human brain. Thus, I hypothesize that acupuncture at ST36 may also treatment disorders related to olfactory functions such as rhinitis. I then purified, inoculate, and sequenced the nasal microbes from rhinitis patients before and after acupuncture treatment at ST36. I found that the colony numbers of *Staphylococcus capitis subsp.urealyticus*, major pathogenic bacterial species, were significantly reduced in all the patients tested. Together, this article has provided a potential biological basis for ST36 acupuncture and demonstrated its clinical application in treating rhinitis.

Keywords:

Acupuncture, Physical Therapy, Rhinitis, Neuroinflammation, Olfactory System.

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Introduction

Inflammation is a defensive stress response process as a reaction to injury or infection. Under pathological conditions, mis-regulated inflammation will lead to damage of human body or inflammation related diseases such as rheumatoid arthritis, rhinitis and Alzheimer's Disease (neural inflammation), which still lack sustainably effective treatment[1].

It is widely appreciated that electroacupuncture stimulation (ES or other physical stimulation of specific body parts can modulate a specific physiological response at another distal body parts. These ES responsive body regions are often known as acupuncture points[2], and the acupuncture stimulation has been used to treat human diseases since ancient ages. Interestingly, emerging evidence has suggested the utility of acupuncture stimulation in modern medicine, with several underlying neurocircuits revealed.

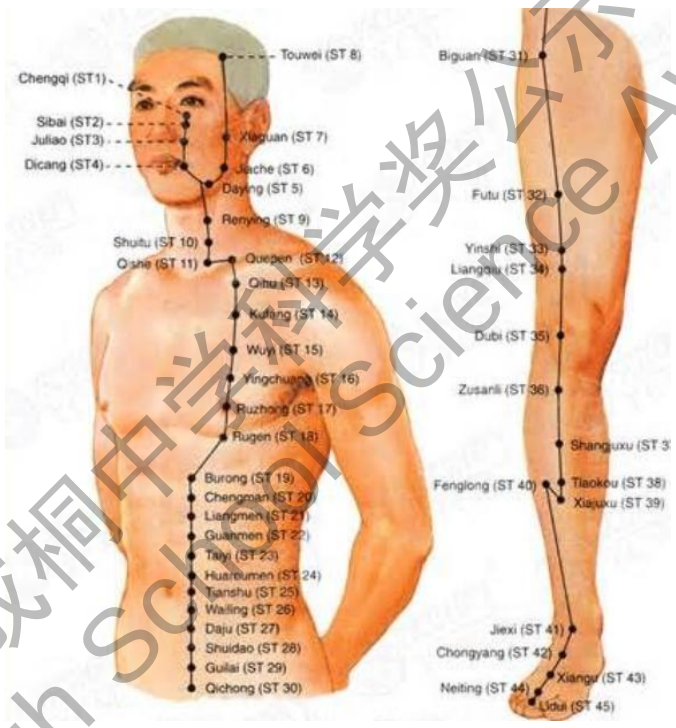

Fig. 1 The Stomach Meridian of Foot-Yangming.

For example, the ES in hindlimb ST36 can result in lower stomach peristalsis enhancing in order to heal stomach pain and abdominal distension[3]. Other studies have shown that acupuncture can also treatment acute bronchitis by altering the gene expression profiles of cells[4]. In order to make connections between specific parts and somato-internal organs, the primitive channel theory is formulated to explain the feasibility of acupuncture. These channels are named for their different locations, such as Foot-Yangming, Hand-Yangming (**Fig. 1**). Although modern anatomical studies have not yet supported the physical presence of such channels, but ES in the specific parts do make a difference to our body. This is the basis of our research.

Recently published studies on have demonstrated that ProkR2 positive neurons play a significant role in drive the vagal-adrenal anti-inflammatory axis in response to the

acupuncture of ST36 (足三里) (**Fig. 2**)[3]. Since the stomach meridian of Foot-Yangming (足阳明胃经) can extend from ST36 all the way to the head, I hypothesize that ES of ST36 might also affect brain functions. However, whether this hypothesis is valid and which body part may be affected by physical stimulation of Foot-Yangming acupuncture points have not been investigated.

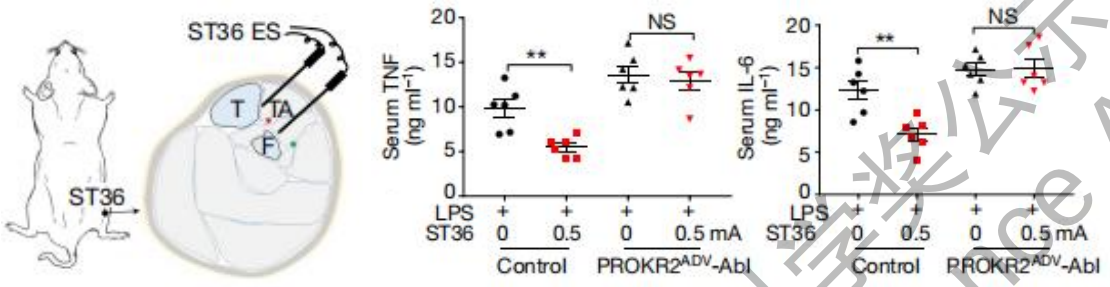

Fig. 2 Electrical stimulation of ST36 on Foot-Yangming alleviate neuroinflammatory responses via Prokr2+ neurons[5].

To address these questions, I first performed brain connectivity mapping of ProkR2 positive neurons. Surprisingly, I found that ProkR2 positive neurons exist in human brain olfactory area (Allen Brain Atlas). Therefore, I designed experiments to answer whether the acupuncture could also heal olfactory system diseases like rhinitis or rhinosinusitis. I then tracked 4 patients who suffered from rhinitis before and after acupuncture therapy. Meanwhile, I purified the nasal microbiomes from the patients (before and after treatment) and cultured them *in vitro*. I recorded both the density and diversity of bacteria colonies, which reflect their individual remission of diseases. I found that the amount of this rhinitis bacterium per unit volume of mucus decreased with the performance of acupuncture treatments.

Methods and Materials

1. Brain Gene Expression and Connectivity Analyses with Allen Brain Atlas

The acupuncture of ST36 is shown to drive the vagal-adrenal axis for releasing anti-inflammatory factor, which relies on ProkR2+ neurons in the peripheral nervous system (PNS). To map the ProkR2 expression in brain circuitry, I employed the single cell sequencing database of cortical neurons from Allen Brain Atlas[6] (WHOLE CORTEX & HIPPOCAMPUS SMART-SEQ, 2019). I first searched the ProkR2 protein expression in both mouse and human brain (SMART-seq) to find out the neurons that show enriched ProkR2 expression. Then I found the feature plot of ProkR2 positive neurons, in order to

visualize the clusters of neurons with various levels of ProkR2 expression. At last, I output the figures to visualize the cortical areas where ProkR2 is expressed. With these analyses, I then compared the similarity of gene expression between mouse Brain Atlas to human Brain Atlas.

2. Clinical Application of Acupuncture and Collection of Patient Nasal Biopsies

For application of acupuncture in the treatment of human diseases, such as rhinitis, I contacted Dr. Mingkuan He, a senior traditional Chinese medicine practitioner in a certified hospital (mentioned in the **Acknowledgement**), who kindly agreed to help me collect patient nasal biopsies. Dr. He treats many patients with rhinitis every day, enabling us to select representative and similar cases for clinical study. To control the independent variables, I selected four patients with the similar rhinitis symptoms and causes. However, in the past, diagnosis of rhinitis severity was based on the subjective feelings from the patients. To examine the effect of acupuncture treatment, I decided to evaluate the effectiveness and severity of acupuncture by culturing and observing the bacteria in the patients' nasal cavity.

2.1 Nasal Biospy Collection

(1) Dr. He collected the patient's snot (~0.2-0.5ml) before the acupuncture treatment. The nasal samples were collected in microbe collection tubes and stored at 4℃ until use.

(2) Dr. He performed acupuncture in specific and fixed locations for treatment (ST 36).

(3) After 3 days after acupuncture treatment and during patients' re-visit, Dr. He collected the patient's snot again (~0.2-0.5ml), following the same procedure as Step (1).

2.2 Microbe Culture Procedures

(4) I performed serial dilutions of collected nasal samples from the patients, either before or after acupuncture treatments, to $1:10^4$, $1:10^5$, $1:10^6$. Then I inoculated the microbe on the solid agar culture plates and incubated them 37℃ for 36 hours. As control groups to rule out the microbe contamination, I used both the plates without inoculation and the plates with "mock" inoculation but without nasal biopsies.

(5) After the designated incubation period, I observed and compared the similarities and differences between the bacteria colonies grown from different snot samples and controls. All culture plates were stored at 4℃ for a short period of time before PCR and sequencing analysis.

3. Identification of Nasal Microbe by PCR and Sequencing

3.1 PCR of nasal microbes

(1) I picked the selected single colony from the culture plate into the sterile PCR tube. Quantitative PCR reactions were conducted on a Bio-Rad iQ5 Real-time PCR system. Each reaction was performed in a 20-μL volume with the selected colony, 8-μL ddHO, 1-μL of each primer (**Table. 1**), and 10-μL Power SYBR Green PCR Master Mix (Takara, Dalian, China). The PCR condition was initiated at 95℃ for 3 min, followed by 35 cycles of 95℃ for 30s, 55℃ for 30s and 72℃ for 1 min. Then, after final extension at 72℃ for 5 min, the samples were subjected to 12℃ for storage. The melting Curves and standard curves were analyzed to ensure specificity of the amplified products.

Fig. 3 Conditions for PCR reaction. The date on the thermal cycler was not the actual date of experiment.

(2) The primers of the PCR used were to target the 16S rRNA gene (**Table. 1**).

Table. 1 Primer sequences of nasal biopsy PCR

Name	Type	Sequence
Eubac 27F	**Forward primer**	**5'-AGAGTTTGATCCTGGCTCAG-3'**
Eubac 1492R	**Reverse primer**	**5'-GGTTACCTTGTTACGACTT-3'**

(3) I sent the tested PCR samples to a professional biometric laboratory. In order to be aware of the type and the function of each bacteria species and function on the plate.

3.2 Analysis of nasal bacteria sequencing results and the association with rhinitis

After receiving the sequencing results from a professional biometric laboratory, I performed Basic Local Alignment Search Tool (BLAST) analysis on the microbial sequences before and after acupuncture treatment. This will allow me to understand the species of nasal microbes. Then, by conducting literature searching on published research articles, I comprehensively explored the effects of each identified nasal microbe species in human diseases ("beneficial species" or "detrimental species").

Results

1. Mapping of acupuncture related ProkR2 neuron connectivity in brain

1.1 Expression and projection of ProkR2 neurons in mouse cortical areas

Previous studies on the mechanism of ST36 stimulation focused on PNS, but I realized that the Foot-Yangming extend further from the foot to the head[5]. Therefore, I asked whether stimulation of ST36 or other Foot-Yangming acupuncture points may affect facial or cortical functions. To test this point of view, I first examined if the ProkR2+ neurons that underlie ST36 mediated anti-inflammation response are present in the mouse cortex, given that the previous studies of ST36 acupuncture utilized *Mus musculus* as the model organism. Thus, I employed the mouse in situ hybridization database from Allen Brain Atlas (**Fig. 4** showing the sagittal view and **Fig. 5** showing the coronal view). Interestingly, I demonstrated that ProkR2 is particularly enriched in the olfactory bulb areas of human brain. Other ProkR2 enriched areas include cortical subplate (CTXsp), MY (medulla), STR (Stratum), and so on.

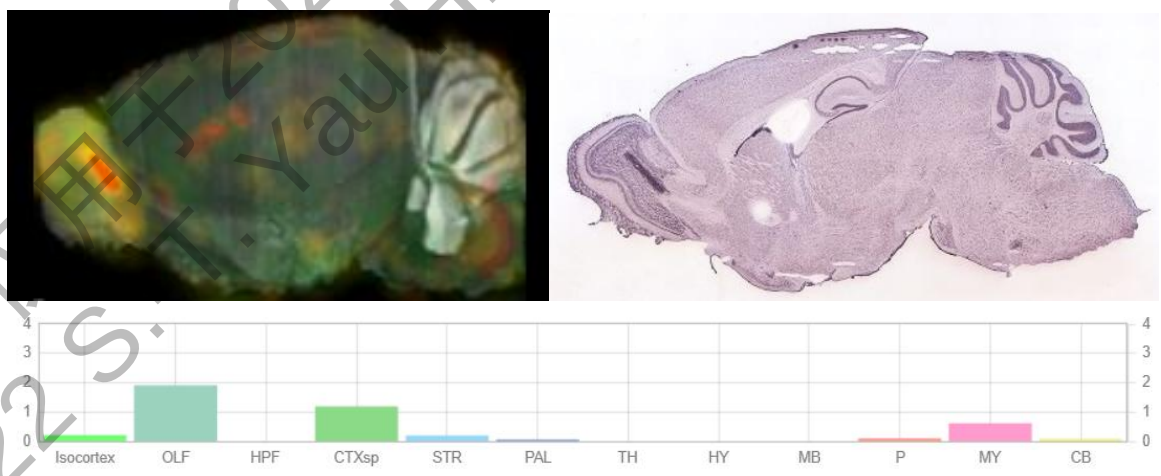

Fig. 4 Sagittal sections showing the expression of ProkR2 in mouse brain. Expression levels are shown as "raw expression values" defined by Allen Brain Atlas.

To understand the link between the characteristics and functions of ProkR2 expressing neurons, I explored the mouse whole cortex single cell RNA-seq data from Allen Brain Atlas. From the expression heatmap of ProkR2 in mouse cortical areas, multiple cell types are confirmed with ProkR2 expression (**Fig. 6**). To better visualize the expression of ProkR2 in different types of cortical cells, I generated the scatter plot of ProkR2 across the entire cortex and hippocampus of mouse (**Fig. 7**). Interestingly, only a few subtypes of neurons express ProkR2, including excitatory and inhibitory neurons (88_SST, 97_SST and 98_SST). Most of these ProkR2 expressing neurons have signature somatostatin (SST) expression. SST is a neuropeptide which is found throughout the brain, inhibiting principal neurons and other interneurons[7]. SST+ cells present in all olfactory areas, and central SST is involved in olfactory information processing[8]. Reduction of SST is strongly associated with the decline of olfactory functions in neurodegenerative diseases, such as Alzheimer's Disease and Parkinson's Disease[7]. These findings are consistent with the brain region maps from in situ hybridization (**Fig. 4** and **Fig. 5**). Overall, given the expression of ProkR2 genes in the mammalian olfactory system, it might be possible to hypothesize if stimulating ST36 may also affect the function related to the olfactory system.

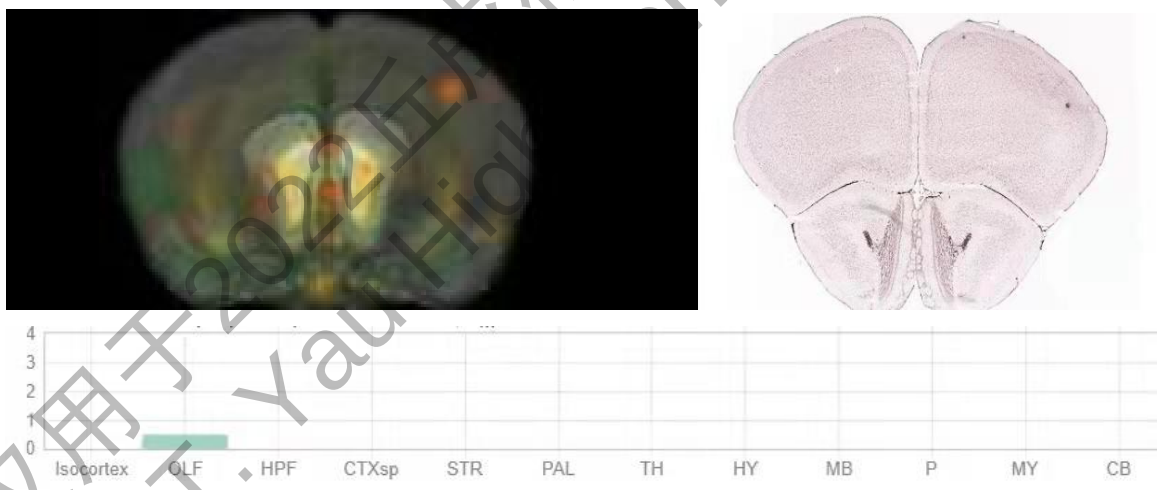

Fig. 5 Coronal sections showing the expression of ProkR2 in mouse brain. Expression levels are shown as "raw expression values" defined by Allen Brain Atlas.

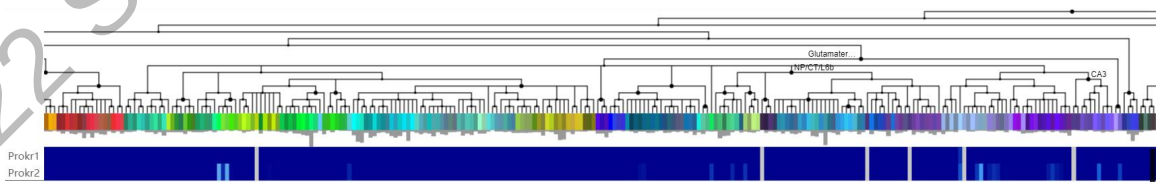

Fig. 6 ProkR2 neurons only represent in a small subset of cortical neurons of mouse cortex

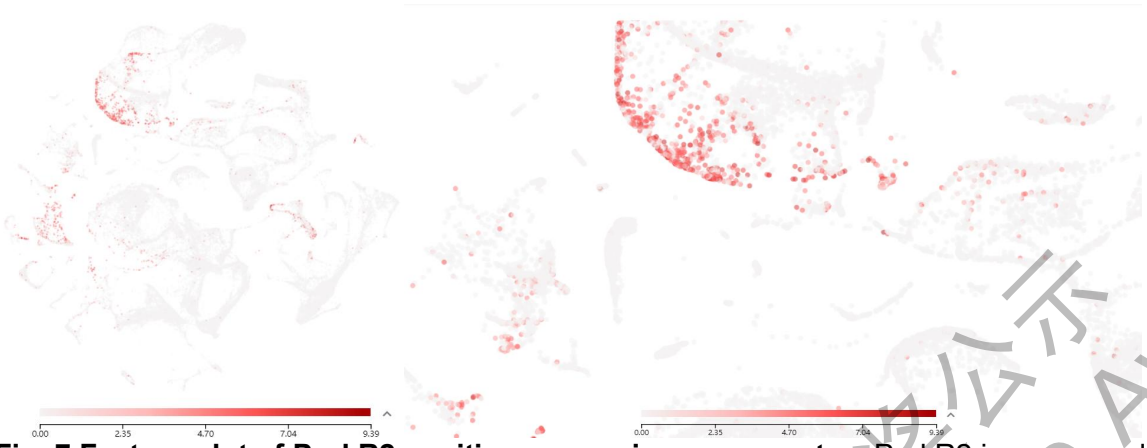

Fig. 7 Feature plot of ProkR2 positive neuron in mouse cortex. ProkR2 is expressed in multiple cortical regions, including excitatory and inhibitory neurons. Left, global view of feature plot; right, zoom-in view of feature plot.

1.2 Expression of ProkR2 neurons in human cortical areas

In order to further explore the connection between the neuroanatomy and function of ProkR2+ neurons in human nervous system, I also generated the heatmap of ProkR2 gene expression from the human Allen Brain Atlas RNA-seq data from MULTIPLE CORTICAL AREAS - SMART-SEQ (2019) (**Fig. 8**). Compared with the heatmap of mouse in Allen Brain Atlas (RNA-seq data WHOLE CORTEX & HIPPOCAMPUS - SMART-SEQ), the human heatmap indicates a less concentrated ProkR2 expression and reveals that the ProkR2 mainly exist in the neuronal type named Exc L6 FEZF2 TBCC. The ProkR2 expression scatter plot further pinpoints that ProkR2 expressing neurons are concentrated in three subgroups of neurons (**Fig. 9** and **Table. 2**), two excitatory neuronal types and one inhibitory neuronal type. Interestingly, it is known that FEZF2 is responsible for olfactory system development, and Exc L6 FEZF2 TBCC neurons have been identified as olfactory system neuron by previously[9]. In concordance with the analyses on mouse cortical maps, it is reasonable to hypothesize if stimulating ProkR2+ neurons or ST36 might promote the secretion of anti-inflammatory factors, thus potentially treating inflammatory diseases related to the olfactory system.

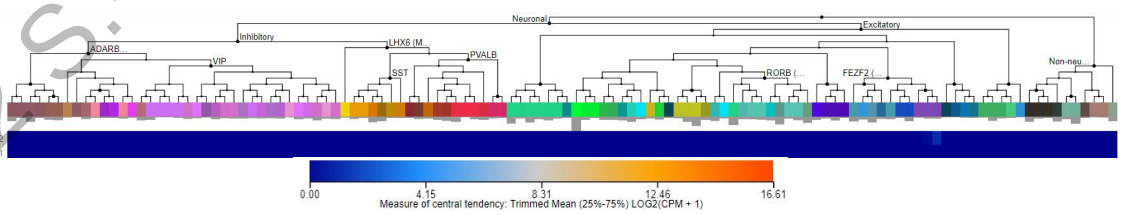

Fig. 8 ProkR2 neurons only represent in a small subset of cortical neurons of human cortex.

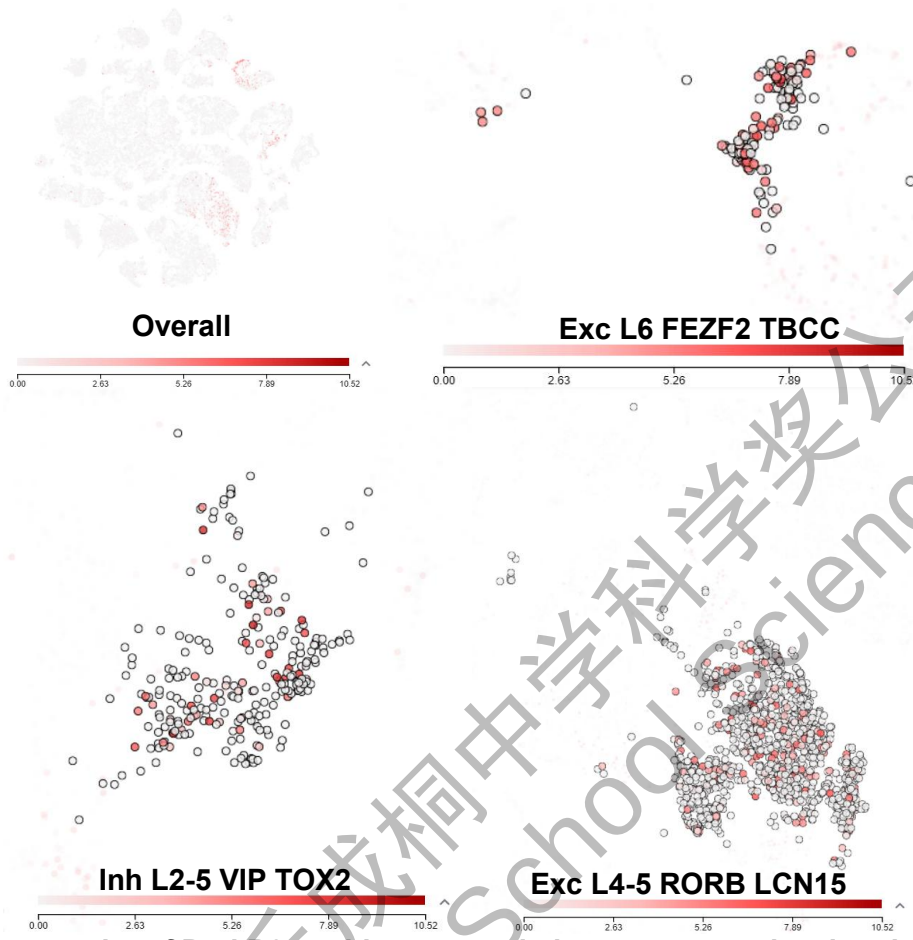

Fig. 9 Feature plot of ProkR2 positive neuron in human cortex showing the highly expressed regions of ProkR2

Table. 2 Expression level of human ProkR2 enriched regions

	Avg	Max
Exc L6 FEZF2 TBCC	2.45	8.15
Inh L2-5 VIP TOX2	0.99	9.12
Exc L4-5 RORB LCN15	0.77	7.22

2. Analysis of nasal bacteria in rhinitis patients before and after acupuncture treatments

2.1 Background information of rhinitis patients

In order to verify whether acupuncture would alter the nasal microbiome of patients with rhinitis, I then sought to culture the bacteria from the nasal cavity and observe the density and diversity of these nasal bacterial species. I select 0.1ml of nasal mucus from each of the 4 patients, before and after acupuncture treatment, whose case reports have

been well documented (**Table. 3**). Despite minor individual difference, I can observe that all patients have almost the same background information and symptoms, which eliminates many uncertain factors for our data analysis and is more conducive to our data analysis and summary of test results. All patients after acupuncture reported relieved symptoms, although it might be difficult to correlate the degree of alleviation with our microbial analysis in this study.

Table. 3 Background information of patients with nasal bacteria investigated

	Patient No.1	Patient No.2	Patient No.3	Patient No.4
Age	25	30	24	23
Gender	male	male	male	male
Symptom	Nasal congestion; diminished sense of smell	Nasal congestion; diminished sense of smell	Nasal congestion; diminished sense of smell	Nasal congestion; diminished sense of smell
Medical history	Bone fracture	Echinophthalmia	No	No
Treatment outcome	nasal congestion resolved, nasal discharge reduced	Sneezing improved, nasal discharge reduced	Sneezing improved, nasal congestion and runny nose resolved	Snoring resolved, sneezing improved, nasal congestion remains

2.2 Nasal bacterial culture and quantification

After acquiring the patient nasal microbial samples before and after ST36 acupuncture, I conduct series dilution (1:10^4, 1:10^5, 1:10^6) and inoculated them on LB culture plates, following standard human microbe culture condition (**Table 4-5**). After 36h of incubation, numbers of colonies in each condition were counted, and at least three independent experiments for each condition were conducted (**Fig. 10-13**).

Table. 4 Colony counts of nasal microbes from rhinitis patients before treatment

Culture condition: 35℃, pH = 7 for 36 hours

Dilution factor	10^4	10^5	10^6	Estimated total
Patient No.1	62±4	34±3	25±1	(9.7±0.4)×10^6
Patient No.2	52±1	43±1	21±2	(3.6±0.7)×10^6
Patient No.3	55±8	29±4	18±2	(7.2±0.8)×10^6
Patient No.4	665±12	197±14	58±8	(28.1±3.1)×10^6

Data are presented as mean±s.d..

As expected, the number of colonies decreased as I increased the dilution factor (**Table. 4-5**). Unexpectedly, most of the colonies before or after treatment exhibited the

same round shaped and clear powder white color (Patient No.1 and No.2 are shown as representatives in **Fig. 10-13**). This lack of diversity may suggest that there is a dominant bacterial species in the nasal microbes. It is interesting to further ask if there is a potential connection between these powder white colonies and rhinitis. However, I observed a very rare yellow colony on the 10^6-dilution plate of Patient No.2 before acupuncture treatment (further analyzed in Part 3 of **Results**).

Table. 5 Colony counts of nasal microbes from rhinitis patients after treatment

Culture condition: 35 ℃, pH = 7 for 36 hours

Dilution factor	10^4	10^5	10^6	Estimated total
Patient No.1	30±1	16±2	9±3	$(3.6±1.0)×10^6$
Patient No.2	34±2	26±1	11±3	$(4.6±1.0)×10^5$
Patient No.3	44±3	24±3	16±1	$(6.3±0.4)×10^5$
Patient No.4	439±30	112±14	27±4	$(14.2±1.9)×10^4$

Data are presented as mean±s.d..

Fig. 10 Bacterial culture result from Patient No.1 before treatment (each represents $1:10^4$, $1:10^5$, $1:10^6$, **from left to right).**

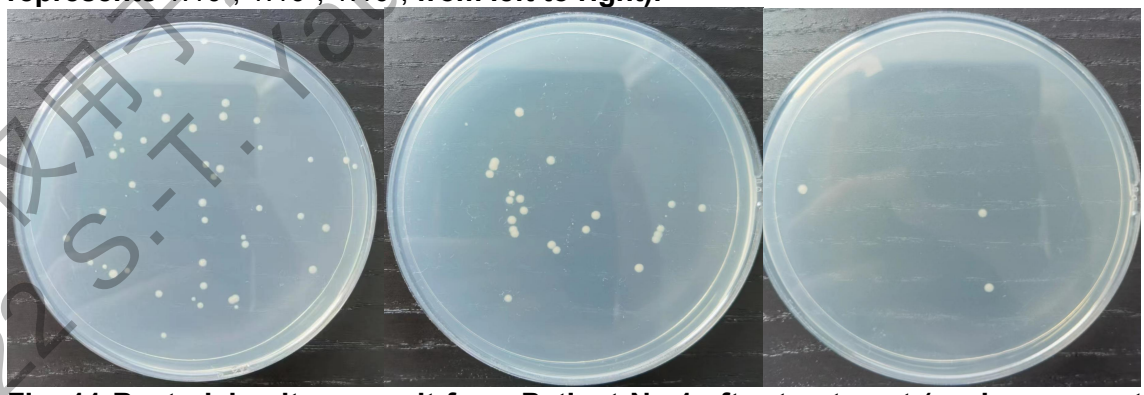

Fig. 11 Bacterial culture result from Patient No.1 after treatment (each represents $1:10^4$, $1:10^5$, $1:10^6$, **from left to right).**

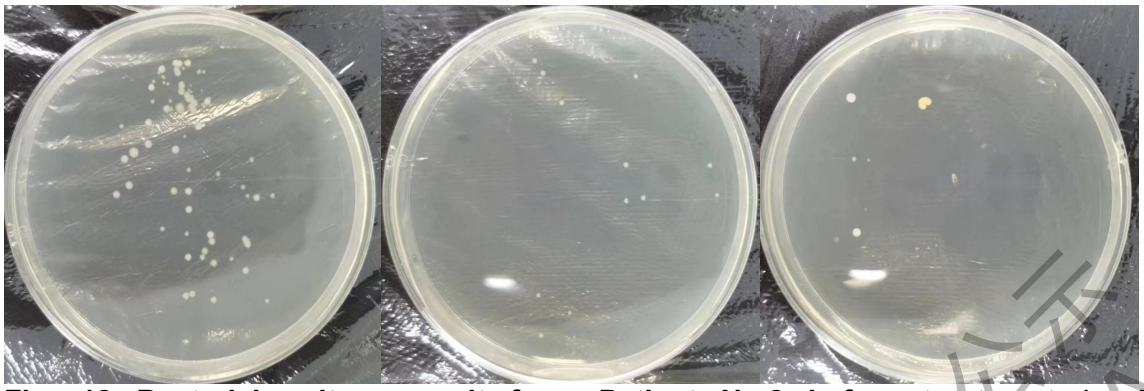

Fig. 12 Bacterial culture result from Patient No.2 before treatment (each represents $1{:}10^4$, $1{:}10^5$, $1{:}10^6$, **from left to right).**

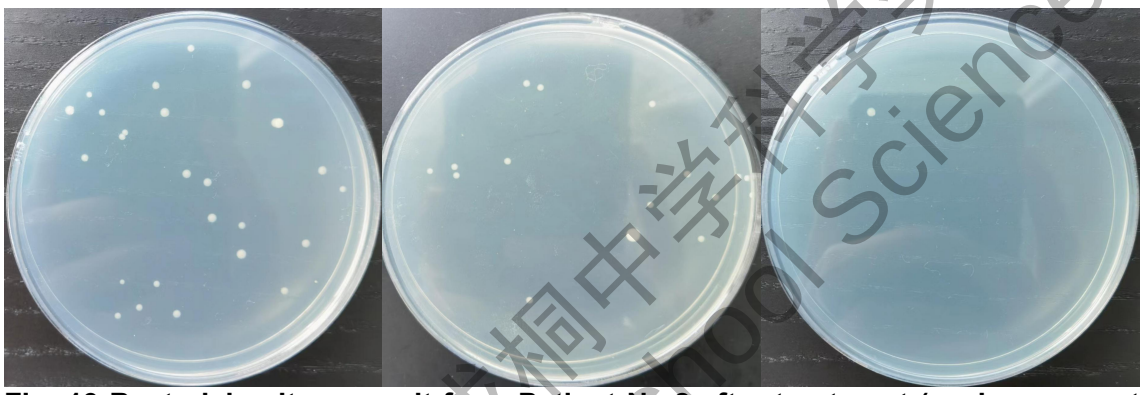

Fig. 13 Bacterial culture result from Patient No.2 after treatment (each represents $1{:}10^4$, $1{:}10^5$, $1{:}10^6$, **from left to right).**

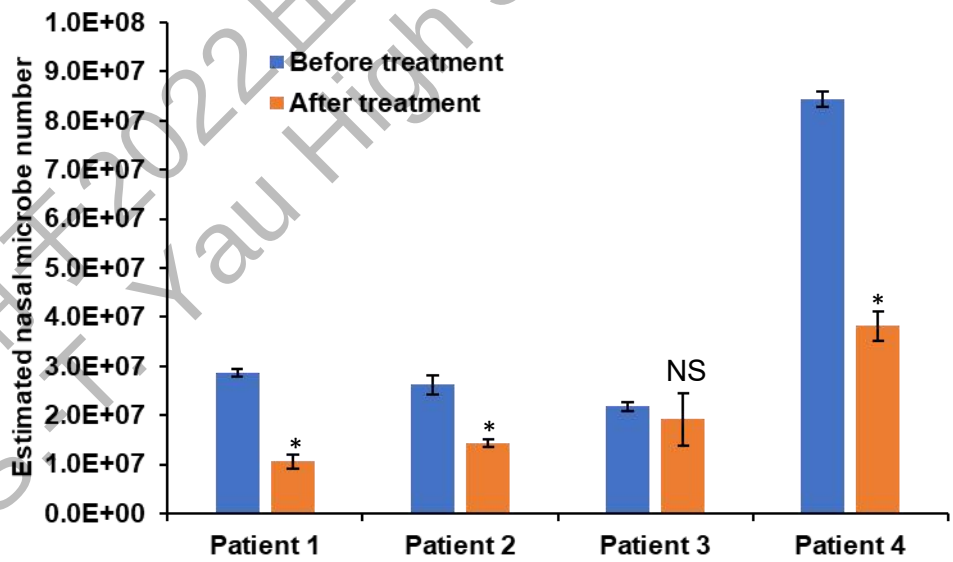

Fig. 14 Microbe counts for patients before and after treatment. Data are presented as mean±s.e.m., and student t tests were used to determine if there are statistically significant difference before and after treatment for each patient. *p<0.05, NS, not significant.

By comparing **Table 4** and **Table 5**, I found that the number of colonies in each patient was reduced after acupuncture treatment, although the colony numbers between patient individuals vary a lot (**Fig. 14**, three out of four patients showed statistically significant reduction of colonies numbers after treatment). Based on the fact that each patient had reported alleviation of their symptoms, our results suggested that this powder white microbial species (dominant nasal microbe species) may play a negative role in nasal health. Acupuncture stimulation of ST36 can effectively reduce this detrimental species. Then, the question is what this nasal microbial species is.

3. Sequencing and taxonomy analysis of nasal microbiome before and after acupuncture treatment

3.1 The dominant nasal species is Staphylococcus capitis subsp. ureolyticus

In order to test our hypothesis that the powder white colonies may contribute to rhinitis, I submit both the colony PCR product and culture plate to a professional biometric laboratory for sequencing. Prior to sequencing, I used electrophoresis to confirm whether the PCR experiments were successful. The PCR results indicate that the correct 16S rRNA genes from the colonies tested were amplified (**Fig. 15**). Then, two independent sequencing experiments based on either the forward primer (27F) or the reverse primer (1492R) were performed, and the same sequencing results were generated (sequence result of 27F shown on **Fig. 16**).

Fig.15 The electrophoresis results of PCR products. Lane 1 represents the DNA marker. Lane 2 is the PCR samples without any colony as negative control. Lane 3 is the white colony (dominant species) before the treatment. Lane 4 is the yellow colony before the treatment. Lane 5 is another DNA marker. Lane 6 is the white colony (dominant species) after the treatment. Lane 7 is the yellow colony after the treatment.

The next question is to confirm the identity of the dominant nasal microbial species based on its sequence. Using BLAST search, I found that *Staphylococcus capitis subsp.*

ureolyticus showed the highest homology to our identified sequence (**Fig. 17**, 98% homology). I then conducted comprehensive Pubmed search for previously published literatures of this bacterial species in rhinitis. *Staphylococcus capitis subsp. ureolyticus* belongs to the coagulase negative and gram positive *Staphylococci*, which often reside on human skin and mucous membrane[10].

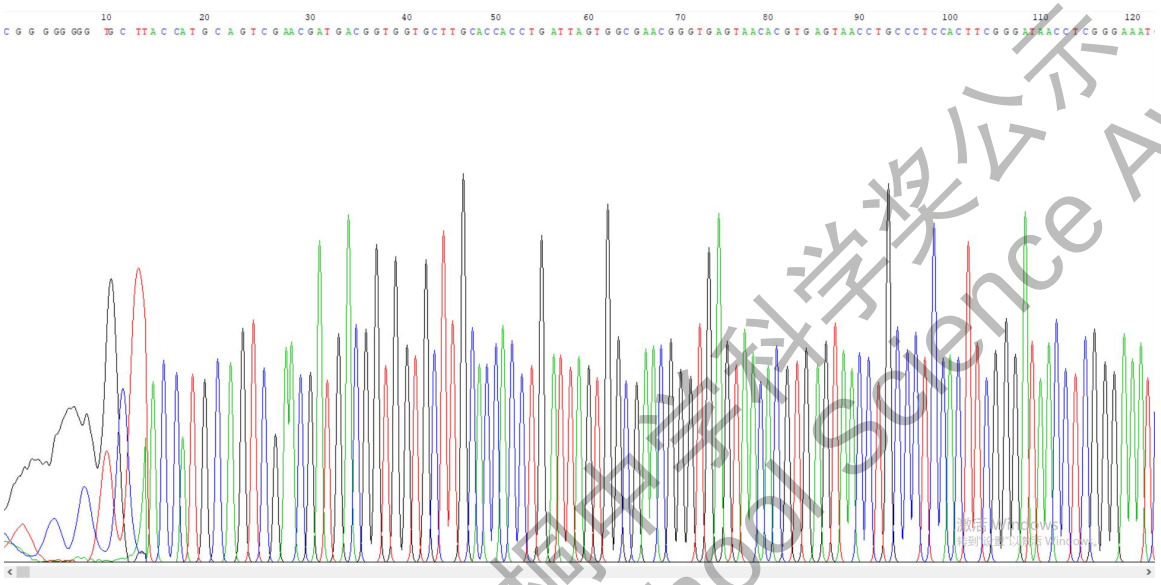

Fig. 16 The result of nasal bacteria sequencing from primer 27F (white colony).

Score	Expect	Identities	Gaps	Strand
2050 bits(1110)	0.0	1167/1191(98%)	18/1191(1%)	Plus/Minus

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Query  1     AATTCTGTTCACCTTCGACGGCTAGCTCCAAA-GGTTACTCCACCGGCTTCGGGTGTTAC  59
             |||| ||||| ||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  1240  AATT-TGGTCA-CTTCGACGGCTAGCTCCAAATGGTTACTCCACCGGCTTCGGGTGTTAC  1183

Query  60    AAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGTAGCAT  119
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1182  AAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGTAGCAT  1123

Query  120   GCTGATCTACGATTACTAGCGATTCCAGCTTCATGTAGTCGAGTTGCAGACTACAATCCG  179
             |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  1122  GCTGATCTACGATTACTAGCGATTCCAGCTTCATATAGTCGAGTTGCAGACTACAATCCG  1063

Query  180   AACTGAGAACAACTTTATGGGATTTGCTTGACCTCGCGGTTTTGCTACCCTTTGTATTGT  239
             |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  1062  AACTGAGAACAACTTTATGGGATTTGCTTGACCTCGCGGTTTCGCTACCCTTTGTATTGT  1003
```

Fig. 17 The BLAST result of nasal bacterial sequencing (white colony).

Although *Staphylococcus capitis subsp. ureolyticus* is not as common as *Staphylococcus aureus*, cases of infection from clinical reports have been increasing more recently[10]. As an important pathogen in inflammatory diseases, *Staphylococcus*

capitis subsp. ureolyticus can induce meningitis, prosthetic valve endocarditis, and delayed sepsis. This bacterium is a subtype of *Staphylococcus cephalus*, and the pathogenesis of the *Staphylococcus capitis subsp. urealyticus* is mainly due to its ability to produce a viscous biofilm, which is consistent with the situation that the nasal lining of the rhinitis patients is often in a viscous condition, and the cause for shortness of breath may also be explained by the detrimental effects from this bacterium. In summary, our sequencing results have suggested that the bacterium species that lead to rhinitis of our patients is *Staphylococcus capitis subsp.urealyticus*.

3.2 The rare colony from Patient No.2 is Brachybacterium paraconglomeratum

Additionally, I also picked the yellow colony and sent it for sequencing. Based on the same species identification scheme as the white colony, I found that this bacterial species is *Brachybacterium paraconglomeratum* (**Fig. 18-19**, 99% homology). In fact, the nasal infection cases of this bacterial species have been rarely reported, except for one 57-year-old Japanese male patient who had uveitis. Interestingly, I confirmed that Patient No.2 indeed had echinophthalmia during the acupuncture treatment. Based on the current understanding of *Brachybacterium paraconglomeratum* in the microbiology field, it is hard to draw the conclusion whether this rare species would also contribute to rhinitis. I could possibly hypothesize that the presence of *Brachybacterium paraconglomeratum* may be related to echinophthalmia from the prior medical history of Patient No.2.

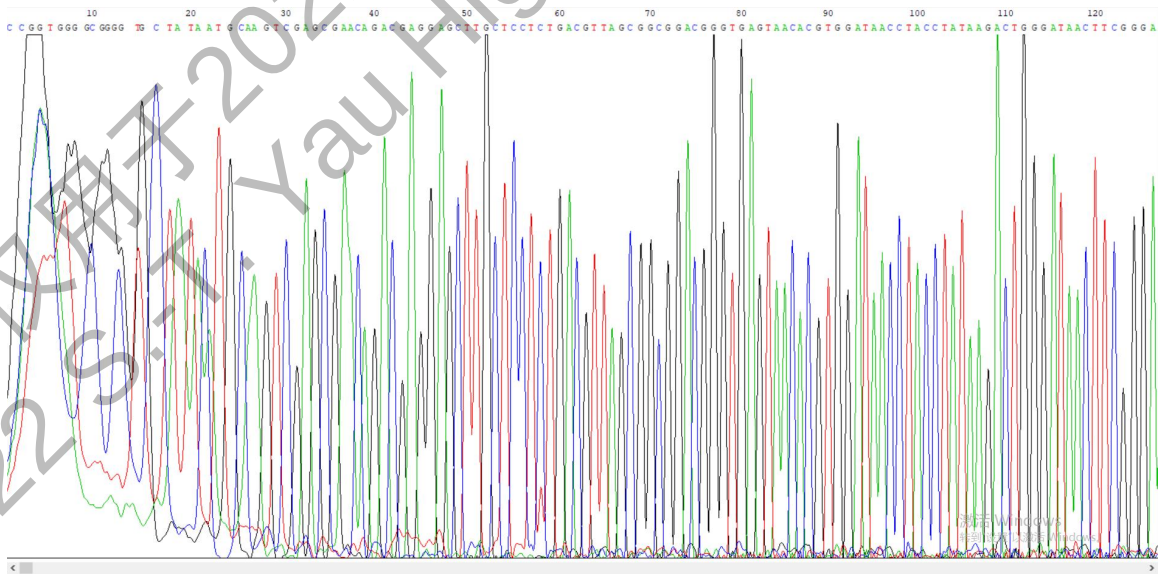

Fig. 18 The result of nasal bacteria sequencing from primer 27F (yellow colony).

Score	Expect	Identities	Gaps	Strand
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Query  62   GGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCTCCACTTCGGGATAACCTCGGGAAA  121
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  65   GGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCTCCACTTCGGGATAACCTCGGGAAA  124

Query  122  TCGTGGCTAATACCGGATATGAGCACTCATCGCATGGTGGGTGCTGGAAAGATTTATTGG  181
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  125  TCGTGGCTAATACCGGATATGAGCACTCATCGCATGGTGGGTGCTGGAAAGATTTATCGG  184
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Fig. 19 The BLAST result of nasal bacterial sequencing (yellow colony).

Discussion

In this study, I attempted to develop new methods (1) to study the underlying mechanisms of acupuncture based physical therapy; and (2) to treat olfactory system diseases such as rhinitis using acupuncture. I managed to establish the connection between acupuncture stimulation and cortical ProkR2+ neurons or neural circuits. In light of previously published results exploring the effects of acupuncture on PNS[5], I hypothesized that acupuncture treatment may act through the anti-inflammatory processes on local neural circuits. I evaluated the therapeutic effects by not only recording the patient's intuition on sternutation and rhinorrhea (**Table. 3**) but also analyzing the microbiome of nasal snot from the patients before and after the treatment. Notably, this study first and creatively assessed nasal microbe flora in rhinitis patients with ST36 acupuncture treatment. I believe further mechanistic studies would be needed to facilitate the development of acupuncture-based therapy into a more targeted disease treatment approach, especially on the treatment of inflammation.

As for the limitations and improvements for this study, I first reason that it is important to consider the rhinitis patients without any treatments (or sham treatment) as control. However, it was not very feasible for us to conduct such clinical studies which requires more complicated patient consents to receive a non-effective treatment. However, future systematic clinical studies should include sham control groups to evaluate the benefits of acupuncture therapy. Secondly, the confirmation of acupuncture treatment outcomes was based on documented symptoms. I have considered more quantitative nasal

function measurements such as nasal resistance. However, the costs to purchase or assemble such devices prevented us to do so, especially during the coronavirus pandemic. As part of my future plan, I will collaborate other medical institutions with more professional instrumental supports to quantify the nasal functions of the patients before and after treatment. Finally, we should carefully consider whether it is plausible to combine colony quantification and sequencing as a standard for rhinitis diagnosis and treatment. I believe that this is a very attractive direction, particularly given the emerging studies on gut microbiota and various human diseases. It would also be interesting to explore the relevance between “nasal microbiota” and inflammation-related diseases[11].

By exploring the mechanisms behind the acupuncture of rhinitis, I realize that its treatment should also be a multifaceted process, perhaps combining physical and pharmacological approaches. This might explain why administration of antibiotics and corticosteroids only transiently relieve the symptoms[12]. Meanwhile, it is also noteworthy that the bacterial quantity and diversity or “nasal microbiota” may play an important role in inflammation, which has not been well studied previously. The 16s rRNA PCR sequencing has revealed that *Staphylococcus capitis subsp. ureolyticus* is the dominant species, which is consistent with previously published studies[13]. Based on the nasal bacterial characterization, my working hypothesis is that the population number of *Staphylococcus capitis subsp. ureolyticus* that cause the rhinitis varies from individual to individual. Similarly, in the inflammation-related studies of gut microbiota, the quantitative alteration in the gut flora may cause acute enteritis and pancreatic necrosis[14]. Further studies on “nasal microbiota” can be performed based on more sensitive sequencing and quantification tools.

Acupuncture is a Traditional Chinese Medicine therapy that has existed for at least two thousand years[15]. Despite its extensive use in Traditional Chinese Medicine, the underlying molecular and neuroanatomical mechanisms of acupuncture is still insufficient. As a side project, I tried to compare the distribution between the “meridian system” and the nervous or circulatory system (**Fig. 20**). Intriguingly, no apparent overlap has been observed. Based on a few recently published studies[5], I postulate that the stimulation of certain acupuncture points may be associated with the regulation of neural activity by certain proteins or neural circuits (such as ProkR2 or release of cytokine). If such specific proteins can be identified following the stimulation of each

acupuncture points, it is possible to translate the “meridian theory” into modern medical science language.

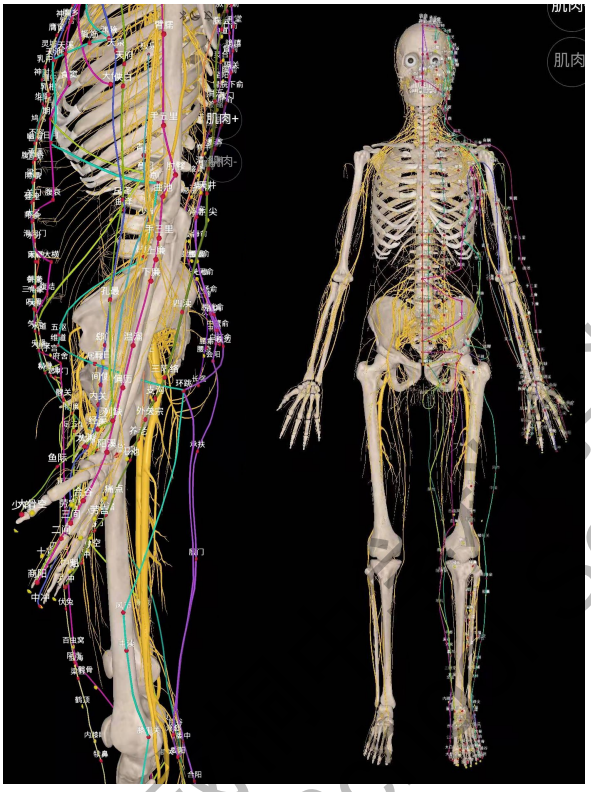

Fig. 20 Anatomical map between meridian system and nervous system (yellow).

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Acknowledgement (致谢)

在 2019 年 9 月 18 日，我经历了一段我人生中迄今为止最痛苦的时间，先是单纯的尾椎骨按压疼痛，到后来我甚至因为尾椎骨在运动中被别的肌肉牵拉所导致的剧痛，使我无法进行正常的站立或行走。在西医的怀疑是强直性脊柱炎的情况下进行几番治疗无果后，我近乎绝望并开始担心我的余生是否得和轮椅度日。抱着试一试的形态，我的父母带着我来到了当地一位有名中医开的诊所，奇迹般地，在两三次的治疗后我的病就以肉眼可见的趋势痊愈了，在感叹自己可以再一次使用双腿后，我也惊讶并好奇于针灸这一项神奇的疗法的原理和逻辑。

September 18, 2019 was the most painful day of my life to date. What began as a simple pain in my coccyx progressed to a severe pain that prevented me from standing. After several conventional modern medicine treatments proved ineffective, I was suspected of suffering from ankylosing spondylitis and was desperately worried about whether I be confined to a wheelchair for the rest of my life. As an alternative option, my parents took me to a local acupuncture clinic run by a famous Traditional Chinese Medicine doctor. After just two or three treatments, there was a steady and almost miraculous alleviation of my symptoms, and it was a real relief to be able to use my legs again. I found this surprising and became interested in the principles and logic that underlie acupuncture.

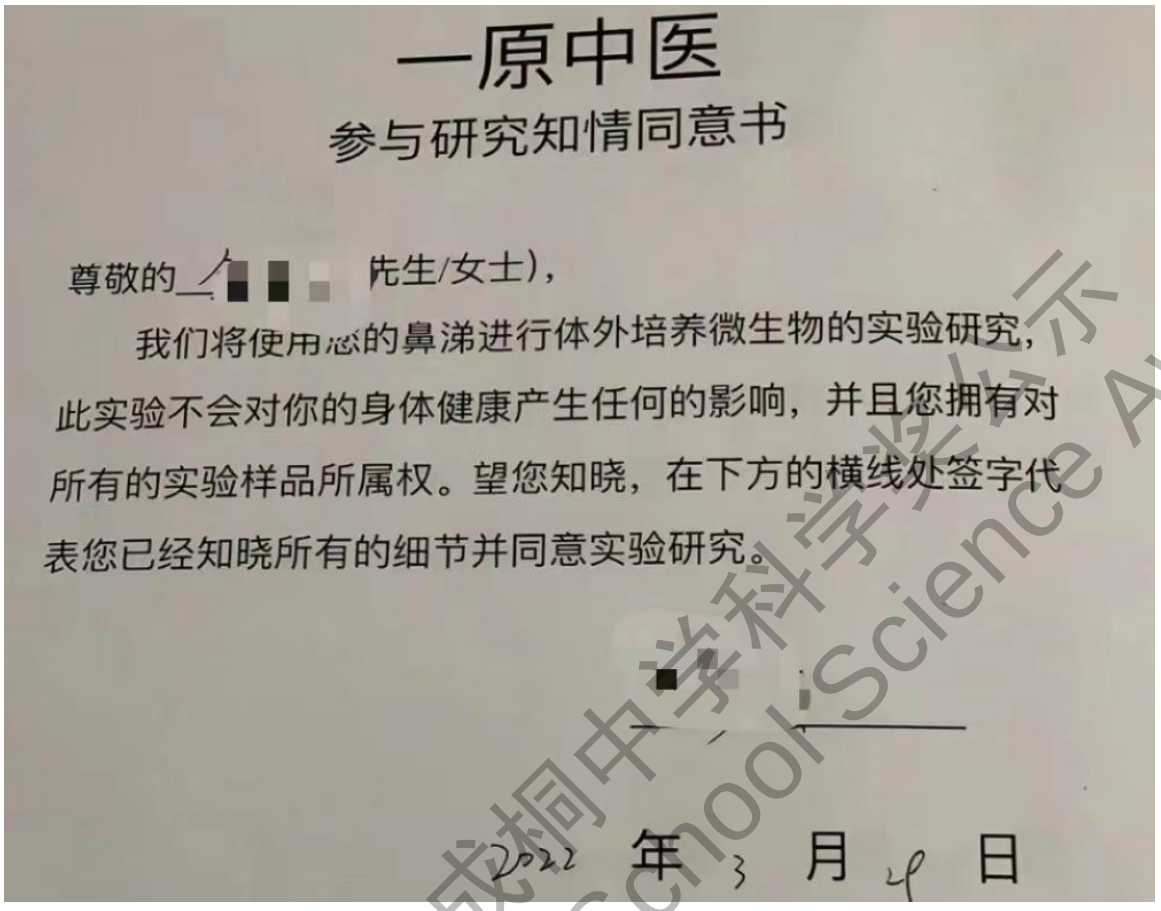
在经过一系列的调查后我发现，针灸疗法却并没有被主流医学界广泛使用。究其原因，中医对针灸原理的解释比较宏观和笼统，而且其很多理论并没有理论医学依据和解剖学基础，所以这种难以让人信服却有用的医学手段目前还没有被广泛使用。在了解到这些信息后我为这项神奇但却还未被解释的医学手段而感到惋惜，因为我认为其中一定存在很多能被医学界发现并利用的理论。所以这些经历也促使我写了这篇关于针灸的研究报告，并决定采取英文作为文章的主要语言。尽管在描述经络等中医的特有名词时我遇到了很大的困难，但我还是坚持完成了英文研究报告的写作，目的是为了能让更多人看到关于针灸的研究，并且试图从转化医学的角度分析针灸背后的机理，希望它能引起更多理论医学界的注意，并且有一天彻底完成从经验疗法到理论疗法的转变。

Through a series of further investigations, I found that acupuncture therapy is not widely used within the modern medicine community. This is because the principles of acupuncture in Traditional Chinese Medicine are relatively general and macroscale. It seems that the practice of Traditional Chinese Medicine is not well supported by anatomical and molecular basis from modern medicine. As a result, it does not widely spread outside China. I felt it was a great pity that this medical method does not receive the attention it deserves. I felt that if the principles by which it functions could be uncovered and codified into theories, it would be of great utility to the broader medical community. My own medical treatment and the lack of wider application of Traditional Chinese Medicine prompted me to compose this research report on acupuncture. Therefore, I decided to write the report in English, in order to share my study with a wider audience. Despite the great difficulties I encountered in describing terms specific to Traditional Chinese Medicine, such as meridians, I felt that English would better serve to attract more attention internationally to acupuncture research. Also, by analyzing exploring the mechanisms underlying acupuncture from the perspective of translational medicine, I hope that this could expedite the eventual transition of acupuncture from empirical therapy to theoretical medicine.

在文章的最后，我要由衷地感谢我的父母！即使在高中繁重的学习压力下，他们依旧支持我完成我的研究和论文。一原中医的何明宽何医生，治好了我的疾病，并协助我完成了鼻炎病人的针灸治疗。我也要感谢我的高中——成都七中对我的研究项目的大力支持，尤其是两位指导老师——袁智城老师和刘浩秋老师，以及成都七中的竞赛主任文宗老师。最后，我想感谢四川大学生命科技学院的王丽教授对我的研究课题提出了一些很有建设性的意见。在你们对我热心与慷慨的帮助下，我才能最终完成这篇文章。我也希望未来我能够去帮助跟多的人，完成这个轮回，就好像你们对我的帮助一样。从我的痛苦到我的重生，从梦的开始到结束，我也希望未来的我也能像你们一样能帮助那些需要的人，从始至终。

I would like to express my sincere gratitude to the people who have been generously supporting me during my research. First, I would like to thank my parents, who encouraged me to complete my research despite the heavy workload of high school. I would also like to thank Dr. HE Mingkuan from the “Yiyuan” clinic, whose effective treatment of my disease kindled my interest in Traditional Chinese Medicine. My high school has also been very supportive. In particular, I am grateful for the intellectual help

provided by my two instructors YUAN Zhicheng and LIU Haoqiu from Chengdu No.7 High School, as well as the Director of Competition of Chengdu No.7 High School, Mr. REN Wenzong. Last but not the least, as a high school student, my research paper has greatly benefited from the useful comments from Professor WANG Li from the School of Life Science and Technology of Sichuan University. It is their continuous and generous support that have made this research possible. I hope that in the future I will have the opportunity to spread this kindness to others and help those in need, just as you all helped me, through my pain and onto my rebirth from the beginning to the completion of my dream.



一原中医

参与研究知情同意书

尊敬的[redacted]先生/女士)，

我们将使用您的鼻涕进行体外培养微生物的实验研究，此实验不会对你的身体健康产生任何的影响，并且您拥有对所有的实验样品所属权。望您知晓，在下方的横线处签字代表您已经知晓所有的细节并同意实验研究。

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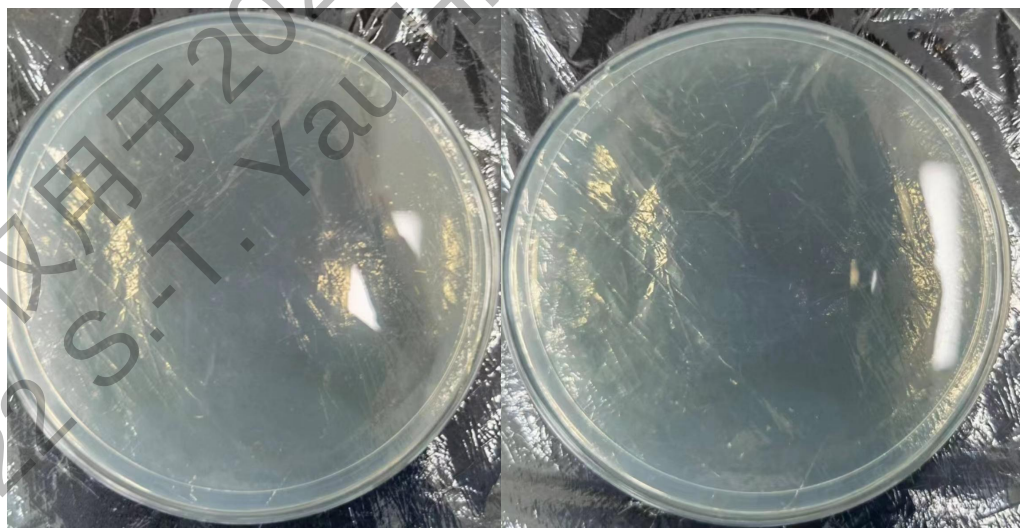
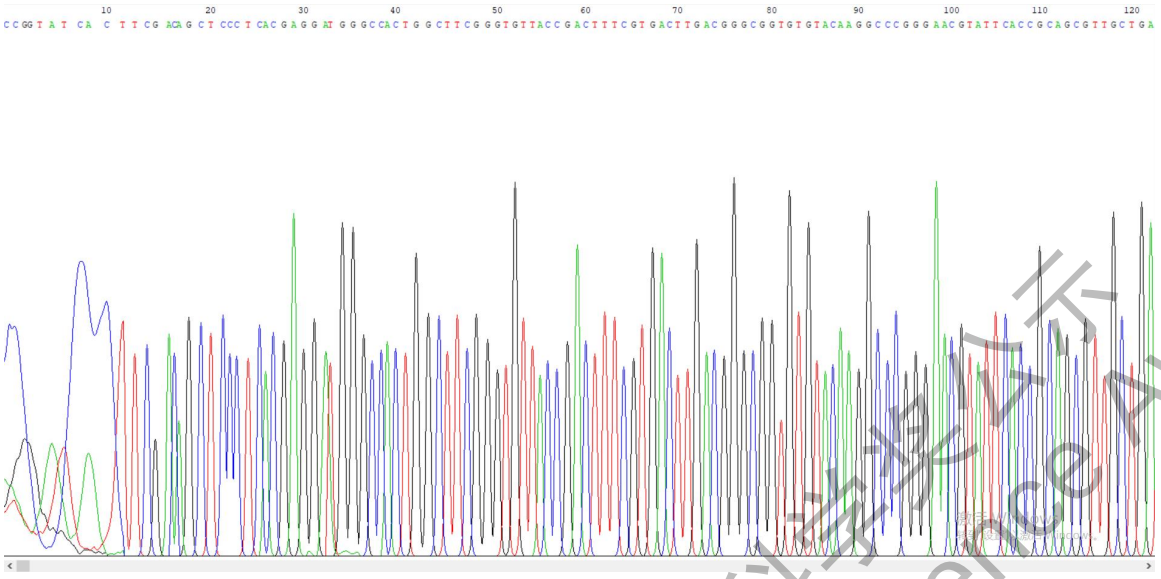
2022 年 3 月 4 日

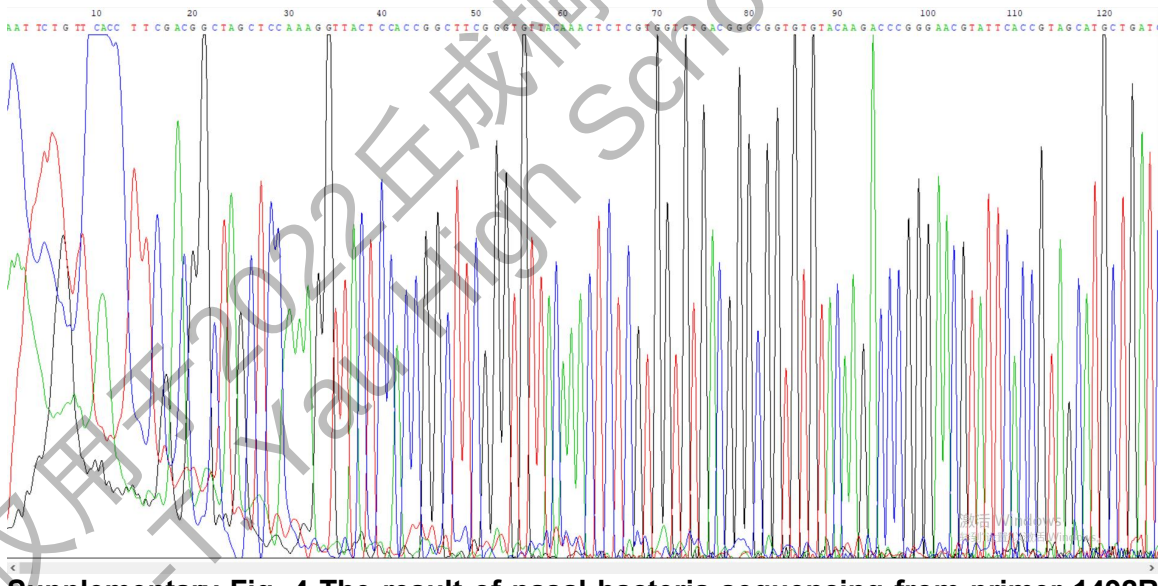
Supplementary Fig. 1 Signed Patient Consent for this study (one representative consent).



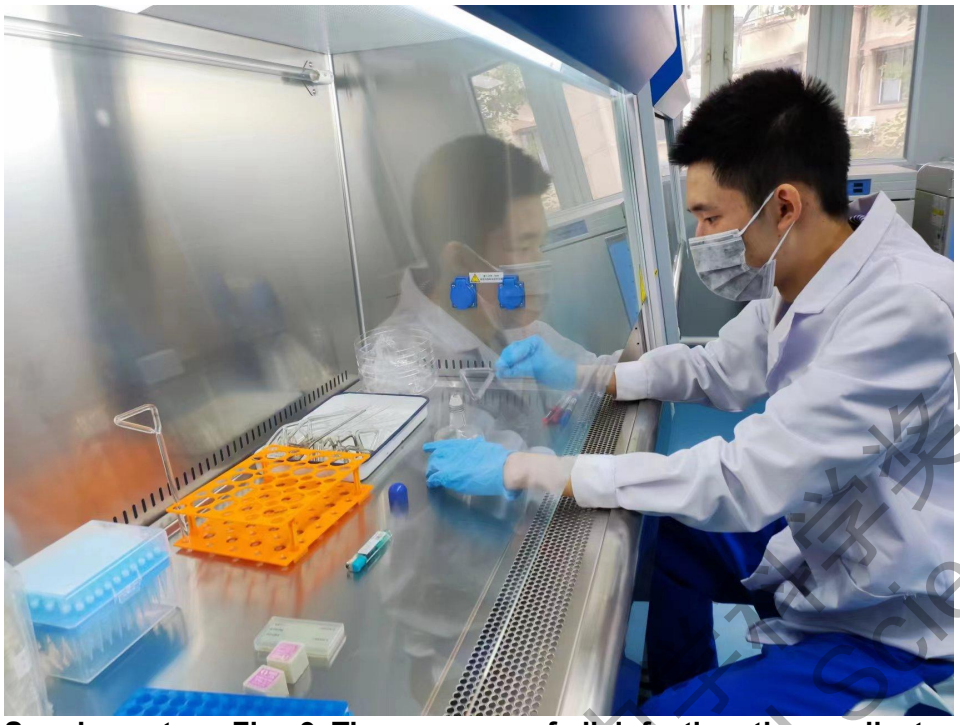
Supplementary Fig. 2 Bacterial culture result from blank control group. (left) No inoculation; (right) inoculated with PBS.



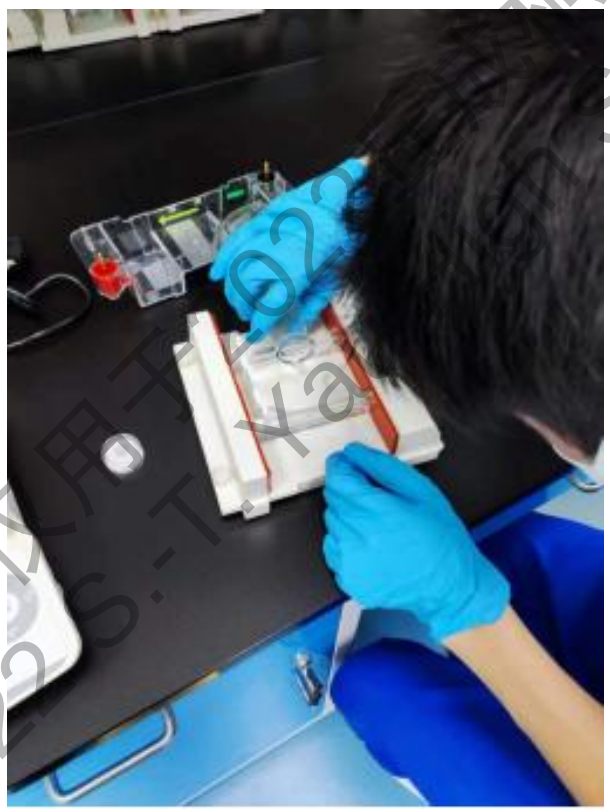
Supplementary Fig. 3 The result of nasal bacteria sequencing from primer 1492R (white colony).



Supplementary Fig. 4 The result of nasal bacteria sequencing from primer 1492R (yellow colony).



Supplementary Fig. 9 The process of disinfecting the applicator on the clean bench.



Supplementary Fig. 10 The process of assembling the gel for the electrophoresis.

Supplementary Fig. 11 The process of making PCR samples.