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研究課題名(和文) Proteomic, metagenomic and genome-wide analyses of oral cavity of ovariectomized rats with psychological stress

研究課題名(英文) roteomic, metagenomic and genome-wide analyses of oral cavity of ovariectomized rats with psychological stress

研究代表者

PAUDEL Durga (Paudel, Durga)

北海道医療大学・先端研究推進センター・助教

研究者番号：20910590

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研究成果の概要(和文)：この研究は、心理的ストレスや閉経後の状態が、唾液や唾液腺を含む口腔内の遺伝子発現、タンパク質発現、微生物叢に影響を与える可能性があるかどうかを調査することを目的としていました。私たちは2つの重要な発見を実証しました：1) 心理的ストレスを軽減または治療するために使用される薬剤は、口腔内の微生物叢に影響を与える可能性があります。2) 心理的ストレスは唾液腺の遺伝子発現とタンパク質発現に影響を及ぼし、唾液の量や流量を変化させる可能性があります。これらの発見は、研究論文として2つの異なる国際ジャーナルに掲載されました。

研究成果の学術的意義や社会的意義

The findings of our study will aid in understanding the effect of psychological stress and drugs used to relieve psychological stress on the oral cavity in gene expression, protein expression, and microbiota.

研究成果の概要(英文)：The study aimed to explore if psychological stress and postmenopausal conditions can affect the gene expression, protein expression, and microbiota in the oral cavity including saliva and salivary glands. We demonstrated two important findings: 1) The drugs used to relieve or treat psychological stress can affect the microbiota in the oral cavity. 2) Psychological stress affects gene expression and protein expression in salivary glands, which can alter salivary rate or flow. These findings have been published in two different international journals as a research paper.

研究分野：Psychosomatic Dentistry

キーワード：psychology stress oral cavity saliva oral microbiota amitriptyline RNA seq salivary glands

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## 1 . 研究開始当初の背景

Post-menopausal females undergo physical and emotional changes as a result of hormonal alteration in their bodies. The menopausal period is characterized by the reduced number of ovarian follicles thereby resulting in reduced responsiveness to follicle stimulating hormone and luteinizing hormone from the pituitary gland. This results in reduced production of sex hormones, estrogen and progesterone. The majority of symptoms such as hot flushes, vaginal atrophy, osteoporosis, and sexual problems in post-menopausal females are linked to estrogen deficiency. Apart from these symptoms, studies show that post-menopausal females experience psychological stress and anxiety more frequently.

Medically unexplained oral symptoms such as burning pain, oral dryness, and taste alterations are most commonly prevalent in post-menopausal females. Previous studies co-relate these symptoms with high psychological stress and low estrogen levels. However, how psychological stress and estrogen deficiency cause these unexplained oral symptoms is yet not clear. Moreover, multiple local and systemic factors in the oral cavity make it difficult to understand the role of psychological stress and estrogen deficiency in post-menopausal females. Therefore, the extensive study of proteins, genes, and microbiomes in the oral cavity of an animal model of a post-menopausal state with psychological stress could elucidate the involved mechanisms.

## 2 . 研究の目的

We used an ovariectomized model of rats and a chronic stress model of rats to achieve the proposed research objective. However, the ovariectomized models of rats did not yield significant results to report. On the other hand, the results from the chronic stress model of rats were interesting. Therefore, we set two final objectives:

1. To demonstrate the effect of psychological stress on the transcriptome of salivary glands
2. To demonstrate if psychotropic drugs such as amitriptyline, commonly used to treat medically unexplained oral symptoms, affect the microbiota of the oral cavity

## 3 . 研究の方法

**For Objective 1:** *To demonstrate the effect of psychological stress on the transcriptome of salivary glands*

The rats were randomly divided into control and stress groups. The stress group rats were enclosed in a restraint tube of 20 cm with ventilation for 4 h per day over 6 weeks. The tube size was such that the rats would sufficiently fit in but could not move freely. After the stress protocol, the rats were returned to their respective cages. The control group rats were left



Chronic restraint stress-  
4hours daily

undisturbed during the stress protocol. RNA sequencing was performed on RNA extracted from the submandibular gland. The differentially expressed genes were identified, and the genes of interest were further validated using qRT-PCR, immunofluorescence, and western blot.

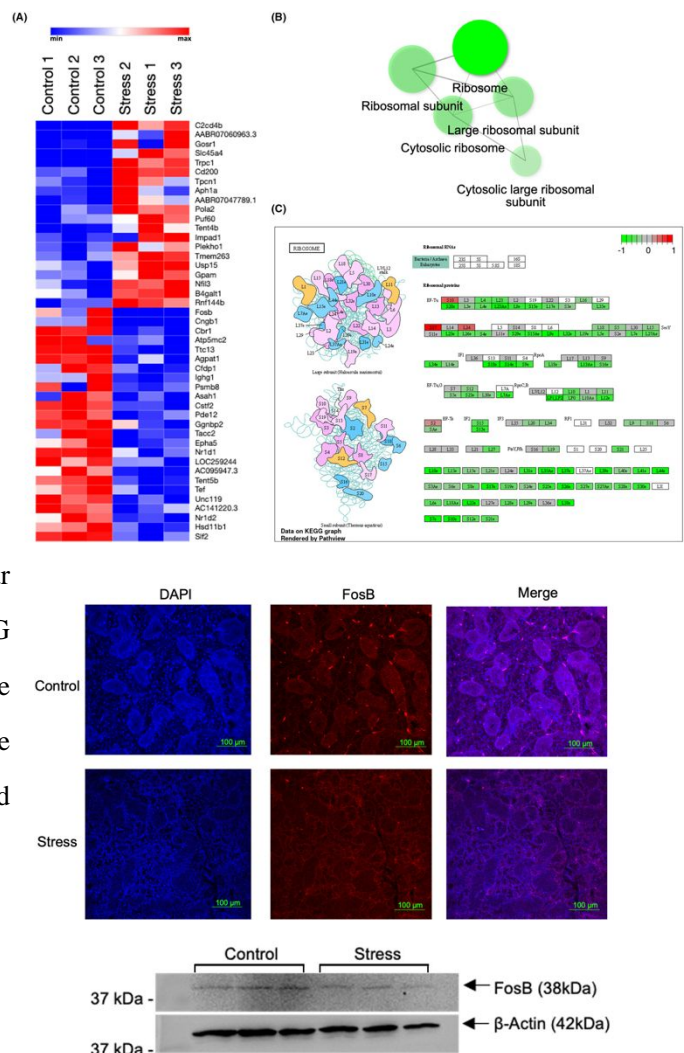
**For Objective 2:** *To demonstrate if psychotropic drugs such as amitriptyline, commonly used to treat medically unexplained oral symptoms, affect the microbiota of the oral cavity*

Sprague-Dawley rats were intraperitoneally injected with amitriptyline for 2 weeks. The DNA extracted from the oral swabs were used to perform 16SrRNA sequencing to evaluate the oral microbiome. Quantitative RT-PCR was performed to evaluate the mRNA levels of antimicrobial peptides in the buccal tissues.

## 4 . 研究成果

**For Objective 1:** *To demonstrate the effect of psychological stress on the transcriptome of salivary glands*

A comparison between control and stress groups showed 45 differentially expressed genes. The top five altered genes in RNA sequencing data showed similar gene expression in qRT-PCR validation. The most downregulated gene in the stress group, FosB, was a gene of interest and was further validated for its protein-level expression using immunofluorescence and western blot. The genesets for gene ontology cellular component, molecular function, and KEGG showed that pathways related to ribosome biosynthesis and function were downregulated in the stress group compared to the control.



**For Objective 2:** To demonstrate if psychotropic drugs such as amitriptyline, commonly used to treat medically unexplained oral symptoms, affect the microbiota of the oral cavity

No significant differences in salivary flow rates were observed between the amitriptyline and control groups. Taxonomic analysis showed significant alterations in bacteria such as *Corynebacterium*, *Rothia*, and *Porphyromonas* due to amitriptyline administration. The beta diversity showed significant differences between the amitriptyline and control groups. Additionally, the predicted metagenome functions were significantly different between the two groups.

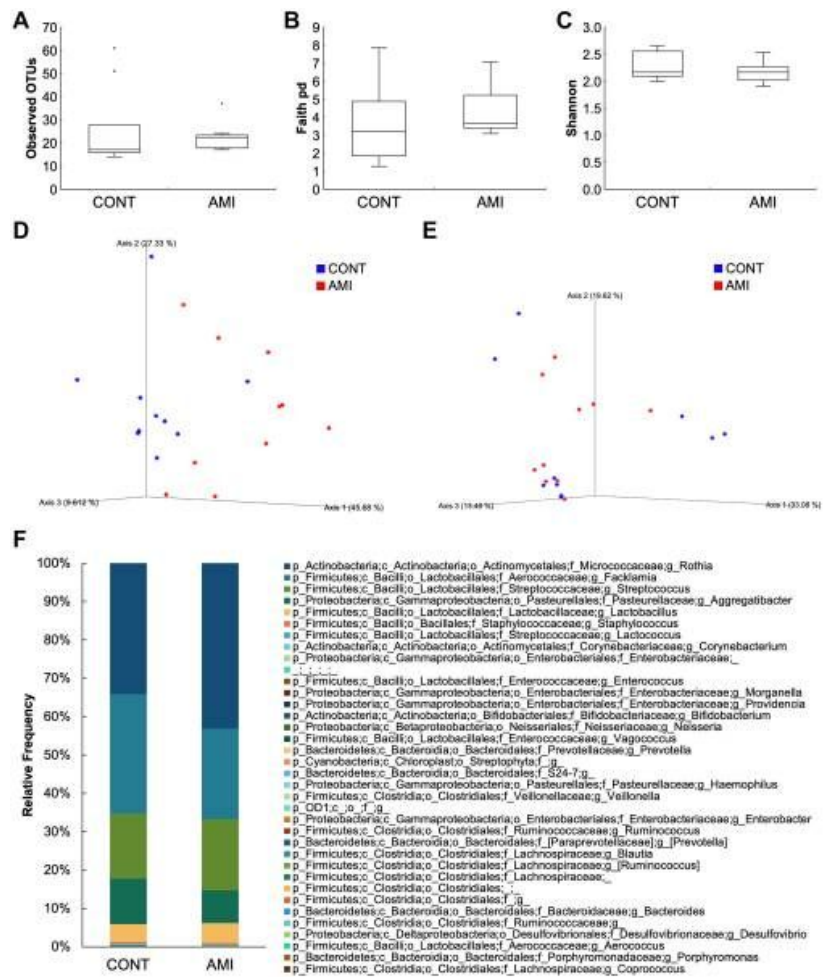
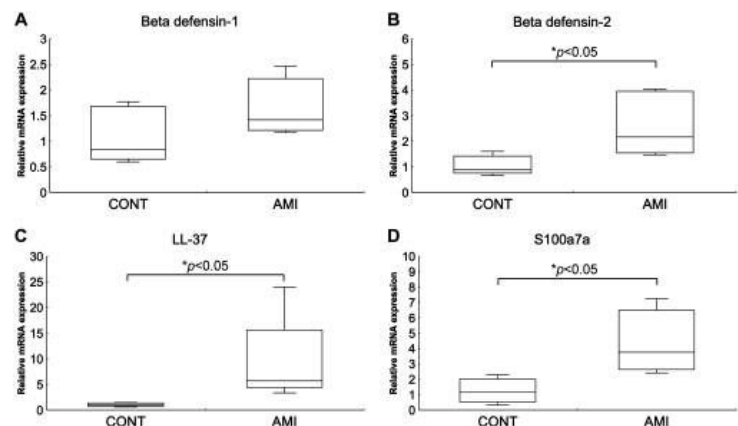


Fig: Metagenomic analysis of the oral cavity. The alpha diversity as evaluated by (A) observed operational taxonomic units, (B) faith phylogenetic diversity, and (C) Shannon index showed no significant differences between the two groups. The PCoA plot to evaluate beta diversity by (D) unweighted UniFrac and (E) weighted UniFrac. The weighted UniFrac was significantly different between the two groups ( $p=0.011$ ; PERMANOVA). (F) The bar graph shows the abundant bacteria at the genus level. Each group was dominated by genera, such as *Rothia*,

The mRNA expression levels of antimicrobial peptides in the amitriptyline group were significantly higher as compared to controls.



5. 主な発表論文等

〔雑誌論文〕 計2件（うち査読付論文 2件 / うち国際共著 2件 / うちオープンアクセス 1件）

〔学会発表〕 計1件（うち招待講演 0件 / うち国際学会 1件）

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|---|
| 1. 発表者名<br>Durga Paudel, Osamu Uehara, Tetsuro Morikawa, Koki Yoshida, Yoshihiro Abiko    |
| 2. 発表標題<br>Effect of Chronic Psychological Stress on Transcriptome of Submandibular Gland |
| 3. 学会等名<br>2023 AADOCR/CADR Annual Meeting & Exhibition, Portland, USA (国際学会)             |
| 4. 発表年<br>2023年   |

〔図書〕 計0件

〔産業財産権〕

〔その他〕

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| Advanced Research Promotion Center<br><a href="https://www.hoku-iryu-u.ac.jp/sentan/">https://www.hoku-iryu-u.ac.jp/sentan/</a> |
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6. 研究組織

| 氏名<br>(ローマ字氏名)<br>(研究者番号) | 所属研究機関・部局・職<br>(機関番号) | 備考 |
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7. 科研費を使用して開催した国際研究集会

〔国際研究集会〕 計0件

8. 本研究に関連して実施した国際共同研究の実施状況

| 共同研究相手国 | 相手方研究機関 |
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