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ABSTRACT

Objective: To identify and characterize the expression of phoenixin receptor GPR173 (G protein-coupled receptor 173) in the rat lacrimal and major salivary glands (parotid, submandibular, and sublingual). GPR173 is associated with hormonal regulation and autoimmunity; however, its expression in exocrine glands has yet to be characterized. Design: Under anesthesia, eight Sprague Dawley rats underwent bilateral dissection of the lacrimal and major salivary glands. GPR173 expression was assessed using Western blotting and immunofluorescence. Immunoreactivity was quantified using ImageJ software and compared using the Friedman test with Dunn-Bonferroni post hoc analysis. Results: GPR173 protein expression was found in all examined exocrine glands. In the major salivary glands, GPR173 was detected in both acinar and ductal cells, while expression was confined to acinar cells in the lacrimal gland. The submandibular and parotid glands showed significantly higher immunoreactivity than the lacrimal gland ($p < .01$ and $p < .05$, respectively). Ductal epithelium showed stronger immunopositivity compared to acinar epithelium. GPR173 immunoreactivity was also observed in periductal stromal tissue in major salivary glands. Conclusion: GPR173 expression was observed in both acinar and ductal cells of all major salivary glands, while in the lacrimal gland, it was restricted to the acinar cells. This differential localization suggests tissue-specific relevance.

Keywords: Receptors, G-Protein-Coupled, Acinar Cells, Salivary Glands, Exocrine Gland, Autoimmune Diseases

1. Introduction

G-protein-coupled receptor 173 (GPR173), also known as the super conserved receptor expressed in brain 3 (SREB3), has attracted significant attention recently due to its involvement in various physiological processes. In humans, GPR173 is widely expressed throughout the body, particularly in the brain, notably the hypothalamus and pituitary gland (McIlwraith & Belsham, 2018). It is found in the ovaries, pancreas, and to a lesser extent in the heart and skin. Recently, the expression of GPR173 has also been shown in the gastrointestinal tract (Kras, Osiak-Wicha, & Arciszewski, 2025). Phoenixin acts as a ligand for GPR173 (Lyu et al., 2013) and exists in two active forms, phoenixin-14 and phoenixin-20. Previous studies have reported expression of the peptide in various central and peripheral tissues across multiple species, including humans, rats, mice, bovine, and zebrafish (McIlwraith, Zhang, & Belsham, 2022). The activation of GPR173 by phoenixin has been linked to the modulation of gonadotropin-releasing hormone and luteinizing hormone, both of which play crucial roles in reproductive health (McIlwraith, Zhang, & Belsham, 2022; Yosten et al., 2013). The interaction of phoenixin with its receptor GPR173 indicates their role in estrous cycle by modulation of sex steroid secretion (Haddock et al., 2020; McIlwraith & Belsham, 2018). Phoenixin also decreases the release of reactive oxygen species and increases the production of the anti-inflammatory molecule glutathione, indicating a possible role in addressing neuroinflammation (Wang et al., 2020). GPR173 has been identified as a novel X-linked susceptibility gene associated with systemic lupus erythematosus, a disease characterized by a significant female predominance (Zhang, Zhang, & Wang, 2018). GPR173 has been linked to autoimmune disease susceptibility, partly due to its location on the X chromosome, which may contribute to female-biased disease prevalence. Current evidence suggests that GPR173 plays roles in inflammatory regulation and may be involved in the pathogenesis of autoimmune disorders (Billert et al., 2020; Zhang, Zhang, & Wang, 2018). GPR173, recognized for its anti-inflammatory properties and connection to autoimmune disorders, may contribute to immune regulation in chronic immune disorders with female dominance such as Sjögren's disease. Spatial expression of GPR173 has been described primarily in the central nervous system and selected endocrine-related tissues, where transcriptomic and limited protein-level data have shown its expression (Stein et al., 2016). However, the spatial localization of GPR173 in peripheral exocrine tissues remains uncharacterized. In particular, no studies to date have mapped its distribution within the lacrimal gland or the major salivary glands, leaving a significant gap in the histological characterization of GPR173 in exocrine tissues. Therefore, this study aims to determine whether the phoenixin receptor GPR173 is expressed and to characterize its localization within

the lacrimal gland and the major salivary glands of the rat, including the submandibular, parotid, and sublingual glands.

2. Materials and methods

2.1. Animals and operative procedures

The study included eight Sprague Dawley rats, comprising five females and three males, aged six months, obtained from the Marmara University Animal Center (DEHAMER), Istanbul, Türkiye. The rats were housed under controlled environmental conditions, with humidity levels ranging from 65% to 70% and a temperature of 22 ± 2°C, maintained under 12-hour light/dark cycle. They received regular rat pellets and had unrestricted access to water. The animals were euthanized under ketamine and chlorpromazine anesthesia (100 and 0.75 mg/kg) by exsanguination after cutting the abdominal aorta. Thereafter, the parotid, submandibular, and sublingual glands, as well as the lacrimal gland, were removed, weighed, frozen (−20°C), and stored (−80°C) until analysis. Tissue samples were fixed in 10% neutral buffered formalin for routine histological evaluation. For immunofluorescence analysis, tissues were fixed in a 4% paraformaldehyde solution.

All animal procedures were conducted in accordance with the Animals (Scientific Procedures) Act 1986, EU Directive 2010/63, and the NIH Guide for the Care and Use of Laboratory Animals.

All experimental protocols complied with Turkish legislation regarding animal research, were approved (13.2019.mar) by the Marmara University Animal Care and Use Committee, and conformed to the principles and norms set forth by the New York Academy of Sciences.

2.2. Western Blot Analysis

Immunoblotting was performed as an initial protein-level verification of GPR173 expression in the lacrimal and major salivary glands prior to spatial localization assessment by immunofluorescence.

Frozen gland tissues were homogenized in lysis buffer supplemented with Mini Protease Inhibitor Cocktail (1000 µl) (Roche, Merck, Germany). The lysates were centrifuged to remove insoluble debris, and the supernatants were collected for protein analysis. Protein samples were stored at -80°C for protein quantification. The protein content of all lysates was determined

using the Pierce BCA Protein Assay Kit (Thermo Scientific, USA), based on the method described by Lowry *et al.* (Lowry et al., 1951).

SDS-PAGE: Protein samples (150 µg) mixed with sample buffer (4X) were denatured at 100°C for 3–5 minutes. Equal amounts of protein (50 µg per lane) were loaded onto 12% SDS-PAGE gels and electrophoresed at 150 volts for 1.5 hours to separate into fractions. Following electrophoresis, the gel, nitrocellulose membrane, filter papers, and sponges were soaked in transfer buffer for 10 minutes. In the gel cassette, the layers were arranged in the following order over the positive electrode (anode): sponge, 3 mm filter paper, nitrocellulose membrane, gel, 3 mm filter paper, and sponge. The cassette was closed and placed in the transfer system. Transfer buffer was added to cover the cassette, and transfer was performed at 80-100 V for 1 hour.

Membranes were blocked in TBS-T blocking buffer containing BSA and incubated overnight at 4°C with the same rabbit anti-GPR173 primary antibody used for immunofluorescence staining (Invitrogen/Thermo Fisher Scientific, catalog no. PA5-106835, Waltham, MA, USA). This antibody targets a synthesized peptide corresponding to amino acids M254–V304 of human GPR173, a region that shares 100% sequence identity with the rat ortholog. After washing with TBS-T, membranes were incubated with HRP-conjugated goat anti-rabbit secondary antibody at a 1:500 dilution at room temperature. Immunoreactive bands were visualized using a Nitro Blue Tetrazolium/5-Bromo-4-Chloro-3-Indolyl Phosphate (NBT-BCIP) substrate buffer. β-actin (Santa Cruz, sc-1616, USA) was used as the protein loading control. Western blotting was conducted on two independent experimental days, with each experiment performed in duplicate using independent biological replicates. Comparable results were obtained through experiments.

2.3. Tissue Preparation for Histological Assessment

Tissue samples were fixed in 10% neutral buffered formalin. Following fixation, the tissues were dehydrated through an ascending series of ethanol concentrations (70%, 90%, 96%, and 100%) and then cleared with xylene. Clearing and paraffin incubation were performed using an automated tissue processor (Thermo Citadel 2000). The tissues were then embedded in paraffin. Sections of approximately 5 µm were cut using a rotary microtome (Thermo Scientific) and, then they were stained with hematoxylin-eosin (H&E) for histological evaluation.

2.4. Immunofluorescence

Tissues were fixed with a 4% paraformaldehyde solution for immunofluorescence analysis. The tissues were embedded in paraffin using a routine embedding procedure. Sections with a thickness of 5 μm were deparaffinized and rehydrated. Then, the sections were washed in PBS (phosphate buffered saline), treated with citrate buffer (pH 6.0) in a microwave oven, and allowed to cool for 15 minutes. To reduce nonspecific binding, sections were incubated in a blocking solution for 10 minutes. The sections were then incubated overnight at 4°C with rabbit anti-GPR173 primary antibody (Invitrogen/Thermo Fisher Scientific, catalog no. PA5-106835, Waltham, MA, USA) at a 1:100 dilution. The same primary antibody was used for both Western blotting and immunofluorescence analysis. This antibody targets a synthesized peptide corresponding to amino acids M254–V304 of human GPR173 (UniProt Q9NS66), a region that shares 100% sequence identity with the rat ortholog. After primary antibody application, sections were washed in PBS and incubated with goat anti-rabbit secondary antibody (Thermo, USA) at a 1:500 dilution at room temperature. All sections were finally incubated with 4′,6-diamidino-2-phenylindole (DAPI) at room temperature in the dark and examined using a confocal microscope (Zeiss LSM 700). Antibody specificity was verified using negative controls in which the primary antibody was excluded and replaced with antibody diluent; these controls showed no immunoreactivity.

GPR173 immunoreactive density was quantified using ImageJ software. Relative protein expression was quantified by measuring the mean fluorescence intensity (MFI) within a defined region of interest (ROI). For each image, the specific tissue area (ROI) was manually outlined using the selection tool. The MFI of the targeted ROI was measured, and the local background fluorescence intensity was obtained from a non-fluorescent area of the same section. To minimize sampling bias and ensure reproducibility, each animal measurement was consistently performed on four distinct regions obtained from 20× magnification images. Quantification was performed by two independent investigators, who were randomized and blinded to ensure an unbiased approach.

2.5. Statistical Analysis

For immunofluorescence quantification, GPR173 immunoreactive density was measured using ImageJ software. For each gland type, immunoreactivity values obtained from the left and right glands of the same animal were averaged to generate a single value per animal. Thus, the

individual animal, rather than each gland side, was considered the independent experimental unit.

Since the data did not show a normal distribution, differences in GPR173 immunoreactivity among the lacrimal, parotid, submandibular, and sublingual glands were analyzed using the non-parametric Friedman test. When a significant overall difference was detected, the Dunn-Bonferroni multiple-comparison test was applied to identify the source of the significant differences. To assess potential sex-related differences in GPR173 immunoreactivity, female rats (n = 5) and male rats (n = 3) were compared using the Mann-Whitney U test. The significance level was set at $\alpha = .05$. Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using R software (RStudio 2025.09.1, Room 1.7.32) and related packages.

3. Results

Expression levels of GPR173 in the lacrimal and major salivary glands (parotid, submandibular and sublingual) were first examined by Western blotting. The representative image of a nitrocellulose membrane acquired from Western blot demonstrated the expression of GPR173 and β-actin in the lacrimal gland and salivary glands of the parotid, submandibular, and sublingual tissues (Figure 1).

In the histological examination, the normal morphology of salivary and lacrimal glands was observed (Figure 2). The acinar and ductal cells showed acinar organization in all examined gland types. In the parotid and lacrimal glands, the parenchyma was composed predominantly of serous acinar cells, consistent with their protein-rich secretory profile. The submandibular gland exhibited a mixed morphology, containing both serous and mucous acini, reflecting its dual secretory function. In contrast, the sublingual gland was characterized mainly by mucous acinar cells, with only a minor contribution from serous elements, in line with its mucin-dominant secretion (Figure 2). To assess potential sex-related differences, GPR173 immunoreactivity was compared between female (n = 5) and male (n = 3) rats. No statistically significant difference was observed between the sexes ($p > .05$).

Figure 3 shows the immunofluorescence expression of GPR173 in the acinar and tubular duct cells of the parotid, submandibular, sublingual tissues. In the lacrimal gland, GPR173 expression was found only in acinar cells. The submandibular and parotid glands exhibited significantly higher immunoreactivity compared to the other glands. Stronger

immunopositivity was particularly evident in the ductal epithelium, whereas the acinar epithelium displayed lower positivity. The sublingual and lacrimal glands showed relatively weak immunopositivity. In addition, GPR173 immunoreactivity was observed in the periductal stromal connective tissue, most prominently in the parotid and lacrimal glands. In negative control staining; in which the primary antibody was excluded, and replaced with antibody diluent, no positive signals were observed in any of the glands studied.

Quantitative analysis showed that mean GPR173 immunoreactivity was highest in the submandibular gland, followed by the sublingual and parotid glands, whereas the lacrimal gland showed the lowest mean value.

The mean ± SD values were 12,544.4 ± 2,229.3 for the submandibular gland, 11,332.5 ± 3,274.4 for the sublingual gland, 11,064.8 ± 3,068.9 for the parotid gland, and 7,194.2 ± 1,595.6 for the lacrimal gland (Figure 4). The Friedman test revealed a significant overall difference in GPR173 immunoreactivity among gland types ($\chi^2(3) = 8.55$, $p = 0.0359$). GPR173 immunoreactivity was significantly lower in the lacrimal gland than in the parotid gland ($p = 0.0079$), submandibular gland ($p = 0.0079$), and sublingual gland ($p = 0.0347$). No statistically significant differences were observed among the three major salivary glands. Sex-related comparisons were insignificant between female and male rats in any gland (see Supplementary Figure 1). For the parotid gland, median values were 11,910.0 in females and 11,153.0 in males ($p = 1.000$). For the submandibular gland, median values were 13,721.0 in females and 13,195.0 in males ($p = 0.551$). For the sublingual gland, median values were 10,137.0 in females and 9,214.0 in males ($p = 0.766$). For the lacrimal gland, median values were 6,592.0 in females and 7,362.0 in males ($p = 1.000$).

4. Discussion

The present study identified the phoenixin receptor GPR173 in both the lacrimal and major salivary glands of the rat. In the salivary glands, receptor expression was observed in both acinar and ductal cells, while in the lacrimal gland it was restricted to the acinar cells. This differential localization suggests that GPR173 may have different functions in secretory and structural roles within exocrine tissues. The lack of ductal localization in the lacrimal gland may be attributed to its simpler ductal structure, which consists primarily of intercalated and excretory ducts (Conrady, Joos, & Patel, 2016). In contrast, salivary glands possess a highly

branched ductal system that includes intercalated, striated, interlobular, and main excretory ducts (Tucker, 2007). This intricate network plays an active role in altering saliva composition, especially through ion transport and pH regulation (Proctor & Carpenter, 2007). Although the lacrimal gland's ductal system is less complex, duct cells also contribute to the modification of tear secretion (Dartt, 2009). The structural differences between salivary and lacrimal glands may point to tissue-specific roles for the GPR173 receptor. Its presence in both acinar and ductal cells of the salivary glands suggest a broader regulatory function for GPR173.

The secretory activities of both the lacrimal (Dartt, 2009) and salivary glands are mainly controlled by the autonomic nervous system of parasympathetic and sympathetic branches (Proctor & Carpenter, 2007). The receptors of these two branches work synergistically at the intracellular level, namely the parasympathetic system via muscarinic type acetylcholine receptors and the sympathetic system via adrenergic receptors (Ekström et al., 2012). They are mainly responsible for both fluid and protein secretion as well as protein synthesis (Tucker, 2007). In addition, other receptors have been identified, including melatonin, cholecystokinin and gastrin in salivary (Aras & Ekström, 2006; Isola et al., 2013), and lacrimal glands (Rossiter, Hölzl, & Kerschbaum, 1993). GPR173 has been detected in central and peripheral tissues of humans, rats, mice, bovine, and zebrafish (Alarma-Estrany & Pintor, 2007; Yosten et al., 2013). It was initially implicated in regulating reproductive function by stimulating luteinizing hormone release from pituitary cells through increasing gonadotropin-releasing hormone levels (Stein et al., 2016). GPR173 is a highly conserved receptor with various roles in physiological processes, including neuroendocrine signaling and reproduction (Kras, Osiak-Wicha, & Arciszewski, 2025; Stein et al., 2016).

The tissue-specific distribution pattern observed for GPR173 in this study is consistent with existing evidence for other GPCRs in exocrine glands. Muscarinic acetylcholine receptor subtypes, for instance, display differential expression across acinar and ductal compartments depending on gland type (Proctor & Carpenter, 2007), and melatonin receptors show tissue-dependent localization in lacrimal and salivary glands (Alarma-Estrany & Pintor, 2007). The parallel between GPR173 and other well-characterized receptors supports the plausibility of tissue-specific GPR173 localization in exocrine glands and further supports the current findings.

The expression of GPR173 mRNA increases in brain regions with age (Kras, Osiak-Wicha, & Arciszewski, 2025; Stein et al., 2016). In females, GPR173 participates in the central regulation

of reproductive hormone secretion and cyclic ovarian function (Stein et al., 2016). The interaction of GPR173, together with its ligand phoenixin, may be influenced by hormonal fluctuations, and also with age related reproductive status (Haddock et al., 2020). Current evidence suggests that GPR173 together with its ligand phoneixin play roles in inflammatory regulation and may be involved in the pathogenesis of female-biased autoimmune disorders (Billert et al., 2020; Zhang, Zhang, & Wang, 2018). Moreover, GPR173 has been identified as a novel X-linked susceptibility gene for systemic lupus erythematosus, a disease with female predominance (Zhang, Zhang, & Wang, 2018). This underscores the need for further research to clarify the role of GPR173 in exocrine gland physiology and to determine its potential involvement in autoimmune exocrinopathies, including Sjögren's disease.

It should be acknowledged that the present study provides initial insights. Given the limited number of animals, the findings should be interpreted with caution and considered exploratory, indicating a need for future functional investigations. Although antibody specificity in the present study could not be confirmed using GPR173 knockout rat models, previous studies employing the same antibody as in the current work have demonstrated GPR173 expression in other tissues, including rat ovaries and periovarian adipose tissue (Kalamon et al., 2020), as well as in bovine gastrointestinal tissues (Kras, Osiak-Wicha, & Arciszewski, 2025). While receptor expression patterns often show strong similarities between rodents and humans, species-specific differences can be evident. This underscores the need for future studies to validate GPR173 expression in human lacrimal and salivary glands. Moreover, the present study focused exclusively on identifying the presence and distribution of GPR173 in rat exocrine glands, without assessing the functional role of the receptor. Future studies are warranted to investigate the functional role of GPR173 in human exocrine glands.

5. Conclusion

This study provides a spatial characterization of GPR173 in the rat major salivary and lacrimal glands. GPR173 displays distinct localization patterns in lacrimal and salivary glands, suggesting tissue-specific relevance and underscoring the need for further investigations in human exocrine glands.

Abbreviation explanation

GPR173, G-protein-coupled receptor 173; SREB3, Super conserved receptor expressed in brain 3; TBS-T, Tris-Buffered Saline with 0.05% Tween-20; NBT-BCIP, Nitro Blue Tetrazolium/ 5-Bromo-4-Chloro-3-Indolyl Phosphate; PBS, Phosphate-Buffered Saline; DAPI, 4′, 6-diamidino-2-phenylindole; H&E, hematoxylin-eosin; TBS-T, tris-buffered saline with Tween-20; ROI, Regions of interest.

CRediT authorship contribution statement

EBG and HCA designed the study, performed the animal surgeries, and drafted the manuscript. MAE carried out the immunofluorescence staining of the glands, IPE performed the western blot analyses, and SA conducted the histological evaluation of the glands. HCA reviewed and finalized the manuscript.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability statement

The raw data of the present study is available from the corresponding author upon request.

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Declaration of generative AI and AI-assisted technologies

During the preparation of this work the authors used AI, Microsoft Copilot for English language editing. After using this tool/service, the authors reviewed and edited the content as needed and they take full responsibility for the content of the published article.

FIGURE LEGENDS

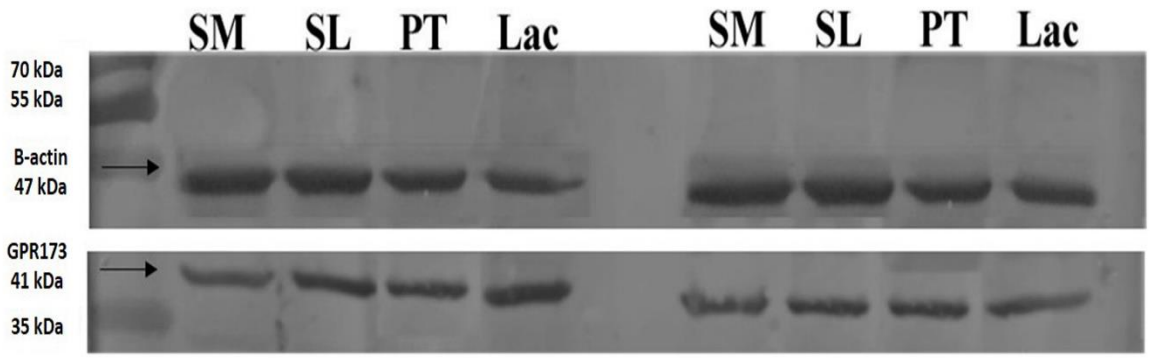

Figure 1. The expression of GPR173 and β-actin in the submandibular, sublingual, parotid, and lacrimal glands was shown by western blot. The same molecular bands for beta-actin and GPR173 were observed in lacrimal and all the major salivary glands. The experiments were repeated 2 times for validation.

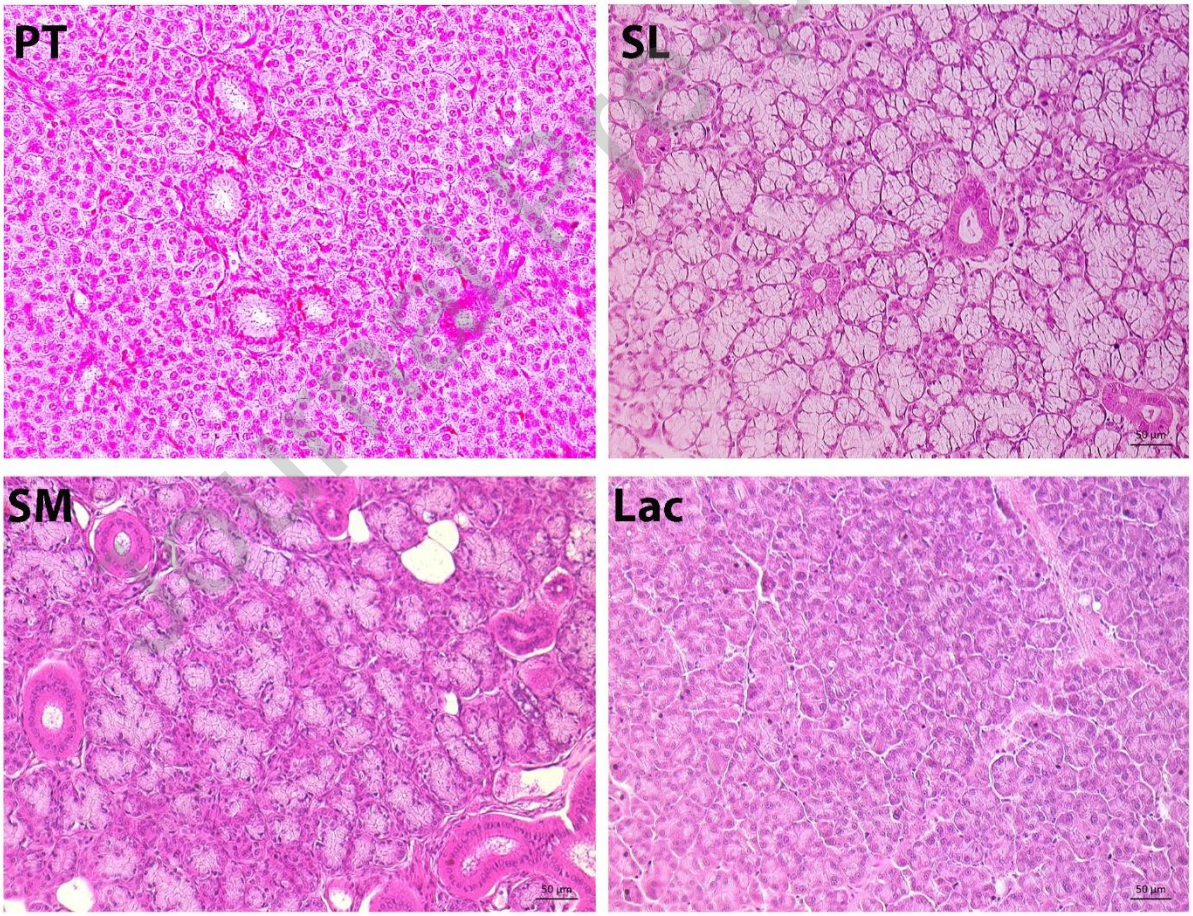

Figure 2. Representative photomicrographs of salivary and lacrimal glands. Normal morphology is observed in the parotid (PT), submandibular (SM), and sublingual (SL) salivary

glands, as well as in the lacrimal gland (Lac). (Hematoxylin and Eosin staining, magnification: ×20)

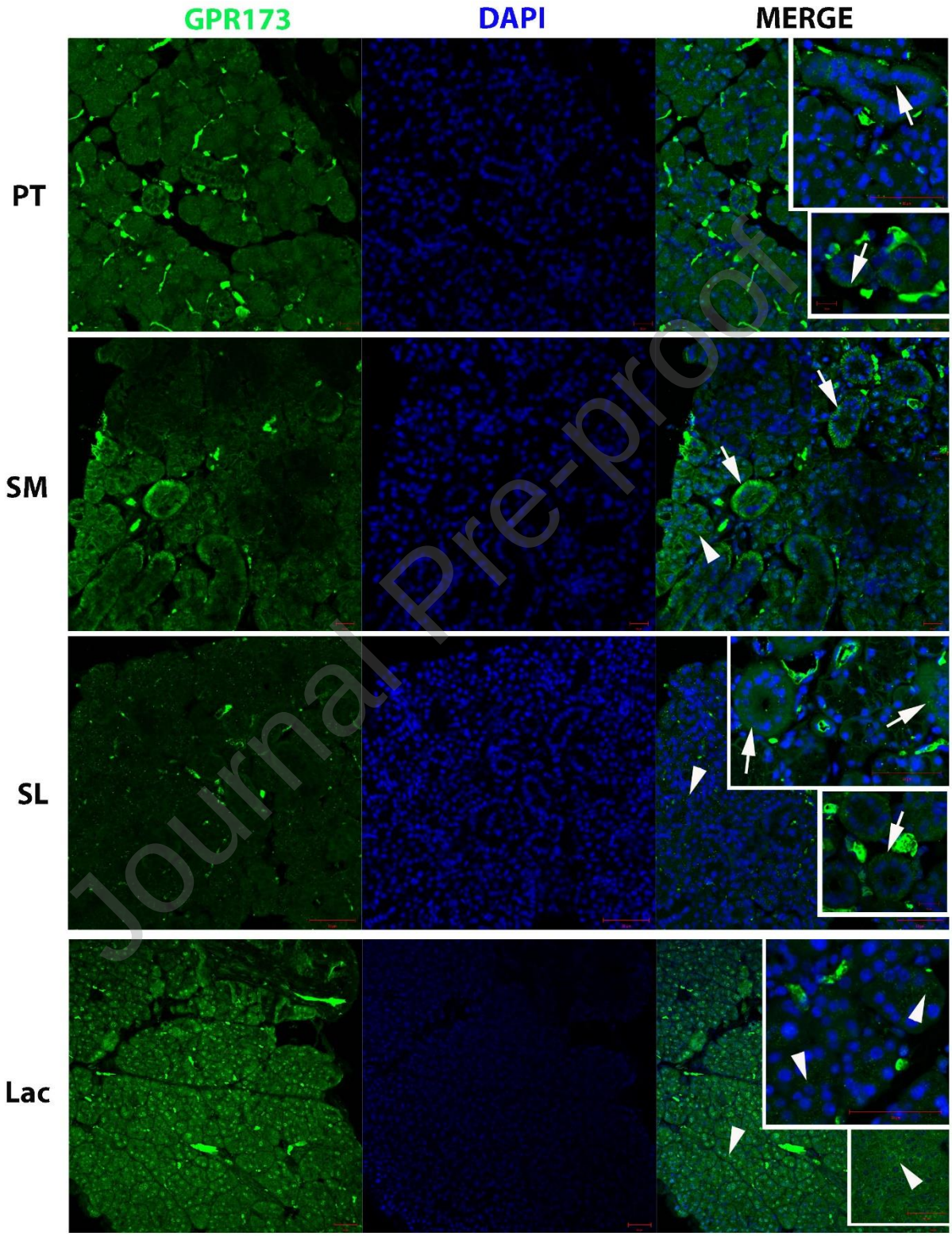

Figure 3. Representative micrographs showing GPR173 immunofluorescence in salivary and lacrimal glands. GPR173 immunopositivity appears in bright green, while nuclei are counterstained with DAPI (blue). In the parotid gland, intralobular intercalated ducts (upper inset, arrow) and striated ducts (lower inset, arrow) exhibited immunopositivity. In the submandibular gland, striated ducts (main image at low magnification and lower inset at higher magnification) and acinar cells (arrowhead) showed immunopositivity. In the sublingual gland, intralobular intercalated ducts (upper inset), striated ducts (lower inset, arrow), and acinar cells (arrowhead) demonstrated immunopositivity. In the lacrimal gland, only acinar cells (arrowhead) exhibited immunopositivity. PT: parotid gland, SM: submandibular gland, SL: sublingual gland, Lac: lacrimal gland. Micrographs were acquired at 20× optical magnification, and the inserts were subsequently digitally zoomed to enhance visualization.

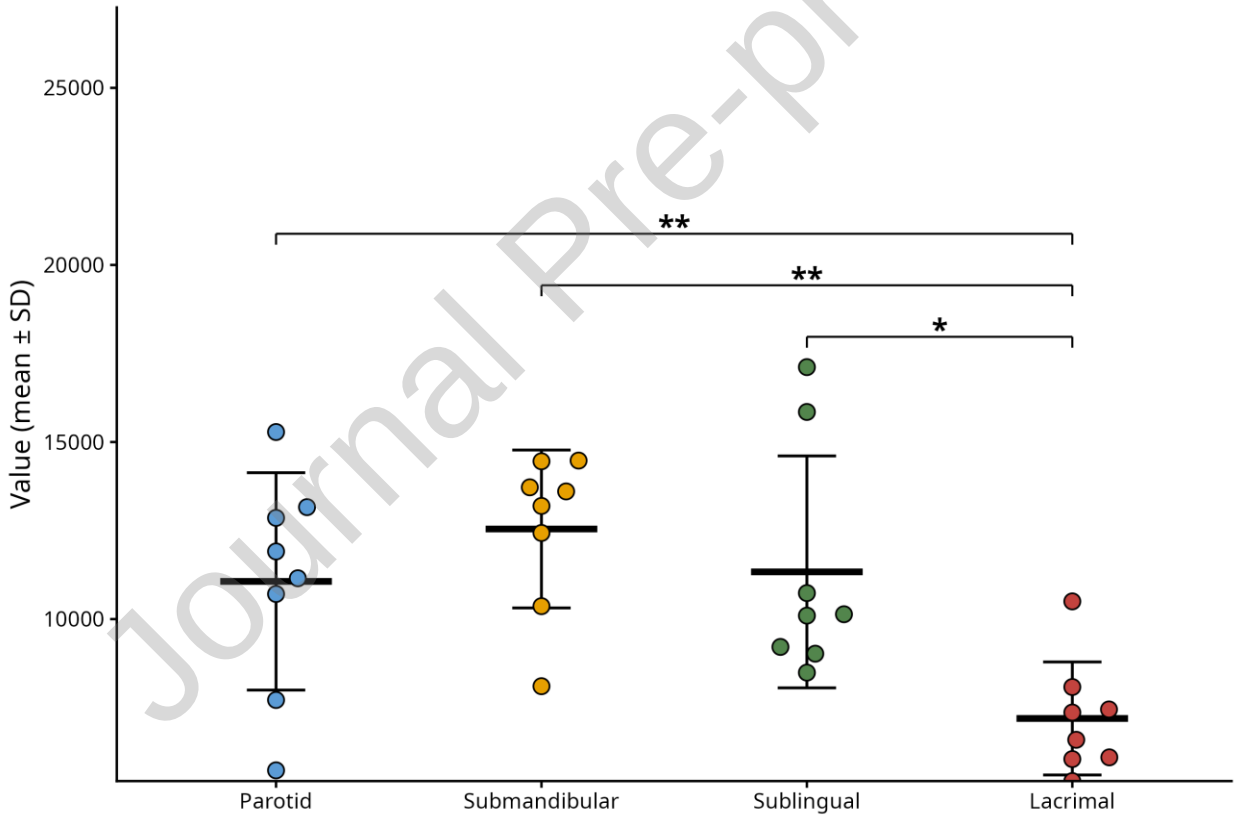

Figure 4. GPR173 immunoreactivity in lacrimal and major salivary glands. Quantitative analysis of GPR173 immunoreactive density in the parotid, submandibular, sublingual, and lacrimal glands. Data are shown as mean ± SD. Statistical analysis was performed using the Friedman test followed by Conover all-pairs post-hoc comparisons. The lacrimal gland showed significantly lower GPR173 immunoreactivity than the parotid, submandibular, and sublingual glands. *$p < 0.05$, **$p < 0.01$.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

- GPR173 was expressed in both acinar and ductal cells of all major salivary glands
- In the lacrimal gland, GPR173 expression was restricted to acinar cells
- The functional role of GPR173 in exocrine regulation requires further study
- GPR173 is a promising target for studies on exocrine dysfunction with autoimmune origin