Received: 14 May 2021 | Revised: 8 December 2021 | Accepted: 25 January 2022

DOI: 10.1111/jop.13290

Journal of Oral Pathology & Medicine WILEY

ORIGINAL ARTICLE

Immunohistochemical analysis of pyroptosis-related protein expression in IgG4-related sialadenitis

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Abstract

Background: NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3)-induced pyroptosis is involved in the development of a variety of autoimmune diseases, but its role in IgG4-related sialadenitis (IgG4-RS) is unclear.

Methods: Salivary gland tissues from 19 patients with IgG4-RS were designated the experimental group, and peritumoral tissues from 20 patients with benign salivary gland tumours were designated the control group. The cell morphology and fibrosis in the IgG4-RS samples were observed by haematoxylin-eosin (H&E) and Masson trichrome (MT) staining. Immunohistochemical (IHC) staining was used to determine pyroptosis-related proteins (NLRP3, ASC (apoptosis-associated speck-like protein containing a CARD), Caspase-1, GSDMD (gasdermin family members, including digestive dermatin D), interleukin 1β (IL-1β), and interleukin 18 (IL-18)) expression levels.

Results: Increased lymphoid follicle proliferation, germinal centre plasma cell infiltration, and irregular fibrosis were observed in the experimental group compared with the control group. The NLRP3, ASC, Caspase-1, GSDMD, IL-1β, and IL-18 levels were significantly higher in the experimental group than in the control group ($p < 0.0001$).

Conclusion: This study suggested that pyroptosis-related proteins might be involved in IgG4-RS pathogenesis. However, the specific cellular pathway involved and whether multiple cell death pathways contribute to the occurrence of IgG4-RS still need to be further studied.

KEYWORDS
IgG4-related diseases, IgG4-related sialadenitis, inflammasomes, pyroptosis

1 | INTRODUCTION

IgG4-related sialadenitis (IgG4-RS), a kind of IgG4-related disease (IgG4-RD), is mainly characterized by the painless enlargement of one or more salivary glands. The submandibular gland is the organ that is most commonly affected, whereas the parotid gland, sublingual gland, and other minor salivary glands are less often affected. The affected sites are usually, but not always, bilateral, and the course of disease usually lasts longer than 3 months.[1] IgG4-RS may often be confused with Sjogren's syndrome (SS) and sometimes with other rare conditions, such as Kimura disease and Castleman's disease.[2–4] Glucocorticoids (GCs) and biological agents are effective treatments, but relapses may occur.[5]

According to research reports, the interaction between innate immunity and acquired immunity seems to be involved in controlling the abnormal pathogenesis of IgG4-RD.[6,7] Therefore, elucidating the

innate immune response related to IgG4-RD is helpful to explore treatment options for this immune disease. In autoimmune diseases, the formation of inflammasomes plays an important role in the response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), and the assembly of inflammasomes, such as the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome, results in multiprotein complexes. Assembly of the NLRP3 inflammasome can result in the activation of Caspase-1 and promote the maturation and release of the inflammatory cytokines interleukin 1β (IL-1β) and interleukin 18 (IL-18), which in turn leads to pyroptosis. The typical characteristics of pyroptosis are the rupture of the plasma membrane and the release of pro-inflammatory substances from cells.[8] Several studies have shown that NLRP3 inflammasome-induced pyroptosis is closely related to a variety of autoimmune diseases, such as SS, systemic lupus erythematosus, and rheumatoid arthritis.[9–11] However, research on IgG4-RS is limited to the involvement of pro-inflammatory cytokines, mainly the IL-1 family members IL-1β and IL-18.[12,13] Therefore, the purpose of this study was to explore the expression of pyroptosis-related proteins in IgG4-RS.

2 | MATERIALS AND METHODS

This study was approved by the Institutional Review Board of the First Affiliated Hospital of Xinjiang Medical University before the study began (K202012-14).

2.1 | Patients and samples

This study examined 39 formalin-fixed paraffin-embedded tissue blocks retrieved from the Oncological Department of Oral & Maxillofacial Surgery and the Department of Pathology at the First Affiliated Hospital of Xinjiang Medical University from 2015 to 2020, and these tissues were divided into the experimental and control groups. The experimental samples clearly included tumour tissues (including submandibular glands (n = 17) and parotid glands (n = 2)). Among these samples, five specimens were obtained by needle puncture biopsy, and the rest were obtained by surgical resection. Paratumoral tissues of benign tumours of salivary glands were included in the control group (n = 20). The inclusion criteria included the following: (i) all patients met the comprehensive diagnostic criteria for IgG4-RD[14,15] and (ii) the patient's data were relatively complete. The exclusion criteria included the following: (i) patients with symptoms similar to those of IgG4-RS but not diagnosed with IgG4-RS; (ii) patients with other infectious diseases, rheumatism, and malignant tumours; and (iii) patients who had used immunosuppressants. According to the comprehensive diagnostic criteria for IgG4-RD,[14,15] 7 (36.84%), 2 (10.53%), and 10 (52.63%) of these 19 patients were diagnosed with definite, probable, and possible IgG4-RD, respectively. There were 10 males and 9 females, with an average age of 60.00 ± 9.93 years. The duration of symptoms before diagnosis was 12 (range 1–120) months.

TABLE 1 Baseline features of 19 patients with IgG4-related sialadenitis (IgG4-RS)

Sex, *n* (%)	
Male	10 (52.63)
Female	9 (47.37)
Age at diagnosis, mean ± SD, years	60.00 ± 9.93
Comprehensive diagnostic criteria, *n* (%)	
Definite	7 (36.84)
Probable	2 (10.53)
Possible	10 (52.63)
Disease duration, median (IQR), months	12 (2–24)
Follow-up time, median (IQR), months	22 (6–40)
No. of organs involved, mean ± SD	3.74 ± 1.48
Main organ involvement, *n* (%)	
Submandibular gland	19 (100)
Lacrimal gland	13 (68.42)
Lymph nodes	12 (63.16)
Sinuses	7 (36.83)
Parotid	4 (21.05)
Treated with glucocorticoids, *n* (%)	15 (78.95)
Not receiving treatment, *n* (%)	4 (21.05)

Abbreviation: IQR, interquartile range.

Submandibular gland swelling was the main symptom (78.95%), followed by parotid gland swelling (10.53%), cough (5.26%), and chest tightness (5.26%). All the patients had two or more organs affected, and the median number of affected organs was 3 (range 2–6); the affected organs included the submandibular gland (100%), lacrimal gland (68.42%), lymph nodes (63.16%), sinuses (36.84%), and some other organs, such as the liver, gallbladder, and lung. Fifteen patients (78.95%) were treated with glucocorticoids (GCs). The median follow-up time was 22 (range 4–58) months, and the cumulative recurrence rate was 40%. The main manifestations were re-enlargement of lacrimal glands and salivary glands or involvement of other organs. Four other patients did not take GCs for personal reasons, three patients failed to follow up, and one patient died (Table 1).

2.2 | Haematoxylin and eosin (H&E) staining

H&E staining of IgG4-RS tissues was carried out to evaluate morphological changes.

2.3 | Masson trichrome (MT) staining

MT staining was performed to examine fibrotic IgG4-RS tissues. Connective and fibrotic tissues were selectively stained blue, nuclei were stained with Weigert's iron haematoxylin and appeared dark brown to black, and the cytoplasm was stained red.

2.4 | Immunohistochemical (IHC) staining analysis

IHC staining was performed on all the specimens to analyse the expression levels of NLRP3, Caspase-1, ASC (apoptosis-associated speck-like protein containing a CARD), GSDMD (gasdermin family members, including digestive dermatin D), IL-1β, and IL-18. The antibodies used included anti-IL-1β (1:200 dilution, catalogue #ab2105; Abcam), anti-NLRP3 (1:200 dilution, catalogue #ab214185; Abcam), anti-ASC (1:200 dilution catalogue #10500-1-AP; Proteintech), anti-Caspase-1 (1:100 dilution, catalogue #ab62698; Abcam), anti-GSDMD (1:50 dilution, catalogue #DF12275; Affinity Biosciences), and anti-IL-18 (1:200 dilution, catalogue #ab68435; Abcam). Primary antibodies against NLRP3, ASC, Caspase-1, GSDMD, IL-1β, and IL-18 were incubated at 4°C overnight. The sections were rinsed three times with phosphate-buffered saline (PBS) and allowed to react with a secondary antibody (Zsbio) for 30 min at room temperature. Colorimetric detection was completed with 3,3′-diaminobenzidine (Zsbio), and the slides were counterstained with haematoxylin. Negative controls were used for each staining group, and PBS was used in place of a primary antibody to establish the negative control.

Images of five different high-power fields (400×) were captured from areas positive for the immunoreactive lymphocytes, and the images were evaluated by two pathologists who had no knowledge of the clinicopathological outcomes. Cells stained brown suggested positivity, and cells not stained brown suggested negative staining. All the images were analysed using Image-Pro Plus software (V.6.0, Media Cybernetics, LP). The positive results were assessed by semiquantitative scoring. The immunohistochemical analysis of pyroptosis-related protein expression in the cytoplasm of salivary glands affected by IgG4-RS was performed using an immunoreactive score (IRS),[16] and the ratio of the number of positive cells to the total number of cells counted was used to quantify the staining.[17] The intensity of expression was scored as follows: 1, weak; 2, moderate; and 3, strong. The criteria for grading the percentage scores were as follows: 0, 0% of cells were positive; 1, 1%–10% of cells were positive; 2, 11%–30% of cells were positive; 3, 31%–50% of cells were positive; 4, 51%–80% of cells were positive; and 5, >80% of cells were positive. The IRS was obtained by multiplying the percentage and intensity subscores.

2.5 | Statistical analysis

All the statistical tests were performed using SPSS 26 and GraphPad 8.0 software. The data that conformed to a normal distribution are expressed as the mean ± standard deviation, and the data that did not conform to a normal distribution are expressed as the median (interquartile range). Count data are expressed as a percentage. For IHC, the Mann–Whitney test was performed to compare different groups. Differences were considered statistically significant when $p < 0.05$.

3 | RESULTS

HE staining showed that there were different degrees of inflammatory cell infiltration, and lymphoid tissue hyperplasia, mainly follicular hyperplasia, different degrees of follicular enlargement, scattered plasma cell infiltration, and fibrous tissue proliferation were observed in the germinal centres in the experimental group. However, there was no lymphoid follicle formation in the control group. MT staining mainly showed storiform fibrosis with extensive ectopic germinal centre formation in the experimental group, while the control group exhibited fibrosis only around the ducts and connective tissue. Compared with that in the normal tissue, the content of collagen fibres in the experimental group tissues increased abnormally, and the staining became deeper.

IHC staining showed that pyroptosis-related proteins (NLRP3, ASC, Caspase-1, IL-18, IL-1β, and GSDMD) were mainly distributed in the cytoplasm of salivary gland epithelial cells, cytoplasm of inflammatory cells, and ducts in the IgG4-RS samples. NLRP3, ASC, Caspase-1, IL-18, IL-1β, and GSDMD staining was positive (Figure 1A–F). Except for part of the catheter area, no brown particles were observed in the control group (Figure 1A–F). In this study, a negative control group was used for each staining, but no positive staining was observed in the negative control group (data not shown), which confirms the accuracy of the IHC staining results. The difference between the IgG4-RS group and the control group was significant (Figure 2A-F, $p < 0.0001$).

The relationships between the presence of factors and the high expression of pyroptosis-related proteins (NLRP3, ASC, Caspase-1, GSDMD, IL-1β, and IL-18) were analysed. All of the results revealed a significant p-value ($p < 0.05$), except for the relationship between GSDMD and IL-1β ($p > 0.05$) (Table 2).

4 | DISCUSSION

IgG4-RS, known as Küttner's tumour, is a chronic fibro-inflammatory salivary gland disease. It mostly occurs in middle-aged and elderly men. In a study by Wang et al, the age of onset of IgG4-RD was 55.2 ± 13.9 years old, and the male:female ratio was 1.56:1, similar to the results in this report.[18] IgG4-RD is a fibro-inflammatory disease that can affect almost any organ and is often accompanied by eosinophilia and elevated serum immunoglobulin E (IgE) and immunoglobulin G4 (IgG4) levels.[19] The pathological features of IgG4-RD are typical fibrotic histological changes.[19] In this study, the pathological manifestation of storiform fibrosis could be seen in more than half of the patients, while the normal tissue exhibited fibrosis only around the duct and connective tissue, and there was no obvious fibrosis in fine needle aspirations, which was consistent with the study by Kamisawa et al.[20] and might be related to the volume and location of puncture tissue. However, pyroptosis was morphologically indistinguishable from other forms of cell injury, further validating the presence of pyroptosis in IgG4-RD. We examined the key molecules involved in pyroptosis.

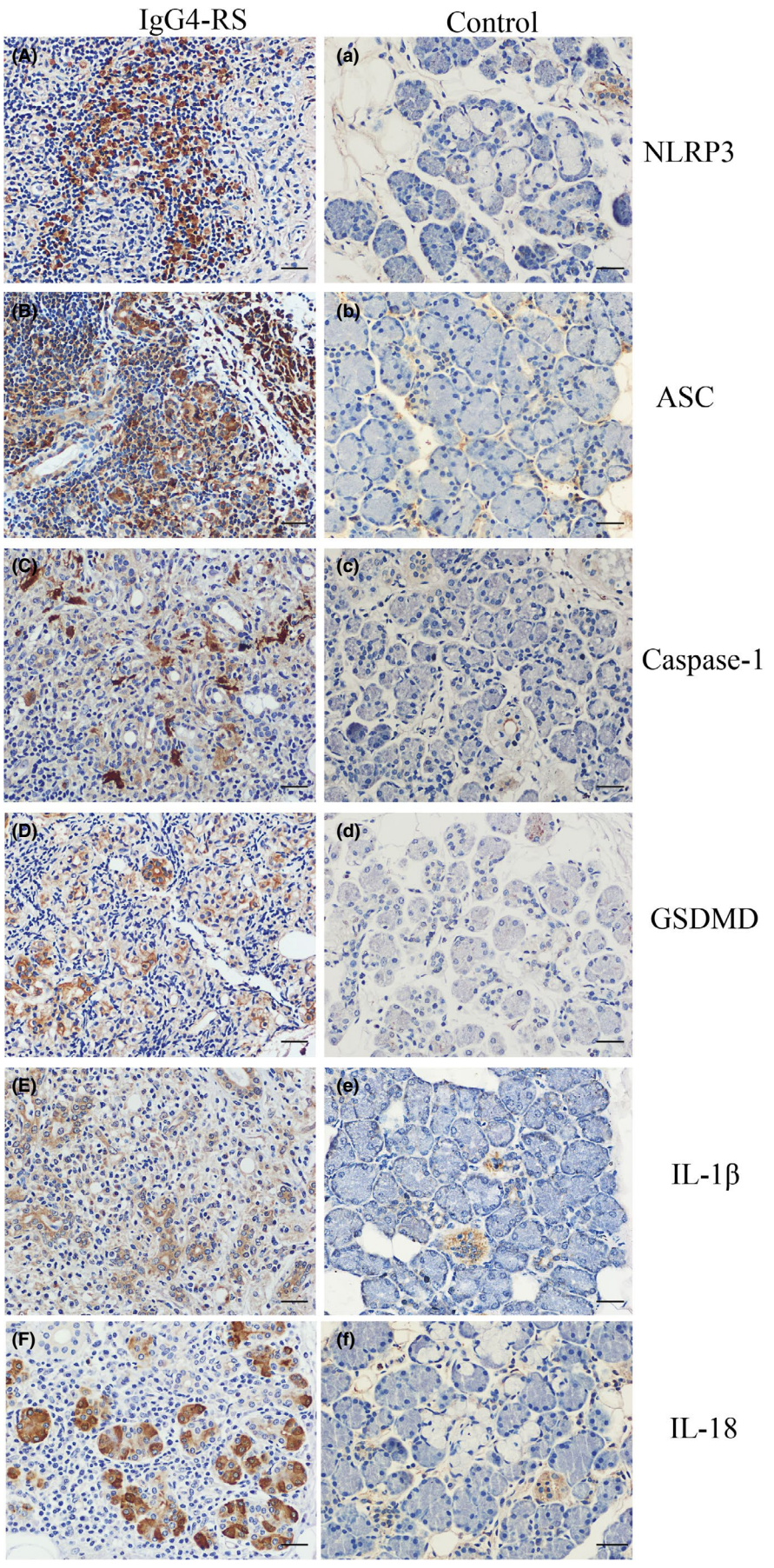

FIGURE 1 Immunohistochemical (IHC) staining of pyroptosis-related proteins in IgG4-related sialadenitis (IgG4-RS) tissues and control tissues (400×). IHC staining of IgG4-RS tissues: (A) NLRP3 (NOD-, LRR- and pyrin domain-containing protein); (B) ASC (apoptosis-associated speck-like protein containing a CARD); (C) Caspase-1; (D) GSDMD (gasdermin family members, including digestive dermatin D; (E) interleukin 1β (IL-1β); (F) interleukin 18 (IL-18). IHC staining of the control tissues: (a) NLRP3; (b) ASC; (c) Caspase-1; (d) GSDMD; (e) IL-1β; (f) IL-18. Scale bars, 100 μm

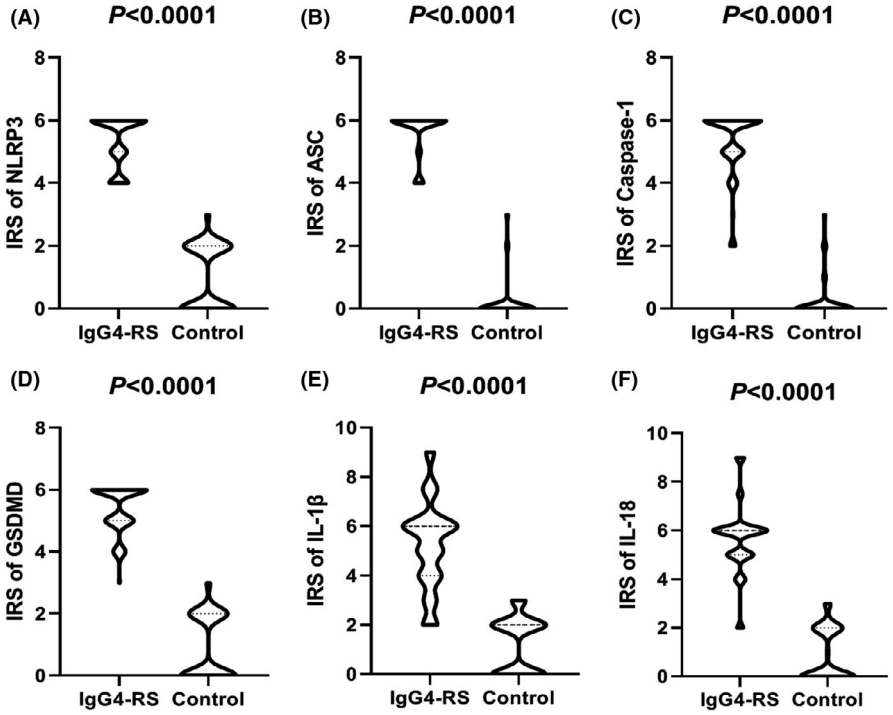

FIGURE 2 Expression of pyroptosis-related proteins in IgG4-related sialadenitis (IgG4-RS) tissues and control tissues. (A–F) The NLRP3 (NOD-, LRR- and pyrin domain-containing protein), ASC (apoptosis-associated speck-like protein containing a CARD), Caspase-1, GSDMD (gasdermin family members, including digestive dermatin D), interleukin 1β (IL-1β), and interleukin 18 (IL-18) levels were significantly higher in the experimental group than in the control group ($p < 0.0001$)

	NLRP3	ASC	Caspase-1	GSDMD	IL-1β	IL-18
NLRP3	1					
ASC	0.731**	1				
Caspase-1	0.641**	0.635**	1			
GSDMD	0.618**	0.640**	0.827**	1		
IL-1β	0.725**	0.644**	0.602**	0.452	1	
IL-18	0.760**	0.644**	0.626**	0.549*	0.714**	1

TABLE 2 The correlations among the pyroptosis-related protein levels in IgG4-related sialadenitis (IgG4-RS) tissues

**The correlation was significant at the 0.01 level (double tail). *The correlation was significant at the 0.05 level (double tail).

As a systemic autoimmune inflammatory disease, the aetiology and molecular mechanism underlying IgG4-RD include many factors, such as heredity, infection, and autoimmune reaction. Among these factors, the role of inflammation-related immune molecules has been widely studied.[21] Pyroptosis is an important immune response of the body, and moderate pyroptosis clears pathogenic microbes, stimulates the adaptive immune response, and enhances host survival, while excessive pyroptosis leads to the occurrence of body diseases.[22] Currently, we found that pyroptosis is widely involved in the occurrence and development of tumours, infectious diseases, metabolic diseases, neurological diseases, and so on. Pyroptosis mainly mediates the activation of Caspase family members (mainly Caspase-1/3/8/4/5/11) through inflammasomes, thereby activating a variety of gasdermin family members, including digestive dermatin D (GSDMD), which causes cell perforation and subsequently cell death.[23]

It has been confirmed in other autoimmune diseases that high levels of NLRP3 inflammatory corpuscles can be seen in the peripheral blood of patients with rheumatoid arthritis.[11] Moreover, in a mouse model of lupus, a P2X7 receptor blocker blocked the NLRP3-ASC-Caspase-1 signalling pathway and reduced the occurrence of pyroptosis in mice with lupus.[10] NLRP3 inflammasome activation and pyroptosis are observed in salivary gland-infiltrating macrophages from patients with Sjogren's syndrome (SS). Hong et al.[9] pointed out that type I interferon (IFN) could increase the NLRP3, ASC, Caspase-1, GSDMD, IL-1β, and IL-18 levels in the salivary glands of patients with primary Sjogren syndrome (SS) and consequently induce pyroptosis. Regardless of the immunohistochemical assessment method used in our study, the results showed that the expression of NLRP3, Caspase-1, ASC, GSDMD, and other molecules closely related to pyroptosis was significantly higher in the experimental group than in the control group, and these molecules were mainly expressed in the salivary gland epithelial cytoplasm, inflammatory cell cytoplasm, and ducts, which indicates that NLRP3, Caspase-1, ASC, and GSDMD are likely to participate in the pathogenesis of IgG4-RS by inducing pyroptotic cell death. It is common knowledge that the pathway by which GSDMD is involved in pyroptosis can be divided into classical pathways and nonclassical pathways.[8] In the classical pathway, cells recognize PAMPs and DAMPs to activate inflammasomes and then activate Caspase-1.[24]

In the nonclassical pathway, Caspase-4/5/11 directly recognizes cytoplasmic lipopolysaccharides through caspase activation and recruitment domains (CARD).[22] However, the terminal links of the above two pathways need to induce the activation of pro-IL-1β and pro-IL-18, resulting in pyroptosis.[22] Previous study by Mattoo confirmed that IL-1β may be an important cause of chronic inflammation and significant fibrosis in IgG4-RD,[25] and Komori et al confirmed that IL-18 is related to the pathogenesis of atypical inflammation in IgG4-RD, stimulating T helper type 1 (Th1) cells to produce T helper type 2 (Th2) cytokines and enhancing the immune reaction of Th2 cytokines in the pathogenesis of IgG4-RD.[25] Similar to these results, we found that IL-1β and IL-18 were highly expressed in IgG4-RS tissues. To further investigate the role of pyroptosis-related protein components, we also evaluated the expression of ASC, Caspase-1, GSDMD, IL-1β, and IL-18. We found an expression pattern similar to that of NLRP3 and confirmed that there was a positive correlation between NLRP3, ASC, Caspase-1, GSDMD, IL-1β, and IL-18. Our results were similar to those of Xue et al.,[26] who indicated that the NLRP3 protein was closely related to inflammasome components. Therefore, by observing the expression of pyroptosis-related proteins in IgG4-RS, we hypothesized that pyroptosis might be involved in the development of IgG4-RS.

However, there are some limitations in this study: (1) the clinical sample size in this study was limited, so the correlation between each indicator of pyroptosis and clinical variables was not determined; (2) this study only preliminarily explored the overall relationship between pyroptosis-related proteins and IgG4-RS in clinical samples, but it is still unknown what specific cellular pathway is involved and whether multiple pyroptotic pathways are jointly involved in the development of IgG4-RS. Therefore, by designing more targeted in vitro and animal experiments to monitor dynamic changes in the gene expression of key molecules related to pyroptosis, our future research will determine the potential pyroptosis-related pathways and will determine patterns of up- and downstream specific molecular regulation.

5 | CONCLUSION

In conclusion, the expression of NLRP3, Caspase-1, and other pyroptosis-related proteins was successfully detected in IgG4-RS, which suggested that pyroptosis-related proteins might be involved in IgG4-RS pathogenesis.

ACKNOWLEDGEMENTS

The author thanks all the patients who provided samples. We thank the Oncological Department of Oral & Maxillofacial Surgery, the First Affiliated Hospital of Xinjiang, and the Uygur Autonomous Region Institute of Stomatology for their help and technical guidance.

CONFLICT OF INTEREST

The authors declare that no potential conflict of interest exists with respect to the research, authorship, and/or publication of this article.

AUTHOR CONTRIBUTIONS

Jiao Pu: Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft; Writing – review & editing. **Mengying Jia:** Conceptualization; Data curation; Formal analysis; Validation; Writing – review & editing. **Wei Shi:** Conceptualization; Formal analysis; Resources; Software; Validation. **Lulu Hu:** Conceptualization; Data curation; Project administration; Resources; Supervision. **Fang Wang:** Conceptualization; Data curation; Investigation; Methodology; Validation. **Yaqi Niu:** Conceptualization; Formal analysis; Investigation; Methodology; Resources. **Qiaoying Tong:** Conceptualization; Investigation; Methodology; Validation. **Zhongcheng Gong:** Conceptualization; Data curation; Funding acquisition; Methodology; Resources; Supervision.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the author upon reasonable request.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/jop.13290.

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REFERENCES

1. Puxeddu I, Capecchi R, Carta F, et al. Salivary gland pathology in IgG4-related disease: a comprehensive review. *J Immunol Res*. 2018;2018:6936727.
2. Tsuboi H, Honda F, Takahashi H, et al. Pathogenesis of IgG4-related disease. Comparison with Sjögren's syndrome. *Mod Rheumatol*. 2020;30:7-16.
3. Kottler D, Barète S, Quéreux G, et al. Retrospective multicentric study of 25 Kimura disease patients: emphasis on therapeutics and shared features with cutaneous IgG4-related disease. *Dermatology*. 2015;231:367-377.
4. Zhang X, Zhang P, Peng L, et al. Clinical characteristics of a concurrent condition of IgG4-RD and Castleman's disease. *Clin Rheumatol*. 2018;37:3387-3395.
5. Lee HW, Moon S-H, Kim M-H, et al. Relapse rate and predictors of relapse in a large single center cohort of type 1 autoimmune pancreatitis: long-term follow-up results after steroid therapy with short-duration maintenance treatment. *J Gastroenterol*. 2018;53:967-977.
6. Liu C, Zhang P, Zhang W. Immunological mechanism of IgG4-related disease. *J Transl Autoimmun*. 2020;3:100047.
7. Watanabe T, Minaga K, Kamata K, et al. Mechanistic insights into autoimmune pancreatitis and IgG4-related disease. *Trends Immunol*. 2018;39:874-889.
8. Gong W, Shi Y, Ren J. Research progresses of molecular mechanism of pyroptosis and its related diseases. *Immunobiology*. 2020;225:151884.
9. Hong S-M, Lee J, Jang SG, et al. Type I interferon increases inflammasomes associated pyroptosis in the salivary glands of patients with primary Sjögren's syndrome. *Immune Netw*. 2020;20:e39.
10. Ma Z-Z, Sun H-S, Lv J-C, et al. Expression and clinical significance of the NEK7-NLRP3 inflammasome signaling pathway in patients with systemic lupus erythematosus. *J Inflamm*. 2018;15:16.
11. Mathews RJ, Robinson JI, Battellino M, et al. Evidence of NLRP3-inflammasome activation in rheumatoid arthritis (RA), genetic

variants within the NLRP3-inflammasome complex in relation to susceptibility to RA and response to anti-TNF treatment. *Ann Rheum Dis*. 2014;73:1202-1210.
12. Komori T, Kondo S, Wakisaka N, et al. IL-18 is highly expressed in inflammatory infiltrates of submandibular glands in patients with immunoglobulin G4-related disease. *Hum Pathol*. 2015;46:1850-1858.
13. Capecchi R, Italiani P, Puxeddu I, et al. IL-1 family cytokines and receptors in IgG4-related disease. *Cytokine*. 2018;102:145-148.
14. Umehara H, Okazaki K, Masaki Y, et al. Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. *Mod Rheumatol*. 2012;22:21-30.
15. Umehara H, Okazaki K, Kawa S, et al. Revised comprehensive diagnostic (RCD) criteria for IgG4-RD. *Mod Rheumatol*. 2020;2020:1-14.
16. Sánchez-Romero C, Carreón-Burciaga R, Gónzalez-Gónzalez R, et al. Perilipin 1 and adipophilin immunoexpression suggests the presence of lipid droplets in tooth germ, ameloblastoma, and ameloblastic carcinoma. *J Oral Pathol Med*. 2021;50:708-715.
17. Cardoso CM, de Jesus SF, de Souza MG, et al. High levels of ANXA2 are characteristic of malignant salivary gland tumors. *J Oral Pathol Med*. 2019;48:929-934.
18. Wang L, Zhang P, Zhang X, et al. Sex disparities in clinical characteristics and prognosis of immunoglobulin G4-related disease: a prospective study of 403 patients. *Rheumatology*. 2019;58:820-830.
19. Chen LYC, Mattman A, Seidman MA, et al. IgG4-related disease: what a hematologist needs to know. *Haematologica*. 2019;104:444-455.
20. Kamisawa T, Zen Y, Pillai S, et al. IgG4-related disease. *Lancet*. 2015;385:1460-1471.
21. Li Y, Huang H, Liu B, et al. Inflammasomes as therapeutic targets in human diseases. *Signal Transduct Target Ther*. 2021;6:247.
22. Lu F, Lan Z, Xin Z, et al. Emerging insights into molecular mechanisms underlying pyroptosis and functions of inflammasomes in diseases. *J Cell Physiol*. 2020;235:3207-3221.
23. Shi J, Gao W, Shao F. Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends Biochem Sci*. 2017;42:245-254.
24. Ball DP, Taabazuing CY, Griswold AR, et al. Caspase-1 interdomain linker cleavage is required for pyroptosis. *Life Sci Alliance*. 2020;3:e202000664.
25. Mattoo H, Mahajan VS, Maehara T. Clonal expansion of CD4(+) cytotoxic T lymphocytes in patients with IgG4-related disease. *J Allergy Clin Immunol*. 2016;138:825-838.
26. Xue YI, Du H-D, Tang DI, et al. Correlation between the NLRP3 inflammasome and the prognosis of patients with LSCC. *Front Oncol*. 2019;9:588-599.

How to cite this article: Pu J, Jia M, Shi W, et al. Immunohistochemical analysis of pyroptosis-related protein expression in IgG4-related sialadenitis. *J Oral Pathol Med*. 2022;51:388–394. doi:10.1111/jop.13290