Ann Rheum Dis 000 (2026) 1–10

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Original Research

Functional insights into autoantibodies targeting muscarinic acetylcholine receptors in Sjögren's disease

Fiona Engelke[1,*], Harald Heidecke[2], Kai Schulze-Forster[2], Heike Bähre[3], Yasmin Liß[3], Tina Hagedorn[3], Thomas Dörner[4], Diana Ernst[1], Jan Gras[5], Luca Quartuccio[6], Jacob Ritter[4], Benjamin Seeliger[5,7], Detlef Neumann[3], Torsten Witte[1]

[1] *Department of Rheumatology and Clinical Immunology, Hannover Medical School, Hannover, Germany*
[2] *CellTrend GmbH, Luckenwalde, Germany*
[3] *Institute of Pharmacology, Hannover Medical School, Hannover, Germany*
[4] *Rheumatology and Clinical Immunology, Charité – Universitätsmedizin Berlin, Berlin, Germany*
[5] *Department of Respiratory Medicine, Hannover Medical School, Hannover, Germany*
[6] *Rheumatology Division, Department of Medicine, Azienda Sanitaria Universitaria del Friuli Centrale, University of Udine, Udine, Italy*
[7] *German Center for Lung Research (DZL), Biomedical Research in End-Stage and Obstructive Lung Disease Hannover (BREATH), Hannover, Germany*

ARTICLE INFO

Article history:
Received 18 August 2025
Received in revised form 17 June 2026
Accepted 17 June 2026

ABSTRACT

Objectives: G protein-coupled receptors (GPCRs) participate in various pathophysiological processes in Sjögren's disease (SjD); in particular, autoantibodies against muscarinic acetylcholine receptor 3 (M_3R) inhibit the secretion of saliva and tears by exocrine glands. We aimed to identify autoantibodies targeting other muscarinic receptors in SjD and assess their potential functional relevance to signalling pathways.

Methods: Autoantibodies against all muscarinic acetylcholine receptor subtypes were investigated in the serum of patients with SjD (n = 347) using enzyme-linked immunosorbent assays (ELISAs). Healthy controls (HCs; n = 50), patients with non-Sjögren's sicca syndrome (NSS; n = 44), and patients with other autoimmune diseases (AIDs; n = 67) served as controls. To analyse the functional role of muscarinic receptor autoantibodies against muscarinic acetylcholine receptors 1 to 3 and 5 (M_1R, M_2R, M_3R, and M_5R, respectively), purified immunoglobulin (Ig) G fractions from patients with SjD (n = 10) and HCs (n = 10) were examined in Chinese hamster ovary (CHO) cells transfected with the specific receptor using calcium mobilisation and cyclic adenosine monophosphate (cAMP) accumulation assays.

Results: IgG autoantibodies against M_1R to M_5R were significantly increased in patients with SjD compared with HCs ($p \leq .05$) and detected in up to 30% of patients. A combination of anti-M_2R, anti-M_3R, and anti-M_5R identified up to 31% of Ro/SSA-negative SjD cases, with 96% and 75% specificity for SjD vs HCs and SjD vs NSS, respectively. Functionally, purified IgG from SjD serum reduced calcium flux by up to 30% in M_1R-, M_3R-, and M_5R-expressing CHO cells, whereas its effects on cAMP accumulation in M_2R-transfected cells were absent.

*Correspondence to Dr Fiona Engelke, Department of Rheumatology and Clinical Immunology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany.

E-mail address: engelke.fiona@mh-hannover.de (F. Engelke).

Detlef Neumann and Torsten Witte contributed equally to this work.

Handling editor Josef S Smolen

https://doi.org/10.1016/j.ard.2026.06.016

Conclusions: The association between autoantibodies targeting M_1R to M_5R in patients with SjD and their functional quantification provides strong evidence that autoantibodies against all muscarinic receptor isotypes may have pathogenic relevance in SjD.

INTRODUCTION

Sjögren's disease (SjD) is a chronic inflammatory autoimmune disorder characterised by lymphocytic infiltration of the lacrimal and salivary glands, resulting in their dysfunction.

What is already known about this topic

- Muscarinic acetylcholine receptors 1 to 5 (M_1R-M_5R) are G protein-coupled receptors found on human labial cells.
- Autoantibodies targeting M_3R suppress intracellular calcium flux and lead to reduced tear and salivary flow in patients with Sjögren's disease (SjD).

What this study adds

- Enzyme-linked immunosorbent assays detected autoantibodies against M_1R to M_5R in patients with SjD.
- Purified immunoglobulin (Ig) G from patients with SjD significantly inhibited calcium flux via $G\alpha_q$-coupled muscarinic acetylcholine receptors.

How this study might affect research, practice or policy

- Detection of muscarinic autoantibodies, combined with functional analysis, enhances understanding of the pathogenesis of SjD and provides a basis for new therapeutic strategies aimed at inhibiting the activity of these functional autoantibodies in order to reduce their suppressive effect and potentially alleviate symptoms in patients with SjD.

As a result, patients with SjD complain about ocular and oral dryness. Furthermore, 40% of patients with SjD develop extraglandular manifestations, including lung involvement, and they also have an increased risk of developing B-cell non-Hodgkin lymphoma [1]. The exact pathophysiological mechanism of SjD is poorly understood. Histopathological findings of the salivary glands of patients with SjD indicate that exocrine glands may retain their structural integrity despite pronounced secretory dysfunction. In addition, acinar tissue may regain its function *in vitro* [2]. Therefore, impaired glandular secretion in SjD cannot be solely a consequence of tissue destruction; other factors must interfere with the secretory process.

Autoantibodies against muscarinic acetylcholine receptor 3 (M_3R) were discovered decades ago [3]. In SjD, autoantibodies targeting the second extracellular loop of M_3R exert an antagonistic effect on human salivary gland cells, suppressing the increase in intracellular calcium. Consequently, tear and salivary flow are reduced in patients with SjD [4–6]. Antibodies against M_3R are not found in all patients with SjD and reduce gland control via muscarinic nerves only partially; therefore, they cannot be the sole cause of glandular hypofunction.

M_3R belongs to the G protein-coupled receptor (GPCR) family, a group of 7-transmembrane receptors that form the largest class of integral membrane proteins in humans [7,8]. The muscarinic acetylcholine receptor subclass consists of 5 members (muscarinic acetylcholine receptors 1-5 [M_1R-M_5R]). Subtypes of muscarinic receptors are found on human labial cells from both healthy and SjD individuals. However, M_2R has been reported at very low levels in both groups [9,10].

The present study aimed to investigate autoantibodies against M_1R to M_5R and to analyse their functional activity in SjD.

METHODS

Study subjects

Serum samples from a total of 347 patients with primary SjD were recruited from the Department of Rheumatology and Immunology at the Hannover Medical School, Germany (n = 171), the Department of Respiratory Medicine at Hannover Medical School, Germany (n = 46), the Department of Medicine/Rheumatology and Clinical Immunology at Charité in Berlin, Germany (n = 72), and the Department of Medicine/Rheumatology Clinic at the University of Udine, Italy (n = 58). Additional serum samples from patients with rheumatoid arthritis (RA; n = 33), systemic lupus erythematosus (SLE; n = 34), non-Sjögren's sicca syndrome (NSS; n = 44) who did not fulfil the 2016 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) classification criteria for SjD, and healthy controls (HCs; n = 48-50) were obtained from the Department of Rheumatology and Immunology at the Hannover Medical School. None of the controls with other autoimmune diseases was diagnosed with secondary SjD; only individuals confirmed to be negative for Ro/SSA antibodies and without sicca symptoms (as verified by Saxon and Schirmer tests) were included. All study participants fulfilled the ACR/EULAR classification criteria for their respective diseases [11–13]. Serum samples were aliquoted and stored at −80°C until use.

The clinical and demographic characteristics of the 289 patients with SjD from Hannover and Berlin, and the controls (RA, SLE, NSS, and HCs), are shown in Table 1. The characteristics of the SjD samples from Udine were not registered.

Quantification of autoantibodies against muscarinic acetylcholine receptors by enzyme-linked immunosorbent assay

Human immunoglobulin (Ig) G autoantibodies against M_1R to M_5R were detected in serum samples using enzyme-linked immunosorbent assays (ELISAs; CellTrend GmbH) according to the manufacturer's instructions. Briefly, GPCR-coated polystyrene plates were incubated with a 1:100 serum dilution for 2 hours at 4°C, followed by incubation with horseradish peroxidase (HRP)-labelled goat anti-human IgG for 1 hour. The autoantibody concentration was quantified in arbitrary units (U) per millilitre using a 5-point standard curve, ranging from 2.5 to 40 U/mL.

Quantification of autoantibodies against muscarinic acetylcholine receptors by Western blot

Recombinantly expressed and purified M_1R to M_5R proteins were provided by CellTrend GmbH. These were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes along with a molecular weight marker (YesBlot Western Marker

Table 1
Characteristics of Sjögren's disease patients and healthy controls in autoantibody quantification and functional analysis, as well as control groups, including non-Sjögren's sicca syndrome, rheumatoid arthritis, and systemic lupus erythematosus, in autoantibody screening.

	Autoantibody quantification						Functional analysis	
	HC n = 50	SjD n = 289		Other AIDs n = 67		NSS n = 44	HC n = 10	SjD n = 10
		Hannover n = 217	Berlin n = 72	RA n = 33	SLE n = 34			
Female, (%)	63.6	77.3	94.3	66.7	76.5	90	70.0	90.0
Age (y) at visit	46.8	61	47	57.8	47.3	54.7	39	64
Age (y) at onset		56	NA					48
Laboratory values, (%)								
ANA ≥ 1:160		77.2	NA	48.5	100.0	52.3		100.0
Rheumatoid factor-positive		31.8	64.2	72.7	2.9	4.5		80.0
Presence of Ro/SSA antibodies		61.6	80.6	0.0	35.3	0.0		60.0
Presence of La/SSB antibodies		21.4	45.8	0.0	5.9	0.0		10.0
Oral and ocular tests, (%)								
Saxon test pathological		49.3	100.0			54.5		20.0
Schirmer test pathological		68.5	33.3			65.9		60.0
Salivary gland biopsy, (%)								
Biopsy performed		42.3	11.4			45.5		80.0
Chisholm-Mason grade ≥3		58.8	62.5			0.0		87.5
ESSDAI, (%)								
Constitutional symptoms		9.7	50.0					10.0
Lymphadenopathy		2.7	1.4					0.0
Glandular involvement		5.3	51.4					0.0
Articular involvement		26.9	31.4					40.0
Cutaneous involvement		3.4	8.6					0.0
Pulmonary involvement		57.2	4.3					10.0
Renal involvement		4.0	0.0					10.0
Muscular involvement		3.4	2.9					10.0
Peripheral nervous system involvement		11.1	5.7					50.0
Central nervous system involvement		2.9	0.0					0.0
Haematological involvement		30.2	42.9					50.0
Biological involvement		15.7	21.4					10.0

AID, autoimmune disease; ANA, antinuclear antibody; ESSDAI, EULAR Sjögren's Syndrome Disease Activity Index; HC, healthy control; NA, not available; NSS, non-Sjögren's sicca syndrome; RA, rheumatoid arthritis; SjD, Sjögren's disease; SLE, systemic lupus erythematosus.

I, SMOBio Technology). Membranes were blocked (ROTI Block, Carl ROTH) and incubated overnight at 4°C with 1:50 dilutions (in ROTI Block) of purified IgG isolated from either HC sera or SjD patient sera (see 'Purification of IgG from patient serum'). Following the washing steps, bound antibodies were detected using HRP-conjugated anti-human IgG (Jackson ImmunoResearch, 1:2000 dilution in ROTI Block) and Westar ECL substrate (Cyanagen). Signal acquisition of chemiluminescent immunoreactive bands was performed using exposure times of 5 and 15 seconds on a Chemostar Plus imager (Intas).

Cell culture of Chinese hamster ovary cells

Chinese hamster ovary (CHO) cells were stably transfected with human muscarinic acetylcholine receptors obtained from the European Collection of Authenticated Cell Cultures (ECACC; CHO-CHRM1, CHO-CHRM2, T02J-7/10 [CHO-CHRM3], and CHO-CHRM5). Cells were cultivated in Ham's F12 medium (Bio&Sell) supplemented with 10% fetal bovine serum (FBS; Bio&Sell), 1% glutamine (Bio&Sell), and 0.5 mg/mL G418 (Gibco).

Cell-surface expression of the muscarinic acetylcholine receptors was verified by a commercial supplier (InSCREENeX GmbH), as described by Schucht et al. [14]. Unpublished data from InSCREENeX GmbH showed that nontransfected CHO cells did not endogenously express muscarinic acetylcholine receptors. In CHO cells transfected with M_4R, receptor expression could not be verified; therefore, no further functional analyses were performed.

Purification of IgG from patient serum

IgG was isolated from patient sera using immobilised Pierce Protein A Agarose (Thermo Fisher Scientific) according to the manufacturer's instructions. The purified IgG was buffered with phosphate-buffered saline (PBS; Bio&Sell) and diluted to a final concentration of 3 g/L.

Calcium mobilisation assay

Intracellular calcium flux was assessed by incubating $G\alpha_q$-coupled muscarinic acetylcholine receptor-transfected cells with Calbryte 520 AM (4 µM; AAT Bioquest) in calcium-free Hanks' balanced salt solution (HBSS) buffer (Sigma-Aldrich) containing 20 mM HEPES (Carl ROTH) for 1 hour at 37°C. After staining, cells were washed, resuspended in HBSS buffer containing purified human IgG, and incubated for 15 minutes at 37°C. The muscarinic receptor agonist carbachol (Merck Millipore) was added at a cell line-specific concentration corresponding to the half-maximal effective concentration (EC50). The EC_{50} values were determined from prior dose-response experiments (CHO-M_1R: 7 nM; CHO-M_3R: 2 nM; CHO-M_5R: 10 nM). Changes in intracellular calcium concentrations were determined using a Synergy HTX reader (BioTek) at 520 nm and calculated from baseline, minimum, and maximum fluorescence intensity signals obtained after the subsequent addition of ethylene glycol tetraacetic acid (EGTA; 600 mM; Sigma-Aldrich) and 30% Triton X-100 (Sigma-Aldrich). All conditions were tested in technical triplicate and validated in 3 independent experiments.

Table 2
Prevalence of muscarinic autoantibodies in Sjögen's disease patients at the study centres Hannover, Berlin, and Udine.

				% Positive patients						
				SjD (n = 347)						
Antigen	*P* value[a]	*P* value[b]	HC n = 50	All	Ro/SSA-negative	H n = 217	B n = 72	U n = 58	NSS n = 44	AID n = 67
IgG_M_1R	$3.52.10^{-05}$	$1.60.10^{-03}$	3.8	10.4	6.7	6.5	22.2	10.3	2.3	11.9
IgG_M_2R	$2.98.10^{-05}$	$1.00.10^{-04}$	3.8	29.7	23.3	21.7	52.8	31.0	15.9	14.9
IgG_M_3R	$3.24.10^{-02}$	$1.00.10^{-04}$	3.8	13.8	12.2	14.7	15.3	8.6	9.1	6.0
IgG_M_4R	$3.22.10^{-04}$	$1.00.10^{-04}$	3.8	16.4	8.8	12.9	26.4	17.2	15.9	11.9
IgG_M_5R	$2.03.10^{-02}$	$1.00.10^{-03}$	1.9	16.7	7.8	12.4	16.7	32.8	0.0	13.4

HCs and patients with NSS, as well as patients with AIDs, including those with rheumatoid arthritis (RA; n = 33) and systemic lupus (SLE; n = 34), served as controls. Patients with a positive status are reported as a percentage and defined using the receiver operating characteristic (ROC) curve, with the optimal likelihood ratio serving as the cutoff.
AID, autoimmune disease; B, Berlin; H, Hannover; HC, healthy control; IgG, immunoglobulin G; M_1R to M_5R, muscarinic acetylcholine receptors 1 to 5; NSS, non-Sjögren's sicca syndrome; U, Udine.
[a] Comparison of *P* values for patients with SjD vs HCs using the Mann-Whitney U test.
[b] Comparison of *P* values for patients with SjD vs HCs using Fisher's exact test.

Cyclic adenosine monophosphate accumulation assay

Cells with Gα_i-coupled muscarinic acetylcholine receptors were preincubated with purified human IgG for 15 minutes at 37°C and then stimulated with a mixture of carbachol and 5 μM forskolin (Sigma-Aldrich) for 10 minutes at room temperature (RT). Forskolin was used to increase intracellular cyclic adenosine monophosphate (cAMP) levels, thereby providing a measurable signal for inhibition. Meanwhile, carbachol acted as an agonist at muscarinic receptors, counteracting the effects of forskolin-induced cAMP accumulation. The half-maximal inhibitory concentration (IC_{50}) value of carbachol in the presence of 5 μM forskolin was quantified in initial dose-response experiments (CHO-M_2R: 5 nM). The concentration of forskolin was selected based on previous studies in CHO cells [15]. This setup allowed assessment of whether IgG interferes with the inhibitory effect of carbachol on forskolin-stimulated cAMP signalling. Following stimulation, the cells were lysed in acetonitrile (Th. Geyer GmbH & Co. KG), methanol (Avantor Performance Materials), and water (2:2:1), supplemented with 25 ng/mL tenofovir (internal standard [IS]; National Institutes of Health). Intracellular cAMP concentrations were analysed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS; Shimadzu Nexera, Shimadzu Scientific Instruments; Sciex QTRAP 5500 System). All conditions were tested in technical triplicate and validated in 3 independent experiments.

Data analysis

The antigen profiles of patients with SjD and HCs were compared using the Mann-Whitney U test, and *p* values of <.05 were considered statistically significant. In addition, the data were binarised using the receiver operating characteristic (ROC) curve based on the optimal likelihood ratio for SjD classification, and further statistically tested with Fisher's exact test. The antigen profile results were visualised using heatmaps and violin plots.

For a comparative analysis of mobilised calcium across measurements, the calcium concentrations observed in the calcium mobilisation assay were normalised to the maximum signal from carbachol-only stimulation. The percentage of calcium in carbachol-activated CHO cells, in the presence of purified IgG from HCs and patients with SjD, was plotted as a scatter plot. Statistical analysis was performed using the Mann-Whitney U test. ROC analysis was used to determine inhibitory reactivity by comparing patients with SjD with HCs. Correlation analyses were performed using Pearson's correlation coefficient to examine the relationship between the calcium assay and ELISA concentrations. The results were visualised as scatter plots with linear regression lines and 95% CIs.

Regarding the data analysis of the cAMP accumulation assay, the observed cAMP concentrations were normalised to the maximum cAMP concentration induced by forskolin, expressed as a percentage, and plotted as a scatter plot. A Kruskal-Wallis test was conducted to compare cAMP concentrations between HCs and patients with SjD.

RESULTS

Autoantibodies against muscarinic receptors in SjD

Autoantibodies against M_1R to M_5R were measured in patients with SjD (n = 347), HCs (n = 48-50), NSS (n = 44), and patients with other autoimmune diseases (AIDs; n = 67) using ELISA.

The concentrations of muscarinic autoantibodies were significantly higher in patients with SjD than that in HCs (Mann-Whitney U test; Table 2). The prevalence of autoantibodies in the study groups was determined using antigen-specific cutoffs defined by the ROC curve and the optimal likelihood ratio. Autoantibodies against M_1R to M_5R were found in 10% to ~30% of patients with SjD (Figs 1 and 2A-E, Table 2, and Supplementary Fig S2).

Using principal component analysis, comparative studies of autoantibody reactivity in patients with SjD across the study centres revealed similarities in global distribution (Supplementary Fig S3). Minor differences were found in anti-M_2R autoantibodies across the study centres. The Berlin cohort had approximately twice as many autoantibodies as the Hannover cohort (Table 2). Furthermore, the autoantibody profiles of patients with SjD and controls (HCs, NSS, and AIDs) showed distinct yet overlapping patterns.

Notably, autoantibodies against muscarinic receptors were more prevalent in patients with SjD than in HCs. Specifically, anti-M_1R, anti-M_2R, anti-M_3R, and anti-M_5R were more frequent in patients with SjD than in patients with NSS, with distinct overlap observed across groups, including shared anti-M_4R reactivity, while anti-M_1R and anti-M_5R were largely absent in patients with NSS (Table 2 and Supplementary Fig S2).

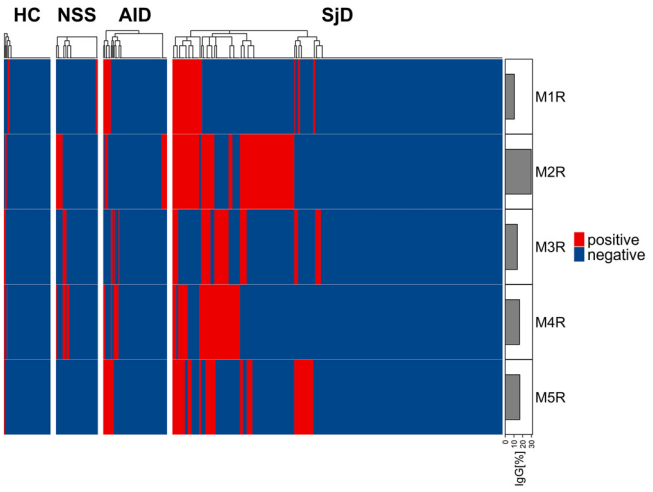

Figure 1. Unsupervised hierarchical cluster analysis of autoantibody reactivity of the muscarinic acetylcholine receptors 1 to 5 (M_1R-M_5R) as antigen targets in Sjögren's disease (SjD; n = 347) compared with patients with healthy individuals (HC, n≤50), non-Sjögren's sicca syndrome (NSS, n = 44) and other autoimmune disease patients (AID, n = 67). Each column represents an individual patient, and each row corresponds to a specific muscarinic autoantibody. The colour code indicates whether individuals are positive (red) or negative (blue) for the specific autoantibody. The bar chart on the right shows the overall prevalence of each muscarinic autoantibody among all patients with SjD, expressed as the percentage of patients with detectable immunoglobulin (Ig) G against that antigen.

Given the diagnostic challenges in identifying Ro/SSA-negative patients with SjD, we investigated the presence of muscarinic autoantibodies in this subgroup (n = 90). These autoantibodies were found in up to 23% of patients in this subcohort, with anti-M_2R demonstrating the highest IgG reactivity (Table 2). Combining all muscarinic autoantibodies enabled the detection of 33% of patients with SjD who were negative for Ro/SSA, with specificities of 92% and 64% are for SjD vs HCs and SjD vs NSS, respectively. The specificity improved when the detection panel consisted only of anti-M_2R, anti-M_3R, and anti-M_5R, enabling the detection of 31% of patients with SjD, with specificities of 96% and 75% are for SjD vs HCs and SjD vs NSS, respectively.

Furthermore, associations between the presence of antibodies against M_1R to M_5R and antinuclear antibodies (ANA), hypergammaglobulinemia, rheumatoid factor and complement depletion, markers of glandular involvement (pathological saliva and tear production and salivary gland biopsy results), and clinical manifestations (selected European Alliance of Associations for Rheumatology Sjögren's Syndrome Disease Activity Index (ESSDAI) domains, disease activity, duration, and onset) were investigated using Fisher's exact test; the 289 patients with SjD from Hannover and Berlin were included in the analysis (Supplementary Table S1). Notably, a positive rheumatoid factor result was associated with the presence of anti-M_2R, anti-M_4R, and anti-M_5R antibodies. Higher IgG concentrations showed a significant positive correlation with anti-M_2R, whereas the haematological domain was positively associated with anti-M_5R. However, none of these associations remained statistically significant after accounting for multiple testing using the Benjamini-Hochberg procedure. Furthermore, the presence of inhibitory autoantibodies was not associated with clinical manifestations, including dryness, as assessed by the Saxon and Schirmer tests.

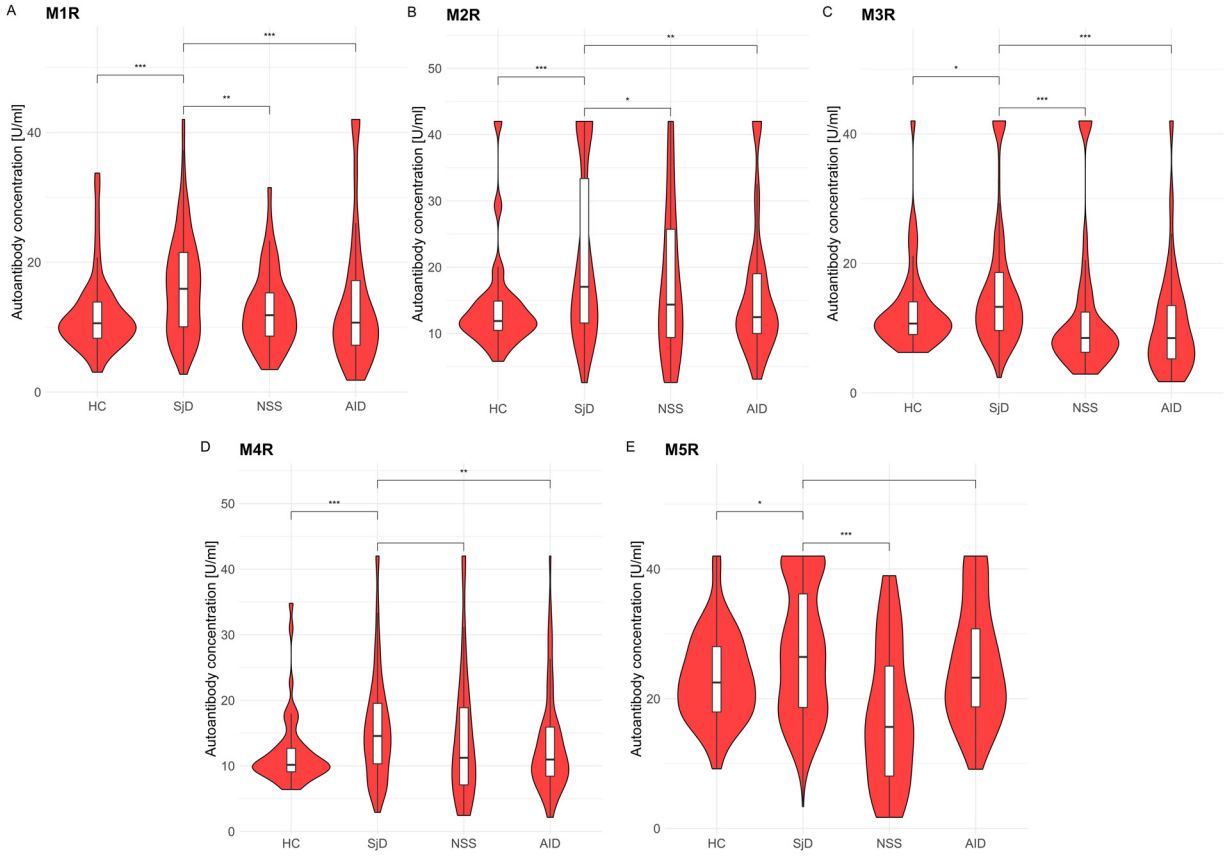

Figure 2. A-E, Violin plots of autoantibody concentration (in U/mL) of muscarinic autoantibodies (muscarinic acetylcholine receptors 1-5 [M_1R-M_5R]) are shown for the individual sample groups (patients with Sjögren's disease [SjD], n = 347; healthy controls [HCs], n ≤ 50; patients with non-Sjögren's sicca syndrome [NSS], n = 44; and patients with other autoimmune disease patients [AIDs], n = 67). Statistical analysis was performed by the Mann-Whitney U test. *$p \leq .05$; **$p \leq .01$; ***$p \leq .001$.

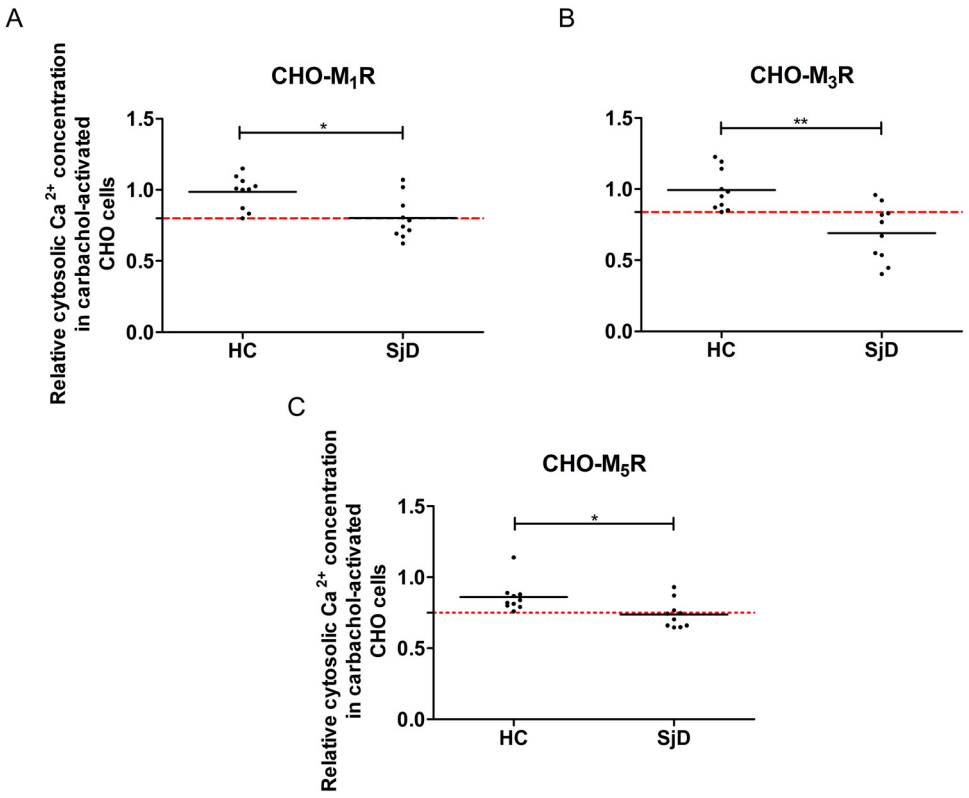

Figure 3. Relative cytosolic calcium concentrations in carbachol-activated Chinese hamster ovary (CHO) cells preincubated with purified human immunoglobulin (Ig) G from Sjögren's disease (SjD; n = 10) patients and healthy controls (HCs; n = 10). Normalisation was performed to the individual maximal calcium concentration in CHO cells (A: CHO-M_1R; B: CHO-M_3R; C: CHO-M_5R) induced by carbachol without preincubation. The mean of 3 replicate experiments per patient or control is represented by a data point. Red dotted lines indicate the inhibitory cutoff. Statistical analysis was performed by the Mann-Whitney U test. *$p \leq .05$; **$p \leq .01$ compared with HCs.

The specificity of the ELISA was confirmed by inhibition and cross-inhibition assays performed with well-characterised antibody-positive and antibody-negative sera, thereby enabling discrimination between specific and nonspecific signal contributions. Preincubating SjD sera on separate ELISA plates coated with identical muscarinic receptor antigens resulted in lower optical densities for the receptor-specific antibodies (anti-M_1R to anti-M_5R) in question (Supplementary Fig S1). Additionally, supplementing the sera with a 10-fold higher concentration of soluble M_3R antigen almost completely inhibited binding to M_3R-coated ELISA plates. This decrease in signal intensity indicates that antigen-specific Igs present in the sera were competitively absorbed during the preincubation step. Cross-inhibition was ruled out, as preincubation with any of the other 4 muscarinic receptor subtypes did not significantly reduce the optical densities.

Despite the demonstrated specificity of the ELISA, a second independent method was established to further verify the presence of muscarinic receptor autoantibodies in SjD sera. To this end, purified recombinant muscarinic receptor proteins were subjected to Western blot analysis and probed with purified IgG fractions from either patients with SjD or HCs. Immunoreactive bands at ~70 kDa were readily detectable in lanes where M_3R and M_4R proteins were loaded. In contrast, in lanes loaded with M_1R, M_2R, and M_5R, only very faint bands appeared at 70 kDa, whereas stronger immunoreactivity was observed at ~15 kDa. However, consistent with the ELISA findings, IgG from patients with SjD, on average, exhibited stronger M_3R- and M_4R-immunoreactive bands compared with IgG from HCs (Supplementary Fig S4), indicating increased antibody reactivity against muscarinic receptor proteins in patients' samples. Thus, Western blot analysis independently confirmed the presence of muscarinic receptor autoantibodies in SjD patient sera, probably at a higher titre and/or reactivity than in HC samples.

Impact of purified serum IgG on CHO cells transfected with $G\alpha_q$-coupled muscarinic acetylcholine receptors

To address the functional relevance of autoantibodies against $G\alpha_q$-coupled M_1R, M_3R, and M_5R in SjD, calcium mobilisation was quantified by measuring cytosolic calcium concentration in CHO cells in the presence of purified IgG from patients with SjD (n = 10) or HCs (n = 10; Table 1). SjD sera were selected based on their positivity in the ELISA. A detailed overview of these results can be found in Supplementary Table S2. Notably, none of the 10 patients tested positive for all muscarinic receptor autoantibodies. Rather, each patient tested positive for only a subset of the antibodies tested.

No intrinsic activity was observed when cells were stimulated with purified IgG alone. Preincubation with purified IgG (final concentration 1.5 g/L) from patients with SjD or HCs, followed by stimulation with carbachol at an EC_{50} concentration, showed that IgG from patients with SjD significantly inhibited calcium flux through all $G\alpha_q$-coupled muscarinic acetylcholine receptors, in contrast to IgG from HCs (Fig 3). Depending on the receptor, calcium flux was inhibited by up to 30% (M_1R: 20%; M_3R: 30%; M_5R: 10%) relative to the mean calcium flux in HC samples. Calcium concentrations ≤75% to 84% were determined as inhibitory with a specificity of 100% using ROC analysis (M_1R: ≤80%; M_3R: ≤84%; M_5R: ≤75%). Accordingly, 60% to 80% of patients with SjD had inhibitory antimuscarinic autoantibodies (M_1R: 60%; M_3R: 80%; M_5R: 70%).

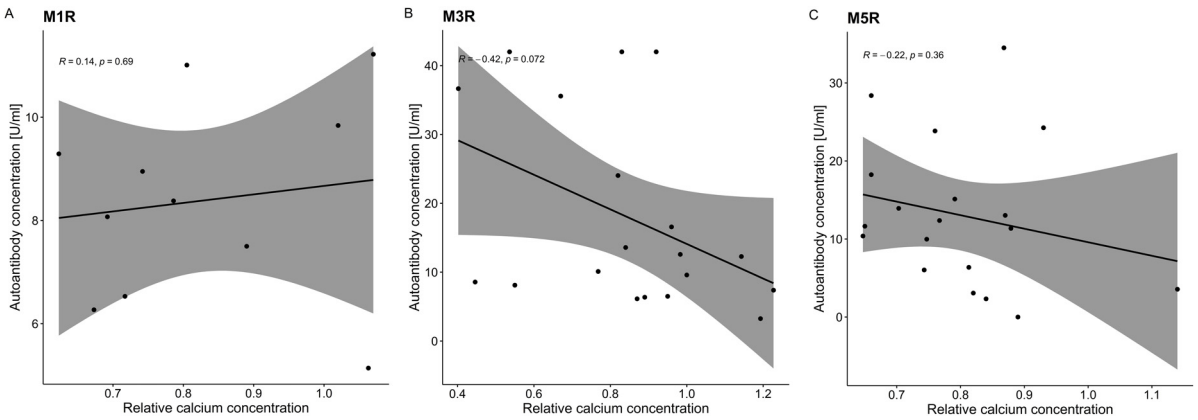

Figure 4. Correlation-based comparison of the muscarinic autoantibody concentration derived from enzyme-linked immunosorbent assay (ELISA) and the relative calcium concentration determined by the calcium mobilisation assay. (A) CHO-M_1R, (B) CHO-M_3R, and (C) CHO-M_5R. M_1R, M_3R, and M_5R, muscarinic acetylcholine receptors 1, 3, and 5, respectively.

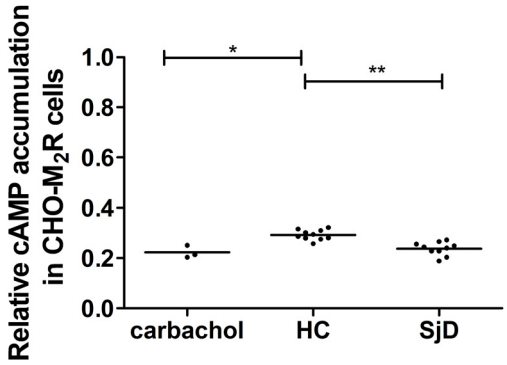

Figure 5. Relative cyclic adenosine monophosphate (cAMP) accumulation in CHO-M_2R cells preincubated with purified immunoglobulin (Ig) G from patients with Sjögren's disease (SjD; n = 10) or healthy controls (HCs; n = 10). To test the effects of IgG, the cells were subsequently stimulated with a mixture of forskolin and carbachol, with forskolin increasing intracellular cAMP levels and carbachol at its half-maximal inhibitory concentration (IC_{50}) confirming M_2R-mediated inhibition. Data were normalised to the maximum forskolin-induced cAMP response. The mean of 3 replicate experiments per patient or control is represented by a data point. Statistical analysis was performed by the Kruskal-Wallis test. *$p \leq .05$; **$p \leq .01$.

Furthermore, the correlation between the autoantibody concentration determined by ELISA and the inhibitory activity of autoantibodies in the calcium mobilisation assay was assessed (Fig 4). A nonsignificant negative association between the 2 assays was observed for M_3R and M_5R, suggesting that increased inhibitory activity in the calcium mobilisation assay was related to a higher autoantibody concentration in the ELISA. In contrast, a nonsignificant positive correlation was observed between the 2 M_1R assays, indicating that a greater inhibitory effect was associated with a lower autoantibody concentration.

Impact of purified serum IgG on CHO cells transfected with Gα_i-coupled M_2R

The functional relevance of autoantibodies against Gα_i-coupled M_2R in SjD was examined using a cAMP accumulation assay. No intrinsic activity of purified IgG alone was observed in CHO-M_2R. To evaluate the impact of IgG fractions on M_2R signalling, forskolin was used to increase intracellular cAMP levels. Carbachol acts as an agonist at muscarinic receptors and inhibits forskolin-induced cAMP accumulation. It was used at its IC_{50} concentration as a positive control to verify receptor-mediated inhibition, thereby providing a functional readout to assess whether IgG interferes with inhibitory signalling. In this system, IgG from HCs partially restored cAMP levels above the minimum induced by carbachol, indicating agonistic or synergistic activity (Fig 5). By contrast, IgG from patients with SjD did not modify cAMP accumulation, which remained at the level suppressed by carbachol.

DISCUSSION

In this study, we systematically investigated autoantibodies targeting all muscarinic acetylcholine receptor subtypes in a large cohort of patients with SjD and in individuals with sicca symptoms. To our knowledge, this is the first study to comprehensively analyse the presence of autoantibodies against multiple muscarinic receptors in patients with SjD, whereas previous reports have focused primarily on M_3R and M_5R [5,16,17]. Additionally, we conducted functional analyses to evaluate the effect of these autoantibodies on receptor activity. While previous studies have primarily focused on M_3R, our work extends these findings by providing the first functional data on M_1R and M_5R.

In the muscarinic autoantibody reactivity study, autoantibodies against M_1R to M_5R were significantly more common in patients with SjD than in HCs. This observation extends previous studies reporting the presence of autoantibodies against M_3R and M_5R in patients with SjD. In addition, anti-M_3R were also found in patients with other AIDs, including systemic sclerosis (SSc) and SLE [18,19]. Since autoantibodies against M_1R to M_5R were also found in the Ro/SSA-negative SjD patient group, they could be used to improve the identification of these patients, which currently relies on invasive salivary gland biopsy. Combining 3 of the 5 muscarinic autoantibodies (anti-M_2R, anti-M_3R, and anti-M_5R) enabled the detection of approximately one-third of Ro/SSA-negative patients with SjD. Furthermore, this study revealed that autoantibodies against M_2R, M_3R, and M_4R were also more common among individuals with sicca symptoms who did not meet the classification criteria for SjD than in HCs. SjD was excluded in these patients because they had no Ro/SSA autoantibodies, and the salivary gland biopsy did not meet the Chisholm-Mason grade 3 or higher criteria. In our cohort, 33/35 biopsies from Ro/SSA-positive patients with SjD revealed a Chisholm-Mason grade of at least 3, indicating a biopsy sensitivity of 94.3% at our institution. However, a

proportion of NSS individuals may have SjD that is not detected on salivary gland biopsy. There may, however, be an additional subset of NSS individuals in whom autoantibodies against muscarinic acetylcholine receptors may contribute to the pathogenesis of reduced glandular function.

In our functional analysis, purified IgG from patients with SjD was found to significantly inhibit calcium flux in CHO-M_3R cells, resulting in a reduction of approximately 30% in calcium signalling. Similar inhibitory effects were observed in CHO-M_1R and CHO-M_5R cells, demonstrating for the first time that autoantibodies against these receptor subtypes can impair Gα_q-coupled receptor signalling. These results corroborate previous studies indicating reduced calcium responses in CHO-M_3R cells and human submandibular gland (HSG) cells when exposed to SjD IgG and support the notion that autoantibodies can interfere with agonist binding to salivary gland membranes [4,20,21]. To the best of our knowledge, this is the first study to show that autoantibodies have an antagonistic effect on M_1R and M_5R, thereby inhibiting their activity and subsequent signalling. As both M_1R and M_5R have been identified in salivary gland tissue, these findings suggest a possible mechanistic link to glandular hypofunction in SjD. Beyond the classical focus on M_3R, which has been extensively studied in the context of impaired calcium-dependent secretion, our data suggest that other muscarinic receptor subtypes may contribute to pathogenesis. Antagonistic autoantibodies against M_1R and M_5R could reduce intracellular signalling via Gα_q-coupled pathways, leading to diminished secretory responses. Furthermore, we assume that glandular hypofunction in SjD is unlikely to result from a single receptor-antibody interaction but rather from a broader imbalance in muscarinic receptor signalling. Antagonistic autoantibodies that target multiple receptor subtypes can simultaneously impair distinct calcium-dependent pathways, leading to reductions in secretory function that are either additive or synergistic. This multifaceted disruption of receptor signalling could explain why clinical manifestations vary among patients, as different combinations or concentrations of autoantibodies may affect glandular activity in distinct ways.

In contrast, no functional role for autoantibodies targeting Gα_i-coupled M_2R in modulating cAMP signalling was observed. While IgG fractions from HCs occasionally showed minor effects on cAMP accumulation, these were likely attributable to background reactivity or nonspecific IgG interactions rather than to disease-related autoantibodies. This is consistent with previous reports showing that M_2R is only minimally expressed in labial salivary gland tissue and is not predominantly localised to secretory epithelial cells, suggesting that this receptor subtype is unlikely to play a physiologically meaningful role in glandular dysfunction. Transfection of M_4R did not result in cell-surface expression; therefore, functional analysis was not possible.

Studies on anti-M_3R autoantibodies have produced conflicting results, with some researchers failing to detect antibodies [22]. These discrepancies may be due to differences in the detection techniques used. Notably, ELISA using intact GPCR proteins, as employed in our initial experiments, is likely to have limited sensitivity to these antibodies, highlighting the challenges in this area of research [23]. Consistent with this, we also observed discrepancies between our ELISA results and those of the functional assays, supporting the view that ELISA is poorly correlated with functional assay positivity. Importantly, this underscores the need to analyse both ELISA-positive and ELISA-negative SjD sera in functional assays to obtain a more reliable assessment of antibody activity.

However, to confirm receptor-binding specificity in ELISA, inhibition and cross-inhibition assays were performed. Preincubating SjD sera with identical muscarinic receptor antigens on separate plates markedly reduced the corresponding optical density values, indicating specific antibody-antigen interactions. Interestingly, preincubation of the sera with a 10-fold excess of soluble M_3R antigen almost completely inhibited subsequent binding to M_3R-coated ELISA plates. By contrast, preincubation with heterologous muscarinic receptor subtypes did not affect the optical density, indicating that the autoantibodies recognised only their respective receptor subtypes and showed no cross-reactivity between different muscarinic receptors.

Despite the demonstrated specificity of the ELISA, as evidenced by inhibition and cross-inhibition experiments, we validated the presence of muscarinic receptor autoantibodies using a second, independent detection method. This was particularly important due to inconsistent findings in anti-M_3R autoantibody detection and the challenges of using ELISA to analyse membrane-bound GPCRs.

To address this, we established a modified Western blot approach. Recombinant muscarinic receptor proteins were separated by SDS-PAGE, transferred onto nitrocellulose membranes, and probed with purified serum IgG fractions serving as primary antibodies. Consistent with the ELISA findings, immunoreactive bands were observed, providing independent evidence of autoantibody reactivity against muscarinic receptor proteins in SjD sera. Moreover, the Western blot approach also confirmed increased reactivity against M_3R and M_4R in SjD patient-derived sera compared with HCs. However, this Western blot system has not yet been fully established and should therefore be interpreted with caution. For M_1R, M_2R, and M_5R, major bands were detected at lower molecular weights than expected, a finding that was also evident in Ponceau S and Coomassie staining of the recombinant protein preparations (data not shown). These low-molecular-weight species may represent degradation products or partial recombinant proteins; however, no detailed information regarding the composition of the protein preparations was available from the manufacturer. Nevertheless, the Western blot approach supports the conclusion that muscarinic receptor autoantibodies are present in SjD sera.

Correlation analysis of oral and ocular dryness, as assessed by the Saxon and Schirmer test, revealed no association with the presence of muscarinic autoantibodies, not even with anti-M_3R. This may be partially explained by the heterogeneity of our cohort. We included both patients with a recent diagnosis of SjD, in whom glands are probably still largely intact and functional autoantibodies are likely relevant, and patients with a long disease duration, in whom glands are likely mostly destroyed and functional autoantibodies will no longer affect glandular secretion. Furthermore, not all muscarinic autoantibodies detected by ELISA may be functionally relevant, since antibodies against the complete proteins, rather than only the acetylcholine-binding domain, are measured. Finally, additional autoantibodies against other water channels involved in glandular secretion, such as aquaporin-5, have been described in SjD and may also be functionally relevant [24].

It will be interesting to see in ongoing studies whether B-cell-directed drugs, such as Ianalumab, or IgG-reducing drugs such as nipocalimab or efgartigimod, improve salivary and lachrymal gland function in patients with SjD with M_1R, M_3R, or M_5R, or with antibodies against water channels, to a greater extent than in patients without these autoantibodies.

This study was strengthened by a 2-step selection and quantification of autoantibodies targeting the muscarinic acetylcholine

receptor, thereby enhancing the reliability of the findings. Furthermore, validation was carried out on a large SjD cohort recruited from 3 different study centres, as well as on disease controls, including individuals with other AIDs and sicca symptoms. Furthermore, CHO cells, as a model system, are well established in *in vitro* studies and allow standardisation through defined cell numbers and uniform environmental conditions, compared with smooth muscle from the bladder or colon as a model system for demonstrating functional antibodies [25]. In addition, unpublished data from InSCREENeX GmbH showed that no endogenous muscarinic acetylcholine receptors were present in the nontransfected CHO-K1 cells. Accordingly, the observed calcium and cAMP signals were entirely generated by the specific transfected receptor.

With regard to the autoantibody quantification, the limitations of this study include that the autoantibody concentration may be affected by drugs such as immunosuppressants, which induce B lymphocyte depletion and thus may alter autoantibody concentrations. Approximately 5% of patients in our study had received rituximab therapy, which may have contributed to reduced antibody levels. In addition, all study participants were recruited from university hospitals, where disease activity and manifestations were more severe than in medical practice, which may introduce a bias into autoantibody reactivity or correlation analyses. Future studies should examine the consistency of autoantibodies over time and include patients from medical practice. In addition, we observed that autoantibody levels can differ between patient groups. These differences could be influenced by preanalytical factors, such as sample collection, processing, and storage conditions. This underscores the importance of including local control groups when interpreting autoantibody measurements to account for potential procedural variability and ensure reliable comparisons across studies. Regarding the functional analysis of muscarinic autoantibodies, this study lacks an age-matched control group, which is an important consideration for future research. Furthermore, it should be noted that functional experiments conducted on CHO cells may not accurately reflect signalling dynamics, eg, in primary human salivary gland cells. Differences in receptor density, expression patterns, and coupling efficiency can affect the magnitude of calcium or cAMP responses and other downstream signalling events. In addition, *in vitro* systems cannot fully replicate the complex *in vivo* environment, in which additional regulatory factors, cell-to-cell interactions, and tissue architecture influence muscarinic receptor function. Therefore, while these experiments provide valuable mechanistic insight, the observed effects may differ under physiological conditions.

A further limitation of the current study is that receptor subtype expression was not systematically assessed at the RNA or signalling levels in minor salivary gland tissue. Importantly, previous studies investigating muscarinic receptor expression in minor salivary glands were limited by small cohort sizes, inconsistent findings, and methodological challenges, particularly regarding the specificity and validation of antibodies against individual muscarinic receptor subtypes. Future studies using transcriptomic or spatial RNA-based approaches may therefore provide a more comprehensive and reliable characterisation of receptor expression patterns and downstream signalling pathways in salivary gland tissue from patients with SjD.

However, a deeper understanding of the functional properties of autoantibodies is crucial for developing and optimising therapeutic approaches for patients with SjD. For instance, pilocarpine, an M_3R agonist, is used to stimulate saliva and tear secretion for symptomatic relief. The efficacy of pilocarpine may be limited in patients with functionally blocking M_3R antibodies, as the receptor is blocked by these antibodies. Without functional characterisation of autoantibodies, resistance to therapy would remain unrecognised. Therefore, analysing antibody function is essential for understanding the disease, planning individualised therapies, and developing targeted treatment strategies.

In conclusion, autoantibodies against muscarinic receptors (M_1R-M_5R) are present in patients with SjD and may be implicated in the pathogenesis of the disease. These findings, along with further functional analysis, could lead to new therapeutic strategies aimed at targeting functional muscarinic autoantibodies and reducing their inhibitory activity, thereby improving symptom outcomes in SjD.

Competing interests

TW reports a relationship with Amgen, Novartis AG, Johnson & Johnson, that includes: speaking and lecture fees and also reports a relationship with Takeda that includes: funding grants. BS reports a relationship with Boehringer Ingelheim GmbH, GlaxoSmithKline Inc, and AstraZeneca that includes: speaking and lecture fees. All other authors declare they have no competing interests.

CRediT authorship contribution statement

Fiona Engelke: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Harald Heidecke:** Writing – review & editing, Resources. **Kai Schulze-Forster:** Writing – review & editing, Resources. **Heike Bähre:** Writing – review & editing, Supervision. **Yasmin Liß:** Writing – review & editing, Methodology. **Tina Hagedorn:** Methodology, Writing – review & editing. **Thomas Dörner:** Writing – review & editing, Resources. **Diana Ernst:** Writing – review & editing, Resources. **Jan Gras:** Writing – review & editing, Resources. **Luca Quartuccio:** Writing – review & editing, Resources. **Jacob Ritter:** Writing – review & editing, Resources. **Benjamin Seeliger:** Writing – review & editing, Resources. **Detlef Neumann:** Writing – review & editing, Supervision. **Torsten Witte:** Writing – review & editing, Supervision, Funding acquisition.

Acknowledgements

We are grateful to all members of the outpatient clinics for their support in patient recruitment. We are deeply grateful to the late Prof Salvatore De Vita (University of Udine, Udine, Italy) for facilitating access to the SjD cohort. We would like to thank Katja Kniesch for performing an additional ELISA to confirm the specificity of the assay.

Parts of this work were previously presented at the European Congress of Rheumatology (June 2024, Vienna, Austria) and the 14th International Congress on Autoimmunity (May 2024, Ljubljana, Slovenia).

Funding

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) and Germany's Excellence Strategy - EXC 2155 (project number 390874280).

Patient consent for publication

Written informed consent from study participants was received for the use of their blood material for research purposes.

Ethics approval

This study adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee (Vote Hannover Medical School ethical committee number 5582, 29.03.2010; Vote Charité Universitätsmedizin Berlin ethical committee numbers EA1/002/16 and EA1/089/08; Vote University of Udine CEUR-2017-Os-027-ASUIUD, 19.04.2017).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Data are available on reasonable request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2026.06.016.

REFERENCES

[1] Mariette X, Criswell LA. Primary Sjögren's syndrome. N Engl J Med. 2018;378(10):931–9. doi: 10.1056/NEJMcp1702514.

[2] Dawson LJ, Field EA, Harmer AR, Smith PM. Acetylcholine-evoked calcium mobilization and ion channel activation in human labial gland acinar cells from patients with primary Sjögren's syndrome. Clin Exp Immunol. 2001;124(3):480–5. doi: 10.1046/j.1365-2249.2001.01526.x.

[3] Bacman S, Sterin-Borda L, Camusso JJ, Arana R, Hubscher O, Borda E. Circulating antibodies against rat parotid gland M3 muscarinic receptors in primary Sjögren's syndrome. Clin Exp Immunol. 1996;104(3):454–9. doi: 10.1046/j.1365-2249.1996.42748.x.

[4] Preuss B, Tunaru S, Henes J, Offermanns S, Klein R. A novel luminescence-based method for the detection of functionally active antibodies to muscarinic acetylcholine receptors of the M3 type (mAchR3) in patients' sera. Clin Exp Immunol. 2014;177(1):179–89. doi: 10.1111/cei.12324.

[5] Tsuboi H, Matsumoto I, Wakamatsu E, Nakamura Y, Iizuka M, Hayashi T, et al. New epitopes and function of anti-M3 muscarinic acetylcholine receptor antibodies in patients with Sjögren's syndrome. Clin Exp Immunol. 2010;162(1):53–61. doi: 10.1111/j.1365-2249.2010.04188.x.

[6] Watson EL, Abel PW, DiJulio D, Zeng W, Makoid M, Jacobson KL, et al. Identification of muscarinic receptor subtypes in mouse parotid gland. Am J Physiol. 1996;271(3 Pt 1):C905–13. doi: 10.1152/ajpcell.1996.271.3.C905.

[7] Flock T, Hauser AS, Lund N, Gloriam DE, Balaji S, Babu MM. Selectivity determinants of GPCR-G-protein binding. Nature. 2017;545(7654):317–22. doi: 10.1038/nature22070.

[8] Venkatakrishnan AJ, Deupi X, Lebon G, Tate CG, Schertler GF, Babu MM. Molecular signatures of G-protein-coupled receptors. Nature. 2013;494(7436):185–94. doi: 10.1038/nature11896.

[9] Ryberg AT, Warfvinge G, Axelsson L, Soukup O, Götrick B, Tobin G. Expression of muscarinic receptor subtypes in salivary glands of rats, sheep and man. Arch Oral Biol. 2008;53(1):66–74. doi: 10.1016/j.archoralbio.2007.07.012.

[10] Ueda H, Mitoh Y, Fujita M, Kobashi M, Yamashiro T, Sugimoto T, et al. Muscarinic receptor immunoreactivity in the superior salivatory nucleus neurons innervating the salivary glands of the rat. Neurosci Lett. 2011;499(1):42–6. doi: 10.1016/j.neulet.2011.05.029.

[11] Kay J, Upchurch KS. ACR/EULAR 2010 rheumatoid arthritis classification criteria. Rheumatology (Oxford) 2012;51(Suppl 6):vi5–9. doi: 10.1093/rheumatology/kes279.

[12] Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Arthritis Rheumatol. 2019;71(9):1400–12. doi: 10.1002/art.40930.

[13] Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. Arthritis Rheumatol. 2017;69(1):35–45. doi: 10.1002/art.39859.

[14] Schucht R, Lydford S, Andzinski L, Zauers J, Cooper J, Hauser H, et al. Rapid establishment of G-protein-coupled receptor-expressing cell lines by site-specific integration. J Biomol Screen. 2011;16(3):323–31. doi: 10.1177/1087057110396371.

[15] Yoon C, Kim D, Lim JH, Lee GM. Forskolin increases cAMP levels and enhances recombinant antibody production in CHO cell cultures. Biotechnol J. 2020;15:2000264. doi: 10.1002/biot.202000264.

[16] Kovács L, Marczinovits I, György A, Tóth GK, Dorgai L, Pál J, et al. Clinical associations of autoantibodies to human muscarinic acetylcholine receptor 3 (213-228) in primary Sjogren's syndrome. Rheumatology (Oxford). 2005;44(8):1021–5. doi: 10.1093/rheumatology/keh672.

[17] Longobardi S, Lopez-Davis C, Khatri B, Georgescu C, Pritchett-Frazee C, Lawrence C, et al. Autoantibodies identify primary Sjögren's syndrome in patients lacking serum IgG specific for Ro/SS-A and La/SS-B. Ann Rheum Dis. 2023;82(9):1181–90. doi: 10.1136/ard-2022-223105.

[18] Reina S, Pisoni C, Eimon A, Carrizo C, Arana R, Borda E. Anti-M3 muscarinic acetylcholine receptor antibodies in systemic lupus erythematosus. Pharmacol Pharm. 2015;6:25–33. doi: 10.4236/pp.2015.61004.

[19] Kawaguchi Y, Nakamura Y, Matsumoto I, Nishimagi E, Satoh T, Kuwana M, et al. Muscarinic-3 acetylcholine receptor autoantibody in patients with systemic sclerosis: contribution to severe gastrointestinal tract dysmotility. Ann Rheum Dis. 2009;68(5):710–4. doi: 10.1136/ard.2008.096545.

[20] Li J, Ha YM, Kü NY, Choi SY, Lee SJ, Oh SB, et al. Inhibitory effects of autoantibodies on the muscarinic receptors in Sjögren's syndrome. Lab Invest. 2004;84(11):1430–8. doi: 10.1038/labinvest.3700173.

[21] Robinson CP, Brayer J, Yamachika S, Esch TR, Peck AB, Stewart CA, et al. Transfer of human serum IgG to nonobese diabetic Igmu null mice reveals a role for autoantibodies in the loss of secretory function of exocrine tissues in Sjögren's syndrome. Proc Natl Acad Sci U S A. 1998;95(13):7538–43. doi: 10.1073/pnas.95.13.7538.

[22] Dawson LJ, Allison HE, Stanbury J, Fitzgerald D, Smith PM. Putative anti-muscarinic antibodies cannot be detected in patients with primary Sjogren's syndrome using conventional immunological approaches. Rheumatology (Oxford). 2004;43(12):1488–95. doi: 10.1093/rheumatology/keh389.

[23] Roescher N, Kingman A, Shirota Y, Chiorini JA, Illei GG. Peptide-based ELISAs are not sensitive and specific enough to detect muscarinic receptor type 3 autoantibodies in serum from patients with Sjogren's syndrome. Ann Rheum Dis. 2011;70(1):235–6. doi: 10.1136/ard.2010.129049.

[24] Wang X, Wu H, Zhong B, Zhang L, Wang Y. Autoantibody against aquaporin-5 may be a new diagnostic biomarker for primary Sjögren's syndrome. Clin Rheumatol. 2024;43(12):3781–7. doi: 10.1007/s10067-024-07190-1.

[25] Waterman SA, Gordon TP, Rischmueller M. Inhibitory effects of muscarinic receptor autoantibodies on parasympathetic neurotransmission in Sjögren's syndrome. Arthritis Rheum. 2000;43(7):1647–54. doi: 10.1002/1529-0131(200007)43:7<1647::AID-ANR31>3.0.CO;2-P.