Chronic rhinosinusitis: assessment of changes in nociceptive neurons

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Background: **Pain is a major symptom of chronic rhinosinusitis (CRS). It is mainly associated with CRS without nasal polyps (CRSsNP) and has a major impact in the decision to move on to surgery. Patients with CRS with nasal polyps (CRSwNP) are characterized by trigeminal hypoesthesia and suffer from less pain. The aim of this study was to investigate whether CRS induces alterations in the peripheral nociceptive neurons, mainly focusing on quantitative changes.**

Methods: **Sinus mucosa and inferior turbinate (IT) samples were obtained from patients with CRS, and IT tissue of healthy patients served as controls. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed for neuronal markers including CNTNAP2, FAM19A1, GFRA2, NEFH, NTRK1, PLXNC1, RET, SCN10A, SCN11A, TRPV1, and PGP 9.5; enzyme-linked immunosorbent assay (ELISA) was performed for KCNK18, SCN10A, MRGPRD, and MAP2. For PGP 9.5, immunohistochemistry was additionally used to analyze tissue slides.**

Results: **We included 35 patients with CRSsNP, 47 patients with CRSwNP, and 18 control patients. No differences in expression of the neuronal markers were observed between CRSsNP, CRSwNP, and controls. SCN10A was the only marker exclusively expressed on nociceptive neurons in sinus tissue. No histological difference in nerve fibers was observed between sinus mucosa of both phenotypes.**

Conclusion: **Our results indicate that the nociceptive nerve density in CRSwNP is not lower than in CRSsNP, as was assumed previously. The nociceptive neurons in sinonasal mucosa cannot be classified into subtypes due to the lack of specificity of the respective marker genes. Our findings question the generally accepted claim that nasal polyp tissue does not contain any nerves.** © 2020 ARS-AAOA, LLC.

Key Words:
chronic rhinosinusitis; olfaction; rhinosinusitis; sinus anatomy; nociceptive neurons

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According to the 2012 European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS),[1] chronic rhinosinusitis (CRS) is defined as inflammation of the nose and paranasal sinuses characterized by 2 or more symptoms for more than 12 weeks and signs on imaging. One of the symptoms should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), together with facial pain/pressure and/or reduction or loss of smell. The signs on imaging can be endoscopic signs of disease and/or computed tomography (CT) changes. Although CRS with and without nasal polyps (CRSwNP and CRSsNP, respectively) are both characterized by secretion and nasal obstruction, it is CRSsNP that is frequently associated with facial pain/pressure/fullness.[2] Patients with CRSwNP, on the other hand, suffer more frequently from hyposmia.[3-6]

TABLE 1. Patient and sample characteristics*

RT-qPCR	Control	CRSsNP	CRSwNP
Subjects, n	17	23	35
Age (years)	32 [14–58]	40 [19–72]	51 [16–62]
Female	8 (47.1)	9 (39.1)	11 (31.4)
Skin prick test-positive	0	8 (34.8)	15 (43.9)
Asthma in history	0	2 (8.7)	17 (48.6)
Aspirine intolerance reported	0	0	6 (17.1)

		CRSsNP		CRSwNP	
ELISA	Control	Ethmoidal mucosa	IT	Ethmoidal polyps	IT
Subjects, n	12	12	12	12	12
Age (years)	38 [22–58]	48 [26–65]	35 [26–44]	53 [22–72]	57 [48–67]
Female	4 (36.4)	5 (41.7)	6 (50.0)	4 (33.3)	1 (50.0)
Skin-prick test–positive	0	5 (41.7)	7 (58.3)	6 (50.0)	7 (58.3)
Asthma in history	0	2 (16.7)	6 (50.0)	6 (50.0)	6 (50.0)
Aspirin intolerance reported	0	1 (8.3)	0 (0.0)	3 (25.0)	1 (50.0)

		CRSwNP		
IHC	CRSsNP	Nasal polyps	Maxillary mucosa	Frontal mucosa
Subjects, n	5	5		
Samples, n	5	5	5	5
Age (years)	50 [39–72]		31 [21–69]	
Female	0 (0.0)		2 (40.0)	
Skin prick test-positive	2 (40.0)		2 (40.0)	
Asthma in history	1 (20.0)		2 (40.0)	
Aspirin intolerance reported	0 (0.0)		0 (0.0)	

*Values are presented as median [range] or n (%). The maxillary and frontal mucosa of CRSwNP patients for IHC refer to nonpolypous mucosa of the respective sinus.
CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; ELISA = enzyme-linked immunosorbent assay; IHC = immunohistochemistry; IT = inferior turbinate; ; RT-qPCR = reverse transcription quantitative polymerase chain reaction.

Recent findings have moreover shown that the intranasal sensitivity threshold is elevated in CRSwNP patients, indicating trigeminal hypoesthesia.[7] The histology of nasal polyps is classically characterized by damaged respiratory epithelium, tissue edema, very few blood vessels and glands, and essentially no nerves.[8-11] The latter finding would be an assumable cause of the hypoesthesia and hyposmia.

Sinus sensibility is provided by the fifth cranial nerve, the trigeminal nerve, whose cell bodies lie in the trigeminal ganglion (TG). It has recently been shown that the classic grouping of nociceptive neurons into Aδ-fibers and C-fibers is undifferentiated.[12] In a large-scale single-cell RNA sequencing study, Usoskin et al.[13] showed that nociceptive neurons can be classified into multiple subtypes. Proposed marker genes were CNTNAP2, FAM19A1, GFRA2, NEFH, NTRK1, PLXNC1, RET, SCN10A, SCN11A, and TRPV1. A combination of these genes allows specific targeting of each nociceptor subtype, but individually they are each not specific for a single subtype. Moreover, the specificity in sinonasal mucosa is unknown. On the other hand, SCN10A, KCNK18, and MRGPRD are genes involved in nociception that are known to have a very high trigeminal specificity.[14,15] The SCN10A or Nav1.8 gene is expressed in nociceptors and is essential for inflammatory pain. The channel is also known to be involved in trigeminal neuropathic pain, and variants have been identified in peripheral and central pain disorders.[16-19] The KCNK18 messenger RNA (mRNA) is exclusively expressed in human TG and it is, together with SCN10A, reported to be the most selective ion channel gene in both the human TG and dorsal root ganglion (DRG).[14,15] A mutation within the KCNK18 gene leads to a nonfunctional protein and has been found to be associated with migraines.[20] MRGPRD is very specifically expressed on non-peptidergic nociceptors of the human TG and DRG.

These receptors are involved in both pain sensation and modulation.[14,15,21,22]

We wanted to investigate whether CRS induces alterations in the local peripheral nociceptive neuronal network. Our primary goal was to assess quantitative and histological neuronal differences in CRSsNP and CRSwNP. In order to analyze the specificity of the gene markers of nociceptor subtypes in peripheral sinonasal tissue, we first performed reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Patients and methods

Patients and sample collection

Patients aged 18 years or older who were undergoing sinonasal surgery were included at the Department of Otorhinolaryngology, Ghent University Hospital, Belgium. The tissue sampling was approved by the local ethical committee and written informed consent was obtained from all patients before collecting material. Sinus and inferior turbinate (IT) mucosa samples were taken from patients with CRS. Samples of IT were obtained from patients without sinus disease who were undergoing (rhino)septoplasty, serving as the only controls. Diagnosis of CRS was based on the history, clinical examination, nasal endoscopy, and CT scan, which is consistent with the 2012 EPOS guidelines.[1] The baseline characteristics of the patients are given in Table 1. Oral corticosteroids were stopped at least 4 weeks before surgery and nasal corticosteroids for at least 2 weeks. An atopic evaluation was done by skin-prick tests for common inhalant allergens.[23] The diagnosis of asthma was based on the Global Initiative for Asthma (GINA) guidelines.[24] After collection, the tissue samples were either immediately embedded in paraffin and/or snap-frozen in liquid nitrogen and stored at –80°C.

Tissue homogenates and extractions

Snap-frozen tissue samples (±30 mg) were homogenized by means of mechanical disruption with a mortar and pestle containing liquid nitrogen as described,[25] yielding interstitial and cytosolic proteins. Membrane protein fractions were prepared by means of a ProteoExtract Native Membrane Protein Extraction kit (CalBiochem/EMD Biosciences, San Diego, CA) according to the manufacturer's guidelines. These fractions were used for protein measurements by enzyme-linked immunosorbent assay (ELISA) (see ELISA section below).

RT-qPCR

The following mRNA markers were selected to specifically target multiple subtypes of nociceptive neurons: CNTNAP2, FAM19A1, GFRA2, NEFH, NTRK1, PLXNC1, RET, SCN10A, SCN11A, TRPV1, and PGP 9.5. The measurements were performed on diseased sinus samples of CRSsNP and CRSwNP, control IT tissue, and on commercially acquired RNA of human DRG to control the efficiency of these mRNA markers (TaKaRa Bio Europe, St-Germain-en-Laye, France). Total RNA isolation was performed using the RNeasy Mini Kit (Qiagen, Antwerp, Belgium) and complementary DNA (cDNA) was synthesized from 1 μg of RNA using the iScript Advanced cDNA Synthesis Kit for RT-qPCR (Bio-Rad, Temse, Belgium) following the respective manufacturers' instructions. Amplifications were performed in a Light Cycler LC480 System (Roche, Vilvoorde, Belgium) by using a specific PrimePCR Assay (Bio-Rad) as given in Table 2.

All results were normalized for transcription and amplification variations against the results of the validated reference genes, which are listed in Table 3. The

TABLE 2. Markers used for the RT-qPCR analysis, ELISA, and IHC*

Abbreviation	Marker
RT-qPCR	
CNTNAP2	Contactin associated protein-like 2
FAM19A1	Family with sequence similarity 19 (chemokine (C-C motif)-like), member A1
GFRA2	Glial cell-derived neurotrophic factor (GDNF) family receptor alpha 2
NEFH	Neurofilament, heavy polypeptide
NTRK1	Neurotrophic tyrosine kinase, receptor, type 1
PLXNC1	Plexin C1
RET	Ret proto-oncogene
SCN10A or Nav1.8	Sodium channel, voltage-gated, type X, alpha subunit
SCN11A or NAV1.9	Sodium channel, voltage-gated, type XI, alpha subunit
TRPV1	Transient receptor potential cation channel, subfamily V, member 1
UCHL1 or PGP 9.5	Ubiquitin C-terminal esterase L1 (ubiquitin thiolesterase) or protein gene product 9.5
ELISA	
Kcnk18	Potassium channel subfamily K member 18 (MBS280172)
MAP2	Microtubule associated protein 2 (MBS703770)
Mrgpr	MAS-related G protein-coupled receptor (MBS9340224)
SCN10A or Nav1.8	Sodium voltage-gated channel alpha subunit 10 (MBS9319177)
IHC	
PGP 9.5	Protein gene product 9.5

*The catalogue number of the ELISA kits (MyBioSource, San Diego, CA) is shown between parentheses after the abbreviation.
ELISA = enzyme-linked immunosorbent assay; IHC = immunohistochemistry; RT-qPCR = reverse transcription quantitative polymerase chain reaction.

TABLE 3. Primer sequences for qPCR[30]

Gene	Forward (5′ → 3′)	Reverse (5′ → 3′)	Amplicon size (bp)	Accession number	Efficiency (%)
EF-1	CTGAACCATCCAGGCCAAAT	GCCGTGTGGCAATCCAAT	59	NM_001402	93.9
SDHA	TGGGAACAAGAGGGCATCTG	CCACCACTGCATCAAATTCATG	86	NM_004168	88.9

bp = base pair; EF-1 = elongation factor 1; qPCR = quantitative polymerase chain reaction; SDHA = succinate dehydrogenase complex flavoprotein subunit A.

normalized relative quantities (NRQs) were calculated from the obtained cycle quantification (Cq) values with qBase + software (Biogazelle, Ghent, Belgium) and the final gene expression results are expressed as the logarithm of NRQs per 5 ng cDNA.

ELISA

Protein concentrations of KCNK18, SCN10A, MRGPRD, and MAP2 were measured on diseased sinus and IT mucosa and on IT of healthy controls by means of ELISA kits (My-BioSource, San Diego, CA) according to the instructions of the manufacturer (Table 2).

Immunohistochemical staining

Nasal polyp tissue and nonpolypous mucosa were gathered separately from patients with CRSwNP. Twenty tissue samples from the last 5 CRSsNP and 5 CRSwNP patients were prepared with PGP 9.5 as primary antibody (Thermo Scientific, Merelbeke, Belgium) in accordance with the manufacturer's instructions. Random fields of 9 mm^2 per sample were systematically analyzed by immunohistochemical (IHC) staining without knowing the diagnosis or clinical data.

Statistical analysis

All statistical analyses were performed with the SPSS statistical package, version 25.0 (IBM Corp., Armonk, NY). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test for normal distribution of the variables. Variables without a normal distribution are presented as median [range]. The mRNA data were used as logarithmic data. To compare continuous variables in 2 groups, we used the Mann-Whitney *U* test or a Student *t* test. Comparisons between more than 2 groups were made by using the Kruskal-Wallis test or the 1-way analysis of variance (ANOVA) test as a parametric test. Confidence interval refers to 95% confidence interval. A 2-sided p value <0.05 was considered to indicate statistical significance. A post hoc power analysis with an alpha level of 0.05 on the SCN10A protein concentration suggests that the current sample size has a statistical power of 71.1%.[26]

Measurements were considered as outliers if the value was not contained by the interval [Q1 – 1.5*IQR, Q3 + 1.5*IQR] and as extreme outliers if the value lay outside [Q1 – 3*IQR, Q3 + 3*IQR] (with Q1 = lower quartile; Q3 = upper quartile; IQR = interquartile range).[27]

Results

Clinical data

We included 35 patients with CRSsNP, 47 patients with CRSwNP, and 18 control patients. All baseline and clinical patient characteristics are shown in Table 1.

RT-qPCR

We found that only SCN10A mRNA was not detectable in the peripheral sinus mucosa samples (see Fig. 1).

ELISA

The IT mucosa of patients without sinus disease was used as control. No significant difference in expression of the neuronal markers KCNK18, SCN10A, MRGPRD, and MAP2 was observed between CRSsNP and CRSwNP sinus mucosa and controls (Fig. 2). Moreover, there was no difference in expression of the markers between the sinus mucosa of CRSsNP or CRSwNP and the nasal mucosa of the respective patient group. None of the markers were significantly differently expressed in the IT mucosa of the healthy controls compared to the IT mucosa of CRSsNP and CRSwNP (Supporting Fig. 1). There was also no significant difference between the nasal tissue of both diseased groups.

IHC

Immunostaining with the pan-neural marker PGP 9.5 was distributed in the sinus tissue of both CRSsNP (Fig. 3A,B) and CRSwNP (Fig. 3C,D). Within the CRSwNP group, neurons were detected both in the nonpolyp mucosa and in nasal polyps. Nerve fibers surrounding mucosal blood vessels and glandular ducts were most likely autonomic fibers (Fig. 3E-G, respectively). No clear difference in appearance of the neurons was observed between groups, but thicker nerve fibers, such as shown in Figure 3A,B, were not found in any of the 10 samples of CRSwNP patients.

Discussion

Facial pain/pressure/fullness is a cardinal symptom of CRS but is mostly seen in patients with CRSsNP.[2] Patients with CRSwNP suffer by contrast more frequently from intranasal hypoesthesia and hyposmia.[3-7] The exact role that nerves play in the inflammation remains an important challenge for CRS researchers.[28] Pain sensation is established through a complex pathway; we especially focused on quantitative differences of nociceptive neurons between

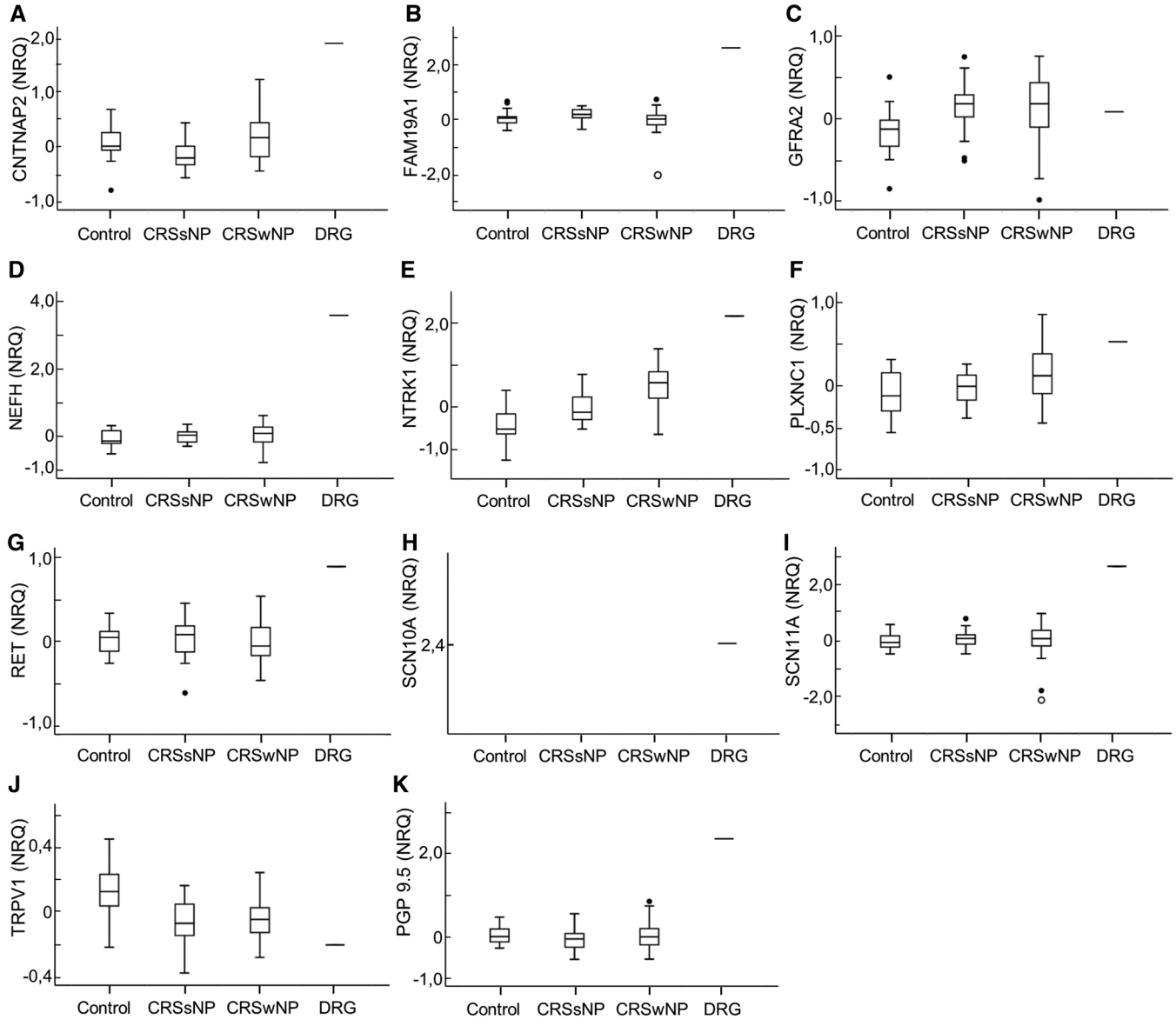

FIGURE 1. RT-qPCR analysis. The RT-qPCR analysis was performed on CRSsNP and CRSwNP sinus mucosa; IT of patients without sinus disease served as controls and DRG samples as control for the marker efficiency. Only SCN10A mRNA was not detectable in sinus mucosa. All mRNA markers were measurable in DRG. (A) CNTNAP2 mRNA, (B) FAM19A1 mRNA, (C) GFRA2 mRNA, (D) NEFH mRNA, (E) NTRK1 mRNA, (F) PLXNC1 mRNA, (G) RET mRNA, (H) SCN10A mRNA, (I) SCN11A mRNA, (J) TRPV1 mRNA, and (K) PGP 9.5 mRNA. Closed circles represent outliers and open circles represent extreme outliers. The boxes indicate the median and IQR. CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; DRG = dorsal root ganglion; IQR = interquartile range; IT = inferior turbinate; mRNA = messenger RNA; NRQ = normalized relative quantities; RT-qPCR = reverse transcription quantitative polymerase chain reaction.

sinonasal mucosa of both CRS phenotypes. Usoskin et al.[13] suggested a classification of nociceptive neurons, in which every subtype is characterized by multiple marker genes. To know what genes are specific for neurons in peripheral sinonasal tissue, we first performed RT-qPCR. Indeed, this mucosa only contains the peripheral process of the neurons and not the neuronal mRNA, which is centrally localized in the TG on the base of the skull. Human DRG-derived mRNA was used to control the efficiency of the mRNA markers and PGP 9.5 mRNA was assessed as a general neuronal marker. Only the mRNA expression of SCN10A was not measurable in the sinonasal mucosa samples, but clearly present in DRG, indicating that SCN10A was the only marker not expressed by other cells than neurons in the mucosal tissue. The mRNA of all other marker genes could be amplified from the sinonasal samples, so these are not specific for sensory neurons.

Therefore, the nociceptor protein markers SCN10A, KCNK18, and MRGPRD were selected based on our qPCR results and on the work of Flegel et al.[14] and Manteniotis et al.[15] Criteria were trigeminal specificity, height of expression, and involvement in nociception and inflammatory pain. By contrast, MAP2 is a more general neuronal marker, not specific for sensory neurons.

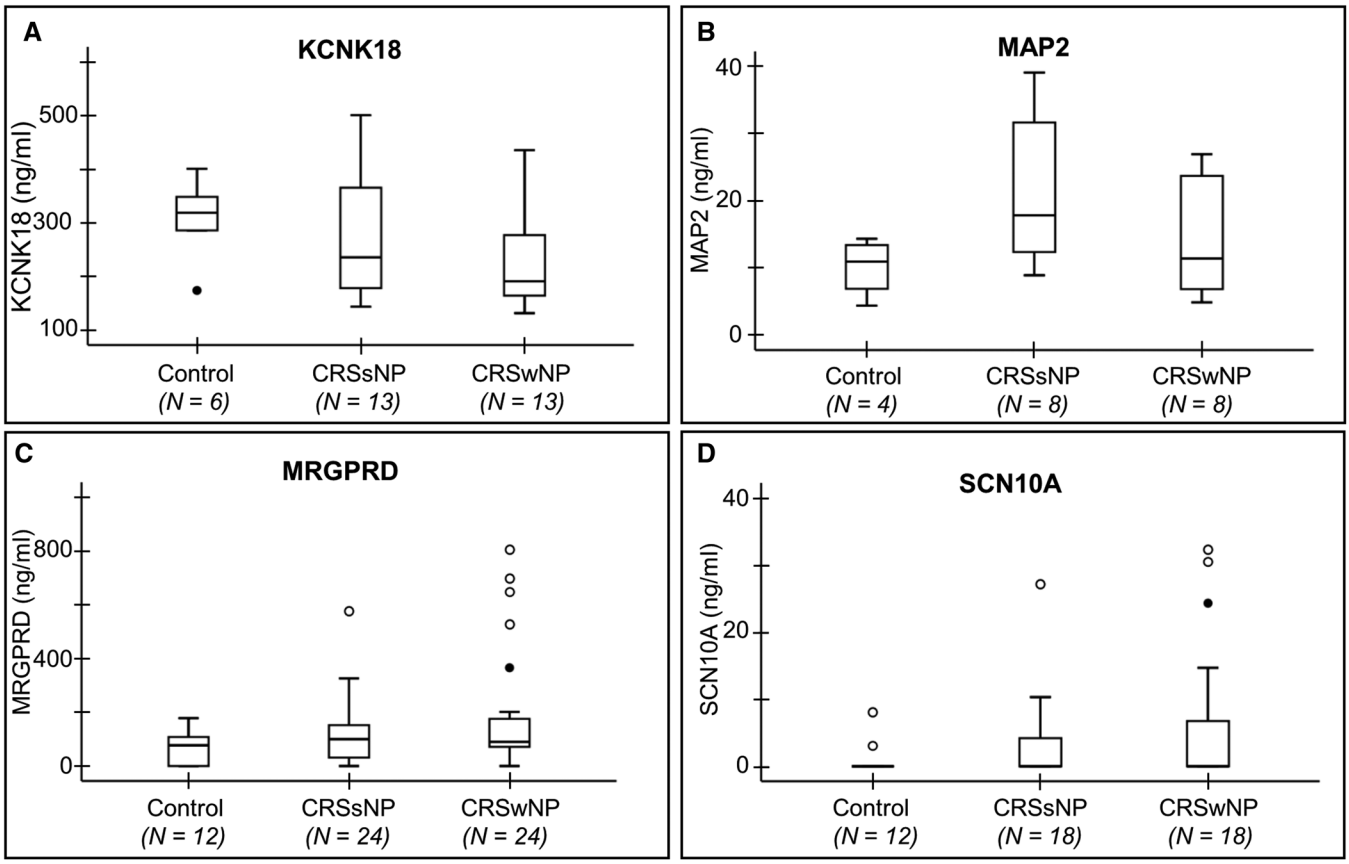

FIGURE 2. ELISA. The graphs show no difference in protein expression of KCNK18, SCN10A, MRGPRD, and MAP2 between CRSsNP and CRSwNP sinus mucosa and IT of patients without sinus disease as controls. (A) KCNK18 membrane fraction, (B) MAP2 soluble fraction, (C) MRGPRD membrane fraction, (D) SCN10A membrane fraction. Closed circles represent outliers and open circles represent extreme outliers. The boxes indicate the median and IQR. CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; IQR = interquartile range; IT = inferior turbinate.

There was no difference in expression levels of the markers on protein level. This indicates that the quantity of neurons and nociceptor neurons is similar in CRSsNP, CRSwNP, and controls. There was moreover no significant difference in concentration of the neuronal protein markers between sinus and nasal tissue in patients with CRSsNP and CRSwNP. These findings do not, however, exclude functional differences between the different subject groups, for example by point mutations or by the degree of phosphorylation of the SCN10A channels.

Nasal polyps are classically characterized by damaged respiratory epithelium, tissue edema, very few blood vessels and glands, and essentially no nerves.[8-11] It is even assumed that the edema is caused by decreased secretory activity of the glands and abnormal vascular permeability due to complete denervation of the nasal polyps. IHC staining with the pan-neuronal marker PGP 9.5, however, showed nerve fibers in the nasal polyps and in the nonpolyp sinus mucosa of CRSwNP patients. Clear differences in appearance of the nerve fibers or density were not observed between CRSsNP and CRSwNP by means of IHC. As a limiting factor, there is a lot of variation in the histological pattern of nasal polyps, both between patients and within the same polyp of a single patient.

In this pilot study, the number of included patients was rather low. The post hoc power analysis is of concern for interpreting the nonsignificant results. Because all patients were included in our tertiary hospital, we cannot exclude a selection bias of a more severe group of patients. Moreover, the presence of pain is an argument for a surgical intervention and thus tissue collection. A useful variable that is lacking in our fundamental pilot study is the clinical presence of pain with its severity and location. Turbinate tissue was used from control patients because no sinus mucosa was obtained. However, it is known that the ITs in CRS develop a similar inflammatory pattern as the sinuses.[29] Moreover, there was no difference in expression of the protein markers between the nasal tissue of controls and both CRS phenotypes. Because this study focused on quantitative neuronal differences, we cannot exclude functional differences between the different subject groups, eg, by point mutations, or by the degree of phosphorylation of the SCN10A channels.

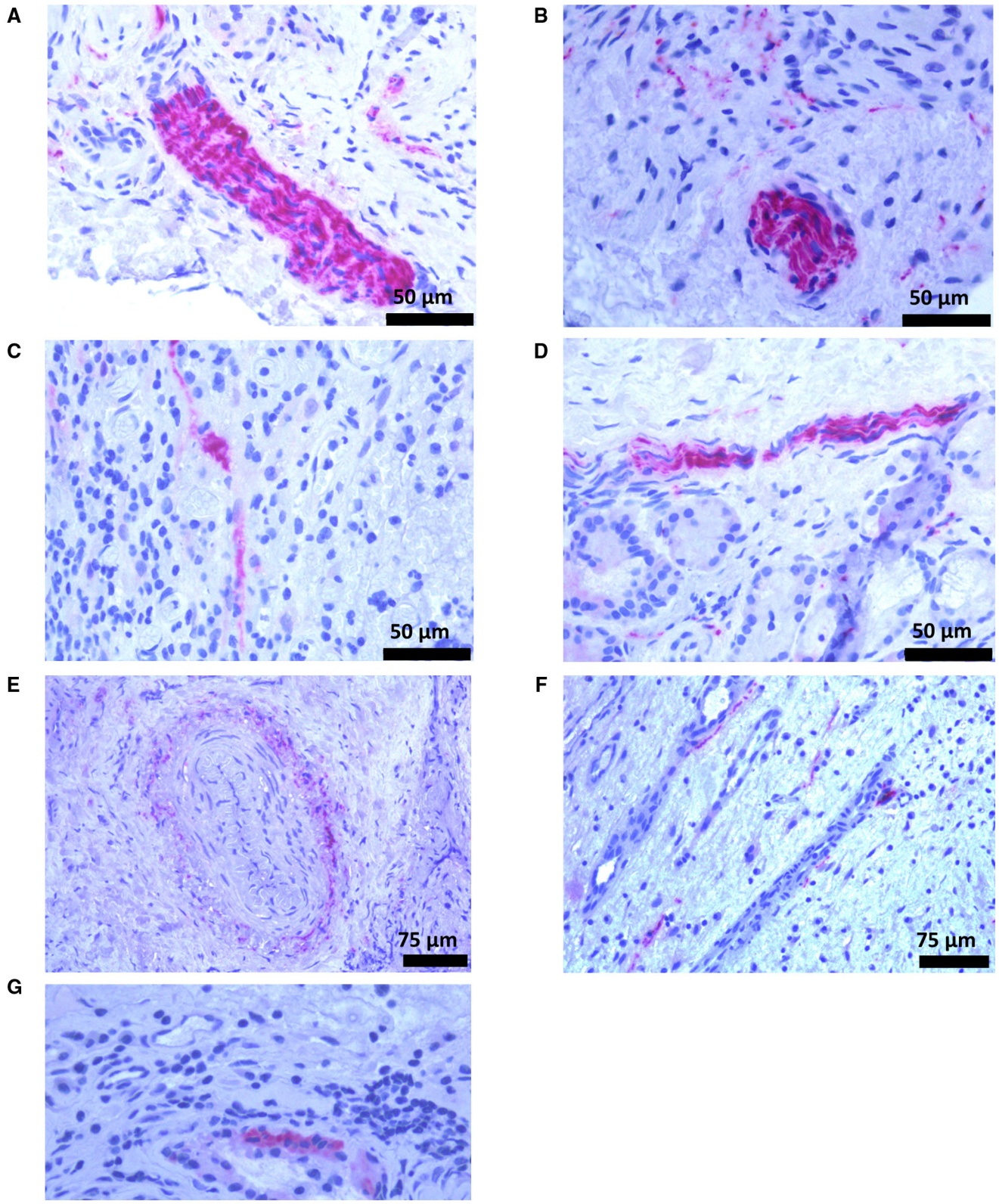

FIGURE 3. Immunolabeling with antibodies directed against PGP 9.5 in sinonasal tissues of patients with CRS. PGP 9.5-immunoreactive fibers in sinus mucosa of a patient with CRSsNP (A,B), in nonpolyp mucosa of a patient with CRSwNP (C), and in a nasal polyp (D). Autonomic fibers around blood vessels in sinus mucosa of a patient with CRSsNP (E), in a nasal polyp (F), and a in glandular duct in a nasal polyp (G). CRS = chronic rhinosinusitis; CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps.

Conclusion

This is the first work that has investigated whether the inflammatory reactions in CRS induces alterations in the local peripheral nociceptor neuronal network. We showed that the nociceptive nerve density is not lower in CRSwNP than CRSsNP, as was assumed previously. Both our findings on protein level and on histology question the generally accepted claim that nasal polyp tissue does not contain any nerves. Nociceptive neurons cannot be quantified by subtype because of the lack of specificity of the respective marker genes. The findings of this pilot study suggest that the difference in sensation is caused by functional, qualitative changes of nociceptive neurons rather than quantitative alterations.

References

1. Fokkens WJ, Lund VJ, Mullol J, et al. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhinology*. 2012;50:1-12.
2. Bachert C, Pawankar R, Zhang L, et al. ICON: chronic rhinosinusitis. *World Allergy Organ J*. 2014;7:25.
3. Gregurić T, Trkulja V, Baudoin T, Grgić M, Šmigovec I, Kalogjera L. Differences in the Sino-Nasal Outcome Test 22 and visual analog scale symptom scores in chronic rhinosinusitis with and without nasal polyps. *Am J Rhinol Allergy*. 2016;30:107-112.
4. Poetker DM, Mendolia-Loffredo S, Smith TL. Outcomes of endoscopic sinus surgery for chronic rhinosinusitis associated with sinonasal polyposis. *Am J Rhinol*. 2007;21:84-88.
5. Banerji A, Piccirillo JF, Thawley SE, et al. Chronic rhinosinusitis patients with polyps or polypoid mucosa have a greater burden of illness. *Am J Rhinol*. 2007;21:19-26.
6. Eweiss AZ, Lund VJ, Barlow J, Rose G. Do patients with chronic rhinosinusitis with nasal polyps suffer with facial pain? *Rhinology*. 2013;51:231-235.
7. Poletti SC, Cuevas M, Weile S, Hummel T. Trigeminal sensitivity in chronic rhinosinusitis: topographical differences and the effect of surgery. *Rhinology*. 2017;55:70-74.
8. Mygind N, Dahl R, Bachert C. Nasal polyposis, eosinophil dominated inflammation, and allergy. *Thorax*. 2000;55(Suppl 2):S79-S83.
9. Bachert C, Gevaert P, Holtappels G, Van Cauwenberge P. Mediators in nasal polyposis. *Curr Allergy Asthma Rep*. 2002;2:481-487.
10. Mygind N, Lund V. Intranasal corticosteroids for nasal polyposis. *Treat Respir Med*. 2006;5:93-102.
11. Settipane GA. Nasal polyps: epidemiology, pathology, immunology, and treatment. *Am J Rhinol*. 1987;1:119-126.
12. Pinho-Ribeiro FA, Verri WA Jr, Chiu IM. Nociceptor sensory neuron–immune interactions in pain and inflammation. *Trends Immunol*. 2017;38:5-19.
13. Usoskin D, Furlan A, Islam S, et al. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci*. 2015;18:145.
14. Flegel C, Schöbel N, Altmüller J, et al. RNA-seq analysis of human trigeminal and dorsal root ganglia with a focus on chemoreceptors. *PLoS One*. 2015;10:e0128951.
15. Manteniotis S, Lehmann R, Flegel C, et al. Comprehensive RNA-Seq expression analysis of sensory ganglia with a focus on ion channels and GPCRs in trigeminal ganglia. *PLoS One*. 2013;8:e79523.
16. Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron*. 2000;26:13-25.
17. Duan G, Han C, Wang Q, et al. A SCN10A SNP biases human pain sensitivity. *Mol Pain*. 2016;12:1744806916666083.
18. Strickland IT, Martindale JC, Woodhams PL, Reeve AJ, Chessell IP, McQueen DS. Changes in the expression of NaV1.7, NaV1.8 and NaV1.9 in a distinct population of dorsal root ganglia innervating the rat knee joint in a model of chronic inflammatory joint pain. *Eur J Pain*. 2008;12:564-572.
19. Dib-Hajj SD, Cummins TR, Black JA, Waxman SG. Sodium channels in normal and pathological pain. *Annu Rev Neurosci*. 2010;33:325-347.
20. Lafreniere RG, Cader MZ, Poulin JF, et al. A dominant-negative mutation in the TRESK potassium channel is linked to familial migraine with aura. *Nat Med*. 2010;16:1157-1160.
21. Wang C, Gu L, Ruan Y, et al. Facilitation of MrgprD by TRP-A1 promotes neuropathic pain. *FASEB J*. 2019;33:1360-1373.
22. Shinohara T, Harada M, Ogi K, et al. Identification of a G protein-coupled receptor specifically responsive to beta-alanine. *J Biol Chem*. 2004;279:23559-23564.
23. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008. *Allergy*. 2008;63:8-160.
24. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. Fontana, WI: GINA; 2020. https://ginasthma.org/. Accessed May 25, 2020.
25. Van Crombruggen K, Holtappels G, De Ruyck N, Derycke L, Tomassen P, Bachert C. RAGE processing in chronic airway conditions: involvement of *Staphylococcus aureus* and ECP. *J Allergy Clin Immunol*. 2012;129:1515-1521.e8.
26. ClinCalc. Post-hoc Power Calculator. https://clincalc.com/stats/Power.aspx. Arlington Heights, IL: ClinCalc, LLC; 2018. Accessed May 25, 2020.
27. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods*. 2009;41:1149-1160.
28. Schleimer RP. Immunopathogenesis of chronic rhinosinusitis and nasal polyposis. *Ann Rev Pathol*. 2017;12:331-357.
29. Van Crombruggen K, Van Bruaene N, Holtappels G, Bachert C. Chronic sinusitis and rhinitis: clinical terminology "chronic rhinosinusitis" further supported. *Rhinology*. 2010;48:54-58.
30. Pattyn F, Robbrecht P, De Paepe A, Speleman F, Vandesompele J. RTPrimerDB: the real-time PCR primer and probe database, major update 2006. *Nucleic Acids Res*. 2006;34(Suppl 1):D684-D688.