Trigeminal cold receptors and airflow perception are altered in chronic rhinosinusitis*

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Rhinology 62: 1, 63 - 70, 2024
https://doi.org/10.4193/Rhin23.128

***Received for publication:**
April 11, 2023
Accepted: September 9, 2023

Abstract

Background: In chronic rhinosinusitis (CRS), nasal obstruction can often be explained by anatomical deformities, polyps, or congested nasal mucosa. However, in cases with little deformity or inflammation, perceived nasal obstruction may result from reduced airflow perception caused by an alteration of the intranasal trigeminal system. The aim of this study was to assess this association.

Methodology: We performed a prospective case-control study of 15 CRS patients, 18 patients with a deviated nasal septum (DNS) and 16 healthy controls. We assessed olfactory function using the Sniffin' Sticks test and Visual Analog Scales (VAS). We used the Trigeminal Lateralization Task (TLT) with eucalyptol and cinnamaldehyde to examine intranasal trigeminal function. Further, we assessed nasal patency with Peak Nasal Inspiratory Flow and VAS. Finally, we measured protein levels of trigeminal receptors (TRPM8, TRPA1 and TRPV1) and inflammatory markers (IL-13, INF-γ and eosinophils) in CRS and DNS patients' mucosal biopsies using Western Blots.

Results: CRS patients had significantly lower olfactory function than DNS and healthy controls. They also had significantly lower TLT scores for eucalyptol than both other groups. CRS patients had significantly lower nasal patency than controls; for DNS patients this was limited to subjective measures of nasal patency. In line with this, CRS patients exhibited significantly higher levels of sTRPM8-18 than DNS patients.

Conclusions: Intranasal trigeminal function is decreased in CRS patients, possibly due to the overexpression of short isoforms of TRPM8 receptors.

Key words: chronic rhinosinusitis, trigeminal system, nasal obstruction, trigeminal receptors, TRPM8

Introduction

Nasal obstruction is a main complaint in chronic rhinosinusitis (CRS) [(1)]. It is often explained by structural deformities such as septal deviation, nasal polyposis, or edematous and inflamed nasal mucosa. However, in some cases, little anatomical deformity or discrete obstructive mucosal inflammation is present even though patients complain of severe nasal obstruction. In these cases, alterations of afferent neural pathways responsible for airflow perception, namely the intranasal trigeminal system may cause reduced subjective nasal patency [(2, 3)]. Nasal patency is perceived via activation of multimodal receptors on the trigeminal nerve located on the nasal cavity's epithelium. These receptors respond to temperature changes (e.g., low temperatures are associated with increased intranasal airflow), but also to chemical stimulation, such as menthol or eucalyptol. Perceptually, exposure to these chemicals causes the same sensation of cooling as does increased airflow [(4)]. In addition, the trigeminal system is engaged in perception of warmth, burning, stinging, or tickling by volatile substances [(5, 6)], in the sensation of pain and in neurogenic inflammation [(7)].

Receptors belonging to the Transient Receptor Potential (TRP) channels family play a key role in perceiving trigeminal stimuli. Particularly, TRPM8 receptors allow for sensations such as coolness [8, 9]. Interestingly, the short isoforms sTRPM8-18 and sTRPM8-6 negatively regulate menthol and cold induced channel activity by stabilizing the closed state of the channel [10]. Other receptors of the TRP family include TRPA1 receptors (responsible for burning sensations following e.g. stimulation with cinnamaldehyde [11]) and TRPV1 receptors (responsible for stinging and burning sensations following e.g., stimulation with capsaicin [12]). TRPA1 and TRPV1 receptors are implicated in neurogenic inflammation [7, 11].

Patients with low intranasal trigeminal sensitivity may be more prone to suffer from nasal obstruction [13]. CRS patients exhibit decreased trigeminal function for eucalyptol, an TRPM8 agonist [3]. However, the mechanism underlying the reduced trigeminal function in CRS is unclear. Our study sought to assess the role of the intranasal trigeminal system in the sensation of nasal obstruction in CRS patients by carrying out a complete nasal chemosensory assessment and analysing the relationship between perception of nasal patency, and expression of trigeminal receptors (TRPA1, TRPV1, sTRPM8-18 and sTRPM8-6) and inflammatory markers (IL-13, INF-γ and eosinophil).

Materials and methods

This prospective study was carried out at Geneva University Hospitals (data collection and nasal mucosal biopsies) and at Université du Québec à Trois-Rivières (protein level quantification). We performed the study according to the Declaration of Helsinki on Biomedical Research Involving Human Subjects; it was approved by the institutional ethics review boards (IRB approval No: 2018-02234 (Geneva) and CER-19-256-10.01 (Trois-Rivières)).

Participants

We recruited 15 patients with CRS (without polyps: n=12, grade 1: n=1, grade 3-4: n=1, unknown: n=1; mean age: 37 ± 13), 18 patients with a deviated nasal septum (DNS) (mean age: 36 ± 13) and 16 healthy controls (mean age: 35 ± 9).

We established CRS diagnosis according to current recommendations [14]. Specifically, CRS patients presented symptoms >12 weeks including two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), with or without facial pain/pressure and reduction or loss of smell in conjunction with signs of paranasal inflammation seen in nasal endoscopy or CT. We determined DNS diagnosis by the presence of chronic nasal obstruction and a deviated septum on nasal endoscopy without signs or other symptoms of CRS. Both groups (CRS, DNS) had a full ENT examination including nasal endoscopy and CT. The healthy control group did not complain of any nasal or paranasal symptoms, and they had a clinical examination to rule out any anatomical deformity or mucosal inflammation.

All participants were recruited at the ENT department of the Geneva University Hospitals. We excluded participants with a complete obstruction due to septal deviation, as this would interfere with the testing regimen (intranasal trigeminal function and nasal patency). CRS and DNS patients included were unsatisfied with medical treatment initially conducted to improve their disease burden; their clinical situation required surgery for further management of their nasal condition. We harvested nasal mucosa biopsies during that surgery.

DNS patients served as controls for expression of trigeminal receptors and inflammatory markers. To avoid a bias in the group comparisons, we specifically compared intranasal trigeminal function scores between healthy controls and the DNS group.

Methods

Psychophysical tests

Intranasal trigeminal function: We assessed trigeminal function using the trigeminal lateralization task (TLT; [15]). Two 250ml squeeze bottles were presented simultaneously to each participant's nostrils. One bottle contained the mixed olfactory-trigeminal stimulus (target: 30 ml (a) eucalyptol; eucalyptus odor, or (b) cinnamaldehyde, cinnamon odor, both, Sigma-Aldrich, Switzerland); the other bottle contained clean air. We delivered a puff of air by pressing the two bottles simultaneously. We applied a total of 40 pseudo-randomized stimuli, at an inter-stimulus interval of 30-40s. Participants were blindfolded to avoid visual cues. After each stimulation, participants identified the stimulated nostril (forced choice). The sum of correct identifications was used to estimate trigeminal sensitivity (score range: 0- 40).

Nasal patency: (A) Objective: We assessed nasal patency using the Peak Nasal Inspiratory Flow (PNIF; [16]). We employed a portable spirometer with a face mask adapted to the participant's mouth and nose. At the end of a maximal expiration followed by three medium deep breaths, we asked participants to perform a forced maximal inspiration with their mouth closed to measure the highest airflow achieved through both nostrils. This was repeated three times, and the highest value was recorded. (B) Subjective: A Visual Analog Scale (VAS; 0: complete obstruction; 100: empty nose) was used to rate nasal patency.

Olfactory function: (A) Objective: We assessed olfactory function using the Sniffin' Sticks test kit (Burghart, Germany) including olfactory threshold, discrimination, and identification [17,18]. In short, this test is based on felt-tip pen-like odor dispensing devices and allows for separate evaluation of odor discrimination, identification, and detection threshold. (B) Subjective: A Visual Analog Scale (VAS; 0: absence of smell; 100: excellent sense of smell) was used to rate olfactory function.

Nasal mucosal biopsy

We intraoperatively took biopsies of the middle turbinate (me-

dial face) from 15 CRS patients (mean age: 37 ± 13) undergoing functional endoscopic sinus surgery and 15 DNS patients (mean age: 36 ± 12) undergoing septoturbinoplasty. We immediately transferred the tissue samples in RNAse free tubes, stored at -80°C in 500µl of TRIzol reagent (Thermo Scientific, Ottawa, ON, Canada).

Protein level quantification

We lysed samples, separated phases (Pub. No. MAN0001271), and isolated the proteins [19]. After lysing samples and obtaining three separated phases (RNA, proteins, and DNA), we removed the aqueous solution containing the RNA and precipitated DNA from the remaining solution with 100% ethanol (3:10 with TRIzol regent). This phenol-ethanol supernatant was moved to a new tube. Next, we precipitated proteins by adding isopropanol (2:1 of TRIzol regent), vortexed, incubated samples for 10 minutes, after which proteins were pelleted by centrifugation (10 mins; 4°C; 12000xg). After discarding the supernatant, pellets were washed (500µL, 95% ethanol) and centrifuged (5 mins; 4°C; 7600xg). This procedure was performed a second time with 250µL of 95% ethanol. Finally, protein pellets were air dried (10 mins).

Next, we homogenized mucosal samples in a solution of RIPA buffer (50mM Tris-HCl, 0.15M NaCl, 1% Triton, 0.25% Sodium deoxycholate, 1mM EDTA and a cocktail of protease and phosphatase inhibitors; Roche, Indianapolis, IN, USA). We quantified protein concentrations by Bradford assay (Bio-Rad, Hercules, CA, USA). We electrophoresed protein lysate (TRPA1 and TRPV1: 6.25µg; sTRPM8-6 and sTRPM8-18: 20µg) on 10-20% SDS-PAGE and transferred on nitrocellulose membranes. Subsequently, we blocked the membranes in 5% BSA/TBS-Tween 0.1% for 1 hour at room temperature and incubated them overnight at 4°C with the primary antibodies diluted in 1% BSA/TBS-Tween 0.1%. We raised rabbit polyclonal antibodies against TRPA1 (1:15000, NB110-40763, Novus Biologicals), TRPV1 (1:1000, PA1-748, Thermo Scientific), sTRPM8-6 and sTRPM8-18 (1:500, ab3243, Abcam, Cambridge, MA), IL-13 (1:500, ab106732, Abcam), INF-γ (1:500, ab9657, Abcam) and eosinophil (1:500, NBP3-03635, Novus Biologicals). Next, we washed the membranes in TBS-Tween 0.1% three times for 5 mins, and incubated them with anti-rabbit IgG, HRP-linked antibody (1:10000, Cell Signaling Technology, Danvers, MA, USA, #7074) diluted in 1% BSA/TBS-Tween 0.1% for 1 hour at room temperature. The membranes were then washed in TBS-Tween 0.1% three times for 5 minutes before visualizing protein bands. We used rabbit monoclonal antibody against β-actin (1:50000, Cell Signaling Technology, #5125) as a loading control. To visualize protein bands, we performed chemiluminescence reactions using SuperSignal West Femto Chemiluminescence Kit (Thermo Scientific) for TRPA1, TRPV1, TRPM8 and IL-13 and we used SuperSignal West Pico PLUS Chemiluminescent Kit (Thermo Scientific) for INF-γ, eosinophil and β-actin. We analyzed the densitometry using Vision work LS software (UVP bioimaging, Upland, CA, USA).

Table 1. Descriptive statistics, mean scores and standard deviation for the psychophysical tests for the three groups.

	CRS (n=15)	DNS (n=18)	Healthy controls (n=16)
Olfactory function			
TDI	27.15 (8.93)	33.15 (5.17)	34.42 (5.97)
VAS olfactory	44.33 (22.59)	72.67 (13.73)	65.00 (19.06)
Intranasal trigeminal function			
TLT eucalyptol	29.33 (6.83)	34.11 (4.65)	35.94 (4.78)
TLT cinnamaldehyde	26.67 (5.67)	25.11 (6.49)	28.38 (4.90)
Nasal patency			
PNIF (L/min)	91.33 (36.81)	124.17 (56.42)	136.25 (51.75)
VAS nasal patency	29.00 (17.75)	37.06 (17.28)	77.19 (12.78)

Statistical analysis

Psychophysical tests analysis

We analysed data with SPSS 28.0 (SPSS Inc., Chicago, IL, USA). Alpha value was set to 0.05.

Intranasal trigeminal function: We performed a repeated-measures (rm) ANOVA on TLT scores with group (3 levels: CRS, DNS, healthy controls) as between subject factors (bsf) and stimulus (2 levels: eucalyptol, cinnamaldehyde) as within subject factors (wsf). To disentangle interactions, we subsequently performed two univariate ANOVA (one by test), with group (wsf; 3 levels: CRS, DSN, healthy controls). For significant effects, we ran post-hoc t tests with Bonferroni-Holm corrections. In order to verify if participants were able to localize both stimuli, we carried out one sample t-tests (vs chance score of 20) for each stimulus and group separately.

Nasal patency: We performed a rmANOVA with group (bsf; 3 levels: CRS, DNS, healthy controls) and test (wsf; 2 levels: PNIF, VAS nasal patency). We subsequently analyzed if there was any group difference on average score for nasal patency. To do so, we performed two univariate ANOVA (one by tests), with group (wsf; 3 levels: CRS, DSN, healthy controls). For significant effects, we ran post-hoc t tests with Bonferroni-Holm corrections.

Interactions between objective and subjective measurements: We examined whether scores for objective and subjective measurements of nasal patency (PNIF and VAS nasal patency) were correlated with Pearson's correlations.

Interactions between intranasal trigeminal function and nasal patency: We examined whether scores for TLT eucalyptol were

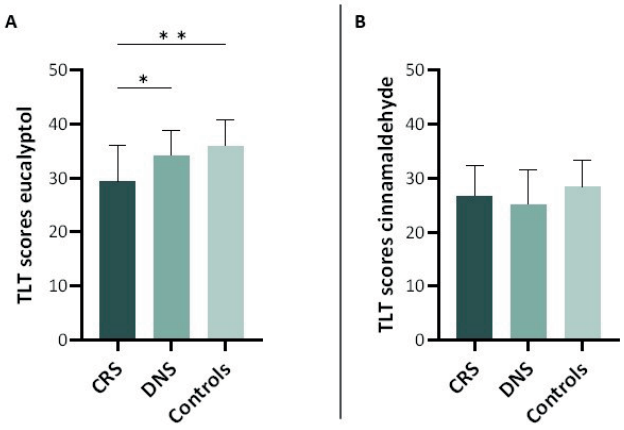

Figure 1. Trigeminal Lateralization Task (TLT) according to the groups (chronic rhinosinusitis - CRS, deviation of the nasal septum - DNS and controls): A) Means of TLT for eucalyptol and B) Means of TLT for cinnamaldehyde. Error bars represent standard deviation (SD).

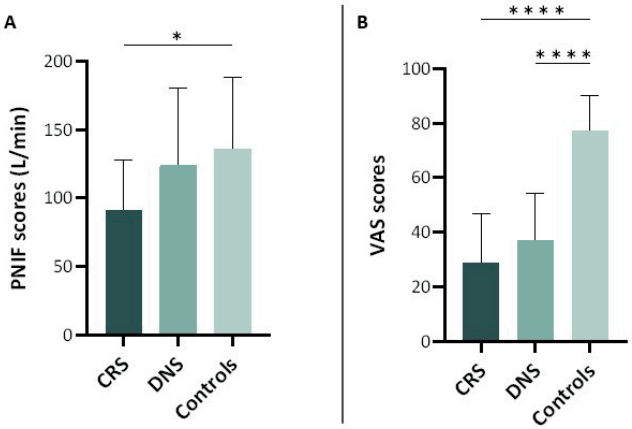

Figure 2. Nasal patency results according to the groups (CRS, DNS and controls): A) Means of Peak Nasal Inspiratory Flow (PNIF) and B) Means of Visual Analog Scale (VAS) for nasal patency. Error bars represent standard deviation (SD).

correlated with objective and subjective measurements of nasal patency (PNIF and VAS nasal patency) with Pearson's correlations.

Olfactory function: We performed a rmANOVA on TDI scores with group (bsf; 3 levels: CRS, DNS, healthy controls) and test (wsf; 2 levels: TDI, VAS olfactory). We subsequently analyzed if there was any group difference on average score for olfactory function, by performing two univariate ANOVA (one by tests), with group (wsf; 3 levels: CRS, DSN, healthy controls). For significant effects, we ran post-hoc t tests with Bonferroni-Holm corrections.

Protein level quantification analysis

We analysed data statistically using GraphPad Prism software (Version 9.4.1, GraphPad Software, San Diego, CA, USA). Data were reported as the mean ± SEM. We set the alpha value to 0.05.

Protein levels of trigeminal receptors and inflammatory markers: Since data of TRPA1, TRPV1 and sTRPM8-6 were not normally distributed, we compared CRS vs DSN groups with Wilcoxon's test. We performed a paired t-test on sTRPM8-18. We then analyzed the protein levels of inflammatory markers IL-13 (paired t-test), INF-γ and eosinophil (not normal; Wilcoxon's test).

Interaction between protein levels of trigeminal receptors and intranasal trigeminal function: For trigeminal receptors with a significant group difference, we examined correlations between the corresponding TLT result and protein levels by computing Spearman's correlations, separately for each group.

Results

Psychophysical tests results

We present descriptive statistics for the psychophysical tests for the three groups separately in Table 1.

For intranasal trigeminal function, the rmANOVA yielded significant effects of stimulus [F(1.46) = 52.043; p<0.001] and stimulus*group [F(2.46) = 4.557; p=0.016]. The effect of group failed to reach significance (p=0.053). To disentangle the interaction stimulus*group, we carried out two separated univariate ANOVA, one for each stimulus. For eucalyptol, the univariate ANOVA revealed a significant effect of group [F(2.46) = 6.054; p=0.005]. Post-hoc comparisons indicated that CRS patients scored significantly lower than both DNS (p=0.047) and healthy controls (p=0.005). For cinnamaldehyde, group had no significant effect (p=0.267) (Figure 1).

For nasal patency function, the rmANOVA yielded significant effects of test [F(1.46) = 92.305; p<0.001] and group [F(2.46) = 11.698; p<0.001]. The factor group had a significant effect on PNIF values [F(2.46) = 3.387; p=0.042]: CRS patients scored significantly lower than healthy controls (p=0.046). No other group difference was observed. Similarly, the factor group also had a significant effect on the nasal patency VAS [F(2.46) = 40.861; p<0.001]. Both CRS patients (p<0.001) and DNS patients (p<0.001) scored significantly lower than healthy controls, with no significant difference between these two groups (Figure 2). Objective (PNIF scores) and subjective (VAS nasal patency scores) measurements of nasal patency were significantly correlated (r=0.287; p=0.045). However, nasal patency measures and psychophysical measures of trigeminal function were not.

For olfactory function, the rmANOVA yielded significant effects of test [F(1.46)=146.938; p<0.001], group [F(2.46)=10.605; p<0.001] and test*group [F(2.46)=7.298; p=0.002]. To disentangle the interaction test*group, we carried out two separated univariate ANOVA, one for each test. For the TDI score, the univariate ANOVA revealed a significant effect of group [F(2.46)=5.101; p=0.01]. Post-hoc comparisons indicated that CRS patients scored significantly lower than DNS (p=0.044) and healthy controls (p=0.013). For olfactory VAS scores, the univariate ANOVA revealed a significant effect of group [F(2.46)=9.99; p<0.001]. Post-hoc comparisons indicated that CRS patients sco-

Table 2. Descriptive statistics, mean scores and standard error of mean (SEM) for the proteins of trigeminal receptors for the two groups.

	CRS (n=15)	DNS (n=15)
Proteins of Trigeminal receptors		
TRPA1 mean ± SEM % of control (TRPA1/β-Actin)	 3.46 (0.71) 139.42 (24.87)	 3.32 (0.76) 100
TRPV1 mean ± SEM % of control (TRPV1/β-Actin)	 4.15 (1.55) 80.24 (15.12)	 8.32 (6.69) 100
sTRPM8-18 mean ± SEM % of control (TRPM8/β-Actin)	 1.87 (0.46) 149.25 (21.29)	 1.46 (0.33) 100
sTRPM8-6 mean ± SEM % of control (TRPM8/β-Actin)	 1.71 (0.49) 136.57 (20.31)	 1.33 (0.30) 100
Proteins of inflammatory markers		
IL-13 mean ± SEM % of control (IL-13/β-Actin)	 3.02 (0.45) 136.57 (20.31)	 2.91 (0.46) 100
INF-γ mean ± SEM % of control (INF-γ/β-Actin)	 2.49 (0.83) 151.94 (45.00)	 3.00 (1.27) 100
Eosinophil mean ± SEM % of control (Eosinophil/β-Actin)	 0.53 (0.10) 230.09 (75.12)	 0.38 (0.07) 100

red significantly lower than DNS (p<0.001) and healthy controls (p=0.01) (Figure 3).

Protein level quantification

We present descriptive statistics for protein level quantification for both groups separately in Table 2.

CRS had significantly higher level of sTRPM8-18 levels than DSN patients (t-test; p=0.039). For sTRPM8-6 levels, there was a trend in the same direction (Wilcoxon's test; p=0.073). We did not observe any significant group difference for TRPA1 (p=0.135) or TRPV1 (p=0.2524). There was no effect of age, sex, or smoking status on protein levels of trigeminal receptors (Figure 4).

Similarly, we did not observe any significant group difference for protein levels of inflammatory markers (IL-13: p=0.9341; INF-γ: p=0.5307; eosinophil: p=0.1514).

Since there were differences in TRPM8 protein levels, we analyzed correlations with TLT eucalyptus scores in both groups. In CRS patients, but not in DNS patients, TLT eucalyptol scores showed a trend with sTRPM8-6 (r=-0.514; p=0.050) and sTRPM8-18 (r=-0.505; p=0.055).

Discussion

The results of this study led us to four main findings. First, CRS patients had a lower objective and subjective nasal patency than controls, and DNS patients scored significantly lower than controls for subjective nasal patency. Second, we found that

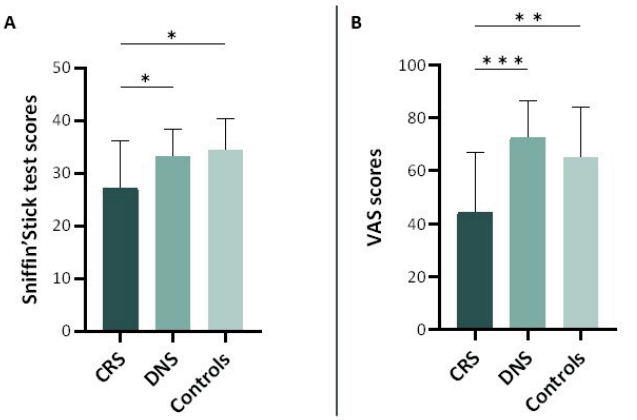

Figure 3. Olfactory results according to the groups (CRS, DNS and controls): A) Mean of Threshold, Discrimination, and Identification (TDI) and B) Means of Visual Analog Scale (VAS) for olfactory function. Error bars represent standard deviation (SD).

CRS patients exhibit a decreased intranasal trigeminal function compared to both DNS patients and controls. Third, CRS patients exhibit higher protein levels of sTRPM8-18 compared to DNS. Fourth, intranasal trigeminal function trended to correlate with levels of short TRPM8 isoforms in CRS patients.

The present results support the involvement of an impaired intranasal trigeminal system in subjective nasal obstruction [2, 3, 20]. Specifically, subjective chronic nasal obstruction may be linked to decreased perception of nasal airflow by alteration of the intranasal trigeminal system rather than physical obstruction [2, 3, 20]. In addition to this, our study shows an upregulation of short TRPM8 isoforms (sTRPM8-18 and sTRPM8-6) in CRS. These isoforms negatively regulate menthol- and cold-induced channel activity by stabilizing the closed state of channels through interaction with their C-terminal regions [10]. Accordingly, intranasal trigeminal function and protein levels of short TRPM8 isoforms are associated. In fact, TLT scores for eucalyptol, a TRPM8 agonist, showed a trend to a negative correlation with protein levels of short TRPM8 isoforms in CRS. In other words, an increase in protein level expression of short TRPM8 isoforms is reflected by a decrease of intranasal trigeminal function. One may therefore hypothesize that upregulation of short isoforms sTRPM8-18 and sTRPM8-6 may cause the inhibition of the sensation of nasal patency in CRS.

Our results could be interpreted that, like other sensory systems, trigeminal perception has (a) conductive, (b) sensorineural, and (c) central components. Accordingly, DNS patients have a conductive impairment, whereas CRS patients with the characteristics of our sample have a sensorineural impairment as highlighted by the TRPM8 upregulation. Finally, psychological disorders, e.g., anxiety can impact nasal patency on a central level [21]. Multiple mechanisms can be put forward to explain this finding. The upregulation of protein levels of short TRPM8 isoforms could be the result of airway inflammation. In fact, inflammation

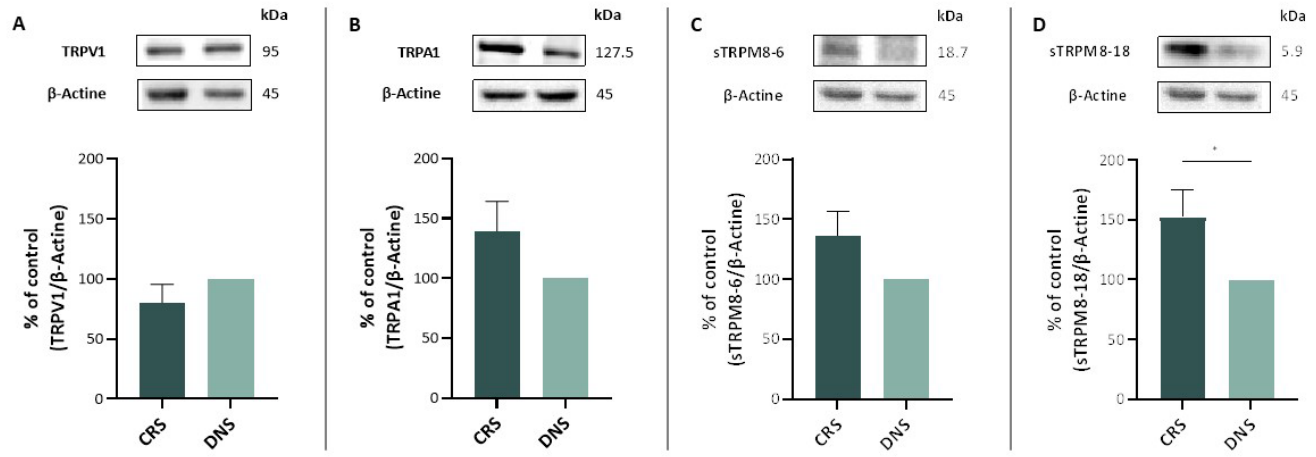

Figure 4. Protein expression of trigeminal receptors according to the groups (CRS and DNS): A) TRPV1 (95 kDa), B) TRPA1 (127.5kDa), C) sTRPM8-6 (18.7kDa) and D)sTRPM8-18 (5.9kDa). Error bars represent standard error of mean (SEM).

can modify the expression or activity of TRP channels [22], as they mediate neurovascular reflexes and have physiological roles in the perception and response to various stimuli. They are also implicated in neurogenic inflammation [22, 23]. However, our results don`t allow us to observe a link between inflammation and TRPM8 isoforms levels as the levels of the inflammatory markers tested weren`t significantly different between groups. Further, sensitivity towards cinnamaldehyde, an agonist of the trigeminal TRPA1 receptor, was similar between groups. An alternative mechanism may involve p53, a tumor suppressor protein, due to its role in apoptosis. The TRPM8 promoter possesses a putative binding site for p53 [24]. Conditions associated with overexpression of p53 such as a variety of cancers including prostate, breast, lung, colon and skin [25] accordingly exhibit increased TRPM8 levels (24). In fact, p53 is overexpressed in CRS with nasal polyps due to the inflammatory cascade [26]. Future studies should investigate how p53 is involved in altered sensitivity to cooling and reduced perception of nasal patency. We don`t yet know to which extent the intranasal trigeminal function is altered on the nasal mucosal level and by which mechanism overexpression of the protein levels of TRPM8 short isoforms is achieved. Activity of the TRPM8 channel needs to be addressed by immunoprecipitation assays. By doing so, the neurobiological underpinning of upregulation of TRPM8 channel could be unveiled, leading to possible novel treatments.

Our results aren't limited to the expression of trigeminal receptors. In fact, our data suggests that reported nasal obstruction in a subset of CRS patients with subjective nasal obstruction that don't have any obvious anatomical deformity, may be linked to a combination of deficient perception of nasal airflow by the intranasal trigeminal system and a mechanical obstruction not visible by endoscopy. Our results support this observation by a lower intranasal trigeminal function in CRS than DSN patients and controls, and a lower nasal patency in CRS than controls. In fact, the PNIF results represent an objective measurement of the intranasal airflow of patients. With this test, it seems that CRS patients had an objective chronic nasal obstruction even though their endoscopy had revealed only little anatomical deformity or discrete obstructive mucosal inflammation. However, PNIF is very sensitive to the presence of nasal valve collapse who is more frequent in Caucasians [27, 28]. Our study population consisted exclusively of Caucasians and the PNIF results may therefore not be generalized to other ethnicities. Therefore, our data may be skewed if nasal valve abnormality was present which we didn't specifically address during the examination.

Our findings are particularly important for clinicians dealing with subjective complaints of nasal obstruction in CRS patients. Our results point towards a potential cause of the subjective nasal obstruction in CRS. Clinicians should be aware that subjective nasal obstruction may be caused by a decrease of intranasal trigeminal function due to an overexpression of the short TRPM8 isoforms. In this subset of patients, if no obvious anatomical deformity explains the sensation of nasal obstruction and medical treatment has failed, investigation of the intranasal trigeminal function should be done. This will help the caregivers and the patients to understand the cause of their complaint of chronic nasal obstruction, and consequently, to avoid repeated visits to clinicians, increasing costs of medications and undergoing multiples surgeries [2, 29]. In future studies, it will be interesting to investigate the possible avenues to treat the overexpression of TRPM8 receptors.

A primary limitation of this study is the biopsy sampling. The nasal mucosal tissues were taken on the middle turbinate (30). Consequently, the results obtain by this study don't represent the total protein levels of trigeminal receptor of the nasal cavity, but only a part of it. A second limitation is the limited size of our groups due to the clinical aspect of the study. A larger sample may have allowed to observe conclusive results about

the correlations. Future studies should be done with a larger sample. A third limitation is the heterogenous nature of CRS patients included. Future studies should consider testing only CRS patients without polyps. A fourth limitation is the absence of validated patient rated outcome measures such as the Nasal Obstruction Symptom Evaluation. The use of this standard questionnaires wasn't included in the standard preoperative workup in the institution. Nevertheless, the VAS allowed us to evaluate subjectively the sensation of nasal obstruction.

Conclusion

Our study suggests that reported nasal obstruction in CRS patients may be linked to a combination of deficient perception of nasal airflow by the trigeminal system, in the absence of obvious anatomical deformity or obstructive mucosal inflammation. In this subset of patients, the protein levels quantification of short TRPM8 isoforms seem to be upregulated (CRS vs DNS). Since these isoforms negatively regulate menthol-and cold-induced channel activity by stabilizing the closed state of the channel, one may hypothesize that subjective nasal obstruction in CRS patients may be linked to this overexpression of short TRPM8 isoforms.

Acknowledgements

The authors thank the ENT doctors who realized the biopsies.

Authorship contribution

Conception or design of the work (all authors); data acquisition (CMB, KL), data analysis (CMB, KL, JF), drafting work (all authors), final approval (all authors).

Conflict of interest

The authors declare no conflict of interest.

Funding

This study was supported by the Fonds de Recherche du Québec – Santé to JF and CMB, and Natural Sciences and Engineering Research Council of Canada (NSERC) to JF (Grant #RG-PIN-2022-04813). Part of the protein levels quantification were supported by the NSERC to MC (Grant #2017-06411).

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