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ORIGINAL ARTICLE

The expression of bitter taste receptor TAS2R38 in patients with chronic rhinosinusitis

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Abstract

Chronic rhinosinusitis (CRS) is a frequent disease with high social impact and multifactorial pathogenesis. Recently, the bitter taste receptor TAS2R38 has been described to play a role in upper airway innate mucosal defense. The aim was to determine the localization and expression of the TAS2R38 in the selected cell lines and tissue collected from patient suffered from CRS as well as to correlate the results with clinical data. Moreover, the purpose was the estimation of the TAS2R38 distribution changes during acute and CRS. Forty-two patients undergoing nasal surgery were enrolled in the study. The TAS2R38 expression was assessed in the collected tissues using immunohistochemistry and immunocytochemistry methods. The western blot analysis was performed on human cell lines HeLa, MCF7, MDA-MB-231 to assess the location of the TAS2R38 protein. Moreover, the HeLa cell line was used as a model of acute inflammation induces by lipopolysaccharide. Immunohistochemistry analysis displayed a statistically significant difference of TAS2R38 level in the patients with CRS compared to healthy control and was different in CRS with and without nasal polyps. The results showed the abundance of TAS2R38 receptor in the cell nucleus in patients with CRS and cell lines. The variance in TAS2R38 receptor expression in two CRS types suggests their different pathogenesis. The first time in literature, we confirmed the presence of plasma membrane TAS2R38 receptor in the cell nuclei in CRS as well as in cell lines, what strongly suggests the different than membrane TAS2R38 function.

Keywords TAS2R38 · Chronic rhinosinusitis · Bitter taste receptor

Abbreviations

CRS	Chronic rhinosinusitis
CRSwNP	Chronic rhinosinusitis with nasal polyps
CRSsNP	Chronic rhinosinusitis sine nasal polyps
EPOS	European position paper on rhinosinusitis
IHC	Immunohistochemistry
ICC	Immunocytochemistry
LPS	Lipopolysaccharide
SNPs	Single-nucleotide polymorphisms

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Introduction

Chronic rhinosinusitis (CRS) is one of the most common health condition affecting nearly 16% of the world's population (Bhattacharyya et al. 2012), and its prevalence has increased over the last decade. CRS has many known contributing factors including environmental exposure, impaired mucociliary clearance, immune abnormalities, bacterial colonization, and allergy (Kern et al. 2008). Nevertheless, the underlying cause and mechanism remain unknown. It has been presumed that genetic predispositions may facilitate the onset of CRS (Qu et al. 2007).

The bitter taste receptor (T2Rs) genes are a newly discovered gene family that might contribute to CRS. Interestingly, T2Rs are expressed not only in gustatory cells but also in many other tissues (Li 2013). T2Rs belong to a group of

G protein-coupled receptors (Rosenbaum et al. 2009). Its activation is connected with innate bacterial defense mechanisms. These generate the calcium-dependent production of nitric oxide (NO), which is known as bactericidal mediator and leads to increased mucociliary clearance (Carey et al. 2016). Genetic variations of the bitter taste receptor TAS2R38, expressed in the cilia of the human sinonasal epithelial cells, appear to be associated with susceptibility to upper respiratory tract infections and CRS (Adappa et al. 2014; Dzaman et al. 2016; Lee et al. 2012). TAS2R38 are particularly unique because of their naturally occurring genetic variants. The activity of the receptor, which correlate with markedly different taste sensitivities to the bitter substance phenylthiocarbamide (Bufe et al. 2005) depends on three common single-nucleotide polymorphisms (SNPs) within the TAS2R38 gene (Lee et al. 2012). SNPs produce changes, generating two common haplotypes: (1) the functional, "taster", and (2) the nonfunctional, "non taster" (Kim et al. 2003). People who are homozygous for the taste allele perceive phenylthiocarbamide as intensively bitter, whereas the same concentration will be imperceptible to those with the non-function allele (Dinehart et al. 2006).

Furthermore, SNPs in this gene have been recently shown to correlate with sinusitis caused by Gram-negative bacteria (Lee and Cohen 2014).

The aim of this study was to assess the TAS2R38 protein abundance in patients with CRS vs healthy control and to determine the subcellular localization of these protein in the selected human cell lines. Moreover, the purpose was evaluation of TAS2R38 expression and localization during lipopolysaccharide (LPS) stimulation of cell lines.

The study was approved by the Local Ethics Committee, Warsaw Medical University, Poland (KB/130/2015).

Materials and methods

Patients

Samples were obtained from 33 CRS patients who underwent functional endoscopic surgery (FESS) (study group) and 9 patients without inflammatory changes in paranasal sinuses who underwent septoplasty surgery. The study group included patients with CRS with nasal polyps (CRSwNP: 15 patients) and CRS sine nasal polyps (CRSsNP: 18 patients). Fresh sinus mucosa was obtained during FESS and septoplasty from the ostiomeatal complex regardless of the study group. Based on EPOS 2012 (Fokkens et al. 2012), the presence of clinical and radiological evidence of CRS and lack of polyps on nasal endoscopy were required to be enrolled. The classification of disease severity was performed using computer tomography scans graded by the Lund–Mackay scoring system (Fokkens et al. 2012). All the tissues were analyzed as formalin-fixed, paraffin-embedded samples.

Immunohistochemistry

Paraffin-embedded samples were cut on 3.5 µm sections and dried at 37 °C overnight. After the deparaffinization according to standard protocol (Nirmalan et al. 2009), immunohistochemistry (IHC) analysis was performed using Envision Flex+, Mouse, High pH Detection System (Dako, Agilent, USA). This method was described by Sarnowska et al. (2017). Incubation with specific primary antibodies against TAS2R38 receptor (Taste Receptor, Type 2, member 38 Antibody, Catalog No OSR00163W, Thermo Fisher Scientific, Poland) was performed for 1 h in room temperature. Intensity and quantity of brown color of horseradish peroxidase–3,3′-diaminobenzidine reaction determine level of protein expression. Semi-quantitative analysis of IHC results was based on the calculation of histological score (H-score). H-score bases on the cells staining intensity (on a scale of 0 to 3, where 0 is missing, 1 is weak, 2 is medium and 3 is very strong staining) and the percentage of stained cells. It is calculated according the following formula:

$$[1 \times (\%\ \text{cells}\ 1+) + 2 \times (\%\ \text{cells}\ 2+) + 3 \times (\%\ \text{cells}\ 3+)]$$

Haematoxylin were used as a nuclear stain, which effects in blue color of cells' nuclei.

Cell culture and LPS treatment

Because of lack of cell lines that could serve as a model of rhinosinusitis, the MCF-7 and MDA-MB-231 and HeLa cell lines were used. The HeLa cell line was used as the model in LPS experiment. Human Hela, MCF-7 and MDA-MB-231 were grown in DMEM medium (Biowest, European Union) with 10% fetal bovine serum (Biowest, South America) under optimized conditions (37 °C, 95% humidity, 5% CO_2). Trypsinization of these adherent cells was performed according to ATCC: The Global Biosource Centre. After collecting, the cell lines were used for further analysis.

For LPS treatment, 40,000 cells of HeLa cell line were transferred into Lab-Tek II Chamber Slides (Catalog No 154526, Nalge Nunc International) and cultured in DMEM medium with 10% FBS for 24 h to get into adherent properties. Then the LPS was added in appropriate concentration (10, 25, 50 µg/ml). Untreated cells were negative control of acute inflammation. The results were observed after 2 and 4 h of incubation with LPS.

Immunocytochemistry

Immunocytochemistry (ICC) on HeLa cell line after LPS treatment was performed as it was described above in *Immunohistochemistry* chapter. Instead of deparaffinization, cross-linking with 4% cold formalin was conducted to fixate the cells on the microscope slides. Then the cells were treated with 0.1% Triton-X for 5 min to retrieve proteins' epitopes and after that—1% bovine serum albumin in phosphate buffered saline was used for 30 min as blocking reagent.

Cells fractionation and western blot

Previously collected cells of HeLa, MCF-7 and MDA-MB-231 cell lines were homogenized in Potter homogenizer with 1 ml of 2× MEB buffer (100 mM Tris-HCl pH 7.5; 20% glycerol, 4 mM DTT, 10 µl/ml proteases inhibitor cocktail), filtrated through polyester membrane (Millipore, Merck, Germany) to remove cellular debris and centrifuged in 2000*g* for 15 min in 4 °C. Supernatant was transferred to a new tube and centrifuged in 10,000*g* for 15 min in 4 °C. Supernatant from this centrifugation was cytoplasmic/microsomal fraction of the cells. Pellet from the first centrifugation was resuspended in 0.5 ml 2 × MEB buffer, centrifuged in 2000*g* for 15 min and this new pellet was homogenized in 0.2 ml 2× MEB buffer, which constituted nucleic fraction of the cells.

Cytoplasmic/microsomal and nucleic fractions of HeLa, MCF-7 and MDA-MB-231 cell lines were resuspended in Laemmli buffer, heated at 95 °C for 10 min and used for SDS-PAGE electrophoresis on 15% polyacrylamide gel. Fifty to 70 µg of protein samples were loaded on gel. Following steps were performed according to standard SDS-PAGE and western blot protocols (Laemmli 1970; Manoussopoulos and Tsagris 2009) TAS2R38 receptor antibody (Taste Receptor, Type 2, member 38 Antibody, Catalog No OSR00163W, Thermo Fisher Scientific, Poland) was used in 1:750 dilution and histone H3 antibody (Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb, Catalog No 9733S, Cell Signaling Technology)—1:1000. The usage of histone H3 antibodies proved the correctness of cells fractionation. Stain-free technology (Gilda and Gomes 2015) was used as a loading and normalization control.

Statistics

Statistical analysis was performed using GraphPad Prism 5.0 by nonparametric Mann–Whitney rank test for independent samples. The $p < 0.05$ was considered as statistically significant.

Results

TAS2R38 is present in cell nucleus

TAS2R38 receptor was found in the cytoplasmic fraction which contains cell membranes and in the nucleus in all examined cell lines (Fig. 1a). Quantification of the signals demonstrated that the MDA-MB-231 cell line had an order-of-magnitude (tenfold) less TAS2R38 expression in the cytoplasmic fraction as the HeLa cell line. The slightly reduced the amount of protein in the cytoplasmic/membrane fraction and a 82% reduced amount of the nuclear fraction in the MCF-7 line was observed compared to HeLa cell line (Fig. 1b).

LPS treatment strengthens TAS2R38 localization in cell nucleus

The immunocytochemical analysis of the TAS2R38 protein in the human HeLa cells after LPS treatment displayed an increase TAS2R38 abundance in cell nuclei in LPS dependent manner (Fig. 2). The TAS2R38 protein was observed in cells nuclei also without stimulation of inflammation with LPS, but after LPS exposure, the amount of these protein was higher.

Immunostaining of patients' tissues

Single tissue sections from 42 patients were analyzed: 9 patients of control group, 16 CRSsNP patients and 17 CRSwNP patients. Immunohistochemical analysis demonstrated that level of TAS2R38 expression had significant difference among CRSwNP and CRSsNP in comparison with control group. There was significantly higher expression of TAS2R38 in cells nuclei than in cytoplasm (Fig. 3).

The relationship between the H-score of control and CRS patients ($p = 0.0175$) was confirmed, as well as the relationship between the percentage of stained cells in both groups of patients (significance level 0.0319). There was also a correlation between the percentage of stained cells and CRS without polyps ($p = 0.0149$). No correlation was observed in the presence of polyps (Fig. 4).

Discussion

CRS is a frequent disease with high social impact and multifactorial pathogenesis. The incidence of CRS is extremely high and treatment is often limited to sinus surgeries, and routine controls. The scientists suggest the genetic background (Mfuna-Endam et al. 2011; Purnell et al. 2019; Wang

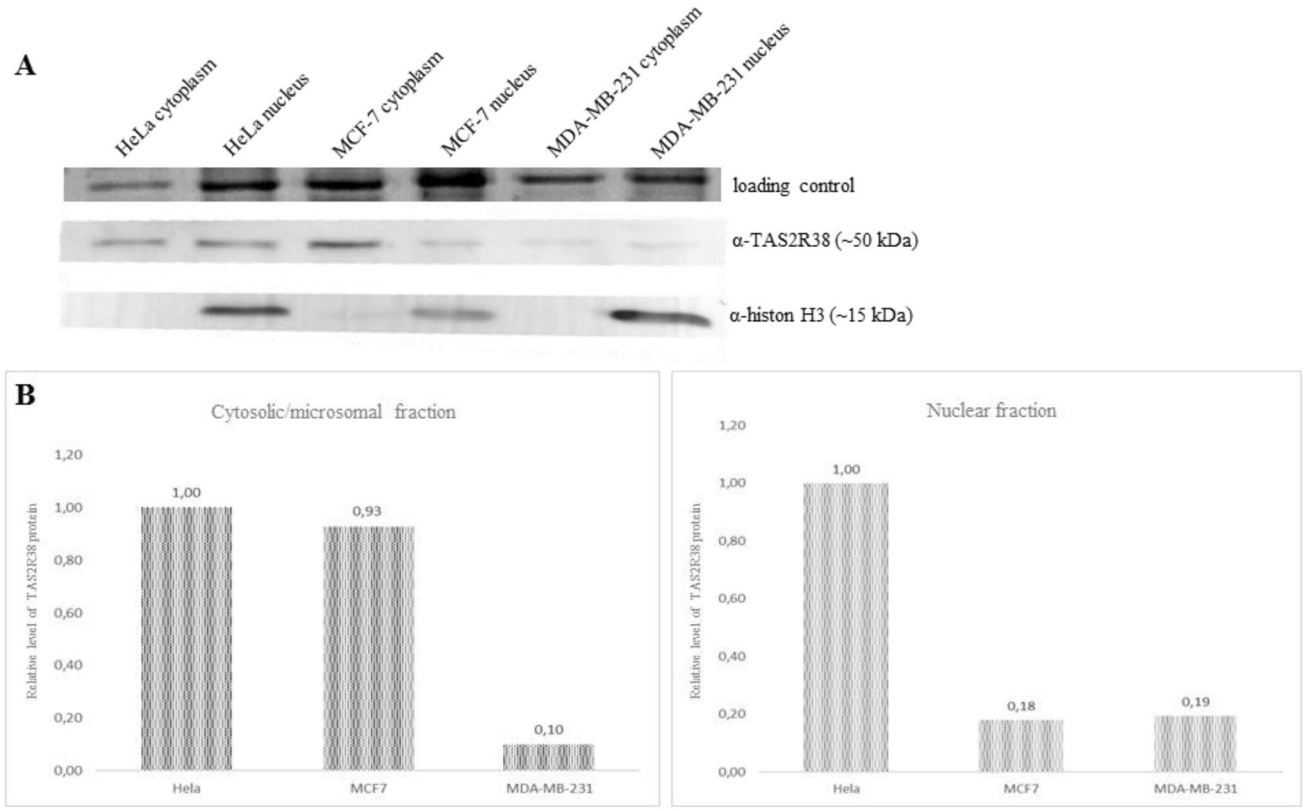

Fig. 1 Localization of TAS2R38 protein in fractionated human cell lines. **a** Western blot against TAS2R38 analysis of HeLa, MCF-7, MDA-MB-231 cell lines after cells fractionation. Receptor was located both in cells cytoplasm/membrane and nuclei. Histon H3 was observed only in nuclear fraction, what proved the correctness of the fractionation. **b** Quantification of the signals showed decrease of the TAS2R38 protein level in MCF-7 and MDA-MB-231 cell lines in comparison to HeLa cell line. Number of experiments, $n = 2$

et al. 2000b) based on the observation of familiar predisposition to the disease of some patients. Understanding the genetic cause of the disease would enable its faster diagnosis and the development of more effective treatment. Researchers identified several genes that may be responsible for the pathogenesis of CRS. One of them is the TAS2R38 gene, encoding the bitter taste receptor TAS2R38 (Mfuna Endam et al. 2014; Wang et al. 2000a), which is also exposed in mucociliary cells of nose mucous tissue (Chaudhari et al. 2000; Tizzano et al. 2011). The performed ICC and IHC analyzes indicated the presence of the TAS2R38 receptor in the cell nucleus (Figs. 2 and 3). As it is known, taste receptors are proteins located in the cell membrane (Chaudhari et al. 2000). In our study, we showed the TAS2R38 in the nucleus of the cells. The abundance of TAS2R38 in the nuclei was increased upon LPS treatment, as well as in CRS patient samples compare to healthy control. This result strongly suggests that TAR2R38 can translocate to the nucleus upon stimulation of inflammation signals. To confirm the nuclear localization of TAS2R38 cells, fractionation and the western blot were performed. The ability for nuclear translocation membrane receptor e.g. epidermal growth factor receptor was already described in the literature (Raper et al. 1987).

To revealed if the TAS2R38 nuclear abundance is typical for sinus or is more common. The western blot was performed and the nuclear expression of TAS2R38 protein in various cell lines was found (Fig. 1). Decreased level of protein expression in breast cancer cells (the MCF7 and MDA-MB-231 lines) relative to the HeLa line (HeLa uterus cancer cells) was observed, both in the cytoplasmic and nuclear fractions. The IHC analysis displayed the increased expression of the TAS2R38 receptor in the cytoplasm and, in the cell nuclei in patients with CRS compare to healthy tissue. Interestingly, in the healthy tissues, the TAS2R38 receptor was found only in the cell membrane and cytoplasm, what can suggest that the chronic inflammation caused the receptor migration to the nucleus. This might be triggered by the interaction of agents or mediators of inflammation (e.g., bacteria or granulocytes) on the receptor as a result of defense mechanism. Statistical tests confirmed the assumption of a direct association of the TAS2R38 protein level with the occurrence of CRS, but no correlation was found with the formation of polyps (Fig. 4). The chronic inflammation

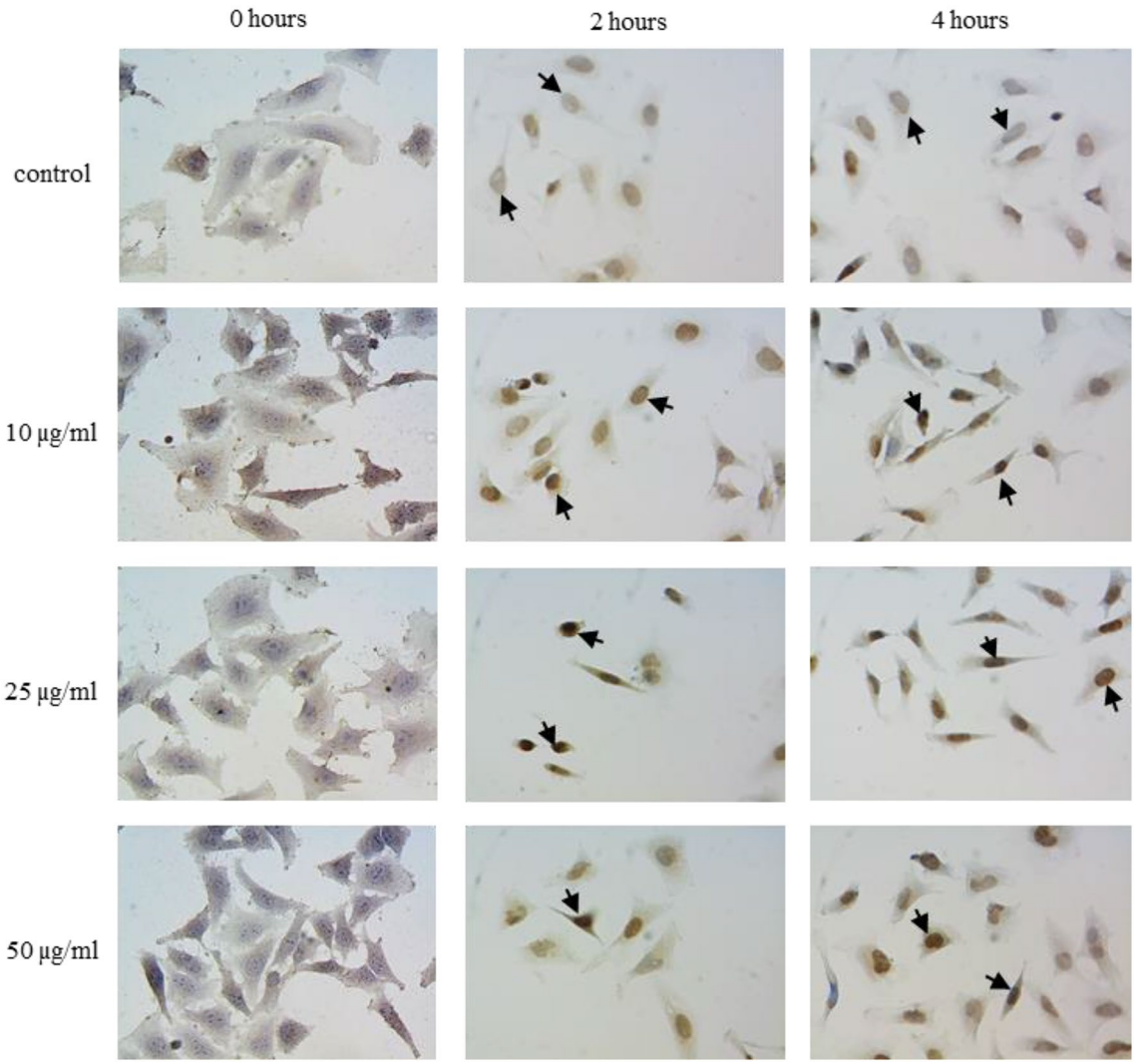

Fig. 2 Immunocytochemical analysis of HeLa cell lines after treatment with LPS. The level of TAS2R38 in cells nuclei increased after LPS treatment. Black arrows indicated the particular cells with very strong TAS2R38 abundance in nucleus. Experimental $n=2$

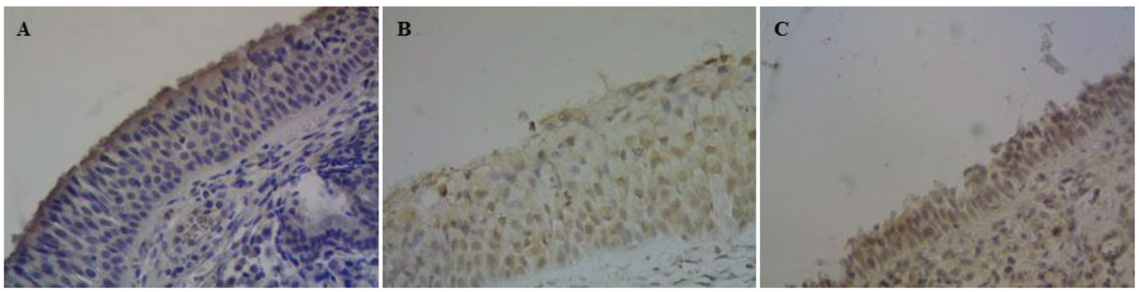

Fig. 3 Immunohistochemical analysis of TAS2R38 expression in mucosal tissues. Individual photos showed the tissue obtained from: **a**—control patients (9 patients), **b**—patients with CRSsNP (16 patients), **c**—patients with CRSwNP (17 patients). There is noticeable increase of receptor expression among patients' wits CRS, especially in cells nuclei

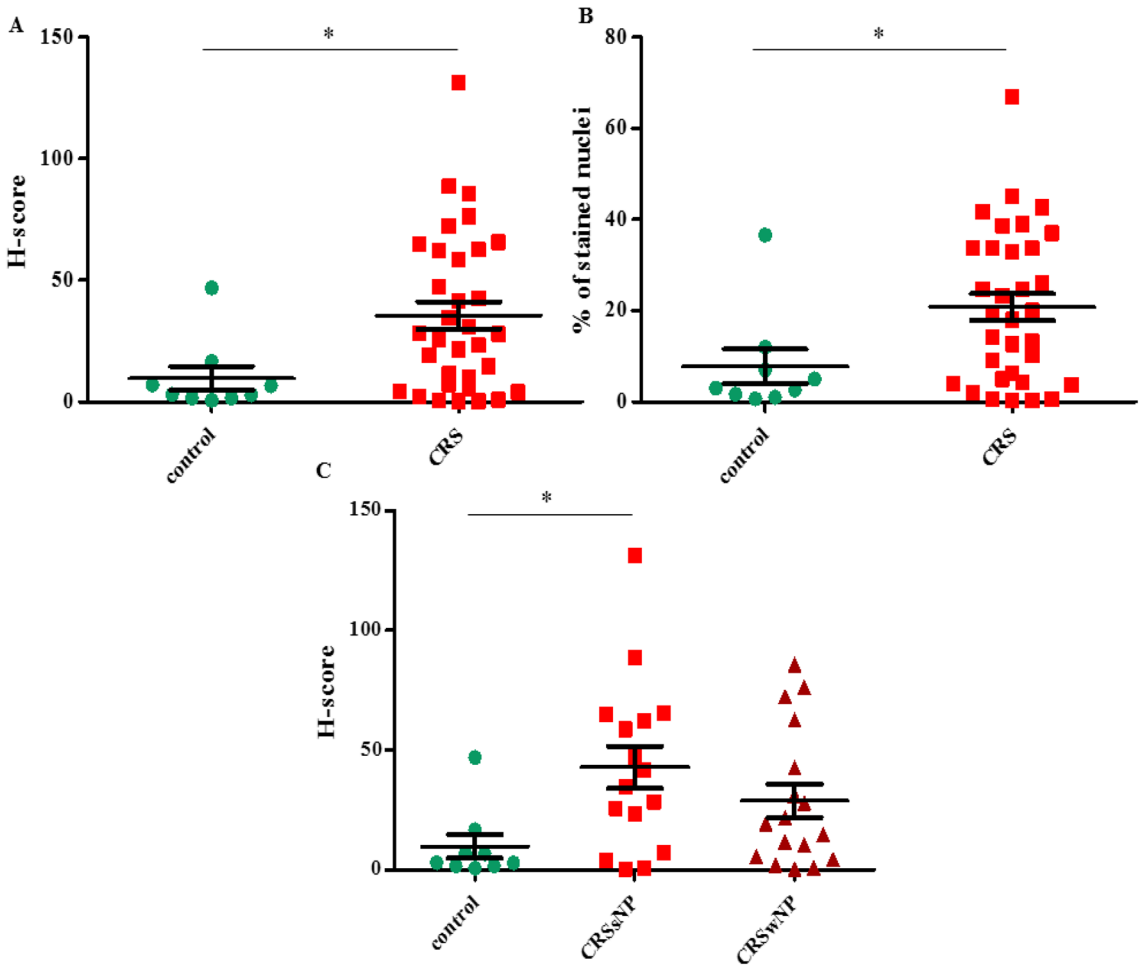

Fig. 4 **a**, **b** All of the patients with CRSsNP and CRSwNP were gathered together. Statistical analysis showed significant relationship between expression of TAS2R38 and CRS prevalence. There was observed great difference in TAS2R38 expression level among patients with CRS ($p=0.0175$) for H-score and 0.0319 for percentage of nuclei staining, respectively. **c** CRS patients were grouped into CRSsNP and CRSwNP. There was no significant difference between nasal polyps occurrence (CRSwNP) and TAS2R38 expression level compare to healthy control. TAS2R38 expression level has significant difference only in CRS without nasal polyps compare to healthy one ($p=0.0149$)

could cause its chronic migration to the nucleus, where they could for e.g. stimulate the expression of proinflammatory genes—although the exact role of TAS2R38 in the nucleus is still unknown and needs further elucidation.

What is more, the elevated staining seemed to be also observed in the control tissue around the cilia of sinonasal epithelial cells. We also noticed that patients with the CRS did not show this kind of staining. However, this hypothesis needs more research from the authors. According to the literature, the T2R38 expression is observed exactly in the apical membrane and cilia of sinonasal respiratory epithelial cells. Lee et al. (2012) found that T2R38 when stimulated by AHLc (acyl-homoserine lactone quorum-sensing molecules secreted by *Pseudomonas aeruginosa* and other Gram-negative bacteria), elicited calcium-dependent NO production that increased mucus and antimicrobial effects. They also demonstrate that upper airway epithelial cells from individuals with nonfunctional T2R38 alleles have significantly blunted NO and ciliary responses following exposure to Gram-negative quorum-sensing molecules, and that these individuals are more likely to be infected. These studies can most clearly explain the fact that among healthy people, antibacterial defence is effective due to expression of TAS2R38 receptors within cilia, which patients with CRS do not show.

As it turned out, not only chronic inflammation process can trigger an increased TAS2R38 receptor response. The cells of the HeLa line were treated with LPS, one of the endotoxins of Gram-negative bacteria causing disease states. Increasing concentrations of LPS—0, 10, 25 and 50 µg/ml—were used for the experiment. It was also noticeable that the receptors migrate to the nucleus, or at least accumulate there of nearby, after treatment with LPS cells (Fig. 2). Perhaps,

a similar situation occurs as in the case of chronic inflammation—the TAS2R38 protein is translocated to the cell nucleus to transmit the signal or as a transcription factor.

The above results bring a new knowledge about subcellular localization of the TAS2R38 receptor in the cell and can serve as a solid basis for further research on the role of this receptor in the pathogenesis of rhinosinusitis. Translocation of membrane protein can be used in modern therapy in CRS in the future. Therefore, the deciphering of the precise mechanism of TAS2R38 action in normal condition as well as in inflammation will be important. This knowledge will provide the new highlights on the TAS2R38 function not only in CRS but also in other inflammatory diseases as well as in cancer.

Conclusion

1. We confirmed the presence TAS2R38 receptor in the cell nucleus in both CRS patients' samples and cancer cell lines.
2. We showed the elevated expression of TAS2R38 in patients with chronic rhinosinusitis compare to healthy control group.
3. The translocation of TAS2R38 occurs upon LPS treatment.
4. The TAS2R38 nuclear abundance was elevated in CRS patients compared to healthy control.

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Author contribution Conceived and designed the experiments: KZP, MS, ES, JS, and KD. Performed the experiments: KZP, MS, and NR. Analyzed the data: KZP, MS, ES, and KD. Contributed reagents/materials/analysis tools: KZP, MS, and JS. Wrote the paper: KZP, MS, ES, KD.

Compliance with ethical standards

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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