Second International Virtual Conference of Biotechnology Research Center (IVCBRC-2021)
Journal of Biotechnology Research Center
Vol.15 No.2 **P-ISSN: 1815-1140**
Published Online: (December) 2021 **https://doi.org/10.24126/jobrc.2021.15.2.618** **E-ISSN: 2708-1370**

Expression of Bitter Taste Receptors in Human Bronchial airways Smooth Muscle Cells by using Immunofluorescence Staining

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Received: 10 /Aug. /2021 , Accepted: 25 /Aug. /2021

Abstract

Asthma and chronic obstructive pulmonary diseases (COPDs) have been identified as one of the serious, public health concerns in the world, which results due to the host as well as the environmental factors. Based on the WHO, this disease will be the 3rdkiller disease worldwide by the year of 2020 following the cancer and heart disease. Concerning the asthma, there have been 389,000,000 individuals worldwide, afflicted by COPD and Asthma. Moreover, there has been a noticeable increase in the asthmatic incidences in the young adults and children by 4% to 5% yearly worldwide. Those two illnesses are fundamental global morbidity and mortality reasons, particularly in the patients that respond poorly to the current treatments. Which is why, there are unmet needs to come up with innovative therapies for the COPD as well as the asthma patients. Bitter taste receptors (TAS2R) are part of the family of the G-protein coupled receptors (GPCR). It was theorized previously that the TAS2R only expression on the tongue's taste buds. The family of the TAS2R includes over 25 different subtypes of the receptors in the humans. Lately, a number of the researches showed that the TAS2R could be representing a new treatment target of the lung diseases. The TAS2R has been expressed in the human air-way smooth muscles (ASMs). It should be noted that TAS2Ractivation by the saccharin and chloroquine results in the induction of air-way relaxation. More significantly, bronchodilatory effects that result from the TAS2R agonists has been found greater compared to the β2 adrenergic receptor agonists, the basic bronchodilator medications in treating asthma. the bronchial airway smooth muscle (BASM) cells have been cultured from COPD (n=3), asthmatic (n=3), and healthy (n=3) individuals. The Immuno-fluorescence staining (with and with no permeabilize cells) have been conducted on 3 groups with the use of the poly-clonal anti-bodies against the (TAS2R10 as well as the TAS2R14) receptors. The immuno-fluorescence assays had shown that the TAS2R10 and TAR2R14 had shown a positive staining of the two receptors in the human ASM cells. It has been discovered as well that those two receptors have been expressed as well in nucleus.

Kewwords: Asthma, COPD , Bitter taste receptors, ASM.

Introduction

COPD and Asthma have been increasingly identified as one of the serious, world-wide public healthcare concerns. In particular, in the last years, due to emergence as well as growing (host and environmental) factors resulting in an increase in the spread of the obstructive lung illnesses (1). The main reason of death and inability of both diseases is airways obstruction, which led to constricted smooth muscle of the bronchi (2). Based on the WHO, this disease will be the 3rd killer disease worldwide by the year of 2020 following the cancer and heart disease. Concerning the asthma, there have been 300,000,000 individuals worldwide, afflicted by COPD and Asthma (3,4). The latest researches had noticed that there has been a noticeable increase in the asthmatic incidences in the young adults and children by 4% to 5% yearly worldwide (5). Both of COPD and asthma have been identified as separate disease with some distinct clinical traits. Despite the obvious differences between the two diseases in structural, functional, pharmacological characteristics, but there is overlap between some asthmatic and COPD patients (2).

The function of the airways smooth muscle has crucial effect on asthma progression many recent studies on patients with asthma have shown that the functional and structural unit of airways

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participate in pathogenesis of asthma particularly ASM(6). Previous studies have noticed that the physical and biological activities of ASM during asthma such as airway obstruction, hypertrophy, and inflammatory mediators' production play significant role in airways inflammation and hyper responsiveness. (7)

The action of current treatment of asthma is relax obstructed airways and diminution airways inflammation, which at the end lead to expand the internal diameter of air ways and improves expiratory flow. Despite the fact that current medications for asthma are fast, efficient and quick-acting but also have side effects on asthmatics (local and systemic impact).The majority of hospital admittances are children and elderly people, patients who suffering from the side effect of medication is most serious, therefore, the health service organizations around the world are facing great pressure in order to discover novel therapeutic target for treating asthma. The major objective of developing new therapy is to find medications having considerable effects upon the inflammations of the air-ways. (8)

It has been hypothesized earlier, that the receptors of the bitter taste are expressed on the tongue only; none-the-less, lately, BTR presence was documented by (9) *etal*., in the human airways smooth muscle. This paper aims at the detection of BTR expression in the human BASM.

Material and method

Procedure of the Cell Culture:

In all of the experimentations that are related to the tissue culture, there was a necessity for performing all of the methods through the use of the sterile equipment for the prevention of the contaminations, even though the laminar facilities of the airflow provide a proper environment. the working place within the hood has to be frequently cleaned by 70% IMS, and all of the stuff have been either disposable or sterilized either by the autoclave (121ºC and 15 Pounds per Inch2 for 15min.) or dry-heating (160ºC for 3hrs in the oven).

The BASM muscle that has been obtained from the Glen field Hospital, the cells have been grown in a DEME medium that has been provided by 1% SP, 10% FBS, 1% N.E.A.A and 1% A.A. The primary cells of the cultured ASM have been obtained from the bronchial biopsy samples from the asthmatic, COPD and non-asthmatic subjects according to Leicestershire Ethics Committee.

Procedure of passing cells (sub culturing, splitting):

This approach begins with the warm up media and the trypsin to 37º in a water bath. After the media have been takeout by the aspirator, a proper amount of the trypsin (5.0ml) has been added for covering the flask's surface area followed by the incubation for 5min - 7min (incubation duration varies based upon the type of the cell), after that, the cells have been observed under a microscope. In the case where cells are entirely detached from the surface of the flask, the fresh media is added (5ml) into the trypsin due to the fact that it's very hash to the cells. After that, the tubes have been centrifuged at 22ºC, 1,400 r.p.m, for 10.min, the pallets of the cells are suspended by 1.0ml of the feeding media to the counting cell (10µl of the cell suspension +10µl of the trypan blue) with the use of the haemto-cytometer chamber, then, the cells are aliquot to new flask based on the recommended ratio for experimentation.

Immunofluorescence staining:

BASM were grown at 8000 cells per well into Lab-TekTM II 8 well chamber slides (Nunc, ThermoFisher). They were placed in an incubator at 37°C in 5% CO2/95% air and grown for 24 hours prior the staining. Cells were then rinsed with 1x PBS (Sigma-Aldrich) and fixed with 100 µl/well of 10% neutral buffered formalin for 15 minutes at room temperature, washed 3 times with 1x PBS, then permeabilized with ice-cold 90% methanol for 15 min at room temperature. To block nonspecific binding sites, cells were incubated with 100 µl/well 3% BSA/PBS for 1 hour at room temperature. Primary antibodies and Isotype controls were diluted in 3% BSA/PBS and incubated overnight at 4 ºC; the chamber slides were placed on moistened tissue in order to ensure they remained humidified during the primary incubation. Following incubation with the primary antibody, cells were washed with 1x Phosphate Buffered Saline (PBS). Alexa Fluor® 488 secondary antibody was diluted in 3% BSA/PBS and incubated for 45 min at room temperature. Cells were washed again following incubation with secondary antibody. Cells were

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mounted using VectaMount with DAPI (Vector Laboratories) and coverslip (Fisher Scientific). Immunofluorescence microscopy was performed with an inverted microscope (Carl Zeiss Inc). Data acquisition and analysis was performed by the Zen 2012 lite software (Carl Zeiss Inc).

Table 1: Summary of Primary and secondary antibodies used for immunofluorescence can be found in

Antibodies	Host species	Dilutions	Manufacturer
TAS2R10	Rabbit	1:50	Novus Biological ® NBP1-71302
TAS2R14	Rabbit	1:50	Novus Biological ® NBP1-71304
Alexa Fluor® 488	Goat	1:300	SantaCruz.biotechnology
Isotype Control	normal rabbit IgG	1:50	Santa Cruz

Results

1-Immunofluorescence assay of Human Bronchial Smooth Muscle cells

The immuno-fluorescence staining in the permeabilized as well as the non permeabilized cells that have been obtained from the healthy individuals, COPD and asthmatic patients has been carried out as well for the purpose of providing more evidence for expressing TAS-10 and TAS14 in BHASM. Those two receptors have been expressed at the ASM's cytoplasmic membrane in the non-permeabilized cells. The nuclear expression of the two receptors has been noticed in the permeabilized cells.

1.1 Expression of TAS2R 10 in non- permeabilized cells by immunofluorescence staining

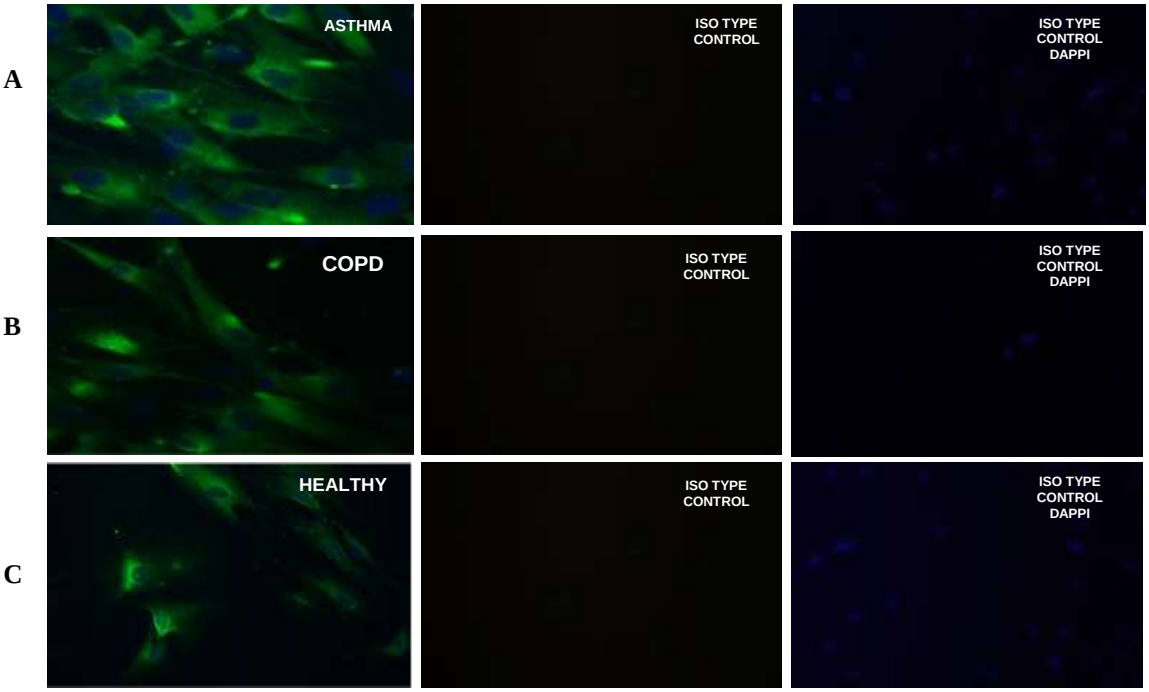

Figure 1.1: Expression of TAS2R 10 in cultured HASM cells stained with primary polyclonal- antibody against TAS2R10 (2µg/ml), FITC-labelled secondary antibody (1:300). Negative control includes Rabbit- IgG antibody. Images have taken by immunofluorescentmicroscope 20X magnification). Panels represents staining in cells from (**A**) Asthmatic subject n= 3, (**B**) COPD subjects n=3, (**C**) Healthy subjects n=3. Overlay pictures of the green (TAS2R10) and blue channels (DAPI) have shown for better visualization of TAS2R10 at plasma membrane of non-permeabilized cells.

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1.2 Expression of TAS2R10 inpermeabilizedcellsby immunofluorescence staining

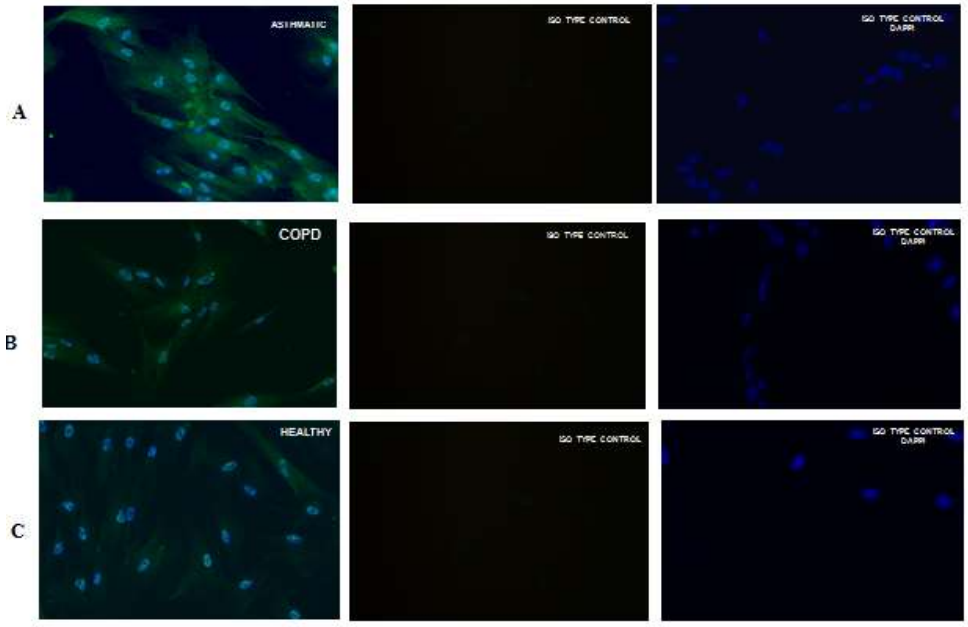

Figure 1.2: Expression of TAS2R 10 in cultured HASM cells stained with primary polyclonal -antibody against TAS2R10 (2µg/ml), FITC-labelled secondary antibody (1:300). Negative control is Rabbit IgG antibody. Images have taken by immunofluorescent microscope 20X magnification). Panels represents staining in cells from (**A**) Asthmatic n= 3,(**B**) COPD n=3, and (**C**) Healthy subjects n=3. Overlay pictures have illustrated clear expression of TAS210 at nucleus and cytoplasmic membrane of permeabilized cells by using 90%of ice cold methanol.

1.3 Expression of TAS2R 14 in non- permeabilized cells by immunofluorescence staining

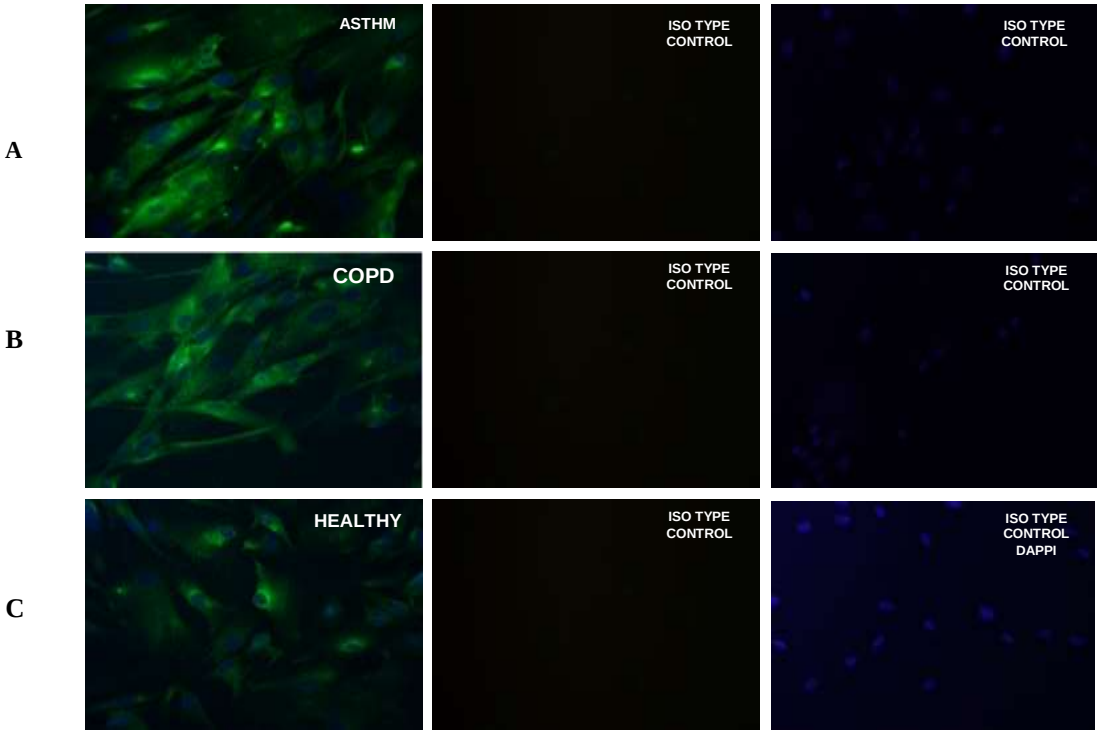

Figure1.3: Expression of TAS2R 14 in cultured HASM cells stained withprimary polyclonal antibody against TAS2R14 (2µg/ml), FITC-labelled secondary antibody (1:300). Negative control includes Rabbit IgG antibody. Images have taken by immunofluorescent microscope (20X magnification). Panels represents staining in cells from (**A**) Asthmatic subject n= 3, (**B**) COPD subjects n=3, and (**C**) Healthy subjects n=3. Overlay images of the green (TAS2R14) and blue channels (DAPI) have detected presence of TAS214 at plasma membrane of non- permeabilized cells.

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1.4 Expression of TAS2R 14 in permeabilized cells by immunofluorescence staining

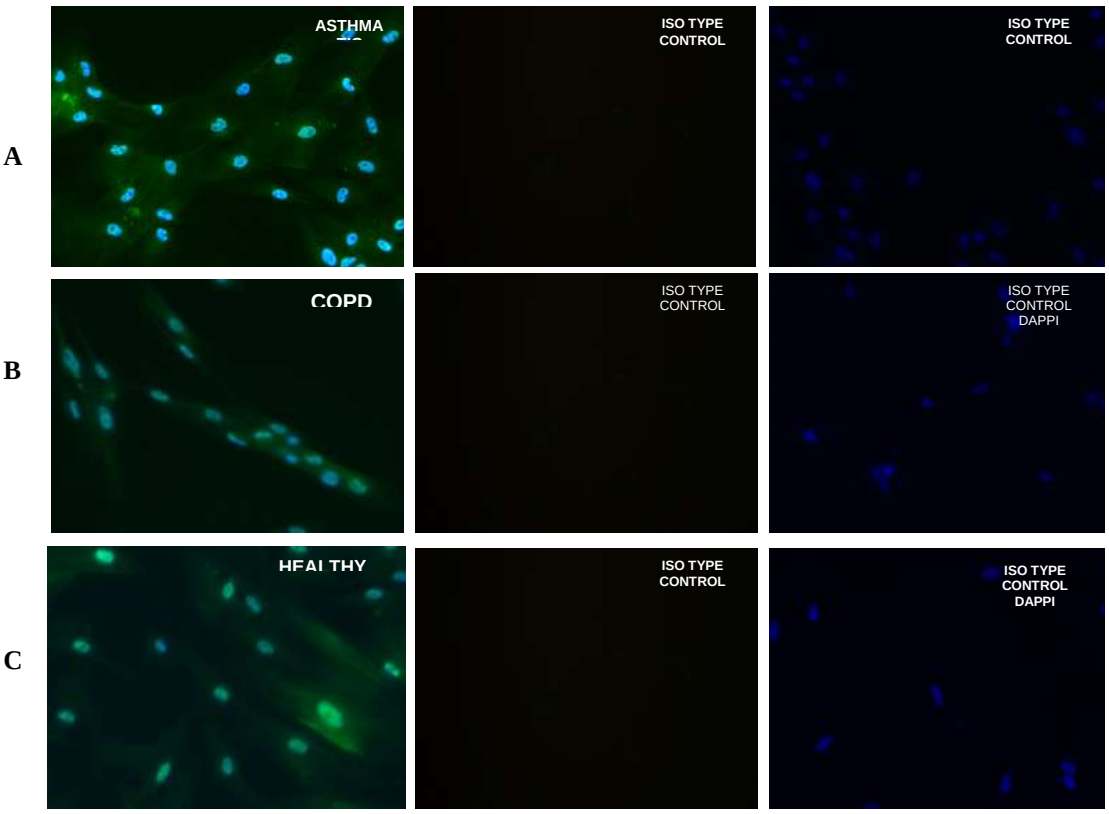

Figure1.4: Expression of TAS2R 14 in cultured HASM cells stained withprimary polyclonal antibody against TAS2R14 (2µ/ml), FITC-labelled secondary antibody (1:300). Negative control includes Rabbit IgG antibody. Images taken by immunofluorescent microscope (X20 magnification). Panels represents staining in cells from (**A**) Asthmatic subject n= 3,(**B**) COPD subjects n=3, and (**C**) Healthy subjects n=3. Overlay pictures of the green (TAS2R14) and blue channels (DAPI) have detected presence of TAS214 at nucleus and plasma membrane of permeabilized cell by by 90%of ice cold methanol.

Disscussion

Presence of BTRs in the respiratory system of the humans:

The unexpected BTR presenceoutside the oral cavity in the respiratory system were lately documented. A research that has been carried out by (10) *etal.*, had highlighted BTR expression on the motile cilia cells of the humans, which had emerged from the air-ways' epithelium tissues.

A study that has been carried out by (9) *etal*., had utilized the quantitative PCR approaches for demonstrating the TAS2R receptor expressions in the HASM cells. Authors have shown that the TAS2R 10, 14, and 31 are the most abundant amongst the genes in the HASM. (11) *etal* , had characterized that the BTRS have been expressed in the bronchial segments of the humans, which have been obtained from the normal individuals and patients that have undergone a surgical lung carcinoma removal.

The latest preliminary data had presented by a conference of the American Thoracic Society (11) *etal*, 2014 had shown that the receptors of the bitter taste have been expressed as well in the parenchyma and macrophages of the human lung that have been separated from lung cancer patients.

Concerning the asthma, (12) *etal*, had discovered that TAS2R-10 has been most highly expressed receptor of the bitter taste in the **peripheral WBCs** that have been separated from the adult asthma patients. Moreover, (12) had researched that TAS2R expression has been higher in the lymphocytes, in comparison to the monocytes and neutrophils.

The immuno-fluorescence staining findings of the present study had indicated the fact that the TAS210 as well as the 14 receptors have been expressed in the **BHASM** cells that have been obtained from the asthmatic, COPD and healthy

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individuals. Those findings have confirmed earlier studies by (9) *etal* that had shown that the receptors of the bitter taste (TAS2R-10, 31, 14, 7, 19, 43 and 38) have been expressed as well in the HASM cells. There has been no obvious difference observed in the expression of the TAS2R-14 in the cells of HASM between the asthmatic, COPD and healthy individuals, even though an increased TAS2R-10 trend might be observed in the asthmatic patients compared with the healthy ones. More experiments are required for confirming that observation. The most interesting one of the findings has been the presence of the TAS2R in nucleus in the case where the cells have been permeabilized with the use of 90 % of the ice cold methanol.; which has suggested that the two receptors might exist in the nuclear membrane in the cytoplasmic cell membrane as it has been stated in literature reviews. (9) The repetition of immuno-fluorescence staining in the non-permeabilized cells had shown no nuclear expressions of the TAS2R-10 and TAS2R14. More precise strategy requires establishing more details concerning the nuclear localization of the receptors of the bitter taste in the ASM like the confocal microscopes.

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التحري عن مستقبلات الطعم المر في خلايا العضلات الملساء للشعب الهوائية البشرية باستخدام التلوين المناعي

نور الهدى فخري العبيدي
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الخلاصة

تم تحديد الربو وأمراض الانسداد الرئوي المزمن (COPDs) كواحد من مخاوف الصحة العامة الخطيرة في العالم، حيث ان كلا المرضين يسبب التهاب مزمن وتضيق في مجرى التنفسي يرجع السبب الى عوامل جينية والبيئية كالتلوث الهواء والتدخين . وفقا لاحصائيات منظمة الصحة العالمية ان هناك مايزيد على 389000000 شخص مصاب بالربو حول العالم لذلك ظهرت حاجة ملحه لايجاد علاجات جديدة خصوصا للاشخاص الذين تكون استجابتهم ضعيفة للادوية المستخدمة حاليا. تعتبر مستقبلات الطعم المر جزءًا من عائلة المستقبلات المقترنة بالبروتين والذي يسمى G-Protein . لقد كان يعتقد سابقا ان هذة مستقبلات متواجدة فقط على براعم الذوقية اللسان. تضم عائلة TAS2R أكثر من 25 نوعًا فرعيًا مختلفًا من المستقبلات في البشر. في الآونة الأخيرة اظهرت عدد من الأبحاث أن TAS2R يمكن أن يمثل هدفًا علاجيًا جديدًا لأمراض الرئة كالربو والانسداد الرئوي المزمن. وتجدر الإشارة إلى أن تنشيط TAS2 بواسطة الناهضات هذة المستقبلات مثل السكرين والكلوروكين يؤدي إلى استرخاء مجرى الهواء. الأهم من ذلك ، أن التأثيرات التوسعة القصبية الناتجة عن ناهضات TAS2R قد وُجدت أكبر مقارنة بمنبهات مستقبلات β2 الأدرينالية ، وهي الأدوية الأساسية لتوسيع الشعب الهوائية في علاج الربو. وقد اضهرت جميع النتائج هذا البحث عن تواجد هذه المستقبلات في العضلات الملساء في مجرى الهواء التنفسي (ASMs) ليس فقط في موقعها الاصلي في الغشاء البلازمي للخلايا انما ايضا بين التصبيغ عن تواجد هذه المستقبلات على الغشاء النووي للخلايا العضلات الملساء والتي بدورها تعلب دور اساسي في تقلص وانبساط القصبة الهوائية.

الكلمات المفتاحية : الربو ، مرض الانسداد الرئوي المزمن ، مستقبلات الطعم المر ، التلوين المناعي.