



RESEARCH ARTICLE

Validation Study: Evaluating Phenotypic Expression of Bitter Taste Receptors

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Abstract

Introduction: Bitter taste receptors (T2Rs) have shown to play a role in sinonasal innate immunity against SARS-CoV-2. A taste strip test kit has been used in prior COVID-19 studies (prototype version), and has shown significant results. This study addresses the validity of this test kit for assessing the genotype/phenotype correlation of T2R expression, with emphasis on the importance of performing phenotypic testing, rather than genotype testing, as a measure for T2R function as a part of innate immunity against SARS-CoV-2, as phenotype expression appears to decline with age.

Method: An investigational device study was performed on 171 subjects, with categorization into 3 groups (High Taster, Moderate Taster, & Low/Non taster) via phenotypic expression of T2Rs. Subjects underwent genotype analysis to evaluation correlation between phenotype and genotype.

Results: 171 (53.2% female) subjects (mean age of 41.56 years) were evaluated, 36 (21.1%) were high tasters, 91 (53.2%) were moderate tasters and 44 (25.7%) were low/non tasters. Genetic analysis of subjects revealed that 39 had PAV/PAV diplotype, 90 had a single PAV allele, and 42 subjects had a non-PAV containing allele. Phenotype using this taste strip test kit was 94.7% (162/171) accurate in predicting genotype (p-value < 0.01). The average age of the discordant results was 61.3.

Conclusion: Findings emphasize the importance of integrating phenotypic expression of T2Rs in evaluating innate immune response to upper respiratory tract infections, as level of expression of T2Rs declines with age. This taste strip test kit is accurate in evaluation of current T2Rs level of expression.

Keywords

Bitter taste receptors, T2R38, COVID-19, Phenotype, Genotype

Introduction

The SARS-CoV-2 coronavirus, etiologic agent of COVID-19 has been responsible for more than 190 million cases of infection and more than 4 million deaths worldwide [1,2]. The presentation and the course of the disease can range from asymptomatic to mild respiratory infections and pneumonia. Some infected patients develop more severe disease with acute respiratory distress several days after onset of symptoms, following a rapid viral replication, increased proinflammatory cytokine production, "cytokine storm", as well as chemokine responses and inflammatory cell infiltrates [3-5]. Younger individuals often are asymptomatic or present mild symptoms and thus might have a crucial role in the spread of the disease [6,7]. It appears that genetic variability plays a crucial role in the prognosis of COVID-19.

Genetic and/or environmental modifiers could contribute, albeit it to different degrees, to the definition of the phenotype throughout life. Regarding bitter taste receptors (T2Rs), changes in gene expression in the development phase or hormonal influences around the time of puberty may account for a different

penetrance of the T2R38 gene, TAS2R38, genotypes at different ages [8]. Consequently, propylthiouracil (PROP) sensitivity should be considered a quantitative rather than a qualitative trait [9].

Bitter taste receptors (T2Rs) are receptors for bitter substances and these are G-protein coupled receptors (GPCRs) [10] originally identified in type II taste receptor cells of the oral cavity [11]. In humans, 25 different T2Rs are known to be expressed [12]. One, the antithyroid-toxin receptor T2R38 responds to compounds which contain a thiourea (N-C = S) moiety such as goitrin or its precursor progoitrin as well as compounds containing an isothiocyanate (N = C = S) moiety [13].

T2Rs are genetically diverse, a phenomenon that helps to explain the wide variety of taste preference both within and between cultures [14]. Many individuals find bitter foods such as coffee or herbs to be detestable, while others do not have an aversive response. This genetic variation of T2Rs is not exclusively found in the tongue; T2R receptor variation in the airway appears to also play a key role in respiratory defense [13].

While only a few of these polymorphisms have well-documented phenotypic effects, hundreds of T2R polymorphisms and several T1R polymorphisms have been noted in humans [15]. The most well-known and well characterized example is the bitter receptor isoform T2R38 [16]. The TAS2R38 gene encoding T2R38 has two common polymorphisms, one encoding a functional receptor and one encoding a nonfunctional receptor. The differences in the resulting proteins are at amino acid positions 49, 262, and 296. The functional T2R38 receptor contains proline (P), alanine (A), and valine (V) residues while the nonfunctional T2R38 contains alanine (A), valine (V), and isoleucine (I) at these positions, respectively [13]. Loss of the valine at the third position in the AVI variant prevents receptor activation [17-19].

These polymorphisms are distributed in a nearly Mendelian ratio in Caucasian populations. Homozygous AVI/AVI individuals (approximately 30% frequency in Caucasian populations) are “non-tasters” for the T2R38-specific agonists’ phenylthiocarbamide (PTC; also known as phenylthiourea or PTU) and PROP. PAV/PAV individuals (approximately 20% frequency in Caucasian populations) are termed “super tasters” for these agonists because they perceive them as intensely bitter, while AVI/PAV heterozygotes have varying intermediate levels of taste [8]. These TAS2R38 polymorphisms also have clinical implications due to the extraoral expression of T2R38.

In the airway, T2Rs were first discovered on the ciliated cells lining the bronchial [20] and sinonasal epithelium and appear to be involved in innate immunity [21-23]. Ciliated cells are integral to airway defense. A thin layer of mucus secreted by airway secretory goblet cells and submucosal glands traps inhaled pathogens

and particulates [24-27].

Stimulation of these receptors by known bitter compounds activates calcium-dependent nitric oxide (NO) production that increases phosphorylation of ciliary proteins through protein kinase G (PKG). This increases ciliary beat frequency to facilitate the movement mucus out of the airway by increasing mucociliary transport rates. The generated NO also diffuses into the airway surface liquid (ASL) and acts as an antibacterial defense mechanism. NO damages bacterial cell walls and DNA and may also damage fungal pathogens and inactivate viral proteins [23,28,29].

Clinical data suggest these rapid T2R responses may be important in chronic rhinosinusitis (CRS). T2R38 was found to be expressed in sinonasal ciliated cells [23,28,30]. Prior study found that sinonasal ciliated cells from patients homozygous for the AVI polymorphism in the TAS2R38 gene resulting in a nonfunctional T2R38 receptor have decreased NO and ciliary beat frequency responses to bacterial AHLs *in vitro*, & subsequently are more susceptible to gram-negative bacterial infection [25], have a higher prevalence of biofilm-forming sinonasal bacteria [31], and are at greater risk for CRS requiring functional endoscopic sinus surgery (FESS) [32,33]. AVI/AVI patients may have worse outcomes after FESS for CRS without nasal polyps compared with patients homozygous for the functional (PAV) allele of TAS2R38 [34].

In a study, Åkerström, et al. [35] found that NO also inhibits the replication of SARS-CoV by two distinct mechanisms. Firstly, NO or its derivatives cause a reduction in the palmitoylation of nascently expressed spike (S) protein which affects the fusion between the S protein and its cognate receptor, angiotensin converting enzyme 2 (ACE2). Secondly, NO or its derivatives cause a reduction in viral RNA production in the early steps of viral replication, and this could possibly be due to an effect on one or both of the cysteine proteases encoded in Orf1a of SARS-CoV. The NO response activated by T2R38 occurs within seconds.

In a retrospective study performed by Barham, et al. [36] on 100 positive cases of COVID-19 confirmed by polymerase chain reaction (PCR), phenotypic expression of T2R38 with taste strip testing appeared to associate with the clinical course and symptomatology specific to each individual, as 100% of the patients requiring inpatient admission were classified as non-tasters. Conversely, supertasters represented 0% of the patient population, suggesting the possibility of innate immunity to SARS-CoV-2. While these results were interesting, the assumption was that the results were potentially confounded by loss of smell and taste in active infection, which is a known symptom of SARS-CoV-2 infection.

Barham, et al. subsequently performed a

prospective study [37] performed on 1935 subjects with occupational exposure to SARS-CoV-2. Participants underwent T2R38 phenotype taste testing to determine their respective taste groups, along with evaluation for lack of infection with SARS-CoV-2 via PCR, IgM and IgG testing. Participants were followed up until confirmation of infection with SARS-CoV-2 via PCR testing. Phenotype of T2R38 was retested after infection with SARS-CoV-2. Two-hundred and sixty-six (266) tested positive for SARSCoV-2, interestingly, non-tasters were significantly more likely to test positive for SARSCoV-2, to be hospitalized, and to be symptomatic for a longer duration. Conversely, supertasters represented only 5.6% of patients infected with SARS-CoV-2, suggesting enhanced innate immune protection, suggesting that T2Rs' allelic variants are associated with innate immune fitness toward SARS-CoV-2 and can be used to correlate with clinical course and prognosis of COVID-19. Genotype/phenotype association was 94.2%. These two previous studies were performed using a prototype of our new taste strip test kit.

Several studies have assessed the effects of individual polymorphisms within the haplotypes of the TAS2R38 taste receptor gene on human bitter taste perception by measuring the PTC and or PROP bitter sensitivity via a taste test. Studies have agreed on the significant genotype/phenotype correlation [38-43].

Accordingly, this can be applied to assessing for an association between the PTC or PROP taste test and sinonasal innate immunity. Prior studies have concluded that the ability to assess airway taste receptor variation with an inexpensive taste test has broad implications because differences in airway taste receptor function may reflect impaired innate immunity and a predisposition to certain respiratory tract infections and inflammatory disorders, and T2R38 functionality in the tongue correlates with nasal symptoms in healthy individuals [44,45].

The genotype/phenotype relationship is modified by age, with heterozygous children being more sensitive to the bitterness of lower concentrations of PROP when compared with adults with the same genotype. Moreover, some individuals who are born sensitive to PROP may become less sensitive with age because of experience, aging, and/or disease [46,47].

Objective

The objective of this study was to evaluate the genotype/phenotype correlation for the T2R expression, with emphasis on the importance of performing the phenotypic expression testing, rather than genotype testing, as a measure for the T2R function as a part of innate immunity against respiratory tract pathogens such as SARS-CoV-2 and Influenza viruses, as phenotype expression appear to vary with age. We postulate that the phenotypic expression would act as a more

truthful indicator of the existing function of T2R, while the genotypic testing could provide false information about the actual level of expression and function of the receptor, especially in the elderly.

Methods

We performed an investigational device study at our outpatient clinical practice and inpatient hospital on 171 patients and health care workers. All subjects were categorized into 3 groups (high tasters, moderate tasters, & low/non-tasters) via phenotypic expression of T2Rs. Subjects underwent genotype analysis to detect SNP in the TAS2R38 gene. Three polymorphisms were genotyped using real-time PCR single nucleotide polymorphism genotyping assays (rs713598, rs1726866, and rs10246939). Correlation between phenotypic expression and genotype was conducted.

Phenotype expression of T2R was evaluated via taste strip tests to evaluate the genetically determined phenotypic taste response of each subject. This study used a prototype general wellness test kit, being developed along with a software function and now owned by Phenomune LLC, designed to be used by persons at home to detect, interpret, record, and produce a trait report describing a person's unique intensity level of phenotypic expression of bitter taste receptors intended to increase a person's awareness to his/her sensitivity to bitter tastes for general improvement to functions associated with a general state of health, such as healthy lifestyle choices to enable wellness monitoring as it relates to dietary choices. A prototype test kit consistent with the Phenomune general wellness test kit was used by the investigator in this study due to its proprietary interpretation system for determining the scaled intensity of expression in order to facilitate a more precise classification of each subject. These taste strip tests included Control (chemical free), PTC, Thiourea and Sodium Benzoate.

In our study, we categorized any subject with two copies of the PAV allele as high taster, those with one copy of PAV allele as moderate taster, and finally, those with no PAV alleles in their genotype were classified as low/nontasters.

Demonstration & Interpretation of the taste strip test

All participants were presented with the taste test strips with the following order:

1. Control strip
2. PTC strip
3. Thiourea strip
4. Sodium Benzoate strip.

Participants were instructed to place the provided litmus paper strip on their tongue until completely

Table 1: Distribution of subjects among the different taste groups according to both phenotype and genotype.

Genotype \ Phenotype	High taster	Moderate Taster	Low/Non Taster	Total	% Accuracy	95% Confidence Interval
PAV/PAV	35	4		39	35/36 (97.2%)	± 0.044
PAV/AVI	1	75	3	79	86/91 (94.5%)	± 0.054
PAV/AAV		11		11		
AVI/AVI			37	37	41/44 (93.2%)	± 0.047
AVI/AAV		1	4	5		
Total	36	91	44	171	162/171 (94.7%)	

moistened, then the next litmus paper strip was provided according to the order stated above. Participants were instructed to comment on the quality of taste they perceived, in addition, to comment on its intensity on a visual analog scale from 0-10, where 0 is (no perception) to 10 (extremely intense quality perceived) as compared to the Control paper. Each participant was oriented to the scale with a verbal explanation prior to proceeding. In between each taste strip provided, participants were allowed to sip water.

Written informed consent was obtained from study participants. All aspects of this study were reviewed and approved by the Baton Rouge General Institutional Review Board (IRB00005439).

Exclusions

Participants with evidence of active infection with SARS-CoV-2 via PCR at study commencement were excluded. Participants with evidence of prior infection with SARS-CoV-2 via IgM and or IgG at study commencement were excluded. Participants were excluded from evaluation with positive results to the Control strip. Participants with prior history of radiation therapy, renal failure, Sjogren's syndrome, and or medications known to influence taste perception were excluded.

Statistical analysis

Statistical analyses were performed using SPSS v 22 (SPSS Statistics for Windows, version 22.0; IBM, Armonk, NY). Descriptive data are presented as percentages and means ± standard deviation (SD). Results were deemed significant with a p value of < 0.05.

Results

One hundred seventy-one patients (171 [53.2%] female) with a mean age of 41.56 years; were assessed with phenotype taste testing. All participants were categorized into 3 groups (high tasters, moderate tasters, and low/nontasters) via phenotypic expression of T2R. Thirty-six (21.1%) were categorized as high tasters. Ninety-one (53.2%) were categorized as moderate tasters. Forty-four (25.7%) were categorized

as low/nontasters (Table 1).

Genetic analysis of the 171 subjects revealed the PAV/PAV diplotype in 39 subjects, while 90 subjects were classified as heterozygotes (PAV/AVI & PAV/AAV) (79 PAV/AVI and 11 PAV/AAV). 42 subjects were categorized in the non-PAV containing group (37 AVI/AVI and 5 AVI/AAV).

When evaluating the relationship between phenotype and genotype, phenotype showed 94.7% (162/171) accuracy in predicting genotype (p-value < 0.01). The average age of the discordant subjects was 61.3. Rate of discordant results in high taster group was lower than the other two groups, with 35/36 (97.2%), with only 1 case testing phenotypically as a high taster but displayed a PAV/AVI genotype. Our study showed discordant results in the moderate taster group (single PAV allele) higher than the other 2 groups, where 86/91 single-PAV carrying subjects (75 PAV/AVI and 11 PAV/AAV) tested phenotypically as moderate tasters (94.5%), with disagreement in 5 cases (4 PAV/PAV and 1 AVI/AAV). Lastly in the low/nontaster group, 41/44 subjects (37 AVI/AVI and 4 AVI/AAV) tested phenotypically in the low/nontaster group (93.2%), with 3 cases that displayed the PAV allele and still categorized phenotypically as a low/nontaster by our taste strip test.

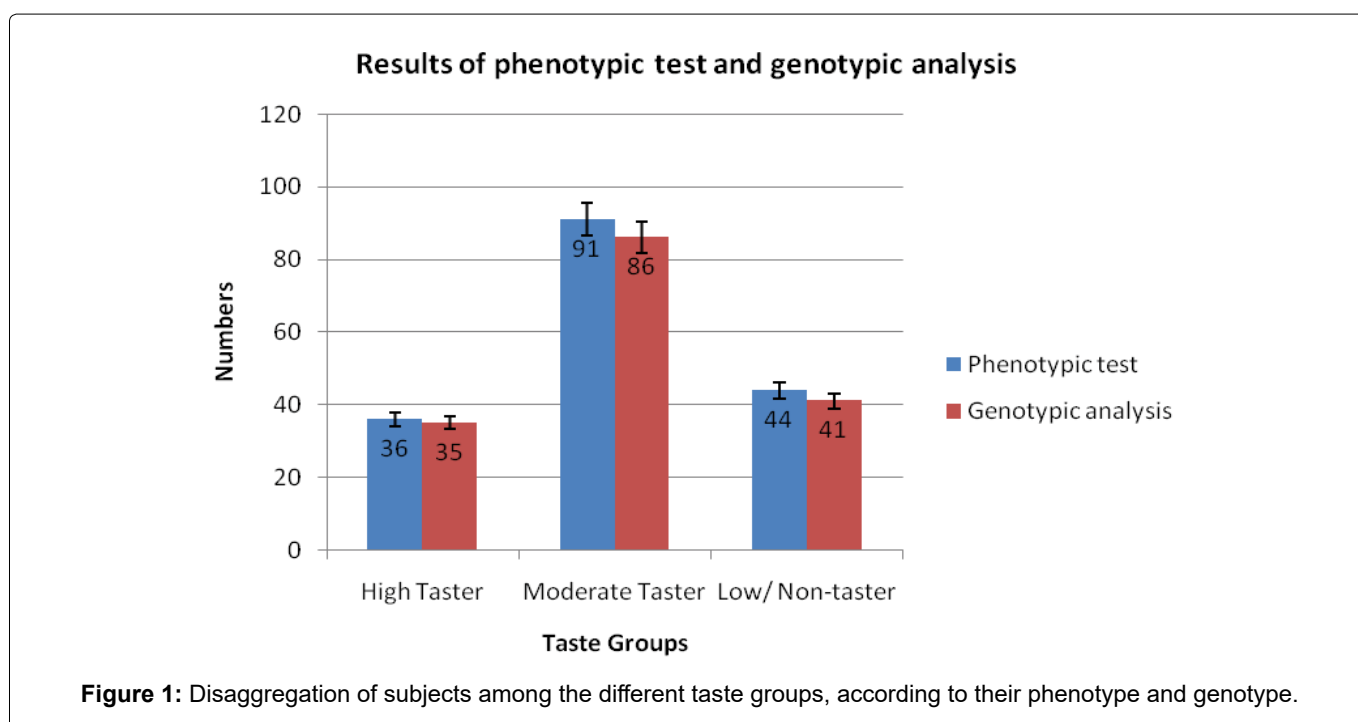
In the nine subjects with discordant results, seven subjects (5 males and 2 females, average age 68.6 years) had a genotype of a higher group (PAV/PAV) but tested phenotypically in the lower group (moderate taster) (Table 2 and Figure 1).

Discussion

Questions remain about the mechanisms controlling gene expression for bitter taste perception: it may be fixed early in life or it may change with development [48] or with exposure to bitter compounds (e.g., caffeinated beverages or vegetables) in the diet. For instance, those with low expression of bitter receptor genes, who find vegetables to taste less bitter, may be more likely to develop a preference for them than those with high expression of these genes. Or the ingestion of bitter vegetables may change gene expression

Table 2: Age, sex and discordant results of the phenotype/genotype correlation of 9 subjects. Seven subjects (*) with lower phenotypic expression of T2Rs in comparison to their genotype.

Number	Age	Sex	Phenotype	Genotype
1	46	Female	High taster	PAV/AVI
2	26	Female	Moderate Taster	AVI/AAV
3*	81	Male	Moderate Taster	PAV/PAV
4*	78	Female	Moderate Taster	PAV/PAV
5*	51	Female	Moderate Taster	PAV/PAV
6*	62	Male	Moderate Taster	PAV/PAV
7*	65	Male	Low/NonTaster	PAV/AVI
8*	70	Male	Low/NonTaster	PAV/AVI
9*	73	Male	Low/NonTaster	PAV/AVI

**Figure 1:** Disaggregation of subjects among the different taste groups, according to their phenotype and genotype.

over time. There is precedence for diet affecting the expression of genes involved in nutrient digestion and metabolism. For example, a high-fat diet results in changes in lipid metabolism and the expression of many genes in human skeletal muscle [49]. Changes in expression with development may also provide an explanation for the observation from studies in children and their parents that the phenotype-genotype relation for PROP sensitivity decreases with age among TAS2R38 heterozygotes [48].

Multiple studies have been performed to correlate the decline of perception of bitter taste of PTC on the tongue with age. From our prior studies, (retrospective & prospective), we displayed a correlation between the intensity of bitterness perception on the tongue with the sinonasal innate immunity against COVID-19, assuming that the innate immunity of sinonasal tract against COVID-19 also declines with age.

In this analysis, we categorized any subject with two copies of the PAV allele as High taster, those with one copy of PAV allele as moderate taster, and finally, those

with no PAV alleles in their genotype were classified as low/nontasters. This categorization was employed as the functionality of the T2Rs and the ability to perceive the bitterness on the tongue along with extraoral sites is dependent on the presence of the PAV allele.

In this study, we evaluated whether the taste strip test (as an indicator of the phenotype) can be used accurately to identify the true expression of T2R involved in innate immunity especially as it appeared to decline with age. In our prior prospective study [32], 2 subjects (81 & 69 years) had a genotype for a higher group (PAV/PAV & PAVI/AVI), while in fact their phenotype test was at the lower groups (PAV/AVI & AVI/AVI), respectively. In the current study, similar results were found, with phenotype/genotype association reaching 94.7% of our included subjects. As expected, the average age of the discordant subjects was high, in which these subjects where phenotypically behaving like those of the lower taste groups compared to their genotype, indicating the importance of phenotype as a more likely predictor of one's T2R role in innate immunity. Our results were

nearly following the Mendelian inheritance in a 1:2:1 pattern.

Multiple studies have evaluated phenotypic perception of PTC/PROP bitter sensitivity occurring over one's lifespan and have shown that sensitivity to the T2R38 agonists decline with age [50-55]. In a recent study conducted by Davies, et al. [56], they used an age-structured mathematical model of data from 6 countries. He estimated that susceptibility to COVID-19 infection in individuals less than 20 years of age is approximately half that of adults aged over 20 years, with symptom manifestation in those aged 10-19 years of 21% which increases to 69% in those above 70 years.

In a prior study [48], subjects were grouped by haplotype and age, as well as sex and race/ethnicity, and then compared to PROP thresholds. Subjects with the same haplotype were similar in bitter threshold regardless of race/ethnicity (all ages) or sex (children and adolescents; all p -values > 0.05) but age was a modifier of the genotype-phenotype relationship. Specifically, AVI/PAV heterozygous children could perceive a bitter taste at lower PROP concentrations than could heterozygous adults, with the thresholds of heterozygous adolescents being intermediate ($p < 0.001$). Similar age effects were not observed for subjects with the PAV/PAV or AVI/AVI homozygous haplotypes ($p > 0.05$). They concluded that there is a change in PROP bitter sensitivity occurring over the lifespan (from bitter sensitive to less so) and it is more common in people with a particular haplotype combination, i.e., PAV/AVI heterozygotes.

In another study [57], a racially diverse group of children (3-10 years-old) and their mothers were tested for the evaluation of the effectiveness of two bitter blockers, sodium gluconate and monosodium glutamate, for five food-grade bitter compounds (quinine, denatonium benzoate, caffeine, PROP, urea) using a forced-choice method of paired comparisons. The blockers reduced bitterness in 7 of 10 bitter-blocker combinations for adults but only 3 of 10 for children, suggesting that perception of bitterness depends on age. They also detected that the bitterness of PROP was not reduced by either blocker in either age group regardless of TAS2R38 genotype.

In a study conducted by the National Institutes of Health (NIH) on 2557 subjects ranging from 12 to 85 years concluded that the intensity of bitterness decreases with subjects' age [58]. Whissell-Buechy and Wills [53] reported that sensitivity to the T2R38 agonist PROP also declines with age.

Concordance between PROP bitter sensitivity and the expected tasting haplotype (PAV) was gradually reached as children approached adolescence. Negri, et al. stated that age-related changes in PROP sensitivity was particularly evident in genetic non-tasters (AVI homozygous) in whom the genotype-phenotype

concordance decreased from 84% in adults to 66% in children. The taster PAV diplotype was less affected by age: The proportions of tasters and supertasters were similar in adults and children with the PAV haplotype. Sensitivity to bitterness was strongly related to the TAS2R38 haplotype, and it was observed to have an intriguing relationship with age. In fact, children were more sensitive than adults with the same TAS2R38 haplotype also within mother-child dyads. The mother-child tasting differences decreased with age and became minimal when children reached adolescence [59].

What causes the developmental shift in taste sensitivity is unknown. One explanation is that the age and diplotype interaction may be due to preferential allele expression, with children over-expressing the taster form rather than the non-taster form of the receptor early in life and then losing this tendency as they age. Adults heterozygous for the TAS2R38 gene do not express mRNA of each haplotype in a one-to-one proportion [60], so it is possible that heterozygous children might have a skewed expression pattern, perhaps over expressing the taster allele.

These results show the importance of integrating the phenotypic expression of T2Rs in evaluating innate immune response to upper respiratory tract infections, as all studies have agreed that the level of expression of T2Rs declines with age. This implies that phenotype is more accurate and cost-effective in predicting T2Rs actual level of expression. This is also based on multiple studies showing association between the paper taste strip results and the incidence of CRS [47,50] along with SARS-CoV-2 [40]. Prior studies have shown evidence for an association between the PTC/PROP taste test and sinonasal innate immunity, concluding that the ability to assess airway taste receptor variation with an inexpensive taste test has broad implications, as differences in airway taste receptor function may reflect impaired innate immunity and predisposition to certain respiratory infections and inflammatory disorders.

Financial Disclosure

The principal investigator, Henry P. Barham, M.D., has a proprietary or financial interest in the Phenomune early prototype general wellness test kit used in this study and has an equity interest in Phenomune LLC whose value cannot be readily determined through reference to public prices; provided, however, Henry P. Barham, M.D. neither received any significant payment paid in support of his activities in this study nor did he enter into any financial arrangement whereby the outcome of this study could affect his compensation for conducting the study, in each case, in an effort to avoid potential investigator bias in this study.

Conflict of Interest

None.

References

- European Centre for Disease Prevention and Control (2021) COVID-19 situation updates.
- WG Dos Santos (2020) Natural history of COVID-19 and current knowledge on treatment therapeutic options. *Biomed Pharmacother* 129: 110493.
- Wang D, Hu B, Hu C, Zhu F, Liu X, et al. (2020) Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. *JAMA* 323: 1061-1069.
- Zou L, Ruan F, Huang M, Liang L, Huang H, et al. (2020) SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med* 382: 1177-1179.
- Liu J, Liu Y, Xiang P, Pu L, Xiong H, et al. (2020) Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. *J Transl Med* 18: 206.
- Guan WJ, Ni Z, Hu Y, Liang WH, Ou CQ, et al. (2020) Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 382: 1708-1720.
- Kronbichler A, Kresse D, Yoon S, Lee KH, Effenberger M, et al. (2020) Asymptomatic patients as a source of COVID-19 infections: a systematic review and meta-analysis. *Int J Infect Dis* 98: 180-186.
- Lipchock SV, Mennella JA, Spielman AI, Reed DR (2013) Human bitter perception correlates with bitter receptor messenger RNA expression in taste cells. *Am J Clin Nutr* 98: 1136-1143.
- Hayes JE, Keast RS (2011) Two decades of supertasting: Where do we stand? *Physiol Behav* 104: 1072-1074.
- Lindemann B (1996) Chemoreception: Tasting the sweet and the bitter. *Curr Biol* 6: 1234-1237.
- Lu P, Zhang C-H, Lifshitz LM, Zhuge R (2017) Extraoral bitter taste receptors in health and disease. *J General Physiol* 149: 181-197.
- Shi P, Zhang J, Yang H, Zhang YP (2003) Adaptive diversification of bitter taste receptor genes in mammalian evolution. *Mol Biol Evol* 20: 805-814.
- Bufe B, Breslin PA, Kuhn C, Reed DR, Tharp CD, et al. (2005) The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. *Curr Biol* 15: 322-327.
- Hayes JE, Wallace MR, Knopik VS, Herbstman DM, Bartoshuk LM, et al. (2011) Allelic variation in TAS2R bitter receptor genes associates with variation in sensations from and ingestive behaviors toward common bitter beverages in adults. *Chem Senses* 36: 311-319.
- Bachmanov AA, Bosak NP, Lin C, Matsumoto I, Ohmoto M, et al. (2014) Genetics of taste receptors. *Curr Pharm Des* 20: 2669-2683.
- Mennella JA, Pepino MY, Reed DR (2005) Genetic and environmental determinants of bitter perception and sweet preferences. *Pediatrics* 115: e216-e222.
- Tan J, Abrol R, Trzaskowski B, Goddard WA (2012) 3D structure prediction of TAS2R38 bitter receptors bound to agonists phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP). *J Chem Inf Model* 52: 1875-1885.
- Biarne's X, Marchiori A, Giorgetti A, Lanzara C, Gasparini P, et al. (2010) Insights into the binding of phenylthiocarbamide (PTC) agonist to its target human TAS2R38 bitter receptor. *PLoS One* 5: e12394.
- Floriano WB, Hall S, Vaidehi N, Kim U, Drayna D, et al. (2006) Modeling the human PTC bitter-taste receptor interactions with bitter tastants. *J Mol Model* 12: 931-941.
- Shah AS, Ben-Shahar Y, Moninger TO, Kline JN, Welsh MJ (2009) Motile cilia of human airway epithelia are chemosensory. *Science* 325: 1131-1134.
- Lee RJ, Chen B, Redding KM, Margolskee RF, Cohen NA (2014) Mouse nasal epithelial innate immune responses to *Pseudomonas aeruginosa* quorum-sensing molecules require taste signaling components. *Innate Immun* 20: 606-617.
- Lee RJ, Cohen NA (2013) The emerging role of the bitter taste receptor T2R38 in upper respiratory infection and chronic rhinosinusitis. *Am J Rhinol Allergy* 27: 283-286.
- Lee RJ, Xiong G, Kofonow JM, Chen B, Lysenko A, et al. (2012) T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *J Clin Invest* 122: 4145-4159.
- Knowles MR, Boucher RC (2002) Mucus clearance as a primary innate defense mechanism for mammalian airways. *J Clin Invest* 109: 571-577.
- McMahon DB, Workman AD, Kohanski MA, Carey RM, Freund JR, et al. (2018) Protease-activated receptor 2 activates airway apical membrane chloride permeability and increases ciliary beating. *FASEB J* 32: 155-167.
- Hariri BM, Cohen NA (2016) New insights into upper airway innate immunity. *Am J Rhinol Allergy* 30: 319-323.
- Cohen NA (2006) Sinonasalmucociliary clearance in health and disease. *Ann Otol Rhinol Laryngol Suppl* 196: 20-26.
- Hariri BM, McMahon DB, Chen B, Freund JR, Mansfield CJ, et al. (2017) Flavones modulate respiratory epithelial innate immunity: anti-inflammatory effects and activation of the T2R14 receptor. *J Biol Chem* 292: 8484-8497.
- Workman AD, Carey RM, Kohanski MA, Kennedy DW, Palmer JN, et al. (2017) Relative susceptibility of airway organisms to antimicrobial effects of nitric oxide. *Int Forum Allergy Rhinol* 7: 770-776.
- Yan CH, Hahn S, McMahon D, Bonislowski D, Kennedy DW, et al. (2017) Nitric oxide production is stimulated by bitter taste receptors ubiquitously expressed in the sinonasal cavity. *Am J Rhinol Allergy* 31: 85-92.
- Adappa ND, Truesdale CM, Workman AD, Doghramji L, Mansfield C, et al. (2016) Correlation of T2R38 taste phenotype and in vitro biofilm formation from nonpolypoid chronic rhinosinusitis patients. *Int Forum Allergy Rhinol* 6: 783-791.
- Adappa ND, Zhang Z, Palmer JN, Kennedy DW, Doghramji L, et al. (2014) The bitter taste receptor T2R38 is an independent risk factor for chronic rhinosinusitis requiring sinus surgery. *Int Forum Allergy Rhinol* 4: 3-7.
- Adappa ND, Howland TJ, Palmer JN, Kennedy DW, Doghramji L, et al. (2013) Genetics of the taste receptor T2R38 correlates with chronic rhinosinusitis necessitating surgical intervention. *Int Forum Allergy Rhinol* 3: 184-187.
- Adappa ND, Farquhar D, Palmer JN, Kennedy DW, Doghramji L, et al. (2016) TAS2R38 genotype predicts surgical outcome in nonpolypoid chronic rhinosinusitis. *Int Forum Allergy Rhinol* 6: 25-33.
- Åkerström S, Gunalan V, Keng CT, Tan YJ, Mirazimi A

- (2009) Dual effect of nitric oxide on SARS-CoV replication: Viral RNA production and palmitoylation of the S protein are affected. *Virology* 395: 1-9.
36. Barham HP, Taha MA, Hall CA (2020) Does phenotypic expression of bitter taste receptor T2R38 show association with COVID-19 severity? *Int Forum Allergy Rhinol* 10: 1255-1257.
37. Barham HP, Taha MA, Broyles ST, Stevenson MM, Zito BA, et al. (2021) Association between bitter taste receptor phenotype and clinical outcomes among patients with COVID-19. *JAMA Netw Open* 4: e2111410.
38. Diószegi J, Llanaj E, Ádány R (2019) Genetic background of taste perception, taste preferences, and its nutritional implications: A systematic review. *Front Genet* 10: 1272.
39. Sollai G, Melis M, Pani D, Cosseddu P, Usai I, et al. (2017) First objective evaluation of taste sensitivity to 6-n-propylthiouracil (PROP), a paradigm gustatory stimulus in humans. *Sci Rep* 7: 40353.
40. Genick UK, Kutalik Z, Ledda M, Souza Destito MC, Souza MM, et al. (2011) Sensitivity of genome-wide-association signals to phenotyping strategy: the PROP-TAS2R38 taste association as a benchmark. *PLoS One* 6: e27745.
41. Duffy VB, Davidson AC, Kidd JR, Kidd KK, Speed WC, et al. (2004) Bitter receptor gene (TAS2R38), 6-n-propylthiouracil (PROP) bitterness and alcohol intake. *Alcohol Clin Exp Res* 28: 1629-1637.
42. Bachmanov AA, Beauchamp GK (2007) Taste receptor genes. *Annu Rev Nutr* 27: 389-414.
43. Khataan NH, Stewart L, Brenner DM, Cornelis MC, El-Sohehy A (2009) TAS2R38 genotypes and phenylthiocarbamide bitter taste perception in a population of young adults. *J Nutrigenet Nutrigenomics* 2: 251-256.
44. Farquhar DR, Kovatch KJ, Palmer JN, Shofer FS, Adappa ND, et al. (2015) Phenylthiocarbamide taste sensitivity is associated with sinonasal symptoms in healthy adults. *Int Forum Allergy Rhinol* 5: 111-118.
45. Workman AD, Brooks SG, Kohanski MA, Blasetti MT, Cowart BJ, et al. (2018) Bitter and sweet taste tests are reflective of disease status in chronic rhinosinusitis. *J Allergy Clin Immunol Pract* 6: 1078-1080.
46. Bartoshuk LM, Duffy VB, Reed D, Williams A (1996) Supertasting, earaches and head injury: Genetics and pathology alter our taste worlds. *Neurosci Biobehav Rev* 20: 79-87.
47. Cowart BJ, Yokomukai Y, Beauchamp GK (1994) Bitter taste in aging: Compound-specific decline in sensitivity. *Physiol Behav* 56: 1237-1241.
48. Mennella JA, Pepino MY, Duke FF, Reed DR (2010) Age modifies the genotype-phenotype relationship for the bitter receptor TAS2R38. *BMC Genet* 11: 60.
49. Cameron-Smith D, Burke LM, Angus DJ, Tunstall RJ, Cox GR, et al. (2003) A short-term, high-fat diet up-regulates lipid metabolism and gene expression in human skeletal muscle. *Am J Clin Nutr* 77: 313-318.
50. Kalmus H, Trotter WR (1962) Direct assessment of the effect of age on PTC sensitivity. *Ann Hum Genet* 26: 145-149.
51. Kaplan AR, Fischer R (1965) Taste sensitivity for bitterness: Some biological and clinical implications. In: Wortis, J, *Recent Advances in Biological Psychiatry*. Plenum, New York, 183-196.
52. Dass U (1976) P.T.C. taste sensitivity among the Mikir of Assam. *Bulletin of the Department of Anthropology Dibrugarh University* 5: 71-73.
53. Whissell-Buechy D (1990) Effects of age and sex on taste sensitivity to phenylthiocarbamide (PTC) in the Berkeley Guidance sample. *Chemical Senses* 15: 39-57.
54. Schiffman SS, Gatlin LA, Frey AE, Heiman SA, Stagner WC, et al. (1994) Taste perception of bitter compounds in young and elderly persons: Relation to lipophilicity of bitter compounds. *Neurobiology of Aging* 15: 743-750.
55. Reed DR, Bartoshuk LM, Duffy V, Marino S, Price RA (1995) Propylthiouracil tasting: Determination of underlying threshold distributions using maximum likelihood. *Chemical Senses* 20: 529-533.
56. Davies NG, Klepac P, Liu Y, Prem K, Jit M, et al. (2020) Age-dependent effects in the transmission and control of COVID-19 epidemics. *Nat Med* 26: 1205-1211.
57. Mennella JA, Reed DR, Roberts KM, Mathew PS, Mansfield CJ (2014) Age-related differences in bitter taste and efficacy of bitter blockers. *PLoS One* 9: e103107.
58. Reed DR, Tanaka T, Mcdaniel AH (2006) Diverse tastes: Genetics of sweet and bitter perception. *Physiol Behav* 88: 215-226.
59. Negri R, Smarrazzo A, Galatola M, Maio A, Iaccarino Idelson P, et al. (2015) Age Variation in Bitter Taste Perception in Relation to the Tas2r38 Taste Receptor Phenotype. *International Journal of Nutrition* 1: 87-98.
60. Robino A, Mezzavilla M, Pirastu N, Dognini M, Tepper BJ, et al. (2014) A population-based approach to study the impact of PROP perception on food liking in populations along the Silk Road. *PLoS One* 9: e91716.