Iraqi Journal of Science, 2023, Vol. 64, No. 10, pp: 5030-5041
DOI: 10.24996/ijs.2023.64.10.12

ISSN: 0067-2904

Genetic and Phenotypic Variations in Phenylthiocarbamide Bitter Taste Receptors in Iraqi Population

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Received: 3/11/2022 Accepted: 28/1/2023 Published: 30/10/2023

Abstract

Bitter substances are identified by protein receptors located on surface of taste cell membranes. Mutational polymorphism of the bitter taste receptor (TAS2R38) is a significant determinant in phenylthiocarbamide (PTC) threshold perception. This research's objectives were to find TAS2R38 polymorphisms in Iraqi people and investigate any correlations between genotype and the PTC taste sensitivity. Bitterness sensitivity was determined by assessing the capacity to differentiate and the responsiveness to a representative strip of PTC. Cheek cells samples were collected for DNA extraction, PCR amplification and genotyping. PCR was performed to amplify the short region of the TAS2R38 gene containing the initial polymorphisms of interest (145G > C, rs713598). Amplified samples were digested by the restriction enzyme (*Hae*III) to study the genetic variations in TAS2R38 which is involved in PTC bitter sensitivity.

This particular study included a total of 32 different cohorts. The phenotypic frequency of PTC strong-tasters and non-tasters was identical at 34.375% which was a greater value than the frequency of weak-tasters (31.25%). While genotypic data analysis showed that weak-tasters had a genotypic frequency of 45.16% which was higher than the genotypic frequencies of strong-taters (22.58%) and non-tasters (32.26%) respectively. In addition to this, 87.1% of the projected phenotypic and genotypic frequencies were in agreement with one another.

Even though more detailed statistical analysis with a bigger group is needed. The results of this study suggest that allelic variation in the single locus TAS2R38 rs713598 works as a crucial genetic marker for bitterness sensitivity and has vital roles in the bitter tasting ability among Iraqi population.

Keywords: Phenylthiocarbamide (PTC); Bitter; Single nucleotide polymorphisms (SNP); TAS2R38

التغايرات الجينية والمظهرية في مستقبلات الطعم المر للفينيل ثيوكاربامايد في العراقيين

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الخلاصة

المركبات ذات الطعم المر يتحسسها الانسان و الحيوان عن طريق مستقبلات البروتين الموجودة على سطح أغشية خلية التذوق و الموجودة على سطح اللسان. يعد تعدد الأشكال الطفري لمستقبلات الطعم المر

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(TAS2R38)عاملاً هامًا في إدراك عتبة تذوق الطعم المر لمركب فينيل ثيوكاربامید. أجريت هذه الدراسة لتقييم التغايرات الجينية في مستقبل الطعم المر (TAS2R38) و البحث في امكانية تاثيرها على تحديد حساسية التذوق للطعم المر لمركب PTC بين مجموعة من العراقيين. قدرت قابلية تحسس المشتركين في التجربة لتذوق الطعم المر واللذين توزعوا ما بين (متحسس قوي، متحسس متوسط، غير متحسس) من خلال تقييم القدرة على التمايز والاستجابة لشدة الطعم المر لشريط مركب فينيل ثيوكاربامید. جمعت عينات من خلايا بطانة الفم (الخد) لاستخلاص الحمض النووي لاستخدامه في تفاعل البوليميراز المتسلسل (PCR) و من ثم دراسة التنميط الجيني. إجري تفاعل البلمرة المتسلسل لتضخيم قطعة من الجين المشفر للمستقبل (TAS2R38) للتحري عن الطفرة الوراثية في تسلسل القاعدة النيتروجينية ,C > G145). (rs713598هضمت عينات ال PCR المضخمة بواسطة إنزيم التقييد (*Hae*III) لدراسة الاختلافات الوراثية في جين المستقبل TAS2R38 والذي له الدور في تحديد شدة تذوق الطعم المر.

شملت هذه الدراسة 32 متطوعا. اظهرت النتائج بان تكرار النمط المظهري لمتذوقين الطعم المر (متحسس قوي) وغير المتذوقين متطابقًا و بنسبة34.375٪ ، وهي قيمة أكبر من تردد المتذوقين للطعم و لكن بمستوى تحسس واطئ و كانت النسبة 31.25٪. بينما أظهرت تحليلات بيانات النمط الجيني أن المتذوقين بمستوى تحسس واطئ لديهم تردد وراثي بنسبة 45.16٪، وهو أعلى من الترددات الوراثية للمتذوقين (متحسس قوي للطعم المر) بنسبة 22.58٪ وغير المتذوقين بنسبة 32.26٪ على التوالي. بالإضافة إلى ذلك، اظهرت النتائج بان الترددات المظهرية والوراثية كانت متوافقة مع بعضها البعض بنسبة 87.1%.

على الرغم من الحاجة إلى تحليلات إحصائية أكثر تفصيلاً لمجموعة أكبر، بينت هذه الدراسة أن التباين الأليلي في الموضع الفردي للجين المسؤول عن تحديد تحسس التذوق للطعم المر TAS2R38 rs713598 يعمل كعلامة جينية حاسمة لحساسية تذوق الطعم المر، كما أن له أدوار حيوية في تحديد قدرة حساسية التذوق المر بين السكان العراقيين.

Introduction

Taste perception is an important part of organism-environment interaction because it offers significant information about the quality and nutritional worth of the food ingested and allows people to recognise potential dietary dangers. Humans are capable of perceiving five distinct tastes (sweet, bitter, sour, salty and umami) [1], [2]. Several studies have discovered a correlation between bitter responses and features such as food preference, alcohol use, smoking habits, thyroid function, Body Mass Index (BMI) and susceptibility to colon polyps. This lends credence to the theory that variations in bitter sensitivity might result in both immediate preferences and long-term downstream consequences [3-5]. As a result, establishing the causes of variations in sensitivity and intake among people and groups can be performed by investigating the processes behind variance in bitter perception [6], [7]. A variety of complicated variables including age, gender, morphology and environment, interact to cause significant individual variances in bitter perception [8]. In the 1930s, Arthur Fox, a chemist at DuPont, synthesized phenylthiocarbamide. While transferring the substance into the bottle, he produced a cloud of PTC dust, causing his lab partner to react dramatically. Although Fox was unable to taste the dust, his colleague found it to be extremely bitter [9].

The TAS2R38 gene (located on chromosome 7) is the most well researched bitter receptor in terms of both functional and epidemiological correlations. It controls taste reactions to thioamides, including the typical biomarker PTC [10]. *Kim et al*. [10] demonstrated the significance of TAS2R38 locus to bitter perception for the first time in 2003. They discovered two common alleles coding for two different sets of amino acids, Proline-Alanine-Valine (PAV) and Alanine-Valine-Isoleucine (AVI), that had radically different functional features in relation to PTC threshold detection. Individual variances in taste sensitivity might be attributed to genetic variants in taste receptors which have been linked to disparities in taste-related behaviours [11-13]. Moreover, relationships between PAV/AVI variations and

ingestion of substances not known to include thioamides imply that TAS2R38 may affect taste reactions to other families of chemicals, although this idea has not been directly verified [14]. Synonymous mutations specify the same amino acid as wild-type alleles because the genetic code is redundant. A nonsynonymous mutation results in a new codon that specifies a distinct amino acid. The CCA proline codon is converted to the alanine-coding sequence GCA via a G to C substitution at position 145 of the TAS2R38 locus. This amino acid substitution modifies the TAS2R38 receptor's ability to bind PTC in a lock-and-key fashion [15].

According to additional study, these genotypes are also based on long-term trends in smoking, eating habits and obesity [16-19]. Study findings, which was carried out in Korea, revealed that the TAS2R38 T allele was linked to a greater fruit consumption and a higher risk of obesity in Korean females [20]. A previous study revealed that adolescents who are sensitive to the bitter taste of 6-n-propylthiouracil (PROP) consume much more sweets and significantly less meat (savoury fats) than adolescents who are not sensitive [21]. The TAS2R38 diplotype has been linked to cruciferous vegetable consumption. Cruciferous vegetables include thiourea-containing glucosinolates which are TAS2R38 agonists [22]. The genetic variable influenced the choice for fruit, sugary meals, fat and alcohol over bitter items [23-26]. Furthermore, bitter taste receptor TAS2R38 polymorphisms is associated to oral microbiota, oral disease and Rheumatoid Arthritis (RH) [27], [28].

In this work, we aimed to further identify the genotypic and phenotypic bases of TAS2R38-mediated PTC bitter reactions by investigating a group of 32 Iraqi individuals by first assessing the capacity to differentiate and the responsiveness to a representative strip of PTC, and then amplifying a portion of the gene using PCR and digestion by restricting enzyme to study PTC-genotype.

Materials and Methods

Subjects

Thirty-two nonsmoking and nonalcohol drinking healthy young female and male students who were studying at the University of Baghdad (Baghdad, Iraq) between April 2022 and May 2022, were selected for this study. All subjects were Arab and from Baghdad, Iraq. No statistical approaches were used to establish sample size in advance. None of the participants were dieting or taking drugs that could impair taste function. As well as, none of the participants suffered from food allergies or got high marks for their eating habits. This trial was listed at Department of Biology (Identification code: 0222/0114). The Ethical Committee of the Department of Biology/College of Science at the University of Baghdad authorised the research methods that were carried out in compliance with the Helsinki Declaration (identifier of Ethical approval code: CSEC/0322/0064). All participants were verbally knowledgeable of the procedure and purpose of the study and signed a consent form after reviewing the information.

Bitter Perception Assessment

A total of 32 students were tested for their sensitivity level to PTC bitter tasting and SNP genotypes. The PTC taste strip (Bartovation, USA) and cheek swabs were used by participants from a variety of sex and age. First strip of PTC control taste paper was positioned in the centre of the participant tongue for several seconds and the taste was recognized. The control paper was removed and one strip of PTC taste paper was positioned as above. In comparison to the control, the following tastes were recorded: strongly bitter, weakly bitter or no flavour other than paper.

Molecular Analysis

Cheek cell samples were collected from the same students as above. The inner surface of student's cheek was gently scraped a few times with the blunt end of a sterilised flat toothpick. The toothpick's blunt end was placed in 0.5mL of sterile 0.9% saline solution. The toothpick was aggressively agitated in the solution to remove the cells. The previous step was repeated two more times (three samples of cheek cells were collected and transferred to tube of saline). Tube was briefly vortexed for 5-10 seconds to break up any cell clumps before centrifuging the samples at 13,000 x g for 90 seconds. A total of 400µl supernatant was aspirated and the tube with pelleted cells was kept. The pelleted cells were resuspended in 250µl of 10% w/v Chelex-100 resin particles (Sigmaaldrich, Germany). The mixture was then placed in 99°C hot block for 10 minutes. The heated solution was resuspended by vigorously shaking for 5 seconds and then the tube was centrifuged at 10,000 x g for 2-5 minutes to precipitate Chelex-100 beads. Without disrupting the bottom beads, 50µl (the extracted DNA) of the solution was pipetted into a fresh tube.

145 SNP Genetic Variation in TAS2R38

Conventional PCR was performed in a T100TM Thermocycler (Bio-Rad, USA) and using OneTaq DNA polymerase (NEB, UK). Using an initial DNA denaturation step at 95°C for 5 minutes followed by 35 cycles at the temperatures: 95°C for 30 seconds, 56°C for 45 seconds, 72°C 30 seconds, followed by 72°C for 10 minutes. The following PCR primers were used in this study: PTC-F 5'-CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG-3' (Forward) and PTC-R 5'-AGGTTGGCTTGGTTTGCAATCATC-3' (Reverse) [29]. A no template reaction (using H_2O) was used as a negative control. PCR products in volume of 10µL were mixed with 6x loading dye [New England Biolabs (NEB), U.K.] and visualized by gel electrophoresis using 2% agarose with EtBr before being visualised using a Dark Reader-Compact Transilluminator (Clare Chemical Research, USA). 10uL of the remaining PCR products was transferred into fresh tube and digested by using 1uL of restriction enzyme [(*Hae*III) (NEB, UK)] and then incubated for 10 minutes at 37°C. The digested products were mixed with 6x loading dye and visualised as mentioned above.

Results

Determination of PTC taster status

Based on the PTC taster status data, out of 32 subjects 11 (34.375%) were same phenotypic frequency for both strong-tasters and non-tasters, while 10 (31.25%) were weak-tasters to PTC (**Figure 1**).

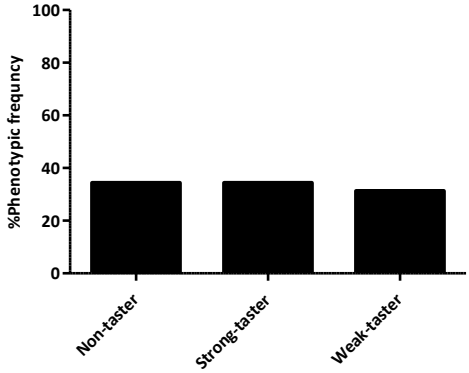

Figure 1: Graph showing phenotype frequencies for PTC tasting ability**.** 32 participants from a variety of sex and age were tested for their sensitivity level to the bitter taste of the PTC-taste strips by placing the taste strip in the center of the tongue for few second. The taste was

noted as compared to the control: strongly bitter (Strong-taster), weakly bitter (Weak-taster), or no taste (Non-taster).

SNP linkage analysis

The TAS2R38 gene, which encodes bitter protein receptors, is located on chromosome 7 in humans. The whole-gene sequencing consists of 1,143 nucleotides (1,002 bp of coding sequence and 141 bp of UTR and generates polypeptides consisting of 334 amino acids). It is known from sequencing data that it contains three SNPs (C145G, C785T, and A886G) that encode three amino acid substitutions. The three SNPs for taster and non-taster alleles are listed in **Table 1**.

Table 1: SNPs encode 3 amino acid substitutions and mediated PTC Taster and Non-taster alleles.

Nucleotide position	Taster (PAV)*	Non-taster (AVI)**
145	CCA Proline	GCA Alanine
785	GCT Alanine	GTT Valine
886	GTC Valine	ATC Isoleucine

PAV taster allele is referred to proline, alanine & valine.
AVI non-taster allele is referred to alanine, valine and isoleucine.

Figure 2: Expected PCR products amplified by PTC-F/R primers are (a) the version of the gene that encodes for the ability to taste PTC (TT allele) and (b) the version of the gene that is unable to bind to PTC (tt allele). The underlined sequence represents the forward and reverse primer annealing positions.

DNA sequence below (**Figure 2A & B**) is shows PCR products with size 221 bp. The forward primer used in this study [(PTC-F 5'-CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG-3' (Forward)] ends with the sequence GG and its bind to nucleotides 101–144 within the TAS2R38 bitter tasting receptor gene. One mismatch exists at locus 143 (lowercase) where the primer contains a G and the gene contains an A. This discrepancy is essential for both alleles (TT and tt) at this locus to be amplified equally well with these primer pairs. The A at this locus is substituted with a G in both PCR products. This generates the initial G for the *Hae*III the sequence of recognition GGCC, letting the PCR product of strong-taster allele (TT) to be cut. However, PCR product of non-taster allele contains sequence GGGC and is uncut. Due to this difference in amplicon digestion profile, we can distinguish between the two alleles at this locus.

PTC-taste Receptor Gene Variation

With these three SNPs that are linked to bitter taste sensitivity, in this study, we only examined one mutation (145 SNP) in the TAS2R38 locus to validate the relationship between SNPs genotype and PTC-bitter tasting phenotype in Iraqi population. The diagram below shows how PCR amplification and restriction digestion are used to identify the G145C SNP in the *TAS2R38* (PTC) locus. The homozygote recessive non-taster allele (tt) is not digested by *Hae*III and, when run on agarose gel (left lane), exhibits a single band in the same place as the undigested control (221 bp), thus indicating that PCR product lacks GGCC sequence. However, the homozygous dominant taster allele (TT) is digested by *Hae*III and associated with strong PTC taste. After running on agarose gel (center lane) displays two bands as follows: 44 bp and 177 bp and that means that the PCR amplicon contains GGCC sequence.

The 44 bp band is likely to be weak (unclear) and the 177 bp band migrates slightly ahead of the control (undigested PCR product). A little amount of undigested product may stay at the 221 bp position due to incomplete digestion process, but this band must be noticeably fainter than the 177 bp band. Furthermore, Tt weak-taster (heterozygous) exhibits the following band: 221 bp, 177 bp, and 44 bp that reflect both alleles (TT and tt). The 221 bp band must be more intense than the 177 bp band (weaker 221 bp band indicates an incomplete TT digest) (**Figure 3**).

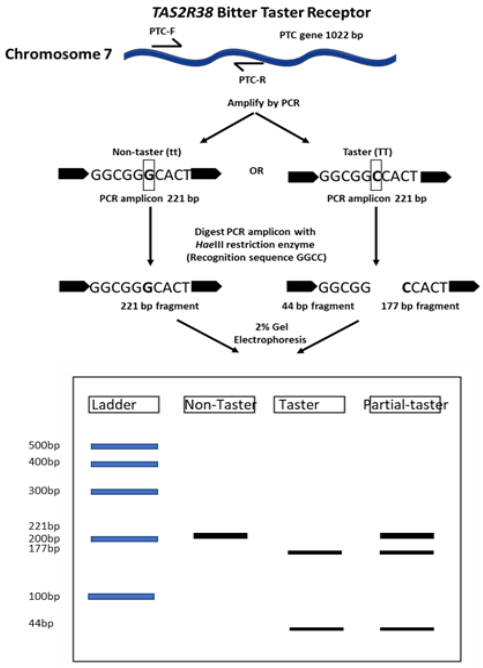

Figure 3: Schematic representation of PCR amplification and restriction digestion to identify the G-C SNP in the TAS2R38 (PTC) locus.

PCR products were visualized on a 2% agarose gel to monitor whether the PCR did work or not before digesting products with restriction enzyme *Hae*III, and also to use these samples as uncut controls by comparing them with restriction digested samples. Products of the expected size were detected for all 32 subjects (predicted PCR product size is 221 bp). All the PCR results are shown in **Figure 4**. To determine the participant's alleles, we compared the digested PCR product (**Figure 5**) with the uncut controls (**Figure 4**). Digested sample 17 was undetectable and for this reason we decided to exclude it from any further analysis. Based on the genotype data analysis, we found the non-tasters samples accounted for 10 (32.26%) of

the total, and they were 4, 8, 10, 12, 13, 16, 18, 22, 26 and 28. The strong-tasters samples were 6, 11, 15, 20, 24, 30 and 32, representing 7 (22.58%) of the total. The weak-taster samples were 1, 2, 3, 5, 7, 9, 14, 19, 21, 23, 25, 27, 29 and 31, representing 14 (45.16%) of the total (**Figure 6**).

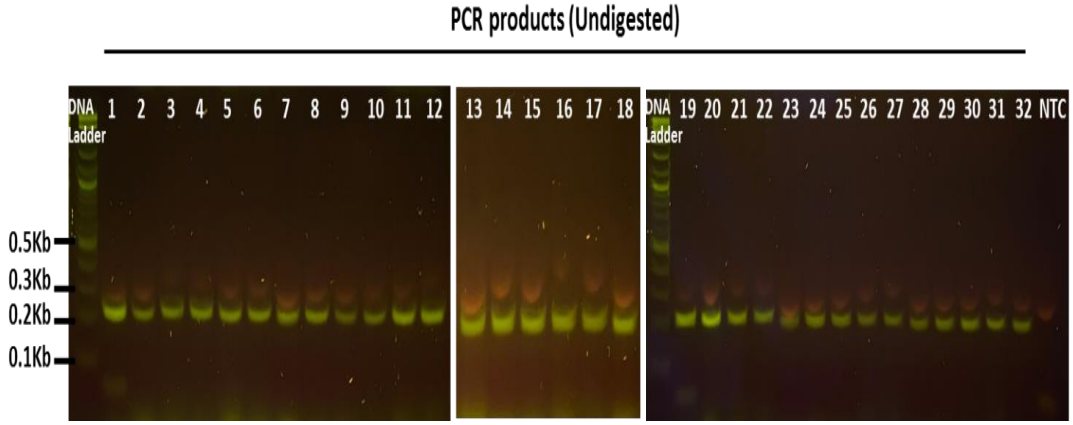

Figure 4: Gel electrophoresis image (2%) shows PCR amplification (undigested) of the 221 bp TAS2R38 bitter taster receptor region in chromosome 7 and the DNA was amplified with primers PTR-F and PTC-R. Each lane is as follows: 1Kb DNA ladder, lane (1-32) DNA extracted from participants cheek cells, (NTC) no template control (H_2O) was used throughout to ensure validity.

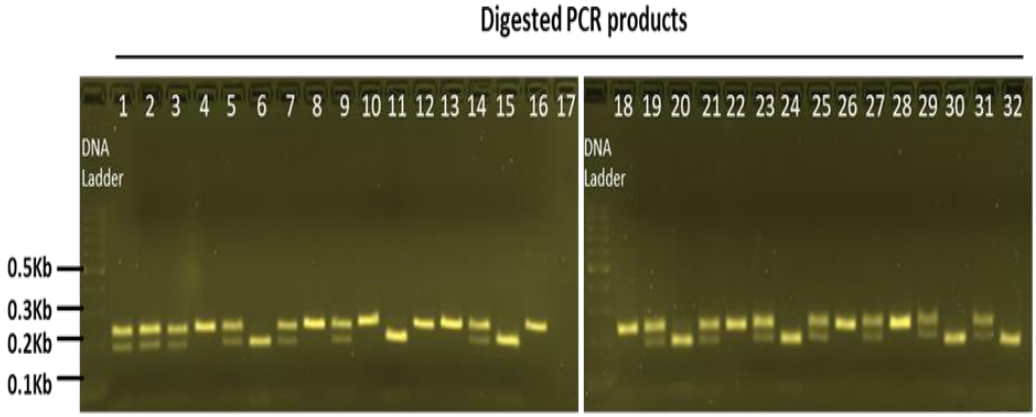

Figure 5: HaeIII digestions of PCR products show the PTC genotypes profile for comparison with the phenotypic data. 1 Kb DNA ladder, Lane 1-32 digested PCR products. The homozygous dominant taster allele (TT) is digested by HaeIII and associated with strong PTC taste shows two fragments as follows: 177 bp and 44 bp. Tt weak-taster (heterozygous) reveals three fragments that represent both alleles (TT and tt)—221 bp, 177 bp, and 44 bp. The homozygote recessive non-taster allele (tt) isn't digested by HaeIII and represents a single fragment in the same position as the uncut control (221 bp band size).

Table 2 summaries the data for phenotypes and genotypes and displays the concordance between the predicted PTC-tasting phenotypes and the *TAS2R38* genotypes profiles. After excluding sample 17 for further analysis, we found that 26 (87.1%) of the total 31 samples represented as matched between PTC-tasting phenotypic and genotypic frequencies. Meanwhile, the percentage of unmatched sample frequencies was 4 (12.9%), and they were as follows: 3, 14, 21, and 31.

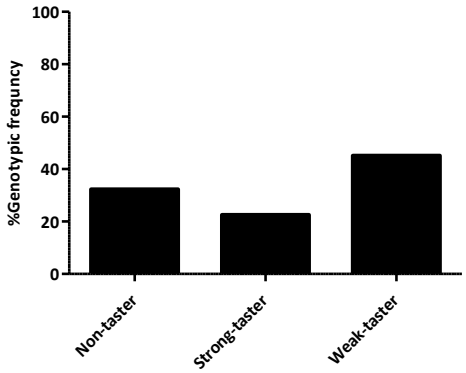

Figure 6: Graph showing genotype frequencies for PTC tasting ability among Iraqi**.**

Table 2: The summary of association between the phenotype and genotype frequency for PTC tasting ability in Iraqi population

Participants	Phenotype	Genotype
1	Weak-taster	Tt
2	Weak-taster	Tt
3*	Strong-taster	Tt
4	Non-taster	Tt
5	Weak-taster	Tt
6	Strong-Taster	TT
7	Weak-taster	Tt
8	Non-taster	Tt
9	Weak-taster	Tt
10	Non-taster	Tt
11	Strong-taster	TT
12	Non-taster	Tt
13	Non-taster	Tt
14*	Strong-Taster	Tt
15	Strong-taster	TT
16	Non-taster	Tt
17	Non-taster	Undetected
18	Non-taster	Tt
19	Weak-taster	Tt
20	Strong-taster	TT
21*	Strong-taster	Tt
22	Non-taster	Tt
23	Weak-taster	Tt
24	Strong-taster	TT
25	Weak-taster	Tt
26	Non-taster	Tt
27	Weak-taster	Tt
28	Non-taster	Tt
29	Weak-taster	Tt

30	Strong-taster	TT
31*	Strong-taster	Tt
32	Strong-taster	TT

**** Indicates an unmatched between the genotypes weaker taste and the predicted PTC strong taste phenotypes.***

Discussion

The initial purpose of this research was to describe the perception of the PTC taste status by evaluating the efficiency of subjects to respond to PTC strips and to determine whether the allelic variation in the single locus TAS2R38 rs713598 works as a crucial genetic marker for bitterness sensitivity. In general, the presence of a T allele (homozygotes) predicts the ability to bitterness taste but poor tasters are more likely to be a heterozygous trait. Even in the most basic genetic systems, such as PTC tasting, it is uncommon for one allele to be completely dominant in comparison to another. This study only considered one of multi mutations in the TAS2R38 locus that affects bitter taste. According to research by *Bufe et al.* [30] variations in the TAS2R38 allele code for functionally distinct receptor types that have a direct impact on how bitterness-containing substances are perceived. The two primary variations of this bitter receptor gene that have been discovered in the majority of the world's populations are the major taster form and the major non-taster form. These two variants differ from one another at positions 49, 262 and 296 of the amino acid position [31].

Taste for the PTC is a typical Mendelian characteristic that has long been seen to vary in the human population. This trait is of genetic, epidemiologic and evolutionary significance that has been linked to a range of food choices, indicating that it has major health consequences for humans [30], [32], [33]. Other studies, on the other hand, have shown that PTC tasting ability is not simply Mendelian in its transmission pattern, and that, rather than being a dichotomous trait, it demonstrates a wide and continuous, although bimodal, distribution of taste sensitivity in the population [34-37].

Importantly, this study provides gene determinations for PTC receptor variants. Despite the small cohort's size, we were able to investigate the effects of specific alleles on PTC-taste measures. The method we employed clearly distinguished tasters from non-tasters, as well as distinguished PTC strong-tasters and PTC moderate tasters. This section examined data from a wide variety of research because regional studies on the capacity to taste PTC in Iraq were either scarce or unavailable. Hence, these a broad studies were described here for comparison [38].

The ratio of tasters to non-tasters varies among every population that contains both tasters and non-tasters. Numerous studies indicate that approximately 70-75% of the global population are PTC tasters and 25-30% are non-tasters [11], [39], [40]. Other study found that the prevalence of non-tasting bitter compounds extent from 3% in West Africa, to 6% in China, 40% in India and 30% in the United States [41]. The data on PTC taste ability is extensive and a considerable deal of research has been conducted worldwide. Previous studies found the percentage frequency of non-tasters for PTC taste ability in Indians is 33.62% [42] which corresponds to 34.375% and 32.26 % (phenotypic and genotypic analyses, respectively) in the current study. Our findings were almost identical to those of this research. Moreover, in this study we found the percentage frequency of 34.375% and 22.58% (phenotypic and genotypic analysis, respectively) for strong-tasters. Other study observed percentage frequency of 42.02% in South Asia [42]. Our research on PTC taste perception discovered that there is similarity percentage frequency of tasters as compared to non-tasters

among cohorts which disagreed with other studies [11], [39], [43]. In addition, we found the percentage frequency of 31.25% and 45.16% (phenotypic and genotypic analysis, respectively) for weak-tasters. Interestingly, there are no bitterness genetic studies that distinguish between PTC strong-tasters and PTC moderate tasters that have been conducted to date. Our finding of the percentage of unmatched sample frequencies was 12.9%, between the PTC-tasting phenotypic status and genotypic analysis was particularly unexpected in light of evidence that allelic variation in the single locus TAS2R38 rs713598 works as a crucial genetic marker for bitterness sensitivity and it has vital roles in the bitter tasting ability. The possible explanations exist for our observation that it may well be that the variance in taste perception is likely influenced by brain processing which involves numerous other genes [34], [44].

Conclusion

Humans have significant variation in taste sensitivity and given the bitter taste receptor genes' extensive sequence variety and variance [10], we think that a large portion of this phenotypic diversity is genetic in origin. Similar to prior investigations, the current research demonstrated that genetic variants in the TAS2R38 bitterness [SNP A49P (145G > C, rs713598)] receptor play a crucial role in the regulation of bitterness perception for PTC in certain Iraqi individuals. More research is needed to determine whether TAS2R38 genetic variations can be used to predict the risk of metabolic disorders and obesity and also to understand the nature of this variation and its relationship to dietary habits and other behaviors including smoking, using drugs, and drinking alcohol.

Conflict of Interest

The author declares no conflict of interest.

Acknowledgements

I appreciate the help of the molecular laboratory staff at the Department of Biology, University of Baghdad, Iraq-Baghdad.

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