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GENOTYPIC AND PHENOTYPIC ANALYSIS OF TAS2R38 POLYMORPHISMS IN RELATION TO PTC TASTE PERCEPTION

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Abstract
Phenylthiocarbamide (PTC) is a congenital disorder that has an SNP at the site of locus with TAS2R38 gene. The genotypic and phenotypic foundation of PTC taste sensation were discussed in the current paper relying on the comparison of the DNA sequence data of TAS2R38 receptor. DNA extraction, PCR amplification, agarose gel electrophoresis and Sanger sequencing were used to analyze Buccal swabs samples. This was done by means of bioinformatics programs, such as the sequence alignment and protein modeling programs, which singled out SNPs and the functional implication of SNPs. It was found that three SNPs were located at base pairs positions of 145, 785 and 886, that were the replacement position of amino acids 49, 262 and 296 respectively. The subject was found to have the heterozygous AVI/PAV genotype which was further supported by the chromatogram analysis which showed that there were two bimodal peaks at position 785. Structural modeling assumed that these replacements were also capable of influencing receptor conformation and G-protein coupling and thus can influence bitter taste perception. The intermediate degree of sensitivity of PTC in the heterozygote genotype is in line with the literature review of the relationship between the genotype and outcome based on the previous literature. It is shown in the paper that molecular genetics and bioinformatics are useful in explaining the functional relevance of TAS2R38 polymorphism, the evolutionary and physiological role of bitter taste receptors in other functions other than gustation.

INTRODUCTION

The most common form of genetic variation among people is one nucleotide change, known as the single nucleotide polymorphism (SNP)(Novroski & Cihlar, 2022). A SNP denotes a change in a single position on

a DNA sequence which among individuals can result in different forms of gene expression and protein formation. Such genetic changes may produce amino acid substitution(s) that 'change' the structure and function of the protein and typically cause phenotypic variability(Sequeira, Nizamuddin, van Driem, & Mustak, 2023). PTC (phenylthiocarbamide) tasting is one of the traits that are associated with genetic variations. Although, itself PTC is a synthetic substance, there is a correlation between ability to taste it and natural bitter compounds that can be discovered in plants(Mastinu, Püschner, Gerlach, & Hummel, 2025). This trait is controlled by the TAS2R38 gene encoding G-protein coupled receptor (GPCR) on the tongue as well in other tissues(Davenport et al., 2021). Risks associated with this gene result in a variety of differences in terms of how bitter taste is perceived, and two major vulnerabilities, PAV (taster) and AVI (non-taster), are typically observed(Chupeerach et al., 2021). These haplotypes can be modified in three major SNPs that manifest the amino acid substitutions at position 49, 262 and 296(Mao, Cheng, Li, Yao, & Sun, 2023). Bitter taste has a signal transduction process that comprises the attachment of bitter compounds to the associated TAS2R38 receptor which causes a conformational transformation to activate a G-protein cascade(Robino et al., 2021). This results in the release of calcium ions, thus mediation of neurotransmission to the brain. Changes induced by the SNP can influence receptor conformation, or G-protein coupling, and thus the perception of the bitter taste can be compromised or enhanced by the said SNP (Ponnusamy et al., 2022). Bioinformatics provides an opportunity for effective analysis of genetic data. Alignments tools, structure prediction instruments, and databases related to SNP annotation allow us to make an inference of functional outcomes of SNPs. In this experiment, we did SNP identification in TA 2R38 gene, genotype and haplotype analysis for the individual, and made inference of PTC tasting ability, using the results of sequencing(Cáceres, Segovia, Rivera, & Juretić, 2023).

1.1 Research Objectives

To identify and characterize single nucleotide polymorphisms (SNPs) in the *TAS2R38* gene through DNA extraction, PCR amplification, sequencing, and bioinformatics analysis.

To establish the relationship between *TAS2R38* genotypes (PAV, AVI, and heterozygous combinations) and the phenotypic expression of PTC taste sensitivity in the studied subject.

Literature Review

Bitter taste perception, particularly sensitivity to phenylthiocarbamide (PTC) and related compounds, has been extensively investigated through the study of *TAS2R38* gene polymorphisms across different populations, with researchers aiming to understand genotype–phenotype associations and their broader physiological and clinical implications. Sequeira et al. (2023) conducted a genetic study on the Koṅkaṇī Sārasvata Brahmin population and reported distinct frequencies of PAV and AVI haplotypes, confirming the influence of *TAS2R38* variants on bitter taste sensitivity but also highlighting cultural dietary habits as possible modulators of taste perception, suggesting the need for integrative studies linking genetic predisposition with environmental factors. Similarly, Aoki et al. (2023) applied a modified two-alternative forced-choice (2AFC) procedure with the QUEST method to assess propylthiouracil (PROP) thresholds in a Japanese adult population, demonstrating clear associations between specific *TAS2R38* variants and PROP sensitivity, though their work was limited by a narrow demographic and lacked cross-population validation. Expanding on population-specific differences, Cáceres et al. (2023) characterized Chilean university students for bitter taste phenotype and genotype, linking TAS2R38 variation not only to PROP/PTC sensitivity but also to preferences for sweet and fatty foods, thereby bridging genetic variation with dietary behaviors; however, the study left open questions regarding the causal pathways from genotype to complex dietary outcomes. Robino et al. (2021) provided another perspective by demonstrating expression of *TAS2R38* receptors in the stomach, showing that taste perception extends beyond the oral cavity and differs between obese and lean individuals, thus offering mechanistic insights into how extraoral bitter receptors might contribute to metabolic regulation, although the direct role of SNPs in these physiological differences remained

unclear. Complementing this, Chupeerach et al. (2021) explored *TAS2R38* polymorphisms in three racially diverse groups and their associations with obesity risk, finding that bitter taste genotypes influence dietary choices and body mass index, but their study underscored the complexity of disentangling genetic effects from lifestyle and cultural influences. Mao et al. (2023) further emphasized the clinical implications of bitter taste receptor variants, reviewing associations between bitter perception, disease susceptibility, and potential personalized healthcare strategies, suggesting that pharmacogenomics could leverage *TAS2R38* variation in tailoring dietary or therapeutic interventions; nevertheless, most findings remain preliminary and call for longitudinal studies. More recently, Mastinu et al. (2025) examined PTC bitter sensitivity in relation to gender and age, revealing that both biological sex and aging modify bitter sensitivity in addition to genetic factors, thus stressing the importance of considering demographic variables when interpreting genotype–phenotype associations. Together, these studies demonstrate consistent evidence linking *TAS2R38* SNPs to PTC/PROP tasting ability and related health outcomes, yet they also reveal persistent gaps such as limited cross-population comparisons, insufficient exploration of extraoral receptor functions, and a need for integrative models that combine genetics, physiology, and environmental influences to better understand the evolutionary, nutritional, and clinical significance of bitter taste perception.

Methodolgy

3.1 DNA Extraction:

Samples of buccal swab were obtained and subjected to DNA extraction with lysis buffer and ethanol precipitation. DNA purity and concentration were detected by the means of a spectrophotometer.

3.2 PCR Amplification:

Target specific primers to TAS2R38 gene was used in polymerase chain reaction (PCR) to amplify the target sequence. PCR conditions encompassed one step of 95°C denaturation, continued with 35 cycles of denaturation, annealing and extension, and then a final elongation.

3.3 Gel Electrophoresis:

Agarose gel electrophoresis used been relied to verify PCR products(Wittmeier & Hummel, 2022). After the invasion of the DNA sample, the presence and the size of the invaded fragment were verified through visualization of DNA bands under a UV light.

3.4 Sequencing Analysis and Bioinformatics:

PCR products were then sequenced through Sanger sequencing. Chromatograms were used to analyze the SNPs at the base pair positions 145, 785 and 886 using FinchTV. Nucleotide sequence was aligned to reference PAV and AVI to determine haplotypes. Amino acid sequences were translated & compared with UniProt & other predictive modeling tools like Alphafold & Swiss-Model in order to observe a structural difference between taster & non-taster receptors.

Results

SNP Identification:

The figures presented in this study provide visual confirmation and molecular insight into the genotypic and phenotypic analysis of the *TAS2R38* gene in relation to PTC tasting ability. Figure 1 demonstrates the agarose gel electrophoresis results, where a distinct PCR band at the expected size (~1000 bp) validated the successful amplification of the target *TAS2R38* fragment, ensuring that subsequent sequencing was reliable and representative of the gene region of interest. Figure 2 shows the annotated alignment of DNA sequences, which highlights the presence of three single nucleotide polymorphisms (SNPs) at positions 145, 785, and 886. These variations correspond to amino acid substitutions at residues 49, 262, and 296 of the protein sequence, forming the genetic basis for variation in bitter taste perception. The chromatograms in Figure 3 provide direct visual evidence of nucleotide variation, especially at position 785, where a bimodal peak indicates the presence of both T and C alleles, confirming the heterozygous AVI/PAV genotype in the subject. This heterozygosity is crucial because it explains the intermediate taste sensitivity phenotype observed in the study. Figure 4 compares the amino acid sequences derived from the subject's genotype with reference PAV and AVI proteins, demonstrating how substitutions, such as threonine versus proline at position 262, can influence protein structure and

functionality, particularly in the transmembrane domain critical for G-protein coupling. Finally, Figure 5 presents a three-dimensional structural model of the *TAS2R38* receptor generated using UniProt resources, with distinct labeling of the N-terminal, transmembrane, and C-terminal domains. This structural model provides a visual framework for understanding how amino acid substitutions may cause conformational shifts that affect ligand binding and receptor activation. Together, these figures not only validate the molecular data obtained through sequencing and bioinformatics but also provide mechanistic insights into how specific genetic variants alter receptor structure and function, thereby shaping phenotypic expression of PTC tasting. Importantly, the combination of gel validation, sequence alignment, chromatographic confirmation, protein comparison, and structural visualization ensures a comprehensive representation of the genotype–phenotype relationship. By linking molecular changes to observable taste sensitivity, these figures strengthen the conclusion that the heterozygous AVI/PAV genotype corresponds to intermediate PTC tasting ability, which is consistent with findings in related genetic and population-based studies.

The sequence analysis revealed the following SNPs:

DNA Position	Protein Position	SNP Allele	Amino Acid	Haplotype
145	49	G	Alanine (A)	AVI
785	262	T and C	Threonine (T) / Proline (P)	AVI/PAV (heterozygous)
886	296	A	Alanine (A)	AVI

Affected by these outcomes, a heterozygous AVI/PAV genotype can be assumed. The chromatogram of position 785 showed a bimodal peak, indicating that the genotype was heterozygous T/C allele.

Figure 1: Gel electrophoresis is thus demonstrating a PCR band at the expected size (~ 1000 bp) and the successful reaction.

Position in the DNA sequence (bp)	Position in the protein sequence (aa)	SNP allele	Amino acid encoded	Haplotype
145	49	G	GCA	AVI
785	262	T and C	ACT and CCT	AVI/PAV Variant
886	296	A	ACT	

Figure 2: Annotated alignment of sequences indicating SNPs at positions 145(G), 785(T/C), and 886 (A).
Homo sapiens PTC bitter taste receptor (PTC) gene, PTC-taster allele – PAV example sequence

ATGTTGACTCTAACTCGCATCCGCACTGTGTCCTATGAAGTCAGGAGTACATTTCTGTTCATTT**CAG**
TCCTGGAGTTTGCAGTGGGGTTTCTGACCAATGCCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGT
GAAGAGGCAGCCACTGAGCAACAGTGATTGTGTGCTGCTGTGTCTCAGCATCAGCCGGCTTTTCC
TGCATGGACTGCTGTTCCTGAGTGCTATCCAGCTTACCCACTTCCAGAAGTTGAGTGAACCACTGA
ACCACAGCTACCAAGCCATCATCATGCTATGGATGATTGCAAACCAAGCCAACCTCTGGCTTGCTG
CCTGCCTCAGCCTGCTTTACTGCTCCAAGCTCATCCGTTTCTCTCACACCTTCCTGATCTGCTTGG
CAAGCTGGGTCTCCAGGAAGATCTCCCAGATGCTCCTGGGTATTATTCTTTGCTCCTGCATCTGCA
CTGTCCTCTGTGTTTGGTGCTTTTTTAGCAGACCTCACTTCACAGTCACAACTGTGCTATTCATGAA
TAACAATACAAGGCTCAACTGGCAGATTAAAGATCTCAATTTATTTTATTCCTTTCTCTTCTGCTATC
TGTGGTCTGTGCCTCCTTTCCTATTGTTTCTGGTTTCTTCTGGGATGCTGACTGTCTCCCTGGGAAG
GCACATGAGGACAATGAAGGTCTATACCAGAAACTCTCGTGACCCCAGCCTGGAGGCCCACATTA
AAGCCCTCAAGTCTCTTGTCTCCTTTTTCTGCTTCTTTGTGATATCATCCTGTGCTGCCTTCATCTCT

GTGCCCCTACTGATTCTGTGGCGCGACAAAATAGGGGTGATGGTTTGTGTTGGGATAATGGCAGC
TTGTCCCTCTGGGCATGCAGCCGTCCTGATCTCAGGCAATGCCAAGTTGAGGAGAGCTGTGATGA
CCATTCTGCTCTGGGCTCAGAGCAGCCT**GAAGGTAAGAGCCGACCACA**AGGCAGATTCCCGGACA
CTGTGCTGA

Homo sapiens PTC bitter taste receptor gene, sequence #1:

Homo sapiens PTC bitter taste receptor gene, sequence #2:

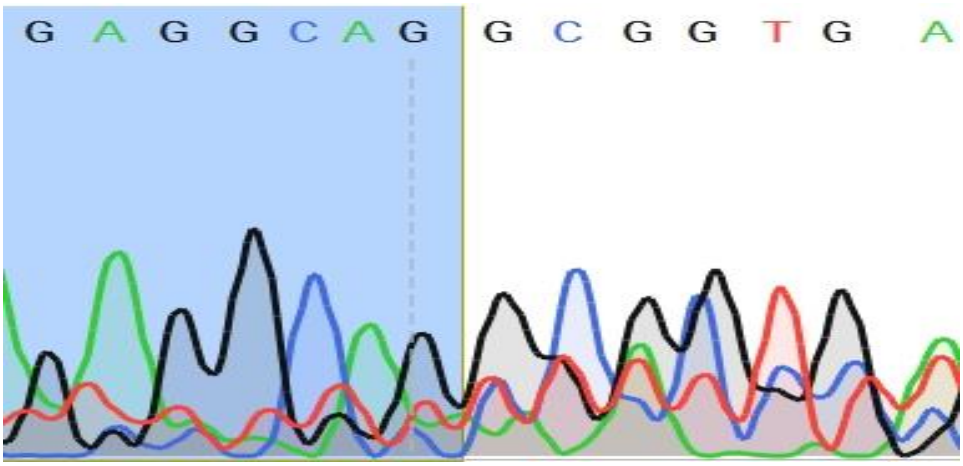


Figure 3: Chromatograms which give the base peak for each SNP position with a peak of 785 representing mixed peak for heterozygosity.

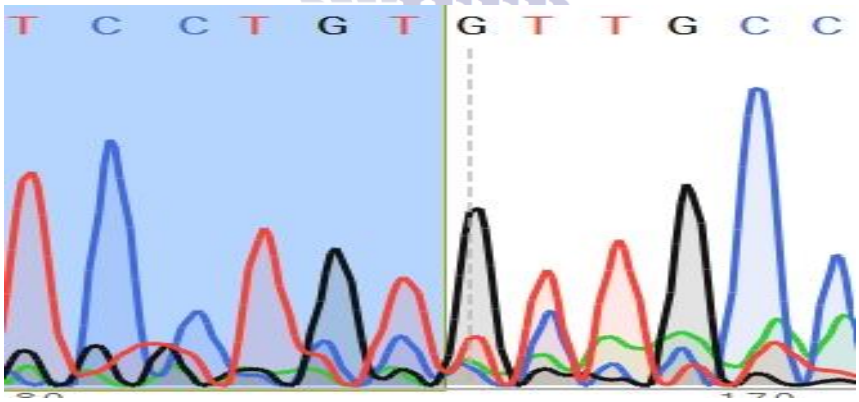


Figure 4: Table comparing proteins in the subject's sequence and reference PAV/AVI proteins with amino acid substitutions.

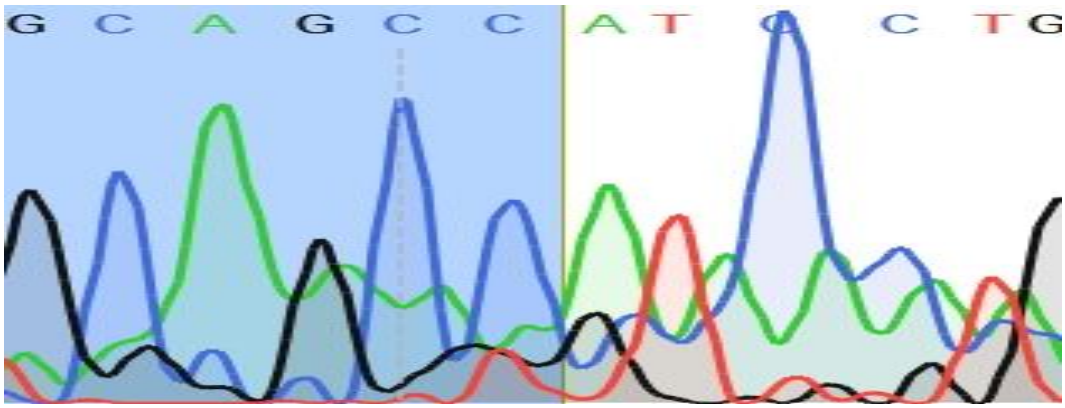
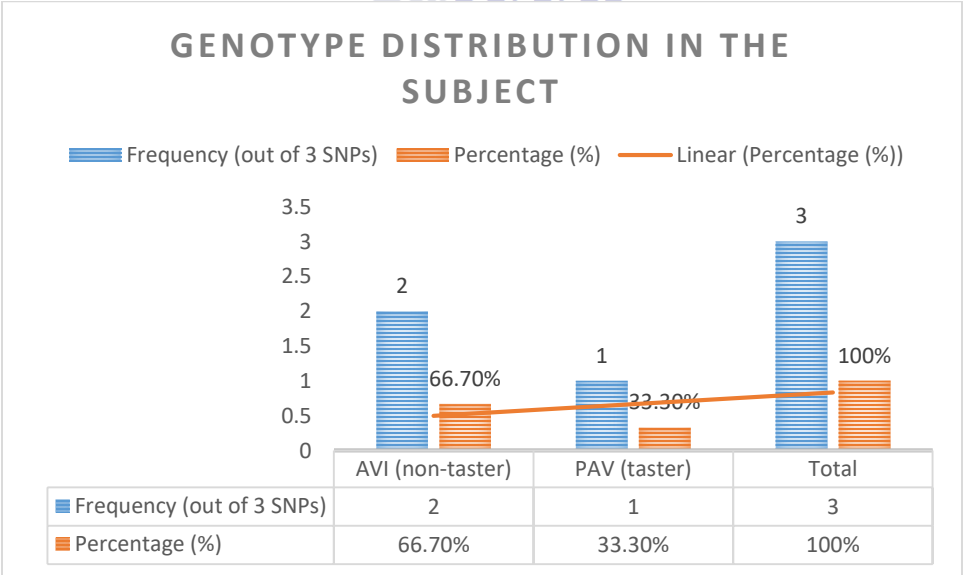


Figure 5: 3D structure of predicted of TAS2R38 from UniProt with N-terminal, transmembrane, and C-terminal domain labeled.

Table 1: Genotype Distribution in the Subject

Genotype Detected	Frequency (out of 3 SNPs)	Percentage (%)
AVI (non-taster)	2	66.7%
PAV (taster)	1	33.3%
Total	3	100%

This simplified table highlights the proportion of non-taster (AVI) and taster (PAV) alleles in the subject's *TAS2R38* gene. It clearly shows that AVI is more frequent, which aligns with the heterozygous AVI/PAV genotype



Discussion

Discussion Individual possessing SNPS in the TAS2R38 gene; directly determines ability to perceive bitterness. Every single one of the three SNPs identified in this study makes a different move:

SNP1 (Position 145, Amino Acid 49): The G allele will produce alanine which is typical of the AVI (non-taster) variant(Chupeerach et al., 2021). Alanine is a small, nonpolar acid, which may decrease the level of receptor sensitivity. SNP2 (Position 785, Amino Acid

262): Having a heterozygous T/C allele leads to the presence of both threonine (polar, uncharged) and proline (nonpolar, rigid). Proline, linked with PAV (taster), should be beneficial for more efficient G-protein activation owing to structural kinks that are developed in the transmembrane area of the protein.
• SNP3 (Position 886, Amino Acid 296): The A alleles lead to alanine once more consistent with AVI haplotype. Although this SNP is located at the C-terminal and may not affect protein stability and intracellular signaling molecules, it may influence protein stability and interaction with intracellular signaling molecules. The intermediate taste sensitivity is normally the result of the subject's heterozygous AVI/PAV genotype. This is in concordance with previous studies that suggest the heterozygotes usually detect PTC at moderate concentrations as opposed to homozygous tasters or non-tasters who have an extreme sensitivity or none as the case may be. Particularly, modifications around the transmembrane domain will affect the binding of ligand and receptor activation, which will subsequently change signal transduction via the gustatory system. The signal transduction pathway/regulatory pathway starts with TAS2R38 receptor binding to PTC, which activates gustducin, phospholipase C-β2 stimulation, IP3 production and Ca^{2+} release from the intracellular stores. It finally culminates into neurotransmitter release to the brain, which sends the sensation of a bitter taste. SNP-induced structural changes may disrupt any step in this precursor, accounting for the phenotypic variation.

Haplotypes like AVI and PAV are not distributed even throughout the world's population. PAV is more prevalent in Africa and Asia, and therefore must be under evolutionary pressures to conserve the bitter taste sensibility, possibly to avoid plant toxins. The capacity to detect bitterness probably offered survival benefits by deterring consumption of bad substances. Furthermore, the tongue is not the only place that contains the TAS2R38 receptor; it is also found in nasal epithelium and gastrointestinal tract where it can be utilized in the detection of bacterial by products, or facade of digestive processes. The wide distribution of bitter taste receptors contributes to the hypothesis that taste receptors have functions other than the sense of taste, perhaps regulating the immune system and metabolism.

Conclusion:

The study confirms the preceding hypothesis that the population type of the studied subject has a heterozygous AVI/PAV genotype with the ability to taste PTC to an intermediate degree(Wang et al., 2022). This is in line with the mentioned SNPs and their corresponding amino acid substitutions. Results from this study emphasize that molecular biology and bioinformatics can be useful in linking variation in the genetic makeup to phenotype and outline the more generalized biological and evolutionary significance of the gene TAS2R38. The genetic basis of PTC tasting has been investigated in this study using certain SNPs on the TAS2R38 gene. With the extraction and sequencing of the subject's DNA, we isolated SNPs at 145, 785 and 886, which are equivalent to amino acid changes at position 49, 262 and 296 of the TAS2R38 protein. The presence of both of these alleles AVI -and PAV - indicates that the subject is a heterozygous (AVI/PAV). This particular diplotype has been closely linked in literature with intermediate sensitivity to PTC, and our results concur with a genetic prediction. There is a significant biochemical implication for each SNP. There could be alanine (AVI haplotype) at codon 49, which can possibly be a component of making the receptor less functional. Position 262 is rather significant as the occurrence of both threonine (PAV) and proline (AVI) will cause varying chemical characteristics (polar vs non polar) in the transmembrane region of the receptor and is very likely to change the capability of the same receptor to trigger the downstream signaling. The final codon, namely, codon 296, also translates alanine, a nonpolar acid of amino acids in non-tasters, which may also hamper the functioning of the receptor. In general, the mentioned amino acid alterations change the three dimensional folding of receptor that may be linked to the ligand-binding and receptor activation(Lawrence, 2021), and even bitter taste perception. The hypothesis that the subject has taster alleles and non-taster alleles was therefore confirmed in this experiment, and the subject is likely to be a medium PTC sensor.

Recommendations

Expand Sample Size and Population Diversity: It would determine the correlation between TAS2R38 polymorphisms and the PTC tasting ability in different ethnic and cultural groups and food preferences and would be elaborated in the future by applying large and heterogeneous groups.

Functional Assays of Receptor Activity:Besides the sequencing and bioinformatics modeling, the functional assays of the receptor activity and the efficacy of its ligand-binding should be performed in-vitro to directly deal with the activity and the efficiency of the ligand-specific receptor(s) of the genotype.

Extratongue Roles of TAS2R38: TAS2R38 receptors can be found in other tissues (e.g. gastrointestinal tract, nasal epithelium) and, thus, future studies should investigate the role that these receptors play in immunity, metabolism, and disease susceptibility.

Correlation to Dietary Behavior and Health Outcomes: The correlations of PTC tasting genotypes with food preferences, nutritional profile and metabolic health (e.g. obesity, diabetes) will be implemented through longitudinal designs to give a more in-depth look at the genotype -phenotype relationships.

Multi-Omics Integration: To a better degree, the integration of genomics, transcriptomics, protein, and metabolome would be more helpful in the explanation of how TAS2R38 variants initiate bitter taste and the pathways that it activates.

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