UNIVERSITY OF CALIFORNIA, MERCED

# Exploring Worldwide Diversity and Variation in Human Gustatory Genes

A dissertation submitted in partial satisfaction of the requirements for
the degree of Doctor of Philosophy

in

Public Health

by

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## Dedication

I dedicate this to my family. To my mother Sylvia, and her husband Arnold. To my father Juan. Mom and Dad, I thank you both for your sacrifices. Mom, thank you for always emphasizing the importance of my education and encouraging my curiosity in science. I dedicate this to my siblings Johnny, Stephanie, Natalie, and Daniel. To my brother-in-law Nick and my niece Noah. You are all my inspiration and I would not be here without your love and support.

I dedicate this work to those who came before me, both in blood and in spirit. To my ancestors, and to those who taught and influenced my mentors. Their influence has brought me to this moment.

Finally, I dedicate this to my god, through whom I have been blessed with this opportunity.

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## Publications

**Journal Publications**

Wooding SP, Ramirez VA. Global population genetics and diversity in the TAS2R bitter taste receptor family. *Front Genet*. 2022;13:952299. Published 2022 Oct 11.

Mackenzie E Hannum, Riley J Koch, Vicente A Ramirez, Sarah S Marks, Aurora K Toskala, Riley D Herriman, Cailu Lin, Paule V Joseph, Danielle R Reed, Taste loss as a distinct symptom of COVID-19: a systematic review and meta-analysis, Chemical Senses, Volume 47, 2022, bjac001

Vicente A Ramirez, Stephen P Wooding, Worldwide diversity, association potential, and natural selection in the superimposed taste genes, CD36 and GNAT3, Chemical Senses, Volume 47, 2022, bjab052

Stephen P Wooding, Vicente A Ramirez, Maik Behrens, Bitter taste receptors: Genes, evolution and health, Evolution, Medicine, and Public Health, Volume 9, Issue 1, 2021, Pages 431–44

Mackenzie E Hannum*, Vicente A Ramirez*, Sarah J Lipson, Riley D Herriman, Aurora K Toskala, Cailu Lin, Paule V Joseph, Danielle R Reed, Objective Sensory Testing Methods Reveal a Higher Prevalence of Olfactory Loss in COVID-19–Positive Patients Compared to Subjective Methods: A Systematic Review and Meta-Analysis, *Chemical Senses*, Volume 45, Issue 9, November 2020, Pages 865–874 *** co-first authorship shared with Dr. Mackenzie Hannum**

**Book Chapters**

Wooding S., Ramirez VA., "Taste Genetics", The Senses: A Comprehensive Reference 2nd Edition, Vol 3 (2021): 264-279

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## Conference Presentations & Posters

Ramirez VA. & Wooding S., Natural Selection and Diversity in Human TAS2R Receptors: Unraveling the Missense of Bitter Taste; Association for Chemoreception Sciences Annual Conference, April 2022

Guerra E, Ramirez V.A., Hunter S, Reed DR, Dalton PH, Parma V. Prevalence of smell and taste loss in youth with COVID-19. Association for Chemoreception Sciences, Bonita Springs, Florida, April 2022. (Evan Guerra Presenting)

Bell KA, Hannum ME, Lin C, Huang A, Kikuchi A, James SL, Ramirez V.A., Nguyen H, Joseph PV, Leszkowicz E, Christensen C, Reed DR. Missing diversity: genetics of bitter taste sensitivity in people of different ancestries. Association for Chemoreception Sciences, Bonita Springs, Florida, April 2022. (Katie Bell Presenting)

Ramirez V.A , “Sniffing though the Evidence: Leveraging Shiny to Conduct Meta Analysis on COVID-19 and Smell Loss”, Evidence Synthesis and Meta Analysis in R Annual Meeting, February 2022

Ramirez V.A., Mainland JD, Dalton P, Reed DR; Assessing the Relationship Between Ambient Air Pollution and Olfactory Function: Revisiting the 1986 National Geographic Smell Survey; International Symposium on Taste and Smell, July 2020

Reed. D.R., Ramirez, V.A., Lin, C., Rawson, N.E., Schwiebert, E.; Bad Taste: Genetics and Cell-Based Assays to Address Global Needs. Pediatric Formulation Development Meeting, Baltimore Maryland, July 2019 (Danielle Reed Presented)

Ramirez V.A, Wallace M., Wooding S. (2019, April), Inia: Scripts for Exploring Localized Regions in the 1000 Genomes Project, Association for Chemoreception Sciences Annual Conference, April 2019

Gharibi H., Ramirez V.A, Entwistle M.,Thao C., Cisneros R., Pesticide and Acute Asthma Attacks in California, USA in 2005 to 2011: A Bidirectional Symmetric Case-Crossover Study; International Society for Environmental Epidemiology Annual Conference, August 2018 (Marcela Entwistle Presented)

Ramirez V.A, Wooding S., Genetic Diversity in Oral Chemosensory Gene Pathways, Association for Chemoreception Sciences Annual Conference, April 2018

Ramirez V.A, Wooding S., Worldwide Variation and Linkage Structure in the Overlapping Orosensation Genes *CD36* and *GNAT3*; Vicente Ramirez & Stephen Wooding PhD, April 2017

## Lectures and Talks

“Assessing the Relationship Between Ambient Air Pollution and Olfactory Function”; Monell Spring Colloquium 2020, April 7 2020, Monell Chemical Senses Center

“Structural Variation in Miscanthus Genomes”; July 2018, Department of Energy Joint Genome Institute

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# Abstract

Gustation, or taste, is a gatekeeper for substances ingested orally. It is hypothesized to have served our ancestors in detecting nutrients from potential food sources and in rejecting potentially toxic substances. Early human migrations following the dispersal from the African continent would have placed ancient populations in novel environments, suggesting the ability to detect nutrients and identify sources of toxins would have been critical to survival to our early ancestors. This role suggests that taste has been subject to selective pressures through the course of evolution. Such pressures contribute to diversity and variance in taste across contemporary populations, suggesting that large differences in taste perception traits are likely the result of holdovers from our evolutionary past. The potential contribution of taste to nutritional behaviors and a variety of diseases, including obesity and diabetes, places taste of interest in taste to health scientist and public health researchers. In chapter 2, I highlight past and recent research examining diversity and genotype-phenotype associations across taste genes. Additionally in this chapter, population genomic data are used to explore diversity and signatures of natural selection across the genes mediating taste. Health and taste researchers alike are interested in such analysis and findings as they describe the underpinnings of natural variability seen among alleles and human phenotypes, and can prioritize targets of future investigation. In chapter 3, I describe Inia, a framework and tool to reproduce such analysis with limited technical expertise. Inia serves as a wrapper around popular analytical tools, providing summaries and rich annotation of variation across local genomic regions. To demonstrate Inia, I examine the putative sour receptor gene *OTOP1*. In chapter 4, I examine the putative fat receptor gene *CD36*, whose transcript is superimposed on the g-protein subunit gene *GNAT3.* CD36 and GNAT3, both of which contribute to taste perception, harbor extensive variation along their locus and such variation may contribute to phenotypic differences. In this chapter I examine potentially functional variants across the region, and explore the extent to which such variation may contribute to phenotypic variance at the population level. This chapter provides an examination of diversity and signatures of selection across CD36 and GNAT3, and provides evidence of important regional trends. Finally, linkage structure across the region is explored given the close proximity of these genes and their dual contribution to taste perception. Together, this dissertation serves to provide insight into the extent and origin of diversity among human populations with regards to the taste genes and the potential contributions to human health and behaviors.

# Chapter 1 Introduction

The origins of the taste genetics story begin as an accidental discovery in 1931, following the unintentional tasting of powdered phenylthiocarbamide (PTC) suspended in the air after a lab accident (Fox, 1932). One of the scientists in the lab noticed the substance was bitter, and the other did not perceive the bitterness. The phenomenon was reproduced in larger samples and studied heavily in the following decades. Among the most important discoveries was the finding that the ability to taste PTC, and the structurally similar molecule propylthiouracil (PROP), was inherited in a mostly Mendelian manner (Snyder, 1931). This discovery would allow the trait to serve as a reliable genetic marker. Bitter taste as a genetic marker was utilized to study several taste and non-taste-related topics such as diabetes(Hardy et al., 1981; Schelling et al., 1965; Terry & Segall, 1947), cancer (Miluničová et al., 1969), psychiatric traits and disorders(Schlosberg & Baruch, 1992; Whittemore, 1990), and many others. However, it was not until over 70 years after the initial discovery of the trait that the gene responsible for the phenomenon was identified. Subtraction hybridization identified taste-specific cDNA clones from subtractive cDNA libraries of rat taste tissues, and ultimately such work would aid in identifying members of the TAS1R family. (Asano-Miyoshi et al., 1998; Hoon & Ryba, 1997). In 1999 a linkage study by Reed and colleagues found that the ability to taste PROP was associated with a region on chromosome 5 and chromosome 7 in humans (Reed et al., 1999). Shortly after, sequence mining and molecular work would lead to the discovery of two GPCR families responsible for sweet and bitter taste: TAS1R and TAS2R (Adler et al., 2000; Chandrashekar et al., 2000; X. Li et al., 2002). Following the 1999 study by Reed, which identified the locus on chromosome 5 that is now TAS2R1, two individual studies using association analysis and linkage analysis would identify the locus accounting for variance in PTC taste to a region chromosome 7 harboring the now-known bitter receptor gene TAS2R38 (Drayna et al., 2003; U. Kim et al., 2003a). Today, at least 25 functional bitter receptors and 11 pseudogenes have been identified in the human genome.

Scientific research focused on understanding the role of genetic variation in shaping human traits and behaviors has sharply increased over the last twenty years. Improvements in sequencing techniques and technologies, such as those found in next-generation sequencing (NGS), have enabled researchers to develop better methods of understanding the genetic underpinnings shaping complex traits and diseases (Goodwin et al., 2016). The discovery of the taste receptor genes was heavily aided by the availability of human and mouse genetic sequencing data at the turn of the century, which allowed for rapid searching of possible taste receptor genes throughout the genome. The completion of the human genome project and the accumulation of large-scale sequencing data have increasingly allowed geneticists and health scientists to investigate the genetic basis of human phenotypic diversity (The 1000 Genomes Project Consortium, 2015; Zheng-Bradley & Flicek, 2017). This abundance of genomic data provides a potential resource for identifying patterns of human genetic variation and diversity in the

genes underlying taste, which may shed light on the evolutionary origins of taste perception, sources of phenotypic variation, and the similarities and differences among human populations. However, the technical skills needed to explore and analyze these data often act as a gatekeeper to producing meaningful findings from such large genomic data. This hinders analyses by nonexperts, including scientists studying the chemosenses.

A primary focus of ongoing work in the field is to identify the receptors responsible for taste and to understand how variation in taste genes shapes individual taste traits such as preferences or perception. Aside from bitterness and the TAS2R family, the receptors responsible for sweet and savory taste have been identified as the TAS1R family of G-protein coupled receptors, which consist of three receptors: TAS1R1, TAS1R2, and TAS1R3 (X. Li et al., 2002; G. Q. Zhao et al., 2003). Additionally, several candidate ion channels and receptors have been identified to mediate salt and sour taste, although the mechanisms for both modalities are still not fully worked out (Dias et al., 2012; Heck et al., 1984; Ishimaru et al., 2006; Ramsey & DeSimone, 2018; Ugawa, 2003; Ye et al., 2016). Relatively recently, the oral detection of fats, which was long believed to be the result of tactile and non-taste sensations, has been established as a distinct taste sensation. The efforts to uncover the genetic underpinnings of fat taste are underway, and current efforts have suggested candidate receptors, including GPR120, GPR40, and CD36 (Cartoni et al., 2010; Ozdener et al., 2014; Running et al., 2015).

The discovery that human taste abilities extend to fat has significant evolutionary implications. It is hypothesized that the ability and drive to obtain fats in the diet played a vital role in the evolution of humans, as the inclusion of high-fat and energy-dense foods likely aided the survival of ancient humans (Leonard et al., 2009). Elucidation of the pressures from natural selection in fat taste may yield novel insights into the role of taste and the dietary value of fats in the origin of humans, as well as relationships between fat perception and health in modern humans.

This dissertation aims to shed light on human taste genetics and the utility of chemosensory science in public health. Chapter 2 shall establish the conceptual basis of taste genetics, offering an extensive review of current and past literature. This chapter focuses on the interspecies diversity among the taste receptors and the genetic diversity observed among human populations. This chapter details previously identified genotype-phenotype associations in taste genes and their context in human health. Finally, this chapter examines patterns of diversity, differentiation, and signatures of natural selection within the genes mediating taste perception through population genetic analysis. These results provide insight into the distribution and diversity of alleles among global populations with regard to taste genes. Chapter 3 presents work on a command line tool, Inia, which extracts and summarizes local variation from the 1000 genomes dataset. This chapter presents a framework, methods, and details scripts devised to aid in identifying and dissecting potential functional variation in human genes using public tools and large-scale sequencing data. As a demonstrating example, I provide the results and discussion of the analysis of the sour taste receptor gene *OTOP1* utilizing the framework and scripts described in Chapter 3. In Chapter 4, I present an analysis of the *CD36* and *GNAT3* locus on chromosome 12, which aims to dissect functional variation and worldwide diversity in

this locus with superimposed taste genes. Finally, chapter 5 discusses the relevant conclusions and significance of these findings in the context of chemosensory genetics and public health.

The conceptual chapter reviews past and recent findings in taste genetics. Among several revelations, this chapter reveals that ongoing efforts to establish and identify sources of variances for taste responses have made significant discoveries over the last twenty years, particularly in our understanding of the biology of bitter taste receptors, but still fall short when addressing the taste of fat, salt, and sourness. While large-scale human genetic studies have aimed to explore the diversity of global variation across taste genes and phenotypes, most studies have focused on bitter and sweet receptor genes. Studies have yet to adequately explore global variation in the genes mediating sour, salty, and fat taste in humans. Examination of global genetic variation reveals that taste genes harbor substantial non-synonymous variation which is indicative of functional differences across haplotypes, however the level of genetic variation is highly variable among taste genes. Population genetic analysis revealed that genetic diversity and population differentiation are variable across taste genes and taste modalities, but most taste genes do not deviate from the genome-wide distributions. As a whole signatures of ongoing selection are largely absent in the global population with regards to taste genes, however several genes reveal deviations from neutral expectations. These findings suggest that selective pressures are relaxed or absent in most of the genes mediating taste perception, at least at the continental level. However, the finding that some taste genes are highly differentiated and reveal departures from neutral expectations suggests that traits associated with taste are likely differentiated among human populations. In total, the current findings highlight the diversity of human taste genes and shine light on how these findings may translate to human health. Further, the findings suggest the need for increased diversity in human cohort studies, given the diversity seen across taste genes.

As described previously, an overarching gatekeeper for taste scientists to explore variation in taste genes, particularly with modern large-scale sequencing data, is the need for computational and technical expertise. This includes installing and managing complex software and computing environments, understanding complex data input formats, and general computational expertise. To address this problem, I have developed Inia, a set of scripts to automate and simplify the extraction and annotation of haplotypes from the 1000 genomes project. These scripts, which require minimal user input, provide individual haplotype data, annotations extracted from the Ensembl database, population genetic statistics, prediction of the effects of single-nucleotide polymorphisms from 2504 individuals in the 1000 genomes dataset, and annotations of polymorphic sites in localized gene regions. At its core, Inia serves as a wrapper around popular genetic tools, including the Variant Effect Predictor, and puts together a framework that generates summaries of human haplotype data that are easily explored and dissected using standard spreadsheet software or popular analysis tools such as R. Thus Inia serves as a potential tool for experts and non-experts to study genes and gene families giving rise to both simple and complex traits.

The best-studied candidate receptor for fat taste is CD36, which interestingly lies near another taste gene — GNAT3. GNAT3 encodes a subunit of the heterotrimeric G-protein essential to the GPCR-mediated bitter, sweet, and savory taste signaling pathways. Interestingly, CD36 and GNAT3 are positioned in an overlapping arrangement in the human genome. The proximity of these genes raises the possibility that the patterns of diversity of these genes are correlated through genomic linkage and thus represent a combination of selective influences. The contribution of both genes in taste perception also raises the possibility that the phenomenon of linkage confounds the associations between polymorphism in the two genes and chemosensory phenotypes. In such a case, nonfunctional genetic variants associated with functional genetic variants may spuriously associate with phenotypes.

In Chapter 4, I investigate the extent of selective pressure on CD36 and GNAT3 by analyzing patterns of diversity across the locus, then identify variants with high potential for association with human phenotypes by computationally predicting their functional effects. I test for deviation of neutral selection using standard population genetics test of natural selection and present newly derived distributions computed from whole genome scans of the 1000 genomes dataset through which to measure the significance of these tests. I have also explored associations between sites in the two genes by analyzing linkage structure across the locus. Thus, I have comprehensively examined and summarized diversity, structure, and the potential contributions of CD36 and GNAT3 to human gustatory phenotypes in Chapter 4. This study has revealed that the CD36-GNAT3 locus harbors several putatively high-impact variants; however, many of these variants are rare, and much of the population-level variance in emerging phenotypes is likely mediated by a handful of variants present at intermediate levels. Our findings suggest that positive selection has acted on CD36 exons consistent with local adaptation, particularly in the African continent, where CD36 exons harbor higher levels of diversity and differentiation. Finally, examination of linkage structure sought to examine whether variation in either gene would spuriously associate with one another, potentially confounding genotype-phenotype associations between variants harbored in either gene, making their contribution to chemosensory traits challenging to distinguish. The present analysis of linkage structure across the region provides evidence that such confounds are unlikely to be present and that known associations between chemosensation and variation harbored in both genes are likely to arise independently.

Given the gaps identified in the conceptual review, the analysis in this dissertation presents a unique exploration into large publicly available human genomic data to uncover meaningful insights into human taste genetics. This dissertation describes new tools to study and summarize global variation in localized genomic regions and focuses on genes related to chemosensory traits. In particular, I have identified the need to better explore variation in the genes encoding human fat taste receptors by examining diversity in large global sequencing data. The findings of this exploration are discussed in the context of public health, focusing on the contribution of fat taste to nutrition. Additionally, the recent discovery of extraoral expression of taste receptors and the implication of taste receptors in multiple non-gustatory physiological roles lends

importance to these findings in the overall context of health. Thus, I close this dissertation with a discussion of the significance of these findings to health science at the individual and population levels.

# Chapter 2 Taste Genetics


## Abstract

Taste is used to receive information about the external world via oral chemicals entering our body. Taste may have guided our ancestors' feeding behaviors, attracting them to nutrient-dense foods and creating aversions toward potential toxins. In modern society, these innate drivers may still modulate our eating behaviors, cravings, and consumption levels. Understanding these drivers and how they vary between individuals may allow us to address significant health issues, such as overconsumption and the increasing prevalence of obesity. This review shall explore the known biological mechanisms that govern taste response and sensitivity, summarize genetic association with variability in taste perception, and explore associations with human health and behaviors. This review will also explore the emerging roles that taste receptors play in extra-oral physiological response, particularly in the airways and the gut. As a culmination of this discussion, I include an analysis of the components discussed with a population genetic analysis of taste genes using large publicly available human sequencing data. Lastly, I will consider the potential for this information to contribute to meaningful health interventions to address significant public health issues.


## Introduction

Taste describes the perception of chemical substances detected upon interaction with receptors on the surface of taste cells in the oral cavity. The observation that individuals vary in their perception of chemicals was documented nearly a century ago. In 1931, in the lab of DuPont chemist Arthur Fox, the discovery that individuals varied in their ability to taste phenylthiocarbamide was discovered by chance. While pouring PTC into a bottle, a laboratory accident released crystallized PTC into the air (Fox, 1932; S. Wooding, 2006). His lab mate, C. R. Noller, described the air as bitter, while Fox tasted nothing. This striking discovery paved the path for studying variation in taste perception. Since this discovery in 1931, many insights have been made into understanding the cell biology, genetics, and molecular evolution of taste.

Over the last two decades, gene families have been discovered that encode cell surface receptor proteins that modulate taste sensation, and genetic variation in these genes across multiple human populations has been extensively studied (Chaudhari & Roper, 2010; Hoon et al., 1999; U. Kim et al., 2003a; Laugerette et al., 2005; X. Li et al., 2002; Sugita, 2006). Variation in genetic loci associated with human gustatory sensitivity and perception may lead to overall variation in the perception of chemical substances, including sugars, fats, and toxins. By exploring this genetic landscape, we may dissect aspects of this phenomenon to better understand how genetic variation shapes our ability to taste and our dietary habits in modern humans.

Gustation, the technical term for taste reception, acts as a gatekeeper for chemicals entering the body orally. A well-studied phenomenon is the perception of bitter

taste derived from many naturally occurring toxic compounds, such as the many toxic compounds found in plants (Drewnowski & Gomez-Carneros, 2000). Our evolutionary ancestors may have used this cue of bitterness to signal unsafe foods, as strong bitter perception causes an aversive response to the food signaling not to ingest (Breslin, 2013; Drewnowski & Gomez-Carneros, 2000). On the other hand, the taste of sweet fruits, protein-rich foods, and calorically dense fats helped our evolutionary ancestors make optimal decisions on what to ingest and may have played critical roles in shaping human evolution (Breslin, 2013; Drewnowski et al., 2012; Thompson et al., 2019). These remnants of our evolutionary past likely shape the dietary habits seen in modern humans.

Highly palatable foods have become readily available with relatively little effort in much of the world's developed societies, which have almost universally strayed away from hunter-gatherer cultures. The biological drivers we possess to make our dietary choices may play a significant role in the food we prefer and how much of these different types of foods we choose to consume (Chamoun, Hutchinson, et al., 2018; Dotson et al., 2010; Drewnowski et al., 2012; Han et al., 2018). The innate preference for desirable foods and aversion to others may be, at least in part, responsible for our dietary choices and preferences. There is much interest in understanding how nutritional preferences are related to the overconsumption of sugary and energy-dense food. This overconsumption may ultimately lead to obesity and a myriad of other health problems.

Taste perception, and its role in shaping dietary behavior, may contribute to obesity and dietary overconsumption. Given that obesity is a risk factor for a myriad of other diseases, including cardiovascular disease and diabetes, it is important to clinicians and health researchers to uncover new mechanisms to address this growing problem (Abbasi et al., 2002; Must et al., 1999). The proportion of adults in the US who are overweight or obese is up to ~70%, with ~38% of all adults considered obese and 7% considered extremely obese. These rates differ across ethnicities, with ~13% of non-Hispanic Asians being considered overweight or obese to ~48% non-Hispanic blacks being considered overweight or obese (Ogden et al., 2014). This highlights the importance of understanding the biology and psychology of nutritional behaviors in modern public health science. The involvement of taste perception in food intake suggests that the taste receptors and the molecular machinery mediating taste perception are potential targets of intervention. Further, the discovery of taste receptor function in extraoral tissues and the emerging physiological mechanisms they contribute to has brought forth a new avenue through which health researchers can consider the effects of chemosensation on human health.

This review is designed to give a brief overview of oral and extraoral chemosensation while highlighting its application in addressing emerging issues in human health. The review is intended to cover the molecular mechanism of taste reception pathways, the molecular evolution of taste receptors, known variations associated with variable taste phenotypes, and the subsequent importance this has on public health. Special focus is given to the population genetics of gustation, with an examination of diversity among global populations.

## Taste Bud Cells and Mechanisms

Taste buds are peripheral organs that sample chemicals entering the oral cavity. Taste buds are clusters of sensory cells, typically 50-100 cells organized in a garlic bulb-shaped fashion, that are distributed primarily across the tongue epithelium and the palate and less densely in other areas of the oral cavity such as the epiglottis, pharynx, and larynx (Roper, 2013; Roper & Chaudhari, 2017). In general, activation of taste receptors on the surface of taste cells triggers a transduction signaling cascade that releases neurotransmitters to afferent sensory fibers. These cells are innervated by the glossopharyngeal nerve, vagus nerve, and the chorda tympani of the facial nerve (Breslin, 2013; Frank, 1991; Lehman et al., 1995; Spector et al., 2003).

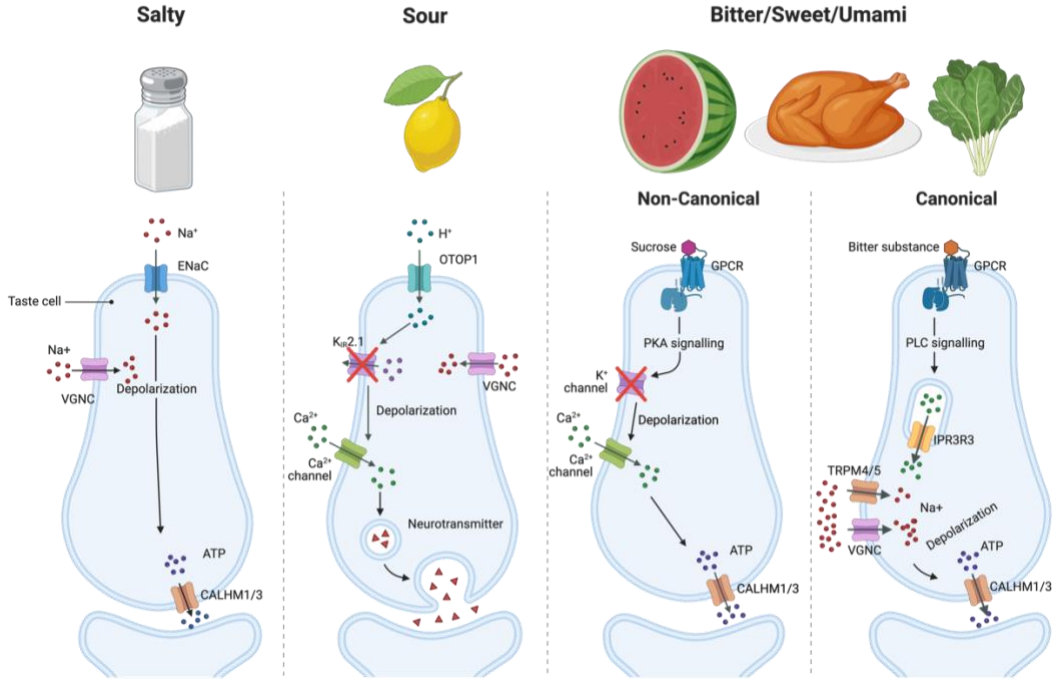


**Figure 2.1: Molecular Process of Taste Transduction** This figure has been adapted from "Taste Transaduction" by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates

The sensory cells of taste buds may be classified into four cell types. Type I cells, the most abundant in the taste bud, are glial-like and play a supporting role in the taste bud (Bigiani, 2001; Roper, 2013; Roper & Chaudhari, 2017). These cells ensheath other taste bud cells, facilitate the elimination of neurotransmitters through ecto-ATPase function and transporters, and are associated with maintaining hyperpolarized resting membrane potential through redistribution of $K^+$ via $K_{IR}1.1$ (also known as ROMK) ion channels (Bartel et al., 2006; Dvoryanchikov et al., 2009; Pumplin et al., 1997). While it was initially suggested that type I cells in mice mediate the amiloride-sensitive salt taste that functions in salt taste, this remains controversial (Vandenbeuch et al., 2008). Type II cells are classified as receptor cells and express GPCRs for sweet, bitter, and umami

compounds (Hoon et al., 1999; Roper & Chaudhari, 2017). They lack synaptic vesicles and communicate via non-vesicular mechanisms (Clapp et al., 2004, 2006; Roper, 2006). By contrast, Type III cells are identified as having identifiable synaptic contacts with gustatory nerves and express synaptic proteins (Roper, 2006). Type III cells are also thought to mediate sour taste response (Y. A. Huang et al., 2008; Roper & Chaudhari, 2017). Lastly, Type IV taste bud cells are basal cells involved in taste bud cell renewal, with recent evidence suggesting that these cells are post-mitotic precursors to all three taste cells(Miura et al., 2014).

In general, the mechanisms for bitter, sweet, and umami taste are mediated by transmembrane proteins known as GPCRs, or G-protein coupled receptors, belonging to the TAS1R and TAS2R family(Adler et al., 2000; Chandrashekar et al., 2000; Hoon et al., 1999; G. Q. Zhao et al., 2003). The binding of stimulus activates the heterotrimeric g-protein, which consists of Gα-gustducin (encoded by *GNAT3*) and its Gβγ (encoded by *GNB1/GNB3* and *GNG13*) subunits (Caicedo et al., 2003; Chaudhari & Roper, 2010; L. Huang et al., 1999; Kinnamon, 2009). Gβγ dissociates from the taste receptor and activates PLCβ2 which cleaves PIP2 into IP3 and DAG, and binding of IP3 to IP3R3 releases intracellular $Ca^{2+}$ stores that activate TRPM4 and TRPM5 (Chaudhari & Roper, 2010; Clapp et al., 2001, 2004; Miyoshi et al., 2001; Rössler et al., 1998). The influx of $Na^{+}$ via TRPM4 and TRPM5 causes activation of voltage-gated ion channels, which depolarize the cell membrane and trigger action potentials to activate voltage-gated ATP channels, specifically CALHM1 and CALHM3 (Banik et al., 2018; Liu & Liman, 2003; Ma et al., 2018; Romanov et al., 2011; Taruno et al., 2013; Z. Zhang et al., 2007). The released ATP binds and activates purinergic receptors P2X2 and P2X3 on afferent gustatory nerve fibers to transmit taste information to the central nervous system(Y. A. Huang, Stone, et al., 2011; Kinnamon & Finger, 2013).

A secondary mechanism has been previously proposed. This mechanism involves Gα-gustducin activation of phosphodiesterase, which decreases cAMP levels and subsequently triggers PKA-mediated inhibition of PLC activity. In this sense, Gα-gustducin regulates the signaling environment. Alternatively, it has been suggested that cAMP levels act on cAMP-dependent ion channels, leading to cell depolarization. This leads to depolarization of the cell and ATP release. In sweet cells, Gα activation of adenylyl cyclase stimulates, leading to changes in cAMP concentration. cAMP activates protein kinase A, which causes phosphorylation of potassium channels, subsequent depolarization of the cell, activation of voltage-gated calcium channels, and neurotransmitter release (Margolskee, 2002; Trubey et al., 2006). While it is clear that Gα-gustducin is necessary for GPCR taste transduction, the non-canonical pathway mechanism is not fully understood, and the role that cyclic nucleotides play in taste transduction remains elusive.

The mechanisms governing other modalities of taste are less understood. Several mechanisms and candidate receptors have been proposed and discarded for sour taste over the years. The prevailing consensus is that type III cells initiate sour taste transduction when protons enter through selective ion channels inhibiting K+ channels and depolarizing the cell, which leads to the firing of action potentials and the release of

neurotransmitters in a calcium-dependent manner (Chang et al., 2010; Y. A. Huang et al., 2008; Y. A. Huang, Pereira, et al., 2011; Richter et al., 2004; Roper & Chaudhari, 2017). Previous support implicated several candidate proteins in mice including acid-sensing ion channels (ASICs), hyperpolarization-activated cyclic-nucleotide gated channels (HCN1 and HCN4), PKD1L3 and PDK2L1, and more recently OTOP1 (Ishii et al., 2009; Ishimaru et al., 2006; Ramsey & DeSimone, 2018; Stevens et al., 2001; Ugawa, 2003). Ye et al. demonstrated the involvement of inward rectifier potassium channels as a signature of the taste transduction mechanism, which acts downstream of proton channels to enhance electrical excitability. Specifically, the authors point to a mechanism in which $K_{IR}2.1$, a $K^+$ channel encoded by *Kcjn2*, is blocked by intracellular acidification and amplifies cell depolarization, thus enhancing sour detection signals (Ye et al., 2016).

The mechanisms governing salt taste are also less understood. However, the consensus is that there are two distinct pathways, an amiloride-sensitive and insensitive pathway (Heck et al., 1984; Roper, 2015; Yoshida et al., 2009). The amiloride sensitive pathway, which is better understood, is characterized suppression of salt taste by amiloride and is thought to be "low salt" taste. The presence of $Na^+$ creates an electrochemical gradient favoring influx of $Na^+$ into the cell through amiloride-sensitive epithelial sodium channels (ENaCs), which depolarizes the taste receptor cell and elicits the release of neurotransmitters (Garty & Palmer, 1997; Heck et al., 1984; Vandenbeuch et al., 2008). While ENaCs have support as salt receptors in mice, they have yet to be established as human salt receptors. The mechanism for the insensitive pathway is poorly understood, although it has been suggested that anion $Cl^-$ may be responsible for gust eliciting a taste response rather than $Na^+$(Roebber et al., 2019).

The cells which respond to salt stimuli are not well-established, although the amiloride-sensitive pathway were initially supported to take place on type I taste cells, this remains controversial. (Vandenbeuch et al., 2008; Yoshida et al., 2009). Recently, Nomura et al demonstrated that an amiloride-sensitive Na+ current was present in both excitable and non-excitable cells ENaC expressing cells, but removal of amiloride induced depolarization and generated action potentials in the excitable cells in a calcium-independent manner (Nomura et al., 2020). These cells express CALHM1/3 channels, suggesting that ATP is a neurotransmitter similar to type II cells. Further, when ENaC in CALHM1 expressing cells was knocked out, nerve response was eliminated. Thus this suggest amiloride-sensitive salt is mediated by a subset of sodium taste cells rather that type I cells, and it is conducted in a calcium-independent manner (Nomura et al., 2020). The amiloride-insensitive responses are suggested to be elicited by subsets of type II and type III cells (Lewandowski et al., 2016). Evidence shows that aversive responses to high salt concentrations are mediated by bitter and sour-sensing taste cells. (Oka et al., 2013). However, a high-salt receptor remains elusive. As a result, salt perception is reported to occur across all three cell types through at least two distinct mechanisms (McCaughey, 2019; Roper & Chaudhari, 2017; Yoshida et al., 2009). These phenomena are still not completely understood in mice, and less understood in humans.

## Evolutionary Perspective of Taste and Modern Implications

The association of taste with nutrient-seeking behaviors and toxin avoidance heavily implies strong contributions of taste to general fitness. The ubiquity of gustatory systems among vertebrates is evidence to the importance of taste in species fitness. Thus, the mechanisms behind the biological origins of taste may shed light on the evolutionary history of mammals, early hominids, and humans and may help us understand the factors shaping modern taste and behavioral traits.

Taste has been significant for the evolution of omnivores, such as humans, in that there is a large potential range of dietary choices compared to species with specialized diets, such as exclusively carnivorous mammals (Breslin, 2013). For instance, felines, which are exclusive carnivores, have pseudogenized (lost function of) versions of the sweet taste receptor gene (*TAS1R2*) but have functional umami taste receptor genes (*TAS1R1 and TAS1R3*), which encode the taste receptor activated by the amino acids found in protein (X. Li et al., 2005, 2006). In contrast, the giant panda, which feeds primarily on vegetation like bamboo, has lost function of its umami taste receptor (*TAS1R1*) (H. Zhao, Yang, et al., 2010). Furthering on this point, aquatic mammals such as dolphins and sea lions, which swallow their food whole and have little opportunity for taste input and response, have lingual epitheliums and oral cavities exhibiting very few taste buds and atrophied lingual papillae (Yoshimura et al., 2002; Yoshimura & Kobayashi, 1997). Jiang et al. reported that the bottlenose dolphin genome contains no intact *TAS2R* bitter receptor genes and loss of function of all *TAS1R* genes. They also report that the sea lion genome has lost function of *TAS1R1* and *TAS1R3* (Jiang et al., 2012). It was later demonstrated that cetaceans, such as whales and dolphins, have undergone extensive loss of taste receptors for sweet, bitter, umami, and sour taste throughout their evolution (Feng et al., 2014; Zhu et al., 2014). This indicates loss of normal gustatory function as evidenced by the genomes of these aquatic mammals, which is likely owed to their specialized diets.

The dietary habits of primates and our early ancestors can shed light on the evolution of taste in these species. For instance, in the context of vitamin C, which primates cannot synthesize themselves due to a loss of a functional gluconolactone oxidase gene, an attraction to sour foods may serve as a guide to vitamin C-rich fruits (Breslin, 2013; Drouin et al., 2011). When combined with sweet taste, sour taste may also help determine the ripeness of fruits (Breslin, 2013). This attraction to sour foods, and the inability to synthesize vitamin C, is still present in modern humans and has shaped our dietary choices to meet our physiological need for vitamin C.

The presence of perceived bitter compounds across plants is essential to omnivores. Bitter perception of these compounds may alert to potential toxins(Breslin, 2013). This bitter response creates an aversion to such compounds, contributing to the rejection or avoidance of food substances containing the offending chemical. For example, strong bitterness is known to trigger nausea, slows gastric emptying, and prepares the body to vomit (Breslin, 2013; Glendinning et al., 2008; Peyrot des Gachons et al., 2011; Wicks et al., 2005). This response may have served our ancestors a

mechanism to halt digestion and expel potential toxins that may have been ingested. The nausea associated with pregnancy has been associated with bitter taste sensitivity and is hypothesized to have arisen to protect the fetus from environmental toxins (Sipiora et al., 2000). Similarly, highly acidic foods and a high concentration of salt may create an aversion response signaling potentially spoiled and non-palatable foods (Oka et al., 2013; Reed & Knaapila, 2010).

Contrasting this, preference for foods is driven by the presence of a viable nutrient source. Sweet taste may drive the detection of foods containing simple carbohydrates, such as those found in fruits (Breslin, 2013). The taste of fats and amino acids may have acted as guides to signal energy-dense and palatable foods (Drewnowski, 1998; Hartley et al., 2019). This innate drive to the taste of protein and fat-rich foods may have had major roles in human evolution. It is hypothesized that a dietary shift to the consumption of animal resources, which is a diet consisting largely of fats and proteins, allowed for early hominid ancestors to meet the caloric needs necessary to develop and maintain a large brain (Thompson et al., 2019). However, these innate preferences are still present in modern humans, and overconsumption of such foods is a contributing factor to obesity.

Salt taste is believed to have evolved from an inherent need for sodium in homeostasis (Hurley & Johnson, 2015). Attraction to sodium-containing substances, such as salt, may have been driven by the relative scarcity of salt-containing foods in the natural environment (Denton, 1982). During instances of true sodium need, animals, including humans, will switch taste preferences to meet homeostatic requirements (Berridge et al., 1984; Denton, 1982). It has been suggested that primate and hominid ancestors, which had largely plant-based diets and lived in low-sodium environments, used orosensory mechanisms to seek sodium-rich foods (Denton, 1982; Hurley & Johnson, 2015). However, instances of true salt need in modern humans are scarcely encountered due to the availability of substances like table salt. Despite this lack of true sodium need, it is common for individuals to have a salt-rich diet and a preference for sodium-rich foods.

Regarding aversions, many plants have mildly toxic compounds that elicit a mild bitter response but are otherwise not harmful and are often beneficial nutrients (Drewnowski & Gomez-Carneros, 2000). Such compounds include various flavonoids, phenols, polyphenols, and glucosinolates found in fruits and vegetables. For example, many cruciferous vegetables, such as brussel sprouts, broccoli, and cabbage can contain high levels of glucosinolates and taste bitter (Drewnowski & Gomez-Carneros, 2000). While large amounts of these compounds have negative health consequences, the consumption of vegetables is a major component of a healthy diet in humans. It would logically follow that an aversion to these compounds can lead to rejection of certain vegetables based on their perceived bitterness, regardless of nutritional benefit (Bell & Tepper, 2006). On the other hand, acceptance and preference of sweet and fat foods may lead to dietary preferences for energy-dense foods, overconsumption, and ultimately, increased risk of obesity (Drewnowski et al., 2012; Han et al., 2018).

## Diversity of Human Taste Receptors

The implication that taste perception plays a key role in guiding nutritional behaviors and avoiding harmful chemicals suggests that the genes mediating taste perception have been under selective pressure. Natural selection has acted upon the genes shaping taste perception through the maintenance of advantageous alleles in the gene pool that confer a higher likelihood of survival and reproduction while driving down the frequency of deleterious alleles. Humans boast a highly diverse set of functional taste genes that are subject to high rates of variation within and between populations (Wooding et al., 2004; Kim et al., 2006; Risso et al., 2016). Patterns in the distribution of alleles in global populations can give insight into driving forces shaping diversity and their effects on phenotypic variability.

The bitter taste receptors, particularly the TAS2R38 gene, are among the most studied taste receptor genes. This is no surprise given the nearly 90 years of inquiry surrounding the PTC phenotype and the genetic mapping of the bitter receptor gene family. Wooding et al. conducted one of the earliest studies examining natural selection in the TAS2R38 gene through examination of Tajima's D and Fu and Li's D and F – which are functions of the abundance of variation, relative diversity, and derived singletons across the locus – to conclude that high allele frequency of the two major *TAS2R38* haplotypes have been maintained through balancing selection (S. Wooding et al., 2004a). They concluded that low differentiation and an excess of variants of intermediate frequency point toward balancing selection. A revisitation to the *TAS2R38* locus by Campbell et al. found that ancient balancing selection likely brought the haplotypes to high frequencies but that recent diversifying selection is likely maintaining rare variants in the African continent (Campbell et al., 2012). Risso et al. revisited the TAS2R38 gene and reported findings similar to Campbell (D. S. Risso et al., 2016). Risso et al. adjusted for population stratification and demographic events and did not find values of Tajima's D that deviated from the neutral expectation with statistical significance. The authors propose that PAV and AVI haplotypes were maintained through ancient balancing selection before the Out-of-Africa event and that the frequencies were maintained in non-African populations through population expansions and bottlenecks, with a relaxation of selection acting on the gene (D. S. Risso et al., 2016). Similarly, they proposed that the AAI haplotype had undergone weak directional selection and balancing selection. The AAI haplotype is almost unique to Africans and has a moderately high frequency (~13%). A recent study has found evidence of departures from neutral expectations and evidence for ongoing balancing selection in Europeans, in which Tajima's D reveals positive values under best-fit demographic models(Valente et al., 2018).

In the case of an allele on TAS2R16, which confers sensitivity to salicin, a positive selection model in Africa has been hypothesized (Soronzo, 2005). Campbell et al. further proposed a complex model for selection suggesting that the allele likely predates the human expansion out-of-Africa and rose to high frequency on multiple haplotype backgrounds, which is an indication that the allele may have risen to high frequency via positive selection on standing variation, e.g., a "soft sweep" (Campbell et al., 2014). The

fixation of the allele in populations outside of Africa is consistent with this scenario. It may have reached fixation due to a selective sweep or through population bottlenecks and expansions during the migration and geographic expansion out of Africa. Further, the study observed that the frequency of non-synonymous mutations on "low sensitivity" salicin haplotypes was low suggesting the ancestral allele of salicin sensitivity is also under purifying selection (Campbell et al., 2014). Thus, TAS2R16 and TAS2R38 likely reflect models of evolution in which multiple selective pressures and demographic influences have acted upon the genes.

In the context of the rest of the bitter receptor family, studies have revealed that the bitter receptors have elevated levels of variation and diversity. Among the *TAS2R* bitter receptor genes, the average level of amino acid substitutions, or fixed non-synonymous mutations, is significantly higher than genome-wide averages. In an analysis of 24 functional bitter-tasting GPCRs, Kim et al. reported that the Ka/Ks ratio, a statistic to measure the proportion of non-synonymous substitutions per non-synonymous site to synonymous substitutions per synonymous site, was more than 5 standard deviations greater than the average for human genes (Kim et al., 2005). Kim et al. also demonstrated that the average level of nucleotide diversity in the *TAS2R* genes was significantly greater than the genome-wide average and coding region average. Lastly, they demonstrated that genetic differentiation at the loci, measured by $F_{ST}$, was significantly greater than the genome-wide average (Kim et al., 2005). This all indicates that the TAS2R gene family has high levels of allelic variation and that among global populations there are greater differences in allele frequencies between populations than the average in most other genes (Kim et al., 2005).

A feature of the taste receptors, at least the bitter receptors, is that species have their own repertoire of receptors and many receptors that were once beneficial become nonfunctional relics in the genome, no longer coding for a functional protein or have completely lost their ability to be transcribed. At least 11 of the human TAS2R gene transcripts are fixed pseudogenes in humans which has abolished their transcription potential into a functional protein (Fischer et al., 2005; Risso et al., 2014). It is likely that these genes no longer offered a benefit to fitness, and in the absence of selective pressure deleterious mutations have reached fixation. However the loss and gain of function of these genes is not so simply straightforward. For instance *TAS2R18P*, which is hypothesized to have undergone pseudogenization prior to the divergence of Hominidae, may have regained function through insertion-deletion variants after the divergence of humans and chimpanzees. In humans, the gene then experienced a second pseudogenization event. Risso et al. point to evidence of ancient balancing selection in *TAS2R18P* and two SNPS carried at variable medium to high frequency across populations as evidence of the possible importance of this gene to ancient humans (D. Risso et al., 2014). On the other hand, *TAS2R2* was originally identified as a pseudogene but later found to exist in a functional and intact form in some individuals. In light of the discovery of function and activating ligands in the receptor, it has recently been termed the "26$^{th}$ bitter receptor"(Lang et al., 2023). Loss of function variants have been found across several bitter taste receptor genes including *TAS2R3, TAS2R7, TAS2R8, TAS2R14,*

*TAS2R19, TAS2R20, TAS2R40, TAS2R41, TAS2R42, TAS2R43, TAS2R46* and *TAS2R60* (Fujikura, 2015). Loss of function variants are also present in *CD36, PKD1L3, PKD2L1,* and *SCNN1D*. These loss of function variants have been found at varying levels in different continental populations, with some variants being population specific (Fujikura, 2015). Additionally, *TAS2R43-TAS2R45* harbor whole gene deletions at high and varying frequencies across global populations. The *TAS2R43* deletion has a population frequency range of 0.21-0.46 with a global frequency of 0.33. The *TAS2R45* deletion has a population frequency range of 0.02-0.64 with a global frequency of 0.18 (Roudnitzky et al., 2016).

Characterizing this diversity gives valuable information to the mechanisms underlying the global distribution of emerging traits related to taste. Among taste genes the bitter taste receptors are the best studied example, where it is hypothesized that plant toxins are a major selective pressure acting on the bitter receptor family. The maintenance of sensitive taste perception to a wide variety of plant toxins and metabolites likely played a key role in the fitness or our ancient ancestors. However, snapshots of genetic diversity in modern humans suggest that the selective pressures acting on gustatory genes have been dynamic. As discussed later, the presence of taste receptor mediated activity in extra oral tissues suggests they have important roles outside of taste in the oral cavity. Given the importance of such physiological functions, these pathways have undoubtedly been subject to selective pressures. Thus, it is likely that several selective forces have acted on the diverse roles of taste receptors, for example allowing for the fine tuning necessary for perception of bitter plant toxins while simultaneously conserving the integrity of the receptor as to not compromise the extra-oral function.

Explorations of diversity is of interest for taste genes and public health intervention. For example, TAS2R agonist that activate airway TAS2Rs have been found to be effective bronchodilators in the treatment of asthma (Deshpande et al., 2010a; Liggett, 2013). However, given the high variability in taste receptor genotypes and phenotypes across populations, applying these findings poses a complex and pressing issue.

## Bitter Taste

Bitter taste has been extensively studied. In humans, the *TAS2R* family encodes 25 functional receptors and 11 pseudogenes associated with bitter tastes (Fischer et al., 2005; He et al., 2004; U. Kim et al., 2006; D. Risso et al., 2014). These receptors, expressed on the surface of type II taste cells are responsible for detection and initiation of signaling cascade to afferent nerve fibers in response to a variety of bitter ligands. Aside from *TAS2R1* residing on chromosome 5, these genes form clusters in the human genome along chromosomes 7, and 12.

Bitter taste genes in the TAS2R family have been associated with binding to a diverse set of ligands mediating bitter taste stimulus. Meyerhof et al. demonstrated redundancy of ligand activation of bitter receptors with naturally occuring ligands such as quinine activating *TAS2R4, -7,-10,- 14, -39, -40, -43, -44, and -46* (Meyerhof et al., 2009).

The same was observed for synthetic ligands such as denatonium, the most bitter compound known, which activates *TAS2R4, -8, -10, -16, -39, -43, -46, -47* (Meyerhof et al., 2009). Among the most broadly tuned are *TAS2R10, TAS2R14,* and *TAS2R46* bitter receptors, which were reported to account for more than half of associated bitter ligands in the study(Meyerhof et al., 2009). Greater than 270 molecules have been identified as associating with at least one human *TAS2R* bitter receptor, and around 500 associations between ligands and bitter receptors have been made among humans. There are around 800 associations between ligands and bitter receptors when combined with non-human species (Dagan-Wiener et al., 2019). Among these receptors, *TAS2R42, -45, -48, and -60* are orphaned receptors, with no identified ligands for activation(Meyerhof et al., 2009; Thalmann et al., 2013).

*TAS2R38*, one of the most widely studied taste receptors, is associated with bitter taste sensitivity to phenylthiocarbamide and PROP (U. Kim et al., 2003b; U. K. Kim & Drayna, 2005). Three single-nucleotide polymorphisms in the coding region of TAS2R38 give rise to distinct haplotypes that account for 55-85% of the variance in PTC sensitivity, which ranges nearly 10,000-fold among individuals (U. Kim et al., 2003b). Rs714598, rs1726866, rs10246939 encode amino acid changes at positions 49 (proline or alanine), 262 (alanine or valine), and 296 (valine or isoleucine). The dominant PAV haplotype, which has a prevalence of ~51%, is associated with the ability to taste PTC and PROP and the AVI haplotype, which has a prevalence of ~43%, is associated with non-tasters (D. S. Risso et al., 2016). Homozygosity for the PAV and AVI haplotypes have been associated with "super tasters" and "non-tasters" respectively, and heterozygous individuals reveal an intermediate phenotype. The prevalence of AAI is 3.39%, AAV is 2.48%, AVV is 0.32%, PAI is 0.18%, and PVI is 0.07%, all of which are associated with intermediate taste sensitivity to PTC and PROP (D. S. Risso et al., 2016). These variations in *TAS2R38* have been associated with sensitivity and preferences to the bitterness of vegetables, with multiple studies showcasing decreased vegetable consumption among the PAV haplotypes than the AVI haplotypes (Calancie et al., 2018; Dinehart et al., 2006; Sandell & Breslin, 2006). Similarly, sensitivity to ethanol, drinking habits, and average alcohol intake have been associated with the *TAS2R38* haplotypes with PAV homozygotes showing decreased consumption of alcohol compared to AVI homozygotes (Allen et al., 2014; Duffy et al., 2004; Wang et al., 2007).

While polymorphisms in *TAS2R38* are the best characterized for bitter ligand sensitivity, other SNPs have been identified. *TAS2R16* has been heavily associated with mediating taste response to beta-glucopyranosides and other naturally occuring bitter compounds (Bufe et al., 2002). The most relevant SNP has been rs846664 which encodes an amino acid substitution at site 172 from asparagine to lysine. Assay screening for TAS2R receptor agonist have shown the Lys172 receptors are less sensitive to plant-derived toxins than Asp172 receptors (Soranzo et al., 2005). This substitution has been shown to associate with alcohol dependence (Hinrichs et al., 2006; Wang et al., 2007). Similarly, in a European-American cohort of individuals, Hayes et al demonstrated that the *TAS2R16* polymorphism rs846672 is associated with higher frequency of consumption of alcohol (Hayes et al., 2011).

*TAS2R31* and *TAS2R43* are taste receptor proteins known to bind to saccharin and acesulfame potassium, two artificial sweeteners, with TAS2R31 playing a much more significant role in the perception of these substances (Kuhn et al., 2004; Roudnitzky et al., 2011). Roudnitsky et al explored the chromosome 12 TAS2R cluster where *TAS2R31* and other *TAS2Rs* lie, generating long-range haplotypes across these genes and found common haplotypes associated with the bitterness of acesulfame K and saccharin (Roudnitzky et al., 2011). Arg35Trp substitution was found to be causal, with Trp35 abolishing receptor response to these two sweeteners (Roudnitzky et al., 2011). Additionally, they found four other amino acid substitutions at sites 45, 237, 276, and 281 severely attenuated or completely abolished receptor function (Roudnitzky et al., 2011).

The bitterness of quinine and grapefruit liking have both been associated with rs10772420, which encodes the Arg299Cys amino acid substitution on *TAS2R19* (Hayes et al., 2011; Reed et al., 2010). However, neither quinine nor the bitterants in grapefruit, limonin and naringin, activate *TAS2R19* (Meyerhof et al., 2009; Thalmann et al., 2013). However, several *TAS2R* genes lie in nearby proximity to *TAS2R19*, giving the possibility that the attributed polymorphism for quinine bitterness may be statistically associated through linkage in the region. As such, it was found that the *TAS2R19* polymorphism was in strong linkage disequilibrium with several *TAS2R31* polymorphisms giving the possibility that the true functional variant may lie outside of *TAS2R19* (Hayes et al., 2015).

PROP tasting status has been associated with increased sensitivity to in other modalities of taste and PROP tasting status has been occasionally used as a marker for overall taste. Interestingly, a possible mechanism for this may arise due to density of fungiform papillae rather than polymorphism at the taste receptor. In the carbonic anhydrase VI gene, also known as gustin, the polymorphism rs2274333, which results in a nucleotide change from A to G, has been associated with changes in fungiform papillae density and PROP threshold (Melis et al., 2013). Specifically, Mellis et al demonstrated that those who carry the homozygous GG allele had thresholds for PROP tasting that were > 10-fold higher than those that carried AA or AG (Melis et al., 2013).

The consequences on behavioral traits have been extensively studied for bitter taste sensitivity. Numerous studies have linked individual taste receptors and polymorphisms to variability in taste response to several substances that are of interest to health researchers. As covered previously, polymorphisms in TAS2R genes have been associated with sensitivity and consumption alcohol, a beverage that is both consumed regularly and has major effects on human health. Several TAS2Rs have been associated with the dietary behaviors including the consumption of fruits and vegetables (Calancie et al., 2018; Dinehart et al., 2006; Hayes et al., 2011; Sandell & Breslin, 2006). However, the relationship between genotype or phenotype and dietary intake is not straight forward. For instance, while the PROP tasting and non-tasting haplotypes, PAV and AVI of *TAS2R38*, are associated with vegetable intake, there have been reported inconsistencies and the relationship has not yet been fully established (Choi et al., 2016; Gorovic et al., 2011). It is likely that cultural impacts and environmental factors have strong effects on these dietary behaviors as well.

## Sweet Taste

In humans the TAS1R receptors are associated with response and sensitivity to sweet stimuli. Similar to bitter taste response, sweet taste is governed by a class of GPCRs that elicit a response upon ligand binding and activation of a downstream signaling cascade (discussed earlier) on the surface of type II taste cells. This is mediated by a heterodimer formed by TAS1R2 and TAS1R3 both of which have genes encoding them on chromosome 1 in humans (X. Li et al., 2002). While both TAS1R2 and TAS1R3 taste receptors take part in sweet taste response, TAS1R2 is unique to sweet taste, but TAS1R3 also mediates umami response (G. Q. Zhao et al., 2003). Known ligands that bind and activate the signaling cascade triggered by these proteins include sugars such as sucrose, glucose, and fructose, glycosides such as stevia, and synthetic sweeteners such as sucralose, aspartame, saccharin, and cyclamates (Masuda et al., 2012).

These receptors are subject to genetic variation, which may lead to changes in sensitivity to these molecules. In particular, *TAS1R2* is highly polymorphic, ranking among the top 5-10% of human genes with reported polymorphism (U. Kim et al., 2006). These polymorphisms include missense variation in the proposed ligand binding domains of the taste receptor proteins. Among these polymorphisms, rs35874116 encodes an amino acid change at residue 191 encoding a change from isoleucine to valine, and is most strongly associated with the consumption of sugars and carbohydrates (Eny et al., 2010). In a cohort of Western Mexican individuals, Ramos-Lopez et al demonstrated that Val/Val was associated with hypertriglyceridemia with an associated of approximately 30% higher triglycerides than the Ile/Val and Ile/Ile genotypes (Ramos-Lopez et al., 2016).

Fushan et al revealed that two C/T polymorphisms, rs307355 and rs35744813, correlate with human taste sensitivity to sucrose, accounting for 16% of population variability in sucrose taste perception (Fushan et al., 2009). Both of these SNPs lie upstream of *TAS1R3* and are associated with a reduction in promoter activity by acting upon a cis-regulatory element that has a strong silencing effect on the *TAS1R3* promoter (Fushan et al., 2009).

Fushan et al (2010), demonstrated SNPs in the *GNAT3* region that were associated with varying sweet taste sensitivity phenotypes. The strongest of these associations were rs2012380 and rs7792845 (Fushan et al., 2010). These polymorphisms lie in an 8kb upstream promoter region of the *GNAT3* coding sequence (Fushan et al., 2010). Additionally, polymorphisms in taste receptors outside of the *TAS1R* family have also been found to influence sweet taste sensitivity and preferences. In relation to bitter taste, the polymorphism rs713598 in *TAS2R38* has been found associate with sweet preferences (Pawellek et al., 2016).

Sweet taste perception has been associated with other relevant health outcomes. The association between higher carbohydrate intake and higher triglyceride concentrations is a major contributor to type two diabetes. The reported association of 30% higher triglyceride levels in those that possess that *TAS1R2* Val/Val haplotype than the Ile/Val and Ile/Ile genotypes then has major implications for the risk and maintenance of diabetic complications. Further, rs35874116 and rs307355 of *TAS1R2* and *TAS1R3* have

been found to be associated with an increase risk of dental caries and tooth decay, with homozygous individuals for the *TAS1R2* haplotype being at high risk (>8 caries) and heterozygous individuals for the *TAS1R3* haplotype being at moderate risk (4-7 caries) (Haznedaroğlu et al., 2015). Lastly, it is likely that sweet taste and perception have influence on other modalities of taste, such as the masking of bitterness, and may play important roles in influencing dietary behaviors (Dinehart et al., 2006; Mennella et al., 2005; Pawellek et al., 2016).

## Umami Taste

Similar to sweet taste response, the taste of umami, or savory taste, is governed by the *TAS1R* receptor family, with TAS1R1 receptor protein being unique to umami taste. In this mechanism a heterodimer complex of T1R1 and T1R3 GPCRs in type II taste cells interact with binding ligands to trigger a signaling cascade that elicits a taste response (X. Li et al., 2002; G. Q. Zhao et al., 2003). Other receptors such as mGluR1(GRM1) and mGluR4 (GRM4) have been implicated in the taste of umami, where they are specific to the binding of glutamate (Yasumatsu et al., 2009). Umami is the taste response to amino acids, such as L-glutamate, and 5-ribonucleotides like 5'-inosinate and 5'-guanylate (Kurihara, 2015). These two ribonucleotides acts synergistically with glutamate to enhance the response of umami taste in the *TAS1R1-TAS1R3* pathway (Kurihara, 2015; X. Li et al., 2002).

Like the other taste receptors, the umami receptors are subject to variation that may give rise to variable phenotypes across individuals. Shigumura et al reported that rs34160967 (Ala372Thr) in *TAS1R1* and rs307377 (Arg757Cys) in *TAS1R3* were associated with varying thresholds of umami taste, with the *TAS1R1* Ala372 and *TAS1R3* Cys757 allele associating with decreased sensitivity for umami (Shigemura et al., 2009). It was then demonstrated in vitro that rs307337 leads to reduced TAS1R1/TAS1R3 response to MSG (Raliou et al., 2011).

Despite known polymorphisms contributing to varying taste thresholds for amino acids, there is no clear relationship for how this effects dietary intake. Han et al demonstrated the individuals with the C/C genotype for rs307355 and rs35744813 of *TAS1R3* consumed more protein than T allele carriers, and that the G/G genotype of rs34160967 (Ala372Thr) in *TAS1R1* consumed more fat and calories as compared to the genotype having the A alleles (Han et al., 2018). However, this study employed only 30 individuals, which is a relatively small sample size to determine genetic associations. Currently, the relationship of how umami receptor polymorphisms shape dietary behaviors is still largely unknown.

## Sour Taste

The mechanisms governing taste receptor function for sour taste in humans has been elusive, with little known about functional receptors in taste transduction. Several candidate receptors have been proposed for acid taste including the transient receptor protein channels *PKD1L3* and *PKD2L1*, acid sensing ion channels, and otopetrin-1 (Horio

et al., 2011; Ishii et al., 2009; Ishimaru et al., 2006, 2010; Ramsey & DeSimone, 2018). More recently $K_{IR}2$, a K+ channel encoded by *KCJN2*, has been implicated in sour taste (Ye et al., 2016). These candidate genes harbor variants that may influence the perception and sensitivity to acidic compounds, however little is known of these associations. In a recent study, Chamoun et al found associations between variants in *KCJN2* and sour taste preference in children and adults (Chamoun, Carroll, et al., 2018). Rs236514 was found to be associated with sour preference in adults, rs173135 with sour preference in children, and rs236512 with sour preference in both adults and children (Chamoun, Carroll, et al., 2018). The association between genetics and sour taste sensitivity and preferences have otherwise been elusive.

## Salt Taste

The mechanisms of salt taste, like sour taste, have been elusive. ENaCs, which are epithelial sodium channels, are among the proteins that have been associated with the taste of salt (Butterworth, 2010; Chandrashekar et al., 2010; Hanukoglu & Hanukoglu, 2016; Roper, 2015). The ENaC channel is a heteromer consisting of α, β, and γ or δ subunits encoded by *SCNN1A, SCNN1B, SCNN1G,* and *SCNN1D* (Hanukoglu & Hanukoglu, 2016). The amiloride sensitive and insensitive pathways were described previously. Less is understood of amiloride insensitive pathways, although candidate receptors have been proposed, namely *TRPV1* (Lyall et al., 2004). However, support for TRPV1 as a salt gustatory receptor on taste cells has been mixed, and mechanisms for TRPV1 association with salt perception have been suggested to be based on somatosensory signals on the chorda tympani rather than gustatory signals(Smith et al., 2012). Nonetheless, polymorphisms in these genes have been associated with differential response to salt in human populations.

Associated polymorphisms for modified taste threshold sensitivity to sodium salt have been identified in *SCNN1B* and *TRPV1.* In *SCNN1B* two intronic polymorphic sites, rs239345 and rs3785368, have been associated with less intense salt perception in homozygous individuals (Dias et al., 2012). In *TRPV1*, homozygous carriers of the rs8065080 polymorphism are more sensitive to salt solutions than non-carriers (Dias et al., 2012). Nonetheless, the understanding of the molecular and genetic mechanisms shaping salt taste has yet to be uncovered and is an active area of research.

## Fat Taste

Evidence has supported the inclusion of fat as a primary taste, and it is now regarded as the sixth modality of taste (Gilbertson, 1998; Running et al., 2015). Although several fat receptors have been proposed, the mechanisms underlying this response are not fully established. Among these candidates, strongly associated receptor candidates include *CD36, GPR120,* and *GPR40*. (Abumrad, 2005; Cartoni et al., 2010; Keller et al., 2012; Ozdener et al., 2014; Sayed et al., 2015).

*GPR40* and *GPR120* are GPCRs that respond to medium and long-chain fatty acids, are expressed in mouse type I and type II taste cells respectively, and knockout of these genes reveals a diminished nerve response to fatty acids (Cartoni et al., 2010) Human gustatory tissues, however, have revealed expression of *GPR120* but not *GPR40* (Liu et al., 2018). *CD36* has the strongest support as a candidate fat receptor and will be the focus of this section.

As with other taste responses, variants in the genes encoding candidate receptors for fat taste can produce variation in sensitivity and response to fatty acids. With a focus on humans, candidate SNPs have been associated with varying fat sensitivity. In *CD36,* rs1761667 has been most frequently associated with the variability in fat detection thresholds and varying sensitivity to fats in humans. This polymorphism is found at a high allele frequency with a global minor allele frequency >40% and varies in frequency from 28% to 53% across continental populations (The 1000 Genomes Project Consortium, 2015). Interestingly rs1761667 is a single nucleotide polymorphism in the intronic region of *CD36* and has been associated with variability of the expression of this gene. Pepino et al. demonstrated that the A-allele of rs1761667 was associated with higher oral fatty acid detection thresholds in obese subjects (Pepino et al., 2012). The same finding was found in a study of obese Tunisian women (Mrizak et al., 2015). Sayed et al. found that rs1761667 A-allele was associated with higher lipid taste perception in obese individuals but not in lean individuals (Sayed et al., 2015). The rs1761667 has also been associated with fat intake, with the A-allele associated with decreased total fat intake, decreased polyunsaturated and monosaturated fatty acids, fatty foods, and vegetable oils in obese children but not in normal weight and lean children (Pioltine et al., 2016). The rs1761667 polymorphism is also associated with a high-fat diet and high serum cholesterol levels (Lopez-Ramos et al., 2005). Additionally, the polymorphism rs1527483 has been associated with fat taste perception. Keller et al found that the T-allele for rs1527483 was associated with increased perception of creaminess and higher fat content rating and rs1761667 with higher fat acceptance (Keller et al., 2012).

This discrepancy between obese and lean individuals may be explained by factors affecting the expression of *CD36* in taste bud cells. Zhang et al demonstrated reduced *CD36* expression in taste bud cells in high-fat diet-induced rats compared to control rats, suggesting an association between fat intake and fat sensitivity (X.-J. Zhang et al., 2011). Costanzo et al employed a co-twin randomized controlled trial in which twin pairs were randomly allocated low-fat and high-fat diets and found that fat taste thresholds had low heritability, suggesting that fat taste is highly influenced by the environment (Costanzo et al., 2018). It is likely that a high-fat diet, and possibly other environmental factors, may have strong effects on fat taste sensitivity.

In all, there have been associations with *CD36* driving fat preference, sensitivity, and food consumption (Laugerette et al., 2005; Martinez-Ruiz et al., 2014). *CD36* and oral and post-oral fat detection present themselves as novel health targets for developing and guiding dietary interventions.

## Chemesthesis: Texture, Pain, and Thermoreception Contribute to Flavor

Complex oral cues, separate from biological taste, shape the perception substances entering the body via the oral cavity. An integral part of encoding flavor profiles of foods and other substances separate from taste and smell is the phenomenon of chemesthesis. Sensation to capsaicin and menthol are, in-part, governed by thermoreceptors and nociceptors expressed in the oral epithelium, such as the transient receptor protein channels TRPV1, TRPM5, and TRPM8 (Immke & Gavva, 2006; Michlig et al., 2016; Ren et al., 2013; Rosenzweig et al., 2008). Notably, these genes belonging to the transient receptor potential channel family of proteins have been associated with other basic taste modalities, as highlighted earlier in this review. Several chemosensory responses are associated with the TRP channels, including response to allyl isothiocyanate and cinnamon by TRPA1, response to allicin by TRPA1 and TRPV1, and response to menthol by TRPM8 (Bautista et al., 2007; Macpherson et al., 2005; Mihara & Shibamoto, 2015).

While somatosensory mechanisms underlying chemesthesis are separate from gustatory mechanisms, they play important roles in food consumption, preferences, and flavor. A rather interesting example comes the Szechuan province of Southwestern China in which the husk of Zanthoxylum sp. seeds, commonly known as Szechuan peppercorn, are used as a common spice in various dishes. This spice is known to give a unique numbing and tingling sensation most often compared to an electric current and paresthesia ("pins and needles") (Bautista et al., 2008; Bryant & Mezine, 1999). A molecularly similar molecule to capsaicin, the agonist of TRPV1, hydroxy-alpha-sanshool has been attributed for causing this unique sensation(Ji et al., 2019; Koo et al., 2007). While hydroxy-alpha-sanshool has been associated as agonist of TRPV1 and TRPA1, it also is associated with inhibiting pH-sensitive two-pore domain $K_{2P}$ channels KCNK3, KCNK9, and KCNK18 (Koo et al., 2007; Richter et al., 2004). Capscaicin (chili peppers), piperine (black peppercorns), 6-gingerol (ginger), and polygodial (mountain pepper) also inhibit these channels(Beltrán et al., 2013).

Similar to Szechuan peppercorns, the splinathol containing jambu plant of Brazil imparts a similar numbness and tingling and is used as a flavor additive in foods and chewing tobacco (Dallazen et al., 2018; Lim, 2014). Menthol, which imparts the sensation of coolness is widely used as a tobacco additive in smokeless tobacco, traditional cigarettes, and more recently electronic cigarettes, to mask the flavor and irritating sensation produced by nicotine (Rosbrook & Green, 2016). An understanding of the sensory perception of these substances may help guide researchers in forming effective interventions to address pressing health issues.

Törnwall et al demonstrated that genetic factors contribute 18-58% of variation in the pleasantness of oral pungency and spicy foods (Törnwall et al., 2012). In terms of ethanol, which elicits a burning sensation, *TRPV1* has three genetic polymorphisms associated with ethanol sensations; rs224547, rs4780521, rs161364 (Allen et al., 2014). The receptors that mediate oral chemesthetic sensations are subject to genetic variation that give rise to variable orosensory phenotypes.

## Emerging Chemosensory Cues

As with the recent inclusion of the taste of fats, other mechanism of chemosensory response have been proposed. For instance, astringency, or perceived oral dryness, is a response to polyphenols in foods (Schöbel et al., 2014). While it has been hypothesized that this is a mechanosensory response to precipitated protein, there has been demonstration that several astringent compounds do not precipitate proteins (Jöbstl et al., 2004; C. A. Lee et al., 2012; C. A. Lee & Vickers, 2012; Schwarz & Hofmann, 2008). Astringent compounds have been shown to stimulate the chorda tympani and bind to taste receptors suggesting it may be a modality related to taste (Schiffman et al., 1992; Soares et al., 2013). However, evidence of astringent perception by non-taste oral tissues suggest this is a somatosensory response (Breslin et al., 1993). Most convincing is that astringency perception is not impaired upon blocking activity of the chorda tympani, but it is impaired when both taste nerve and trigeminal nerve activity is blocked (Schöbel et al., 2014). While the mechanisms mediating astringency are still very much unknown, there is strong evidence it is a chemosensory experience independent of taste response. Alternatively, astringency may involve more than one mechanism of action.

Several other taste cues have been proposed including the response to calcium, which has been proposed as an independent taste modality. TAS1R3 and calcium sensitive receptors have been proposed as potential receptors for calcium detection in both mice and humans (Tordoff et al., 2012). Kokumi, the sense of heartiness or mouthfulness, has been associated with the detection of calcium and y-glutamyl peptides and has also been proposed as a modality of taste (Kuroda & Miyamura, 2015). A proposed receptor for kokumi is CaSR a calcium-sensing receptor in which gluthianone, a kokumi substance, is an agonist (Kuroda & Miyamura, 2015). The taste of complex carbohydrates, described as starchiness, has recently been supported as an independent taste from TAS1R sweet tasting, and the mechanism governing this is an active area of research (Lapis et al., 2016; Low et al., 2017). Other chemesensory cues have also been proposed such as the taste of metal and carbonation.

## Extraoral Taste GPCRs

Recent studies have revealed expression of taste receptors and taste signaling molecules outside of taste bud cells and the oral epithelium. The most notable of these has been the discovery of the expression of TAS2R receptors in the gastrointestinal tract, respiratory tissues, reproductive tissues, and immune cells.

Extraoral taste receptor pathways are characterized by at least three known mechanisms; paracrine regulation, endocrine regulation, and autocrine regulation (Gilca & Dragos, 2017). The paracrine signaling pathway is characterized by activation of T2R and subsequent intracellular calcium concentration, followed by the release of hormones that activate transduction cascades in nearby cells and nerve fibers (Lu et al., 2017). In solitary chemosensory cells from the nasal cavity, stimulation from TAS2R agonist

promotes acetylcholine release, which activates nearby nicotinic cholinergic receptors and induces neurogenic inflammation of the nasal cavity (Lu et al., 2017; Saunders et al., 2014; Tizzano et al., 2010). The endocrine pathways are characterized by similar intracellular calcium activity upon activation of TAS2Rs but releases hormones that are circulated into the bloodstream, such as the release of GLP-1 by enteroendocrine cell upon bitter receptor activation by denatonium (K.-S. Kim et al., 2014; Lu et al., 2017). The autocrine, or cell-autonomous, regulation has been described previously in ciliated cells of the airways in which a dose-dependent activation of TAS2R increases calcium concentration and ciliated beat frequency to eliminate noxious substances entering the airways (Lu et al., 2017; Shah et al., 2009).

Previous studies in mice and humans have revealed expression of TAS2Rs in the upper airway, sinonasal, and bronchial epithelial tissues (R. J. Lee et al., 2012; Shah et al., 2009; Tizzano et al., 2010; Wölfle et al., 2016). For instance, TAS2R38 is expressed in human sinus epithelium and activated by acyl-homoserine lactones, which are generated as quorum sensing molecules by bacteria and are indicative of ongoing infection (R. J. Lee et al., 2012; Lu et al., 2017). In ciliated cells TAS2R38 is activated by noxious compounds through a pathway involving PLCB2 and TRPM5, nitric oxide is increased through a calcium dependent fashion (R. J. Lee, Chen, et al., 2014; Yan et al., 2017). Nitric oxide plays a role as both a bactericide and accelerator of the ciliary beat frequency. The proposed mechanism is that this is accomplished through nitric oxide activation of guanylyl cyclase, production of cGMP, activation of protein kinase G, and phosphorylation of ciliary proteins (R. J. Lee & Cohen, 2013). Additionally, ciliated airways have been shown to express TAS2R -4, 43, and 46 and are found to accelerate ciliary beat frequency in a calcium-dependent manner (Shah et al., 2009). This ultimately promotes the clearance of mucus and particles out of the airway removing microorganisms out of the sinus epithelium during this process. There has also have been found to express TAS2R -4, -14, -16 in the sinonasal cavity and elicit a nitric oxide response (Hariri et al., 2017; Yan et al., 2017). Interestingly, quorum-sensing molecules are generated as microbial communication network molecules for the formation, growth, and maintenance of biofilms in many gram-negative bacteria such as Pseudomonas aeruginosa, an opportunistic human respiratory pathogen (Parsek et al., 1999). The presence of these molecules would signal ongoing colonization of pathogenic microbes on affected tissues. Similar to the detection of toxins and harmful substances entering the oral epithelium, bitter receptor activity provides mechanism for detecting and expelling pathogens and harmful substances in the airways.

Solitary chemosensory cells (SCCs) are specialized chemosensory cells expressing taste receptors such as the TAS1R and TAS2R GPCRs, are found in epithelial tissues in the body, including the nasal cavity (Finger et al., 2003). These cells express other characteristics of taste cells including expression alpha-gustducin, PLCß2, and TRPM5 (Gulbransen et al., 2008). In the human sinonasal cavity SCCs express bitter taste receptors including T2R -4, -10, -14, -30, -46 (Barham et al., 2013; R. J. Lee, Kofonow, et al., 2014a). Their activation, in the presence of markers for infection, results in the secretion of ß-defensin 1 and ß-defensin 2 from surrounding epithelial cells, which act as

antimicrobial molecules against invading bacteria (R. J. Lee, Kofonow, et al., 2014b). Interestingly, TAS1R sweet receptor activity also moderates this mechanism through inhibition TAS2R-mediated activity in response to normal glucose thresholds (R. J. Lee, Kofonow, et al., 2014b; Maina et al., 2018). A signature of infection is a reduced glucose level resulting from the utilization of glucose in bacterial metabolism, and the sweet receptors play a key role in detecting this signature and regulating the TAS2R-mediated immune response in SCCs.

Epithelial cells of the gut have been found to also express taste proteins, most notably TRPM5, gustducin, and PLCß2, and recent evidence has pointed towards a GPCR chemosensory receptor-mediated pathways in microbial monitoring and immune system function in tuft cells, a subset of chemosensory cells, in the gut (Bezençon et al., 2008; Howitt et al., 2016; Lu et al., 2017; Luo et al., 2019). This notion has been strengthened by the finding that loss of TRPM5 disrupts the expansion of tuft cells, goblet cells, eosinophils, and type 2 innate lymphoid cells during parasite colonization (Howitt et al., 2016). This phenomenon is suggested to occur through disruption of the production of IL-25 by tuft cells, which promotes secretion of IL-13 by innate lymphoid cells and leads to hyperplasia of tuft cells and goblet cells (Howitt et al., 2016; Luo et al., 2019). Adding to this, Feng et al demonstrated that the knockout of gustducin leads to aggravated colitis in IBD (Feng et al., 2018). Gustducin knockout mice had reduced IL-13, IL-5, and IL-10 expression and increased production of inflammatory cytokines. Overall there is strong evidence of taste chemosensory pathways playing key roles in gut immunity and inflammation (Feng et al., 2018). Moreover, taste receptor activity has been associated with the secretion of hormones by enteroendocrine cells in the gut, such as in the response to ingested food. Evidence has pointed to this being mediated, at least in part, by taste GPCRs related mechanisms (K.-S. Kim et al., 2014; Kojima & Nakagawa, 2011; Latorre et al., 2016; Shirazi-Beechey et al., 2014; Xie et al., 2018).

TAS2R pathway protein expression and associated activity has been found across a wide variety of tissues not already mentioned in this review. TAS2R activity is implicated in both the male and female reproduction system and, in mice, loss of TAS2R105 function leads to smaller testes or complete infertility (F. Li & Zhou, 2012; Lu et al., 2017). In the smooth airway tissues, TAS2R activity is associated with the relaxation of precontracted airway muscles, and evidence has demonstrated that bitter compounds may act as potent bronchodilators (Deshpande et al., 2010a; Liggett, 2013; Nayak et al., 2019). There is strong evidence of activity in the reproductive system tissues, the urethra, the kidneys, several types of immune cells, the thyroid, the skin, and other tissues(Lu et al., 2017). A review previously published by Lu et al. covers the abovementioned topics and previously discussed extraoral receptors in much greater detail.

## Methods

Large scale sequencing efforts have generated rich data which can be used explore variation in human taste genes. A dive into the 1000 genomes project reveals that the genes contributing to taste have wide ranging levels of variation and diversity. The 1000

genomes project catalogues whole genome sequencing data from 2504 unrelated and self-reported healthy individuals from 26 subpopulations across 5 superpopulations assigned by their continental location; America, Africa, Europe, Eastern Asia, and Southern Asia.

80 genes contributing to taste pathways were examined. The genomic coordinates for these genes were extracted from the Ensembl Biomart and confirmed in the UCSC Genome browser with human reference sequence release GRCh37/hg19. Variant call format (VCF) files from the Phase III release of the 1000 genomes data, which are aligned to human reference GRCh37, were downloaded and gene-specific VCF files for gene locus were extracted using Tabix (H. Li, 2011b; The 1000 Genomes Project Consortium, 2015). VCF files were preprocessed to remove sites that are invariant (e.g. AC=0), multiallelic sites, and variants that resulted in a change in the sequences length (e.g. insertion, deletions and structural variants). The remaining biallelic single nucleotide polymorphisms were annotated using the Ensembl Variant Effect Predictor (McLaren et al., 2016). Annotations were used to classify variant types based on their location in the gene untranslated region, intron, or exon. If the variant resides in an exon, annotations were used to classify the variant as a nonsynonymous or synonymous variant relative to the transcript referenced. The Popgenome package in R was used to calculate $\pi$ and Tajima's D and vcftools was used to calculate $F_{ST}$ for the global sample (Pfeifer et al., 2014). Tajima's D is a common test of natural selection, however because it is sensitive to population history and structure it was computed for each super-population (Tajima, 1989).

Extreme values of the tested statistics suggest deviance from neutrality and shift in the site frequency spectrum, however, these affects are similarly seen during population bottlenecks, expansions, and as a result of migrations. Therefore, deciphering these signals from those generated by genetic drift and demography is difficult. However, the effects of demography would be apparent across the genome, whereas natural selection acts on local regions of the genome harboring advantageous or deleterious polymorphisms.

Since 98% of our genome is non-coding it is assumed that most variants are selectively neutral, offering no advantage or disadvantage to fitness, and typically do not contributing to phenotypic variance. Thus the distribution of population statistics across the genome would provide the null distribution through deviations from neutrality can be tested while accounting for the effects of demography. Empirical distributions for the population statistics were computed using 1kb, 10 kb, and 100kb non-overlapping windows. To determine significance, cutoff values at a=0.05 corresponding to the 2.5% and 97.5% cutoffs on the empirical density curve

This analysis is an expansion of a similar analysis previously published by Wooding and Ramirez using the same data and similar methods on a more expansive list of gene regions (S. P. Wooding & Ramirez, 2022).

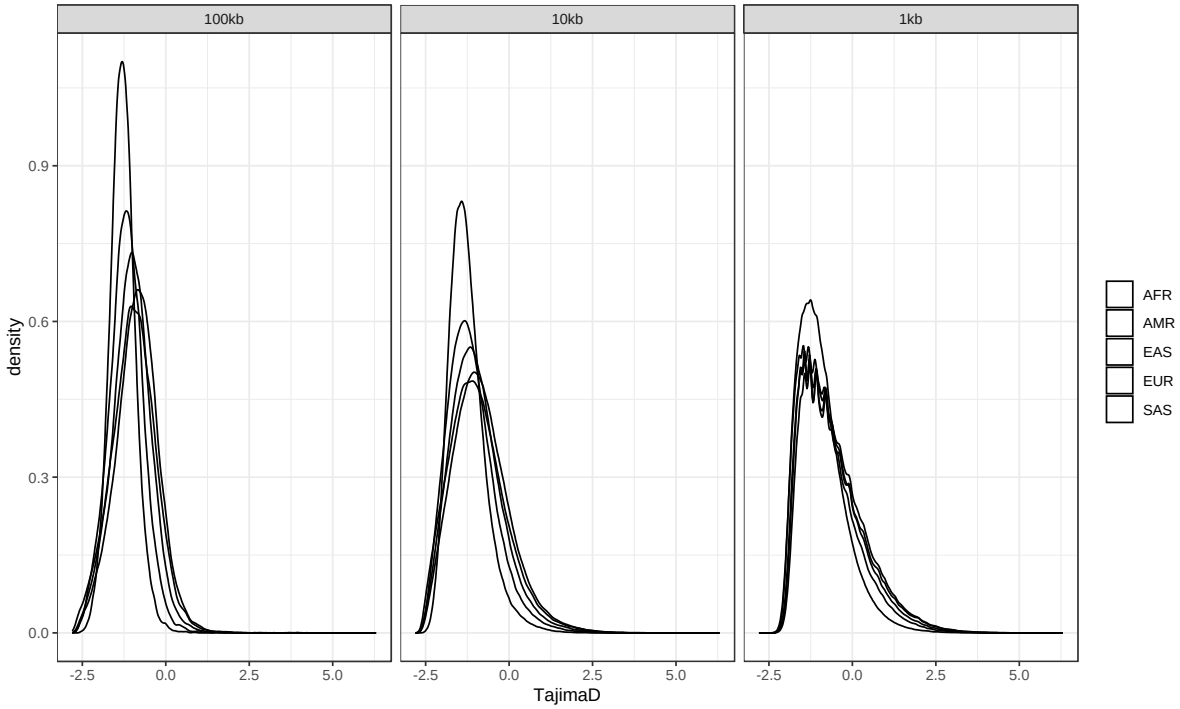


**Figure 2.2: Empirical Distribution Curve of Tajima's D on 5 Super Populations**: Window size was calculated for 1kb, 10kb, and 100kb. Gene's were compared to the empirical distribution based on the length of their transcript.

## Results

All of the genes examined harbored substantial variation. The level of nonsynonymous variation per gene ranged from 3 nonsynonymous polymorphisms in REEP3 to 117 in ITPR3. The level of diversity varied greatly among our genes, with transduction genes such as PRKAR2A at π=0.016% to genetic diversity at levels π=>0.03% in TAS2R20 and TAS2R42. Remarkably *TAS2R39* harbors substantial variation, but low levels of diversity, suggesting most SNPs in this gene are low frequency. Examining the 1000 genomes dataset reveals that among the 32 biallelic variants present in *TAS2R39*, 21 are singletons. $F_{ST}$ can measure if populations are differentiated for each taste gene locus. Several genes have $F_{ST}$ estimates much higher than previously estimated genomic averages. *TAS2R20* has a value of $F_{ST}$=0.258 and *TAS2R43* locus on the other hand represented the top 0.1% of all 1kb windows in the genome with a value with a value of $F_{ST}$ =0.44. Several TAS2Rs had marginally significant levels of differentiation including *TAS2R8, TAS2R13, TAS2R42, and TAS2R50.*

## Table 2.1 Genetics and Diversity of Human Gustatory Genes

| | Gene | Location | $S_{Tot}(S_N,S_S)$ | π(%tile) | $F_{ST}$(%tile) |
|---|---|---|---|---|---|
| Sour | *ASIC1* | 12:50451331-50477394 | 630(13,22) | 0.00047(14.70%) | 0.08002(46.81%) |
| | *ASIC2* | 17:31340105-32501983 | 35171(20,18) | 0.00108(69.76%) | 0.07499(41.29%) |
| | *ASIC3* | 7:150745379-150749843 | 168(52,23) | 0.00039(25.81%) | 0.03386(19.71%) |
| | *ASIC4* | 2:220378892-220403494 | 744(42,28) | 0.00086(52.12%) | 0.08703(53.91%) |
| | *HCN1* | 5:45259349-45696253 | 11173(17,17) | 0.00035(7.08%) | 0.06549(30.53%) |
| | *HCN4* | 15:73612200-73661605 | 1402(53,58) | 0.00070(37.15%) | 0.08857(55.34%) |
| | *KCNJ2* | 17:68164814-68176189 | 267(8,26) | 0.00042(11.53%) | 0.12982(82.4%) |
| | *OTOP1* | 4:4190530-4228616 | 1320(54,21) | 0.00167(91.60%) | 0.08435(51.29%) |
| | *PKD1L3* | 16:71963441-72033877 | 2477(0,0) | 0.00155(89.22%) | 0.08828(55.06%) |
| | *PKD2L1* | 10:102047903-102090243 | 1223(69,33) | 0.00093(58.99%) | 0.09120(57.79%) |
| Salt | *SCNN1A* | 12:6456009-6486896 | 984(54,27) | 0.00117(75.35%) | 0.10110(65.99%) |
| | *SCNN1B* | 16:23289552-23392620 | 3019(42,22) | 0.00090(56.35%) | 0.12472(80.2%) |
| | *SCNN1D* | 1:1215816-1227409 | 598(100,58) | 0.00139(84.85%) | 0.15246(89.61%) |
| | *SCNN1G* | 16:23194036-23228204 | 967(34,21) | 0.00103(66.13%) | 0.07405(40.24%) |
| Fat | *CD36* | 7:79998891-80303725 | 8438(71,14) | 0.0010(63.97%) | 0.08777(54.59%) |
| | *FFAR1* | 19:35842445-35843367 | 29(20,8) | 0.00031(20.88%) | 0.00031(20.88%) |
| | *GPR120* | 10:95326422-95349829 | 689(17,19) | 0.00089(55.57%) | 0.09053(57.18%) |
| Sweet/Umami | *TAS1R1* | 1:6615241-6639817 | 815(76,26) | 0.00080(46.94%) | 0.10641(69.78%) |
| | *TAS1R2* | 1:19166093-19186176 | 742(81,49) | 0.00174(92.60%) | 0.06183(26.36%) |
| | *TAS1R3* | 1:1266694-1270686 | 255(108,66) | 0.00056(37.91%) | 0.07798(57.96%) |
| Bitter | *TAS2R1* | 5:9629109-9630463 | 46(21,6) | 0.00062(42.13%) | 0.17937(92.73%) |
| | *TAS2R2P* | 7:12530721-12531630 | 32(0,0) | 0.00197(90.23%) | 0.05887(42.66%) |
| | *TAS2R3* | 7:141463897-141464997 | 30(17,9) | 0.00104(65.22%) | 0.09566(68.9%) |
| | *TAS2R4* | 7:141478242-141479235 | 31(23,7) | 0.00160(84.05%) | 0.14327(86.46%) |
| | *TAS2R5* | 7:141490017-141491166 | 51(25,8) | 0.00108(67.30%) | 0.12528(81.47%) |
| | *TAS2R7* | 12:10954131-10955226 | 42(31,9) | 0.00033(22.46%) | 0.04541(30.38%) |
| | *TAS2R8* | 12:10958650-10959892 | 45(26,6) | 0.00072(48.40%) | 0.21367(95.95%) |
| | *TAS2R9* | 12:10961693-10962767 | 33(27,4) | 0.00058(39.14%) | 0.16932(91.36%) |
| | *TAS2R10* | 12:10977916-10978957 | 37(27,5) | 0.00030(20.77%) | 0.14101(85.92%) |
| | *TAS2R12P* | 12:11047542-11048481 | 18(0,0) | 0.00008(6.06%) | 0.00828(2.41%) |
| | *TAS2R13* | 12:11060525-11062161 | 34(16,1) | 0.00065(44.03%) | 0.22739(96.79%) |
| | *TAS2R14* | 12:11090005-11091862 | 51(21,9) | 0.00077(51.59%) | 0.14111(85.94%) |
| | *TAS2R15P* | 12:11117024-11117951 | 30(0,0) | 0.00309(97.57%) | 0.00760(2.15%) |
| | *TAS2R16* | 7:122634759-122635754 | 39(20,16) | 0.00115(70.48%) | 0.21548(96.07%) |
| | *TAS2R18P* | 12:11311384-11312293 | 19(0,0) | 0.00280(96.61%) | 0.26865(98.41%) |
| | *TAS2R19* | 12:11174218-11175219 | 32(25,6) | 0.00160(84.05%) | 0.10064(71.48%) |
| | *TAS2R20* | 12:11149094-11150474 | 50(27,9) | 0.00358(98.58%) | 0.25782(98.09%) |
| | *TAS2R30* | 12:11285557-11287243 | 46(22,11) | 0.00144(79.97%) | 0.06535(48.21%) |
| | *TAS2R31* | 12:11182986-11184006 | 42(36,5) | 0.00232(93.84%) | 0.06260(45.9%) |
| | *TAS2R38* | 7:141672431-141673573 | 33(22,10) | 0.00136(77.67%) | 0.07789(57.89%) |
| | *TAS2R39* | 7:142880512-142881528 | 32(23,9) | 0.00005(3.46%) | 0.00874(2.59%) |
| | *TAS2R40* | 7:142919130-142920162 | 30(16,12) | 0.00020(14.92%) | 0.02718(14.02%) |
| | *TAS2R41* | 7:143174966-143175889 | 31(18,13) | 0.00078(51.68%) | 0.08974(65.6%) |
| | *TAS2R42* | 12:11338599-11339543 | 36(24,12) | 0.00335(98.19%) | 0.22240(96.51%) |
| | *TAS2R43* | 12:11243886-11244912 | 34(26,7) | 0.00159(83.71%) | 0.43365(99.9%) |
| | *TAS2R46* | 12:11213964-11214893 | 32(25,7) | 0.00119(71.77%) | 0.05426(38.55%) |
| | *TAS2R50* | 12:11138512-11139511 | 31(16,12) | 0.00123(73.43%) | 0.23369(97.11%) |
| | *TAS2R60* | 7:143140546-143141502 | 34(23,11) | 0.00051(33.35%) | 0.10356(72.9%) |
| | *TAS2R62P* | 7:143134128-143135066 | 28(0,0) | 0.00146(80.48%) | 0.06155(45.%) |
| | *TAS2R63P* | 12:11200931-11201855 | 23(0,0) | 0.00195(89.97%) | 0.05582(39.96%) |
| | *TAS2R64P* | 12:11229915-11230841 | 32(0,0) | 0.00014(10.92%) | 0.00713(1.98%) |
| | *TAS2R67P* | 12:11332269-11333061 | 14(0,0) | 0.00125(74.05%) | 0.25529(98.%) |

| | | | | | |
|---|---|---|---|---|---|
| Transduction | *GNAT3* | 7:80087987-80141336 | 1428(15,13) | 0.00075(42.11%) | 0.15067(89.15%) |
| | *GNB3* | 12:6949118-6956557 | 241(25,19) | 0.00055(22.25%) | 0.13934(85.86%) |
| | *GNG13* | 16:848041-850733 | 162(7,6) | 0.00174(86.71%) | 0.05834(42.19%) |
| | *CALHM1* | 10:105213144-105218645 | 175(43,15) | 0.00074(40.93%) | 0.06347(28.24%) |
| | *CALHM3* | 10:105232561-105238997 | 164(19,11) | 0.00055(21.82%) | 0.11009(72.22%) |
| | *ITPR3* | 6:33588142-33664351 | 2169(117,125) | 0.00110(71.16%) | 0.12194(78.88%) |
| | *PDE1A* | 2:183004763-183387919 | 10258(20,11) | 0.00093(58.86%) | 0.07882(45.51%) |
| | *PLCB2* | 15:40570377-40600136 | 844(61,37) | 0.00073(39.40%) | 0.10340(67.71%) |
| | *PRKACA* | 19:14202500-14228896 | 638(5,9) | 0.00034(6.62%) | 0.09184(58.35%) |
| | *PRKACB* | 1:84543745-84704181 | 3877(6,4) | 0.00052(18.78%) | 0.04324(8.58%) |
| | *PRKACG* | 9:71627469-71629039 | 42(16,11) | 0.00070(47.56%) | 0.05189(36.39%) |
| | *PRKAR1A* | 17:66507921-66547460 | 1190(4,12) | 0.00083(49.50%) | 0.04293(8.37%) |
| | *PRKAR1B* | 7:588834-767287 | 6821(15,32) | 0.00103(66.28%) | 0.06519(30.2%) |
| | *PRKAR2A* | 3:48782030-48885279 | 2219(22,10) | 0.00016(1.38%) | 0.11959(77.68%) |
| | *PRKAR2B* | 7:106685094-106802256 | 2987(8,11) | 0.00058(24.77%) | 0.09530(61.36%) |
| | *TRPM4* | 19:49660998-49715093 | 1788(86,43) | 0.00116(74.83%) | 0.07805(44.68%) |
| | *TRPM5* | 11:2425745-2444275 | 924(101,73) | 0.00156(89.34%) | 0.05528(19.27%) |
| Modifiers | *CA6* | 1:9005926-9035151 | 979(26,22) | 0.00137(84.14%) | 0.07831(44.95%) |
| | *PRB1* | 12:11504757-11508525 | 182(27,10) | 0.00156(83.0%) | 0.06096(44.49%) |
| | *REEP1* | 2:86441122-86565206 | 3398(3,10) | 0.0010(64.10%) | 0.14368(87.21%) |
| | *REEP2* | 5:137774706-137782658 | 213(9,9) | 0.00095(60.10%) | 0.08003(46.81%) |
| | *REEP3* | 10:65281123-65384883 | 2372(6,7) | 0.00065(32.22%) | 0.09042(57.08%) |
| | *RTP3* | 3:46538981-46542439 | 109(7,7) | 0.00122(72.83%) | 0.11489(77.77%) |
| | *RTP4* | 3:187086120-187089864 | 104(21,5) | 0.00111(68.49%) | 0.11452(77.63%) |
| Trigeminal | *TRPA1* | 8:72932152-72987852 | 1575(59,36) | 0.00139(84.82%) | 0.05797(22.12%) |
| | *TRPM8* | 2:234826043-234928166 | 3118(61,51) | 0.00104(67.35%) | 0.12033(78.06%) |
| | *TRPV1* | 17:3468738-3512705 | 1540(57,40) | 0.00157(89.57%) | 0.21390(97.49%) |

Test of natural selection by Tajima's D revealed a number of genes which were above the 97.5% threshold on the empirical distribution, suggesting they are strong candidates for experiencing selective pressure. *TAS2R20* has significantly high Tajima's D above in the Latina America, Europe, and Southern Asia, and is marginally high in Eastern Asia in the top 10% of 1kb regions. *TAS2R42* was highest in Latin America and Europe falling in the 98$^{th}$-99$^{th}$ percentile. On the opposite extreme of the distribution curve lies in the bottom 2.5% are *TAS1R3* and *PRKAR2B*, suggesting selective pressure against these genes. Several genes have signatures in one continent and can be seen in **Table 1.2** below.

**Table 2.2 Tajima's D Statistic for Taste Genes**

| Name | Africa | America | Eastern Asia | Europe | Southern Asian |
|---|---|---|---|---|---|
| *ASIC1* | -1.7(20.21%) | -1.33(43.14%) | -1.81(15.09%) | -1.12(39.88%) | -1.8(13.58%) |
| *ASIC2* | -1.18(62.94%) | -0.88(72.79%) | -0.59(72.4%) | -0.53(70.72%) | -0.68(73.18%) |
| *ASIC3* | -2.07(0.35%) | -1.76(8.37%) | -1.96(0.76%) | -1.77(3.3%) | -1.95(1.05%) |
| *ASIC4* | -1.17(62.39%) | -1.27(46.92%) | -1.33(35.06%) | -1.11(40.04%) | -0.92(57.86%) |
| *HCN1* | -2.1(1.47%) | -2(6.05%) | -1.8(10.67%) | -1.87(6.16%) | -1.93(6.26%) |
| *HCN4* | -1.58(29.3%) | -1.42(37.79%) | -1.32(35.75%) | -1.3(30.97%) | -1.45(29.26%) |
| *KCNJ2* | -1.14(64.31%) | -1.82(16.43%) | -1.98(9.73%) | -1.69(14.98%) | -1.99(7.68%) |
| *OTOP1* | -0.7(85.71%) | -0.59(79.99%) | -0.18(82.48%) | 0.54(93.58%) | -0.3(82.69%) |
| *PKD1L3* | -0.68(93.92%) | -0.42(93.12%) | -0.49(77.2%) | 0.17(95.06%) | -0.44(85.24%) |
| *PKD2L1* | -1(72.81%) | -1.36(41.44%) | -1(50.79%) | -1.31(30.5%) | -0.86(60.93%) |
| *SCNN1A* | -1.16(62.51%) | -1.02(61.44%) | -0.54(70.99%) | -0.56(66.36%) | -0.94(56.79%) |

| | | | | | |
|---|---|---|---|---|---|
| *SCNN1B* | -1.06(74.7%) | -1.63(18.5%) | -0.96(49.89%) | -1.36(20.85%) | -1.18(38.5%) |
| *SCNN1D* | -1.24(56.61%) | -1.66(24.06%) | -1.34(34.76%) | -2.29(1.64%) | -1.65(19.66%) |
| *SCNN1G* | -1.1(67%) | -0.47(83.72%) | -1.33(35.34%) | 0.14(87.6%) | -1.22(41.49%) |
| *CD36* | -0.97(81.22%) | -1.13(54.44%) | -0.32(84.75%) | -0.72(58.77%) | -0.82(64.46%) |
| *FFAR1* | -1.96(1.76%) | -1.38(28.18%) | -0.85(47.86%) | -1.33(21.98%) | -1.1(37.15%) |
| *GPR120* | -0.95(75.11%) | -1.72(21.27%) | -1.62(21.95%) | -1.28(31.7%) | -1(53.6%) |
| *TAS1R1* | -1.29(53.37%) | -1.89(13.42%) | -1.45(29.53%) | -1.8(11.39%) | -1.76(15.44%) |
| *TAS1R2* | -0.66(86.82%) | -0.59(80.16%) | -0.55(70.81%) | 0.37(91.5%) | -0.5(76.57%) |
| *TAS1R3* | -2.01(0.96%) | -2.27(0.02%) | -2.2(0.02%) | -2.36(0%) | -2.23(0.01%) |
| *TAS2R1* | -1.89(3.24%) | -1.33(30.9%) | -0.66(55.96%) | -1.23(27.17%) | -1.54(15.88%) |
| *TAS2R2P* | 0.5(95.7%) | -0.45(70.95%) | 0.09(79.26%) | 1.23(94.79%) | 0.22(84.33%) |
| *TAS2R3* | -1.11(47.25%) | 0.06(84.61%) | -0.21(71.57%) | -0.29(66.55%) | -0.27(71.97%) |
| *TAS2R4* | -1.08(49.14%) | 0.58(92.47%) | 0.5(87.32%) | -0.27(67.28%) | 0.69(91.59%) |
| *TAS2R5* | -1.35(31.63%) | -1.19(37.97%) | -0.92(44.87%) | -0.28(66.98%) | -0.9(47.29%) |
| *TAS2R7* | -2.07(0.38%) | -1.62(14.89%) | -1.09(37.21%) | -1.71(5.16%) | -1.56(14.83%) |
| *TAS2R8* | -1.39(29.55%) | -1.39(27.35%) | -1.1(36.13%) | -1.47(15.13%) | -1.44(20.74%) |
| *TAS2R9* | -1.6(16.59%) | -1.26(34.58%) | -0.5(62.1%) | -0.5(59.26%) | -1.15(34.96%) |
| *TAS2R10* | -1.57(18.33%) | -1.72(9.96%) | -1.62(10.84%) | -1.26(25.97%) | -1.6(12.36%) |
| *TAS2R12P* | -1.82(5.9%) | -1.83(5.46%) | -1.22(31.35%) | -1.47(14.92%) | -1.15(34.92%) |
| *TAS2R13* | -1.23(39.84%) | 0.02(83.62%) | -1.01(41.27%) | 0.04(76.5%) | -0.44(66.32%) |
| *TAS2R14* | -0.97(55.47%) | -1.01(47.33%) | -0.77(51.59%) | -0.97(39.29%) | -1.2(32.98%) |
| *TAS2R15* | 0.99(98.23%) | 1.38(97.85%) | 0.69(89.91%) | 1.46(96.22%) | 1.33(96.7%) |
| *TAS2R16* | 0.03(90.35%) | -0.91(52.15%) | -1.31(26.17%) | -0.07(73.38%) | -1.62(11.52%) |
| *TAS2R18* | 1.22(98.82%) | 2.9(99.86%) | 0.46(86.77%) | 1.23(94.79%) | 1.11(95.42%) |
| *TAS2R19* | -0.25(84.66%) | -0.25(77.07%) | -0.01(77.02%) | 0(75.27%) | -0.56(61.78%) |
| *TAS2R20* | -1.08(48.55%) | 1.68(98.66%) | 0.94(92.79%) | 2.09(98.57%) | 2.46(99.49%) |
| *TAS2R30* | -0.03(89.33%) | 1.19(97.06%) | 0.19(81.5%) | 0.4(84.44%) | 0.2(84%) |
| *TAS2R31* | -0.06(88.74%) | 1.35(97.71%) | -0.73(53.47%) | 1.04(93.22%) | -0.36(69.22%) |
| *TAS2R38* | -0.64(71.74%) | 0.47(91.25%) | 0.07(78.85%) | 1.48(96.36%) | -0.39(68.05%) |
| *TAS2R39* | -1.84(4.81%) | -1.86(4.59%) | -1.65(9.46%) | -1.75(3.82%) | -1.68(8.78%) |
| *TAS2R40* | -1.4(28.94%) | -1.4(26.94%) | -1.65(9.38%) | -1.63(8.07%) | -1.42(21.75%) |
| *TAS2R41* | -2.02(0.8%) | -0.28(76.29%) | -0.56(59.86%) | -0.73(49.97%) | 0.44(88.33%) |
| *TAS2R42* | 1.1(98.54%) | 2.27(99.53%) | -0.71(54.22%) | 1.18(94.44%) | 1.11(95.4%) |
| *TAS2R43* | -1.65(13.58%) | -0.76(58.71%) | -0.8(50.34%) | -0.63(53.84%) | -1.21(32.46%) |
| *TAS2R46* | -1(53.53%) | -0.39(72.72%) | -0.86(47.36%) | 0.35(83.59%) | -0.75(53.9%) |
| *TAS2R50* | -1.06(50.3%) | -0.21(78.19%) | -0.6(58.22%) | 1.12(93.9%) | -0.45(65.94%) |
| *TAS2R60* | -1.42(27.68%) | -0.96(49.64%) | -1.49(17.34%) | -0.77(48.13%) | -1.12(36.63%) |
| *TAS2R62P* | -0.17(86.54%) | 0.18(86.84%) | -0.52(61.24%) | -0.08(73.26%) | 0.39(87.46%) |
| *TAS2R63P* | 0.11(91.62%) | 0.12(85.81%) | 1.39(96.09%) | 2.37(99.11%) | 0.62(90.82%) |
| *TAS2R64P* | -1.86(4.28%) | -1.93(2.56%) | -1.81(3.42%) | -1.42(17.99%) | -1.67(9.31%) |
| *TAS2R67P* | -0.02(89.58%) | -0.3(75.46%) | -0.18(72.15%) | 0.01(75.59%) | -0.17(74.81%) |
| *GNAT3* | -0.94(76.02%) | -1.8(17.33%) | -1.28(37.58%) | -1.53(20.63%) | -1.66(19.61%) |
| *GNB3* | -1.79(14.13%) | -1.78(18.01%) | -0.87(57.03%) | -1.88(9.09%) | -2.14(4.34%) |
| *GNG13* | -1.41(28.23%) | -1.54(19.71%) | -1.11(35.68%) | -1.59(9.55%) | -0.73(54.92%) |
| *CALHM1* | -1.9(8.47%) | -1.03(60.92%) | -1.25(38.93%) | -0.57(65.98%) | -1.05(50.92%) |
| *CALHM3* | -0.5(90.76%) | -1.76(19.29%) | -1.66(20.47%) | -1.84(10.29%) | -1.92(9.65%) |
| *ITPR3* | -1.25(55.48%) | -0.8(77.78%) | -1.3(29.71%) | -0.53(70.47%) | -1.04(48.14%) |
| *PDE1A* | -1.36(43.71%) | -0.91(70.76%) | -0.75(63.4%) | -0.46(74.58%) | -0.69(72.5%) |
| *PLCB2* | -1.36(47.37%) | -1.48(34.22%) | -1.38(32.56%) | -1.3(30.67%) | -1.42(31.24%) |
| *PRKACA* | -1.82(12.56%) | -1.85(15.2%) | -2.17(5.2%) | -2.04(5.44%) | -2.18(3.61%) |
| *PRKACB* | -1.72(12.16%) | -1.4(32.96%) | -1.34(27.78%) | -1.21(27.91%) | -1.55(17.21%) |

| | | | | | |
|---|---|---|---|---|---|
| *PRKACG* | -0.46(78.37%) | -0.62(64.55%) | -0.82(48.87%) | 0.18(79.73%) | -1.14(35.72%) |
| *PRKAR1A* | -1.48(37.38%) | -1.27(46.89%) | -1.14(44.19%) | -0.63(63.17%) | -0.78(64.71%) |
| *PRKAR1B* | -1.49(29.73%) | -1.64(18.22%) | -1.11(40.86%) | -1.19(29.2%) | -1.49(19.97%) |
| *PRKAR2A* | -2.34(0.18%) | -2.39(0.71%) | -2.62(0.27%) | -2.09(3.13%) | -2.54(0.27%) |
| *PRKAR2B* | -1.82(7.52%) | -1.63(18.69%) | -1.24(33.36%) | -1.19(28.8%) | -1.33(28.7%) |
| *TRPM4* | -0.87(79.26%) | -1.53(31.57%) | -1.02(50.21%) | -0.87(52.16%) | -0.92(58.15%) |
| *TRPM5* | -1.15(63.12%) | -0.82(70.96%) | -0.84(58.33%) | -0.66(61.88%) | -0.94(57.03%) |
| *CA6* | -0.71(85.43%) | -0.93(65.93%) | -0.45(74.32%) | -0.68(61.13%) | -0.6(72.67%) |
| *PRB1* | -0.96(55.67%) | -1.12(41.85%) | -1.18(32.84%) | -0.8(46.3%) | -0.84(49.94%) |
| *REEP1* | -1.22(58.27%) | -1.29(41.64%) | -0.76(62.69%) | -0.75(56.72%) | -0.83(63.56%) |
| *REEP2* | -1.04(70.19%) | -0.39(85.96%) | -0.89(56.19%) | -0.46(70.55%) | -0.32(82.17%) |
| *REEP3* | -1.44(34.65%) | -1.03(62.38%) | -1.14(39.05%) | -0.87(49.02%) | -1.15(40.82%) |
| *RTP3* | -1.46(24.76%) | -0.23(77.44%) | -0.64(56.78%) | 0.81(90.75%) | 0.45(88.42%) |
| *RTP4* | -1.19(41.77%) | -0.88(53.5%) | -0.87(46.93%) | -0.61(54.83%) | -0.38(68.26%) |
| *TRPA1* | -0.63(87.65%) | -0.38(86.03%) | -0.2(81.98%) | 0.34(91.01%) | 0.38(94.57%) |
| *TRPM8* | -1.35(44.15%) | -0.98(66.24%) | -0.51(76.38%) | -1.3(23.3%) | -0.79(66.19%) |
| *TRPV1* | -0.95(75.15%) | -0.48(83.37%) | -0.8(60.47%) | -0.6(64.68%) | -0.78(64.65%) |

## Conclusion and Future Directions

As has been detailed, the contribution of taste to overall health is both wide and significant. I have briefly summarized the evolutionary origins, the molecular mechanism governing taste perception, the association of polymorphisms and dietary behaviors, and the attribution taste may play in human health.

Variation in chemosensory cues, like the classic example of PTC and PROP tasting, has been associated with human behavioral traits including dietary preferences and ethanol intake (Dotson et al., 2010; Laugerette et al., 2005; Martinez-Ruiz et al., 2014; Schembre et al., 2013). Taste may contribute to the risk for various diseases and pressing issues in population health such as obesity, cardiovascular disease, diabetes, and substance use disorders. I have assessed the importance of the emerging roles taste genes play in physiological phenomena outside of the domains of taste. Relatively recently, scientists have discovered that taste receptors and the proteins involved in their activation pathways are expressed in various extraoral tissues and have major physiological roles and contributions to disease (Lu et al., 2017). These emerging discoveries have already demonstrated that they have major implications for human health and disease, as detailed in this work.

However, there are large gaps in our understanding of these mechanisms. Much of our understanding comes from animal models, but their chemosensory systems translate differently to humans. Additionally, taste receptors mediating salt taste have yet to be confirmed, and our general understanding of mechanisms driving chemosensation of this taste modality is incomplete. While studies have focused on bitter and sweet taste, much less is currently understood about the biology and genetics of fat, salty, and sour taste. Moreover, the contribution that genetics have on taste sensitivity, food preferences, and dietary choices is still largely unknown, despite great strides that have been made.

This work emphasized the importance of capturing genotypic and phenotypic diversity of these phenomenon across global ancestries. Characterizing this diversity and understanding the processes that have shaped genetic diversity can help scientists to make sense of the distribution of global variation in complex traits, such as diverse taste phenotypes seen in modern populations. Bitter taste is the best studied example, where it is hypothesized that plant toxins are a major selective pressure acting on the bitter receptor family. Thus, maintaining sensitive taste perception to a wide variety of plant toxins and metabolites may have played a key role in our ancient ancestors' fitness. However, snapshots of genetic diversity in modern humans suggest that these roles have been rather dynamic throughout human evolution, and the selective pressures may be more relaxed in modern humans. During different time periods and in different populations, selective processes may have acted on our taste genes, improving fitness and adapting relative to the exposures in the external environment.

In contemporary human populations there has been suggestion that the selective constraints acting on taste have relaxed. It is hypothesized that humans rely less on taste than our ancient ancestors, and therefore selective optimizations of taste genes to detect substances in our environment do not garner the same improvement on fitness as they did for our ancestors. Our analysis of 80 taste genes found that several genes, including one of the PKA subunits, *TAS1R3, TAS2R20,* and *TAS2R42* show signatures of recent selection acting on their locus. Compared to the genome-wide average, taste genes show higher levels of diversity and appear to show greater rates of differentiation suggesting the effect of selective processes over the course of evolution and human migrations. For instance *TAS2R13* has a $F_{ST}$ in the 96$^{th}$ percentile, suggesting that it is highly differentiated with respect to human continental populations, but within its coding region the sample carried only one synonymous polymorphism and the rest were nonsynonymous or residing in the 3’ and 5’ untranslated regions. Thus it is likely that *TAS2R13* is differentiated with regards to phenotypes as well. This aligns with the finding variation in *TAS2R13* is associated with alcohol use, but suggests the effect is not blanketed across the global population. Our findings further highlight that selective pressures have been pervasive on the multiple taste genes, but have also acted in a complex manner on particular haplotype backgrounds, in particular geographies, and across time. While previous work has highlighted patterns of natural selection in *TAS2R16* and *TAS2R38*, the current results suggest that natural selection has acted on multiple taste receptor genes across multiple modalities of oral sensation. Nonetheless, while many genes displayed higher diversity and differentiation than genome-wide averages most of them are within expectations of neutrality suggesting selective pressures have either relaxed or are absent on most of the genes mediating taste. However, the analysis did not examine subpopulations within continents, nor did it differentiate between exons and introns in large genes. This aggregation surely biases these findings. For instance, previous studies examining *TAS2R38* that have aggregated populations between African’s and non-Africans on the same sample have found Tajima’s D to not deviate from the null, however our analysis revealed that D is in the >95$^{th}$ percentile in European populations. Thus, this warrants further analysis, and serves to only draw hypotheses.

It is important to note that while it is hypothesized that dietary processes and nutrient seeking have been the major selective pressure on taste receptor genes, we can only ponder on this factor in light two key points: novel physiological functions of taste receptors are currently being discovered such as the non-oral functions of the bitter taste receptors, and the mechanism mediating taste perception and the ligands for taste receptors are not well characterized in humans. Nonetheless, the current analysis allows new hypotheses to be drawn about evolutionary processes that have acted on taste genes and the distribution of alleles and phenotypes we see in contemporary human populations with regard to taste.

The contributions of taste perception to dietary behavior and health have important implications for public health. With recently emerging details about the physiological roles of taste genes outside of the oral cavity the potential for chemosensory science in health interventions is promising. An immediate example is the use of non-nutritive and low-calorie sweeteners that mimic the taste of sugar in soft drinks, such as stevia, provide an alternative to the large amounts of sugar in soft drinks (Arora et al., 2010). Such interventions aimed at lowering sugar consumption provide an potential avenue to improve adherence to dietary regimens or limiting the risk of obesity and metabolic disorders. Blocking bitterness, such as the bitterness of medicine, can be a more effective avenue for getting children to comply with treatment regimens (Mennella et al., 2013). Palatability of drugs contributes to adherence of treatment regimens in pediatric patients, as children will often spit out bitter and unpalatable medicines (Mennella et al., 2015; Yeka & Harris, 2010). Masking of bitter taste can effectively improve adherence for anti-malaria and HIV medication in pediatric patients (Baguley et al., 2012; Yeka & Harris, 2010). Lastly, targeting of extraoral reception may give a means to developing novel pharmaceutical interventions, such as the treatment of asthma or persistent nasal inflammation.

The quantitative findings here highlight the importance of diversifying modern genetic association studies, which will serve better capture of alleles likely shaping phenotypic variation seen in human traits. Findings from studies seeking to observe associations between genetic polymorphisms and phenotypes of interest are often not generalizable due to underrepresentation of ethnic groups in genomic association studies, and inherently miss functional variants (Bentley et al., 2017; Medina-Gomez et al., 2015; “Non-European Populations Still Underrepresented in Genomic Testing Samples,” 2017). For instance, White et al reported that only 5% of polymorphisms associated with asthma could be replicated in African American samples (White et al., 2016). Capturing this global genotypic and phenotypic diversity is important for dissecting the nature of this phenomenon. Given the elevated levels of genetic diversity and differentiation in taste genes, it important that diverse surveying is a priority in order to better cover mutational landscape to trait and disease susceptibility translate these findings to tangible and effective human health intervention. In doing so, there can be assurance that the innovations and interventions based on taste will be applicable to populations of diverse ancestries and genetic backgrounds and done.

# Chapter 3 Inia: Scripts for Extracting and Summarizing Local Regions in the 1000 Genomes Project


## Abstract

` Whole genome datasets can shed important light on human evolution and inherited disease. However, fully realizing their potential requires integrating information from heterogeneous sources including raw genetic data, computation, and reference to published research and clinical databases, which is laborious. Inia facilitates investigation by providing tools to extract, organize, and summarize variants in the 1000 Genomes Project (1000GP) repository, one of the largest whole-genome sequence databases currently available. Given an input file specifying regions to be analyzed, Inia extracts data from the 1000GP repository, calculates key population genetic measures, and integrates annotations and predictions of functional effects catalogued in the Ensembl database (via Ensembl VEP).

**Availability**
Inia is available at https://github.com/vramirez4/Inia.


## Introduction and Motivation

The past two decades have witnessed a revolution in genomics, which has provided a deeper understanding of the molecular basis of human traits and diseases. Following the successful completion of the Human Genome Project, the development of next- and third-generation sequencing techniques has enabled whole genome sequencing projects aimed at population samples, revealing patterns of variation at high resolution. Simultaneously, empirical and computational strategies for assessing and predicting the functional impact of discovered variants have increased in precision and accuracy.

The availability of high-resolution genomic data provides new and unique opportunities for studying patterns of population genetic and phenotypic variation. Traditionally, population genetic studies addressed patterns of variation in localized genomic regions in small numbers of subjects, limiting their scope. Technological advancements now allow whole genome sequencing in large numbers of subjects, providing a more comprehensive and complex perspective on diversity. This offers opportunities to study processes such as natural selection and population structuring on new, large scales. It also allows assessments of clinically important mutations, particularly their frequencies in different populations, which can shed light on population-specific disease susceptibility.

Another valuable source of information about the underpinnings of phenotypic diversity and its evolutionary origins is functional prediction. For instance, in studies of human evolution, tests for natural selection frequently detect signatures in general

genomic regions but face the obstacle of determining exactly which variant is responsible. Because natural selection can only operate on functional variants, predictions of whether a particular variant has functional effects or not can narrow down or even pinpoint responsible mutations. Information on functional effects is valuable in gene mapping, as well. For instance, one of the most widely used and successful tools for identifying variants underlying disease phenotypes is the genome wide association study (GWAS). The general approach of GWAS is to characterize variants across the genome, testing for associations between each variant and the phenotype of interest. The incorporation of functional information can be used to prioritize candidate variants, increasing statistical power by effectively reducing the number of tests. Information on such effects can be obtained both computationally and from extensive empirical literature and clinical databases. However, the size and heterogeneity of these sources makes integrating them laborious.


**Figure 3.1 Graphic for Inia Package** - The tile graphic demonstrates the namesake of the Inia package, Inia geofrennis which is more commonly known as the pink Amazonian River dolphin or “boto” dolphin.

To facilitate investigations of population genetics and functional variation in genomic data, we created the Inia software package (ref. Figure 2.1). With a namesake derived from the Amazonian river dolphins Inia geofrennis, that dive to the depths of the murky Amazon river to hunt their food. In a similar manner, the Inia software package dives into large public sequence data in order to extract and summarize key features and information in the localized regions of the genome. Inia provides scripts that extract sequence data from the 1000 Genomes Project (1000GP), the largest publicly available sample of global human diversity published to date, provides important annotation of variants, and computes basic population genetic analysis. Given genomic coordinates specified by the user in a csv file, Inia generates a comprehensive description of diversity and functional polymorphism including annotations of alternate alleles, codon contexts, exon positions, population genetic measures, computed and assayed functional

predictions, PubMed references, and known clinical associations (Adzhubei et al., 2010; Kumar et al., 2009; Landrum et al., 2018; Ng & Henikoff, 2003). Output is in the form of a readily parsed, human readable csv file.

## Methodology

Inia utilizes haplotypes from 2504 unrelated individuals from the 1000 genomes phase III dataset. These data consist of individuals from 26 subpopulations that are grouped into 5 geographic superpopulations: Africa, Eastern Asia, Southern Asia, Europe, and Admixed Americas. Sequences in the dataset were aligned to GRCh37 release of the human reference genome sequence prior, and variant calls are stored in the 1000 genomes ftp repository. The data consist of single nucleotide polymorphisms, small insertions and deletions, and complex structural variants e.g., copy number variants, large deletions and insertions, and translocations.

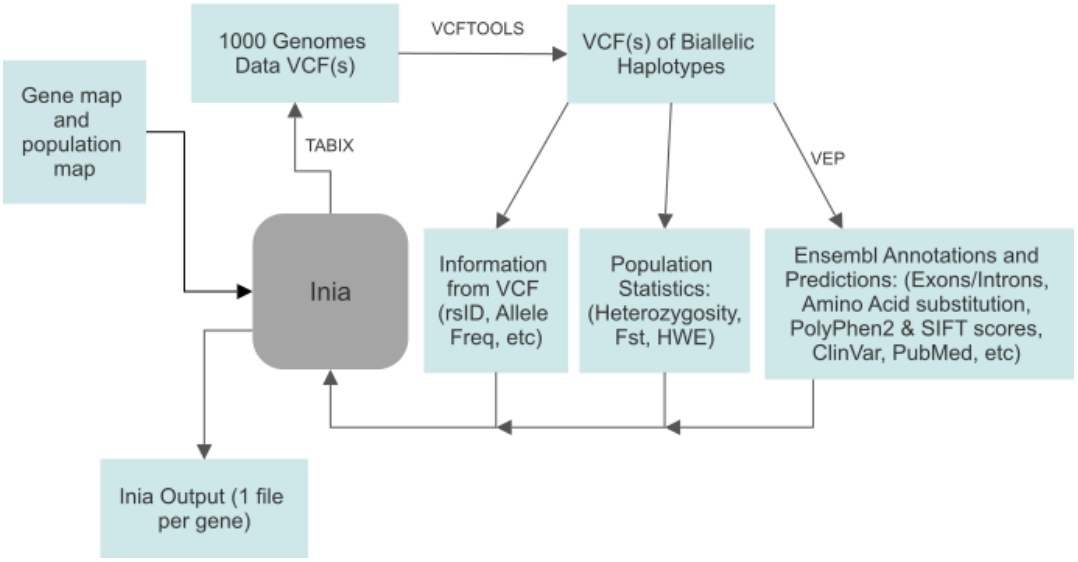


**Figure 3.2 Framework for Analysis** - Inia relies on commonly used bioinformatic and computational tools to summarize, annotate, and analyze variation in the 1000 genomes dataset.

Input to Inia is given as a csv file with lines consisting of 6 columns: gene/feature name, chromosome number, start site, end site, Ensembl gene ID, and Ensembl transcript ID. Multiple genes can be specified in the same file. Inia uses these parameters to query the Phase III repository of the 1000GP ftp server using Tabix and stores the returned vcf files locally (H. Li, 2011b). Finally, Inia imports the 1000GP vcf files into R using the VariantAnnotation package of Bioconductor.

After import, Inia ascertains three widely utilized population genetic statistics, which describe levels of diversity and population structure: per site nucleotide diversity (π), genetic distance ($F_{ST}$), and departure from Hardy-Weinberg equilibrium. π is calculated as the mean pairwise nucleotide difference between sequences and the equation is summarized below.

$$\frac{n}{n-1}\sum_{ij} x_i\, x_j \pi_{ij} = \frac{n}{n-1}\sum_{i=2}^{n}\sum_{j=1}^{i-1} 2x_i\, x_j \pi_{ij}$$

Where $x_i$ and $x_j$ are the frequencies of the ith and jth sequence, and $\pi_{ij}$ refers to the proportion of sites with differences. However, for the output of Inia, per-site π is given. Thus this serves as a measurement of heterozygosity per site.

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As a demonstrating for 2504 individuals (5008 sequences), three SNPs are found across a 30 nucleotide stretch of sequences that have MAF = (0.4, 0.3, 0.2).

SNP 1 $\frac{2(5008)}{(5008-1)}0.4(1-0.4) = 0.480$

SNP 2 $\frac{2(5008)}{(5008-1)}0.3(1-0.3) = 0.420$

SNP 3 $\frac{2(5008)}{(5008-1)}0.2(1-0.2) = 0.320$

$\pi = \frac{0.480+0.420+0.320}{30} = 0.04667$

$F_{ST}$ is obtained using vcftools' implementation of Weir-Cockerham's $F_{ST}$ calculation (Danecek et al., 2011; H. Li, 2011a)(Weir & Cockerham, 1984). The calculation of $F_{ST}$ briefly is calculated using the following equation

$$a = \frac{\overline{n}}{n_c}\left\{s^2 - \frac{1}{n-1}\left[\overline{p}(1-\overline{p}) - \frac{r-1}{r}s^2 - \frac{1}{4}\overline{h}\right]\right\}$$

$$b = \frac{\overline{n}}{\overline{n}-1}\left[\overline{p}(1-\overline{p}) - \frac{r-1}{r}s^2 - \frac{2\overline{n}-1}{4\overline{n}}\overline{h}\right]$$

$$c = \frac{1}{2}\overline{h}$$

where:

$$\overline{n} = \sum_i n_i / r$$

$$n_c = \frac{r\overline{n} - \sum_i {n_i}^2 / r\overline{n}}{r-1}$$

$$\overline{p} = \sum_i \frac{n_i \tilde{p}_i}{r\overline{n}}$$

$$s^2 = \sum_i n_i (\tilde{p}_i - \overline{p})^2 / \overline{n}(r-1)$$

$$\overline{h} = \sum_i n_i \tilde{h}_i / r\overline{n}$$

Where p is defined as the frequency of an allele and h is the proportion of heterozygotes in a sample of size n from ith population (i= 1, 2.. r). Then $F_{ST}$ can be estimated by $\hat{\theta}$ for a single locus:

$$\hat{\theta} = \frac{a}{a+b+c}$$

In the case of multiple locus Weir and Cockerham two methods are utilized to compute each $F_{ST}$ for each jth (j=1,2..L) and combined across loci by taking an average value across the number of loci examined

$$\hat{\theta} = \frac{\sum_j \frac{a_j}{a_j + b_j + c_j}}{L}$$

or computing the weighted $F_{ST}$

$$\hat{\theta} = \frac{\sum_j a_j}{\sum_j a_j + b_j + c_j}$$

Hardy-Weinberg equilibrium is calculated using the standard equations (Hartl & Clark, 2007). $F_{ST}$ is calculated at the global level with each superpopulation input as a parameter. Population files are provided by Inia.

The predicted functional effects of variants are obtained by passing the stored VCF files to Ensembl Variant Effect Predictor, which extract Ensembl annotations and computationally predicts the functional effects of non-synonymous variants using the SIFT and PolyPhen2 scoring implemented in VEP. SIFT predicts potential impact of amino acid substitution based on evolutionary conservation among sequence homologs, and physiochemical similarity between the amino acids substituted. PolyPhen-2 predicts the impact of an amino acid substitution based on conservation, sequence homology, the biochemical characteristics of the amino acid change, sequence based features of the protein, and predicted changes to the three dimensional structure of the protein. Both tools return a quantitative and qualitative rating of the substitution. PolyPhen-2 returns a score ranging 0-1 that estimates probability the substitution is damaging and qualitatively rates the variant based on the probability of a false positive rating from the classifier model. For the Ensembl Polyphen2 annotations the cutoffs are 0-0.446 (benign), 0.446-0.908 (possibly damaging), and 0.908 (probably damaging). SIFT returns a score ranging from 0-1 normalized probability that the amino acid substitution would be encountered in that position where SIFT < 0.05 is qualitatively categorized as deleterious and SIFT > 0.05 is categorized as tolerated. These data are merged into a single output file and stored on the local machine.

As a final step, Inia removes all temporary files, preserving only the output table (1 file per gene in the gene map) and the extracted vcf files containing the 1000GP haplotypes.

## Usage

The general usage of Inia is documented below:

```
Inia.R [options]

--input -i Gene Coordinates csv

--Fst -F Population File

--out -o Directory for output
```

An example of such a command is:

```
Inia.R -i TAS2RMAP.csv -F ~/Inia/Populations.csv -o ~/Output/
```

Inia was tested on a mid-2012 MacBook Pro with 8GB of DDR3 1600MHz RAM, 2.5 GHz Intel Core i5 dual-core CPU, and running R-3.3.3 and MacOS Mojave. Subsequent test were conducted on M1 MacBook Air running MacOS Ventura and PC running Ubuntu.

## Analysis

Gene coordinates for *OTOP1* were retrieved from the UCSC genome browser for GRCh37. Transcript coordinates were received from the Biomart and confirmed in the Ensembl browser. Post-processing of Inia output files was conducted in the R environment.

## Results

As a demonstration I have queried a local genomic region using Inia. The input file and output files can be found in the Supplementary Files. I specified coordinates for the sour taste receptor gene *OTOP1*.

In *OTOP1* a total of 1379 variants were present, however removing sites that were monomorphic (AF=1 or AF=0) and filtering for biallelic sites only 1360 remained. Interestingly only 78 of the 1360 variants catalogued occurred in exons, and 21 of these variant sites were synonymous substitutions. Of the remaining variants there are 3 nonsense variants encoding premature stop codons, 1 in-frame deletion, 1 frameshift deletion, 1 variant predicted to affect splicing, and 51 missense variants. 26 variants were predicted to be damaging by either SIFT or PolyPhen-2; 2 by only SIFT, 3 by only PolyPhen-2, and 21 by both tools. In total, 33 variants have high potential the protein coding sequence of *OTOP1*. A total of 5 of these variants have a minor allele frequency > 1% in any of the five super populations present in the 1000 genomes project. Of these 5 variants, 1 is an in-frame deletion are 4 are missense substitutions. Three of these missense substitutions were predicted by both SIFT and Polyphen-2 to be damaging to protein function; rs145781170, rs201894404, and rs556627325. See **Table 2.1** for a summary of these variants. The in-frame deletion was absent in Eastern Asian populations (0%) but present at low to moderate frequency among other super populations (4.5-15.85%). In the most common potentially functional SNP, rs145781170, heterozygosity ($\pi$) and differentiation were measured at $\pi$=3.03% and $F_{ST}$=0.11. The allele was to be most common among the Americas (maf=10.66%) and virtually absent in other global populations (maf<0.2%).

**Table 3.1 Putatively High Impact Variants in OTOP1**

| Variant ID | Position | Protein Position | Amino Acid | Exon | Variant Type | SIFT | PolyPhen-2 |
|---|---|---|---|---|---|---|---|
| rs188805040 | 4:41900536 | 611/612 | K/N | 6 | missense variant | deleterious (0.02) | possibly damaging (0.783) |
| rs149188671 | 4:41900586 | 595/612 | I/L | 6 | missense variant | tolerated (0.41) | benign (0.09) |
| rs568991103 | 4:41900619 | 584/612 | I/L | 6 | missense variant | deleterious (0.02) | possibly damaging (0.815) |
| rs538042112 | 4:41900625 | 582/612 | P/T | 6 | missense variant | tolerated (0.37) | probably damaging (0.976) |
| rs554408396 | 4:41900672 | 566/612 | R/Q | 6 | missense variant | deleterious (0) | probably damaging (0.977) |
| rs145781170 | 4:41908907 | 552/612 | L/F | 5 | missense variant | deleterious (0) | probably damaging (0.984) |
| rs112623841 | 4:41908948 | 536-538/612 | GNA/A | 5 | inframe deletion | - | - |
| rs547040796 | 4:41908949 | 538/612 | A/T | 5 | missense variant | tolerated (0.57) | benign (0.011) |
| rs566824701 | 4:41908969 | 531/612 | P/L | 5 | missense variant | tolerated (0.2) | benign (0.003) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| rs534646106 | 4:41908975 | 529/612 | R/H | 5 | missense variant | tolerated (0.14) | benign (0.003) |
| rs199964022 | 4:41909000 | 521/612 | W/R | 5 | missense variant | tolerated (0.48) | benign (0) |
| rs142378612 | 4:41909037 | 508/612 | S/R | 5 | missense variant | tolerated (0.52) | benign (0) |
| rs536856664 | 4:41909054 | 503/612 | V/M | 5 | missense variant | tolerated (0.45) | benign (0.006) |
| rs34666677 | 4:41909083 | 493/612 | K/T | 5 | missense variant | tolerated (0.56) | benign (0.015) |
| rs148773760 | 4:41909215 | 449/612 | R/Q | 5 | missense variant | tolerated (0.05) | benign (0.192) |
| rs11736799 | 4:41909261 | 434/612 | V/M | 5 | missense variant | tolerated (0.06) | possibly damaging (0.536) |
| rs200557787 | 4:41909264 | 433/612 | I/F | 5 | missense variant | deleterious (0) | probably damaging (0.93) |
| rs142612164 | 4:41909266 | 432/612 | A/V | 5 | missense variant | tolerated (1) | benign (0.003) |
| rs151007916 | 4:41909302 | 420/612 | R/P | 5 | missense variant | tolerated (1) | benign (0) |
| rs199980035 | 4:41909311 | 417/612 | G/D | 5 | missense variant | tolerated (0.41) | benign (0.003) |
| rs561441189 | 4:41909365 | 399/612 | S/W | 5 | missense variant | deleterious (0.01) | probably damaging (0.945) |
| rs367576928 | 4:41909402 | 387/612 | R/C | 5 | missense variant | deleterious (0) | probably damaging (0.986) |
| rs547101628 | 4:41909404 | 386/612 | A/V | 5 | missense variant | deleterious (0) | possibly damaging (0.857) |
| rs566782000 | 4:41909435 | 376/612 | E/* | 5 | stop gained | - | - |
| rs552247079 | 4:41909480 | 361/612 | M/L | 5 | missense variant | deleterious (0) | probably damaging (0.994) |
| rs138644838 | 4:41909514 | 349/612 | M/I | 5 | missense variant | tolerated (0.08) | benign (0.076) |
| rs536918492 | 4:41909515 | 349/612 | M/T | 5 | missense variant | tolerated (0.1) | benign (0.025) |
| rs145321134 | 4:41909549 | 338/612 | R/C | 5 | missense variant | tolerated (0.06) | benign (0.042) |
| rs137952827 | 4:41909575 | 329/612 | V/G | 5 | missense variant | deleterious (0) | probably damaging (0.956) |
| rs140788465 | 4:41909600 | 321/612 | V/M | 5 | missense variant | deleterious (0.03) | probably damaging (0.934) |
| rs561501386 | 4:41909618 | 315/612 | A/T | 5 | missense variant | tolerated (0.97) | benign (0.001) |
| rs115843191 | 4:41909624 | 313/612 | V/L | 5 | missense variant | tolerated (0.82) | benign (0.007) |
| rs201019958 | 4:41909625 | 312/612 | M/I | 5 | missense variant | tolerated (0.65) | benign (0.009) |
| rs2916414 | 4:41909634 | 309/612 | D/E | 5 | missense variant | tolerated (0.96) | benign (0.076) |
| rs552563487 | 4:41909733 | 276/612 | E/D | 5 | missense variant | deleterious (0.01) | probably damaging (0.989) |
| rs530747449 | 4:41909754 | 269/612 | Y/* | 5 | stop gained | - | - |
| rs536026630 | 4:41909812 | 250/612 | P/L | 5 | missense variant | deleterious (0.02) | probably damaging (0.973) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| rs28394859 | 4:4204184 | 241/612 | I/V | 4 | missense variant | tolerated (0.16) | benign (0.007) |
| rs116668089 | 4:4204:4253 | 218/612 | V/I | 4 | missense variant | tolerated (0.1) | possibly damaging (0.644) |
| rs536797077 | 4:4204:4270 | 212/612 | L/P | 4 | missense variant | deleterious (0) | probably damaging (1) |
| rs556627325 | 4:4204:4283 | 208/612 | F/I | 4 | missense variant | deleterious (0.01) | possibly damaging (0.881) |
| rs57982980 | 4:4204:4292 | 205/612 | H/D | 4 | missense variant | deleterious (0) | probably damaging (1) |
| rs148385670 | 4:4207799 | 200/612 | R/K | 3 | missense variant/ splice region variant | deleterious (0.02) | benign (0.181) |
| rs527769643 | 4:4228190 | 134/612 | R | 1 | splice region variant / synonymous variant | - | - |
| rs200694692 | 4:4228192 | 134/612 | R/S | 1 | missense variant | deleterious (0.01) | possibly damaging (0.525) |
| rs201894404 | 4:4228207 | 129/612 | G/S | 1 | missense variant | deleterious (0.02) | probably damaging (0.911) |
| rs545416749 | 4:4228209 | 128/612 | A/X | 1 | frameshift variant | - | - |
| rs371584407 | 4:4228251 | 114/612 | S/T | 1 | missense variant | tolerated (0.43) | benign (0.212) |
| rs569097165 | 4:4228264 | 110/612 | Y/D | 1 | missense variant | deleterious (0) | probably damaging (0.998) |
| rs531426133 | 4:4228265 | 109/612 | W/* | 1 | stop gained | - | - |
| rs548341243 | 4:4228297 | 99/612 | L/F | 1 | missense variant | deleterious (0) | probably damaging (0.996) |
| rs568093695 | 4:4228377 | 72/612 | A/E | 1 | missense variant | deleterious (0.04) | possibly damaging (0.878) |
| rs553397205 | 4:4228459 | 45/612 | R/W | 1 | missense variant | deleterious (0.01) | possibly damaging (0.639) |
| rs2980146 | 4:4228464 | 43/612 | A/V | 1 | missense variant | tolerated (0.09) | benign (0) |
| rs556462448 | 4:4228467 | 42/612 | P/L | 1 | missense variant | deleterious (0.03) | benign (0.007) |
| rs576654018 | 4:4228468 | 42/612 | P/S | 1 | missense variant | tolerated (0.32) | benign (0.132) |
| rs201197069 | 4:4228513 | 27/612 | A/S | 1 | missense variant | tolerated low confidence (0.73) | benign (0.007) |

## Conclusion and Future Considerations

Modern genetic studies have generated hundreds of thousands of associations to simple and complex traits. Similarly in evolutionary studies, identification for loci which

may deviate from the assumption of neutrality may identify loci of interest. However, it is often that case that the variant(s) responsible are ambiguous. One strategy to prioritize candidate variants is through computational or so-called “in silico” predictions of variants on function.

Public genomic data contain an abundance of information valuable for dissecting the underpinnings of human traits and diseases, but it can be difficult to extract due to the large size of datasets and the challenge of integrating different sources of information. Inia addresses these barriers by generating integrated datasets obtained by querying major repositories, extracting and collating raw data, and calculating key statistics for any genomic region of interest. Further, Inia’s ease of use as a simple command line tools with minimal input make it a user-friendly method for extracting and annotating data from the 1000 genomes project that can be utilized by users with little to no background in bioinformatics or computational specialization.

I have demonstrated the functionality of Inia to explore and summarize variation in genome through examination of taste receptor genes. I have examined variation the sour taste receptor candidate gene *OTOP1.* I have summarized the presence of variation in both genes with potential functional effects. In *OTOP1*, I have identified three individual SNPs of potential high impact, suggesting these variants may shape phenotypic variance observed in sour taste sensation. In general, the presence of missense variation in *OTOP1* was limited, where most missense variants were of low frequency globally and completely missing in exon 2. The presence of *OTOP1* across a wide variety of species and low levels of varation suggest the gene is evolutionarily conserved (Tu et al., 2018). For the SNP rs145781170, a single study has referenced genetic association between the variant and the oral microbiome (de Jesus et al., 2022). Given the relevance of oral health to taste sensation, this makes rs145781170 a candidate variant for varying sensory phenotypes and future studies should consider this variant as a potential genetic marker to be tested. In all, the results from Inia have generated rich summaries of the genes examined and have provided new and relevant hypotheses.

The reach of genome research into new areas of investigation continues to broaden rapidly, and we plan ongoing development of Inia’s ability to integrate disparate databases and analyses, enhancing their accessibility and usefulness to informatics nonspecialists.

# Chapter 4 Worldwide diversity, association potential, and natural selection in the superimposed taste genes, CD36 and GNAT3


## Abstract

CD36 and GNAT3 mediate taste responses, with CD36 acting as a lipid detector and GNAT3 acting as the α subunit of gustducin, a G protein governing sweet, savory, and bitter transduction. Strikingly, the genes encoding CD36 and GNAT3 are genomically superimposed, with *CD36* completely encompassing *GNAT3*. To characterize genetic variation across the *CD36-GNAT3* region, its implications for phenotypic diversity, and its recent evolution, we analyzed from ~2,500 worldwide subjects sequenced by the 1000 Genomes Project (1000GP). *CD36-GNAT3* harbored extensive diversity including 8,688 single-nucleotide polymorphisms (SNPs), 414 indels, and other complex variants. Sliding window analyses revealed that nucleotide diversity and population differentiation across *CD36-GNAT3* were consistent with genome-wide trends in the 1000GP ($\pi$ = 0.10%, $P$ = 0.64; $F_{ST}$ = 9.0%, $P$ = 0.57). In addition, functional predictions using SIFT and PolyPhen-2 identified 60 variants likely to alter protein function and they were in weak linkage disequilibrium ($r^2$ < 0.17), suggesting their effects are largely independent. However, the frequencies of predicted functional variants were low ($\bar{p}$ = 0.0013), indicating their contributions to phenotypic variance on population scales are limited. Tests using Tajima's *D* statistic revealed that pressures from natural selection have been relaxed across most of *CD36-GNAT3* during its recent history (0.39 < $P$ < 0.67). However, *CD36* exons showed signs of local adaptation consistent with prior reports ($P$<0.035). Thus, *CD36* and *GNAT3* harbor numerous variants predicted to affect taste sensitivity, but most are rare and phenotypic variance on a population level is likely mediated by a small number of sites.


## Introduction

Taste perception is a fundamental mechanism of diet selection and control. By allowing animals to evaluate the nutritional properties and safety of foods before they are consumed, taste provides a powerful means of enhancing health and evolutionary fitness (Lindemann, 2001; Reed & Knaapila, 2010; Roper & Chaudhari, 2017). For instance bitter sensations, which are triggered by plant toxins, signal the presence of noxious components, allowing avoidance. Sweet sensations, which are triggered by sugars, signal carbohydrate richness. Salty, sour, and umami/savory sensations signal the presence of electrolytes, acidity indicative of ripeness, and protein content. Together these modalities provide a nutrient profile that can be used to guide intake, a major foraging advantage. The significance of this role is evident in the diversity of taste receptors found throughout (Antinucci & Risso, 2017; Baldwin et al., 2014; Behrens et al., 2021; Feng et al., 2014;

Fischer et al., 2005; Jiang et al., 2012; D. Li & Zhang, 2014; S. Wooding, 2011; S. Wooding et al., 2006; H. Zhao et al., 2015; H. Zhao, Zhou, et al., 2010).

A key feature of taste perception in humans is that it varies due to polymorphism in genes encoding receptors and other signaling components (Bachmanov et al., 2014; U.-K. Kim et al., 2004)). For example, *TAS2R38*, which encodes a bitter receptor, harbors alleles associated with taste responses to goitrin, a thyroid toxin synthesized by plants in the Brassicaceae family (S. Wooding et al., 2010). Similar associations are found between variants in *TAS1R3* (an umami receptor subunit) and monosodium glutamate, and between *CA6* variants (a salivary carbonic anhydrase) and sodium salt (Chen et al., 2009; Feeney & Hayes, 2014). Polymorphism in taste pathways also associates with preferences and consumption of foods such as alcoholic beverages and cruciferous vegetables, as well as health measures such as body mass index, susceptibility to colorectal cancers, and kidney disease (Allen et al., 2014; Barontini et al., 2017; Basson et al., 2005; Behrens et al., 2013; Choi et al., 2016; Greene, 1974; Hayes et al., 2015; S. P. Wooding et al., 2012). These affect evolutionary fitness, and taste genes in humans harbor signatures of natural selection including evidence of local adaptation, balancing pressures, and purifying effects (Campbell et al., 2012, 2014; Drayna, 2005; U. Kim et al., 2005, 2006; D. Risso et al., 2018; D. S. Risso et al., 2016; S. Wooding et al., 2004b). Thus, modern patterns of variation in taste sensitivity, nutrition, and health reflect ancient evolutionary influences on taste.

Mounting evidence suggests that human taste abilities extend to the detection of fats, particularly long chain fatty acids (LCFAs), and that fat taste sensitivity varies from person to person as the result of genetic polymorphism. In psychophysical assays, subjects are capable of discriminating fat content in controlled preparations even when non-gustatory cues are masked, supporting a role for taste (Cartoni et al., 2010; Chale-Rush et al., 2007; Mattes, 2011). In addition, like other taste signals, neural signals generated by oral fat exposure originate in taste receptor cells and travel via the chorda tympani and glossopharyngeal nerves in mice (Gaillard et al., 2007). Oral fat exposure also activates the brain's insular cortex, which is activated during sweet perception (De Araujo & Rolls, 2004). Several lines of evidence indicate that CD36, a fatty acid translocase, is the receptor accounting for these effects. It localizes to taste receptor cells, natively responds to fatty acids in vitro, and knockout of *CD36* in rats and mice alters their preferences for fat-containing solutions and foods (Laugerette et al., 2005). *CD36* also harbors alleles associated with both orosensory detection of fats and preferences for them (Keller et al., 2012; Pepino et al., 2014). In addition, CD36 plays known roles beyond taste, contributing to immune system function, lipid metabolism, and cell adhesion (Pepino et al., 2014) These findings raise questions about the extent of genetic polymorphism at CD36 and its effects on fat perception and other phenotypes.

The potential contributions of CD36 to fat taste also raise evolutionary questions. The high nutritional value of lipids, which are calorically rich but environmentally scarce, suggests that CD36's role as a taste sensor placed it under selective pressures in the course of human evolution. In particular, humans' population expansion and migration out of Africa 50-60 thousand years ago introduced them to new physical and nutritional

environments that likely altered the advantages of fat perception and metabolism. For instance, they may have been shaped by factors such as the accessibility of fats when hunting and foraging, or climate, which poses thermoregulatory challenges. They could also have arisen from CD36's non-gustatory roles in processes such as cell adhesion, which makes it vulnerable to exploitation by pathogens (Silverstein & Febbraio, 2009). Such pressures leave signatures in genetic diversity including effects on allele frequencies and population differentiation (Bamshad & Wooding, 2003). Thus, modern patterns of diversity in *CD36* may provide clues to the evolutionary factors driving responses to fats.

Strikingly, *CD36* genomically encompasses a second gene participating in taste perception*, GNAT3* (**Figure 4.1**). *GNAT3* encodes a G-protein subunit mediating detection of sweet, savory, and bitter substances and, like CD36, harbors variants associated with taste sensitivity (Farook et al., 2012; Fushan et al., 2010; Pepino et al., 2012). GNAT3 plays non-gustatory roles as well, such as the detection of foreign compounds in the gut and airways (Deshpande et al., 2010b; Egan & Margolskee, 2008). The nested arrangement of the two genes suggests that patterns of diversity in them may be correlated due to their genetic linkage and shared evolutionary histories. If so, GNAT3-mediated taste responses (bitter, sweet, and umami) and CD36-mediated responses (fat) may be correlated. However, the *CD36-GNAT3* region is sufficiently large (~305kb) that linkage disequilibrium may not be high across its entirety. Linkage disequilibrium can also be shaped by natural selection, which can affect its range and magnitude. Establishing the structure of genetic variation across *CD36-GNAT3* has the potential to reveal the extent of such effects.

We addressed these issues in a population genetic analysis of *CD36-GNAT3* in >2,500 subjects from the 1000 Genomes Project (1000GP) (H. Li, 2011b; The 1000 Genomes Project Consortium, 2015). To establish the extent of diversity at *CD36-GNAT3* and its implications for genotype-phenotype associations, we comprehensively identified variable sites in the region, their allele frequencies in worldwide populations, and their linkage structure. We then used computational prediction to detect sites likely to have functional effects, and evolutionary analyses to determine the role of natural selection in shaping this variability. Our results shed light on the architecture of diversity in *CD36* and *GNAT3*, its potential contributions to taste and metabolism, and its evolutionary origin

**Table 4.1. Population Sample**

| Super Population | Population |
|---|---|
| Africa (N = 661) | African Caribbeans in Barbados (N = 96) |
| | Americans of African Ancestry in SW USA (N = 61) |
| | Esan in Nigeria (N = 99) |
| | Gambian in Western Divisions in the Gambia (N = 113) |
| | Luhya in Webuye, Kenya (N = 99) |
| | Mende in Sierra Leone (N = 85) |
| | Yoruba in Ibadan, Nigeria (N = 108) |
| Americas (N = 347) | Colombians from Medellin, Colombia (N = 94) |
| | Mexican Ancestry from Los Angeles, USA (N = 64) |
| | Peruvian from Lima, Peru (N = 85) |
| | Puerto Rican in Puerto Rico (N = 104) |
| East Asia (N = 504) | Chinese Dai in Xishuangbanna, China (N = 93) |
| | Han Chinese in Beijing, China (N = 103) |
| | Japanese in Toyko, Japan (N = 104) |
| | Kinh in Ho Chi Minh City, Vietnam (N = 99) |
| | Southern Han Chinese (N = 105) |
| Europe (N = 503) | British in England and Scotland (N = 91) |
| | Finnish in Finland (N = 99) |
| | Iberian population in Spain (N = 107) |
| | Toscani in Italia (N = 107) |
| | Utah residents (CEPH) with European ancestry (N = 99) |
| South Asia (N = 489) | Bengali from Bangladesh (N = 86) |
| | Gujarati Indian from Houston, TX (N = 103) |
| | Indian Telegu from the UK (N = 102) |
| | Punjabi from Lahore, Pakistan (N = 96) |
| | Sri Lankan Tamil from the UK (N = 102) |

## Methods

We examined genetic variation across *CD36-GNAT3* in 2504 subjects included in Phase 3 of the 1000 Genomes Project (1000GP) (The 1000 Genomes Project Consortium, 2015). The 1000GP subjects comprise a random, demographically representative sample of 26 worldwide populations in five superpopulations, providing a diverse hierarchical perspective on human genetic variation (**Table 4.1**)

The genomic structure of the *CD36-GNAT3* region was determined from the Ensembl GRch37 human genome assembly, the reference for the 1000GP. These placed CD36 (Ensembl ENSG00000135218, ENST00000435819) at position 7:79998891-7:80308593 (~305kb) and GNAT3 (ENSG00000214415, ENST00000398291) at position 7:80087987-7:80141336 (53kb), with GNAT3 located in introns 1 and 2 of *CD36*. Data for the region were extracted from 1000GP databases in variant call format (vcf) using the Tabix software package (H. Li, 2011b)

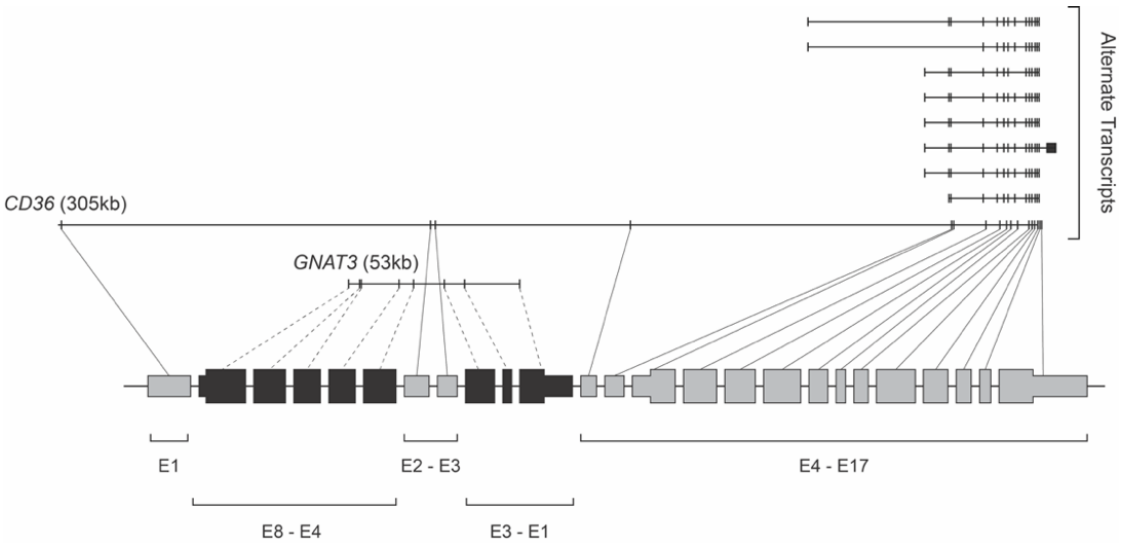


**Figure 4.1 Genomic organization of CD36 and GNAT3**: The *CD36-GNAT3* region is ~305 kb in length. *GNAT3* is nested within *CD36,* with exons 1–3 located in *CD36* intron 3 and exons 4–8 located in CD36 exon 1.

Genetic variation was assessed with respect to three factors, allelic polymorphism, population substructure, and linkage disequilibrium. Nucleotide diversity (π), the mean pairwise nucleotide difference among sequences normalized to sequence length, was calculated across CD36-GNAT3 as well as separately for *CD36* exons, *CD36* introns, *GNAT3* exons, and *GNAT3* introns (Tajima, 1983) Population substructure was measured within and across 1000GP superpopulations using Weir and Hill's weighted $F_{ST}$ (Weir & Hill, 2002). These calculations were performed using vcftools and the R packages PopGenome, pegas, hierfstat, and adgenet (Danecek et al., 2011; GOUDET, 2005; Jombart & Ahmed, 2011; Paradis, 2010; Pfeifer et al., 2014). Linkage disequilibrium (LD) was assessed for variants with frequencies >0.05 using two measures, D' and $r^2$ (Mueller, 2004; Slatkin, 2008). D', a measure of correlation between sites relative to the maximum possible given their allele frequencies, was used to determine the extent to which recombination has shaped diversity across the region. A raw measure of correlation among genetic markers, $r^2$, was used to determine the extent to which sites are expected to exhibit similar genotype-phenotype associations. These measures were calculated and visualized using the VariantAnnotation and Ldheatmap packages in the R statistical analysis environment and Bioconductor library (Gentleman et al., 2004; Ihaka & Gentleman, 1996; Obenchain et al., 2014; Shin et al., 2006).

Two algorithms were used to predict the potential functional impact of exon variants, PolyPhen-2 and SIFT (Adzhubei et al., 2010; Kumar et al., 2009). PolyPhen-2 predicts the impact of amino acid changes on protein function on the basis of the location of the changed site within the protein structure, level of conservation relative to homologous genes, and the biochemical characteristics of the substituted amino acids. It denotes the impact of substitutions on a scale from 0.0 (benign) to 1.0 (damaging). SIFT

predicts whether amino acid substitutions affect protein function on the basis of probabilities estimated from gene homologues. It denotes impact on a scale from 0.0 to 1.0, categorizing scores below 0.05 as deleterious and higher scores as tolerated. Both scores were obtained using the Variant Effect Predictor (VEP) software package (McLaren et al., 2016). Regulatory variants were identified by using VEP to query the Ensembl Regulatory Build.

Tests for natural selection were performed using Tajima's D statistic (Tajima, 1989). D compares the number of variable sites and mean nucleotide difference between alleles in a sample, which are differentially affected by selective processes. It is designed to test for selective effects nonrecombining genomic regions, but is applicable with elevated conservativeness to regions with recombination. These analyses were performed using the PopGenome R package. As with pi estimates, these were calculated both overall and with respect to *CD36* and *GNAT3* exons and introns.

Because the vast majority of the human genome (>98%) is noncoding it is can be assumed to be evolving neutrally or nearly so with respect to natural selection. Therefore, to provide a neutral baseline for evaluating diversity measures in the CD36-GNAT3 we generated empirical distributions for three measures ($\pi$, $F_{ST}$, and D) using sliding window analyses. These were obtained by iteratively calculating each measure in ~270,000 adjacent 10kb windows spanning the length of the 1000GP genomes, excluding known unstable and repetitive regions such as telomeres. The probability of the observed values given genome wide trends was then determined by comparing the values observed in *CD36-GNAT3* with their genome-wide distributions. We denoted P-values from these empirical tests $P_E$ to distinguish them from P values obtained in parametric tests.

## Results

The *CD36-GNAT3* region harbored extensive variation . A total of 9111 polymorphic sites were identified. The majority (95.3%) were SNPs (8653 biallelic and 32 multiallelic). A smaller number (4.5%) were insertion/deletion (indel) polymorphisms (390 biallelic and 21 multiallelic). The remainder (<0.2%) were rare complex variants, including three sites with both SNP and indel alleles, 11 copy number variants (CNVs), and one Alu insertion. *CD36* exons, which totaled 2365bp in length, contained 112 SNPs (all biallelic) and 8 indels, which occurred in 7 exons (**Figure 4.2**). Four of the eight were frameshift deletions, two were 1bp insertions in untranslated regions, and two were in-frame deletions. *GNAT3* exons, which totaled 1159bp in length, contained 28 SNPs (all biallelic), with one indel in the 5' untranslated region of exon 1. The number of variants also differed among exons. No polymorphism was found in *GNAT3* exon 2, 17 SNPS were present in *CD36* exon 17, and the mean across exons was 6. The number of SNPs per nucleotide ranged from 0.0 (GNAT3 exon 2) to 0.1 (CD36 exon 12), with an average of 0.037.

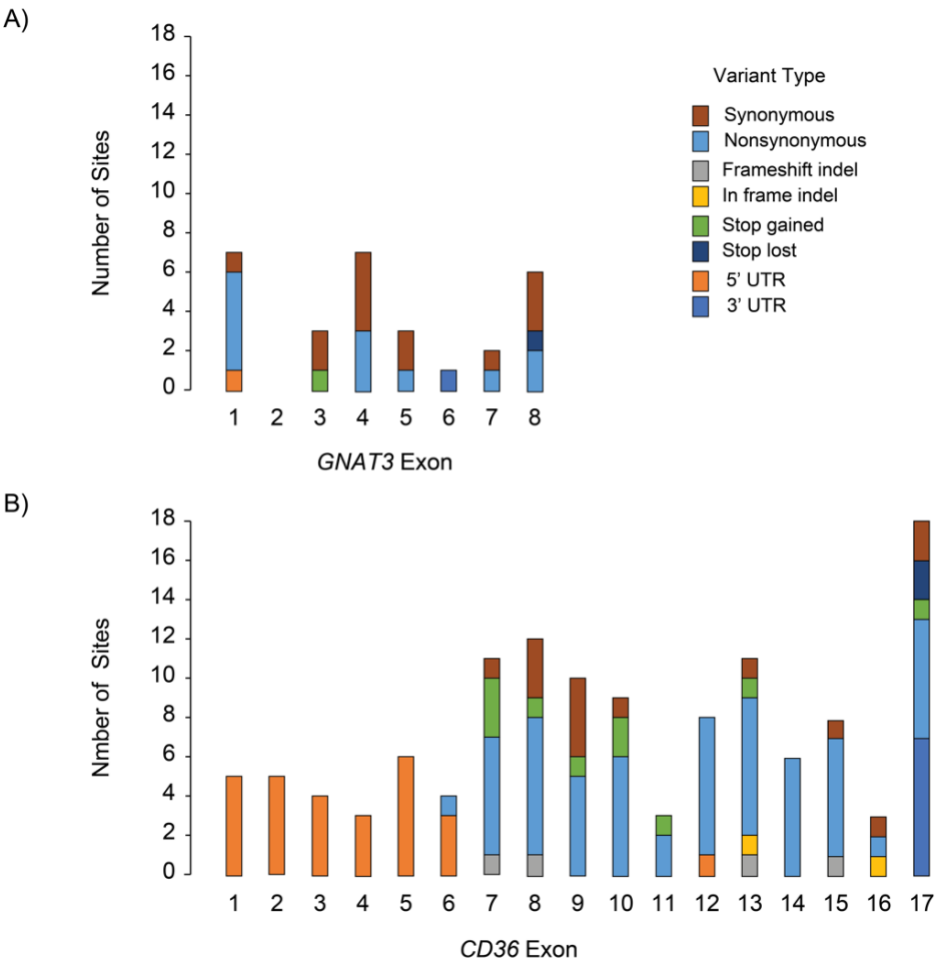


**Figure 4.2 Variant types and their frequencies across CD36 and GNAT3 exons**: Both *GNAT3* and *CD36* harbored extensive variation, including numerous variants likely to affect function.

Among the 140 SNPs in *CD36* and *GNAT3* exons, PolyPhen and SIFT detected 57 likely to alter function in the CD36 and GNAT3 proteins. Fifty-one had PolyPhen scores of Possibly or Probably Damaging and 51 had SIFT scores of Deleterious. The scores were largely in agreement between the two measures. Forty variants were scored as Possibly/Probably Damaging by PolyPhen and Deleterious by SIFT, and a further 15 were sites scored as Benign by PolyPhen and Tolerated by SIFT. Predictions disagreed for 12 sites, with 6 scored as tolerated by SIFT but Possibly/Probably Damaging by PolyPhen, and 6 scored as Deleterious by SIFT but tolerated by PolyPhen. Sites without SIFT and PolyPhen scores but likely to have functional impact were also found. Eighteen variants in *CD36* were indels causing frame shifts (4 sites) stop gains (10 sites), stop losses (2 sites), or in-frame deletions (2 sites). *GNAT3* harbored one stop gain (in exon 3) and one stop loss (in exon 8). For further analyses, the 60 sites with variants scored as possibly damaging by PolyPhen and Deleterious by SIFT, frameshifts, and those occurring in start

or stop codons were denoted putatively high impact (PHI) sites, and their derived alleles denoted PHI alleles (**Table 4.2**).

### Table 4.2 Putative high impact (PHI) sites

| Gene | rsid | Exon | Variant Type | Reference Codon | Alternate Codon | Reference Amino Acid | Alternate Amino Acid |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| *GNAT3* | rs533524866 | 1 | Ns | aCc | aAc | T | N |
| *GNAT3* | rs573082324 | 3 | Sg | tTg | tAg | L | Stop |
| *GNAT3* | rs200010494 | 4 | Ns | Gat | Aat | D | N |
| *GNAT3* | rs571120313 | 4 | Ns | cTg | cCg | L | P |
| *GNAT3* | rs570030158 | 5 | Ns | ttG | ttT | L | F |
| *GNAT3* | rs186877232 | 8 | Sl | Taa | Caa | Stop | Q |
| *GNAT3* | rs534902139 | 8 | Ns | Ttc | Gtc | F | V |
| *CD36* | rs559876270 | 6 | Ns | gGg | gAg | G | E |
| *CD36* | rs75326924 | 7 | Ns | Cct | Tct | P | S |
| *CD36* | rs139067066 | 7 | Sg | tgG | tgA | W | Stop |
| *CD36* | rs150037612 | 7 | Ns | aCg | aTg | T | M |
| *CD36* | rs534577878 | 7 | Ns | tgG | tgC | W | C |
| *CD36* | rs545489204 | 7 | Sg | Cag | Tag | Q | Stop |
| *CD36* | rs556181210 | 7 | Ns | aTc | aAc | I | N |
| *CD36* | rs571975065 | 7 | Fs | Aaa | aa | K | na |
| *CD36* | rs574416705 | 7 | Sg | taC | taG | Y | Stop |
| *CD36* | rs70961715 | 8 | Ns | cGt | cCt | R | P |
| *CD36* | rs201765331 | 8 | Ns | tCa | tTa | S | L |
| *CD36* | rs548507859 | 8 | Ns | Tca | Cca | S | P |
| *CD36* | rs556438655 | 8 | Sg | Gaa | Taa | E | Stop |
| *CD36* | rs563097847 | 8 | Ns | Ctc | Ttc | L | F |
| *CD36* | rs572295823 | 8 | Fs | aAC | a | N | na |
| *CD36* | rs201759307 | 9 | Ns | Tgg | Cgg | W | R |
| *CD36* | rs568503917 | 9 | Ns | gGc | gTc | G | V |
| *CD36* | rs569959776 | 9 | Sg | taT | taG | Y | Stop |
| *CD36* | rs35776095 | 10 | Ns | gGa | gAa | G | E |
| *CD36* | rs373829578 | 10 | Sg | Aaa | Taa | K | Stop |
| *CD36* | rs200067322 | 10 | Ns | Gga | Aga | G | R |
| *CD36* | rs535150936 | 10 | Ns | gGt | gTt | G | V |
| *CD36* | rs201245766 | 10 | Sg | agg | agGTAAg | R | Stop |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *CD36* | rs149178142 | 11 | Ns | aCa | aTa | T | I |
| *CD36* | rs149985988 | 11 | Sg | tgC | tgA | C | Stop |
| *CD36* | rs557732736 | 11 | Ns | aTt | aAt | I | N |
| *CD36* | rs142186404 | 12 | Ns | Ttt | Gtt | F | V |
| *CD36* | rs145908803 | 12 | Ns | cCa | cTa | P | L |
| *CD36* | rs199681631 | 12 | Ns | aGg | aCg | R | T |
| *CD36* | rs201155452 | 12 | Ns | cCt | cAt | P | H |
| *CD36* | rs535549168 | 12 | Ns | tTg | tCg | L | S |
| *CD36* | rs3211938 | 13 | Sg | taT | taG | Y | Stop |
| *CD36* | rs200757788 | 13 | Ns | aGa | aTa | R | I |
| *CD36* | rs554019170 | 13 | Ns | gAc | gGc | D | G |
| *CD36* | rs558115067 | 13 | Fs | ctG | ct | L | na |
| *CD36* | rs567491856 | 13 | Ns | tGt | tTt | C | F |
| *CD36* | rs571553184 | 13 | If | aAAGaa | aaa | KE | K |
| *CD36* | rs147903735 | 14 | Ns | Cat | Tat | H | Y |
| *CD36* | rs370701210 | 14 | Ns | Gca | Cca | A | P |
| *CD36* | rs371884082 | 14 | Ns | cAt | cGt | H | R |
| *CD36* | rs376311045 | 14 | Ns | Cca | Tca | P | S |
| *CD36* | rs564971571 | 14 | Ns | Cct | Tct | P | S |
| *CD36* | rs148910227 | 15 | Ns | Cgg | Tgg | R | W |
| *CD36* | rs200194486 | 15 | Ns | aCt | aGt | T | S |
| *CD36* | rs200906462 | 15 | Ns | Act | Cct | T | P |
| *CD36* | rs201355711 | 15 | Ns | cAg | cTg | Q | L |
| *CD36* | rs551607784 | 15 | Fs | Gca | ca | A | na |
| *CD36* | rs550565800 | 16 | If | taTATTGTGCCTATt | tat | YIVPI | Y |
| *CD36* | rs201558608 | 17 | Ns | Ggt | Agt | G | S |
| *CD36* | rs550163799 | 17 | Sl | Taa | Gaa | Stop | E |
| *CD36* | rs559916528 | 17 | Ns | gGt | gCt | G | A |
| *CD36* | rs563772337 | 17 | Sg | Caa | Taa | Q | Stop |
| *CD36* | rs570171917 | 17 | Sl | tAa | tCa | Stop | S |

Potential regulatory variation was also found. VEP identified 48 regulatory regions and 10 features as transcription factor binding sites, which together harbored 685 variants annotated by Ensembl as expression modifiers. One hundred and thirty-one of these were most proximal to *GNAT3* exons and 554 were most proximal to *CD36* exons. The majority (667; >95%) were SNPs, but other variant types were also present. Ten short insertions and 16 short deletions were found, along with six copy number and structural

variants ranging from ~2kb to ~130kb in length, which spanned numerous regulatory and transcription factor binding sites.

As expected given that the majority of the *CD36-GNAT3* region is intronic, most variants (7827) occurred in noncoding regions (**Table 4.3 A**). A smaller number, 686, occurred in regulatory regions. In addition, consistent with their relative lengths, *CD36* harbored more segregating sites than did *GNAT3* (112 vs 28). Derived allele frequencies at noncoding sites ranged from 0.0002 (singletons) to 0.9998 with a mean of 0.033. Consistent with the low mean, the majority of alleles were rare, with 92% having frequencies below 0.05 and 83% having frequencies below 0.01 (**Table 4.4**). Alleles with intermediate frequencies accounted for a small proportion of sites, with 4% having frequencies between 0.25 and 0.75. These patterns extended to *CD36* and *GNAT3* exons and regulatory regions, with >80% of alleles having frequencies below 0.01 in all three cases.

**Table 4.3 Genetic diversity in populations and superpopulations**. Numbers in parentheses indicate nonsynonymous variants.

A)

| ***S*** | | | | | | |
|---|---|---|---|---|---|---|
| **Site Category** | Worldwide | Africa | Americas | East Asia | Europe | South Asia |
| Noncoding | 7827 | 3885 | 2624 | 2124 | 2120 | 2443 |
| CD36 Exons | 112 (98) | 43 (38) | 22 (19) | 30 (27) | 21 (17) | 24 (19) |
| GNAT3 Exons | 28 (15) | 10 (6) | 10 (6) | 5 (2) | 6 (2) | 9 (5) |
| PHI Sites | 60 | 24 | 10 | 24 | 8 | 14 |
| Regulatory Sites | 686 | 333 | 237 | 180 | 194 | 227 |

B)

| ***π (%)*** | | | | | | |
|---|---|---|---|---|---|---|
| **Site Category** | Worldwide | Africa | Americas | East Asia | Europe | South Asia |
| Noncoding | 0.100 | 0.121 | 0.087 | 0.092 | 0.079 | 0.087 |
| CD36 Exons | 0.045 | 0.054 | 0.038 | 0.042 | 0.041 | 0.042 |
| GNAT3 Exons | 0.036 | 0.038 | 0.035 | 0.026 | 0.033 | 0.034 |
| PHI Sites | - | - | - | - | - | - |
| Regulatory Sites | - | - | - | - | - | - |

C)

| $F_{ST}$ | | | | | | |
|---|---|---|---|---|---|---|
| **Site Category** | Worldwide | Africa | Americas | East Asia | Europe | South Asia |
| Noncoding | 0.090 | 0.015 | 0.019 | 0.015 | 0.002 | 0.005 |
| CD36 Exons | 0.045 | 0.079 | 0.000 | 0.011 | 0.004 | 0.000 |
| GNAT3 Exons | 0.093 | 0.006 | 0.030 | 0.001 | 0.007 | 0.015 |
| PHI Sites | 0.066 | 0.108 | 0.000 | 0.015 | 0.000 | 0.007 |
| Regulatory Sites | 0.080 | 0.016 | 0.031 | 0.003 | 0.001 | 0.007 |

Consistent with the abundance of low frequency alleles, π across noncoding sites was low, 0.10% (**Table 4.3 B**). It had a $P_E$ of 0.64 in the sliding window analysis, indicating that is consistent with expectations given the 1000GP sample. Values observed within superpopulations were similar to π values across the sample as a whole, ranging from 0.08 in Europe to 0.12 in Africa. Little difference in diversity was observed with respect to *CD36* and *GNAT3* exons within superpopulations, with π in *CD36* ranging from 0.04% (in Europe) to 0.05% (in Africa) and π in *GNAT3* ranging from 0.03% (in East Asians) to 0.04% (in Africans). Differences in diversity between introns and exons at both GNAT3 and *CD36* were also small, with *CD36* having π values of 0.08% and 0.05% in introns and exons respectively and *GNAT3* having values of 0.07% and 0.04%.

**Table 4.4. Allele frequency quantiles**.

| **Frequency Quantile** | | | | | | |
|---|---|---|---|---|---|---|
| **Site Category** | < 0.01 | < 0.02 | < 0.03 | < 0.04 | < 0.05 | 0.05 < |
| Noncoding | 0.83 | 0.04 | 0.02 | 0.02 | 0.01 | 0.09 |
| CD36 Exon | 0.96 | 0.01 | 0.00 | 0.01 | 0.00 | 0.02 |
| GNAT3 Exon | 0.96 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 |
| PHI | 0.97 | 0.02 | 0.00 | 0.02 | 0.00 | 0.00 |
| Regulatory | 0.83 | 0.05 | 0.02 | 0.01 | 0.00 | 0.09 |

The frequencies of PHI alleles ranged from 0.0002 to 0.031 with a mean of 0.0013. Thus, while the minimum frequency of PHI alleles was identical to that across noncoding sites, the mean was substantially lower (0.0013 vs 0.0333). Nucleotide diversity could not be calculated for PHI sites or regulatory variants because the denominator in π calculations, the length of the analyzed sequence, is indeterminate because not all sites in the genome have potential to harbor PHI or regulatory variants. However, heterozygosity estimates were consistent with the distributions of allele frequencies at both noncoding and PHI sites, with 80% of noncoding and 97% of PHI sites having heterozygosities below 0.025. As with π, this pattern extended to superpopulations. Alleles scored as modifiers in regulatory regions ranged in frequency from 0.0002 to 0.970 with a mean of 0.037, and 83% having frequencies below 1%.

**Table 4.5. Neutrality Tests**

| **Region** | **Tajima's *D*** | ***P*** | ***$P_E$*** |
|---|---|---|---|
| All Sites | -1.87 | < 0.001*** | 0.608 |
| *CD36* Exons | -2.41 | < 0.001*** | 0.035* |
| *CD36* Introns | -1.86 | < 0.001*** | 0.620 |
| *GNAT3* Exons | -1.81 | < 0.001*** | 0.665 |
| *GNAT3* Introns | -2.05 | < 0.001*** | 0.390 |

Tests rejecting the null hypothesis with $P < 0.001$ are indicated by ∗∗∗. Tests rejecting the null hypothesis with $P < 0.05$ are indicated by ∗.

The overall $F_{ST}$ of *CD36-GNAT3* among superpopulations was 9.0% with a $P_E$ of 0.57 (**Table 4.3 C**). Pairwise $F_{ST}$ values ranged from a low of 1.1% (between Europe and the Americas) to a high of 13.2% (between Africa and Europe). $F_{ST}$ values among populations within superpopulations were smaller, ranging from 0.003 in Europe to 0.018 in the Americas. The $F_{ST}$ among superpopulations was lower for both PHI sites (6.6%) and regulatory sites (8.0%) than for noncoding sites (9.0%) (**Table 4.3 C**). This pattern held for pairwise $F_{ST}$s between superpopulations, in which $F_{ST}$ for noncoding variants was always higher than $F_{ST}$ for PHI and regulatory sites. However, $F_{ST}$ within superpopulations did not follow this pattern (**Table 4.3 C**). $F_{ST}$ for PHI sites was similar to or less than $F_{ST}$ for noncoding sites in four populations (Americas, East Asia, Europe, and South Asia). In contrast, $F_{ST}$ in Africa was substantially higher for *CD36* exons than for noncoding sites (7.9% vs 1.5%) and higher still for PHI sites, 10.8%.

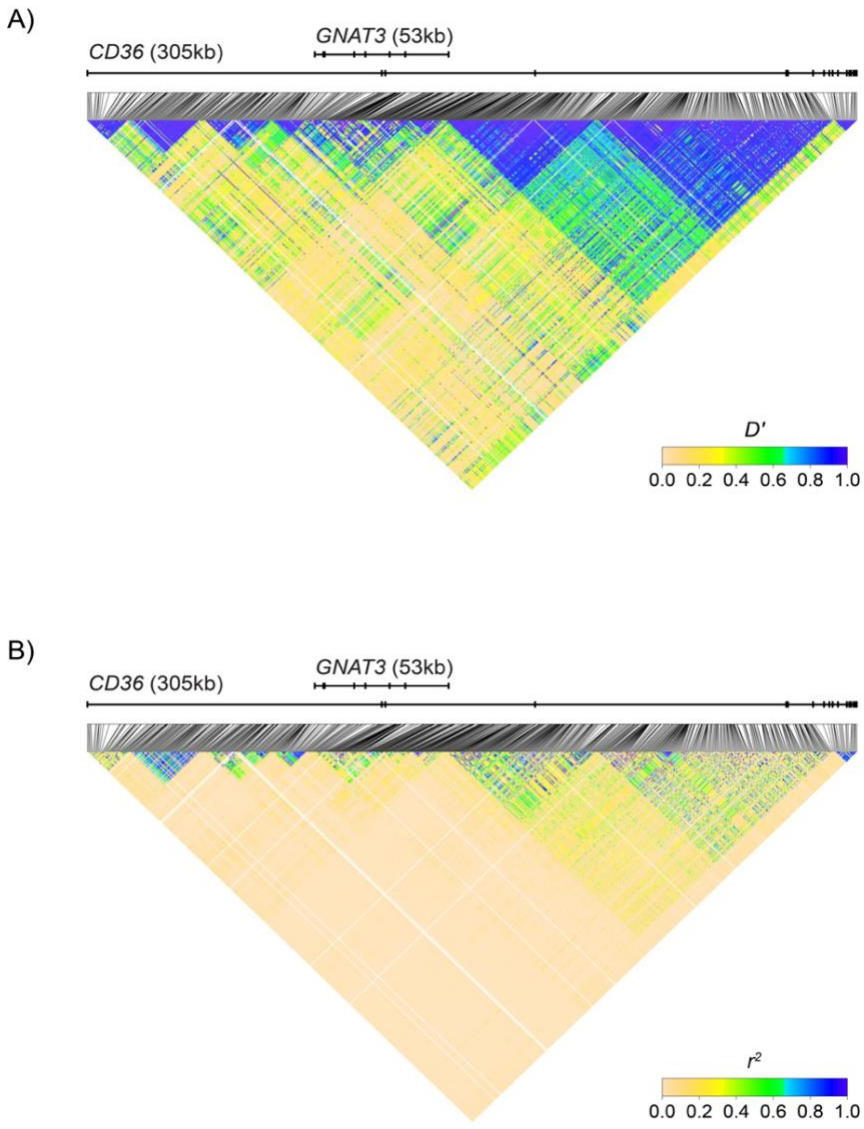


**Figure 4.3 Linkage disequilibrium across CD36-GNAT3**. (A) Pairwise *D′*. (B) Pairwise $r^2$.

D' values across *CD36-GNAT* were high (0.7-1.0) across localized regions separated by regions of lower LD (<0.5), pointing to the presence of 7 haplotype blocks ranging from ~10kb to ~100kb in length (**Figure 4.3**). A ~70kb D' block was centered on GNAT3 and spanned its full length (53kb). *CD36* exons were distributed across 5 of the 7 blocks, which contained exons 1, 2-3, 4, 5-8, and 9-17 respectively. In contrast to D', $r^2$ was low across *CD36-GNAT3* with the exception of highly localized areas. *CD36* exons 10-17 were located in a block with $r^2$ near 1.0. LD calculated among all PHI sites was consistent with patterns expected when allele frequencies are low. In general, pairwise LD measures between rare variants take on extreme values, with D' values near 1 and $r^2$ values near 0. This pattern held across all but one pair of PHI sites. No pair of PHI sites had an $r^2$ above 0.015 or D' below 0.995 with the exception of rs563097847 and rs558115067, which had an $r^2$ of 0.17.

Tajima's D values were strongly negative for the primary site categories (all sites, exons, and introns) (**Table 4.5**). However their statistical significance depended on the test used. While the standard D test rejected neutrality at a high level of significance ($P < 0.001$), comparisons with the sliding window distribution yielded different results. In the sliding window analyses, only the D value of *CD36* exons departed from expectations, and marginally so with $P_E = 0.035$. Across the other four categories $P_E$ ranged from 0.390 to 0.665, well within expectations (**Table 4.5**).

## Discussion

Contemporary human populations are the product of a rapid expansion of small ancestral populations out of Africa 50-60 thousand years ago (Bergström et al. 2021). This process is evident in diversity patterns in human genes, which harbor signatures of ancient demography and natural selection (Bamshad & Wooding, 2003; Marth et al., 2003). Most obvious on a genome-wide scale are a paucity of variation and downward skew in allele frequencies, patterns attributed to a combination of early population bottlenecks and pervasive pressure from purifying selection (Cvijović et al., 2018; Rogers, 1995). However, while prevalent, these processes can act simultaneously with others, and patterns of diversity in individual genes can reveal the evolutionary underpinnings of specific traits (Hancock & Rienzo, 2008). For instance, unexpectedly high LD and $F_{ST}$ in the lactase (*LCT*) gene indicate that positive selection favored variants conferring lactase persistence in early herding peoples, where ability to digest milk was a fitness advantage (Bersaglieri et al., 2004). Conversely, high diversity and low LD in calpain-10 (*CAPN10*) signal the presence of long-term balancing selection at a locus implicated in energy use and storage, with the explanation being that more than one allele has been selectively maintained for an extended period and LD has decayed (Vander Molen et al., 2005). The overlapping structures of *CD36* and *GNAT3* together with their roles in taste and metabolism raise questions about the extent of diversity at these loci, its potential impact on phenotypes, and the roles of demography and natural selection in shaping it.

## Worldwide diversity across CD36-GNAT3

We found that patterns of nucleotide diversity across *CD36-GNAT3* were consistent with prevailing genome-wide trends and inferences about human origins. Diversity in noncoding regions, $\pi$ = 0.10%, was well within expectations given the empirical distribution ($P_E$ = 0.64) and similar to numerous prior estimates, which vary depending on the populations sampled but are typically 0.075-0.125% (**Table 4.3 B**)(Consortium et al., 2010). Such values fall far below theoretical expectations given humans' current population size, instead agreeing with paleoanthropological and genetic evidence that ancient populations sizes were small and attained their current size relatively recently (Bergström et al., 2021; Henn et al., 2012; Osada, 2015). Nucleotide diversity within continents was similarly consistent with contemporary findings on human evolution. In particular, it was somewhat higher in Africa ($\pi$ = 0.12%) than in other superpopulations ($\bar{\pi}$ = 0.09%). This pattern is widely observed and usually ascribed to the antiquity of African populations, which have diverged over a longer period than populations founded during humans' recent expansion (Bergström et al., 2021; Campbell & Tishkoff, 2008; Henn et al., 2012; Yu et al., 2002).

Patterns of population differentiation with respect to noncoding variation in our sample were also consistent with genome-wide trends and human origins. $F_{ST}$ among superpopulations with respect to noncoding sites, 9%, was typical for values in the 1000GP ($P_E$ = 0.57) and similar to previous estimates among continents, which are typically near 10% (**Table 4.3 C**) (Holsinger & Weir, 2009). For instance, it is only slightly higher than the mean observed in HapMap phase 3 data (8.0%), which have a similar population composition (Elhaik, 2012). Pairwise $F_{ST}$ values between superpopulations were also consistent with previous estimates, varying widely depending on the continents being compared (1.1%-13.2%) (Elhaik, 2012). The mean within-superpopulation $F_{ST}$ in our sample, 1.2%, was similarly consistent with previous genome-wide estimates, such as the 1% reported in HapMap phase 3 populations by Elhaik (2012). As with $\pi$, these values reflect expectations following humans' history of population growth and dispersal, with groups near each other having more recent common ancestry and higher rates of intermigration than populations farther apart.

The presence of nonsynonymous SNPs and PHI sites in *CD36* and *GNAT3* exons suggests that functional polymorphism is present in both genes, which could affect fat taste and metabolism as well as non-gustatory phenotypes. However, patterns of diversity at PHI sites indicated that their contributions to phenotypic variance on a population scale are limited. Of the 113 coding changes we found, only 60 were scored as PHI variants. Further, the frequencies of PHI alleles were low, with only two of the 60 having frequencies above 1% and the most common having a frequency of 3% (**Table 4.4**). These patterns extended to diversity within superpopulations, with the number of PHI sites being lower than the number of nonsynonymous variants on all continents (**Table 4.3 A**). These patterns indicate that while polymorphism affecting phenotypes is almost certainly present, it is low in frequency.

$F_{ST}$s within superpopulations revealed potentially important regional trends. In particular, $F_{ST}$s were far higher in Africa than elsewhere for two site categories (**Table 4.3 C**). The $F_{ST}$ of *CD36* in Africa was 7-fold greater than in any other superpopulation (0.079 versus 0.011 in East Asia), and 5-fold greater than in noncoding sites in Africa (0.079 versus 0.015). The $F_{ST}$ of PHI sites in Africa was also 7-fold greater than in any other superpopulation (0.108 vs 0.015 in East Asia) and 5-fold greater than in noncoding sites. These patterns suggest that populations may be differentiated with respect to functional variation and, if so, it is likely most pronounced with respect to *CD36* in Africa. However, it is important to note that while patterns in $F_{ST}$ with respect to PHI sites point to the presence of phenotypic differences among populations they provide little information about the magnitude of the differences, which depend not just on allele frequencies, but on effect sizes as well.

## Signatures of natural selection

The high calorie content of fats makes them highly valuable nutritionally, so the mechanisms underlying their detection must be under strong selective pressure. This is a familiar issue in human evolutionary biology, most famously as part of Neel's "thrifty gene" hypothesis, which posits that ability to store fat provides fitness advantages (Neel 1962; Reales et al. 2017). Under the thrifty gene hypothesis, the advantages of perceiving and metabolizing fats are evident. However, its implications specifically for *CD36* and *GNAT3* are not. On the one hand, the roles of *CD36* and *GNAT3* in sensory signaling, lipid metabolism, and other processes imply that they are intolerant of novel mutations and under pressure from purifying natural selection. However, humans' diffusion out of Africa placed *CD36* and *GNAT3* into novel environments, which could have produced pressures to adapt. Moreover, a single genomic region can be under more than one selective pressure at a time. For instance, selective pressures on the lactase (LCT) gene appear to have been strong in some parts of Europe but not others (Bersaglieri et al. 2004). Similarly, selective pressures on *TAS2R38* have been stronger on some haplotype backgrounds than others (Risso et al. 2016).

The results of our Tajima's D tests across *CD36-GNAT3* as a whole were most consistent with an absence of selective pressure during its recent history. As expected given the low diversity and downward skew in allele frequencies, observed D values were strongly negative (**Table 4.5**). This can result from two common selective pressures: purifying selection and positive selection. However, it can also be caused by rapid population growth, during which genetic drift slows and new variants accumulate (Adams and Hudson 2004; Wooding 2003). In our study, the observed D was below expectations at a high level of statistical significance ($P < 0.001$). However, this did not reveal whether the cause of the shift was selection, demography, or both. D tests using the empirical distribution in the 1000GP clarified the probable cause. Because the vast majority of the human genome (>98%) is noncoding it is can be assumed to be evolving neutrally or nearly so with respect to selection, but it is still shaped by demography. Thus, the empirical

distribution of D we obtained from the 1000GP represents expectations adjusted for demography while the standard distribution does not. We found that when compared with the empirical distribution, D values were well within expectations for four of the five site categories we analyzed ($0.390 < P_E < 0.665$) (**Table 4.5**). The exception was in *CD36* exons, where the observed D was significantly lower than expected, albeit marginally so ($D = -2.41$, $P_E = 0.035$). This is consistent with localized purifying or weak positive selection. In our view the empirical tests are the more convincing and conclude that on a worldwide scale selective pressures have been largely absent across *CD36-GNAT3* during its recent history with exception of exonic regions in *CD36*.

Evidence for selection in *CD36* exons in the 1000GP is consistent with prior findings by Fry et al. (2009). In a study of *CD36* in Africa, Fry et al. detected signatures of positive selection favoring a premature stop allele at rs3211938, which is located in *CD36* exon 13. Fry et al.'s investigation centered on resistance to malaria infection, which is hypothesized to be affected by variation in *CD36* because the receptor is an antigenic target for Plasmodium falciparum. The study found signatures of selection surrounding rs3211938 but rejected the malaria resistance hypothesis because association studies failed to detect correlations with malaria severity. However, the selective signatures were robust; suggesting that factors other than malaria resistance must be responsible. Evidence that lipid perception and metabolism are mediated by CD36 offers a possible explanation. In clinical studies variation in *CD36* associates with lipid perception and metabolic phenotypes, and rs3211938 is specifically implicated as the source of the associations (Love-Gregory et al., 2008, 2011). This supports the hypothesis that *CD36* harbors genotypes affecting responses to fats, which could exert selective pressures on genes mediating fat taste, metabolism, or both. Thus, a speculative explanation for the high frequency of the rs3211938 stop allele in localized areas of Africa is that it was favored by the nutritional environment, and contemporary associations are a holdover from those pressures.

Our findings on population differentiation are also consistent with Fry et al.'s (2009) proposal that selection has promoted differentiation among African populations. In our data, $F_{ST}$ with respect to noncoding sites in Africa ($F_{ST}$ = 1.5%) was similar to or slightly greater than in other superpopulations ($F_{ST}$ =0.2%-1.8%), indicating they are differentiated to roughly the same extent with respect to neutral variation (**Table 4.3 C**). However, $F_{ST}$ with respect to *CD36* exons was far higher in Africa than in other superpopulations (7.9% versus <1.5%), suggesting that some factor has driven differentiation specifically at that locus. Moreover, the trend was amplified in PHI sites, which is expected under local adaptation because selection most affects sites with functional effects. Further, differentiation in Africa was greatest specifically with respect to the premature stop in *CD36* (rs3211938), a major mutation particularly likely to experience selective effects. These patterns are consistent with the effects of local adaptation and that evolutionary pressures on fat responses had important effects on diversity in the continent.

**Table 4.6. PHI and regulatory sites with reported genotype-phenotype associations.**

| | | Allele Frequency | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **rsid** | **Effect** | Worldwide | Africa | Americas | East Asia | Europe | South Asia | **Reports** |
| rs1527479 | Expression modifier | 0.35 | 0.22 | 0.46 | 0.31 | 0.54 | 0.30 | Bokor et al. 2010 |
| | | | | | | | | Jayewardene et al. 2016 |
| | | | | | | | | Lecompte et al. 2011 |
| rs1534314 | Expression modifier | 0.26 | 0.26 | 0.19 | 0.44 | 0.07 | 0.30 | Ghosh et al. 2011 |
| rs3211883 | Expression modifier | 0.64 | 0.34 | 0.76 | 0.62 | 0.90 | 0.69 | Bokor et al. 2010 |
| | | | | | | | | Ghosh et al. 2011 |
| | | | | | | | | Heni et al. 2011 |
| rs3211938 | Stop gained (CD36 Exon 13) | 0.03 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | Fry et al. 2009 |
| | | | | | | | | Love-Gregory et al. 2011 |
| | | | | | | | | Love-Gregory et al. 2008 |
| rs6960369 | Expression modifier | 0.23 | 0.33 | 0.13 | 0.34 | 0.07 | 0.28 | Ghosh et al. 2011 |
| rs12706912 | Expression modifier | 0.56 | 0.60 | 0.45 | 0.59 | 0.44 | 0.68 | Ghosh et al. 2011 |

## Implications for association studies

Our finding that *CD36-GNAT3* harbors numerous sites with putative functional effects supports predictions that the region bears variants affecting lipid perception and metabolism. To date more than 80 variants in *CD36* and *GNAT3* have been reported to associate with both gustatory and non-gustatory phenotypes, and the potential for connections of *CD36* with obesity and related diseases draws ongoing interest (MacArthur et al. 2017). Our diversity estimates and functional predictions support these findings. They specifically buttress evidence for associations at eight sites identified in six previous studies (**Table 4.6**). Cross tabulating our list of PHI and regulatory sites against the list of previously reported associations revealed six with putative effects: rs1527479, rs1534314, rs3211883, rs3211938, rs12706912 and rs6960369. And of these, three (rs1527479, rs3211883, and rs3211938) showed evidence of associations in more than one study. One (rs3211938, the premature stop in *CD36*) showed evidence of being under

pressure from positive natural selection, which can only act on functional sites. Thus, these are particularly strong candidates for future investigation.

Statistical power is a critical consideration in association studies aimed at dissecting phenotypic effects, and our findings predict that it will be weak in *CD36-GNAT3*. Although numerous sites predicted to affect protein function and gene expression were present in the region, their frequencies were low. For instance, 83% of regulatory alleles had frequencies below 1%, and 97% of PHI alleles had frequencies below 1%. This is important because even when such alleles have functional impact they cannot contribute much phenotypic variance overall. Conversely, it suggests that if *CD36-GNAT3* does harbor alleles contributing substantial variance, they must be limited to a small number of sites where allele frequencies are elevated. This pattern is evident in the cross tabulation of PHI and regulatory sites against reported associations, in which all alleles exhibiting associations were found at high frequencies (>10%) in at least one superpopulation (**Table 4.6**).

Patterns of LD in our sample also have implications for association studies. When high, LD can produce false associations where noncausal variants cosegregate with causal ones, making their effects difficult to discriminate. However, it can enhance efforts to dissect associations by reducing the density of genotyping needed to localize the sources of effects, which can subsequently be pinpointed using fine mapping strategies. In contrast, when LD is low, sites' independent effects on phenotypes can be pinpointed directly through dense genotyping; however, this approach is vulnerable to missing effects if marker density is insufficient. In the case of *CD36* and *GNAT3* the structure of LD is a particularly important consideration because the two genes underlie similar chemosensory traits. Thus, if present, high LD could cause *GNAT3*- and *CD36*-mediated phenotypes to spuriously associate with variants in both genes, making their functional contributions difficult to distinguish. We saw low potential for such effects in *CD36-GNAT3*. While the high D' values we observed are consistent with a risk of confounds, $r^2$ was low or 0 between almost all site pairs, including PHI and regulatory sites. This makes confounds unlikely.

# Chapter 5 Conclusions and Future Considerations

This dissertation details an exploration of genetic variation and global diversity in human taste genes. By doing so, the current results highlight novel insights into the genetic underpinnings shaping diversity amongst global populations with regards to taste genes.

Chapter 2 presents an extensive review of past and present research in the field of taste genetics. This chapter highlights the basic molecular biology of the taste pathways, the evolution of taste, the genotypic diversity of taste gene families, and the association of genotypic variation to human taste phenotypes. This chapter highlights the gaps in understanding the genetic underpinnings contributing to phenotypic variation for most taste modalities, as much of the literature on human genotypic diversity in taste genes is focused on bitter taste. The chapter details evidence of natural selection acting on taste receptor genes across species, highlighting the importance of taste on fitness among mammals. Species have lost taste genes, differentiated into new alleles, and duplicated taste genes, resulting in unique repertoires of taste receptors that are seen across species. Among the human evolutionary timeline, the frequent migrations accompanying the human expansion out of Africa placed humans in new environments and under new selective pressures, which likely contributed to the phenotypic variance and genetic differences we see among global populations.

In recent times, the accumulation of genetic data from large sequencing projects and biobanks has allowed researchers to easily conduct examinations of human genetic diversity and genetic variation across global populations. In Chapter 2, I provide an analysis of human population genetic diversity and examine signatures of natural selection in an extensive catalog of the genes encoding the components of multiple taste pathways. To my knowledge, this analysis is one the largest, if not the most comprehensive, examination of taste genes, including newly identified taste receptor genes and several genes that encode proteins that carry out transduction and signaling in the downstream taste pathway. This analysis revealed that several genes show signatures of selection, lending evidence to the prevailing hypothesis that selection has been pervasive on human taste throughout human evolution. However, these signatures are often isolated to individual continental populations and largely absent globally, which supports reports of relaxed selection of sensory genes in contemporary humans. Natural selection acts upon functional regions of the genome, where selective pressure from the environment favors the fixation of alleles that produce a higher probability of survival and reproduction and drive down the frequency of alleles which results in lower fitness. Regarding taste, the finding that departures from neutrality tend to be isolated to individual populations suggests that such changes resulted from local adap Such findings highlight the importance of including diverse cohorts when designing future studies and interventions.

While there is ubiquitous recognition of the utility of genetic-based approaches in public health science, traditional public health scientists' integration of such approaches

has its challenges. Among the most evident is the recognition that much of this data requires specific computational expertise and knowledge of obscure data formats and software tools. To this note, this work has provided a general framework and tool for parsing, summarizing, and prioritizing variation in localized regions of the human genome. Inia, presented in chapter 3 of this dissertation, utilized popular bioinformatic tools and allows users to access data cataloged in the 1000 genomes project database without extensive computational expertise (The 1000 Genomes Project Consortium, 2015). Inia provides a set of scripts that extract and summarize localized user-specified regions of the 1000 genomes project, provides relevant annotation and predictions of variant effects, and conducts basic population genetic analysis. Additionally, Inia provides the user with haplotype calls extracted from the 1000 genomes project database, allowing the user to conduct further downstream analysis. In totality, Inia serves as a user-friendly tool that provides researchers with a means to extract, summarize, and perform basic analysis on data from the 1000 genomes project without the need for computational expertise or prior knowledge of the data structure. To demonstrate Inia, I have applied the tool to examine taste receptor genes.

Examining genes associated with taste perception and sensitivity can shed light on the physiological and psychological drives affecting nutritional behaviors and disease risk. Despite nearly one hundred years of scientific inquiry into the genetics of taste perception, there is still much ambiguity regarding the influence of genetic variants on dietary traits. Moreover, much of our understanding surrounds a narrow number of bitter taste receptors and has focused on the association of bitter taste perception, and to a lesser extent sweet perception, and food behaviors. Recent molecular studies have uncovered new mechanisms through which the modalities of taste perception function in mammals, including the identification of putative sour receptors and the establishment of fat taste sensation (Running et al., 2015). These provide new avenues for which to better understand the global diversity in taste perception genotypes and phenotypes, and the role this plays in shaping human health and dietary traits. As a result, Chapter 3 provides an examination of the putative sour receptor gene *OTOP1.*

Results from Inia highlight variants with the potential to have functional effect on the sour receptor gene *OTOP1*. Across *OTOP1* most non-synonymous variants occurred at low frequency, and some exons lack variation among all individuals examined. This finding is consistent with reports of *OTOP1* conservation across species from nematodes to humans (Tu et al., 2018). Several variants were predicted to have functional effect by SIFT or Polyphen-2, but only a handful of these were predicted by both tools. Given both the low occurrence and low allele frequency of these variants that are predicted to potentially have high impact on OTOP1 protein structure and function, it is likely that only a few variants have significant effect on the distribution of sour tasting phenotypes, with respect to OTOP1. From the generated catalogue of putatively high impact variants, only a single variant was previously referenced in the literature. Interestingly this variant has been previously associated in studies characterizing the oral microbiome (de Jesus et al., 2022).

Chapter 4 presents findings on the putative fat receptor *CD36*, whose transcript is superimposed on the taste gene *GNAT3*. These findings suggest that both of these genes contain a number of variants with potentially important functional effects, which could influence taste perception. The results in this work suggest that most putative high impact variants are low frequency and often constrained to individual populations and subpopulations, suggesting that only a handful of variants in *CD36* contribute to phenotypic variance at the population level. This chapter presents a test of natural selection across the *CD36-GNAT3* locus through the Tajima's D statistic, revealing significance in *CD36* exons, but not in noncoding regions of the gene. This suggests that pressures of natural selection likely have been limited or absent across the *CD36-GNAT3* locus, except on *CD36* exons. Examination of differentiation, measured through $F_{ST}$, revealed elevated genetic differentiation in the African continent for CD36 exons and high impact sites. It is likely that local adaptation has contributed to genetic diversity and phenotypic variance, and such differences are most pronounced in the African continent. Further, these results suggest differences between African and non-African populations. Finally, scans of linkage disequilibrium across the locus were examined due to the close proximity of *CD36* and *GNAT3*, both of which associate with tasting phenotypes. Strong linkage between the genes may confound genotype-phenotype associations, making it difficult to distinguish the mediating variant and gene. The present findings, however, do not reveal strong linkage between *CD36* and *GNAT3* variants. Despite the close proximity of the two genes, associations arising from either gene are likely independent of each other.

Together, the current work seeks to better understand the underpinnings of human genetic and phenotypic diversity with regards to taste perception. With the advent of recent large scale sequencing data, examinations of diversity and signatures of natural selection may serve to prioritize genetic variation which are both biologically relevant and clinically relevant. Further, examinations of population diversity give a better understanding of the global distribution factors contributing to human traits and disease risk. While decades of research have detailed the genetic underpinnings of bitter taste, much less work has been undertaken on the other modalities of taste. Thus the analysis of *OTOP1* and *CD36* adds to current knowledge surrounding genetic contributions to sour and fat taste phenotypes. Further, the work provides an example of utilizing functional prediction alongside measures of population diversity and differentiation to prioritize and decipher variants of interest, such as those returned by genome wide association studies. Conversely this work provides a list of high priority variants that can be tested in future genetic association studies involving fat and sour taste. The distribution of the workflow used in these analyses as an open-source package provides interested health researchers with the capability to quickly and easily apply such an analysis to any gene or list of genes with minimal user input and expertise. As a result, the detailed work here extends well beyond the realms of taste genetics and can be applied across health science.

The current work does not stand without its own limitations. The algorithms which are used to predict the effects of non-synonymous variants have their own biases which are prone to prediction error. Further, the identification of variants of high interest in

regulatory regions is based on annotations of the human genome, which are imperfect. For example, rs1761667, which is widely suggested to be a regulatory variant in the CD36 intronic region, was not identified as a variant of interest (Aguenaou et al., 2020; Lopez-Ramos et al., 2005; Love-Gregory et al., 2011). This peculiarity is unsurprising and may suggest that the variant is simply linked to another variant which is responsible for the associations or that it lies in a regulatory region that is yet to be correctly annotated. In the latter case, it is generally understood that the identification of regulatory regions in the human genome is far from complete and thus prone to such errors.

Despite an abundance of diverse haplotype data the current work is limited by the ability to test the findings presented in this work. While the quantitative analysis present suggests that variants identified in our work are suggested to have effects on taste protein structure and function, the lack of qualitative data, e.g., phenotypic data, does not allow for these effects to be associated to human phenotypes. The current results can only speculate that the high-impact variants identified may contribute to the variance seen among tasting phenotypes. It is notable that much of these variants are low in frequency, and those that are identified in the literature and in previous association studies only represent a small number of the overall list of variants identified in the current work. Thus, large sample populations with both phenotypic and genotypic data are ideal for addressing such an issue. At present no such data exist, and primary collection of such data during the timeline of the current research was not feasible.

The health and medical community has long shied away from viewing taste or smell with the same level of importance as other sensory traits, such as sight or hearing. However, similar to our utilization of sight, we rely on chemosensation in our daily lives to give us context into our environment. Despite the importance of our senses of taste and smell, routine assessment of chemosensory function has never been normalized in the clinical setting. In recent years, however, there has been a shift in interest in chemosensation from the medical and public health community. This has been partially owed to the discovery that chemoreceptors mediating taste play key roles not only in the oral cavity but manifest and carry important functions across multiple tissues, as previously covered in this work. Similarly, this shift in interest can be attributed to increased recognition that deficits in chemosensation are a tell-tale sign of disease, including neurocognitive diseases such as Parkinson's disease (Doty, 2014). During the COVID-19 pandemic a massive global interest in human taste and smell spurred globally after recognition that chemosensory deficits are common symptoms in those infected with early variants of the SARS-CoV-2 virus (Hannum and Ramirez et al., 2020). However, this further demonstrated the lack of standardized testing of chemosensation, particularly in clinically testing of taste function where there are few standardized protocols to measure taste sensation that were employed in a healthcare setting during the pandemic (Hannum etal., 2022). Recent endeavors to collect large amounts of phenotypic and whole genome data, including the All of Us and UK Biobank programs, give promise to the future (Sudlow et al., 2015; The All of Us Research Program Investigators, 2019). However, these projects do not currently collect chemosensory information and instead collect information on food behaviors including intake and liking.

The NHANES program's inclusion of genotypic data in previous years and recent inclusion of chemosensory information has offered promise for future large-scale endeavors to collect both genotype and chemosensory phenotype collections in large scale populations (Rawal et al., 2015). These endeavors, and the increased recognition of chemosensory health as a tool for public health, offer a glimpse into the future. While the current work suffers from limited ability to test the identified variants, a clear path has been painted forward with a strong candidate list for future investigations into fat and sour taste.

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