BITTER TASTE GENETICS AND ASSOCIATION WITH ORAL HEALTH AND COVID-19 OUTCOMES IN THE CANADIAN LONGITUDINAL STUDY ON AGING

By

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DISCLAIMER

This modified sandwich thesis comprises two multi-authored manuscripts. These manuscripts contain the majority of the original work contributed by the author of this thesis, as demonstrated by their first-authorship. Collaborating colleagues contributed to various parts of the study. The list of individual author contributions is provided on the next page.

AUTHOR CONTRIBUTIONS

1. Bitter taste genetics and oral health in Canadian Longitudinal Study on Aging. **Marziyeh Shafizadeh**, Vikram Bhatia, Samah Ahmed, Britt Drögemöller, Chrysi Stavropoulou, Philip St. John, Rajinder P. Bhullar, Prashen Chelikani, Carol A. Hitchon. bioRxiv 2024.11.12.623243; doi: https://doi.org/10.1101/2024.11.12.623243

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2. Genetic variations in bitter taste receptors and COVID-19 in Canadian Longitudinal Study on Aging. **Marziyeh Shafizadeh**, Mohd Wasif Khan, Britt Drögemöller, Chrysi Stavropoulou, Philip St. John, Rajinder P. Bhullar, Prashen Chelikani, Carol A. Hitchon.

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ABSTRACT

Bitter taste receptors (T2Rs, encoded by *TAS2R* genes) are expressed in various tissues, including the mucosal and bronchial epithelium and immune cells, where they play a crucial role in the early response to pathogens. Research on single nucleotide polymorphisms (SNPs) in *TAS2R*s has focused on *TAS2R38*, while other *TAS2R* genes and pseudogenes remain largely unexplored.

This thesis investigated SNPs in 25 *TAS2R* genes and 12 *TAS2R* pseudogenes in individuals of European ancestry within the Canadian Longitudinal Study on Aging (CLSA). The first part of the thesis compared the allele distribution of *TAS2R* SNPs with those reported in the 1000 Genomes Project (1KGP) database and examined the association between *TAS2R* SNPs and self-reported oral health outcomes in the CLSA cohort. The second part assessed the relationship between *TAS2R* SNPs and COVID-19 infection and antibody seroconversion in individuals with and without at-risk chronic medical conditions, including diabetes, immune-mediated inflammatory disease (IMID), respiratory disease, and cardiovascular disease.

The analyses identified fifteen SNPs in *TAS2R8, 9, 13, 14, 20,* and *50* as significantly associated with self-reported sore jaw muscles, a symptom commonly linked to temporomandibular disorders (TMDs). *TAS2R20* exhibited the highest number of associated SNPs. Structure-function analysis suggested that variants in *TAS2R20* may contribute to this symptom by altering ligand interactions. Additionally, in the COVID-19 Questionnaire Study (N = 14,073), rs117458236 (C) in *TAS2R20* (OR = 1.95, $P = 0.039$) was significantly associated with COVID-19 infection. In the COVID-19 Antibody Study (N = 8,313), three SNPs were associated with seroconversion: rs2234235 (G) in *TAS2R1* with anti-nucleocapsid (OR = 1.55, $P = 0.018$) and anti-spike (OR = 0.74, $P = 0.033$); rs2234010 (A) in *TAS2R5* with anti-nucleocapsid (OR = 1.56, $P = 0.014$); and rs34039200 (A) in *TAS2R62P* with anti-spike (OR = 0.86, $P = 0.013$). Subgroup analyses suggested an association between rs2234235 (G) in *TAS2R1* and lower odds of spike antibody response, as well as increased risk of SARS-CoV-2 infection among individuals with IMID or respiratory disease.

These findings highlight the potential of *TAS2R* genetic screening to identify individuals at elevated risk for TMD and COVID-19, suggesting applications in personalized medicine and vaccination strategies for high-risk groups.

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DEDICATION

I dedicate this work to my family for their unconditional love and support.
I also dedicate it to the brave people of Iran, and to all those who suffered or lost loved ones due to COVID-19.

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1KGP	1000 Genomes Project
ACE2	Angiotensin converting enzyme 2
AMP	Antimicrobial peptide
AVI	Alanine, valine, isoleucine
cAMP	Cyclic adenosine monophosphate
CCDSS	Canadian Chronic Disease Surveillance System
CLSA	Canadian Longitudinal Study on Aging
COVID-19	Coronavirus disease 2019
DMFT	Decayed, missing, and filled teeth
ECL	Extracellular loop
GPCR	G-protein coupled receptors
GWAS	Genome-wide association study
HUGO	Human Genome Organization
IBD	Inflammatory bowel disease
ICL	Intracellular loop
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-1β	Interleukin-1β
IMID	Immune-mediated inflammatory disease
LD	Linkage disequilibrium
lncRNA	Long noncoding RNA
MAF	Minor allele frequency
NO	Nitric oxide
PAV	Proline, alanine, valine
PCA	Principal component analysis
PTC	Phenylthiocarbamide
PROP	6-n-propylthiouracil
PSR	Periodontal Screening and Recording

QSM	Quorum-sensing molecule
RA	Rheumatoid arthritis
RANK	Nuclear factor-κB
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SNP	Single nucleotide polymorphism
T1R	Taste 1 Receptor (Sweet/umami taste receptor)
T2R	Taste 2 Receptor (Bitter taste receptor)
TAS2R	Bitter taste receptor gene
TM	Transmembrane
TMD	Temporomandibular joint disorder
TMPRSS	Transmembrane protease serine 2
TNF-α	Tumor necrosis factor alpha
TWAS	Transcriptome-wide association study
UTR	Untranslated region
VEP	Variant Effect Predictor
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 BITTER TASTE RECEPTORS

In mammals, taste perception is mediated by two distinct families of receptors. Specialized ion channels detect sour and salty tastes (Heck et al. 1984, Kinnamon et al. 1988, Avenet and Lindemann 1988). G-protein coupled receptors (GPCRs), a large superfamily of more than 800 transmembrane receptors in humans, detect a wide range of extracellular ligands, including sweet, umami, and bitter tastants (Lander 2001, Venter et al. 2001, Schiöth and Fredriksson 2005, Lagerström and Schiöth 2008, Striem et al. 1989, Chaudhari et al. 2000, Wong et al. 1996).

The Taste 1 Receptor (T1R) subfamily includes three members that form heterodimeric complexes to detect umami (T1R1–T1R3) and sweet tastes (T1R2–T1R3) (Nelson et al. 2001, Toda et al. 2013). Bitter taste receptors, also known as Taste 2 Receptors (T2Rs), comprise a distinct subgroup of GPCRs specialized for sensing bitter compounds. T2Rs are composed of an extracellular N-terminus, seven transmembrane (TM) α-helices, three extracellular loops (ECLs), three intracellular loops (ICLs), and an intracellular C-terminus (Figure 1.1) (Hoon et al. 1999).

Compared to other GPCRs, T2Rs are characterized by a relatively short N-terminal extracellular domain and ligand-binding sites located in the transmembrane helices (Chandrashekar et al. 2000, Pydi et al. 2012, Brockhoff et al. 2010, Sakurai et al. 2010). T2Rs can recognize structurally diverse bitter compounds, including bacterial metabolites and antibiotics, through a ligand-binding pocket formed primarily by residues within extracellular loop 2 and specific transmembrane helices (Jaggupilli, Singh, et al. 2019, Medapati, Singh, et al. 2021, Jaggupilli et al. 2018).

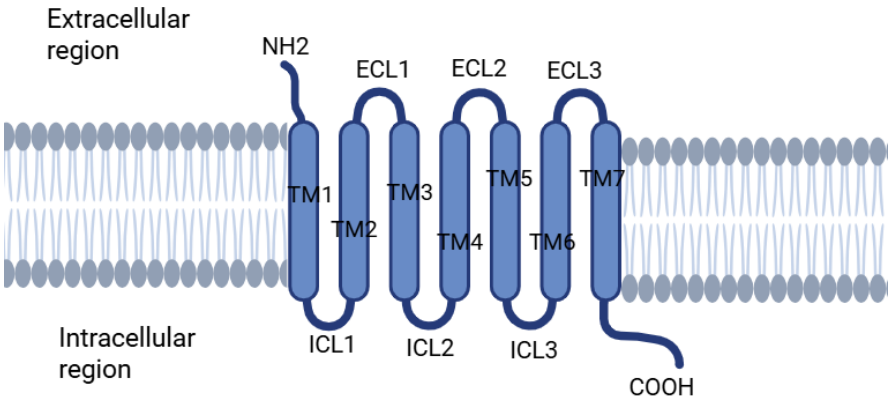


Figure 1.1 2D representation of T2Rs.
T2Rs consist of a short N terminus, seven transmembrane (TM) helices, three intracellular loops (ICLs), three extracellular loops (ECLs), and an intracellular C terminus. Created in BioRender.

T2Rs are expressed in various extraoral tissues, including the gastrointestinal tract, respiratory system, and immune cells, and contribute to physiological processes beyond taste perception (Jaggupilli et al. 2017, Maurer et al. 2015, Tran et al. 2018, Sternini and Rozengurt 2025). In these tissues, T2Rs can bind to environmental compounds and metabolites produced by pathogens, triggering signaling cascades that lead to protective responses such as increased ciliary movement, airway reflex protection, and antimicrobial activity (Shah et al. 2009, Krasteva et al. 2011, Deshpande et al. 2011, Deshpande et al. 2010, Orsmark-Pietras et al. 2013, Finger et al. 2003). Collectively, T2Rs act beyond mere taste receptors and serve as multifunctional receptors with extensive physiological roles, particularly in innate immunity, which will be discussed in the following sections.

1.1.1 Bitter taste receptor genes and pseudogenes

T2Rs are encoded by relatively small genes of approximately 1,000 base pairs, referred to as taste 2 receptors (*TAS2R*s) according to the Human Genome Organization (HUGO) Nomenclature (Braschi et al. 2019). The number of T2R types varies greatly across species (Shi and Zhang 2006, Li and Zhang 2013). This diversity may result from evolutionary pressure to

recognize a wide array of potentially toxic or harmful compounds, which are perceived as bitter taste. For example, herbivores have a greater number of T2Rs than carnivores, likely because toxic compounds are more common in plant tissues than in animal tissues (Li and Zhang 2013). This underscores the importance of T2Rs in survival and suggests that dietary differences among species may have played a role in their evolution (Li and Zhang 2013).

In humans, 25 potentially functional *TAS2R* genes have been identified, each responsive to several specific bitter compounds (Behrens et al. 2007, Meyerhof et al. 2010, Hoon et al. 1999, Conte et al. 2002). Additionally, 12 nonfunctional genes with sequence similarity, known as pseudogenes, have been identified (Conte et al. 2002, Adler et al. 2000). *TAS2R* genes and pseudogenes are located on three chromosomes. Except for *TAS2R1*, which is located on chromosome 5, the remaining genes are clustered at five specific loci on chromosomes 7 and 12 (**Figure 1.2**). Different *TAS2R* types exhibit sequence homology at certain conserved amino acid residues (**Table 1.1**).

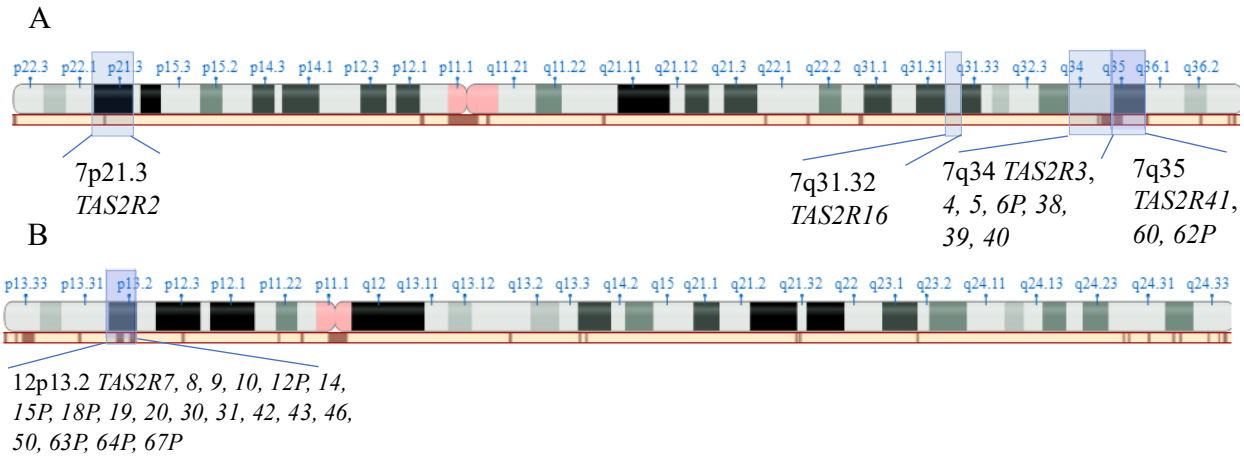


Figure 1.2 Location of *TAS2R* genes on chromosomes 7 (A) and 12 (B).
Adopted from the NCBI's Genome Data Viewer (O'Leary et al. 2024).

Table 1.1 Sequence similarity between *TAS2R* genes and pseudogenes.
Based on NCBI BLAST sequence alignment. See also Figure S1.1.

Pseudogene	Pseudogene Location	Gene(s)	Gene Chromosome	Total Score	Query Coverage	Percent identity
TAS2R6P	7q34	*TAS2R5*	7	247	96%	66.82
TAS2R12P	12p13.2	*TAS2R7*	12	156	25%	73.38
		TAS2R8	12	130	21%	79.12
		TAS2R9	12	78.8	35%	65.99
		TAS2R42	12	59.9	7%	77.33
		TAS2R10	12	59.9	7%	78.57
		TAS2R60	7	36.5	7%	71.23
		TAS2R5	7	35.6	2%	91.67
TAS2R15P	12P13.2	*TAS2R30*	12	1098	98%	85.24
		TAS2R43	12	1086	97%	85.27
		TAS2R50	12	1084	98%	84.7
		TAS2R46	12	1058	92%	85.93
		TAS2R31	12	1044	94%	85.01
		TAS2R45	12	1014	91%	84.94
		TAS2R19	12	982	98%	82.59
		TAS2R20	12	958	97%	82.14
		TAS2R14	12	185	77%	65.13
		TAS2R13	12	132	68%	64.75
		TAS2R10	12	71.6	7%	81.08
		TAS2R41	7	76.5	12%	71.43
		TAS2R39	7	42.8	6%	74.6
		TAS2R9	12	32.8	2%	88
		TAS2R1 5	5	30.1	4%	75.61
TAS2R18P	12P13.2	*TAS2R42*	12	395	97%	69.77
		TAS2R7	12	164	34%	72.15
		TAS2R10	12	181	47%	68.39
		TAS2R9	12	149	55%	65.55
		TAS2R20	12	83.3	19%	71.67
		TAS2R46	12	82.4	21%	70.77
		TAS2R30	12	167	31%	70.26
		TAS2R43	12	70.7	18%	70.66
		TAS2R14	12	70.7	19%	68.75
		TAS2R8	12	67.1	35%	65.02
		TAS2R45	12	60.8	18%	69.64
		TAS2R31	12	59.9	11%	73.39
		TAS2R19	12	58.1	14%	70.68
		TAS2R13	12	85.5	21%	70.09
		TAS2R50	12	46.4	4%	85
		TAS2R39	7	45.5	5%	78.85
		TAS2R3	7	43.7	5%	80.43
TAS2R22P	12	-	-	-	-	-
TAS2R62P	7q35	*TAS2R41*	7	138	37%	73.81
		TAS2R3	12	34.6	8%	69.14
		TAS2R8	12	34.6	8%	70.59
TAS2R63P	12P13.2	*TAS2R19*	12	1282	100%	88.22
		TAS2R30	12	939	99%	80.89
		TAS2R20	12	913	100%	80.43

		TAS2R43	12	876	94%	80.46
		TAS2R31	12	852	96%	79.61
		TAS2R46	12	847	92%	80.34
		TAS2R50	12	799	98%	78.05
		TAS2R45	12	787	89%	79.73
		TAS2R14	12	87.8	49%	64.97
		TAS2R10	12	46.4	5%	76.67
		TAS2R41	7	38.3	8%	69.88
		TAS2R39	7	33.7	3%	81.82
TAS2R64P	12p13.2	*TAS2R19*	12	1415	100%	91.23
		TAS2R30	12	1028	100%	82.72
		TAS2R43	12	1005	95%	83.16
		TAS2R31	12	962	96%	82.02
		TAS2R20	12	952	99%	81.46
		TAS2R46	12	942	92%	82.6
		TAS2R50	12	932	99%	80.78
		TAS2R45	12	884	88%	82.07
		TAS2R13	12	182	81%	65.83
		TAS2R14	12	178	82%	65.6
		TAS2R10	12	61.7	8%	75.28
		TAS2R39	7	58.1	9%	73.4
		TAS2R41	7	55.4	15%	69.62
		TAS2R38	7	35.6	4%	77.27
TAS2R67P	12p13.2	*TAS2R42*	12	315	98%	68.03
		TAS2R7	12	73.4	68%	63.62
		TAS2R8	12	72.5	8%	79.27
		TAS2R9	12	71.6	12%	73.17
		TAS2R10	12	67.1	32%	66.88
		TAS2R13	12	130	15%	76.39
		TAS2R14	12	46.4	5%	78.18
		TAS2R3	7	79.2	10%	74.29
		TAS2R50	12	35.6	8%	71.08
TAS2R68P	12	*TAS2R19*	12	1295	99%	90.48
		TAS2R30	12	940	99%	82.48
		TAS2R20	12	908	99%	81.71
		TAS2R43	12	900	98%	81.41
		TAS2R46	12	883	97%	81.28
		TAS2R31	12	842	98%	80.09
		TAS2R50	12	838	97%	80.19
		TAS2R45	12	827	94%	80.63
		TAS2R14	12	139	42%	68.3
		TAS2R13	12	113	64%	64.95
		TAS2R10	12	68	12%	72.5
		TAS2R39	7	44.6	9%	70.21
		TAS2R41	7	30.1	15%	66.44
		TAS2R3	7	30.1	2%	84.62

Based on the HUGO definition, a pseudogene is defined as a DNA sequence that is highly homologous to a functional gene but lacks the ability to produce a functional protein (Braschi et al. 2019). Several characteristics of pseudogenes cause a loss of function: lack of transcriptional and translational initiation sequences, early stop codons, absence of introns, and frameshifts in the coding sequence (Proudfoot and Maniatis 1980).

Pseudogenes are classified into four groups based on their structure and origin. Unitary pseudogenes are those without a functional parental gene. They originate from the pseudogenization of the parental gene itself (Zhang et al. 2010, Nishikimi et al. 1992). The second group, polymorphic pseudogenes, are similar in origin to unitary pseudogenes in that they arise from the pseudogenization of a functional gene. However, in this case, the functional version of the gene still exists within the population (Xue et al. 2006).

The third group, duplicated pseudogenes, result from unequal crossing over during DNA duplication (Sen et al. 2010). These pseudogenes consist of an intron–exon structure similar to the parental gene but have lost functionality due to truncations or errors in the duplication process. For example, *CYP21A1P* is a duplicated pseudogene of *CYP21A2* with accumulated mutations (Lee 2014). Moreover, as these pseudogenes are redundant in the genome, they are not subject to evolutionary pressure, and they may accumulate a variety of mutations resulting in loss of function. Duplicated pseudogenes are often found in clusters in proximity to their parental genes (Salmena 2021).

The last group, processed pseudogenes, comprises the majority of pseudogenes (Zhang et al. 2004, Torrents et al. 2003). They resemble the mRNA sequence of their parental gene: they lack intron-like sequences and include the 3’ end polyadenylation (Vanin 1984, 1985). These pseudogenes emerged as a result of the retrotranscription of mRNA and the subsequent insertion of the cDNA product into DNA (Salmena 2021). Pseudogenization occurs when the sequence is inserted into a region lacking a promoter sequence. These genes are invariably located on chromosomes other than that of the parental gene (Salmena 2021). Since *TAS2R*s lack intron structures, it is challenging to determine the classification and origin of their pseudogenes. Nonetheless, because *TAS2R* clusters are mainly located on the same chromosome as the parental

genes and lack a poly A/T structure, they appear to have arisen as a result of the duplication process.

Several hypotheses have been proposed for the evolutionary drivers of *TAS2R* pseudogenization, including the overlap in bitter tastants recognized by different T2Rs. For example, *TAS2R2* has been previously identified as a pseudogene due to an early stop codon mutation. However, Go et al. showed that a functional version of *TAS2R2* is present in certain populations, including the Adyge (Eastern European), Biaka (African Pygmies), and Mbuti (African Pygmies) populations (Go et al. 2005). Lang et al. (2023) evaluated bitter compounds recognized by T2R2, and showed that, except for one compound (phenylbutazone), all other agonists are also recognized by other T2Rs (Lang et al. 2023). This suggests that the redundancy of this gene might have led to an evolutionary pressure for its pseudogenization in some populations. The presence of a functional version in certain populations may be due to specific toxic bitter compounds in the local environment. Another hypothesis is that these populations may have lost the function of another *TAS2R* gene, and the functional version of *TAS2R2* compensates for that loss (Lang et al. 2023).

Despite lacking protein-coding ability, growing evidence indicates that pseudogenes can still be transcribed and may play important regulatory roles in gene expression (Balakirev and Ayala 2003, Tam et al. 2008, Ma et al. 2021). About 10% of pseudogenes are transcribed at levels above what could be considered accidental (Talyan et al. 2021). There are signs indicative of their role in regulating gene expression, such as the presence of transcription factor binding sites and their location within active chromatin (Sisu et al. 2014). More recent studies have suggested that these pseudogenes should be regarded as a group of functional long noncoding RNAs (lncRNAs) due to their biological roles (Pink et al. 2011, Salmena 2021).

1.1.2 Role of bitter taste receptors in immunity

T2Rs play a crucial role in the innate immune response to oral pathogens. They are expressed not only in taste buds but also in various tissues throughout the body, including the mucosal epithelium, solitary chemosensory cells in the oral epithelium and respiratory tract, and immune cells (Jaggupilli et al. 2017, Maurer et al. 2015, Tran et al. 2018). Jaggupilli et al. (2017) showed that *TAS2R* expression varies significantly across different tissues. *TAS2R20* and *TAS2R14* exhibit the

highest expression levels in extraoral tissues, including the bronchial epithelium and airway smooth muscle (Jaggupilli et al. 2017).

T2Rs detect and respond to various quorum-sensing molecules, such as acyl-homoserine lactones (AHLs) and hydroxyquinolones, which are secreted by many Gram-negative bacteria (Medapati, Singh, et al. 2021, Medapati, Bhagirath, et al. 2021). Quorum-sensing molecules are small signaling compounds crucial for bacterial communication, interaction with the microenvironment, and biofilm formation (Fuqua et al. 1994, Singh et al. 2000).

Taste signaling is initiated once a bitter compound binds to its receptor. This ligand–receptor interaction induces a conformational change in the receptor, which activates the heterotrimeric G-protein complex ($G\alpha\beta\gamma$) (Chaudhari and Roper 2010). In gustatory tissues, this G-protein is composed of the $G\alpha_{gustducin}$ subunit and the $\beta_3\gamma_{13}$ dimer (Caicedo et al. 2003, Wong et al. 1996). Activated $G\alpha_{gustducin}$ dissociates from the complex, triggering two signaling cascades: In one pathway, $G\alpha_{gustducin}$ activates phosphodiesterase, which hydrolyzes cyclic nucleotides, including cyclic adenosine monophosphate (cAMP), a key intracellular second messenger (Caicedo et al. 2003). Decreased cAMP levels enhances phagocytosis (Gopallawa et al. 2021).

In the other pathway, the $\beta_3\gamma_{13}$ dimer activates phospholipase $C\beta_2$ ($PLC\beta_2$), an enzyme that hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) (Zhang et al. 2003). IP3 then binds to its receptors on the endoplasmic reticulum, stimulating calcium release (Ca^{2+}) (Chaudhari and Roper 2010). In taste buds, this calcium surge activates specific cation channels in the membrane, resulting in cell depolarization, neurotransmitter release, and signal transmission to the brain (Hofmann et al. 2003). The released Ca^{2+} also acts as a second messenger and interacts with intracellular proteins, including calmodulin, to propagate the signal transduction cascade (Clough et al. 2002, Elsaraj and Bhullar 2008).

The intracellular calcium surge activates the production of nitric oxide and calcium-mediated antimicrobial peptides, both of which exert direct antimicrobial effects (**Figure 1.4**) (Jaggupilli, Singh, et al. 2019, Jaggupilli, Howard, et al. 2019, Jaggupilli et al. 2018). Additionally, nitric

oxide increases cGMP levels, which in turn contributes to the induction of acute phagocytosis (Gopallawa et al. 2021).

In addition to their direct antimicrobial effects, T2Rs regulate the adaptive immune response by modulating the production of inflammatory cytokines (Tuzim and Korolczuk 2021). Activation of T2Rs in macrophages can suppress the release of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and Interleukin-6 (IL-6), while promoting the production of anti-inflammatory cytokines such as Interleukin-10 (IL-10) (Grassin-Delyle et al. 2019). Since these cytokines are essential for B cell activation, T2Rs may indirectly impact the adaptive immune response (Grassin-Delyle et al. 2019, Tran et al. 2018, Vazquez et al. 2015). Significantly higher levels of T2R38 have been observed in activated lymphocytes compared to controls, as well as in memory cells compared to naïve cells (Tran et al. 2018). This immunomodulatory role is particularly important during viral infections, where an overactive inflammatory response can lead to tissue damage. By modulating cytokine production, T2Rs may mitigate the excessive inflammatory response and limit tissue damage (Grassin-Delyle et al. 2019).

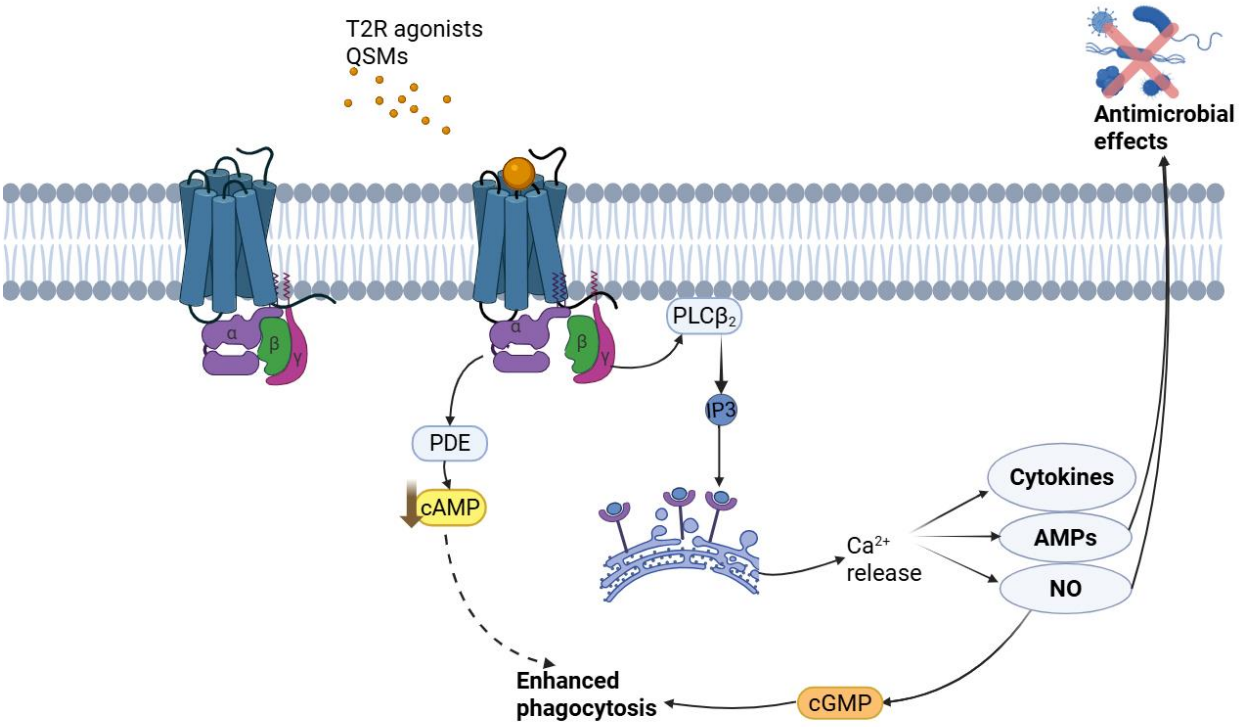


Figure 1.3 Role of T2Rs in the immune response.

T2Rs bind to a variety of ligands, including bacterial quorum-sensing molecules (QSMs), and initiate a signaling cascade that leads to the release of antimicrobial peptides (AMPs) and nitric oxide (NO), both of which have direct antimicrobial effects. Additionally, this signaling pathway modulates cytokine release and enhances phagocytosis, contributing to the immune response. Created in BioRender.

1.1.3 Genetic variations in T2Rs

TAS2R genes show extensive mutational diversity, primarily single nucleotide polymorphisms (SNPs), which are defined as single-nucleotide variations present in at least 1% of the population (Brookes 1999, Kim et al. 2005). These polymorphisms are traditionally classified as synonymous (no amino acid change), nonsynonymous (missense), or nonsense substitutions, any of which may impact receptor expression or function (Gil et al. 2015, Kim et al. 2005, Shastry 2009). While synonymous variants preserve the amino acid sequence, they may alter mRNA secondary structure and stability, potentially influencing post-transcriptional regulation of gene expression (Hunt et al. 2014, Spencer et al. 2012). Emerging evidence supports reclassifying these as "unsense" variants to reflect their functional impact (Vihinen 2023). Missense variants result in the substitution of one amino acid for another in the polypeptide chain, which may destabilize the protein, disrupt its three-dimensional structure, or confer gain or loss of function (Petrosino et al. 2021).

Nonsense variants are defined as nucleotide substitutions that introduce a premature stop codon. Such mutations can substantially disrupt gene functionality and may result in a nonfunctional protein (Conte et al. 2002). For instance, *TAS2R2* was previously identified as a pseudogene due to an early stop codon (TGG -> TAG) (Lang et al. 2023). However, a functional version of *TAS2R2*, lacking this stop codon, has been identified in Adyge (Eastern European), Biaka (African Pygmies), and Mbuti (African Pygmies) populations (Lang et al. 2023, Go et al. 2005, Research 1953). As a result, *TAS2R2* may not be considered a pseudogene in these populations. This finding suggests that intact, functional versions of other *TAS2R* pseudogenes may also exist in specific human populations and exert biological effects.

Genetic variations in *TAS2R* genes have been associated with a variety of clinical outcomes (Mao et al. 2023). Some of these variants are linked to sweet food intake and medication compliance, particularly in pediatric patients (Pawellek et al. 2016, Riccio et al. 2018, Cont et al. 2019, Mennella et al. 2015). Additionally, several *TAS2R* polymorphisms are associated with caffeine and alcohol consumption, as well as smoking behaviors (Hayes et al. 2011, Dotson et al. 2012, Duffy et al. 2004, Hinrichs et al. 2006, Baker et al. 2018, Risso et al. 2017). Due to these connections with nutrition and lifestyle, *TAS2R* variants have also been implicated in metabolic

conditions, including obesity and type 2 diabetes mellitus (Chupeerach et al. 2021, Khan et al. 2020, Lee and Shin 2023). The *TAS2R38* non-taster genotype is associated with an increased risk of Parkinson's disease (Cossu et al. 2018). Variants in *TAS2R3* and *TAS2R4* have been linked to altered thyroid function and a higher risk of papillary carcinoma (Choi et al. 2018). T2R activation in cancer cells demonstrated anti-cancer effects, including suppression of cell proliferation, invasion, metastasis, and angiogenesis, as well as enhanced apoptosis (Singh et al. 2020, Martin et al. 2019, Seo et al. 2017, Stern et al. 2018, Salvestrini et al. 2020). Increasing evidence also indicates that *TAS2R* polymorphisms may be associated with oral health conditions and respiratory diseases (Khimsuksri et al. 2022, de Jesus et al. 2022b, Chisini et al. 2021, Barham et al. 2021, Parsa et al. 2021).

Among the 37 human *TAS2R* genes and pseudogenes, *TAS2R38* is the most studied, primarily due to its two common haplotypes that influence the ability to taste 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC), bitter compounds commonly used in genetic studies to assess individual differences in taste perception (Kim, Jorgenson, et al. 2003). These haplotypes result from three common SNPs in the *TAS2R38* gene: rs713598 C/G-145, rs1726866 C/T-785, and rs10246939 G/A-866. The functional haplotype encodes proline-49, alanine-262, and valine-296 (PAV) at specific amino acid positions, while the non-functional haplotype encodes alanine-49, valine-262, and isoleucine-296 (AVI). The PAV haplotype confers the ability to taste PROP and PTC. Individuals with the PAV/PAV genotype are classified as "supertasters", those with the AVI/AVI genotype as "non-tasters", and individuals with the PAV/AVI genotype have intermediate tasting ability (Wooding et al. 2004).

Genetic variation in *TAS2R* genes differs significantly across populations, which may influence disease associations (Wooding and Ramirez 2022a). Studies have focused on *TAS2R38*, because the tasting phenotype can be easily assessed clinically using taste strips. The T2R38 phenotype is highly correlated with its genotype; however, PROP tasting ability tends to decline with age and can be affected by the assessment methods (Mennella et al. 2010, Rossella et al. 2015). Therefore, confirming the *TAS2R38* genotype is particularly important in studies involving older adults, such as those included in the Canadian Longitudinal Study on Aging (CLSA).

1.2 BITTER TASTE RECEPTORS AND ORAL HEALTH

1.2.1 Oral health in Canadian Longitudinal Study on Aging (CLSA)

The CLSA is a national cohort of 51,338 individuals aged 45–85 from all 10 Canadian provinces (Raina, Wolfson, Kirkland, Griffith, et al. 2009). The study collects data on diverse aspects of participants' lives, including sociodemographic characteristics, lifestyle factors, medical history, medication use, and genetic variations, to evaluate their influence on health and disease development (Raina, Wolfson, Kirkland, Griffith, et al. 2009, Forgetta et al. 2022). The CLSA includes approximately 20 oral health-related questions adapted from the Canadian Community Health Survey 2.1. These questions assess aspects such as teeth and denture status, eating difficulties, dental or denture-related problems, general oral health, oral hygiene practices, and temporomandibular pain.

Several studies have analyzed oral health data within the CLSA, reporting that 92% of participants rated their oral health as excellent to good, and 79% had used dental services within the previous year. Approximately 8% of the cohort were edentulous, and over 10% experienced discomfort while eating due to oral health problems (Bassim, MacEntee, et al. 2020, De Rubeis et al. 2023). Over 30% of the cohort experienced two or more oral health issues (De Rubeis et al. 2023). The number of oral health problems was found to be associated with diet and frailty, highlighting the impact of oral health on general health and quality of life (Bassim, Mayhew, et al. 2020). Factors associated with poor oral health in the CLSA cohort included poor general health, smoking, low income, depression, low physical activity, poor diet, and lack of dental visits (Bassim, Mayhew, et al. 2020, Bassim, MacEntee, et al. 2020). Although these studies investigated various risk indicators, the relationship between oral health and genetic variations has yet to be explored.

1.2.2 Oral health and T2Rs

Oral diseases are often multifactorial, influenced by environmental and lifestyle factors, genetic predisposition, and the microbial composition and activity (Pierce et al. 2019, Schroth et al. 2021). The oral cavity is colonized by a diverse and dynamic microbial community, including over 700 bacterial and 100 fungal species (Dewhirst et al. 2010, Peters et al. 2017). Many of

these microorganisms coexist with the host in homeostatic balance; however, a shift in the composition or function of these polymicrobial communities, a condition known as dysbiosis, can disrupt this balance, leading to the onset and progression of oral pathology (Lamont et al. 2018).

Dental caries result from a shift in the microbial biofilm toward cariogenic pathogens on the enamel surface. These bacteria metabolize fermentable carbohydrates, producing acids that lead to enamel demineralization (Zero 1999). Similarly, periodontal diseases arise from a transition from aerobic to anaerobic conditions in the dental biofilm (Socransky et al. 1998). This dysbiosis stimulates an excessive innate immune response, marked by the release of pro-inflammatory cytokines such as interleukin-1β (IL-1β), prostaglandins, and tumor necrosis factor. The resulting inflammation of the gingiva, or gingivitis, is characterized by redness, swelling, and bleeding (Silva et al. 2015). As the disease advances, the adaptive immune system becomes involved, activating the receptor activator of nuclear factor-κB (RANK) pathway, which stimulates osteoclasts and alveolar bone loss, presented clinically as periodontitis (Hajishengallis and Lambris 2011). The precise mechanisms underlying these dysbiotic interactions are still not fully recognized.

Taste signaling genes are associated with the abundance and diversity of microbial communities in the oral cavity (de Jesus et al. 2021, de Jesus et al. 2022a). The buccal microbial composition differs between individuals with the *TAS2R38* supertaster genotype (PAV/PAV) and those with non-taster genotype (AVI/AVI) (de Jesus et al. 2021). This variation may be partly mediated by the ability of T2Rs to detect and respond to bacterial molecules. An *in vitro* study showed that *TAS2R14* is highly expressed in gingival epithelial cells and can detect competence-stimulating peptides secreted by the cariogenic bacterium *Streptococcus mutans*, triggering calcium signaling and the secretion of cytokines such as IL-8, TNF-α, and IL-6 (Medapati, Singh, et al. 2021). Another study found that T2R14 exerts inhibitory effects on Gram-positive bacteria, particularly *Staphylococcus aureus* and *S. mutans*, by mediating their internalization in gingival epithelial cells (Medapati, Bhagirath, et al. 2021). This is especially important because the upper respiratory tract and epithelial cells are the first line of defense against pathogens. Therefore, T2Rs can influence the bacterial balance in the oral cavity, suggesting that genetic variants affecting their expression or function may contribute to increased susceptibility to oral diseases.

Additionally, evidence indicates a relationship between T2Rs and various lifestyle habits, including smoking and alcohol consumption, which are closely related to oral health conditions such as periodontal disease (Keller et al. 2013, Mao et al. 2023, Pitiphat et al. 2003). Taste genes are also associated with dietary choices and food preferences, including sugar intake, thereby affecting susceptibility to dental caries and periodontitis (Dotson et al. 2010, Eriksson et al. 2019, Yang et al. 2024, Petrenya et al. 2024, Shetty et al. 2014). Furthermore, T2Rs may contribute to tooth structure and alveolar bone health, as GPCR components and calcium signaling cascades play essential roles in tooth and bone development, as well as in the prevention of bone resorption (Luo et al. 2019, Parry et al. 2016, Seymen et al. 2022).

Several studies have examined the relationship between oral diseases and *TAS2R* genetic variants, primarily focusing on *TAS2R38* and dental caries, though results have been inconsistent. A meta-analysis of genome-wide association studies (GWAS) and transcriptome-wide association studies (TWAS) combined genetic and transcriptomic data to identify genes associated with early childhood caries (Orlova et al. 2023). The TWAS meta-analysis revealed a significant association between early childhood caries and the expression of three *TAS2R* genes, including *TAS2R14*. However, the GWAS meta-analysis did not identify any significant association with *TAS2R* genetic variants. Similarly, AlMarshad et al., in a cohort of 360 preschool children, reported no significant association between the *TAS2R38* rs713598 variant and early childhood caries (AlMarshad et al. 2021). In contrast, Kiliç et al. evaluated decayed, missing, and filled permanent/primary teeth (DMFT/dmft) scores in a cohort of 178 children and observed increased DMFT scores among non-tasters (AVI/AVI genotype) (Kiliç et al. 2022).

In another study, de Jesus et al. (2022) investigated genetic association in 176 children and found that variants in *TAS2R3*, *TAS2R4*, *TAS2R5*, and *TAS2R60* were associated with severe early childhood caries. Although they did not identify a significant association between *TAS2R38* variants and childhood caries, they did report a link between *TAS2R38* rs1726866 and *Candida albicans*, a fungal species implicated in dental caries development (de Jesus et al. 2022b). Furthermore, a systematic review and meta-analysis of SNPs in taste genes showed that the heterozygous genotype of rs713598 (CG) was associated with protection against dental caries (OR: 0.17; 95% CI [0.03–1.04]) (Chisini et al. 2021).

In addition, *TAS2R* genes are expressed in gingival solitary chemosensory cells and appear to be associated with periodontal health. An animal study of ligature-induced periodontitis demonstrated that mice lacking gingival solitary chemosensory cells or taste signaling molecules exhibited higher bacterial load, decreased bacterial diversity with a shift toward pathogenic species, and an increased risk of alveolar bone loss (Zheng et al. 2019). Nevertheless, limited research is available on *TAS2R* variants and periodontitis. Khimsuksri et al. found the *TAS2R38* AVI/AVI genotype associated with a lower risk of periodontal disease (Khimsuksri et al. 2022). Similarly, an *in vitro* study showed that gingival epithelial cells with the AVI/AVI genotype displayed altered cytokine release in response to periodontal pathogens (Gil et al. 2015). In contrast, Kaur et al identified no association between *TAS2R38* variants and periodontal health (Kaur et al. 2021).

1.3 BITTER TASTE RECEPTORS AND COVID-19

1.3.1 Coronavirus disease 2019 (COVID-19)

COVID-19 has affected over 777 million individuals and caused more than 7 million deaths worldwide (World Health Organization (WHO) February 2025). Despite the widespread availability of effective vaccines and the subsequent lifting of most public health restrictions, COVID-19 remains a significant public health concern, particularly for older adults and those with compromised immune systems (Gallo et al. 2022). There is considerable variation in infection severity and duration both within these high-risk groups and the general population (Gallo et al. 2022).

Epidemiological and cohort studies have clearly identified older adults and individuals with comorbidities as being at greater risk for severe COVID-19 (Gallo et al. 2022). In older adults, age-related remodeling of innate and adaptive immune system components, along with a progressive state of chronic low-grade inflammation, contributes to increased susceptibility to infection, disease severity, and altered vaccine responses (Ciabattini et al. 2018, Osterholm et al. 2012). Additionally, older adults have a higher prevalence of chronic conditions, such as diabetes, respiratory diseases, and cardiovascular conditions, that are known to increase COVID-19 risk and severity (CDC COVID-19 Response Team 2020, Marfella et al. 2022). Immunocompromised individuals, including those with immune-mediated inflammatory diseases

(IMIDs) who may have immune dysregulation and the use of immunomodulatory medication, are also at greater risk of severe COVID-19 infection (Grainger et al. 2022, Simpson-Yap et al. 2022, Maddur et al. 2010). The underlying reasons are not fully understood but may relate to early anti-viral mucosal immune responses.

1.3.2 COVID-19 and T2Rs

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, is predominantly transmitted through inhalation. Nasopharyngeal and oral mucosal immune responses play a critical role in effective antiviral defenses against airborne pathogens. The primary receptors for SARS-CoV-2 binding and cellular entry, angiotensin converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS) are expressed in nasal, salivary, and intestinal epithelia (Hamming et al. 2004). SARS-CoV-2 is detectable in the saliva of infected patients even after symptom resolution (Huang et al. 2021). Saliva can be used to detect SARS-CoV-2 infection with comparable accuracy to nasopharyngeal swabs for the Wuhan variant and even greater sensitivity for the Omicron variant (Marais et al. 2021, To, Tsang, Leung, et al. 2020, To, Tsang, Yip, et al. 2020). Collectively, these findings suggest that nasopharyngeal tissues and salivary glands may be potential reservoirs for SARS-CoV-2 during asymptomatic infection and contribute to ongoing transmission.

Innate immune responses in the nasopharyngeal and oral mucosa are essential for early defense against airborne pathogens such as SARS-CoV-2. As previously mentioned, T2Rs play an important role in the innate immune response in respiratory and oral epithelia. They exert their effects through multiple mechanisms, including the stimulation of nitric oxide and anti-microbial peptide release. Nitric oxide inhibits SARS-CoV-2 replication by impairing the interaction between the viral Spike protein and host receptors, as well as by reducing viral RNA synthesis (Åkerström et al. 2009). Anti-microbial peptides further inhibit the virus by destabilizing the viral envelope (Currie et al. 2013). Interestingly, taste loss has been recognized as a common symptom in COVID-19 patients, with 39.2% reporting taste dysfunction (Hannum et al. 2021), which may be partially mediated by T2Rs. These receptors are also expressed in bronchial smooth muscle cells and ciliated epithelial cells, where they contribute to airway clearance by

promoting airway reflex protection, increased ciliary movement, and bronchodilation (Shah et al. 2009, Krasteva et al. 2011, Deshpande et al. 2011, Deshpande et al. 2010, Orsmark-Pietras et al. 2013, Finger et al. 2003). In addition, T2Rs in pulmonary macrophages modulate cytokine production, thereby reducing inflammation and potentially limiting tissue damage caused by an overreaction to virus (Grassin-Delyle et al. 2019).

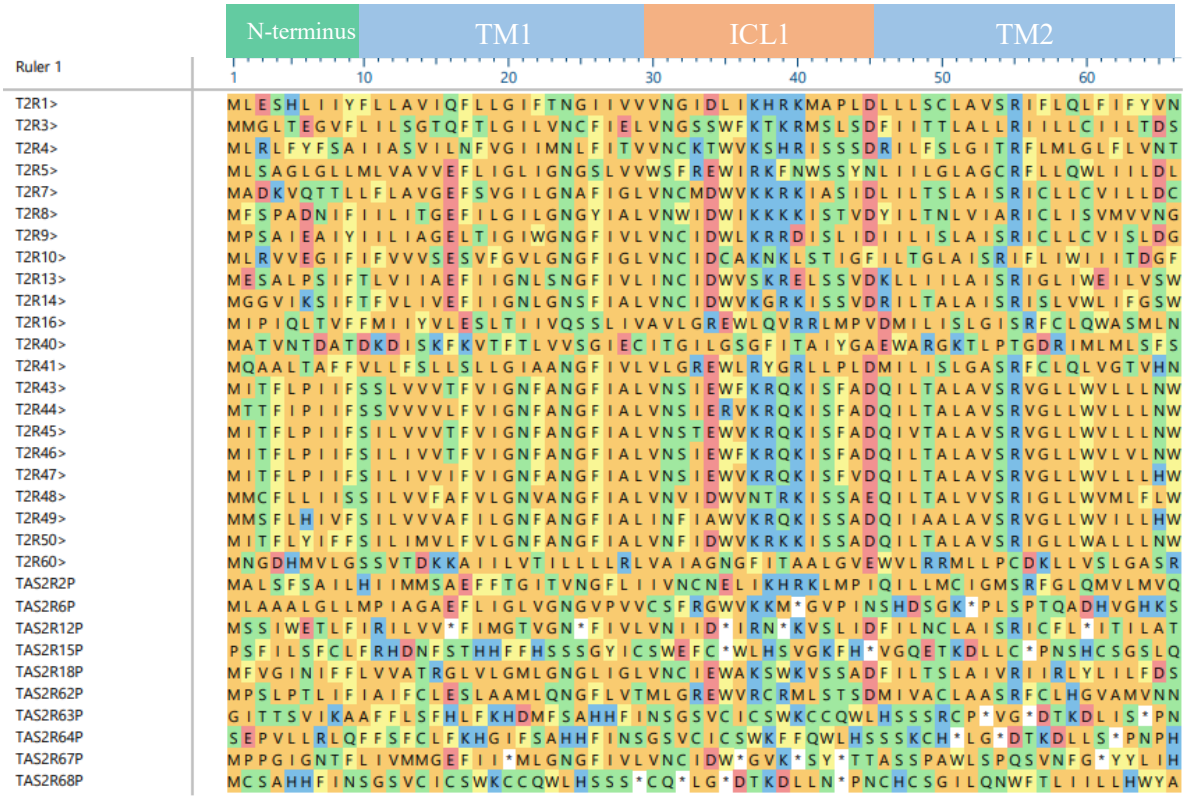
Emerging evidence suggests a relationship between the *TAS2R38* genotype and susceptibility to upper respiratory infections and chronic rhinosinusitis (Gallo et al. 2016, Lee et al. 2012). The *TAS2R38* supertaster genotype (PAV/PAV) is linked to higher expression of nitric oxide and anti-microbial peptides, as well as a lower risk of chronic rhinosinusitis compared to the non-taster genotype (AVI/AVI) (Lee et al. 2012). Additionally, research has indicated that the *TAS2R38* non-taster (AVI/AVI) genotype may be associated with an increased risk of COVID-19 (Barham et al. 2021, Parsa et al. 2021). A prospective cohort study of 1,935 patients and exposed healthcare providers assessed T2R38 phenotype and found that, compared to tasters and super-tasters (functional T2R38), non-tasters (non-functional T2R38) were 10 times more likely to test positive for COVID-19 via polymerase chain reaction (PCR), 3.9 times more likely to require hospitalization if tested positive, and experienced a longer duration of symptoms (Barham et al. 2021). A follow-up treatment trial based on T2R38 phenotypes involving 747 COVID-19 PCR-positive patients from the same research group reported milder symptoms in supertasters, mainly headache and nasal congestion, whereas severe symptoms such as high fever and dyspnea and hospitalization were more common among non-tasters (Taha et al. 2021). Furthermore, a global correlation study examined regional distributions of *TAS2R38* genotypes alongside online national COVID-19 mortality data, identifying weak correlations between the prevalence of the PAV allele and lower COVID-19 mortality (Parsa et al. 2021).

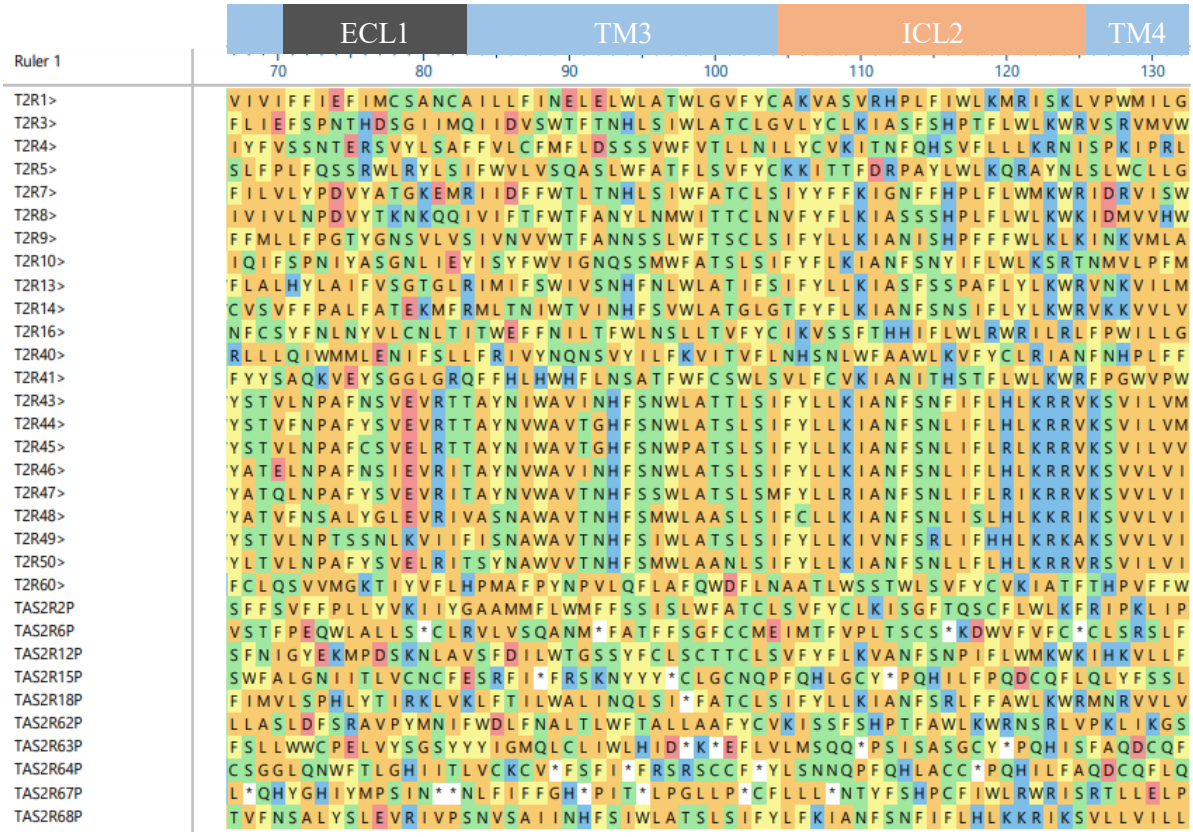
In contrast, a small Italian study of only 54 genotyped healthy individuals found no significant association between *TAS2R38* genotype and COVID-19 severity (Risso et al. 2022). A recent study using the data from the CLSA dataset analyzed the association between *TAS2R38* genotypes and both self-reported COVID-19 PCR-positive cases and serologically confirmed cases (those testing positive for nucleocapsid antibody), reporting no significant association. However, among the self-reported PCR-positive cases, individuals with the AVI/AVI genotype were more

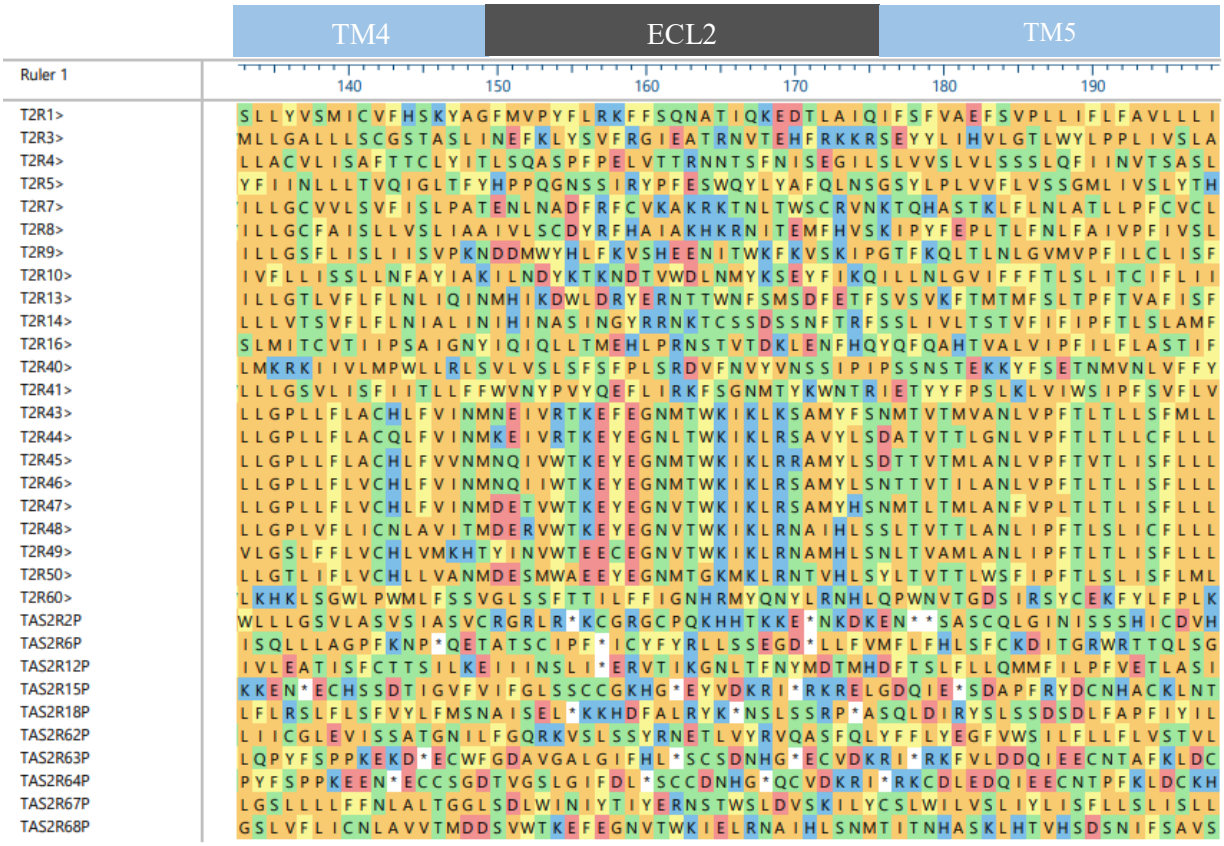
likely to report sinus pain compared to those with the AVI/PAV genotype. Among the serologically confirmed cases (n=177), individuals with the AVI/AVI genotype were less likely to experience a sore or scratchy throat compared to those with the PAV/PAV genotype; nevertheless, both findings were non-significant after correction for multiple comparisons (Meng and Nielsen 2024).

Overall, variations in *TAS2R*s may impact the immune response to SARS-CoV-2 and, consequently, affect disease susceptibility. Additionally, since T2Rs are expressed in immune cells, they may impact serological responses to both infection and vaccination. This may contribute to an increased risk of post-vaccine infection, particularly among immunocompromised patients and older adults who generally have reduced vaccine immunogenicity. Current research in this area is limited and has primarily focused on the association with *TAS2R38*, which is known to have low expression in extraoral tissues (Jaggupilli et al. 2017). Moreover, previous studies did not account for many groups at high risk for severe COVID-19, and their findings have been inconsistent. To date, there is no published data on the relationship between T2Rs and post-infection or post-vaccination serological responses. Additionally, no research has examined the association between COVID-19 infection and other *TAS2R* genes or pseudogenes, including those with higher levels of expression in extraoral tissues.

1.4 SUPPLEMENTARY MATERIAL



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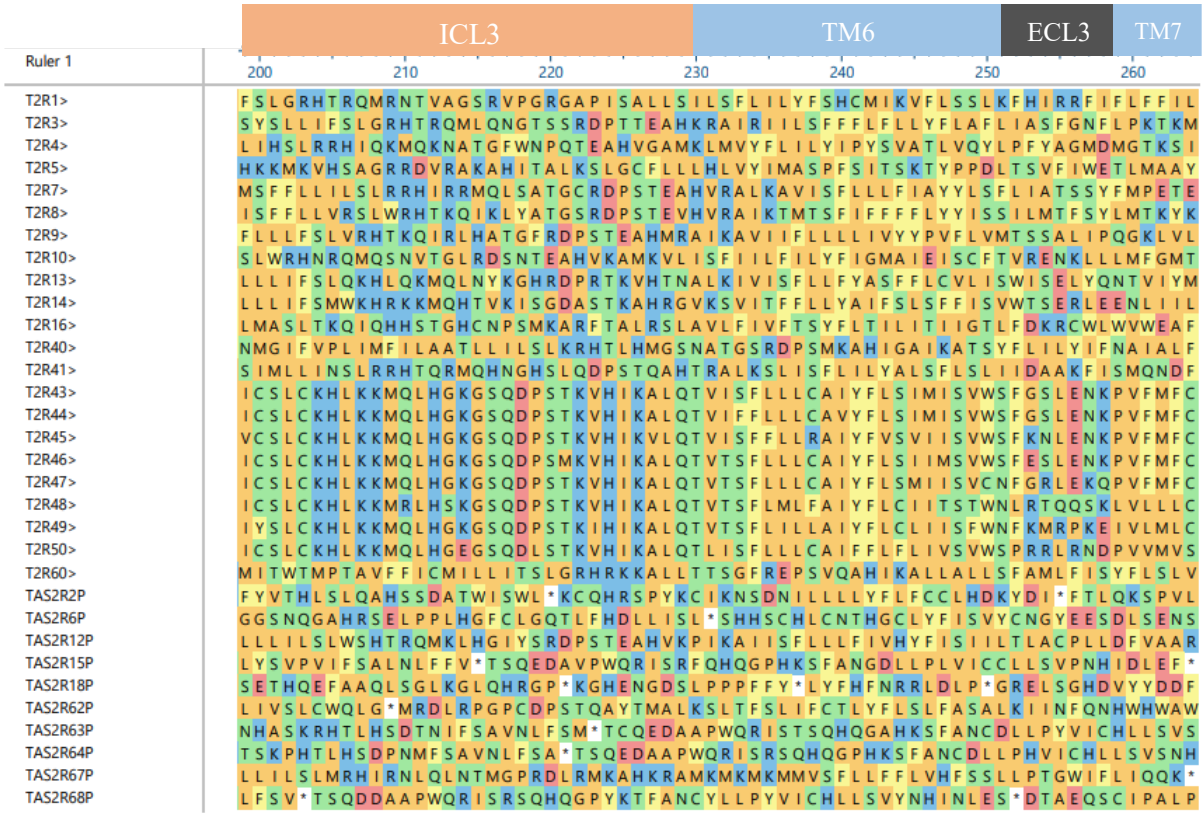
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Figure S1.1 Amino acid sequence alignments of *TAS2R* genes and pseudogenes. Amino acids with similar properties, such as hydrophobicity and charge, are color-coded for easier identification. Premature stop codons are indicated by an asterisk (*). Created using DNASTAR software (DNASTAR, Inc., Madison, USA). TM: transmembrane helix; ICL: intracellular loop; ECL: extracellular loop.

CHAPTER 2: HYPOTHESIS AND OBJECTIVES

2.1 Study rationale

T2Rs detect bacterial molecules and activate intracellular signaling cascades that lead to the release of nitric oxide and antimicrobial peptides, which exert direct antimicrobial effects. This signaling also enhances phagocytosis and modulates inflammatory cytokine release. Thus, T2Rs play a crucial role in the innate immune response, the first-line defense, against oral microbial dysbiosis and airborne pathogens such as SARS-CoV-2. Furthermore, because T2Rs contribute to airway clearance, dietary preferences, smoking, and alcohol use, they may be associated with susceptibility to oral diseases, general health, and COVID-19 infection.

Current literature on the association between *TAS2R* variants and oral health or COVID-19 has primarily focused on *TAS2R38*, with inconsistent findings. The involvement of other *TAS2R* genes and pseudogenes remains largely unexplored. Some studies addressing COVID-19 have relied on phenotypic evaluation of *TAS2R38*, which is less reliable than genotyping, especially in older individuals, who may exhibit decreased PROP sensitivity and are potentially at higher risk of COVID-19 (Mennella et al. 2010, Rossella et al. 2015). There is a significant lack of data regarding other T2Rs that are known to impact mucosal immunity or are highly expressed in extra-oral tissues (Jaggupilli et al. 2017).

There is currently no data on the association between *TAS2R* variants and serological response to SARS-CoV-2 infection or vaccination. Substantial knowledge gaps remain regarding the role of oral and nasal mucosal immune responses in SARS-CoV-2 transmission. Given their role in modulating adaptive immune responses, variations in *TAS2R*s may influence the vaccine effectiveness, particularly in older adults with reduced vaccine immunogenicity. Understanding the association between *TAS2R* variations and serological response may support personalized vaccination strategies and enhance our understanding of individual differences in vaccine efficacy and the risk of breakthrough infections. Such insights may also inform early defense mechanisms against other airborne pathogens.

Additionally, identifying *TAS2R* genetic variations linked to COVID-19 outcomes could inform preventive protocols and help identify high-risk groups, including individuals with medical comorbidities (Gallo et al. 2022, CDC COVID-19 Response Team 2020, Marfella et al. 2022, Grainger et al. 2022, Simpson-Yap et al. 2022). Mapping allele distributions across the Canadian adult population and comparing them to those reported by the 1000 Genomes Project (1KGP) could lead to customized clinical strategies and public health policies.

2.2 Hypothesis

TAS2R variants are significantly associated with oral health and COVID-19 outcomes in the CLSA cohort.

2.3 Objectives

The following objectives were pursued to test the above hypothesis.

2.3.1 Objective 1

To determine the allele frequencies of *TAS2R* SNPs in the CLSA cohort of European ethnicity and compare them with those of the European population reported by the 1KGP.

2.3.2 Objective 2

To assess the relationship between *TAS2R* SNPs and oral health symptoms in the CLSA cohort.

2.3.3 Objective 3

To evaluate the association between *TAS2R* variants and COVID-19 infection in the CLSA cohort.

2.3.3 Objective 4

To assess the relationship between *TAS2R* SNPs and SARS-CoV-2 antibody response in the CLSA cohort.

2.3.4 Objective 5

To determine whether the associations between *TAS2R* SNPs and COVID-19 infection and sero-conversion differ in older individuals with or without at-risk chronic medical conditions in the CLSA cohort.

CHAPTER 3: GENERAL METHODS

3.1 Study population and data preprocessing

Our team received ethics approval from the University of Manitoba's Health Research Ethics Board (HREB HS26021-H2023:173) and obtained access to the CLSA Comprehensive Baseline Dataset (version 7.0) and Genome-wide Genetic Data Release version 3 (CLSA Application ID: 2006008). Details of the CLSA protocol, including inclusion/exclusion criteria, sampling strategy, data collection, and procedures, are available for download under the Researchers section of the CLSA website (www.clsa-elcv.ca). In brief, individuals aged 45-85 at baseline were recruited from multiple sites across Canada and followed with standardized data collection, including demographics and lifestyle/behavioral measures. A subset consented to provide biosamples, including blood for genotyping. Baseline and genetic data were used for this analysis. All CLSA participants consented to the use of their data, which is securely stored on our lab server. The genetic dataset includes information from 26,622 participants, who were genotyped for 794,409 variants using the Affymetrix Axiom array, along with an additional 308 million imputed variants from the TOPMed reference panel (Forgetta et al. 2022). The positions of *TAS2R* genes in the GRCh38 genome assembly were obtained from the National Center for Biotechnology Information (NCBI) Gene database (Sayers et al. 2022).

3.2 Quality control and eligibility criteria

Sample and marker quality control was applied using PLINK (v1.9) according to protocols established in prior CLSA publications (Forgetta et al. 2022). Genetic markers were excluded based on the following criteria: genotype frequency discordance between batches and across control replicates; deviation from Hardy-Weinberg equilibrium (HWE) with a p-value threshold of 3.15×10^{-10}; insertions/deletions (indels); genotype missingness greater than 5%; and variants with minor allele frequency (MAF) ≤ 0.01. Sample exclusions were based on the following: individuals with familial relatedness of 3rd degree or closer (determined by identity-by-descent (IBD) analysis); heterozygosity outliers; discordance between self-reported and chromosomal sex; and samples with more than 5% missing genotypes. Furthermore, imputed variants with a quality score above 0.8 were included in the analysis (Han et al. 2021). To minimize the impact of population stratification in this study, only individuals of European ancestry were included, as

previously identified by the CLSA based on genetic principal component analysis (PCA) (Forgetta et al. 2022). SNPs within *TAS2R* genes were extracted using PLINK (v1.9) (Purcell et al. 2007), based on their GRCh38 coordinates retrieved from the National Center for Biotechnology Information (NCBI) Gene database (Sayers et al. 2022). Annotations were retrieved using the Ensembl Variant Effect Predictor (VEP) (McLaren et al. 2016).

CHAPTER 4: Bitter taste genetics and oral health in Canadian Longitudinal Study on Aging

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4.1 Abstract

This study aimed to investigate the association of SNPs in 25 *TAS2R* genes and 12 *TAS2R* pseudogenes with self-reported oral health outcomes in the CLSA cohort. Following quality control, 124 SNPs with a minor allele frequency > 0.01 and 21,991 individuals of European ancestry were included in the analysis. Fifteen SNPs in *TAS2R8, 9, 13, 14, 20,* and *50* were significantly associated with self-reported sore jaw muscles, a symptom commonly linked to temporomandibular disorders (TMDs). *TAS2R20* exhibited the highest number of associated SNPs. Structure-function analysis suggests that variants in *TAS2R20* may contribute to this symptom by altering ligand interactions. These findings highlight the potential for *TAS2R* genetic screening to identify individuals at elevated risk for TMD, supporting the development of personalized treatment strategies and advancing our understanding of TMD genetic risk factors.

4.2 Introduction

Given the role of T2Rs in the immune response to oral pathogens, genetic variants in *TAS2R* genes can influence the composition of oral microbial communities and, consequently, susceptibility to oral diseases (de Jesus et al. 2022b, de Jesus et al. 2021). Additionally, T2Rs affect food preferences and dietary habits, which are closely related to oral health issues such as dental caries and periodontal disease (Dotson et al. 2010, Eriksson et al. 2019, Yang et al. 2024, Petrenya et al. 2024). A previous transcriptome-wide association study identified a significant relationship between the expression of certain T2Rs and the occurrence of early childhood caries (Orlova et al. 2023). Furthermore, variants in *TAS2R*s are linked to alcohol consumption and smoking, which are known risk factors for periodontal diseases (Keller et al. 2013, Mao et al. 2023, Pitiphat et al. 2003). A previous study demonstrated that individuals with specific genotypes of *TAS2R*38 have a lower risk of periodontitis (Khimsuksri et al. 2022).

Few studies are available on the relationship between *TAS2R* genetic variants and oral health conditions. Available studies focused primarily on dental caries, while other conditions remain largely unexplored (de Jesus et al. 2022b, Orlova et al. 2023, Kiliç et al. 2022, Wendell et al. 2010, Khimsuksri et al. 2022, AlMarshad et al. 2021). Most of these studies have concentrated on the *TAS2R*38 variants (Kiliç et al. 2022, Wendell et al. 2010, Khimsuksri et al. 2022, AlMarshad et al. 2021). Research on the association between dental caries and other *TAS2R* types is limited and has yielded conflicting results (de Jesus et al. 2022b, Orlova et al. 2023). Furthermore, the association between other oral health problems and *TAS2R* variants remains poorly understood.

This study investigated the allele distributions of *TAS2R* SNPs in the CLSA dataset and evaluated their association with various self-reported oral health symptoms within the cohort. Additionally, a structure–function analysis of T2R20 was conducted to explore the potential impact of the variants on ligand interactions.

4.3 Materials and Methods

4.3.1 Oral health data in CLSA

The study design and statistical analysis process are summarized in **Figure 4.1**. Oral health data were obtained from the CLSA Comprehensive Baseline Dataset, collected between 2011 and 2015. The CLSA oral health questionnaire, derived from the Canadian Community Health Survey 2.1, is based on self-reported oral health indicators with demonstrated good construct and concurrent validity, internal consistency, and reliability greater than 0.7 (Locker and Miller 1994, Bassim, MacEntee, et al. 2020). The questions and response values are summarized in **Table 4.1**. In addition to the 20 variables directly obtained from the questionnaire, periodontal disease was defined as the report of at least one loose tooth and/or bleeding gingiva. This definition was validated against prevalence data from the literature. For the genetic association analyses, using PLINK, categorical variables with more than two categories were dichotomized as follows: general oral health (good: excellent, very good, good vs. poor: fair, poor); and avoided eating and uncomfortable eating (yes: often, sometimes vs. no: rarely, never).

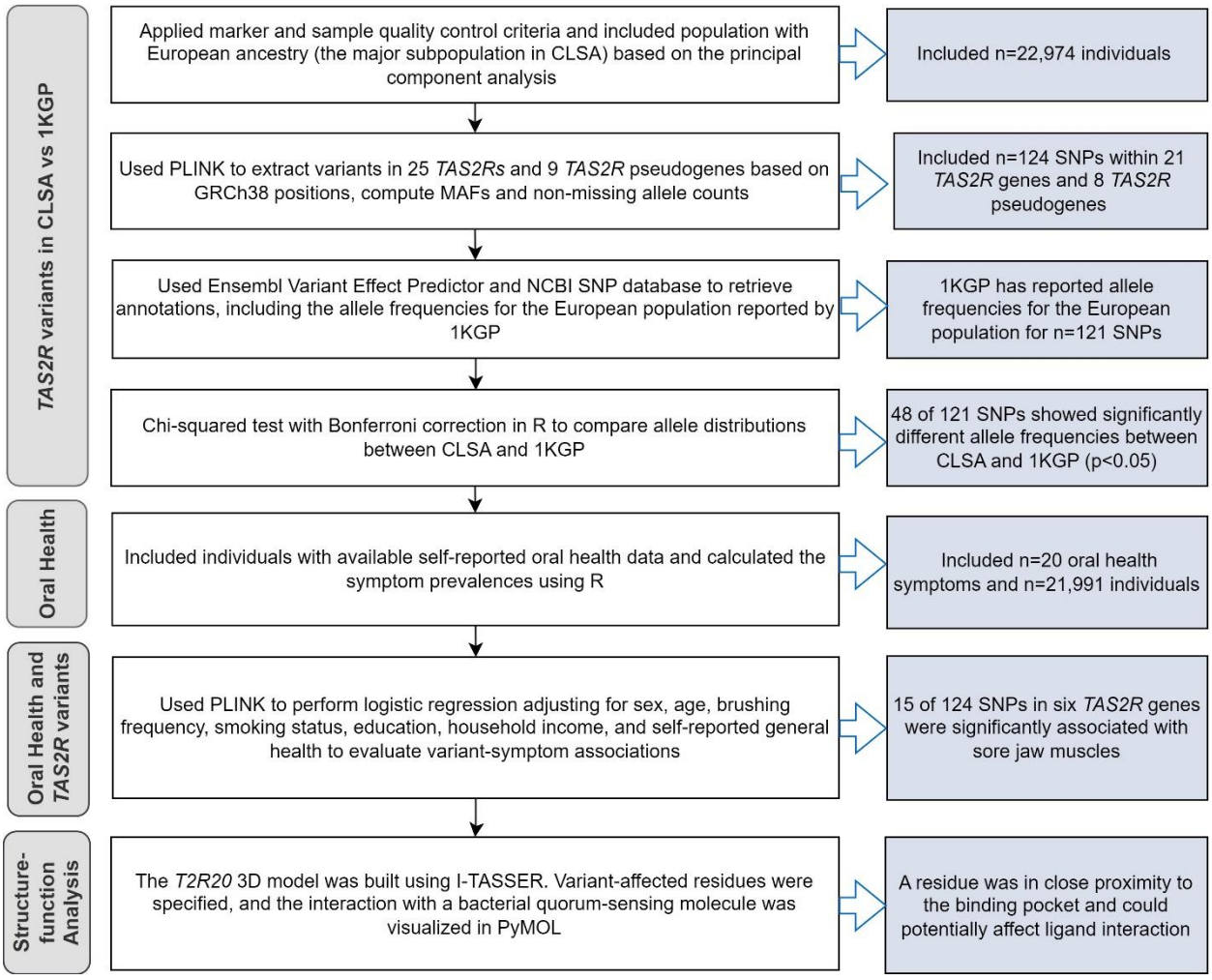

Figure 4.1 Study pipeline for analysis of *TAS2R* genetic variants in CLSA.

SNP: single nucleotide polymorphism; MAF: minor allele frequency; 1KGP: 1000 Genomes Project.

Table 4.1 CLSA questionnaire for assessing oral health symptoms.

Oral health symptoms	Answers	Question[a]
General oral health[b]	1=excellent, 2=very good, 3=good, 4=fair, 5=poor	In general, would you say the health of your mouth is excellent, very good, good, fair, or poor?
Oral health problems - Toothache	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Cannot chew adequately	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Swelling in mouth	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Dry mouth	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Burning mouth	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Sore jaw muscles	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Painful jaw joints	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Decayed natural tooth	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Loose natural tooth	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Broken natural tooth	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Sore gums around natural teeth	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Bleeding gums around natural teeth	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Denture-related sores	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Dirty teeth or dentures	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems - Bad breath	1=yes, 0=no	Have you experienced this in the past 12 months?
Oral health problems – No oral health problems	1=yes, 0=no	Have you experienced this in the past 12 months?
Has one or more of the original teeth	1=yes, 2=no	Do you have one or more of your original teeth?
Uncomfortable eating in last 12 months[b]	1=often, 2=sometimes, 3= rarely, 4= never	In the past 12 months, how often have you found it uncomfortable to eat any food because of problems with your mouth?
Avoided eating in last 12 months[b]	1=often, 2=sometimes, 3= rarely, 4= never	In the past 12 months, how often have you avoided eating particular foods because of problems with your mouth?

[a] Questions asked from the participants for each of the variables.

[b] For statistical analyses, categorical variables with more than two values were dichotomized as follows: general oral health: good (excellent, very good, good) vs. poor (fair, poor); avoided eating and uncomfortable eating: yes (often, sometimes) vs. no (rarely, never).

4.3.2 Statistical analysis

4.3.2.1 *TAS2R* allele distributions in CLSA and 1KGP

Allele frequencies and non-missing allele counts were calculated using PLINK for the CLSA population of European ancestry. Chi-squared tests were applied using R (v4.3.2) (R Core Team 2021) to compare the allelic distributions with those reported for the European population in the 1000 Genomes Project (1KGP) (Auton et al. 2015). Bonferroni correction was applied by multiplying p-values by the number of independent markers in linkage equilibrium ($r^2 < 0.1$ within a 1000 kb window, n=35) (Forgetta et al. 2022). $P<0.05$ was considered statistically significant. Scatter plots of allele frequencies were generated using the ggplot2 package in R (Wickham 2016). Pairwise linkage disequilibrium (LD) between the SNPs was visualized using the corrplot package.

4.3.2.2 *TAS2R* SNPs and oral health symptoms

Individuals with available oral health data were included in the genetic association analysis, with 87 excluded due to conflicting data, as they reported both an absence of natural tooth and tooth-related problems simultaneously. The prevalence of oral health symptoms was summarized using R. Patients with edentulism were excluded from the analysis of tooth-related phenotypes. Logistic regression analysis was conducted using an additive genetic model in PLINK to identify variant-disease associations, adjusting for sex, age, smoking status, brushing frequency, self-reported general health, education, and household income. Correlation analysis was performed to ensure the absence of multicollinearity between the covariates. Manhattan plots were generated using ggplot2 package to visualize the results. P-values were adjusted using Bonferroni correction based on the number of independent markers multiplied by the number of independent phenotypes, with a significance threshold of $P<0.05$. Given the moderate to strong correlations observed among most phenotypes (Phi coefficient > 0.1, **Figure 4.2**), the number of independent phenotypes was considered as 1 for the Bonferroni correction (Akoglu 2018). RNAsnp web server was used to predict the impact of the variants on mRNA structure (RTH). To validate the findings in an independent cohort, the associated variants were examined in genome-wide association study (GWAS) results from the UK Biobank Imputed Version 3 dataset, generated by the Neale Lab, to determine whether they are linked to relevant phenotypes (Bycroft et al. 2018).

4.3.3 Structure-function analysis

To illustrate the impact of the variants on protein structure, a 2-dimensional model of T2R20 was used from a previous publication (Jaggupilli, Singh, et al. 2019), highlighting the amino acids affected by the variants. Additionally, a 3-dimensional model of T2R20 was used, constructed using I-TASSER as described in a previous study (Jaggupilli et al. 2018). I-TASSER is a hierarchical method for predicting protein structure (Yang and Zhang 2015). Energy minimization of the T2R20 structure and ligand docking with the bacterial quorum sensing molecule 3-oxo-C12-Acyl homoserine lactone (AHL) were performed using Maestro v11.0 (Schrödinger, LLC, New York), as previously described (Jaggupilli, Singh, et al. 2019). The interactions between T2R20 residues and AHL within the binding pocket, along with residues affected by the CLSA variants, were visualized using PyMOL molecular graphics software v3.0 (Schrödinger, LLC, New York). The distances of residues from the binding site were calculated using the measurement wizard in PyMOL.

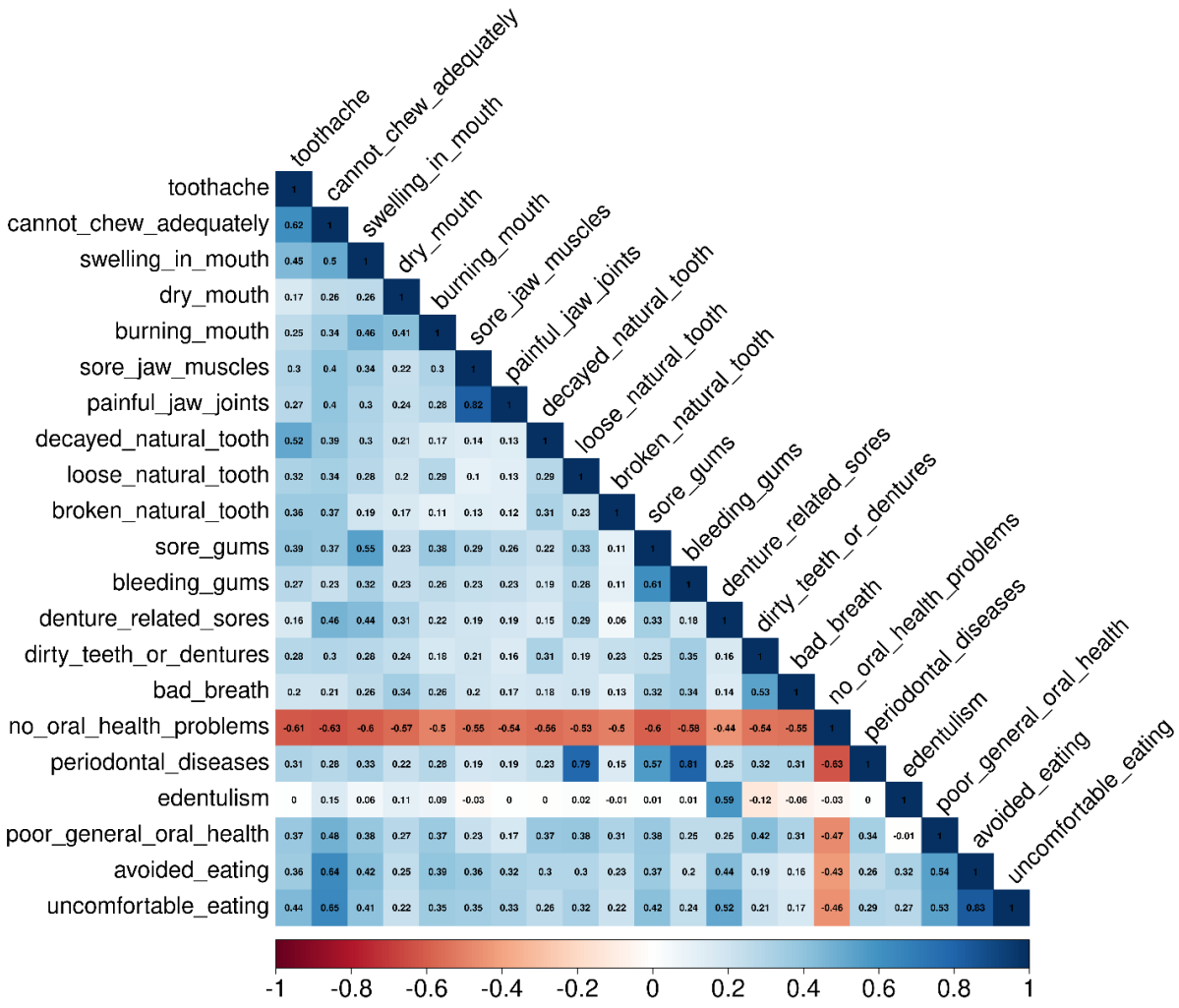

Figure 4.2 Correlation between the included outcomes.

Values are based on the Phi Coefficient (Mean Square Contingency Coefficient) for binary phenotypes.

4.4 Results

4.4.1 Allele distributions of *TAS2R* SNPs in CLSA and 1KGP

A total of 1,171 non-monomorphic variants occurring within *TAS2R* genes and pseudogenes were extracted from the CLSA. Following the application of quality control criteria, 124 SNPs and 22,974 individuals of European ancestry were included in the allele distribution analyses. Of these SNPs, 87 were located in *TAS2R* genes and 37 in *TAS2R* pseudogenes. Detailed annotations are provided in **Table 4.2**. Notably, *TAS2R20* had the highest number of SNPs (n=15), followed by *TAS2R4* (n=12), *TAS2R42* (n=8), *TAS2R14* (n=7), and *TAS2R15P* (n=7). The distribution of functional consequences was as follows: missense (n=38), 3' untranslated region (UTR) (n=20), synonymous (n=19), 5' UTR (n=9), and stop-gained (n=1; in *TAS2R19*).

Allele frequencies for 121 out of 124 SNPs identified in the CLSA dataset were available for the European population in 1KGP. Minor allele frequencies in CLSA and the p-values from the chi-squared tests comparing them with the corresponding allele frequencies in the 1KGP European population are illustrated in **Figure 4.3** with details provided in **Table S4.1**. Chi-squared analysis revealed significant differences in allele frequency for 48 out of 121 SNPs between the two databases (**Table 4.2**). Among these, 15 were missense variants, 14 were located in pseudogenes, 7 in untranslated regions, and 12 were synonymous variants. The highest proportion of variants with significantly different allele distributions was observed in *TAS2R15P*, where all seven variants exhibited differences (**Table 4.3**). *TAS2R20* also showed a substantial proportion, with significant differences in 11 out of 15 variants (73%). Pairwise linkage disequilibrium between the variants is shown in heatmaps in **Figures 4.4-4.6**.

Table 4.2 *TAS2R* SNPs with different allele distributions in CLSA compared to 1KGP (adjusted $P < 0.05$).
Data are presented as minor allele frequencies (MAF) in CLSA European ethnicity and corresponding allele frequencies (AF) in 1KGP European population.

rs ID	SNP[a]	MA[b]	Gene	Consequence	Amino Acid Change	CLSA MAF	1KGP AF	Adjusted p-value[c]
rs2234235	chr5:9629183:A:G	G	*TAS2R1*	Synonymous	Leu 284	0.03	0.05	0.021
rs619381	chr12:10801659:C:T	T	*TAS2R7*	Missense	Met 304 Ile	0.12	0.09	<0.0001
rs1548803	chr12:10806432:C:T	C	*TAS2R8*	Synonymous	Leu 183	0.41	0.32	<0.0001
rs3741845	chr12:10809516:A:G	A	*TAS2R9*	Missense	Ala 187 Val	0.40	0.32	<0.0001
rs11054065	chr12:10895780:G:A	A	*TAS2R12P*	NA	-	0.02	0.01	0.023
rs1015442	chr12:10908096:T:C	T	*TAS2R13*	3 prime UTR	-	0.43	0.38	0.000
rs1015443	chr12:10908523:T:C	T	*TAS2R13*	Missense	Ser 259 Asn	0.43	0.38	0.000
rs3851584	chr12:10937478:G:T	G	*TAS2R14*	3 prime UTR	-	0.43	0.38	0.000
rs3851585	chr12:10937794:C:G	G	*TAS2R14*	3 prime UTR	-	0.02	0.03	0.010
rs3936285	chr12:10937839:T:A	A	*TAS2R14*	3 prime UTR	-	0.02	0.03	0.010
rs16925868	chr12:10938952:T:C	C	*TAS2R14*	Missense	Thr 86 Ala	0.02	0.03	0.012
rs7138535	chr12:10939094:T:A	A	*TAS2R14*	Synonymous	Gly 38	0.25	0.22	0.006
rs11054092	chr12:10964560:T:C	C	*TAS2R15P*	NA	-	0.25	0.22	0.007
rs11054093	chr12:10964615:G:C	C	*TAS2R15P*	NA	-	0.25	0.22	0.007
rs11054094	chr12:10964693:G:A	A	*TAS2R15P*	NA	-	0.25	0.22	0.007
rs4763599	chr12:10964722:A:G	G	*TAS2R15P*	NA	-	0.25	0.22	0.007
rs17810798	chr12:10964833:G:C	C	*TAS2R15P*	NA	-	0.25	0.22	0.007
rs11054095	chr12:10965032:G:C	C	*TAS2R15P*	NA	-	0.25	0.22	0.008
rs11054096	chr12:10965197:T:C	C	*TAS2R15P*	NA	-	0.25	0.22	0.007
rs7296270	chr12:11158991:A:T	A	*TAS2R18P*	NA	-	0.16	0.13	0.011
rs61928604	chr12:11159348:C:T	C	*TAS2R18P*	NA	-	0.16	0.13	0.018
rs2290318	chr12:11159359:G:C	C	*TAS2R18P*	NA	-	0.32	0.36	0.005
rs2290319	chr12:11159427:C:A	A	*TAS2R18P*	NA	-	0.32	0.36	0.005
rs1450839	chr12:10996933:A:G	G	*TAS2R20*	3 prime UTR	-	0.33	0.38	0.000
rs10845279	chr12:10997112:C:A	A	*TAS2R20*	Missense	Arg 255 Leu	0.33	0.38	0.000
rs10845280	chr12:10997121:A:G	G	*TAS2R20*	Missense	Phe 252 Ser	0.33	0.38	0.000
rs10845281	chr12:10997170:T:C	C	*TAS2R20*	Missense	Ile 236 Val	0.33	0.38	0.000
rs12226919	chr12:10997434:G:T	T	*TAS2R20*	Missense	His 148 Asn	0.33	0.38	0.000
rs12226920	chr12:10997447:G:T	T	*TAS2R20*	Missense	His 143 Gln	0.33	0.38	0.000
rs79420812	chr12:10997455:C:T	T	*TAS2R20*	Missense	Val 141 Ile	0.19	0.22	0.001
rs11054142	chr12:10997615:G:A	A	*TAS2R20*	Synonymous	Ala 87	0.33	0.38	0.000
rs11054143	chr12:10997720:T:C	C	*TAS2R20*	Synonymous	Ala 52	0.33	0.38	0.000

rs ID	SNP[a]	MA[b]	Gene	Consequence	Amino Acid Change	CLSA MAF	1KGP AF	Adjusted p-value[c]
rs1463237	chr12:10997980:C:T	C	*TAS2R20*	5 prime UTR	-	0.16	0.14	0.049
rs7301234	chr12:10998285:G:A	A	*TAS2R20*	5 prime UTR	-	0.33	0.38	0.000
rs112849631	chr12:11133780:T:C	C	*TAS2R30*	Synonymous	Thr 155	0.02	0.03	0.004
rs116737741	chr12:11030592:T:C	C	*TAS2R31*	Synonymous	Ser 248	0.15	0.12	0.002
rs76518630	chr7:143478409:C:T	T	*TAS2R41*	Synonymous	Tyr 179	0.01	0.02	0.035
rs1650017	chr12:11186007:G:C	C	*TAS2R42*	Missense	Pro 311 Ala	0.16	0.13	0.011
rs1669411	chr12:11186008:G:A	A	*TAS2R42*	Synonymous	Asn 310	0.16	0.13	0.011
rs1669413	chr12:11186175:A:C	C	*TAS2R42*	Missense	Trp 255 Gly	0.16	0.13	0.011
rs5020531	chr12:11186351:A:G	A	*TAS2R42*	Missense	Ser 196 Phe	0.41	0.35	<0.0001
rs1650019	chr12:11186377:C:T	T	*TAS2R42*	Synonymous	Leu 187	0.16	0.13	0.011
rs35969491	chr12:11186414:T:A	T	*TAS2R42*	Missense	Phe 175 Tyr	0.41	0.35	<0.0001
rs10772397	chr12:10986084:C:T	C	*TAS2R50*	Synonymous	Pro 259	0.40	0.34	<0.0001
rs1376251	chr12:10986253:C:T	T	*TAS2R50*	Missense	Cys 203 Tyr	0.32	0.36	0.003
rs66679979	chr12:10986336:T:C	C	*TAS2R50*	Synonymous	Ser 175	0.15	0.13	0.018
rs319269	chr12:11179986:A:C	C	*TAS2R67P*	Downstream gene (SMIM10L1)	-	0.16	0.13	0.011
rs34648613	chr12:11180118:T:A	T	*TAS2R67P*	Downstream gene (SMIM10L1)	-	0.41	0.35	<0.0001

[a] SNP = single nucleotide polymorphism; Position in GRCh38 shown as chromosome:base pair position: reference allele: alternative allele

[b] Minor allele in CLSA

[c] Chi-squared test with Bonferroni correction.

CLSA = Canadian Longitudinal Study on Aging; 1KGP = 1000 Genomes Project.

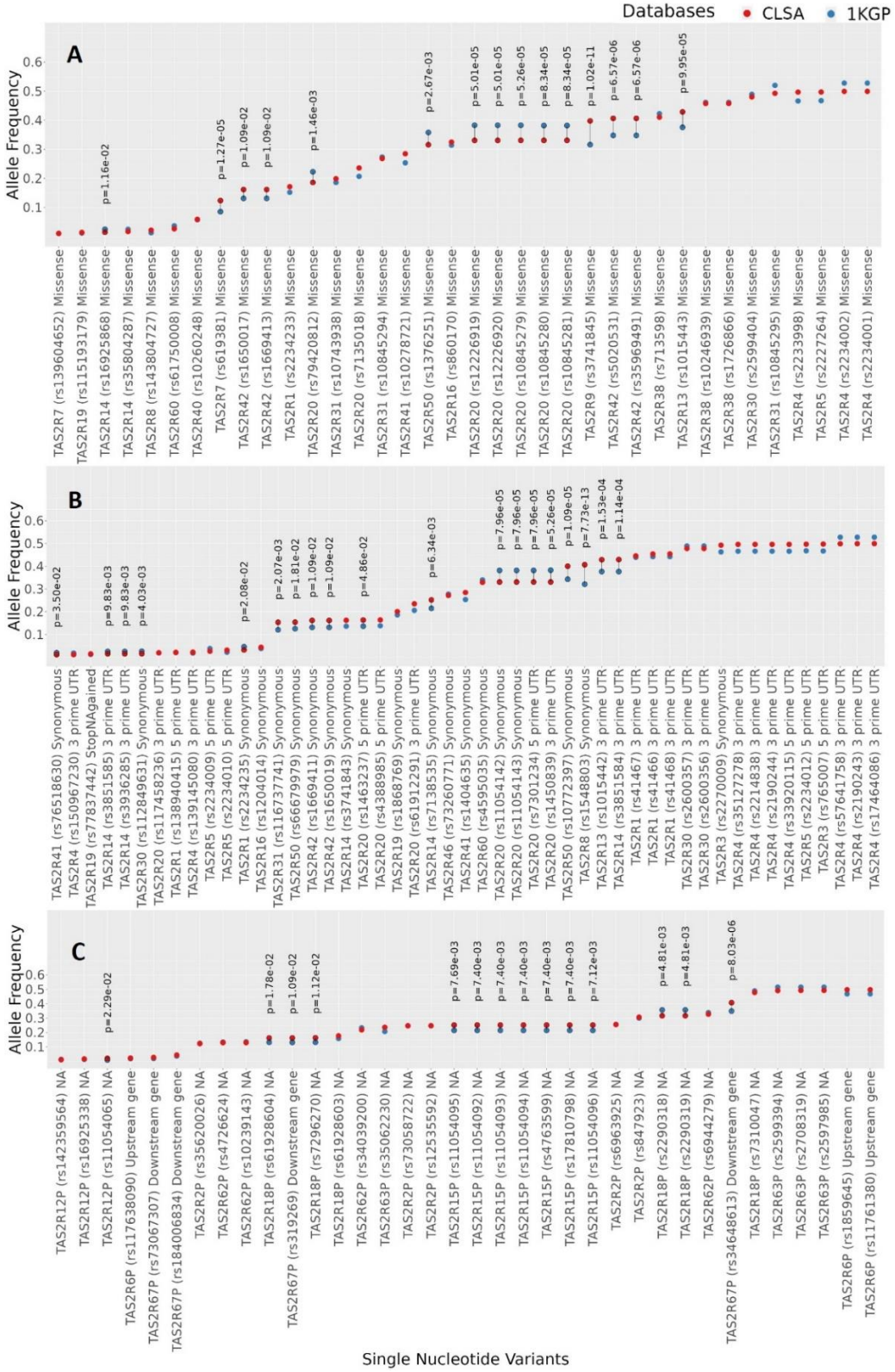

Figure 4.3 Allele distributions of *TAS2R* SNPs in CLSA and 1KGP European populations. Dots represent the MAF of missense (A), Non-missense (B), and pseudogene variants (C). Significant differences (adjusted p-value < 0.05, chi-squared test) are marked with a black line.

Table 4.3 Count of SNPs with different allele distributions in CLSA compared to 1KGP.

Gene	Count of SNPs in CLSA	Count of SNPs with different allele distributions[a]	Proportion of SNPs with different allele distributions[b]
TAS2R16	2	0	0.00
TAS2R19	3	0	0.00
TAS2R2	5	0	0.00
TAS2R3	2	0	0.00
TAS2R38	3	0	0.00
TAS2R4	12	0	0.00
TAS2R40	1	0	0.00
TAS2R46	1	0	0.00
TAS2R5	4	0	0.00
TAS2R60	2	0	0.00
TAS2R62P	4	0	0.00
TAS2R63P	4	0	0.00
TAS2R6P	3	0	0.00
TAS2R7	2	0	0.00
TAS2R8	2	0	0.00
TAS2R9	1	0	0.00
TAS2R1	6	1	0.17
TAS2R67P	5	1	0.20
TAS2R30	4	1	0.25
TAS2R31	4	1	0.25
TAS2R12P	3	1	0.33
TAS2R41	3	1	0.33
TAS2R42	8	4	0.50
TAS2R50	3	2	0.67
TAS2R18P	6	4	0.67
TAS2R14	7	5	0.71
TAS2R20	15	11	0.73
TAS2R13	2	2	1.00
TAS2R15P	7	7	1.00

[a] The number of variants with significantly different allele distributions compared to 1KGP (Chi-squared test with Bonferroni correction)

[b] Count of variants with different allele distributions divided by the total count of variants in CLSA.

SNP = single nucleotide polymorphism; CLSA = Canadian Longitudinal Study on Aging; 1KGP = 1000 Genomes Project.

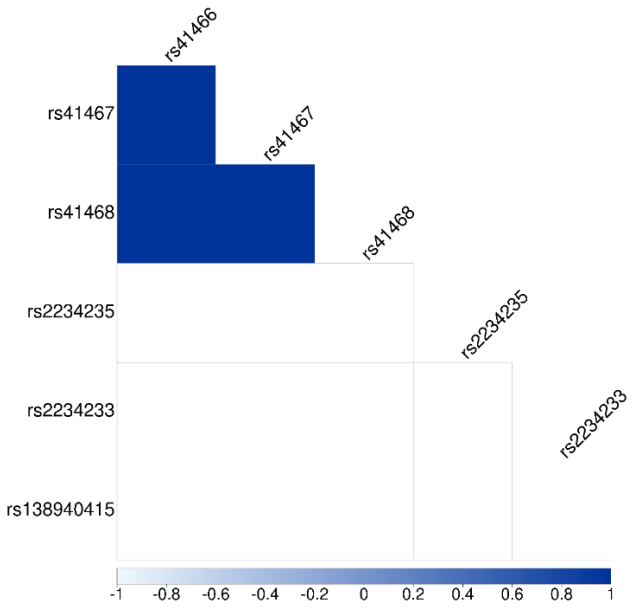

Figure 4.4 Pairwise LD between *TAS2R* variants on chromosome 5.

The color intensity indicates the strength of LD, with r^2 values ranging from 0.2 to 1, while white cells represent $r^2 < 0.2$. Variants associated with sore jaw muscles are highlighted in red.

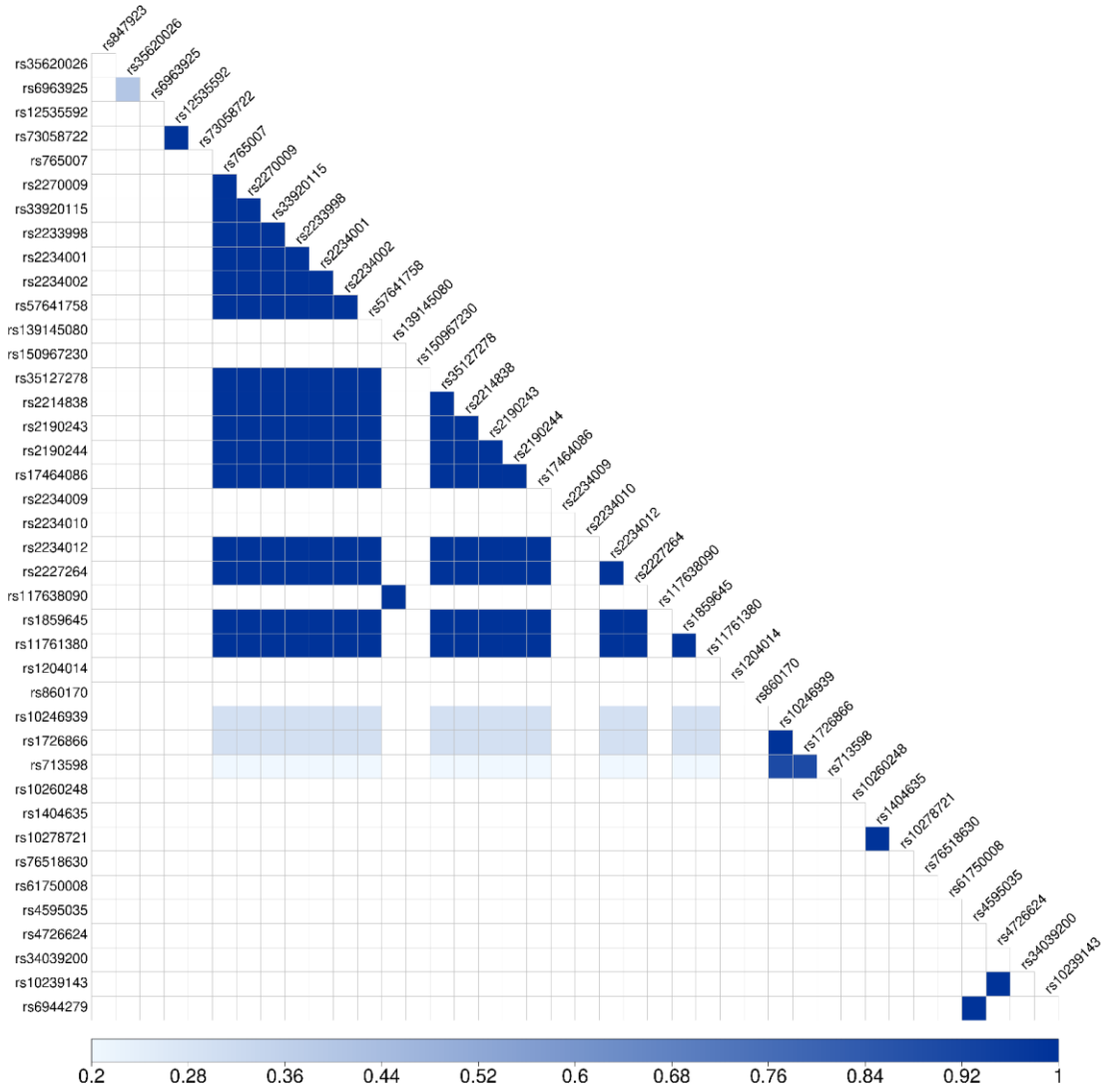

Figure 4.5 Pairwise LD between *TAS2R* variants on chromosome 7.

The color intensity indicates the strength of LD, with r^2 values ranging from 0.2 to 1, while white cells represent $r^2 < 0.2$. Variants associated with sore jaw muscles are highlighted in red.

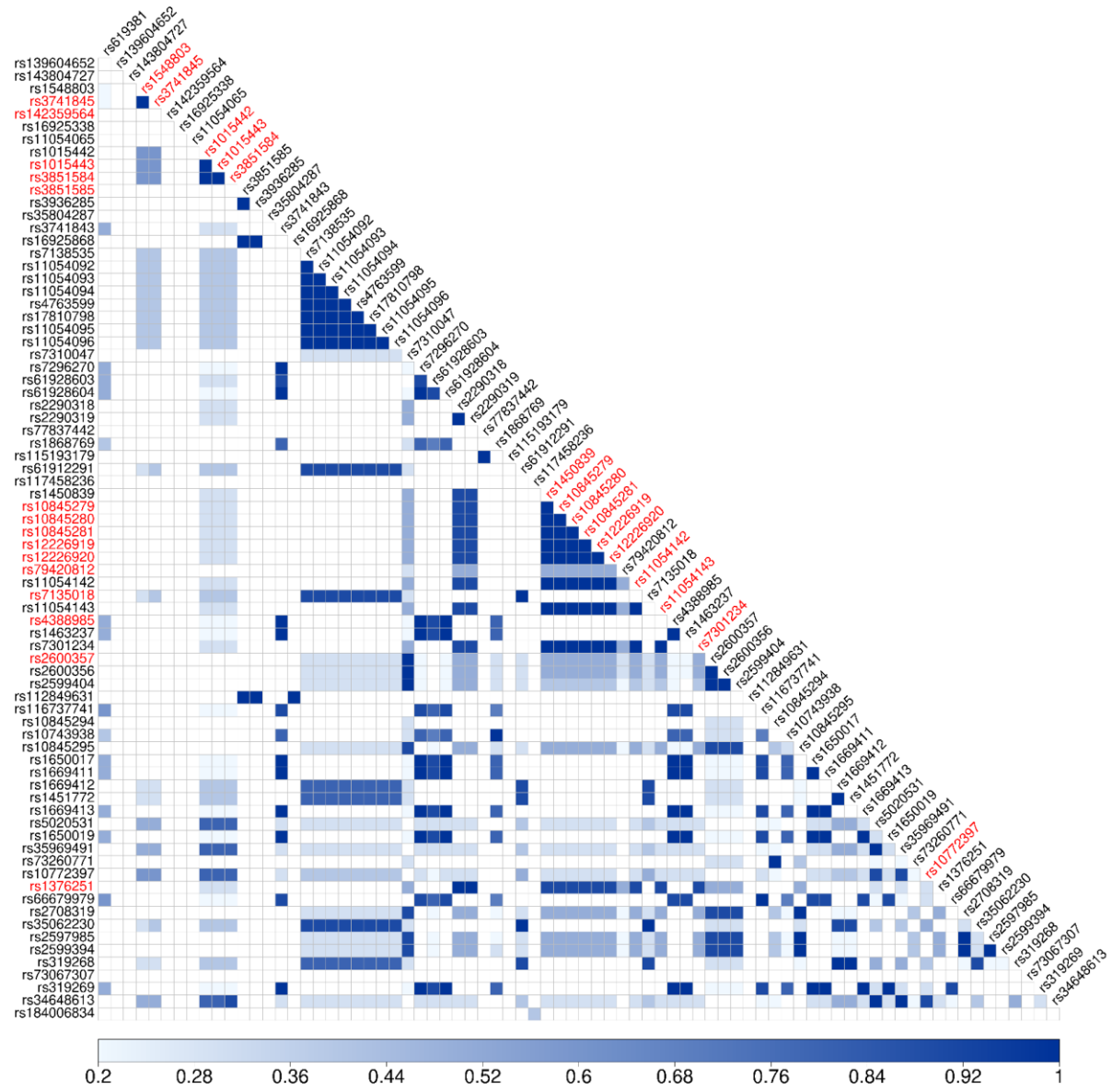

Figure 4.6 Pairwise LD between *TAS2R* variants on chromosome 12.

The color intensity indicates the strength of LD, with r^2 values ranging from 0.2 to 1, while white cells represent $r^2 < 0.2$. Variants associated with sore jaw muscles are highlighted in red.

4.4.2 Prevalence of oral health symptoms in the cohort

Following quality control of the cohort with available oral health data, 21,991 individuals were included, with a mean age of 63 years. The cohort consisted of 10,920 males and 11,071 females, as summarized in **Table 4.4**. Among the participants, 8.5% identified as current smokers, 44.3% as former smokers, and 47.1% as never having smoked. **Table 4.5** provides a summary of the prevalence of oral health symptoms in the study cohort. Nearly 42.6% of the population reported no oral health problems. The most prevalent condition in the cohort was dry mouth, affecting 17.7% of individuals, followed by periodontal disease (14.6%) and decayed tooth (14.3%). Overall, 94.2% of the cohort reported their general oral health as excellent, very good, or good, while 5.8% rated it as fair or poor.

Certain conditions were significantly more prevalent in females ($p<0.05$), including discomfort while eating due to mouth problems, swelling in the mouth, periodontal diseases, denture-related sores, dry mouth, burning mouth, painful jaw joints, and sore jaw muscles. Notably, the latter three conditions were nearly twice as prevalent in females compared to males. Conversely, some conditions were more common in males ($p<0.05$): decayed tooth, broken tooth, toothache, bad breath, and dirty teeth or dentures. The prevalence of the remaining symptoms, including edentulism, loose tooth, and the overall presence or absence of symptoms, did not significantly differ between the sexes ($p>0.05$).

Table 4.4 Study population characteristics.

Characteristic	Males (N = 10,920)[a]	Females (N = 11,071)[a]	P-value[b]
Age	63 (55, 71)	62 (55, 70)	**0.001**
Smoking			**<0.001**
Never smoker	4,816 (44%)	5,553 (50%)	
Former smoker	5,089 (47%)	4,659 (42%)	
Current smoker	1,013 (9.3%)	859 (7.8%)	
Self-reported general health			**<0.001**
Excellent	2,228 (20%)	2,377 (21%)	
Very good	4,528 (42%)	4,836 (44%)	
Good	3,235 (30%)	2,976 (27%)	
Fair	763 (7.0%)	726 (6.6%)	
Poor	155 (1.4%)	149 (1.3%)	
Education			**<0.001**
Less than secondary school	507 (4.7%)	610 (5.5%)	
Secondary school graduation	908 (8.3%)	1,177 (11%)	
Some post-secondary education	793 (7.3%)	852 (7.7%)	
Post-secondary degree/diploma	8,689 (80%)	8,419 (76%)	
Household income			**<0.001**
Less than $20,000	335 (3.2%)	638 (6.2%)	
$20,000 to less than $50,000	1,761 (17%)	2,657 (26%)	
$50,000 to less than $100,000	3,779 (36%)	3,618 (35%)	
$100,000 to less than $150,000	2,356 (23%)	1,826 (18%)	
$150,000 or more	2,185 (21%)	1,502 (15%)	
Brushing frequency			**<0.001**
Less than once daily	1,043 (9.6%)	843 (7.6%)	
Once daily	2,802 (26%)	1,472 (13%)	
Twice daily or more	7,059 (65%)	8,749 (79%)	

[a] Median (IQR); n (%)

[b] Wilcoxon rank sum test; Pearson's Chi-squared test. Significant values are shown in bold ($p < 0.05$).

Table 4.5 Prevalence of oral health symptoms in the CLSA European ancestry cohort.

Characteristic	Males (N = 10,920) [a]	Females (N = 11,071) [a]	P-value [b]
General oral health			**<0.0001**
Excellent	3,414 (31%)	3,958 (36%)	
Very good	4,407 (40%)	4,318 (39%)	
Good	2,399 (22%)	2,210 (20%)	
Fair	545 (5.0%)	447 (4.0%)	
Poor	141 (1.3%)	120 (1.1%)	
Uncomfortable eating in last 12 months			**<0.0001**
Often	193 (1.8%)	296 (2.7%)	
Sometimes	717 (6.6%)	931 (8.4%)	
Rarely	2,135 (20%)	2,197 (20%)	
Never	7,857 (72%)	7,631 (69%)	
Avoided eating in last 12 months			**<0.0001**
Often	143 (1.3%)	213 (1.9%)	
Sometimes	399 (3.7%)	634 (5.7%)	
Rarely	1,031 (9.4%)	1,313 (12%)	
Never	9,330 (85%)	8,893 (80%)	
Edentulism	629 (5.8%)	686 (6.2%)	0.1160
Toothache	1,410 (13%)	1,306 (12%)	**0.0460**
Cannot chew adequately	799 (7.3%)	933 (8.4%)	**0.0013**
Swelling in the mouth	465 (4.3%)	535 (4.8%)	**0.0269**
Dry mouth	1,689 (15%)	2,196 (20%)	**<0.0001**
Burning mouth	102 (0.9%)	207 (1.9%)	**<0.0001**
Sore jaw muscles	396 (3.6%)	788 (7.1%)	**<0.0001**
Painful jaw joints	404 (3.7%)	868 (7.8%)	**<0.0001**
Decayed natural tooth	1,798 (16%)	1,352 (12%)	**<0.0001**
Loose natural tooth	510 (4.7%)	522 (4.7%)	0.7087
Broken natural tooth broken	1,278 (12%)	1,124 (10%)	**0.0017**
Sore gums around natural teeth	754 (6.9%)	894 (8.1%)	**0.0006**
Bleeding gums around natural teeth	1,094 (10%)	1,315 (12%)	**<0.0001**
Denture-related sores	203 (1.9%)	262 (2.4%)	**0.0062**
Dirty teeth or dentures	386 (3.5%)	239 (2.2%)	**<0.0001**
Bad breath	823 (7.5%)	735 (6.6%)	**0.0258**
Periodontal disease (bleeding gums or loose tooth)	1,508 (14%)	1,700 (15%)	**0.0007**
No oral health problems	4,754 (44%)	4,618 (42%)	0.1600

[a] N (%)

[b] Pearson's Chi-squared test. Significant values are shown in bold ($p < 0.05$).

4.4.3 *TAS2R* SNPs are associated with sore jaw muscles

Logistic regression analysis, controlling for sex, age, brushing frequency, smoking status, education, household income, and self-reported general health, revealed a significant association between the 15 variants and sore jaw muscles, as summarized in **Table 4.6** and visualized in **Figure 4.7**. Other variants and symptoms showed no significant association after adjusting for P-values (**Figure S4.1**). The associated variants were examined in the GWAS results from the UK Biobank dataset, which identified six alleles associated with rheumatoid arthritis (**Table 4.7)**. The RNAsnp web server predicted that 7 of the 15 associated SNPs may affect mRNA secondary structure (**Figure 4.8**). The remaining 8 SNPs were not predicted to impact mRNA secondary structure (**Figure S4.2**).

Table 4.6 Significant association between *TAS2R* variants and self-reported sore jaw muscles. Based on the logistic regression results adjusted for sex, age, smoking status, tooth brushing, self-reported general health, education, and income.

rs ID	Gene	Allele[a]	Consequence	P[b]	OR [95% CI]	Predicted Effect
rs1548803	*TAS2R8*	C	Synonymous	0.031	1.16[1.06,1.27]	LD (rs3741845 r^2=0.96)
rs3741845	*TAS2R9*	A	Missense Ala187Val	0.041	1.16[1.06,1.26]	mRNA, Protein
rs1015442	*TAS2R13*	T	3'UTR	0.039	1.16[1.06,1.26]	mRNA, LD (rs1015443, r^2=0.96)
rs1015443	*TAS2R13*	T	Missense Ser259Asn	0.043	1.15[1.06,1.26]	mRNA, Protein
rs3851584	*TAS2R14*	G	3'UTR	0.032	1.16[1.06,1.26]	mRNA, LD (rs1015443, $r^2\approx1$)
rs10772397	*TAS2R50*	C	Synonymous	0.023	1.16[1.07,1.27]	mRNA
rs1450839	*TAS2R20*	G	3'UTR	0.036	0.85[0.78,0.94]	mRNA, LD ($r^2\approx1$)[c]
rs10845279	*TAS2R20*	A	Missense Arg255Leu	0.036	0.85[0.78,0.94]	Protein
rs10845280	*TAS2R20*	G	Missense Phe252Ser	0.035	0.85[0.78,0.94]	Protein
rs10845281	*TAS2R20*	C	Missense Ile236Val	0.036	0.85[0.78,0.94]	Protein
rs12226919	*TAS2R20*	T	Missense His148Asn	0.036	0.85[0.78,0.94]	Protein
rs12226920	*TAS2R20*	T	MissenseHis143Gln	0.036	0.85[0.78,0.94]	mRNA, Protein
rs11054142	*TAS2R20*	A	Synonymous	0.037	0.85[0.78,0.94]	LD ($r^2\approx1$)[c]
rs11054143	*TAS2R20*	C	Synonymous	0.037	0.85 [0.78, 0.94]	LD ($r^2\approx1$)[c]
rs7301234	*TAS2R20*	A	5'UTR	0.037	0.85 [0.78, 0.94]	LD ($r^2\approx1$)[c]

[a] Effect allele

[b] P-value adjusted with Bonferroni correction

[c] All nine variants in *TAS2R20* exhibit LD with one another ($r^2\approx1$).

OR: odds ratio; CI: Confidence interval; LD: Linkage disequilibrium. Detailed LD information is provided in Figures 4.4-6, and predicted changes in mRNA secondary structures are shown in Figures 4.9-10.

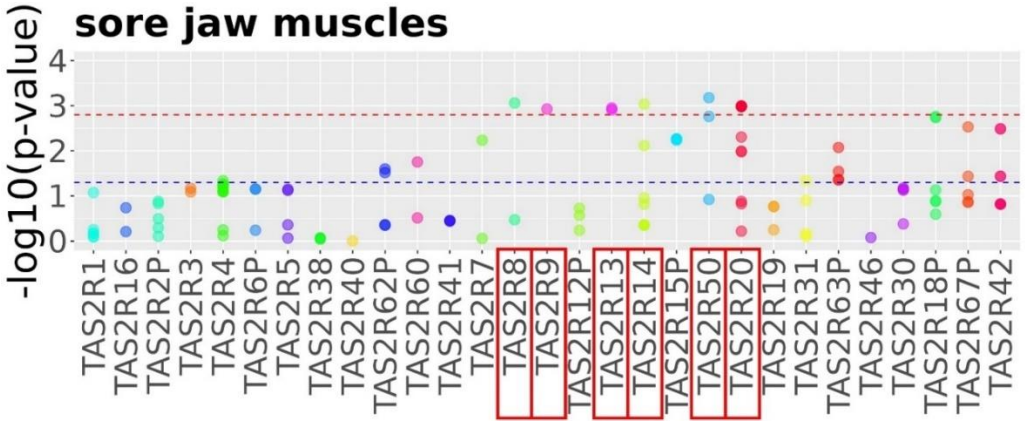

Figure 4.7 Association between *TAS2R* SNPs and self-reported sore jaw muscles in CLSA. The blue line represents the significance threshold (p = 0.05), while the red line marks the Bonferroni-corrected threshold, with dots above this line indicating SNPs with significant associations (logistic regression). Genes containing these associated SNPs are highlighted with red rectangles.

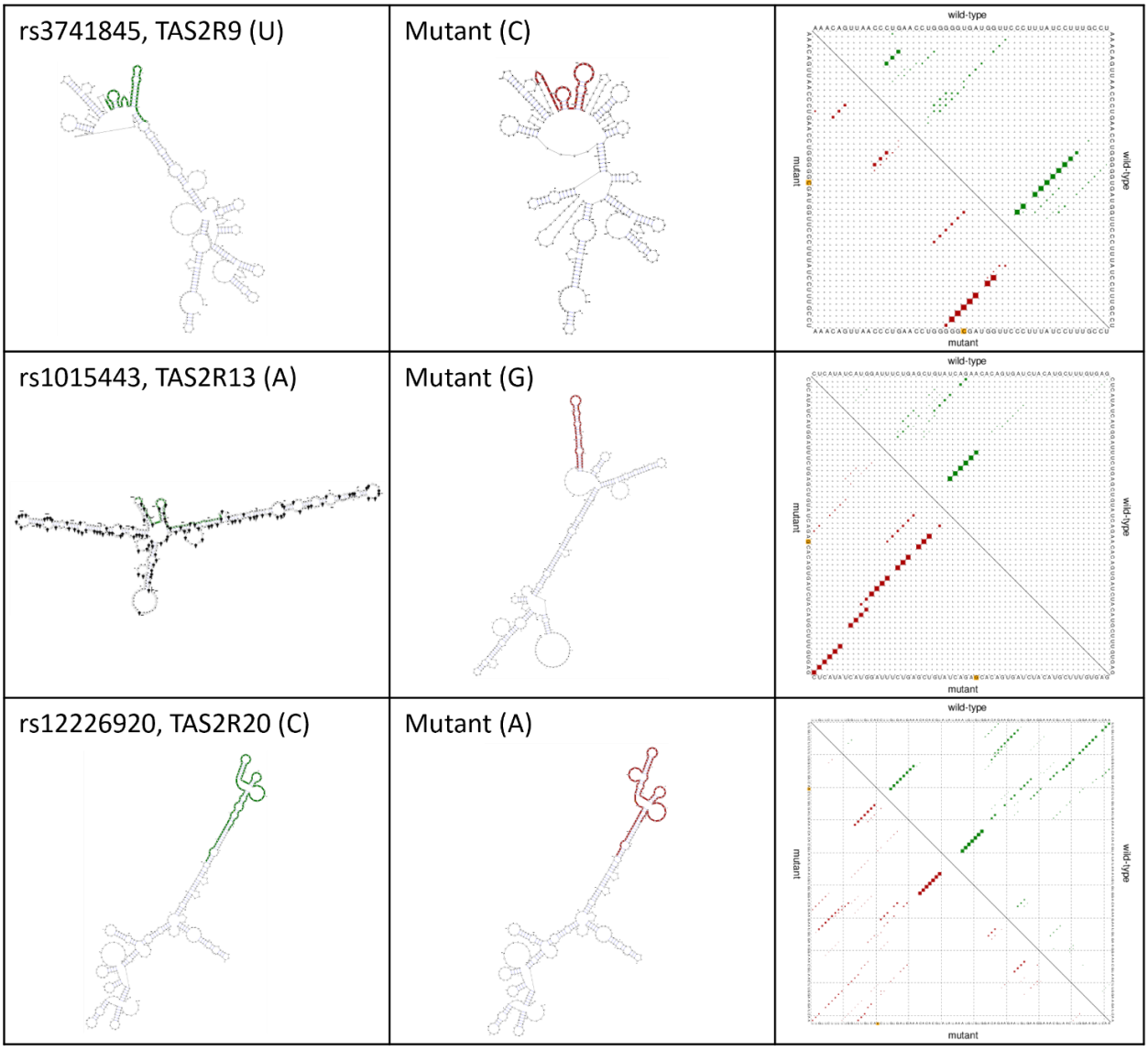

Figure 4.8 RNAsnp predicted changes in mRNA secondary structure for seven variants.

The upper triangle of the matrices represents base pair probabilities for the wild-type local region, and the lower triangle represents those for the mutant sequence.

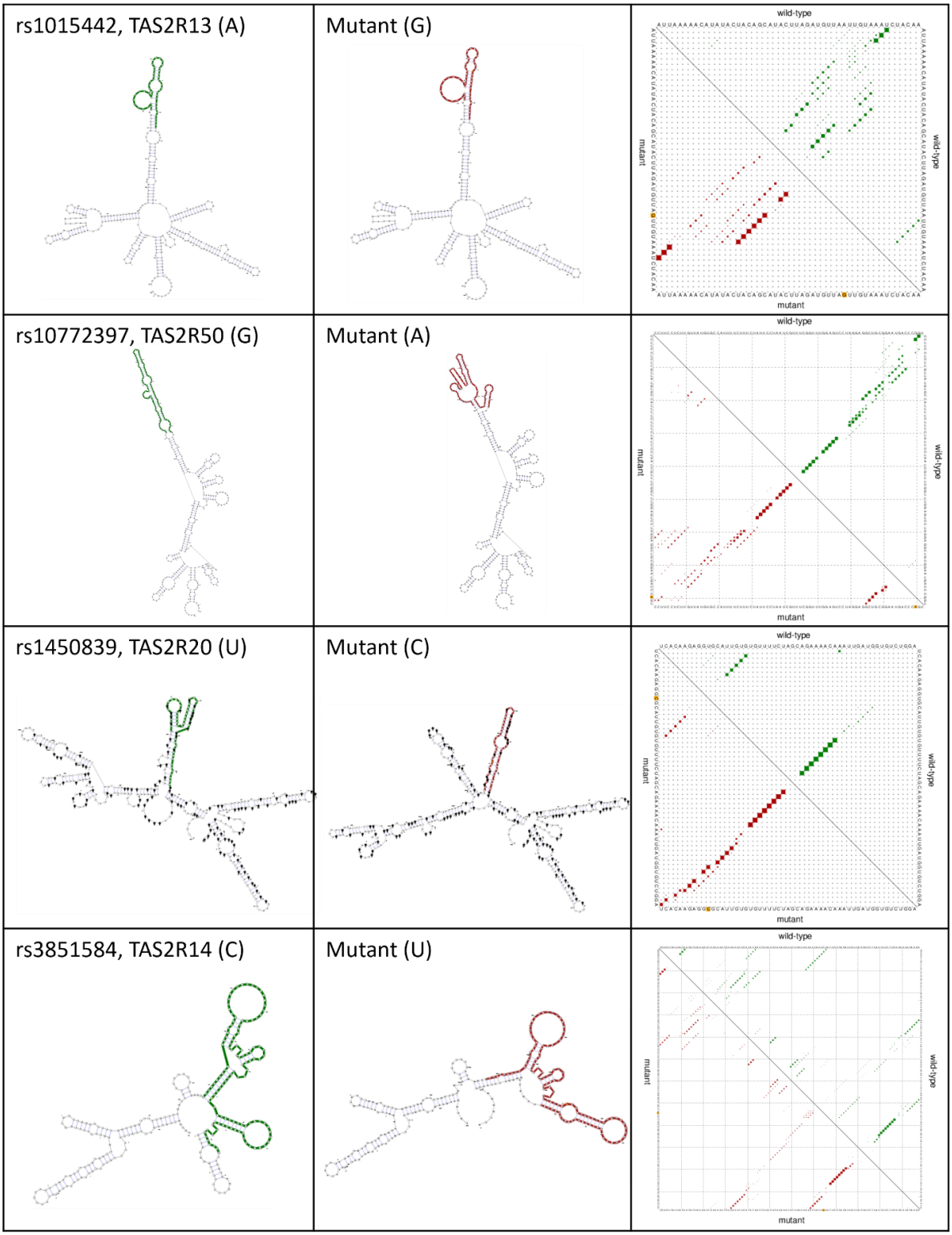

Figure 4.8 (continued) RNAsnp predicted changes in mRNA secondary structure for seven variants.

The upper triangle of the matrices represents base pair probabilities for the wild-type local region, and the lower triangle represents those for the mutant sequence.

Table 4.7 Association of *TAS2R* SNPs linked to sore jaw muscles in the current study with rheumatoid arthritis in UK Biobank (Imputed Version 3).

rs ID	Gene	Minor allele	MAC[a]	Expected MAC[a]	beta	Standard error	P-value[b]
rs1548803	*TAS2R8*	C	4852.47	3313.85	0.000774	0.000251	**0.00202**
rs3741845	*TAS2R9*	A	4933	3238.03	0.000806	0.000252	**0.001361**
rs1015442	*TAS2R13*	T	4646.85	3502.98	0.000665	0.000249	**0.007495**
rs1015443	*TAS2R13*	T	4649	3503.51	0.000679	0.000249	**0.006297**
rs3851584	*TAS2R14*	G	4643.76	3507.59	0.000674	0.000249	**0.006744**
rs10772397	*TAS2R50*	C	4874	3283.05	0.000717	0.000251	**0.004253**
rs1450839	*TAS2R20*	G	2594	2556.37	0.000232	0.000265	0.379467
rs10845279	*TAS2R20*	A	2596	2557.2	0.000241	0.000265	0.362629
rs10845280	*TAS2R20*	G	2595	2557.28	0.000233	0.000265	0.377791
rs10845281	*TAS2R20*	C	2593	2556.32	0.000226	0.000265	0.392459
rs12226919	*TAS2R20*	T	2593	2554.69	0.000237	0.000265	0.369795
rs12226920	*TAS2R20*	T	2594	2556.36	0.000233	0.000265	0.379405
rs11054142	*TAS2R20*	A	2594	2556.36	0.000233	0.000265	0.379405
rs11054143	*TAS2R20*	C	2594	2556.42	0.000232	0.000265	0.380261
rs7301234	*TAS2R20*	A	2594	2556.42	0.000232	0.000265	0.380261

[a] Minor allele count in cases

[b] Statistically significant values are bolded.

4.4.4 Structure-function analysis of T2R20

Five of the seven missense SNPs associated with sore jaw muscles were in *TAS2R20*, prompting a focus on T2R20 for structure-function analysis. **Figure 4.9** presents 2D and 3D models of T2R20, highlighting the amino acids affected by the missense variants. The 3D model and ligand docking analysis revealed the locations of the CLSA variants within T2R20. Phenylalanine 252 was found in close proximity to the binding pocket (4.5 Å), potentially impacting ligand interactions, while the other variants were located approximately 10 Å from the binding site.

Variant	Association with sore jaw muscles
Lys 79 Glu	Not significant
Val 141 Ile	Not significant
His 143 Gln	Decreased risk
His 148 Asn	Decreased risk
Ile 236 Val	Decreased risk
Phe 252 Ser	Decreased risk
Arg 255 Leu	Decreased risk

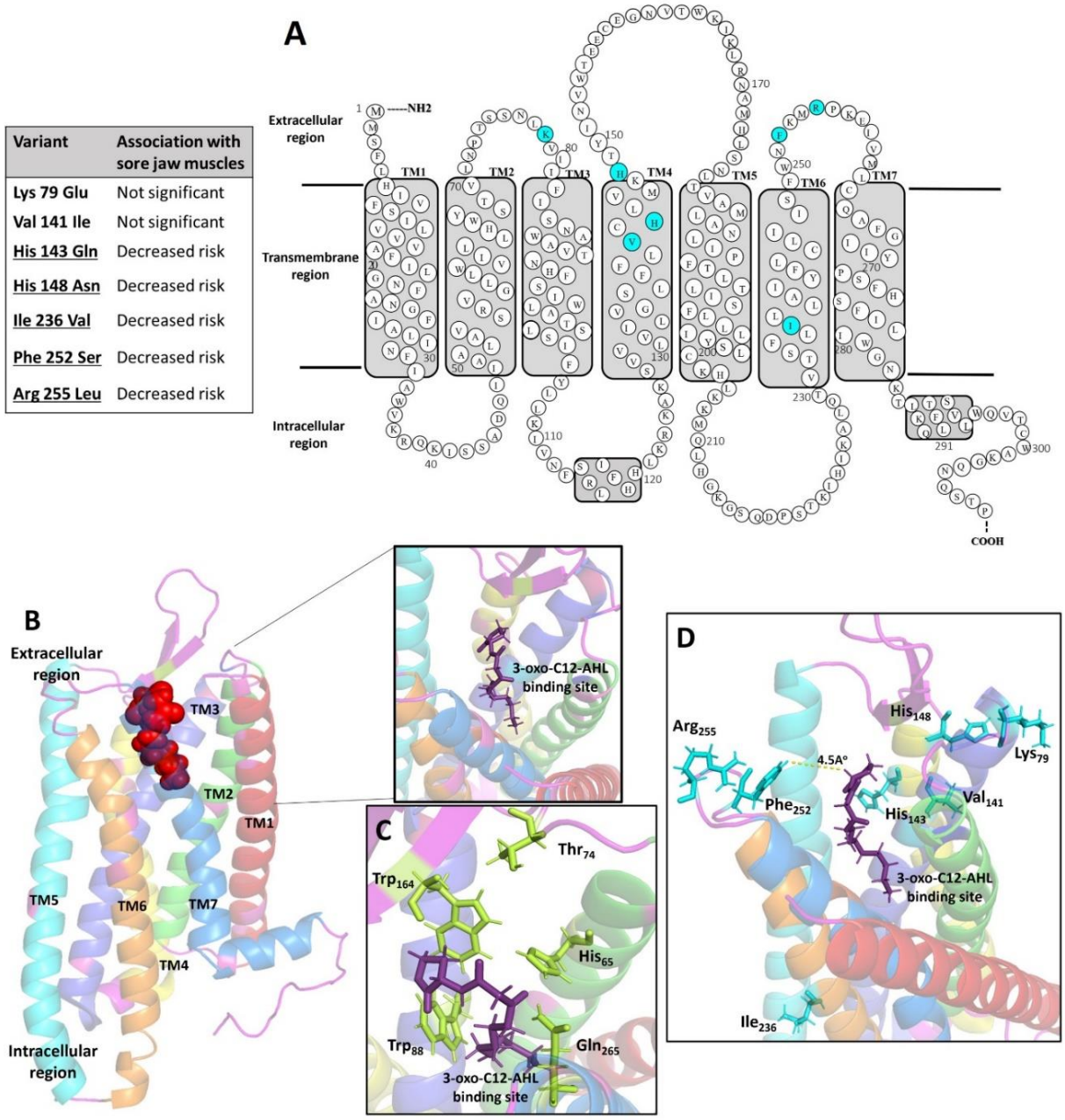

Figure 4.9 Structure-function analysis of T2R20, models adapted from previous studies (Jaggupilli et al. 2018, Jaggupilli, Singh, et al. 2019)

(A) 2D model highlighting amino acids affected by the CLSA SNPs. Underlined variants are in LD and associated with protection against sore jaw muscles. (B-C) 3D model showing the binding site for bacterial AHL (red sphere) and the interacting amino acid residues. (D) T2R20 variants in the CLSA and their proximity to the binding site. Phenylalanine 252 is the closest residue to the binding site (4.5Å), with other variants within approximately 10 Å.

4.5 Discussion

This study investigated the allele distributions of 124 common *TAS2R* variants within the CLSA European ancestry cohort, identifying 48 SNPs with allele distributions differing from those in the 1KGP. Significant associations were identified between *TAS2R* variants and self-reported sore jaw muscles, particularly for *TAS2R20*. Structural analysis of T2R20 revealed two variants close to the binding site, suggesting that these genetic variants may influence the symptom by altering ligand interactions.

Extensive variation within *TAS2R* genes was observed, with *TAS2R20* exhibiting the highest number of variants and *TAS2R15P* showing the greatest count of variants among the pseudogenes. These results align with those reported by Wooding et al., who analyzed 1KGP data from approximately 2,500 individuals worldwide, excluding *TAS2R* pseudogenes (Wooding and Ramirez 2022b). They reported the highest nucleotide diversity in *TAS2R20* and the lowest in *TAS2R39*. Additionally, our results corroborate a previous study on 911 young Canadian adults, which focused on rs713598 in *TAS2R38*—a tag SNP used to identify the supertaster genotype (Khataan et al. 2009). They reported a frequency of the A allele at 55% among Caucasians, closely aligning with the present finding of 59% among individuals of European ancestry.

The comparison of *TAS2R* genetic variants between the CLSA and 1KGP revealed generally similar allele distributions, with some differences, including in 11 missense variants. Six of these missense variants were in *TAS2R20*, all showing lower minor allele frequencies compared to the 1KGP. Previous studies have suggested a decrease in evolutionary pressure on human *TAS2Rs* during recent stages of evolution (Kulichová et al. 2022, Roudnitzky et al. 2016, Wooding and Ramirez 2022a). Therefore, these differences between the cohorts could be attributed to factors such as population structure, founder effects, genetic bottlenecks, or genetic drift (Roudnitzky et al. 2016, Kim and Drayna 2005, Kulichová et al. 2022). Interestingly, five of the six *TAS2R20* alleles with lower frequencies in the CLSA were associated with a reduced risk of self-reported sore jaw muscles.

The findings of this study align with previous reports on oral health in the CLSA population (Bassim, Mayhew, et al. 2020, De Rubeis et al. 2023). This study identified dry mouth as the most prevalent oral health issue among older Canadian adults, with a prevalence of 15% in men and 20% in women, followed by periodontal disease in 14% of men and 15% of women. Since early-stage periodontal disease is often asymptomatic, only severe cases could be identified through self-reported symptoms, defined as gingival bleeding or loose teeth (Gasner and Schure 2024). The 15% prevalence observed in this study aligns with a 2014 meta-analysis, which reported a 15-20% prevalence of severe periodontitis among North American adults aged 45 to 85 (Kassebaum et al. 2014).

Sex differences in oral health symptoms were notable, with females experiencing a higher prevalence of xerostomia, burning mouth syndrome, denture-related sores, sore jaw muscles, and painful jaw joints. These differences may be attributed to underlying conditions such as medication use, hormonal changes, and autoimmune disorders such as Sjögren's syndrome, which is more common in females (Fillingim et al. 2009, Craft 2007, Qin et al. 2015, Retamozo et al. 2021, Gabriel 2001). Decreased estrogen levels in perimenopausal women can lead to oral mucosal degeneration, increasing the risk of burning mouth syndrome (Gurvits and Tan 2013). Sjögren's syndrome, an autoimmune disorder causing oral cavity dryness, predominantly affects women (Qin et al. 2015, Retamozo et al. 2021). Xerostomia is also linked to *Candida* species, which can predispose individuals to denture-induced stomatitis (Samaranayake et al. 1989). This is consistent with the higher prevalence of denture-related sores among females. Furthermore, sore jaw muscles and painful jaw joints were twice as prevalent in females, aligning with previous studies that report a greater prevalence of temporomandibular joint disorder (TMD) among females (Lövgren et al. 2016, Campello et al. 2022, Sharma et al. 2011). These differences may partly be due to sex hormones, which can influence pain sensitivity (Fillingim et al. 2009, Craft 2007). Additionally, TMD is associated with juvenile idiopathic arthritis, an autoimmune disease that has a greater incidence in female compared to male children and can lead to micrognathia and TMD in later adulthood. TMD is also linked to rheumatoid arthritis, an autoimmune disease that is two to three times more common in women (Scott et al. 2010, Kroese et al. 2021, Gabriel 2001, Barut et al. 2017, Larheim and Haanaes 1981).

The analysis of 124 SNPs identified significant associations between sore jaw muscles, the most prevalent manifestation of TMD (Research 2023), and 15 SNPs across six genes. This aligns with the GWAS data from the UK Biobank, which showed that six of these alleles, spanning five genes, were significantly more frequent in individuals with rheumatoid arthritis (Bycroft et al. 2018). The remaining variants were all located in *TAS2R20* and in LD (**Table 4.7**). Among the 15 associated SNPs, four synonymous and four UTR variants were identified, consistent with growing evidence that synonymous variants are linked to various diseases and can have effects comparable to those of nonsynonymous variants (Chen et al. 2010, Sauna and Kimchi-Sarfaty 2013). Synonymous variants may impact mRNA structure, potentially altering its stability and translational speed, thus regulating post-transcriptional gene expression (Hunt et al. 2014, Spencer et al. 2012). Indeed, four of the associated synonymous and UTR variants may impact mRNA structure (**Figures 4.9-10**). The remaining were in LD with the associated missense variants ($r^2 > 0.9$), suggesting that their phenotypic effects may be due to the multiple amino acid changes in the polypeptide caused by the linked missense variants.

Among the five missense variants in *TAS2R20* linked to protection against sore jaw muscles, three were located in extracellular loops, where specific residues directly interact with ligands such as antibiotics and bacterial quorum-sensing molecules (Jaggupilli, Singh, et al. 2019, Jaggupilli et al. 2018). 3D models revealed that Phe252, a residue in the third extracellular loop, is near the binding site and can potentially affect ligand interactions. Phe252 is in LD with four other variants, meaning they are inherited together, which might significantly affect protein structure. The His148Asn variant is located in the second extracellular loop, at the interface with the transmembrane helix. This substitution might make the local structure more rigid due to Asn's smaller and less flexible side chain compared to His, potentially affecting the movement and positioning of the extracellular loop and ligand interactions.

TMD encompasses a range of neuromuscular and musculoskeletal disorders impacting the temporomandibular joint, masticatory muscles, and/or related tissues, resulting in symptoms such as pain, difficulty chewing, and, in severe cases, restricted jaw movement (Research 2023, Schiffman et al. 2014, Ferrillo et al. 2022). It is influenced by multiple risk factors, including malocclusion, oral parafunctions such as bruxism, psychological factors like depression and

anxiety, and iatrogenic injuries (Sharma et al. 2011). Elevated inflammatory markers, including interleukins and TNF-α, have been detected in the blood, masseter muscle extracellular fluid, and saliva of individuals with TMD.(Campello et al. 2022, Farré-Guasch et al. 2023). Additionally, specific bacterial species, particularly *Staphylococcus aureus*, have been implicated in TMD (Kim, Park, et al. 2003, Henry et al. 2000).

The SNPs associated with sore jaw muscles were located in *TAS2R8*, *9, 13, 14, 20,* and *50,* potentially due to the specific roles and expression patterns of these receptors. *TAS2R13, 14, 20*, and *50* are expressed at moderate to high levels in extraoral tissues (Jaggupilli et al. 2017, Kim et al. 2024). Notably, T2R20 and T2R14 detect bacterial quorum-sensing molecules and mediate immune response (Jaggupilli et al. 2018, Medapati, Singh, et al. 2021). A previous study showed that T2R14 responds to molecules from *S. aureus* by inducing inflammatory markers and inhibiting bacterial growth (Medapati, Bhagirath, et al. 2021). Thus, *TAS2R*s may contribute to TMD pathogenesis by modulating the immune response to pathogens and triggering the release of inflammatory biomarkers such as TNF-α and interleukins (Medapati, Bhagirath, et al. 2021, Medapati, Singh, et al. 2021, Jaggupilli, Singh, et al. 2019, Jaggupilli, Howard, et al. 2019, Jaggupilli et al. 2018).

This study found no significant association with other self-reported oral health symptoms. Research on this subject is limited and primarily focuses on dental caries and *TAS2R38* variants, which have yielded contradictory results (Wendell et al. 2010, Kiliç et al. 2022, Khimsuksri et al. 2022, AlMarshad et al. 2021, de Jesus et al. 2022b). A recent meta-analysis of genome-wide association studies also found no significant association between *TAS2R* SNPs and early childhood caries (Orlova et al. 2023). However, their transcriptome-wide analysis identified a significant association with the expression of three *TAS2R*s, including *T2R14*, which was associated with sore jaw muscles in this study. In terms of periodontal disease, Khimsuksri et al. found a lower risk of periodontitis (pocket depth ≥ 5 mm) in individuals with the *TAS2R38* AVI non-taster genotype (Khimsuksri et al. 2022). Conversely, Kaur et al. observed no significant association between *TAS2R* variants and the Periodontal Screening and Recording (PSR) index, which evaluates gingival bleeding and pocket depth, consistent with the findings of this study

(Kaur et al. 2021). Periodontal disease was defined as the presence of gingival bleeding and/or loose teeth, supporting the comparable outcomes.

There are several limitations to the current study. Although the comparison with 1KGP was conducted due to its availability, the small sample sizes of subpopulations limited the ability to account for potential outliers. While some differences were identified between the datasets, exploring their evolutionary origins was not within the scope of this study. Additionally, the analyses focused on individuals of European ancestry, limiting the extension of the findings to other ethnic groups. The self-reported data may introduce potential bias, as symptoms could not be validated through clinical exams due to the large sample size. Nevertheless, these oral health indicators have demonstrated adequate validity and reliability, and the large sample size enhances the study's statistical power (Locker and Miller 1994, Suresh and Chandrashekara 2012). Another limitation is the cross-sectional design, which prevents determining causality between the variants and the symptom. This limitation could be addressed in future longitudinal studies as more CLSA follow-up data become available. Furthermore, the number of SNPs detected in different genes varied, which may contribute to the differing number of associated SNPs. While the study identified significant associations, it did not account for potential interactions with environmental factors or other genetic variants. Finally, although the structure-function analysis is suggestive, further experimental validation is required to confirm the underlying biological mechanisms.

Findings of this study enhance our understanding of genetic risk factors for oral health and highlight the potential role of *TAS2R*s in the pathogenesis of TMD. These receptors, especially the disease-associated residues, could be promising targets for the development of new pharmacological agents. Moreover, identifying genetic variants linked to these conditions could aid in recognizing patients at higher risk, enabling more personalized medicine and tailored treatment plans.

4.6 Disclaimer

The opinions expressed in this manuscript are the author's own and do not reflect the views of the Canadian Longitudinal Study on Aging.

4.7 Supplementary materials

Table S4.1 Allele distributions of *TAS2R* SNPs in CLSA and 1KGP.
Data are presented as minor allele frequencies (MAF) in CLSA European ethnicity and corresponding allele frequencies (AF) in 1KGP European population.

rs ID	SNP[a]	MA[b]	Gene	Consequence	Amino Acid Change	CLSA MAF	1KGP AF	Adjusted p-value[c]
rs41466	chr5:9627670:C:T	C	*TAS2R1*	3 prime UTR	-	0.45	0.44	1
rs41467	chr5:9627977:A:C	A	*TAS2R1*	3 prime UTR	-	0.45	0.44	1
rs41468	chr5:9627996:G:A	G	*TAS2R1*	3 prime UTR	-	0.46	0.44	1
rs2234235	chr5:9629183:A:G	G	*TAS2R1*	Synonymous	Leu 284	0.03	0.05	**0.021**
rs2234233	chr5:9629417:G:A	A	*TAS2R1*	Missense	Arg 206 Trp	0.17	0.15	0.919
rs138940415	chr5:9630121:G:A	A	*TAS2R1*	5 prime UTR	-	0.02	0.02	1
rs847923	chr7:12491252:G:C	C	*TAS2R2*	NA	-	0.31	0.30	1
rs35620026	chr7:12491542:C:T	T	*TAS2R2*	NA	-	0.12	0.13	1
rs6963925	chr7:12491554:C:T	T	*TAS2R2*	NA	-	0.26	0.26	1
rs12535592	chr7:12491615:A:G	G	*TAS2R2*	NA	-	0.25	0.25	1
rs73058722	chr7:12491991:A:G	G	*TAS2R2*	NA	-	0.25	0.25	1
rs765007	chr7:141764114:T:C	C	*TAS2R3*	5 prime UTR	-	0.50	0.47	0.277
rs2270009	chr7:141764965:C:T	T	*TAS2R3*	Synonymous	Gly 269	0.49	0.46	0.349
rs33920115	chr7:141777387:G:A	A	*TAS2R4*	5 prime UTR	-	0.50	0.47	0.300
rs2233998	chr7:141778508:T:C	C	*TAS2R4*	Missense	Phe 7 Ser	0.50	0.47	0.277
rs2234001	chr7:141778774:G:C	G	*TAS2R4*	Missense	Leu 96 Val	0.50	0.53	0.472
rs2234002	chr7:141779000:G:A	G	*TAS2R4*	Missense	Asn 171 Ser	0.50	0.53	0.449
rs57641758	chr7:141779930:A:G	A	*TAS2R4*	3 prime UTR	-	0.50	0.53	0.427
rs139145080	chr7:141779944:A:G	G	*TAS2R4*	3 prime UTR	-	0.02	0.02	1
rs150967230	chr7:141780098:C:T	T	*TAS2R4*	3 prime UTR	-	0.01	0.02	0.184
rs35127278	chr7:141780293:G:T	T	*TAS2R4*	3 prime UTR	-	0.50	0.47	0.307
rs2214838	chr7:141780413:A:G	G	*TAS2R4*	3 prime UTR	-	0.50	0.47	0.307
rs2190243	chr7:141780676:C:G	C	*TAS2R4*	3 prime UTR	-	0.50	0.53	0.483
rs2190244	chr7:141780772:A:G	G	*TAS2R4*	3 prime UTR	-	0.50	0.47	0.307
rs17464086	chr7:141781136:G:A	G	*TAS2R4*	3 prime UTR	-	0.50	0.53	0.508
rs2234009	chr7:141790231:C:T	T	*TAS2R5*	5 prime UTR	-	0.03	0.04	0.058
rs2234010	chr7:141790232:G:A	A	*TAS2R5*	5 prime UTR	-	0.03	0.02	0.854
rs2234012	chr7:141790307:A:G	G	*TAS2R5*	5 prime UTR	-	0.50	0.47	0.406
rs2227264	chr7:141790438:G:T	T	*TAS2R5*	Missense	Ser 26 Ile	0.50	0.47	0.332
rs117638090	chr7:141787930:G:A	A	*TAS2R6P*	Upstream gene	-	0.02	0.02	1
rs1859645	chr7:141788088:A:G	G	*TAS2R6P*	Upstream gene	-	0.50	0.47	0.417
rs11761380	chr7:141788474:A:C	C	*TAS2R6P*	Upstream gene	-	0.50	0.47	0.417
rs619381	chr12:10801659:C:T	T	*TAS2R7*	Missense	Met 304 Ile	0.12	0.09	**<0.0001**

rs ID	SNP[a]	MA[b]	Gene	Consequence	Amino Acid Change	CLSA MAF	1KGP AF	Adjusted p-value[c]
rs139604652	chr12:10801984:A:T	T	*TAS2R7*	Missense	Val 196 Glu	0.01	0.01	1
rs143804727	chr12:10806297:T:G	G	*TAS2R8*	Missense	Glu 228 Asp	0.02	0.01	0.319
rs1548803	chr12:10806432:C:T	C	*TAS2R8*	Synonymous	Leu 183	0.41	0.32	**<0.0001**
rs3741845	chr12:10809516:A:G	A	*TAS2R9*	Missense	Ala 187 Val	0.40	0.32	**<0.0001**
rs142359564	chr12:10895096:C:T	T	*TAS2R12P*	NA	-	0.01	0.01	1
rs16925338	chr12:10895290:T:A	A	*TAS2R12P*	NA	-	0.02	0.01	1
rs11054065	chr12:10895780:G:A	A	*TAS2R12P*	NA	-	0.02	0.01	**0.023**
rs1015442	chr12:10908096:T:C	T	*TAS2R13*	3 prime UTR	-	0.43	0.38	**0.000**
rs1015443	chr12:10908523:T:C	T	*TAS2R13*	Missense	Ser 259 Asn	0.43	0.38	**0.000**
rs3851584	chr12:10937478:G:T	G	*TAS2R14*	3 prime UTR	-	0.43	0.38	**0.000**
rs3851585	chr12:10937794:C:G	G	*TAS2R14*	3 prime UTR	-	0.02	0.03	**0.010**
rs3936285	chr12:10937839:T:A	A	*TAS2R14*	3 prime UTR	-	0.02	0.03	**0.010**
rs35804287	chr12:10938607:G:A	A	*TAS2R14*	Missense	Leu 201 Phe	0.02	0.03	0.468
rs3741843	chr12:10938833:C:T	C	*TAS2R14*	Synonymous	Arg 125	0.16	0.14	0.074
rs16925868	chr12:10938952:T:C	C	*TAS2R14*	Missense	Thr 86 Ala	0.02	0.03	**0.012**
rs7138535	chr12:10939094:T:A	A	*TAS2R14*	Synonymous	Gly 38	0.25	0.22	**0.006**
rs11054092	chr12:10964560:T:C	C	*TAS2R15P*	NA	-	0.25	0.22	**0.007**
rs11054093	chr12:10964615:G:C	C	*TAS2R15P*	NA	-	0.25	0.22	**0.007**
rs11054094	chr12:10964693:G:A	A	*TAS2R15P*	NA	-	0.25	0.22	**0.007**
rs4763599	chr12:10964722:A:G	G	*TAS2R15P*	NA	-	0.25	0.22	**0.007**
rs17810798	chr12:10964833:G:C	C	*TAS2R15P*	NA	-	0.25	0.22	**0.007**
rs11054095	chr12:10965032:G:C	C	*TAS2R15P*	NA	-	0.25	0.22	**0.008**
rs11054096	chr12:10965197:T:C	C	*TAS2R15P*	NA	-	0.25	0.22	**0.007**
rs1204014	chr7:122994789:C:T	T	*TAS2R16*	Synonymous	Thr 282	0.04	0.04	1
rs860170	chr7:122994970:C:T	C	*TAS2R16*	Missense	His 222 Arg	0.33	0.31	1
rs7310047	chr12:11158921:G:A	G	*TAS2R18P*	NA	-	0.48	0.49	1
rs7296270	chr12:11158991:A:T	A	*TAS2R18P*	NA	-	0.16	0.13	**0.011**
rs61928603	chr12:11159188:C:T	C	*TAS2R18P*	NA	-	0.18	0.16	1
rs61928604	chr12:11159348:C:T	C	*TAS2R18P*	NA	-	0.16	0.13	**0.018**
rs2290318	chr12:11159359:G:C	C	*TAS2R18P*	NA	-	0.32	0.36	**0.005**
rs2290319	chr12:11159427:C:A	A	*TAS2R18P*	NA	-	0.32	0.36	**0.005**
rs77837442	chr12:11021687:C:T	T	*TAS2R19*	Stop-gained	Trp 295 *	0.01	0.01	1
rs1868769	chr12:11022154:G:A	G	*TAS2R19*	Synonymous	Leu 140	0.20	0.19	1
rs115193179	chr12:11022246:T:G	G	*TAS2R19*	Missense	Lys 109 Thr	0.01	0.01	1
rs61912291	chr12:10996791:T:G	G	*TAS2R20*	3 prime UTR	-	0.24	0.21	0.100
rs117458236	chr12:10996860:C:T	T	*TAS2R20*	3 prime UTR	-	0.02	0.02	1
rs1450839	chr12:10996933:A:G	G	*TAS2R20*	3 prime UTR	-	0.33	0.38	**0.000**

rs ID	SNP[a]	MA[b]	Gene	Consequence	Amino Acid Change	CLSA MAF	1KGP AF	Adjusted p-value[c]
rs10845279	chr12:10997112:C:A	A	*TAS2R20*	Missense	Arg 255 Leu	0.33	0.38	**0.000**
rs10845280	chr12:10997121:A:G	G	*TAS2R20*	Missense	Phe 252 Ser	0.33	0.38	**0.000**
rs10845281	chr12:10997170:T:C	C	*TAS2R20*	Missense	Ile 236 Val	0.33	0.38	**0.000**
rs12226919	chr12:10997434:G:T	T	*TAS2R20*	Missense	His 148 Asn	0.33	0.38	**0.000**
rs12226920	chr12:10997447:G:T	T	*TAS2R20*	Missense	His 143 Gln	0.33	0.38	**0.000**
rs79420812	chr12:10997455:C:T	T	*TAS2R20*	Missense	Val 141 Ile	0.19	0.22	**0.001**
rs11054142	chr12:10997615:G:A	A	*TAS2R20*	Synonymous	Ala 87	0.33	0.38	**0.000**
rs7135018	chr12:10997641:T:C	C	*TAS2R20*	Missense	Lys 79 Glu	0.24	0.21	0.098
rs11054143	chr12:10997720:T:C	C	*TAS2R20*	Synonymous	Ala 52	0.33	0.38	**0.000**
rs4388985	chr12:10997952:G:A	G	*TAS2R20*	5 prime UTR	-	0.16	0.14	0.090
rs1463237	chr12:10997980:C:T	C	*TAS2R20*	5 prime UTR	-	0.16	0.14	**0.049**
rs7301234	chr12:10998285:G:A	A	*TAS2R20*	5 prime UTR	-	0.33	0.38	**0.000**
rs2600357	chr12:11133155:G:A	G	*TAS2R30*	3 prime UTR	-	0.48	0.49	1
rs2600356	chr12:11133203:G:T	G	*TAS2R30*	3 prime UTR	-	0.48	0.49	1
rs2599404	chr12:11133489:A:C	A	*TAS2R30*	Missense	Leu 252 Phe	0.48	0.49	1
rs112849631	chr12:11133780:T:C	C	*TAS2R30*	Synonymous	Thr 155	0.02	0.03	**0.004**
rs116737741	chr12:11030592:T:C	C	*TAS2R31*	Synonymous	Ser 248	0.15	0.12	**0.002**
rs10845294	chr12:11030687:G:C	C	*TAS2R31*	Missense	Gln 217 Glu	0.27	0.27	1
rs10743938	chr12:11030852:A:T	A	*TAS2R31*	Missense	Met 162 Leu	0.20	0.19	1
rs10845295	chr12:11031233:G:A	G	*TAS2R31*	Missense	Trp 35 Arg	0.49	0.52	0.615
rs10246939	chr7:141972804:T:C	C	*TAS2R38*	Missense	Ile 296 Val	0.46	0.46	1
rs1726866	chr7:141972905:G:A	G	*TAS2R38*	Missense	Val 262 Ala	0.46	0.46	1
rs713598	chr7:141973545:C:G	G	*TAS2R38*	Missense	Ala 49 Pro	0.41	0.42	1
rs10260248	chr7:143222638:C:A	A	*TAS2R40*	Missense	Ser 187 Tyr	0.06	0.06	1
rs1404635	chr7:143478061:G:A	A	*TAS2R41*	Synonymous	Thr 63	0.29	0.25	0.096
rs10278721	chr7:143478252:C:T	T	*TAS2R41*	Missense	Pro 127 Leu	0.29	0.25	0.090
rs76518630	chr7:143478409:C:T	T	*TAS2R41*	Synonymous	Tyr 179	0.01	0.02	**0.035**
rs1650017	chr12:11186007:G:C	C	*TAS2R42*	Missense	Pro 311 Ala	0.16	0.13	**0.011**
rs1669411	chr12:11186008:G:A	A	*TAS2R42*	Synonymous	Asn 310	0.16	0.13	**0.011**
rs1669412	chr12:11186063:T:C	T	*TAS2R42*	Missense	Arg 292 Gln	0.25	NA	NA
rs1451772	chr12:11186144:C:T	C	*TAS2R42*	Missense	Tyr 265 Cys	0.25	NA	NA
rs1669413	chr12:11186175:A:C	C	*TAS2R42*	Missense	Trp 255	0.16	0.13	**0.011**

rs ID	SNP[a]	MA[b]	Gene	Consequence	Amino Acid Change	CLSA MAF	1KGP AF	Adjusted p-value[c]
rs5020531	chr12:11186351:A:G	A	*TAS2R42*	Missense	Gly Ser 196 Phe	0.41	0.35	**<0.0001**
rs1650019	chr12:11186377:C:T	T	*TAS2R42*	Synonymous	Leu 187	0.16	0.13	**0.011**
rs35969491	chr12:11186414:T:A	T	*TAS2R42*	Missense	Phe 175 Tyr	0.41	0.35	**<0.0001**
rs73260771	chr12:11061761:C:T	T	*TAS2R46*	Synonymous	Thr 178	0.27	0.28	1
rs10772397	chr12:10986084:C:T	C	*TAS2R50*	Synonymous	Pro 259	0.40	0.34	**<0.0001**
rs1376251	chr12:10986253:C:T	T	*TAS2R50*	Missense	Cys 203 Tyr	0.32	0.36	**0.003**
rs66679979	chr12:10986336:T:C	C	*TAS2R50*	Synonymous	Ser 175	0.15	0.13	**0.018**
rs61750008	chr7:143443582:G:A	A	*TAS2R60*	Missense	Val 44 Met	0.03	0.04	0.254
rs4595035	chr7:143444382:T:C	T	*TAS2R60*	Synonymous	Arg 310	0.33	0.34	1
rs4726624	chr7:143437150:G:A	A	*TAS2R62P*	NA	-	0.13	0.13	1
rs34039200	chr7:143437188:G:A	A	*TAS2R62P*	NA	-	0.22	0.23	1
rs10239143	chr7:143437699:C:G	G	*TAS2R62P*	NA	-	0.13	0.14	1
rs6944279	chr7:143437702:A:T	T	*TAS2R62P*	NA	-	0.33	0.34	1
rs2708319	chr12:11048442:A:G	A	*TAS2R63P*	NA	-	0.49	0.52	1
rs35062230	chr12:11048811:G:A	A	*TAS2R63P*	NA	-	0.24	0.21	0.102
rs2597985	chr12:11048888:T:C	T	*TAS2R63P*	NA	-	0.49	0.52	1
rs2599394	chr12:11049030:G:A	G	*TAS2R63P*	NA	-	0.49	0.52	1
rs319268	chr12:11179591:T:A	T	*TAS2R67P*	Downstream gene (SMIM10L1)	-	0.25	NA	NA
rs73067307	chr12:11179960:A:G	G	*TAS2R67P*	Downstream gene (SMIM10L1)	-	0.03	0.02	1
rs319269	chr12:11179986:A:C	C	*TAS2R67P*	Downstream gene (SMIM10L1)	-	0.16	0.13	**0.011**
rs34648613	chr12:11180118:T:A	T	*TAS2R67P*	Downstream gene (SMIM10L1)	-	0.41	0.35	**<0.0001**
rs184006834	chr12:11180133:G:A	A	*TAS2R67P*	Downstream gene (SMIM10L1)	-	0.04	0.04	1

[a] SNP = single nucleotide polymorphism; Position in GRCh38 shown as chromosome:base pair position: reference allele: alternative allele

[b] Minor allele in CLSA

[c] Chi-squared test with Bonferroni correction.

CLSA = Canadian Longitudinal Study on Aging; 1KGP = 1000 Genomes Project.

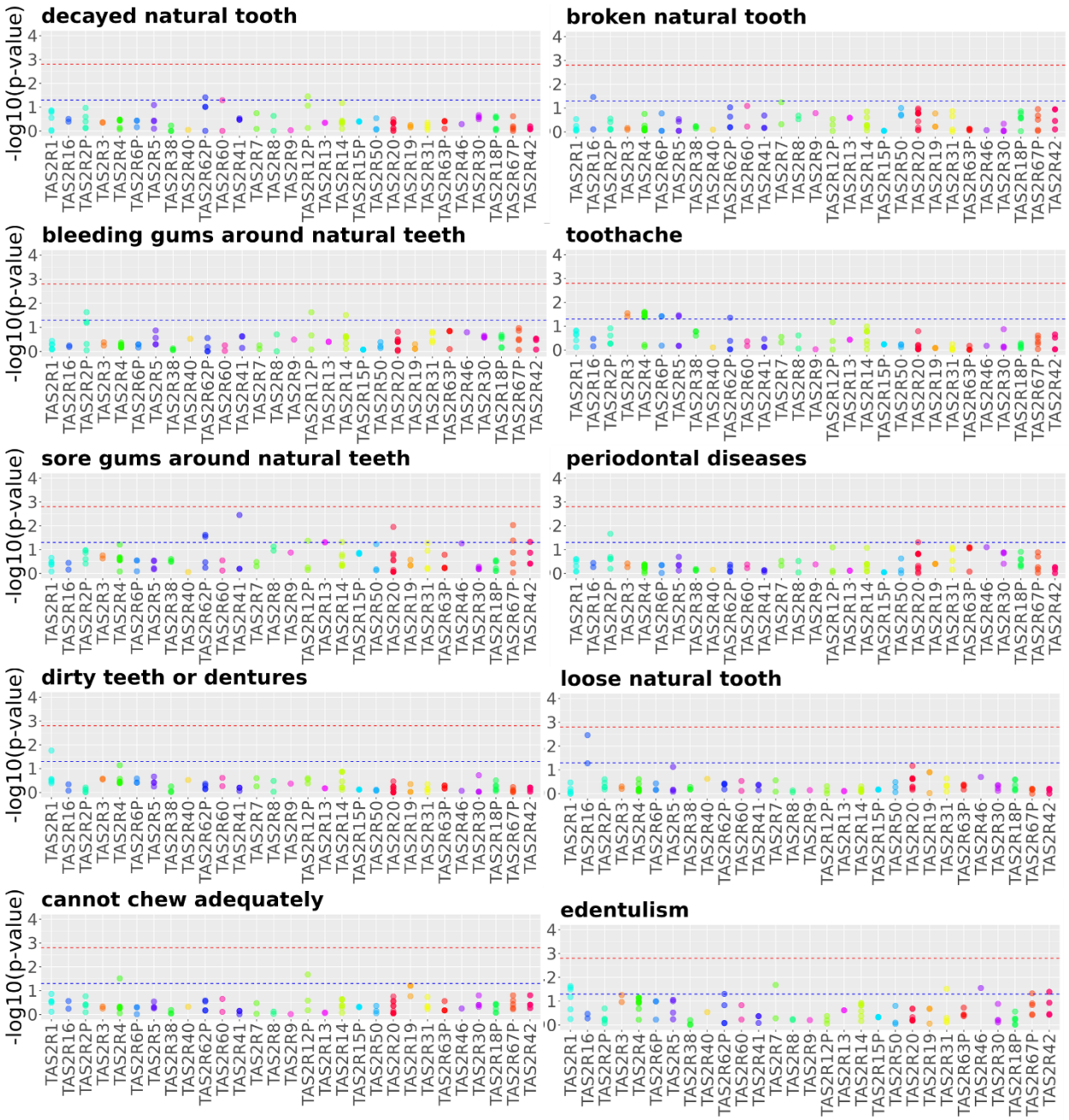

Figure S4.1 Association between *TAS2R* SNPs and self-reported oral health symptoms.

The blue line represents the significance threshold (p = 0.05), while the red line marks the Bonferroni-corrected threshold, with dots above this line indicating SNPs with significant associations (logistic regression).

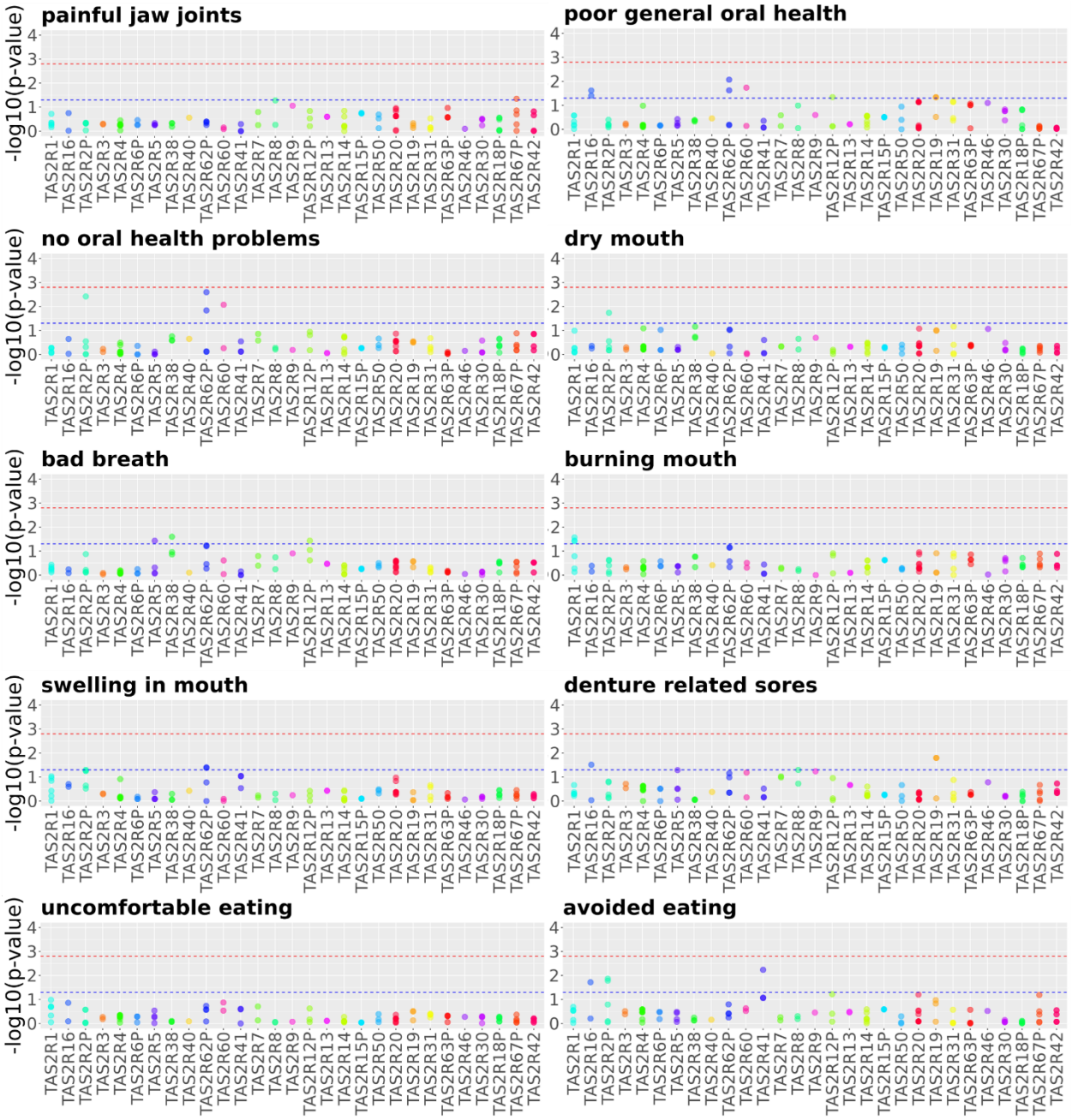

Figure S4.1 (continued) Association between *TAS2R* SNPs and oral health symptoms. The blue line represents the significance threshold (p = 0.05), while the red line marks the Bonferroni-corrected threshold, with dots above this line indicating SNPs with significant associations (logistic regression).

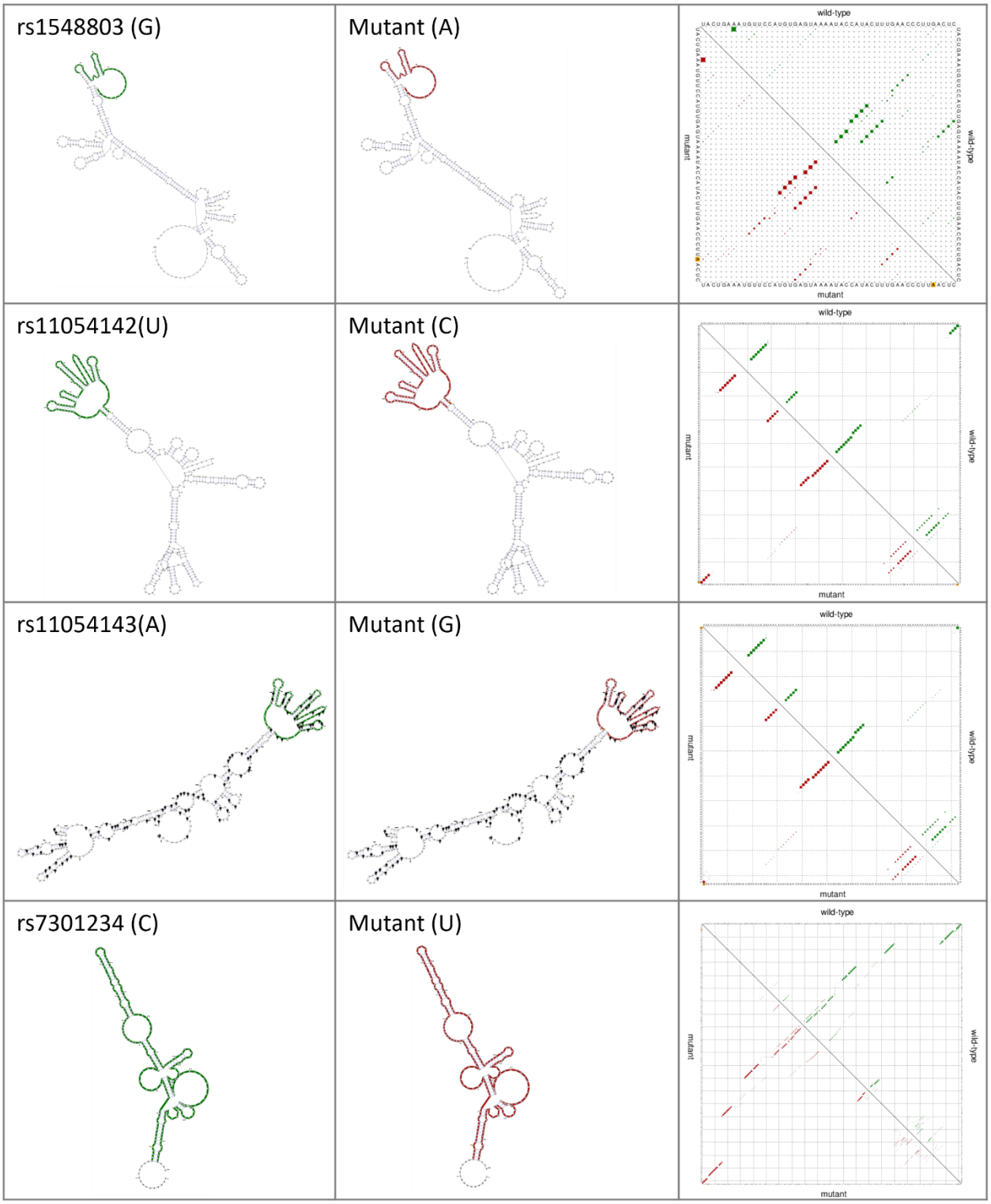

Figure S4.2 RNAsnp predicted no changes in mRNA secondary structure for eight variants. The upper triangle of the matrices represents base pair probabilities for the wild-type local region, and the lower triangle represents those for the mutant sequence.

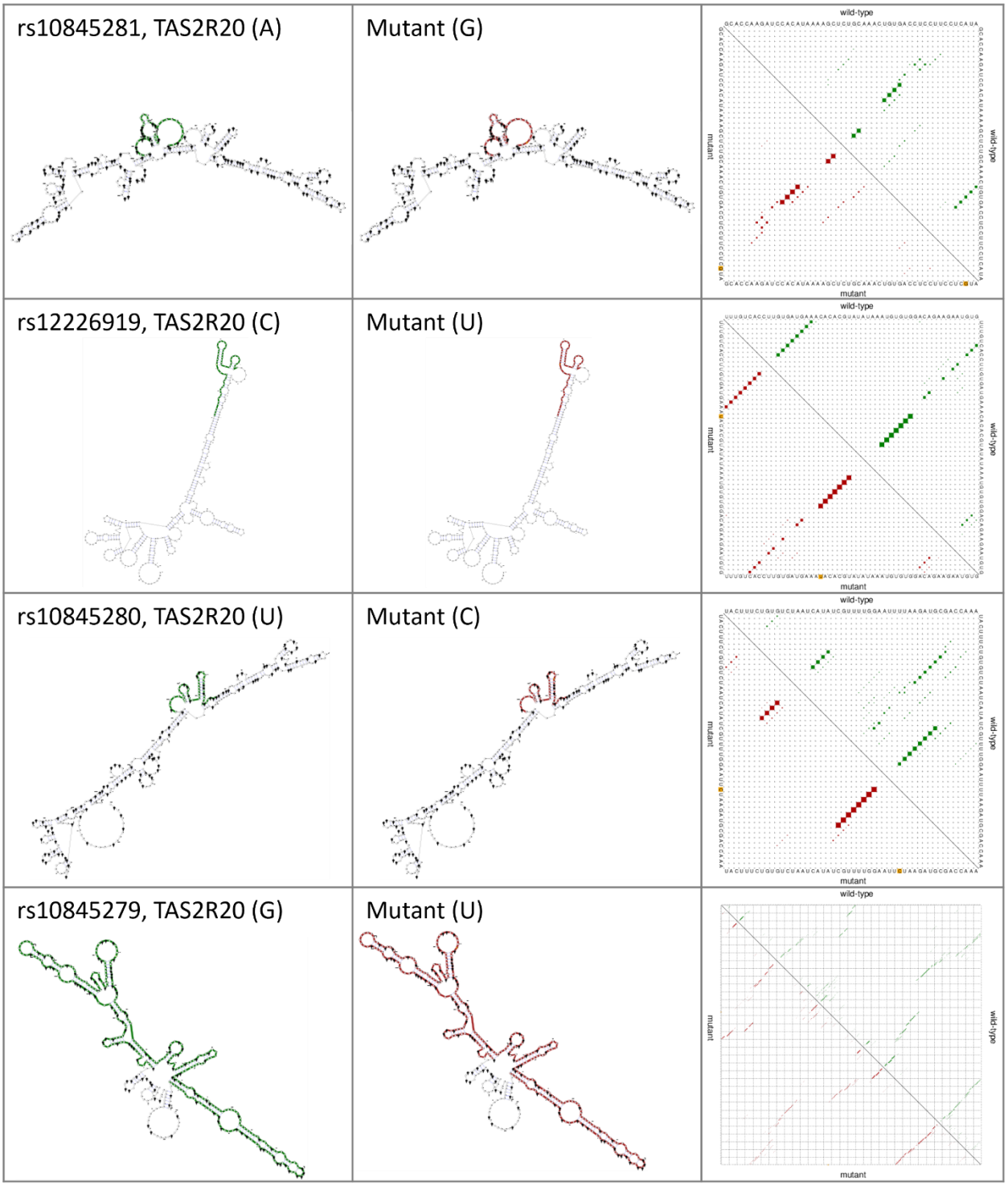

Figure S4.2 (continued) RNAsnp predicted no changes in mRNA secondary structure for eight variants.

The upper triangle of the matrices represents base pair probabilities for the wild-type local region, and the lower triangle represents those for the mutant sequence.

Bridge to Chapter 5

The receptors for SARS-CoV-2 binding and cellular entry are expressed in the nasal and salivary epithelium (Hamming et al. 2004). Furthermore, nasopharyngeal tissues and salivary glands may serve as reservoirs for the virus (Huang et al. 2021, Marais et al. 2021, To, Tsang, Leung, et al. 2020, To, Tsang, Yip, et al. 2020). SARS-CoV-2 can be detected in the saliva of infected individuals even after symptom resolution (Marais et al. 2021, To, Tsang, Leung, et al. 2020, To, Tsang, Yip, et al. 2020). Interestingly, elevated inflammatory markers, such as interleukins and TNF-α, have been detected in the blood and saliva of individuals with TMD (Campello et al. 2022, Farré-Guasch et al. 2023). It is plausible that T2Rs are associated with TMD through their impact on cytokine release. Previous research has shown that T2Rs can suppress the production of pro-inflammatory cytokines, including TNF-α and IL-6, while promoting the release of anti-inflammatory cytokines (Grassin-Delyle et al. 2019). Given that saliva is a reservoir for SARS-CoV-2, and considering the potential influence of T2Rs on inflammatory markers, T2Rs may also impact susceptibility to COVID-19 infection.

Additionally, TMD can result from autoimmune diseases involving the joints, such as juvenile idiopathic arthritis and rheumatoid arthritis (Scott et al. 2010, Kroese et al. 2021, Gabriel 2001, Barut et al. 2017, Larheim and Haanaes 1981). Notably, individuals with autoimmune diseases are also at greater risk of severe COVID-19 infection, potentially due to immune dysregulation or immunomodulatory medications (Grainger et al. 2022, Simpson-Yap et al. 2022, Maddur et al. 2010, Akiyama et al. 2021).

Given the role of T2Rs in immune response, cytokine production, and airway clearance, along with their association with TMD found in the first study, *TAS2R* variants may influence susceptibility to SARS-CoV-2 infection and antibody response, particularly in high-risk individuals with underlying medical conditions. To test this hypothesis, Chapter 5 evaluates the associations between *TAS2R* SNPs and both COVID-19 infection and seroconversion, examining the entire CLSA cohort as well as subgroups stratified by the presence or absence of chronic medical conditions.

CHAPTER 5: Genetic variations in bitter taste receptors and COVID-19 in Canadian Longitudinal Study on Aging.

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[to be submitted to *Vaccines*]

5.1 Abstract

This study evaluated the associations between the SNPs in *TAS2R* genes and pseudogenes and COVID-19 infection and antibody seroconversion in individuals of European ancestry within the CLSA. Logistic regression analyses were conducted under an additive model, controlling for sociodemographics, genetic principal components, smoking, vaccine doses, and high-risk chronic medical conditions, including diabetes, immune-mediated inflammatory disease (IMID), respiratory disease, and cardiovascular disease. In the COVID-19 Questionnaire Study (n=14,073), the rs117458236(C) variant in *TAS2R20* was significantly associated with COVID-19 infection (OR=1.95, *P*=0.039). In the COVID-19 Antibody Study (n=8,313), three SNPs were associated with seroconversion: rs2234235(G) in *TAS2R1* with anti-nucleocapsid (OR=1.55, *P*=0.018) and anti-spike (OR=0.74, *P*=0.033); rs2234010(A) in *TAS2R5* with anti-nucleocapsid (OR=1.56, *P*=0.014); and rs34039200(A) in *TAS2R62P* with anti-spike (OR=0.86, *P*=0.013). Subgroup analyses of participants with and without chronic conditions suggested that rs2234235(G) in *TAS2R1* may be associated with a decreased spike antibody response to infection or vaccination in individuals with IMID or respiratory disease and increased risk of SARS-CoV-2 infection. These findings suggest that *TAS2R* variants are associated with COVID-19 infection and vaccine response, suggesting potential implications for personalized medical practice and targeted vaccination strategies for at-risk individuals.

5.2 Introduction

COVID-19 has affected over 4.71 million Canadians and has resulted in more than 53,297 deaths (World Health Organization (WHO) February 2025). Despite the availability of effective vaccines and the subsequent lifting of public health restrictions, COVID-19 remains a significant public health concern. Globally, over 65,000 new cases and 3,500 deaths are reported each month, posing particular risks to older adults and individuals with compromised immune systems (World Health Organization (WHO) February 2025, Gallo et al. 2022).

Identifying *TAS2R* variants associated with COVID-19 outcomes could guide more effective preventive protocols and help identify individuals who may require early interventions, including those with chronic medical conditions that increase their risk (CDC COVID-19 Response Team 2020, Marfella et al. 2022, Grainger et al. 2022, Simpson-Yap et al. 2022, Gallo et al. 2022). Moreover, understanding the relationship between *TAS2R* variants and serological response to SARS-CoV-2 could contribute to the development of personalized vaccination strategies.

Few studies have assessed the association between *TAS2R* genetic variants and the risk or severity of COVID-19 (Barham et al. 2021, Parsa et al. 2021, Risso et al. 2022, Meng and Nielsen 2024). Those studies that have examined these associations have largely focused on *TAS2R38*, did not account for high-risk groups, such as those with chronic medical conditions, and reported inconsistent findings. Moreover, *TAS2R38* has relatively low extra-oral tissue expression, suggesting that other *TAS2R* genes with higher expression in extra-oral tissues may have a stronger association with COVID-19 outcomes (Jaggupilli et al. 2017). To date, no research has examined the association between COVID-19 outcomes and other *TAS2R* genes or pseudogenes. Additionally, no published data exist on the association between *TAS2R* variants and post-infection or post-vaccination serology.

This study aimed to evaluate the association between *TAS2R* SNPs and COVID-19 outcomes and seroconversion and to determine whether the association differs in high-risk groups in the CLSA. The primary objective was to evaluate the association between *TAS2R* SNPs and COVID-19 infection. The second objective was to assess the relationship between *TAS2R* SNPs and post-infection and post-vaccination antibody response. Finally, this study aimed to determine whether

these associations with COVID-19 outcomes and seroconversion differ among individuals with or without at-risk chronic medical conditions, including diabetes, cardiovascular disease, respiratory disease, and immune-mediated inflammatory disorders (IMIDs).

5.3 Methods

5.3.1 Study population and data preprocessing

The following CLSA datasets were used: Genome-wide Genetic Data Release (version 3), Comprehensive Baseline Dataset (2011–2015), Comprehensive Follow-up 2 Dataset (version 1.1, 2018–2021), COVID-19 Questionnaire Study (2020), and COVID-19 Seroprevalence (Antibody) Study (2020), under CLSA Application ID: 23CA016. This study was approved by the University of Manitoba's Health Research Ethics Board (HREB HS26021-H2023:173).

5.3.2 Case definition for chronic medical conditions

Diabetes, respiratory disease, and cardiovascular conditions were included in logistic regression models as covariates and in subgroup analyses since they are associated with increased risk of severe COVID-19 infection and are among the most common chronic conditions in Canada (Canada 2019, Haybar et al. 2020). Immunocompromised individuals, such as those with immune mediated inflammatory diseases (IMIDs) are also at greater risk of severe COVID-19 infection, potentially due to immune dysregulation or immunomodulatory medications (Grainger et al. 2022, Simpson-Yap et al. 2022, Maddur et al. 2010, Akiyama et al. 2021). Among IMIDs, rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) were the focus, due to their high prevalence in Canada, overlapping use of immunomodulatory agents, and evidence of association with COVID-19 severity (Xiang et al. 2023, Cooper and Stroehla 2003, Hassen et al. 2024, Coward et al. 2023, Macaluso et al. 2023, Wang et al. 2022). Case definitions were generated for these conditions using self-reported data and medication use, based on algorithms developed through an extensive literature review (**Figure 5.1** and **Table 5.1**) (Raina, Wolfson, Kirkland, Keshavarz, et al. 2009, Andreacchi et al. 2023, Oremus et al. 2013, Sakib et al. 2023, MacNeil et al. 2024, Fishbook et al. 2022, Sekhon et al. 2019, Liu et al. 2019, Husein et al. 2021, Duong et al. 2022, Odimba et al. 2023, Taunque et al. 2023, O'Mahony et al. 2024, Wolfson et al. 2019, Dal Bello-Haas et al. 2024). To confirm the relative accuracy of the definitions, the prevalences for each condition were compared with those reported by the Canadian Chronic

Disease Surveillance System (CCDSS) and other CLSA publications (Canada , Sakib et al. 2023, Freeman et al. 2022, Duong et al. 2022, O'Mahony et al. 2024).

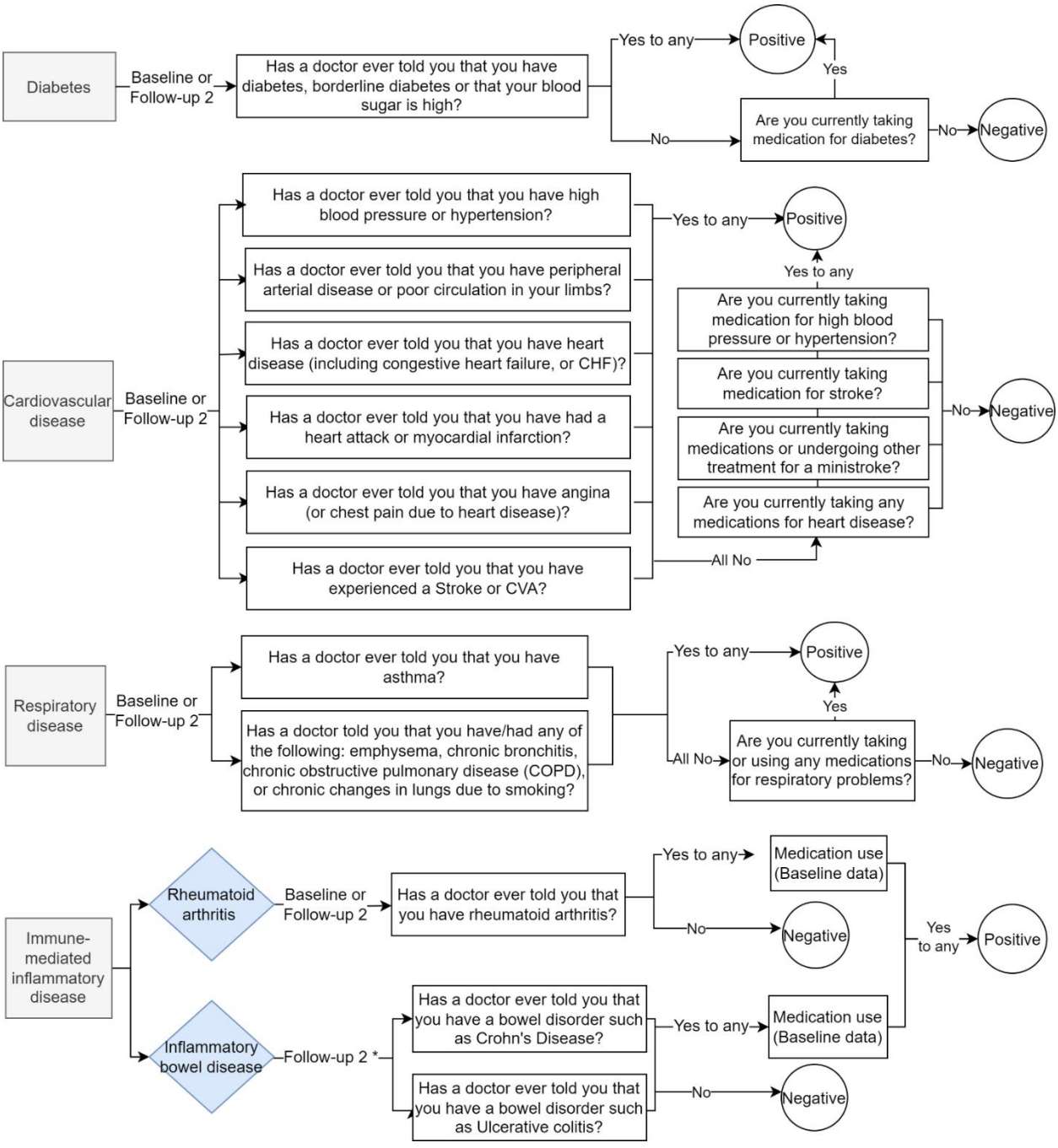

Figure 5.1 Algorithms for defining chronic conditions in CLSA.

Follow-up 2 data was used to determine medication use, based on responses to the question: "Do you use any medications for …?" For rheumatoid arthritis and inflammatory bowel disease, medication data (listed in Table 2) was obtained from the Baseline dataset, as this information was not available in the Follow-up 2 data.

Table 5.1 List of medications for rheumatoid arthritis and inflammatory bowel disease, excluding steroids.
Drug identification numbers (DINs) were retrieved from the Health Canada Drug Product Database.

Disease	Immunomodulators	Immunosuppressants	Biologics and small molecules
Rheumatoid arthritis	Cyclosporin Hydroxychloroquine Minocycline Sulfasalazine	Azathioprine Cyclophosphamide Leflunomide Methotrexate Mycophenolate	Infliximab Adalimumab Etanercept Certolizumab Golimumab Rituximab Abatacept Tocilizumab Tofacitinib Baricitinib
Inflammatory bowel disease	Mesalazine or mesalamine (5-ASA, Mezavant, Salofalk, Asacol, Pentasa, Mesasal, Mezera, Octasa) Thiopurines (Thioguanine) Sulfasalazine Dipentum	Azathioprine 6-mercaptopurine Methotrexate	Infliximab Adalimumab Golimumab Ustekinumab Vedolizumab

*The generic compound is Mesalazine or mesalamine, marketed under different brands.

5.3.3 COVID-19 infection

Data from the CLSA COVID-19 Questionnaire Study (N = 28,559) were used to assess the association between *TAS2R* variants and COVID-19 infection. Data were collected at baseline, weekly, biweekly, monthly, and at the exit time. Two outcomes were evaluated: A. Confirmed infection: Participants who reported a positive nucleic acid amplification test for COVID-19. B. Probable/confirmed infection: Participants who either reported a positive nucleic acid amplification test or were told by a healthcare professional that they had COVID-19 without a confirmed test. The probable cases were included as many individuals were not tested in the early pandemic (Li et al. 2020). Individuals were coded as positive for infection if they reported at least one positive response at any time point during the questionnaire; otherwise, they were coded as negative. Those with missing data at all time points were excluded from the analysis.

5.3.4 COVID-19 antibodies

The CLSA COVID-19 Seroprevalence (Antibody) Study (N ≈ 18,500) was used to evaluate the association between *TAS2R* variants and the presence of nucleocapsid antibody (a marker of serologically confirmed COVID-19 infection) and spike antibody (anti-receptor binding domain, a marker of COVID-19 infection or vaccine seroconversion). To assess the relationship between *TAS2R* SNPs and the serological response to COVID-19 vaccination, participants who had received at least one vaccine dose and showed no evidence of prior infection (i.e., no detectable nucleocapsid antibody) were selected. Within this subgroup, the association between *TAS2R* variants and the presence of spike antibody was investigated, with cases defined as individuals with positive spike antibody and controls as those with negative spike antibody status.

5.3.5 Statistical analysis

Study population characteristics were summarized using the gtsummary package in R. *TAS2R* variants were initially tested in univariable models with chi-squared tests using PLINK (v.1.9) to identify variants associated with the outcomes of interest (COVID-19 infection; COVID-19 seroconversion). Independent SNPs ($r^2 < 0.1$ within a 1000 kb window, n=35) (Forgetta et al. 2022) were then selected for inclusion in multivariable logistic regression under an additive genetic model. The list of covariates that were initially included is provided in **Table 5.2**. Assessment of multicollinearity using Pearson correlation analysis revealed no covariate pairs exceeding 0.6; therefore, all covariates were retained for further analysis (**Figure 5.2**). Stepwise

variable selection was then applied to identify the best-fitting models, and the selected variables were included in the final analyses. The final models included the following variables: Age, sex, top 10 principal components for the European ancestry subset released by the CLSA (ePC1-10), smoking status, dwelling area (available only in the Questionnaire Study), dwelling ownership, number of household residents, number of vaccine doses (available only in the Antibody Study), postsecondary education, diabetes, respiratory disease, cardiovascular disease, and IMID. In the COVID-19 Questionnaire analyses, dwelling ownership and number of household residents were excluded due to a high rate of missing data.

The analyses were conducted separately using data from the COVID-19 Questionnaire and Seroprevalence (Antibody) studies, as these datasets include overlapping but nonidentical populations. Subgroup analyses were performed for each outcome, stratifying individuals based on the presence or absence of chronic medical conditions. Statistical significance was set at $P < 0.05$, and Bonferroni correction was applied to adjust for multiple comparisons, considering the number of independent outcomes as 1 due to the high correlation between outcomes (Phi coefficient > 0.1) (Akoglu 2018).

Table 5.2 Variables initially extracted for logistic regression.

Variable	Dataset	Question	Response values/categories
Sex	Genetic data	-	Female/male (chromosomal)
ePC1-10 [1]	Genetic data	-	Continuous variable
Dwelling area	Covid-19	-	Dichotomized: urban core, rural/not urban core
Number of household residents	Antibody	How many people, including yourself, live in your residence? (sleep there at least 3 nights per week)	Integer
Smoking status	Covid-19 and Antibody	At the present time, do you smoke cigarettes daily, occasionally, or not at all?	Dichotomized: daily/occasionally, not at all
Education	Baseline	What is your highest level of education?	Dichotomized: with or without post-secondary education/graduation
Province of residence	Follow-up 2	-	Newfoundland and Labrador, Prince Edward Island, Nova Scotia, New Brunswick, Quebec, Ontario, Manitoba, Saskatchewan, Alberta, British Columbia, Yukon, Northwest Territories, Nunavut
Alcohol consumption	Follow-up 2	About how often during the past 12 months did you drink alcohol?	Never, 2-3 times per month or less, 1-3 times per week, more than 3 times per week
Systemic comorbiditie s	Follow-up 2 and Baseline	Based on the case definitions explained in Figure 2S	Diabetes, cardiovascular disease, respiratory disease, immune-mediated inflammatory disease
Age (years)	Follow-up	-	Integer
Body mass index (BMI)	Follow-up 2	-	Decimal (pregnant women were excluded)
Marital/partn er status	Follow-up 2	What is your current marital/partner status?	Single (never married/lived with a partner), married or in a common-law relationship, widowed, divorced, separated
Retirement status	Follow-up 2	At this time, do you consider yourself to be completely retired, partly retired, or not retired?	Completely retired, partly retired, not retired, never had a paid job
Dwelling status	Follow-up 2	Do you or your partner own or rent your dwelling?	Own, rent, other
Received vaccine	Antibody	Have you received at least one dose of a COVID-19 vaccine?	Yes, No
Number of vaccines received	Antibody	How many doses of COVID-19 vaccine have you received so far?	Integer
Type of vaccine	Antibody	Which vaccine did you receive?	Pfizer and BioNTech mRNA vaccine, Moderna mRNA vaccine, AstraZeneca Oxford, Other

[1] Top 10 components of the principal component analysis (PCA) from the CLSA European ancestry population

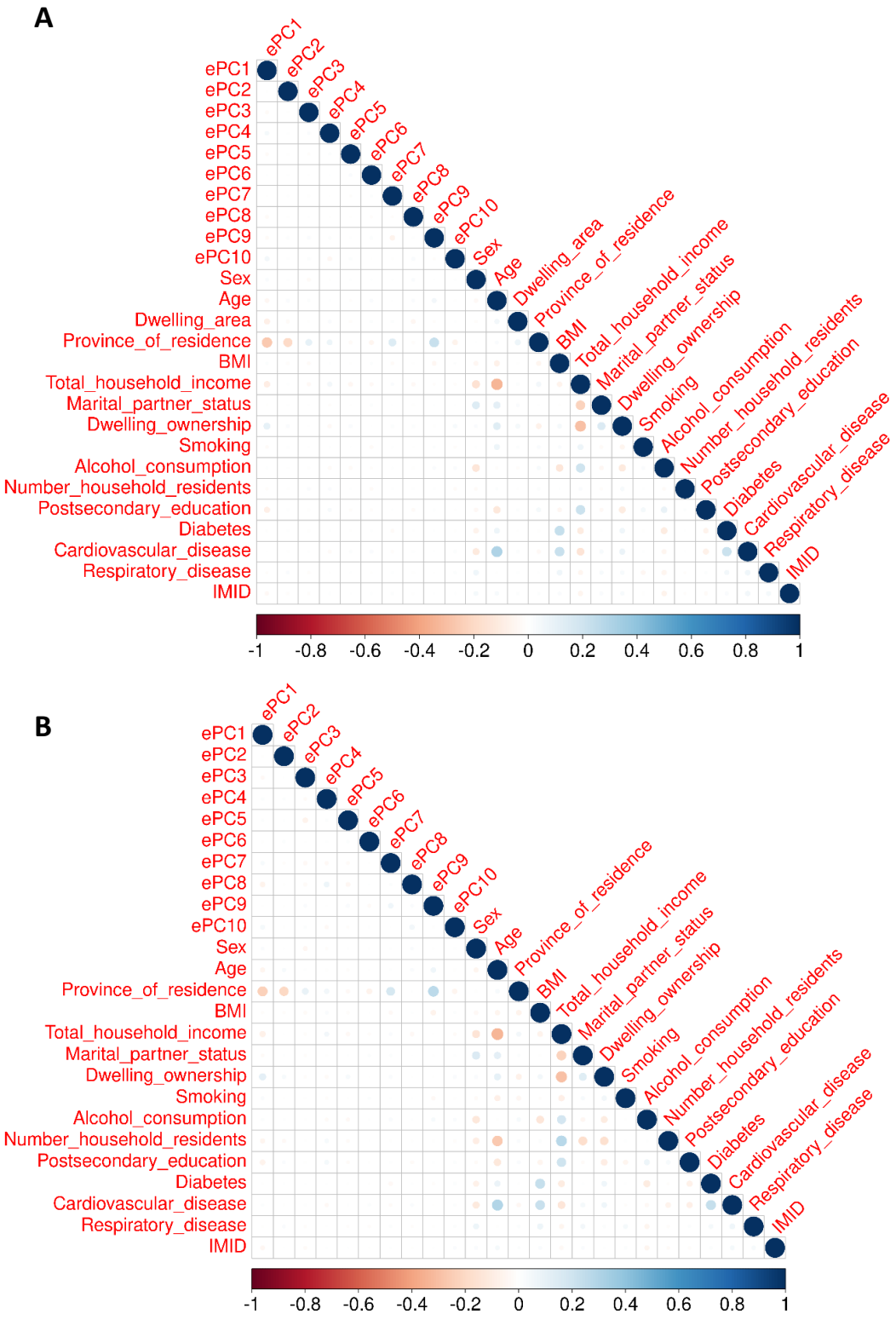

Figure 5.2 Correlation plots of covariates.

(A) COVID-19 cohort (n=14,073). **(B)** COVID-19 Antibody cohort (n=8,313). IMID: immune-mediated inflammatory disease.

5.4 Results

After applying eligibility criteria, 14,073 individuals from the COVID-19 Questionnaire Study and 8,313 from the COVID-19 Antibody Study were included. **Figure 5.2** provides an overview of the sample selection, data analysis, and results. Population characteristics are summarized in **Table 5.3**. Chi-squared tests identified 16 SNPs with differing allele distributions between cases and controls (**Table 5.4**). Among these, eight SNPs were independent ($r^2 < 0.1$) and were included in logistic regression analyses.

5.4.1 CLSA COVID-19 Questionnaire Study

In the Questionnaire Study, 2,299 (16.33%) participants were tested for COVID-19, with 45 (1.96% of those tested) having a positive result (confirmed cases). The total number of confirmed/probable cases was 153 (1.09% of the cohort). Confirmed/probable COVID-19 infection was significantly associated with age (OR: 0.97, P <0.001), female sex (OR: 1.80, P < 0.001), rental dwelling status (OR: 1.66, P = 0.017), and respiratory disease (OR: 1.78, P = 0.001) (**Table 5.5**). The rs117458236 variant, located in the 3′ untranslated region (UTR) of *TAS2R20*, was associated with increased odds of confirmed/probable infection (OR: 1.95, P = 0.039). No significant association was found between confirmed infection and *TAS2R* variants (**Table 5.5**).

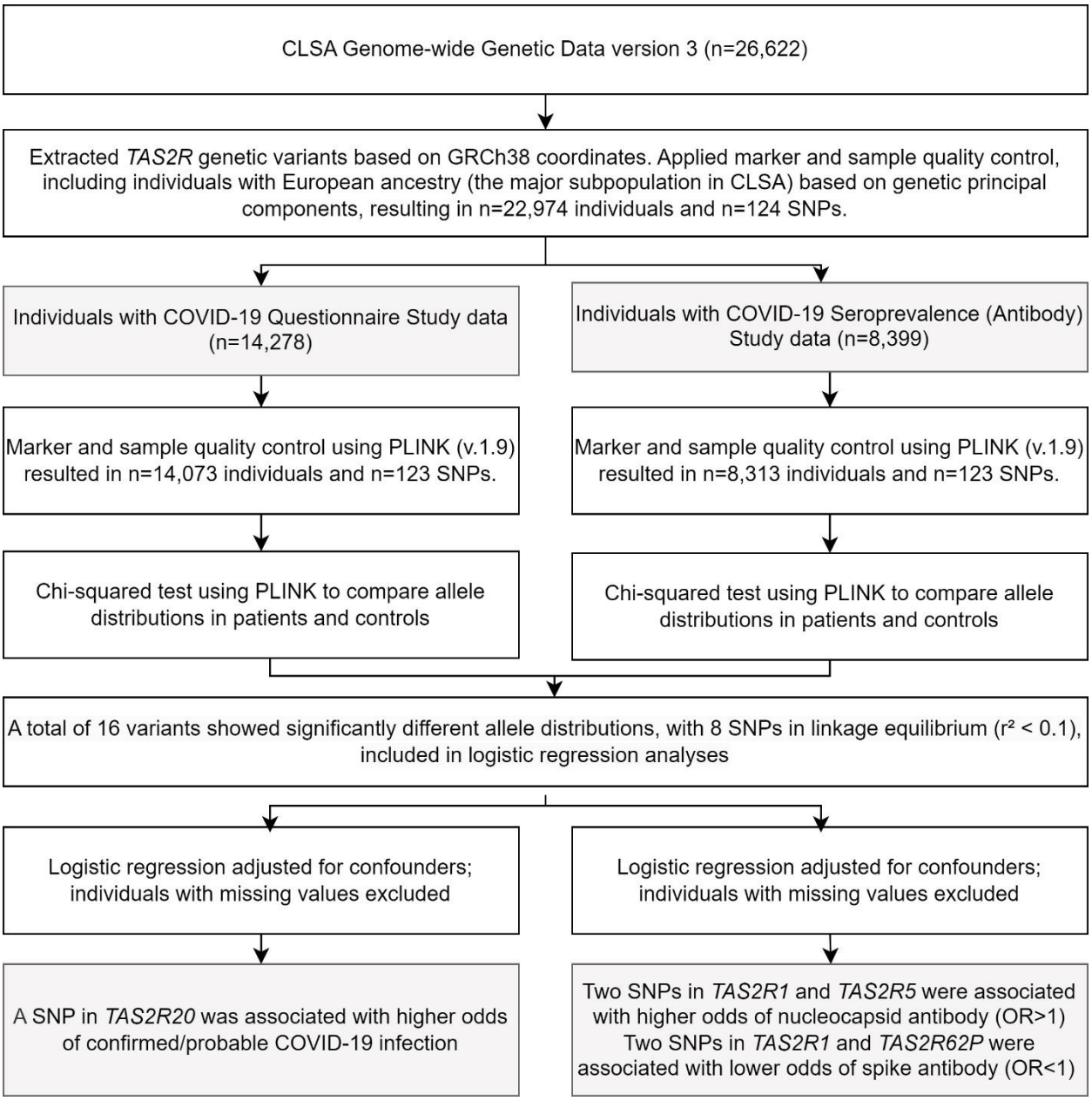

Figure 5.3 Flow diagram of the sample selection and data analysis.

Table 5.3 Population characteristics of the study cohorts.

Characteristic	COVID-19 Questionnaire (N = 14,073)[1]	COVID-19 Antibody (N = 8,313)[1]
Sex		
Male	6,831 (49%)	4,133 (50%)
Female	7,242 (51%)	4,180 (50%)
Age	67 (61, 75)	67 (60, 75)
BMI	26.9 (24.1, 30.5)	27.1 (24.3, 30.5)
Marital partner status		
Single/never married	1,113 (7.9%)	617 (7.4%)
Married/in a common-law relationship	9,728 (69%)	5,816 (70%)
Widowed/divorced/separated	3,201 (23.2%)	1,857 (22.3%)
Dwelling ownership		
Own	11,824 (85%)	7,075 (86%)
Rent	2,064 (15%)	1,125 (14%)
Smoking	857 (6.2)	401 (4.9%)
Alcohol consumption		
Never	1,843 (13%)	1,039 (13%)
Less than 4 times per month	3,725 (27%)	2,182 (26%)
1-3 times per week	4,405 (31%)	2,635 (32%)
More than 3 times per week	4,080 (29%)	2,445 (29%)
Number household residents	2 (2, 2)	2 (2, 2)
Postsecondary education	12,307 (88%)	7,321 (88%)
Diabetes[2]	3,099 (22%)	1,828 (22%)
Cardiovascular disease[3]	7,229 (51%)	4,204 (51%)
Respiratory disease[4]	2,890 (21%)	1,723 (21%)
Immune-mediated inflammatory disease[5]	829 (5.9%)	491 (5.9%)
Tested for COVID-19[6]	2,299 (16%)	2,510 (31%)
Self-reported positive test for COVID-19	45	78

[1] n (%); Median (IQR); Percentages are calculated based on respondents, excluding missing data.
[2] Diabetes, borderline diabetes, or high blood sugar
[3] Hypertension, peripheral arterial disease or poor circulation in limbs, heart disease, heart attack or myocardial infarction, angina, or history of stroke or CVA
[4] Asthma, emphysema, chronic bronchitis, chronic obstructive pulmonary disease (COPD), or chronic pulmonary changes due to smoking
[5] Rheumatoid arthritis or inflammatory bowel disease
[6] Self-reported nucleic acid amplification test

Table 5.4 *TAS2R* variants with differing allele frequencies by COVID-19-related phenotypes. Only variants with $P < 0.05$ are presented (chi-squared test).

Phenotype	rs ID	Gene	Allele [1]	OR (95% CI)[2]	Consequence	P-value	Adjusted P-value
Confirmed or probable infection	rs117458236	*TAS2R20*	T	2.13 (1.19, 3.82)	3′ UTR[3]	0.009	0.072
	rs3851584	*TAS2R14*	G	1.30 (1.03, 1.62)	3′ UTR	0.024	0.192
	rs1548803	*TAS2R8*	C	1.29 (1.03, 1.62)	Synonymous 183	0.028	0.224
	rs184006834	*TAS2R67p*	A	1.64 (1.05, 2.57)	Downstream (SMIM10L1)	0.028	0.224
	rs1015443	*TAS2R13*	T	1.28 (1.02, 1.61)	Missense (Ser 259 Asn)	0.030	0.240
	rs1015442	*TAS2R13*	T	1.28 (1.02, 1.61)	3′ UTR	0.031	0.248
	rs10772397	*TAS2R50*	C	1.28 (1.02, 1.61)	Synonymous 259	0.031	0.248
	rs3741845	*TAS2R9*	A	1.27 (1.01, 1.59)	Missense (Ala 187 Val)	0.038	0.304
Nucleocapsid antibody	rs2234010	*TAS2R5*	A	1.60 (1.13, 2.27)	5′ UTR	0.008	0.064
	rs2234235	*TAS2R1*	G	1.57 (1.10, 2.24)	Synonymous	0.013	0.104
	rs10246939	*TAS2R38*	C	0.85 (0.73, 0.99)	Missense (Ile 296 Val)	0.034	0.272
	rs1726866	*TAS2R38*	G	0.85 (0.74, 0.99)	Missense (Val 262 Ala)	0.040	0.320
	rs713598	*TAS2R38*	G	0.85 (0.73, 0.99)	Missense (Ala 49 Pro)	0.040	0.320
	rs77837442	*TAS2R19*	T	0.44 (0.18, 1.06)	Stop-gained (Trp 295)	0.059	0.472
Spike antibody	rs34039200	*TAS2R62p*	A	0.92 (0.85, 0.99)	Pseudogene	0.026	0.208
	rs2234009	*TAS2R5*	T	1.22 (1.00, 1.48)	5′ UTR	0.050	0.400

[1] Effect Allele
[2] OR = Odds Ratio, CI = Confidence Interval
[3] UTR = Untranslated region

Table 5.5 Association between *TAS2R* variants and COVID-19 infection in the CLSA COVID-19 Questionnaire cohort with available genetic data.

Characteristic	**Confirmed/probable COVID-19 (N=13,536)**[1]			**Confirmed COVID-19 (N=2,176)**[2]		
	Controls (N=13,389)	**Cases (N=147)**		**Controls (N=2,134)**		**Cases (N=42)**
	OR[3]	**95% CI**[3]	**P-value**	**OR**	**95% CI**	**P-value**
Age	**0.97**	**0.95, 0.99**	**<0.001**	1.02	0.98, 1.06	0.27
Sex (female)	**1.80**	**1.28, 2.58**	**<0.001**	1.59	0.82, 3.21	0.18
Smoking	0.75	0.33, 1.45	0.44	0.00	0.00, 16,640,631,130	0.99
Dwelling area (urban core)	1.48	0.91, 2.56	0.14	1.42	0.59, 4.27	0.48
Dwelling ownership (rent)	**1.66**	**1.08, 2.49**	**0.017**	0.88	0.37, 1.91	0.76
Postsecondary education	1.44	0.83, 2.71	0.22	1.15	0.48, 3.20	0.77
Diabetes	1.40	0.94, 2.03	0.088	1.09	0.49, 2.25	0.82
Respiratory disease	**1.78**	**1.24, 2.52**	**0.001**	1.08	0.50, 2.16	0.84
Cardiovascular disease	0.84	0.59, 1.20	0.35	0.90	0.46, 1.76	0.75
IMID[4]	1.33	0.69, 2.34	0.35	2.30	0.75, 5.75	0.10
rs2234235, *TAS2R1* (A/G)	1.48	0.80, 2.52	0.18	1.00	0.24, 2.77	>0.99
rs2234009, *TAS2R5* (C/T)	1.09	0.49, 2.07	0.81	1.32	0.31, 3.83	0.66
rs2234010, *TAS2R5* (G/A)	0.89	0.40, 1.70	0.74	0.80	0.13, 2.80	0.77
rs1726866, *TAS2R38* (A/G)	1.09	0.86, 1.38	0.47	1.08	0.68, 1.70	0.74
rs34039200, *TAS2R62P* (G/A)	1.17	0.89, 1.52	0.26	1.42	0.86, 2.29	0.16
rs3851584, *TAS2R14* [5] (T/G)	1.26	0.99, 1.60	0.058	1.20	0.76, 1.90	0.43
rs77837442, *TAS2R19* (C/T)	0.75	0.18, 2.02	0.63	0.00	0.00, 6,515,699,880,253,166	0.99
rs117458236, *TAS2R20* (C/T)	**1.95**	**0.98, 3.51**	**0.039**	2.20	0.50, 6.77	0.22

[1] Defined as a "yes" response to either of the following questions: "Have you had a positive test result?" or "Have you been told by a healthcare provider that you have COVID-19, but you did NOT have a test to confirm this?"

[2] Defined as a "yes" response to the question, "Have you had a positive test result?"

[3] Logistic regression, OR = Odds Ratio, CI = Confidence Interval

[4] IMID = Immune-mediated inflammatory disease

[5] In linkage disequilibrium with: rs10772397 in TAS2R50 (r2=0.83) and rs1015443 and rs1015442 in *TAS2R13* (r2>0.99)

5.4.2 CLSA COVID-19 Seroprevalence (Antibody) Study

Vaccination history and antibody response data are summarized in **Table 5.6**. A total of 370 (4.45%) participants had detectable nucleocapsid antibody, indicating serologically confirmed infection, while 3,773 had detectable spike antibody, which could result from infection or vaccination. Logistic regression results are summarized in **Table 5.7**. Spike antibody was positively associated with female sex (OR: 1.25, $P = 0.002$), number of vaccine doses (OR: 90.2, $P < 0.001$), and negatively associated with IMID diagnosis (OR: 0.65, $P = 0.003$). Three *TAS2R* variants were associated with antibody response: rs2234235, a synonymous variant in *TAS2R1*, with higher odds of nucleocapsid antibody (OR: 1.55, $P = 0.018$) and lower odds of spike antibody (OR: 0.74, $P = 0.033$); rs2234010, located in the 5′ UTR in *TAS2R5*, with higher odds of nucleocapsid antibody (OR: 1.56, $P = 0.014$); and rs34039200 in *TAS2R62P* (pseudogene) with lower odds of spike antibody (OR: 0.86, $P = 0.013$).

Among the 8,313 participants, 4,267 had received at least one vaccine dose, with Pfizer-BioNTech being the most commonly administered (n = 3,901). Logistic regression in vaccinated individuals without detectable nucleocapsid antibody (**Table 5.8**) confirmed the association of rs2234235 in *TAS2R1* and rs34039200 in *TAS2R62P* with lower odds of spike antibody in vaccinated individuals (OR: 0.72, $P = 0.023$; OR: 0.86, $P = 0.018$, respectively).

Table 5.6 COVID-19 vaccination history and antibody response in the COVID-19 Antibody Study cohort with available genetic data.

Characteristic	Number (N = 8,313)
Number of doses of COVID-19 vaccine[2]	
0	1,438
1	4,267
2 or more	869
Unknown	1,739
Vaccine type[3]	
Pfizer BioNTech	3,901
Moderna	600
AstraZeneca	695
Other type	28
Nucleocapsid antibody response	370
Spike antibody response	3,773
Antibody result interpretation	
Prior SARS-CoV-2 infection	151
Prior SARS-CoV-2 infection and/or vaccination	3,554
Prior SARS-CoV-2 infection OR infection and vaccination	219
No antibodies detected	3,757

[1] "Have you received at least one dose of a COVID-19 vaccine?"

[2] "How many doses of COVID-19 vaccine have you received so far?"

[3] "Which vaccine did you receive?"

Table 5.7 Association between *TAS2R* variants and SARS-CoV-2 seroconversion in the CLSA COVID-19 Antibody cohort with available genetic data.

Characteristic	**Nucleocapsid antibody (N=7,475)**			**Spike Antibody (N=7,475)**		
	Controls (N=7,115)	**Cases (N=360)**		**Controls (N=3,818)**		**Cases (N=3,657)**
	OR[1]	**95% CI**[1]	**p-value**	**OR**	**95% CI**	**p-value**
Age	0.99	0.98, 1.01	0.30	1.00	0.99, 1.01	0.81
Sex (female)	0.87	0.70, 1.08	0.22	**1.25**	**1.08, 1.43**	**0.002**
Smoking	0.84	0.47, 1.39	0.54	0.94	0.68, 1.33	0.74
Dwelling ownership (rent)	0.94	0.67, 1.30	0.73	0.93	0.76, 1.15	0.50
Number of household residents	1.05	0.95, 1.12	0.27	1.05	0.98, 1.12	0.21
Number of vaccine doses	0.98	0.82, 1.16	0.79	**90.2**	**72.6, 114**	**<0.001**
Postsecondary education	0.84	0.61, 1.17	0.29	1.16	0.93, 1.43	0.19
Diabetes	0.94	0.71, 1.23	0.67	0.89	0.75, 1.06	0.19
Respiratory disease	1.08	0.82, 1.40	0.57	0.93	0.79, 1.10	0.40
Cardiovascular disease	0.96	0.76, 1.21	0.72	0.90	0.78, 1.04	0.16
IMID[2]	1.16	0.73, 1.76	0.50	**0.65**	**0.49, 0.86**	**0.003**
rs2234235, *TAS2R1* (A/G)	**1.55**	**1.06, 2.20**	**0.018**	**0.74**	**0.57, 0.98**	**0.033**
rs2234009, *TAS2R5* (C/T)	1.01	0.62, 1.56	0.96	1.33	0.99, 1.80	0.064
rs2234010, *TAS2R5* (G/A)	**1.56**	**1.08, 2.19**	**0.014**	1.26	0.95, 1.69	0.11
rs1726866, *TAS2R38* (A/G)	0.89	0.76, 1.03	0.13	1.04	0.94, 1.14	0.46
rs34039200, *TAS2R62P* (G/A)	0.99	0.82, 1.19	0.93	**0.86**	**0.77, 0.97**	**0.013**
rs3851584, *TAS2R14* **(T/G)**	0.96	0.82, 1.12	0.63	1.02	0.93, 1.13	0.65
rs77837442, *TAS2R19* (C/T)	0.45	0.16, 0.99	0.080	0.91	0.63, 1.34	0.63
rs117458236, *TAS2R20* (C/T)	0.99	0.53, 1.68	0.96	0.94	0.67, 1.33	0.73

[1] Logistic regression, OR = Odds Ratio, CI = Confidence Interval
[2] IMID = Immune-mediated inflammatory disease

Table 5.8 Association between *TAS2R* variants and SARS-CoV-2 spike antibody in individuals who received at least one dose of vaccine and did not have detectable nucleocapsid antibody (N = 4,404).

Characteristic	**Spike Antibody**		
	OR[1]	**95% CI**[1]	**p-value**
Age	1.00	0.99, 1.01	0.72
Sex	**1.31**	**1.13, 1.53**	**<0.001**
Smoking	0.92	0.65, 1.34	0.66
Dwelling ownership (rent)	0.90	0.73, 1.13	0.37
Number household residents	1.06	0.98, 1.16	0.17
Number doses	**8.26**	**5.71, 12.5**	**<0.001**
Vaccine type			
Moderna	**2.33**	**1.34, 4.05**	**0.003**
Pfizer BioNTech	1.52	0.91, 2.51	0.11
AstraZeneca	1.25	0.73, 2.14	0.42
Postsecondary education	1.14	0.90, 1.43	0.28
Diabetes	**0.85**	**0.71, 1.02**	**0.078**
Respiratory disease	0.87	0.72, 1.04	0.12
Cardiovascular disease	**0.87**	**0.74, 1.01**	**0.074**
IMID[2]	**0.62**	**0.46, 0.83**	**0.001**
rs2234235, *TAS2R1* (A/G)	**0.71**	**0.54, 0.95**	**0.021**
rs2234009, *TAS2R5* (C/T)	1.26	0.92, 1.75	0.17
rs2234010, *TAS2R5* (G/A)	1.17	0.86, 1.62	0.34
rs1726866, *TAS2R38* (A/G)	1.02	0.92, 1.14	0.69
rs34039200, *TAS2R62P* (G/A)	**0.86**	**0.76, 0.97**	**0.017**
rs3851584, *TAS2R14* (T/G)	1.05	0.95, 1.17	0.34
rs77837442, *TAS2R19* (C/T)	0.95	0.64, 1.43	0.79
rs117458236, *TAS2R20* (C/T)	0.88	0.62, 1.27	0.47

[1] Logistic regression, OR = Odds Ratio, CI = Confidence Interval

[2] IMID = Immune-mediated inflammatory disease

5.4.3 Subgroup analysis

In the Questionnaire Study, subgroup analysis was performed for confirmed/probable infection due to the larger sample size (**Table 5.9**). The rs117458236 variant in *TAS2R20* remained associated with infection in individuals with diabetes (OR: 3.27, $P = 0.035$) or cardiovascular disease (OR: 2.69, $P = 0.016$). Additionally, this variant was associated with increased risk of infection in individuals without IMID (OR: 2.15, $P = 0.018$), but not in those with IMID ($P > 0.05$). Additionally, rs2234235 in *TAS2R1*, was linked to confirmed/probable infection in individuals with respiratory disease (OR: 2.65, $P = 0.018$) or IMID (OR: 7.39, $P = 0.012$), as well as in those without diabetes (OR: 2.08, $P = 0.013$). Additionally, rs1726866, a missense variant (Val 262 Ala) in *TAS2R38* was associated with infection in individuals with respiratory disease (OR: 1.86, $P = 0.004$).

Table 5.9 Association between *TAS2R* variants and confirmed/probable COVID-19 infection in patients with chronic medical conditions and in corresponding control groups, defined as individuals without the specific condition under analysis.

Characteristic	Diabetes				Cardiovascular disease			
	Controls (N=10,560) OR (95% CI)[1]	p-value	Patients (N=2,976) OR (95% CI)[1]	p-value	Controls (N=6,613) OR (95% CI)[1]	p-value	Patients (N=6,923) OR (95% CI)[1]	p-value
Age	**0.97 (0.95, 0.99)**	**0.011**	**0.95 (0.91, 0.99)**	**0.018**	**0.97 (0.94, 0.99)**	**0.014**	**0.97 (0.94, 1.00)**	**0.023**
Sex (female)	**1.89 (1.25, 2.90)**	**0.003**	1.69 (0.88, 3.31)	0.12	**2.20 (1.35, 3.76)**	**0.002**	1.49 (0.91, 2.47)	0.11
Smoking	0.39 (0.09, 1.04)	0.11	1.99 (0.66, 4.92)	0.17	0.64 (0.19, 1.56)	0.39	0.93 (0.28, 2.32)	0.90
Dwelling area (urban core)	1.60 (0.90, 3.09)	0.13	1.15 (0.48, 3.43)	0.77	1.53 (0.81, 3.20)	0.22	1.48 (0.71, 3.60)	0.34
Dwelling ownership (rent)	1.62 (0.95, 2.64)	0.063	1.98 (0.91, 4.04)	0.071	**1.88 (1.02, 3.26)**	**0.032**	1.48 (0.78, 2.64)	0.21
Postsecondary education	1.42 (0.74, 3.06)	0.33	1.62 (0.62, 5.53)	0.38	0.81 (0.42, 1.71)	0.55	**3.61 (1.32, 14.9)**	**0.032**
Diabetes	-	-	-	-	1.20 (0.61, 2.16)	0.56	1.55 (0.93, 2.54)	0.088
Cardiovascular disease	0.77 (0.50, 1.16)	0.22	1.11 (0.55, 2.35)	0.78	-	-	-	-
Respiratory disease	**2.16 (1.42, 3.22)**	**<0.001**	1.00 (0.47, 2.00)	>0.99	**1.81 (1.09, 2.91)**	**0.018**	**1.72 (1.01, 2.85)**	**0.039**
IMID[2]	1.10 (0.46, 2.25)	0.80	1.72 (0.57, 4.23)	0.28	1.19 (0.41, 2.72)	0.72	1.39 (0.57, 2.90)	0.42
rs2234235, *TAS2R1*	**2.08 (1.11, 3.57)**	**0.013**	0.00 (0.00, 15,096,353)	0.98	1.88 (0.87, 3.57)	0.078	1.01 (0.31, 2.45)	0.98
rs2234009, *TAS2R5*	1.41 (0.59, 2.86)	0.38	0.42 (0.02, 1.92)	0.39	0.77 (0.19, 2.07)	0.65	1.43 (0.51, 3.16)	0.44
rs2234010, *TAS2R5*	0.74 (0.26, 1.65)	0.52	1.21 (0.29, 3.37)	0.76	1.22 (0.47, 2.60)	0.64	0.49 (0.08, 1.56)	0.32
rs1726866, *TAS2R38*	1.15 (0.88, 1.52)	0.31	0.93 (0.58, 1.47)	0.75	1.19 (0.86, 1.64)	0.29	0.97 (0.68, 1.37)	0.85
rs34039200, *TAS2R62P*	1.22 (0.88, 1.65)	0.22	1.05 (0.59, 1.78)	0.87	1.23 (0.85, 1.74)	0.27	1.08 (0.71, 1.62)	0.70
rs3851584, *TAS2R14*	1.28 (0.97, 1.68)	0.086	1.14 (0.71, 1.80)	0.59	**1.38 (1.00, 1.90)**	**0.050**	1.13 (0.79, 1.60)	0.52
rs77837442, *TAS2R19*	0.66 (0.11, 2.11)	0.56	0.97 (0.05, 4.87)	0.98	1.39 (0.33, 3.82)	0.59	0.00 (0.00, 50.4)	0.98
rs117458236, *TAS2R20*	1.65 (0.68, 3.38)	0.21	**3.27 (0.93, 8.91)**	**0.035**	1.29 (0.38, 3.22)	0.63	**2.69 (1.10, 5.65)**	**0.016**

[1]Logistic regression, OR = Odds Ratio, CI = Confidence Interval

[2]IMID = Immune-mediated inflammatory disease

Table 5.9 (Continued) Association between *TAS2R* variants and confirmed/probable COVID-19 infection in patients with chronic medical conditions and in corresponding control groups, defined as individuals without the specific condition under analysis.

Characteristic	Respiratory disease				Immune-mediated inflammatory disease			
	Controls (N=10,757) OR (95% CI)[1]	p-value	Patients (N=2,779) OR (95% CI)[1]	p-value	Controls (N=12,739) OR (95% CI)[1]	p-value	Patients (N=797) OR (95% CI)[1]	p-value
Age	**0.97 (0.94, 0.99)**	**0.005**	**0.96 (0.93, 1.00)**	**0.034**	**0.96 (0.94, 0.98)**	**<0.001**	1.00 (0.93, 1.07)	0.95
Sex (female)	**1.83 (1.21, 2.82)**	**0.005**	1.79 (0.96, 3.52)	0.075	**1.73 (1.21, 2.50)**	**0.003**	4.15 (0.97, 29.4)	0.087
Smoking	0.46 (0.11, 1.25)	0.19	1.32 (0.44, 3.15)	0.57	0.82 (0.36, 1.59)	0.59	0.00 (-, -)	>0.99
Dwelling area (urban core)	1.31 (0.75, 2.48)	0.38	2.12 (0.84, 7.18)	0.16	1.56 (0.93, 2.80)	0.11	0.85 (0.19, 6.28)	0.85
Dwelling rent	1.62 (0.94, 2.68)	0.070	1.74 (0.83, 3.42)	0.12	**1.78 (1.14, 2.71)**	**0.009**	0.82 (0.11, 3.75)	0.82
Postsecondary education	1.91 (0.94, 4.61)	0.10	0.94 (0.41, 2.55)	0.89	1.52 (0.85, 3.03)	0.19	1.23 (0.28, 8.95)	0.81
Diabetes	**1.73 (1.08, 2.71)**	**0.020**	0.90 (0.43, 1.76)	0.77	1.32 (0.87, 1.96)	0.19	3.21 (0.82, 12.5)	0.086
Cardiovascular disease	0.83 (0.53, 1.28)	0.40	0.91 (0.49, 1.68)	0.75	0.84 (0.58, 1.21)	0.35	0.80 (0.20, 3.41)	0.76
Respiratory disease	-	-	-	-	**1.93 (1.33, 2.77)**	**<0.001**	0.77 (0.16, 2.79)	0.71
IMID[2]	1.83 (0.85, 3.49)	0.092	0.67 (0.16, 1.88)	0.50	-	-	-	-
rs2234235, *TAS2R1*	0.99 (0.39, 2.08)	0.99	**2.65 (1.08, 5.61)**	**0.018**	1.24 (0.61, 2.24)	0.52	**7.39 (1.36, 33.8)**	**0.012**
rs2234009, *TAS2R5*	0.72 (0.22, 1.72)	0.52	2.22 (0.67, 5.57)	0.13	1.19 (0.53, 2.27)	0.64	0.00 (-, -)	>0.99
rs2234010, *TAS2R5*	0.88 (0.34, 1.83)	0.75	0.86 (0.14, 2.90)	0.84	0.85 (0.36, 1.70)	0.69	1.03 (0.05, 6.89)	0.98
rs1726866, *TAS2R38*	0.86 (0.64, 1.14)	0.30	**1.86 (1.22, 2.88)**	**0.004**	1.10 (0.86, 1.40)	0.45	0.94 (0.39, 2.25)	0.89
rs34039200, *TAS2R62P*	1.14 (0.81, 1.57)	0.43	1.22 (0.74, 1.94)	0.43	1.17 (0.87, 1.54)	0.29	1.18 (0.38, 3.21)	0.75
rs3851584, *TAS2R14*	1.22 (0.91, 1.63)	0.19	1.36 (0.89, 2.08)	0.15	**1.30 (1.01, 1.67)**	**0.038**	1.04 (0.42, 2.54)	0.92
rs77837442, *TAS2R19*	0.75 (0.12, 2.42)	0.70	0.67 (0.04, 3.23)	0.69	0.82 (0.20, 2.20)	0.74	0.00 (-, -)	>0.99
rs117458236, *TAS2R20*	2.09 (0.92, 4.13)	0.052	1.81 (0.42, 5.34)	0.34	**2.15 (1.08, 3.88)**	**0.018**	0.00 (-, -)	>0.99

[1]Logistic regression, OR = Odds Ratio, CI = Confidence Interval
[2]IMID = Immune-mediated inflammatory disease

In the Antibody Study, subgroup analysis confirmed the association of rs2234235 in *TAS2R1* and rs2234010 in *TAS2R5* with nucleocapsid antibody (OR>1) in most healthy subgroups (**Table 5.10**). Additionally, rs77837442 in *TAS2R19* was associated with nucleocapsid antibody in individuals without diabetes (OR: 0.23, $P = 0.036$). No significant association was found with other variants. The association of rs34039200 in *TAS2R62P* with spike antibody remained significant in individuals with diabetes, as well as those without cardiovascular disease, respiratory disease, or IMID (OR<1, **Table 5.11**). Although rs2234235 in *TAS2R1* was associated with the spike antibody in the entire cohort (OR: 0.74, $P = 0.033$), subgroup analysis revealed significant association only in patients with respiratory disease (OR: 0.48, $P = 0.012$) or IMID (OR: 0.37, $P = 0.058$). Furthermore, the two SNPs in 5′ UTR of *TAS2R5*, which were not associated with spike antibody in the overall cohort, showed significant association in patients with diabetes (OR>2, P<0.05). Lastly, rs77837442, a stop-gained variant (Trp 295) in *TAS2R19*, was associated with spike antibody in patients with diabetes (OR: 0.44, $P = 0.054$).

Table 5.10 Association between *TAS2R* variants and SARS-CoV-2 nucleocapsid antibody in patients with chronic medical conditions and in corresponding control groups, defined as individuals without the specific condition under analysis.

Characteristic	Diabetes				Cardiovascular disease			
	Controls (N=5,875) OR (95% CI)[1]	p-value	Patients (N=1,600) OR (95% CI)[1]	p-value	Controls (N=3,759) OR (95% CI)[1]	p-value	Patients (N=3,716) OR (95% CI)[1]	p-value
Age	1.00 (0.99, 1.02)	0.83	**0.96 (0.93, 0.99)**	**0.007**	0.99 (0.97, 1.01)	0.37	1.0 (0.98, 1.01)	0.56
Sex (female)	0.87 (0.68, 1.11)	0.26	0.80 (0.48, 1.31)	0.38	0.86 (0.64, 1.16)	0.32	0.87 (0.63, 1.20)	0.40
Smoking	0.95 (0.51, 1.63)	0.87	0.45 (0.07, 1.56)	0.29	0.99 (0.46, 1.89)	0.98	0.76 (0.29, 1.62)	0.52
Dwelling rent	0.90 (0.60, 1.32)	0.61	1.08 (0.53, 2.04)	0.81	**0.53 (0.27, 0.94)**	**0.045**	1.28 (0.83, 1.91)	0.25
Number of household residents	1.05 (0.95, 1.13)	0.28	1.04 (0.80, 1.25)	0.73	1.06 (0.94, 1.16)	0.25	1.02 (0.87, 1.14)	0.75
Number of vaccine doses	1.00 (0.83, 1.21)	>0.99	0.88 (0.60, 1.29)	0.53	1.02 (0.81, 1.29)	0.85	0.93 (0.72, 1.19)	0.58
Postsecondary education	0.88 (0.61, 1.32)	0.53	0.66 (0.36, 1.28)	0.20	0.85 (0.53, 1.47)	0.54	0.84 (0.55, 1.31)	0.41
Diabetes	-	-	-	-	0.96 (0.59, 1.50)	0.87	0.93 (0.66, 1.29)	0.67
Cardiovascular disease	0.92 (0.71, 1.19)	0.52	1.08 (0.64, 1.87)	0.79	-	-	-	-
Respiratory disease	1.00 (0.73, 1.35)	0.98	1.36 (0.78, 2.29)	0.26	1.04 (0.69, 1.51)	0.85	1.11 (0.77, 1.59)	0.56
IMID[2]	1.31 (0.78, 2.07)	0.27	0.82 (0.24, 2.08)	0.71	0.64 (0.25, 1.35)	0.29	1.60 (0.91, 2.64)	0.081
rs2234235, *TAS2R1*	**1.72 (1.13, 2.52)**	**0.007**	0.94 (0.32, 2.20)	0.89	**2.04 (1.25, 3.17)**	**0.003**	1.03 (0.52, 1.81)	0.93
rs2234009, *TAS2R5*	0.92 (0.52, 1.51)	0.75	1.47 (0.52, 3.33)	0.40	1.12 (0.58, 1.98)	0.71	0.94 (0.42, 1.79)	0.87
rs2234010, *TAS2R5*	**1.71 (1.15, 2.47)**	**0.006**	0.88 (0.26, 2.26)	0.82	1.38 (0.81, 2.23)	0.21	**1.75 (1.01, 2.86)**	**0.033**
rs1726866, *TAS2R38*	0.85 (0.71, 1.01)	0.071	1.00 (0.71, 1.41)	0.99	0.84 (0.68, 1.04)	0.12	0.94 (0.75, 1.18)	0.60
rs34039200, *TAS2R62P*	0.95 (0.77, 1.17)	0.66	1.18 (0.77, 1.76)	0.44	0.91 (0.69, 1.17)	0.45	1.10 (0.83, 1.43)	0.50
rs3851584, *TAS2R14*	0.97 (0.82, 1.15)	0.74	0.92 (0.64, 1.30)	0.62	1.00 (0.81, 1.24)	0.97	0.92 (0.74, 1.16)	0.49
rs77837442, *TAS2R19*	**0.23 (0.04, 0.71)**	**0.036**	1.43 (0.33, 4.30)	0.57	0.40 (0.07, 1.28)	0.21	0.47 (0.11, 1.28)	0.21
rs117458236, *TAS2R20*	0.85 (0.40, 1.60)	0.65	1.45 (0.42, 3.86)	0.50	0.55 (0.17, 1.33)	0.24	1.54 (0.71, 2.97)	0.23

[1]Logistic regression, OR = Odds Ratio, CI = Confidence Interval

[2]IMID = Immune-mediated inflammatory disease

Table 10 (Continued) Association between *TAS2R* variants and SARS-CoV-2 nucleocapsid antibody in patients with chronic medical conditions and in corresponding control groups, defined as individuals without the specific condition under analysis.

Characteristic	Respiratory disease				Immune-mediated inflammatory disease			
	Controls (N=5,964)		Patients (N=1,511)		Controls (N=7,047)		Patients (N=428)	
	OR (95% CI)[1]	p-value	OR (95% CI)[1]	p-value	OR (95% CI)[1]	p-value	OR (95% CI)[1]	p-value
Age	0.99 (0.98, 1.01)	0.31	0.99 (0.97, 1.02)	0.67	0.99 (0.98, 1.01)	0.36	0.98 (0.91, 1.04)	0.46
Sex (female)	0.87 (0.68, 1.11)	0.25	0.86 (0.53, 1.39)	0.53	0.91 (0.72, 1.13)	0.38	0.49 (0.16, 1.40)	0.19
Smoking	1.07 (0.57, 1.83)	0.82	0.30 (0.05, 1.02)	0.11	0.78 (0.42, 1.33)	0.40	2.91 (0.36, 15.6)	0.25
Dwelling ownership (rent)	0.96 (0.65, 1.38)	0.83	0.82 (0.35, 1.68)	0.61	0.98 (0.69, 1.37)	0.92	0.44 (0.02, 2.70)	0.46
Number of household residents	0.98 (0.86, 1.09)	0.75	**1.20 (1.04, 1.40)**	**0.012**	1.05 (0.96, 1.13)	0.19	0.85 (0.39, 1.71)	0.66
Number of vaccine doses	0.96 (0.79, 1.17)	0.70	1.00 (0.69, 1.46)	0.98	0.97 (0.81, 1.15)	0.72	0.96 (0.43, 2.07)	0.92
Postsecondary education	0.98 (0.68, 1.46)	0.92	**0.47 (0.25, 0.95)**	**0.026**	0.79 (0.57, 1.11)	0.16	4.40 (0.72, 88.1)	0.19
Diabetes	0.88 (0.63, 1.20)	0.43	1.24 (0.71, 2.10)	0.43	0.99 (0.75, 1.31)	0.96	0.54 (0.14, 1.70)	0.33
Cardiovascular disease	0.96 (0.74, 1.24)	0.74	0.95 (0.57, 1.59)	0.84	0.90 (0.71, 1.14)	0.38	2.45 (0.78, 8.61)	0.14
Respiratory disease	-	-	-	-	1.09 (0.82, 1.42)	0.56	1.04 (0.33, 2.97)	0.94
IMID[2]	1.16 (0.67, 1.88)	0.56	1.20 (0.45, 2.67)	0.68	-	-	-	
rs2234235, *TAS2R1*	**1.90 (1.26, 2.76)**	**0.001**	0.50 (0.12, 1.38)	0.25	**1.54 (1.03, 2.23)**	**0.026**	1.59 (0.31, 5.96)	0.52
rs2234009, *TAS2R5*	1.08 (0.63, 1.74)	0.76	0.77 (0.19, 2.04)	0.65	1.10 (0.67, 1.69)	0.70	0.00 (0.00, inf)	>0.99
rs2234010, *TAS2R5*	**1.67 (1.10, 2.44)**	**0.011**	1.16 (0.44, 2.53)	0.73	**1.49 (1.00, 2.14)**	**0.041**	**4.19 (1.06, 15.2)**	**0.031**
rs1726866, *TAS2R38*	0.90 (0.75, 1.07)	0.22	0.81 (0.58, 1.15)	0.24	0.91 (0.77, 1.06)	0.23	0.68 (0.33, 1.39)	0.30
rs34039200, *TAS2R62P*	0.95 (0.77, 1.17)	0.62	1.23 (0.81, 1.81)	0.31	1.00 (0.83, 1.21)	>0.99	0.65 (0.24, 1.60)	0.37
rs3851584, *TAS2R14*	1.00 (0.84, 1.18)	0.96	0.88 (0.61, 1.24)	0.46	0.99 (0.85, 1.16)	0.93	0.55 (0.24, 1.17)	0.14
rs77837442, *TAS2R19*	0.48 (0.15, 1.15)	0.15	0.38 (0.02, 1.77)	0.34	0.51 (0.18, 1.11)	0.14	0.00 (-, -)	>0.99
rs117458236, *TAS2R20*	0.86 (0.40, 1.62)	0.67	1.47 (0.42, 3.92)	0.48	0.91 (0.46, 1.61)	0.76	1.84 (0.22, 10.1)	0.52

[1]Logistic regression, OR = Odds Ratio, CI = Confidence Interval
[2]IMID = Immune-mediated inflammatory disease

Table 5.11 Association between *TAS2R* variants and SARS-CoV-2 spike antibody in patients with chronic medical conditions and in corresponding control groups, defined as individuals without the specific condition under analysis.

Characteristic	Diabetes				Cardiovascular disease			
	Controls (N=5,875) OR (95% CI)[1]	p-value	Patients (N=1,600) OR (95% CI)[1]	p-value	Controls (N=3,759) OR (95% CI)[1]	p-value	Patients (N=3,716) OR (95% CI)[1]	p-value
Age	1.00 (0.99, 1.01)	0.89	1.00 (0.98, 1.01)	0.63	1.00 (0.99, 1.01)	0.96	1.00 (0.99, 1.01)	0.82
Sex (female)	**1.37 (1.17, 1.60)**	**<0.001**	0.92 (0.69, 1.24)	0.60	**1.38 (1.12, 1.69)**	**0.002**	1.17 (0.97, 1.41)	0.11
Smoking	0.92 (0.63, 1.37)	0.67	1.01 (0.50, 2.10)	0.97	0.83 (0.52, 1.34)	0.42	1.09 (0.68, 1.80)	0.72
Dwelling ownership (rent)	1.01 (0.79, 1.29)	0.94	0.79 (0.53, 1.17)	0.24	1.02 (0.74, 1.42)	0.92	0.89 (0.68, 1.16)	0.39
Number of household residents	1.05 (0.97, 1.14)	0.28	1.02 (0.89, 1.20)	0.74	1.03 (0.94, 1.14)	0.59	1.07 (0.97, 1.20)	0.19
Number of vaccine doses	**110 (84.8, 145)**	**<0.001**	**58.9 (39.5, 91.3)**	**<0.001**	**130 (93.9, 185)**	**<0.001**	**64.2 (48.3, 87.4)**	**<0.001**
Postsecondary education	1.09 (0.84, 1.41)	0.49	1.27 (0.85, 1.89)	0.23	1.08 (0.75, 1.54)	0.66	1.20 (0.91, 1.57)	0.19
Diabetes	-	-	-	-	0.80 (0.59, 1.10)	0.16	0.93 (0.76, 1.13)	0.45
Cardiovascular disease	0.86 (0.73, 1.02)	0.080	1.02 (0.73, 1.42)	0.92	-	-	-	-
Respiratory disease	0.99 (0.81, 1.21)	0.90	0.76 (0.54, 1.07)	0.12	1.02 (0.78, 1.34)	0.87	0.88 (0.71, 1.10)	0.25
IMID[2]	**0.63 (0.45, 0.88)**	**0.006**	0.68 (0.40, 1.17)	0.16	**0.59 (0.38, 0.93)**	**0.021**	**0.70 (0.49, 1.01)**	**0.055**
rs2234235, *TAS2R1*	0.80 (0.58, 1.11)	0.17	0.59 (0.34, 1.04)	0.067	0.79 (0.54, 1.19)	0.25	0.70 (0.48, 1.03)	0.066
rs2234009, *TAS2R5*	1.13 (0.81, 1.60)	0.47	**2.19 (1.17, 4.24)**	**0.017**	1.26 (0.81, 2.02)	0.32	1.36 (0.92, 2.05)	0.13
rs2234010, *TAS2R5*	1.07 (0.78, 1.48)	0.68	**2.15 (1.13, 4.16)**	**0.022**	1.24 (0.82, 1.91)	0.32	1.28 (0.87, 1.91)	0.22
rs1726866, *TAS2R38*	1.02 (0.91, 1.14)	0.78	1.13 (0.92, 1.39)	0.25	1.03 (0.89, 1.20)	0.69	1.04 (0.91, 1.18)	0.60
rs34039200, *TAS2R62P*	0.91 (0.80, 1.04)	0.16	**0.72 (0.56, 0.92)**	**0.008**	**0.84 (0.71, 1.00)**	**0.051**	0.87 (0.75, 1.02)	0.095
rs3851584, *TAS2R14*	1.02 (0.91, 1.14)	0.72	1.05 (0.86, 1.29)	0.62	1.03 (0.89, 1.20)	0.66	1.01 (0.89, 1.16)	0.84
rs77837442, *TAS2R19*	1.10 (0.72, 1.72)	0.68	**0.44 (0.19, 1.02)**	**0.054**	1.31 (0.71, 2.53)	0.41	0.75 (0.47, 1.22)	0.25
rs117458236, *TAS2R20*	0.95 (0.65, 1.42)	0.81	0.92 (0.46, 1.89)	0.81	0.80 (0.49, 1.33)	0.38	1.05 (0.66, 1.71)	0.83

[1]Logistic regression, OR = Odds Ratio, CI = Confidence Interval
[2]IMID = Immune-mediated inflammatory disease

Table 5.11 (Continued) Association between *TAS2R* variants and SARS-CoV-2 spike antibody in patients with chronic medical conditions and in corresponding control groups, defined as individuals without the specific condition under analysis.

Characteristic	Respiratory disease				Immune-mediated inflammatory disease			
	Controls (N=5,964)		Patients (N=1,511)		Controls (N=7,047)		Patients (N=428)	
	OR (95% CI)[1]	p-value	OR (95% CI)[1]	p-value	OR (95% CI)[1]	p-value	OR (95% CI)[1]	p-value
Age	1.00 (0.99, 1.01)	0.99	1.00 (0.98, 1.01)	0.62	1.00 (0.99, 1.01)	>0.99	0.98 (0.95, 1.02)	0.28
Sex (female)	**1.33 (1.14, 1.56)**	**<0.001**	1.01 (0.74, 1.38)	0.94	**1.25 (1.08, 1.44)**	**0.003**	1.24 (0.69, 2.21)	0.47
Smoking	1.12 (0.75, 1.71)	0.58	0.60 (0.32, 1.13)	0.11	0.87 (0.62, 1.24)	0.43	2.21 (0.56, 9.47)	0.27
Dwelling ownership (rent)	0.86 (0.69, 1.09)	0.22	1.19 (0.76, 1.88)	0.45	0.94 (0.76, 1.16)	0.54	0.86 (0.36, 2.04)	0.72
Household size	1.02 (0.95, 1.10)	0.64	**1.18 (1.00, 1.41)**	**0.053**	1.05 (0.98, 1.13)	0.21	1.10 (0.76, 1.61)	0.62
Number of vaccine doses	**98.0 (76.5, 128)**	**<0.001**	**75.5 (48.4, 124)**	**<0.001**	**95.5 (76.2, 122)**	**<0.001**	**54.3 (25.4, 135)**	**<0.001**
Postsecondary education	**1.26 (0.99, 1.59)**	**0.058**	0.88 (0.52, 1.47)	0.63	1.11 (0.89, 1.39)	0.36	**2.50 (1.00, 6.43)**	**0.052**
Diabetes	0.96 (0.79, 1.17)	0.70	**0.74 (0.52, 1.05)**	**0.091**	0.89 (0.75, 1.06)	0.20	1.12 (0.58, 2.16)	0.74
Cardiovascular disease	0.92 (0.78, 1.09)	0.33	0.82 (0.59, 1.13)	0.22	0.89 (0.76, 1.03)	0.12	1.12 (0.60, 2.07)	0.73
Respiratory disease	-	-	-	-	0.93 (0.78, 1.10)	0.39	0.93 (0.48, 1.77)	0.81
IMID[2]	**0.62 (0.45, 0.86)**	**0.004**	0.77 (0.44, 1.36)	0.36	-	-	-	-
rs2234235, *TAS2R1*	0.84 (0.62, 1.16)	0.29	**0.48 (0.27, 0.85)**	**0.012**	0.77 (0.58, 1.03)	0.078	**0.37 (0.13, 1.04)**	**0.058**
rs2234009, *TAS2R5*	1.32 (0.95, 1.85)	0.10	1.50 (0.75, 3.10)	0.26	**1.38 (1.01, 1.91)**	**0.044**	1.04 (0.34, 3.25)	0.95
rs2234010, *TAS2R5*	1.29 (0.94, 1.79)	0.12	1.14 (0.61, 2.21)	0.68	1.22 (0.91, 1.66)	0.18	1.40 (0.47, 4.32)	0.55
rs1726866, *TAS2R38*	1.03 (0.92, 1.15)	0.64	1.03 (0.83, 1.28)	0.78	1.04 (0.94, 1.15)	0.50	1.00 (0.65, 1.54)	0.99
rs34039200, *TAS2R62P*	**0.86 (0.76, 0.99)**	**0.030**	0.84 (0.65, 1.10)	0.20	**0.87 (0.77, 0.98)**	**0.027**	0.70 (0.41, 1.16)	0.17
rs3851584, *TAS2R14*	1.02 (0.91, 1.14)	0.74	1.06 (0.86, 1.32)	0.58	1.04 (0.94, 1.15)	0.50	0.86 (0.57, 1.30)	0.48
rs77837442, *TAS2R19*	0.91 (0.59, 1.41)	0.66	1.13 (0.54, 2.47)	0.76	0.90 (0.61, 1.34)	0.59	1.02 (0.29, 3.78)	0.98
rs117458236, *TAS2R20*	0.90 (0.62, 1.34)	0.60	1.05 (0.52, 2.20)	0.90	1.01 (0.71, 1.46)	0.96	0.76 (0.23, 2.41)	0.64

[1]Logistic regression, OR = Odds Ratio, CI = Confidence Interval

[2]IMID = Immune-mediated inflammatory disease

5.5 Discussion

This study evaluated the association between *TAS2R* genetic variants and COVID-19 outcomes in individuals of European ancestry followed in the CLSA cohort. The prevalence of confirmed/probable COVID-19 infection (1.09%) and serologically confirmed infection (4.45%) was consistent with findings from other studies on the CLSA dataset (Meng and Nielsen 2024, Griffith et al. 2023, MacNeil et al. 2024, Oremus et al. 2024). One allele in *TAS2R20* was associated with an increased risk of confirmed or probable infection. Additionally, two alleles in *TAS2R1* and *TAS2R5* were associated with the presence of nucleocapsid antibody, and two alleles in *TAS2R1* and *TAS2R62P* were linked to lower odds of spike antibody seroconversion following COVID-19 vaccination or infection.

This work expands on prior studies assessing *TAS2R* variants and COVID-19 outcomes focused on *TAS2R38* (Barham et al. 2021, Parsa et al. 2021, Risso et al. 2022, Meng and Nielsen 2024). These prior studies primarily focused on three variants (rs1726866, rs713598, and rs10246939) that give rise to the two common haplotypes. A recent study using CLSA data found no significant association between *TAS2R38* haplotypes and COVID-19 infection or symptoms, aligning with the findings of this study on *TAS2R38* (Meng and Nielsen 2024, Akoglu 2018). Similarly, Risso et al. (2022) found no significant association between the *TAS2R38* phenotype and either the presence or severity of SARS-CoV-2 infection (Risso et al. 2022). In contrast, Barham et al. reported an association between the nontaster phenotype and more severe COVID-19 symptoms in a cohort of 100 patients (Barham et al. 2020). Similarly, Parsa et al. in a 2021 systematic review and meta-analysis, found the PAV haplotype (C allele in rs10246939) to be associated with lower COVID-19 mortality (Parsa et al. 2021). Another study by Barham et al. on a cohort of 1,935 individuals, found that non-tasters were more likely to test positive for COVID-19, need hospitalization after infection, and experience prolonged symptom duration (Barham et al. 2021).

The SNP associated with COVID-19 infection identified in this study was located in *TAS2R20*. Additionally, the rs3851584 in *TAS2R14* showed a borderline significant association with the infection ($P = 0.058$). *TAS2R*s are expressed in the bronchial epithelium and airway smooth muscles, where they play a role in airway clearance (Shah et al. 2009, Krasteva et al. 2011,

Deshpande et al. 2011, Deshpande et al. 2010, Orsmark-Pietras et al. 2013, Finger et al. 2003). Additionally, they are expressed in immune cells, such as macrophages, monocytes, and neutrophils, and contribute to the innate immune response against viral infections through nitric oxide production and calcium-mediated antimicrobial peptides (Jaggupilli, Singh, et al. 2019, Jaggupilli, Howard, et al. 2019, Jaggupilli et al. 2018, Åkerström et al. 2009, Tuzim and Korolczuk 2021). *TAS2R20* and *TAS2R14* exhibit the highest expression levels in the bronchial epithelium (Jaggupilli et al. 2017), which may explain the association with SARS-CoV-2 infection. The two variants linked to infection in this study were 3' untranslated region (UTR) variants. The rs3851584 SNP in *TAS2R14* is in nearly complete LD *(*r2>0.99) with rs1015443 in *TAS2R13*, a missense variant that results in a serine-to-asparagine substitution at position 259. This alteration may impact polypeptide structure, potentially contributing to the observed phenotypic effect. Moreover, genetic variants in UTRs can influence post-transcriptional gene regulation by affecting mRNA secondary structure, stability, translation efficiency, and localization (Schuster and Hsieh 2019, Steri et al. 2018).

Three SNPs in *TAS2R1*, *TAS2R5*, and *TAS2R62P* were associated with the SARS-CoV-2 antibody response. Growing evidence suggests that, in addition to their role in innate immunity, T2Rs regulate the adaptive immune response (Tuzim and Korolczuk 2021). *TAS2Rs* are expressed in lymphocytes, with higher levels in younger adults than in older adults (Tuzim and Korolczuk 2021, Tran et al. 2018). Tran et al. reported significantly higher levels of T2R38 in activated lymphocytes compared to controls, as well as in central and effector memory cells compared to naïve cells (Tran et al. 2018). Grassin-Delyle et al. showed that T2R5 activation in human macrophages suppresses pro-inflammatory cytokine secretion (Grassin-Delyle et al. 2019). Since these cytokines are crucial for B cell activation and proliferation, T2Rs may indirectly impact the antibody response to SARS-CoV-2 (Grassin-Delyle et al. 2019, Tran et al. 2018, Vazquez et al. 2015). The SNPs identified to be associated with antibody seroconversion in this study include synonymous, 5' UTR, and pseudogene variants. Although pseudogenes do not produce functional proteins, they are transcribed into RNA and contribute to gene expression regulation (Tam et al. 2008, An et al. 2017). Additionally, synonymous variants may regulate post-transcriptional gene expression by affecting mRNA stability and translation speed (Hunt et al. 2014, Spencer et al. 2012).

Logistic regression results of this study showed a significant association between respiratory diseases and SARS-CoV-2 confirmed or probable infection. This may be because probable cases, defined as those diagnosed by a physician, were more likely to present symptoms, and thus, respiratory disease appears to be associated with symptomatic COVID-19, which is consistent with previous research (Aveyard et al. 2021, Sanchez-Ramirez and Mackey 2020). Interestingly, results from the UK Biobank Imputed Version 3 dataset, generated by the Neale Lab (Bycroft et al. 2018), support a connection between the same *TAS2R* variants and respiratory diseases: rs3851584 (G) in *TAS2R14* and respiratory disease symptoms ($P < 0.001$; effect size reported for T allele: -0.054); rs117458236 (T) in *TAS2R20* and pulmonary inflammation or edema ($P = 0.02$; effect size: 1.5); rs2234010 (A) in *TAS2R5 and* dependence on respirator ($P = 0.001$; effect size: 0.72). On the other hand, rs2234235 (G) in *TAS2R1*, identified in this study as a risk allele for nucleocapsid antibody response, was linked to a decreased risk of chronic airway obstruction in the UK Biobank data ($P = 0.01$; effect size: -0.097) (Bycroft et al. 2018).

In addition, consistent with previous research, a significant association was found between IMID and lower odds of spike antibody response (OR [95% CI]: 0.65 [0.49, 0.86], $P = 0.003$), which is likely due to medication use and immune system dysregulation in IMID patients (Simpson-Yap et al. 2022, Maddur et al. 2010, Simon et al. 2022, Hitchon et al. 2024). Patients with autoimmune diseases, such as rheumatoid arthritis, have approximately twice the risk of COVID-19, primarily due to glucocorticoid medication (Akiyama et al. 2021). The first study on 21,991 CLSA participants showed an association between *TAS2R* genetic variants and temporomandibular symptoms, which can occur in rheumatoid arthritis involving the temporomandibular joint (Shafizadeh et al. 2024, Scott et al. 2010, Kroese et al. 2021, Gabriel 2001, Barut et al. 2017, Larheim and Haanaes 1981). Nine of the fifteen variants linked to temporomandibular joint syndromes were located in *TAS2R20* (Shafizadeh et al. 2024). A different SNP in the same gene was found to be associated with COVID-19 infection in the present study. Furthermore, the SNP rs3851584 in *TAS2R14*, which showed a borderline association with COVID-19 infection in this study, was also linked to temporomandibular joint symptoms in the previous study (Shafizadeh et al. 2024).

Subgroup analyses may clarify the interplay between *TAS2R* variants, COVID-19 outcomes, IMID, and respiratory disease. The rs2234235 (G) variant in *TAS2R1* was associated with higher odds of anti-nucleocapsid and lower odds of anti-spike response (Table 4), despite showing no association with confirmed/probable infection in the overall cohort (Table 2). However, it was significantly associated with higher odds of confirmed/probable infection in individuals with respiratory disease (OR: 2.65, $P = 0.018$) or IMID (OR: 7.39, $P = 0.012$). Additionally, although this variant was linked to reduced odds of anti-spike in the overall cohort (OR: 0.74, $P = 0.033$), subgroup analysis revealed a stronger effect in patients with respiratory disease (OR: 0.48, $P = 0.012$) or IMID (OR: 0.37, $P = 0.058$). These findings suggest that the G allele of rs2234235 may impair antibody responses, potentially increasing susceptibility to SARS-CoV-2 infection in at-risk individuals. This is further supported by GWAS results from the UK Biobank COVID-19 dataset available through the GRASP portal (Thibord et al. 2022), which found a significant association between the G allele of rs2234235 and severe COVID-19 outcomes in the European population ($P = 0.001$, $\beta = 0.44$, OR ≈ 1.55).

Self-reported diagnosis can be challenging to validate. A systematic review of systemic conditions in the CLSA found that some conditions, such as respiratory disease, cardiovascular disease, and diabetes could be accurately diagnosed using algorithms for the questionnaire data (Raina, Wolfson, Kirkland, Keshavarz, et al. 2009). However, validating IMID diagnosis, including inflammatory bowel disease and musculoskeletal conditions such as rheumatoid arthritis, poses challenges, as necessary clinical variables are not included in the CLSA (Raina, Wolfson, Kirkland, Keshavarz, et al. 2009). To improve the accuracy of IMID diagnosis, the specific medications used for these conditions were incorporated into the algorithms. Another study on the CLSA cohort found that incorporating medication data into chronic disease assessment can resolve over 93% of inconsistent responses (Andreacchi et al. 2023). Additionally, Oremus et al. demonstrated that an ischemic heart disease algorithm could achieve optimal sensitivity and specificity using self-reported data alone, without ECG confirmation (Oremus et al. 2013). The prevalence estimates for systemic conditions in this study were comparable to those reported by the Canadian Chronic Disease Surveillance System (CCDSS) for individuals aged 45 to 84 years and other CLSA studies (Sakib et al. 2023, Freeman et al. 2022, Duong et al. 2022, O'Mahony et al. 2024, Canada).

This study has some limitations. First, it included only individuals of European ancestry, which limits the extension of the findings to other populations. Second, only 16% of the Questionnaire cohort reported a nucleic acid amplification test for COVID-19, resulting in a small sample size for the confirmed infection outcome. Although a larger population was included in the confirmed/probable category, some individuals may not have been examined by a physician or may have been asymptomatic. Additionally, the data were skewed toward individuals without COVID-19 infection. To enhance statistical power, future studies should aim to recruit a more balanced number of cases and controls. While multiple potential confounders were accounted for, environmental factors and variants in other genes could not be accounted for. The CLSA cohort consists of individuals who volunteered to participate, which may result in the underrepresentation of populations at higher risk for COVID-19. Furthermore, as this study focused solely on COVID-19, it remains unclear whether the identified variants are also associated with other viral or bacterial infections. Finally, the potential impact of medications known to affect serological responses, particularly B cell-targeted therapies used in IMIDs, could not be accounted for in this study. Further research is needed to investigate the role of T2Rs in B lymphocyte activation and antibody production, as well as to explore the functional impact of the associated variants on T2Rs and their potential causal relationship with SARS-CoV-2 and other airborne pathogens.

This study highlights *TAS2R* variants as potential contributors to COVID-19 infection and immune response. Several *TAS2R* variants were found to increase the risk of infection while reducing the antibody seroconversion following vaccination. Given that COVID-19 continues to generate significant health care burdens, particularly for older adults and persons with chronic disease, these data may inform vaccination strategies for COVID-19 and may provide insights into early innate immune responses to emerging and endemic airborne pathogens. Furthermore, identifying specific *TAS2R* genetic variants that increase the risk of infection could facilitate personalized medical practice and vaccine considerations for individuals at higher risk for serious infections.

5.6 Disclaimer

The opinions expressed in this manuscript are the author's own and do not reflect the views of the Canadian Longitudinal Study on Aging.

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Conclusions

In the first study, allele distributions of 124 common *TAS2R* variants were examined in a European-ancestry cohort from the CLSA. Forty-eight SNPs showed distribution differences compared to 1KGP, including 11 missense variants, six of which were in *TAS2R20*. Associations between the 124 variants and self-reported oral health symptoms, including dental issues, denture-related problems, TMD, and overall oral health, were assessed using multivariate models adjusted for sociodemographic, general health, and oral hygiene variables. Fifteen SNPs across six *TAS2R* genes (*TAS2R8*, *9*, *13*, *14*, *20*, and *50*) were significantly associated with sore jaw muscles, a common TMD symptom, with the strongest associations observed for *TAS2R20* variants. RNA secondary structure predictions indicated that seven of these SNPs may impact mRNA secondary structure. Structural modeling of the missense variants suggested that two *TAS2R20* variants are in close proximity to the ligand-binding site and could affect receptor-ligand interactions. Given the role of inflammatory markers and bacterial species such as *Staphylococcus aureus* in TMD pathogenesis, and the moderate to high expression of *TAS2R13*, *14*, *20*, and *50* in extraoral tissues, these findings suggest that T2Rs may influence TMD via immune modulation and inflammatory signaling.

The second study evaluated the association between *TAS2R* variants and COVID-19 outcomes, including infection status and antibody response. One *TAS2R20* allele was associated with increased infection risk. Additionally, SNPs in *TAS2R1*, *TAS2R5*, and *TAS2R62P* were linked to the presence of nucleocapsid antibodies and reduced spike antibody seroconversion post-infection or vaccination. Given that *TAS2R20* is highly expressed in the bronchial epithelium, their involvement in SARS-CoV-2 susceptibility is biologically plausible. Subgroup analyses further revealed interactions between *TAS2R* variants, COVID-19 outcomes, and chronic conditions such as respiratory disease and IMIDs. Notably, the G allele of rs2234235 in *TAS2R1* may impair antibody response, increasing susceptibility among individuals with IMIDs or respiratory disease.

This research extends prior work by exploring a broader array of *TAS2R* genes beyond *TAS2R38* and underscores the potential involvement of T2Rs in both TMD and COVID-19. Specific TAS2R variants were associated with increased infection risk and reduced antibody seroconversion, highlighting their relevance in host-pathogen interactions. The outcomes may provide insights into early innate immune responses to airborne pathogens. These findings could inform vaccination strategies, particularly for vulnerable populations such as older adults or those with chronic illness, and suggest that T2Rs could be targets for novel therapeutics. Moreover, the findings of this study may help identify high-risk individuals and guide more effective preventive strategies.

6.2 Future Directions

While this study identified associations between *TAS2R* variants and both TMD and COVID-19, its cross-sectional design limits the ability to establish causality. Future longitudinal studies using subsequent waves of CLSA data could better assess causal relationships, particularly for TMD, which could be a persistent or chronic condition. Although large-scale studies are resource-intensive, the variants highlighted here, particularly in *TAS2R14* and *TAS2R20*, offer promising candidates for further investigation in larger cohorts. It is also critical to assess whether these variants contribute to disease severity, symptom profiles, or the duration of symptoms, areas that were limited in this study due to the small sample size of hospitalized individuals for COVID-19. Furthermore, this study focused solely on oral diseases and COVID-19, and it remains unclear whether the identified variants are associated with other viral or bacterial infections. Further studies are needed to investigate whether the same variants are implicated in susceptibility to other bacterial or viral infections.

Future large-scale studies should incorporate clinical and laboratory-confirmed diagnoses of TMD and COVID-19 to increase the number of participants with verified test results and to address the limitations associated with self-reported data, including the potential omission of asymptomatic cases. In the current study, the data were skewed toward individuals without COVID-19 infection, which may have reduced the ability to detect associations. To enhance statistical power, future studies should aim to recruit a more balanced number of cases and controls. Additionally, as the CLSA cohort is composed of volunteer participants, there may be

an underrepresentation of populations at higher risk for COVID-19. Future studies involving larger independent cohorts are needed to validate these findings in more diverse populations.

In addition, cell-based studies are needed to elucidate the mechanisms by which T2R polymorphisms may influence cellular responses to pathogens and environmental stimuli. Future experiments should explore the effects of T2R agonists and antagonists on downstream signaling, cytokine production, B and T lymphocyte activation, and antibody production. Moreover, although the structure-function analysis in this study is suggestive, experiments involving site-directed mutagenesis are essential to confirm the underlying biological mechanisms, including ligand interaction. Some of the effective variants were synonymous or located in untranslated regions, and cell-based assays could confirm whether such variants impact gene expression. Integrating genetic, clinical, and functional data will provide a more comprehensive understanding of T2R-mediated immune mechanisms, ultimately enabling more effective and personalized strategies for the prevention and treatment of infectious and inflammatory diseases.

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