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Genetic variation in taste perception and its role in food liking and health status

MED/03 Genetica Medica

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ABSTRACT

Taste has been described as the body's “nutritional gatekeeper”, affecting the identification of nutrients and toxins and guiding food choices. Genetic variation in taste receptor genes can influence perception of sweet, umami and bitter tastes, whereas less is known about the genetics of sour and salty taste. Differences in taste perception, influencing food selection and dietary behavior, have also shown important long-term health implications, especially for food-related diseases such as obesity, diabetes, cardiovascular diseases. To date, a lot of studies are focused on taste receptor genes and function but further investigations are needed to better understand which factors, including genetic ones, are involved in influencing taste and food preferences and the corresponding connections with health status.

The aim of this thesis is to understand the genetic bases of taste perception and its relationship to food preferences and health outcomes. Data from ~3500 subjects coming from isolated villages located in Italy, Caucasus and Central Asia were collected. The ability to taste PROP (6-n-propylthiouracil) bitterness and NaCL saltiness, food liking and intake were measured. Additional information such as clinical parameters, professional activity, lifestyle, eating habits and family history were also collected. To learn more about taste biology the following steps were performed in this thesis: 1) genome-wide association studies (GWAS) of bitter and salty taste perception; 2) analysis of the possible impact of bitter taste perception on food preferences; 3) investigation of the relationship between differences in taste perception genes, food preferences and dental caries, as example of health outcome.

The main specific results emerging from this PhD thesis work are: 1) GWAS revealed two SNPs closed to TRPV7 and KCNA5 genes associated to salty perception; 2) always through GWAS a SNP closed to GHRL gene, encoding for ghrelin and obestatin, was found to be associated to PROP bitter perception. An additional SNP closed to the 5’ region of the T2R38 gene showed association to the same phenotype; 3) ability to perceive PROP could be a marker for general perception of taste stimuli suggesting that differences in taste perception may be a driver of food liking; 4) the risk to develop dental caries is associated to genetic differences in sweet taste genes. In addition, sweet food liking but not sugar intake

results linked to dental caries prevalence, suggesting that food preferences may predictive of health outcomes better than food intake.

Overall, these data represent a starting point to better understand how chemosensory differences may interact to influence and predict food choices and human nutritional behavior.

ABSTRACT (italiano)

Il gusto può essere considerato il “guardiano alimentare” del corpo, permettendo l’identificazione di sostanze nutritive o tossiche e guidando le scelte alimentari. Variazioni genetiche nei geni che codificano per i recettori del gusto possono influenzare la percezione del gusto dolce, umami e amaro, mentre poco conosciuta è la genetica del gusto acido e salato. Differenze nella percezione gustativa, incidendo sulla scelta del cibo e sul comportamento alimentare, hanno anche mostrato importanti implicazioni a lungo termine per la salute, specialmente per malattie relate alla dieta come l’obesità, il diabete e le malattie cardiovascolari.
Finora, molti studi si sono focalizzati sui geni e la funzione dei recettori del gusto, ma ulteriori indagini sono necessarie per comprendere meglio, quali fattori, inclusi quelli genetici, possono influenzare gusto e preferenze alimentari e il corrispondente legame con lo stato di salute.
Lo scopo di questa tesi è di comprendere le basi genetiche della percezione del gusto e la sua connessione con le preferenze alimentari e lo stato di salute. Sono stati raccolti dati su ~3500 soggetti provenienti da villaggi isolati situati in Italia, Caucaso e Asia centrale. Sono stati misurati la capacità di percepire l'amarezza del PROP (6-n-propylthiouracile) e il gusto salato del NaCL, le preferenze e i consumi alimentari. Sono stati anche raccolti ulteriori informazioni come parametri clinici , attività professionale, stile di vita, abitudini alimentari e storia familiare. Per comprendere meglio la biologia del gusto in questa tesi sono stati svolti i seguenti steps: 1) studi di associazione su tutto il genoma (GWAS) volti a identificare nuovi geni coinvolti nella percezione del gusto amaro e salato; 2) analisi del possibile impatto della percezione del gusto amaro sulle preferenze alimentari; 3) studio della relazione tra differenze genetiche nella percezione del gusto, preferenze alimentari e carie dentale, come esempio di relazione con lo stato di salute.
Le principali scoperte emerse da questa tesi sono: 1) uno studio GWA ha identificato due SNPs vicini ai geni TRPV7 e KCNA5 associati alla percezione del gusto salato; 2) sempre attraverso GWAS uno SNP vicino al gene GHRL, che codifica per la grelina e l’obestatina, è stato trovato associato alla percezione amara del PROP. Un ulteriore SNP localizzato vicino alle regione 5' del gene T2R38 mostra, inoltre, associazione con lo stesso fenotipo PROP; 3) la capacità di

percepire il PROP potrebbe essere un marker per la percezione generale degli stimoli gustativi, suggerendo che le differenze nella percezione del gusto possono rappresentare un “driver” del gradimento del cibo; 4) il rischio di sviluppare carie dentali è associato a differenze nei geni che codificano per il gusto dolce. Inoltre, la preferenza per i cibi dolci, ma non il consumo di zuccheri, risulta associata alla prevalenza di carie dentale, suggerendo che le preferenze alimentari possano risultare migliori predittori dello stato di salute rispetto ai consumi alimentari.

Complessivamente, questi dati rappresentano un punto di partenza per capire meglio come le differenze chemio-sensoriali possono interagire nell’influenzare e prevedere le scelte alimentari e il comportamento alimentare nell’uomo.

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CHAPTER I

General Introduction

GENERAL INTRODUCTION

a. Taste system

Taste is a sensory modality essential for nutrition and survival, allowing to evaluate nutritious content of food and to prevent the ingestion of toxic substances. The world “taste” is defined as the sensations arising from the mouth and is commonly confused with “flavor”. Taste includes only gustatory sensations originate in organs of the oral cavity -taste buds- and elicited by water-soluble compounds that interact with the epithelial cells of taste buds. In contrast, flavor indicates the combined sensory experience of olfaction and gustation and is generated by the integration of taste and smell signals in the orbitofrontal and other areas of the cerebral cortex to generate flavors and mediate food recognition (Rolls & Baylis, 1994; Small & Prescott, 2005). Taste is also frequently confused with somatosensory sensations evoked by foods, such as coolness, pungency, burning. In contrast to taste signals, sensations such us the cool of menthol or the spicy of chili peppers are elicited by the stimulation of ion channels in somatosensory nerve fibers in the tongue and taste buds (Caterina et al., 1997; McKemy, Neuhausser & Julius, 2002). The taste system allows recognizing and distinguishing five basic tastes: salty, sour, sweet, bitter and umami. Each of these taste represent different nutritional or physiological requirements. Salty taste controls intake of Na^+ and other minerals, which play a central role in maintaining the body’s water balance and blood circulation. Sour taste detects the presence of acids, avoiding ingesting spoiled foods. Sweet taste signals sugars and carbohydrates, usually indicating energy rich nutrients. Umami taste, elicited by L-glutamate and a few other L-amino acids, reveals the protein content in food. Finally, bitter taste protects against ingesting toxins and poisons in foods, many of which taste bitter (Chaudhari & Roper, 2010). Recent evidences have shown the presence of an additional quality, the fat taste, essential to detect the presence of fatty acids in foods (Stewart et al., 2010).

The sense of taste system is mediated by taste receptor cells (TRCs), which are organized in taste buds located within gustatory papillae. In humans, there are ~5,000 taste buds in the oral cavity, situated on the superior surface of the tongue, on the palate and on the epiglottis. Four types of papillae have been described:

fungiform papillae, mostly located on the dorsal surface in the anterior two-thirds of the tongue; foliate papillae, present on lateral margins towards the posterior part of the tongue; circumvallate papillae, arranged in a V-shaped row at the back of the tongue. Finally filiform papillae are found all over the surface of the tongue and do not contain taste buds. They are considered to have a mechanical function and to be not directly involved in taste sensation (Figure 1). TRCs project microvilli to the apical surface of the taste bud, where they form the 'taste pore'; this is the site of interaction with tastants (Jayaram Chandrashekar, Hoon, Ryba, & Zuker, 2006).

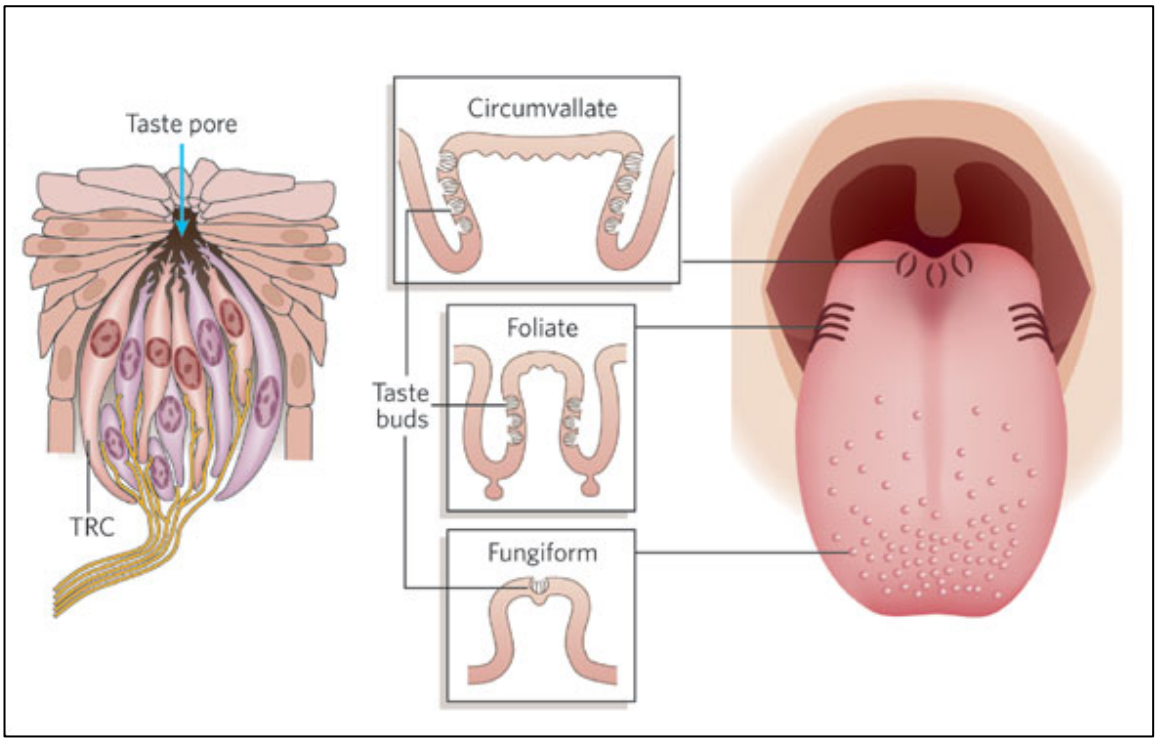

Figure 1. Taste-receptor cells, buds and papillae (Chandrashekar, Hoon, Ryba, & Zuker 2006).

Taste buds exhibit different cell types with very distinct morphological features and cellular functions: type I, II, and III taste cells and basal cells (Roper, 2006) (Figure 2). Basal cells are undifferentiated cells regulating taste cells turnover. **Type I taste cells** are termed "glial like" because their primary function is to support other taste cell types (Finger, 2005). They appear to be involved in terminating synaptic transmission and restricting the spread of transmitters, a role performed in the central nervous system by glial cells (Bartel et al., 2006; Dvoryanchikov, Sinclair et al., 2009). Finally, Type I cells may exhibit ionic currents implicated in salt taste transduction (Vandenbeuch, Clapp & Kinnamon, 2008). **Type II taste cells** are thought to be the actual taste receptor cells. These cells express all of the

elements of the taste transduction cascade for sweet, umami and bitter (Finger, 2005). **Type III taste cells** express synaptic proteins and are characterized by morphologically identifiable synaptic contacts with the gustatory nerve fibers, implicating these cells in transmission of information to the nervous system (Finger, 2005). In addition, these cells also respond directly to sour taste stimuli and carbonated solutions and are presumably the cells responsible for signaling these sensations (Huang et al., 2006; Chandrashekar et al., 2009).

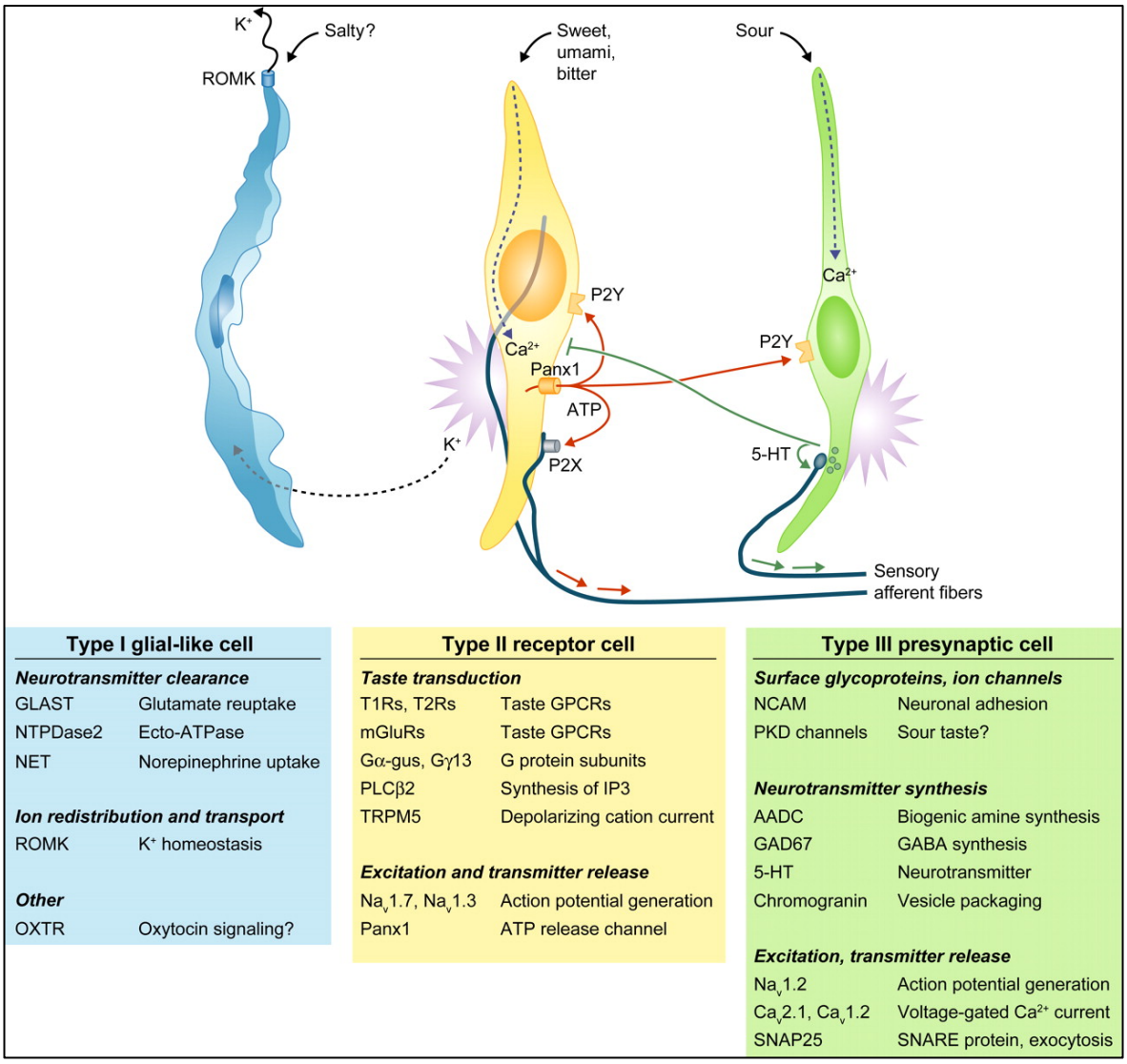

Figure 2. The three types of taste cells.
This classification incorporates ultrastructural features, patterns of gene expression, and the functions of each of Types I, II and III taste cells (Chaudhari & Roper, 2010).

TRCs make synapses with primary sensory axons that run in the three cranial nerves, VII (facial), IX (glossopharyngeal), and X (vagus), which innervate the taste buds. The central axons of these primary sensory neurons in the respective

cranial nerve ganglia project to the nucleus of the solitary tract in the medulla. Gustatory information is then transferred from the nucleus of the solitary tract to the thalamus, and then to gustatory areas of the cortex (Figure 3A). This wide representation of taste information in the brain probably serves to integrate it with interoceptive (hunger, satiety, appetites) and exteroceptive (vision, olfaction, somatosensation) signals and to generate behavioral responses to taste stimuli (Figure 3B) (Purves et al., 2001).

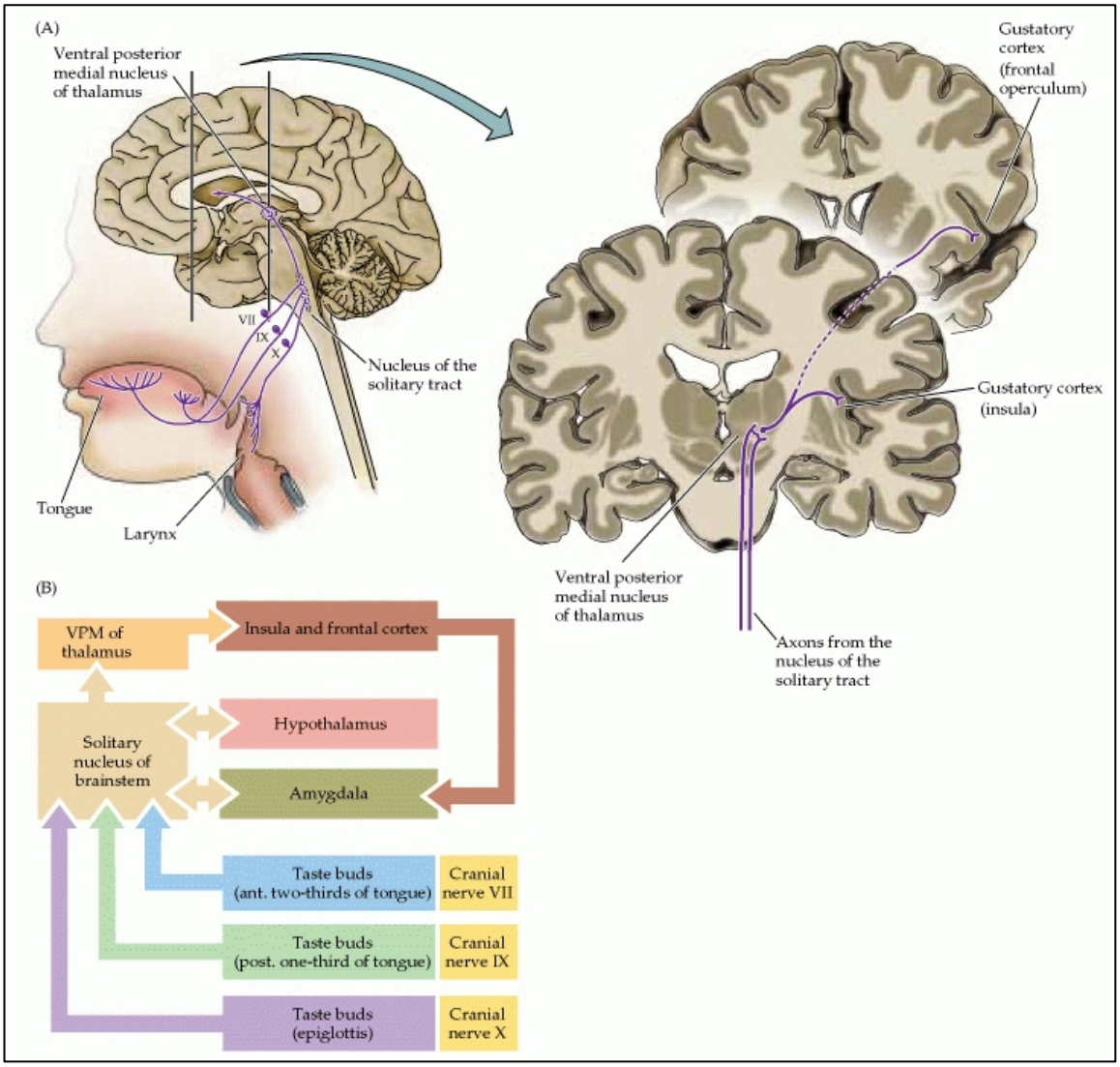

Figure 3. Organization of the human taste system.
(A) The relationship between the gustatory system and the nucleus of solitary tract and cortex in the brain (B) Diagram of taste information pathways (Purves et al., 2001).

b. Taste receptors

The presence of different taste qualities implies for each taste quality the existence of a specific mechanism of signal transduction mediated by specialized taste receptors. Reception of sweet, umami, and bitter taste involves proteins from the T1R and T2R families, while only candidate receptors have been proposed for salty and sour taste. A summary of taste receptors and signal transduction mechanism is reported in table 1.

Taste	Receptor(s)	Signal Transduction
Bitter	T2Rs	G-protein-coupled receptors activation
Sweet	T1R2/T1R3	G-protein-coupled receptors activation
Umami	T1R1/T1R3	G-protein-coupled receptors activation
Salt	ENaC	Ion channels
Sour	PKD2L1	Ion channels
Fat	CD36	Fatty acid transporter

Table 1. Summary of taste receptors and their signal transduction mechanism.

Bitter taste is mediated by a family of G-protein-coupled receptors (GPCRs), named taste 2 receptors (T2Rs or TAS2Rs) (Adler et al., 2000; Chandrashekar et al., 2000, Behrens et al., 2007). Depending on the species, vertebrate genomes contain between 3 T2R genes in chickens and up to 50 in amphibians (Shi & Zhang, 2009). Twenty-five T2Rs located on chromosomes 5, 7 and 12 were identified in the human genome (Conte et al., 2002). This small number of T2R genes raises the question as to how can perceive as bitter such a large number of chemically diverse bitter substances with such a limited number of receptors. Meyerhof and colleagues have suggested that our ability to perceive the enormous number of bitter substances with a limited number of sensors is linked to the molecular receptive ranges of T2R bitter taste receptors. In fact, they showed in a recent work that many bitter receptors respond to different bitter substances, some others instead recognize one or really few compounds. In addition, while one compound can activate several receptors, some compounds activate only one receptor (Meyerhof et al., 2010).

The **sweet taste** receptor is a heterodimer of two G-protein-coupled receptors, T1R2 and T1R3**.** Functional expression studies revealed that T1R3 combines with T1R2 form a sweet taste receptor that responds to all classes of sweet tastants, including natural sugars, artificial sweeteners, d-amino acids and sweet proteins (Li et al., 2002; Nelson et al., 2001).

Studies of *T1r2*- and *T1r3*-knockout mice showed also that homozygous mutants for either receptor subunit show a loss of sweet taste (Zhao et al., 2003; Jiang et al., 2004). Similar studies on **umami taste** established the T1R1 and T1R3 heteromeric GPCR complex as the umami taste receptor (Nelson et al., 2002; Zhao et al., 2003). Metabotropic glutamate receptors mGluR1 and mGluR4 have also been proposed as detectors of umami tastants (Chaudhari, Pereira, & Roper, 2009; Yasumatsu et al., 2012).

Several receptors and mechanisms have been proposed to be responsible for **sour taste**. These include the activation of hyperpolarization-activated cyclic-nucleotide-gated (HCN) channels (Stevens et al., 2001), acid-sensing ion channels (ASICs) (Ugawa et al., 1998), potassium (K2P) channels (Lin et al., 2004) and H^+-gated calcium channels (Waldmann et al., 1997), as well as the involvement of Na^+/H^+ exchangers (Lyall et al., 2004) and acid inactivation of K^+ channels (Cummings & Kinnamon, 1992). However, recent studies have demonstrated that a member of the TRP ion-channel family, PKD2L1, demarcates sour-sensing TRCs. PKD2L1 is selectively expressed in a population of TRCs distinct from those mediating sweet, umami and bitter tastes (Huang et al., 2006).

A number of studies suggested that the receptor for **salt taste** is an epithelial amiloride-sensitive sodium channel, ENaC (Heck, Mierson, & DeSimone, 1984; Avenet & Lindemann, 1988). In humans, there are four ENaC channel subunits, α, β, γ, and δ. In addition, a variant of a vanilloid receptor-1, TRPV1, has been proposed as an amiloride-insensitive salt taste receptor in rodents (Lyall et al., 2004). However, the evidence for ENaC or other candidate salt taste receptors is not as convincing as it is for the T1R and T2R receptors.

As regard **fat taste**, recent data suggest that the fatty acid transporter CD36 is expressed in TRCs and may be involved in oral detection of fatty acids (Fukuwatari et al., 1997; Laugerette et al., 2005).

The receptors for sweet, bitter and umami taste show a common pathway to transduce tastant recognition into cell activation. Tastant binding to T1Rs or T2Rs activates the heterotrimeric G proteins gustducin or transducin leading to the release of the Gβγ subunits and the subsequent stimulation of phospholipase Cβ2 (PLC-β2). Activation of PLC-β2 hydrolyses phosphatidylinositol-4,5-bisphosphate to produce the two intracellular messengers diacylglycerol and inositol-1,4,5-trisphosphate (IP3), which opens the IP3R3 ion channels releasing Ca++ and leads to the gating of the transient receptor potential channel (TRPM5). The combined action of elevated Ca^{2+} and membrane depolarization of TRPM5 results in the release of ATP, which acts as a neurotransmitter linking taste buds to the nervous system. ATP secreted from receptor (type II) cells, in fact, excites primary sensory afferent fibers and probably also stimulate presynaptic (type III) cells to release 5-HT and norepinephrine. On the contrary, salty and sour tastes use a different signaling pathway and operate independently of sweet, umami and bitter tastes, being both detected through ion channels (Purves et al., 2001; Zhang et al., 2003).

c. Variation of taste genes and its role in individual variation in taste responses

Perception of taste may vary between individuals depending on genetic variations in taste receptor genes. Genetic variation in taste perception was reported in humans for sweet, umami and bitter taste (Table 2), while less is known about the genetic variability of salt and sour taste (Kim & Drayna, 2005; Mainland & Matsunami, 2009; Shigemura et al., 2009).

Gene	SNP	Association mechanism	Taste quality
T1R1	A372T	T associated with high sensitivity	Umami
	G1114A	A associated with high sensitivity	Umami
	C329T	T associated with low sensitivity	Umami
T1R3	R757C	C associated with lower sensitivity	Umami
	R247H	H associated with increased sensitivity	Umami
	A5T	A associated with heightened sensitivity	Umami
	C2269T	T more frequent in non tasters	Umami
	C1266T	T alleles result in reduced promoter activity	Sweet
	C1572T	T alleles result in reduced promoter activity	Sweet
T2R16	G516T	G associated with low sensitivity	Bitter
T2R38	P49A	P associated with high sensitivity	Bitter
	A262V	A associated with high sensitivity	Bitter
	V296I	V associated with high sensitivity	Bitter
T2R43	W35S	W associated with high sensitivity	Bitter
T2R44	W35R	W associated with high sensitivity	Bitter

Table 2. Single nucleotide polymorphisms (SNP) in T1R and T2R genes with known functional variation in sweet, umami and bitter perception (modified from Feeney et al 2010).

A number of single nucleotide polymorphisms (SNPs) have been identified in T1Rs genes. Some of these have been linked to variation in taste perception of both umami and sweet tastes. Recent studies suggested that two C/T SNPs within the promoter regions of the T1R3 gene (situated at position 1266 and 1572) were associated with sweetness perception (Fushan et al., 2009). Individuals with T alleles at both loci had reduced sweetness perception compared to those who were homozygous for the C allele at both loci.

As regard variations in umami taste perception, Shigemura and coworkers (Shigemura et al., 2009) showed that the T1R1-372T variant is associated to an increased sensitivity to umami and T1R3-757C results in a reduced sensitivity.

Additional works have identified others SNPs accounting for a part of the interindividual variance in umami perception (Raliou et al., 2009; Chen et al., 2009).

Very recent, data suggested that variations in TRPV1 and SCNNB1 genes might modify salt taste perception in humans. In the SCNN1B gene, 2 SNPs in intronic regions of the gene modified salt taste sensitivity. Those homozygous for the A allele of the rs239345 (A>T) polymorphism and the T allele of the rs3785368 (C>T) polymorphism perceived salt solutions less intensely than carriers of the T or C alleles. In the TRPV1 gene, the rs8065080 (C>T, Val585Ile) polymorphism modified taste sensitivity where carriers of the T allele were significantly more sensitive to salt solutions than the CC genotype (Dias et al., 2013).

Differences in bitter taste perception are the most studied genetic variations in oral

sensations. Several variations have been observed in the T2R gene family, encoding for the bitter receptors. The known example of this variation is the hT2R38 gene, associated to differences in the ability to taste the synthetic compounds phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) (more details are reported in CHAPTER II and III). Additional examples are provided by functional polymorphisms in hT2R16 as well as in hT2R43 and hT2R44. The human T2R16 receptor responds to compounds containing the beta-glucopyranoside moiety such as salicin and amygdalin, including some with a highly toxic cyanogenic activity (Bufe et al., 2002). T2R16 gene contains several polymorphic loci and the G516 variant was associated with a lower sensitivity to salicin, arbutin, and different cyanogenic glycosides (Soranzo et al., 2005). T2R43 and T2R44 genes are activated from several compounds, including saccharin and aloin (Kuhn et al., 2004). Pronin and colleagues demonstrated that T2R43-W35 allele is associated to higher sensitive to aloin and aristolochic acid and both T2R43 and T2R44 are responsible for increased sensitivity to the bitterness of saccharin (Pronin et al., 2007).

d. Implications of variations in taste perception for nutrition and health

Several studies have linked genetic variation in taste receptors to risk of disease. This can occur through differences in taste perception, which may lead to differences in food preferences and food intake. This variation in food intake may, in turn, affect nutritional and health status, as well as the risk of chronic disease (Figure 4).

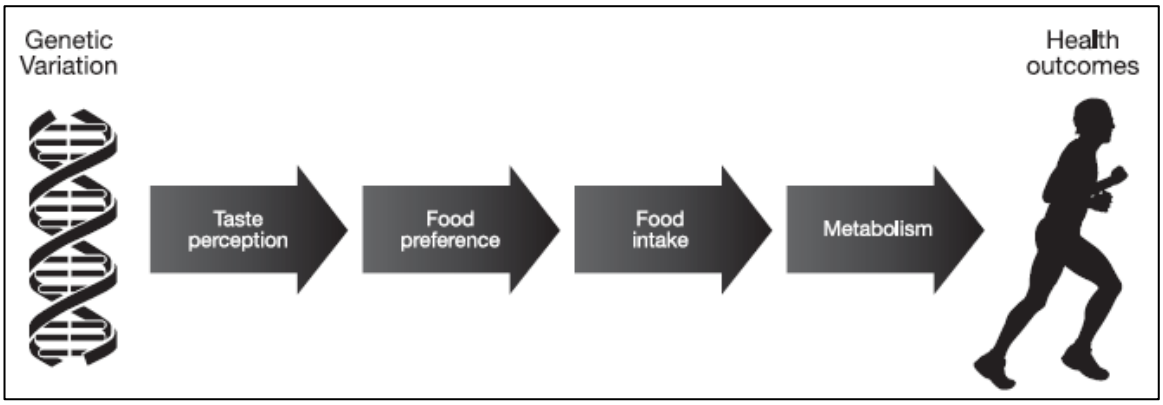

Figure 4. The link between genetic variation in taste perception and health status (Garcia-Bailo et al. 2009).

Different examples for the role of taste receptor variation in human nutrition and health were provided in the last years. A variety of studies have taken into account the influence of bitter taste perception of PTC and PROP, mediated by the *T2R38* gene, on food preferences and intake. In particular, an inverse relationship between bitter PROP perception and preference for different foods such as citrus fruit, Brussels sprouts, cabbage, spinach, asparagus, curly kale, coffee, beer and overall fruit and vegetable consumption has been reported (Keller et al., 2002; Ullrich et al., 2004; Dinehart et al., 2006; Tepper, 2008; Tsuji et al., 2012). PROP bitter taste has also been observed to associate with preference for soy products and green tea (Gayathri Devi, Henderson, & Drewnowski, 1997), sweet and fatty foods (Hayes & Duffy, 2008). Additionally, variations of the T2R38 gene were associated with a nutrient intake pattern indicative of healthy eating, or rather fiber consumption and intakes of thiamine, vitamin B6 and folate (Feeney et al., 2011).

A recent study has shown that polymorphisms in or near T2R genes may influence the sensations, liking or intake of common beverages that contain phytochemicals and other pharmacologically active elements linked to chronic diseases such as cardiovascular disease and cancer. Specifically, T2R16 and T2R38 polymorphisms were associated to differences in alcohol intake. The haploblock formed by SNPs in T2R3, T2R4, and T2R5 were linked to coffee bitterness, while T2R19 variation influenced grapefruit juice bitterness and liking (Hayes et al., 2011).

The perception of bitter taste has also been associated with a number of health effects. For example, higher sensitivity to ethanol bitterness may protect against excess alcohol consumption (Duffy, 2004; Wang et al., 2007). PROP-tasting has shown also relationship with consumption of calories and high-fat foods energy intake (Shafaie et al., 2013), body mass index and adiposity (Tepper & Ullrich, 2002; Tepper et al., 2008; Goldstein, Daun, & Tepper, 2007).

Difference in the risk of colorectal cancer, which is mediated in part by diet, has been reported across T2R38 polymorphic groups (Basson et al., 2005). Furthermore, the risk of developing dental caries, presumably as consequence of higher preference for sugar-containing foods, was linked to variations in bitter perception (Lin, 2003; Wendell et al., 2010).

Association between variation in bitter taste and cardiovascular disease risk was

also hypothesized, by dietary behaviors that increase the risk such as higher alcohol intake, greater preference and intake of high-fat and sweet foods, higher blood pressure, less favorable serum lipids (Duffy, 2004).

Evidences of a relation between taste perception, food choices and health implications have been reported also for others taste quality.

Sweet perception may influence food preferences, as individuals with an increased sweet perception tend to have a lower preference for sugar than less sensitive individuals (Looy, Callaghan, & Weingarten, 1992). Genetic variation in T1R2 gene was linked to habitual consumption of sugars in overweight and obese individuals (Eny et al., 2010). Differences in sweet taste have been also related to alcoholism (Mennella et al., 2010), caries development (Kulkarni et al., 2013) and body mass index, with a reduced threshold observed in obese children (Donaldson et al., 2009).

Common variants in the CD36 gene have been associated with fat preferences for added fats and oils, with individuals with higher sensitivity to fat perception showing greater liking of these foods (Keller et al., 2012). In addition, several report have found relationship between CD36 polymorphisms and body mass index (Bokor et al., 2010; Heni et al., 2011; Yun et al., 2007). In others studies, genetic variations in CD36 gene were also linked to higher free fatty acids, triglyceride levels (Ma et al., 2004; Madden et al., 2008) and metabolic syndrome (Farook et al., 2012).

Overall these data highlight how inter-individual genetic differences may have important implications for individual food preferences and intake, therefore for nutrition and health.

e. Taste is not just for taste buds

Several evidences showed that taste receptors and taste cascade elements are also expressed throughout the gastrointestinal and respiratory tracts (Höfer, Püschel & Drenckhahn, 1996; Wu et al., 2002; Kaske et al., 2007) (Figure 5).

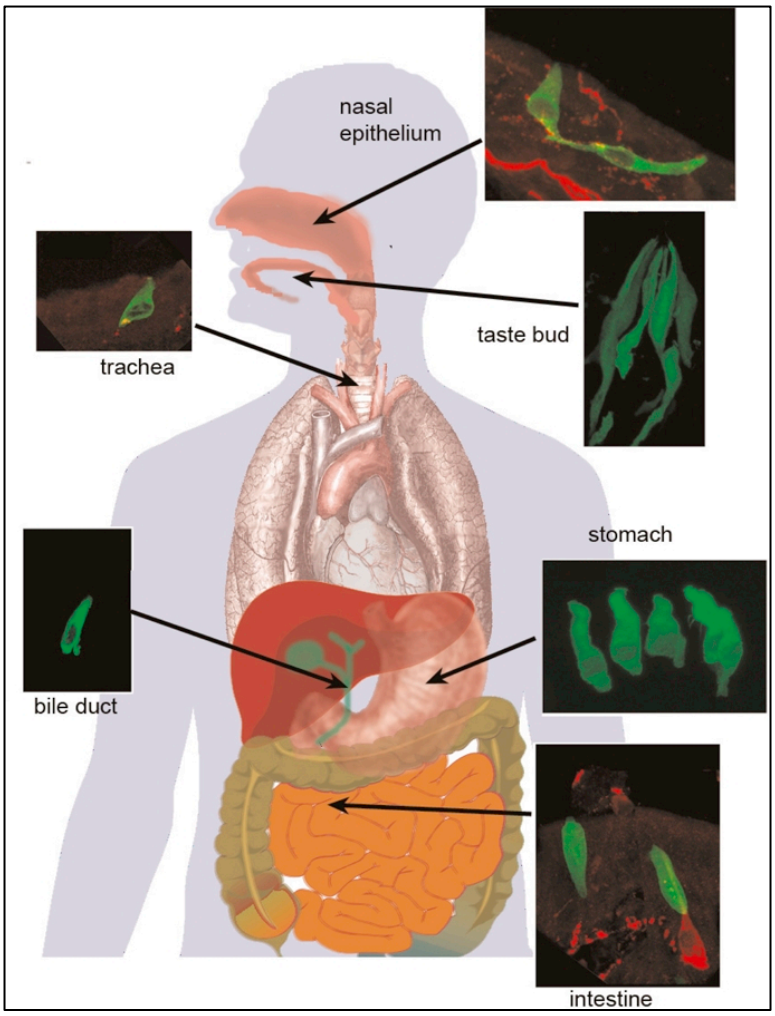

Figure 5. Sites in the body where cells express the canonical taste receptor cascade
(Finger and Kinnamon, 2011)

In contrast to taste receptors in the mouth, taste receptors in the gut do not induce sensations of taste, but rather initiate molecular pathways that help guide the digestion or rejection of food substances traveling through the intestines.

Likewise, the existence of taste pathways in human airway cells is involved in defensive responses to inhaled foreign and potentially toxic substances (Finger & Kinnamon, 2011). The existence of T1R receptors in the gut is responsible of the regulation of digestive functions. In fact, these receptors detect sweet substances and respond by secreting the glucagon-like peptide GLP-1, which in turn stimulates the release of insulin from pancreatic ß-cells, promoting the uptake of glucose. In addition, activation of the sweet receptors in the gut drives the insertion of the glucose transporters SGLT-1 and GLUT2 into the membranes of cells lining

the intestines, facilitating uptake of glucose (Mace et al., 2007; Margolskee et al., 2007).

Less clear is the function of T2R bitter receptors in the gastrointestinal tract. The activation of T2R receptors results in release of the peptide hormone cholecystokinin (CCK), which can reduce gut motility. Thus, intake of a potential toxin that activates the T2R pathway should decrease the rate at which food passes through the stomach and lower the drive for continued eating (Glendinning et al., 2008). However, in the colon activation of T2R receptors similarly appears to combat toxins, inducing the secretion of anions and water, which leads to fluid secretion into the intestine, resulting in diarrhea that flushes out the colon (Kaji et al., 2009).

In the upper airway activation of T2R receptors generate an intracellular cascade to affect the release of the neurotransmitter acetylcholine and to activate nearby nerve fibers, inducing protective reflexes such as apnea (to prevent further inhalation) and sneezing (Tizzano et al., 2010). Interestingly, a recent work showed that T2R38 is an upper airway sentinel in innate defense and that genetic variation contributes to individual differences in susceptibility to respiratory infection. In fact, T2R38 is expressed in human upper respiratory epithelium and is activated in response to acyl-homoserine lactone quorum-sensing molecules secreted by gram-negative bacteria. Receptor activation regulates calcium-dependent NO production, resulting in direct antibacterial effects. Moreover, common polymorphisms of the T2R38 gene were linked to significant differences in the ability of upper respiratory cells to clear and kill bacteria. Lastly, T2R38 genotype correlated with human sinonasal gram-negative bacterial infection (Lee et al., 2012).

In airway smooth muscle cells of the lungs bitter compounds activate the T2R pathway and cause calcium potassium channels activation, allowing the outflow of K+, which produces hyperpolarization and subsequent relaxation of the muscle cells and reduction of airway obstruction. Given the need for efficacious bronchodilators for treating obstructive lung diseases, this pathway can be exploited for therapy with the thousands of known synthetic and naturally occurring bitter tastants (Deshpande et al., 2010).

Furthermore, in the lungs T2R receptors on ciliated airway epithelial cells bind

bitter compounds, initiating the G protein-mediated pathway that results in an increase in ciliary beat frequency, which serves to sweep irritants away from the surface of the cell (Shah et al., 2009).

d. Aims of the thesis

The aims of this thesis were:

a) identify new loci involved in bitter and salt perception through genome-wide association studies (GWAS) and meta-analysis of data coming from 3 different Italian populations (Chapter II).

b) investigate the possible effect of bitter taste perception on food preferences using a population-based approach, based on comparisons between distance matrices (Chapter III).

c) analyse the relationship between differences in taste perception genes, food preferences and health outcomes. In particular, the effect of sweet taste genes and sweet liking on dental caries prevalence was taken into account (Chapter IV).

An outline of the objectives of this thesis is shown in Figure 6.

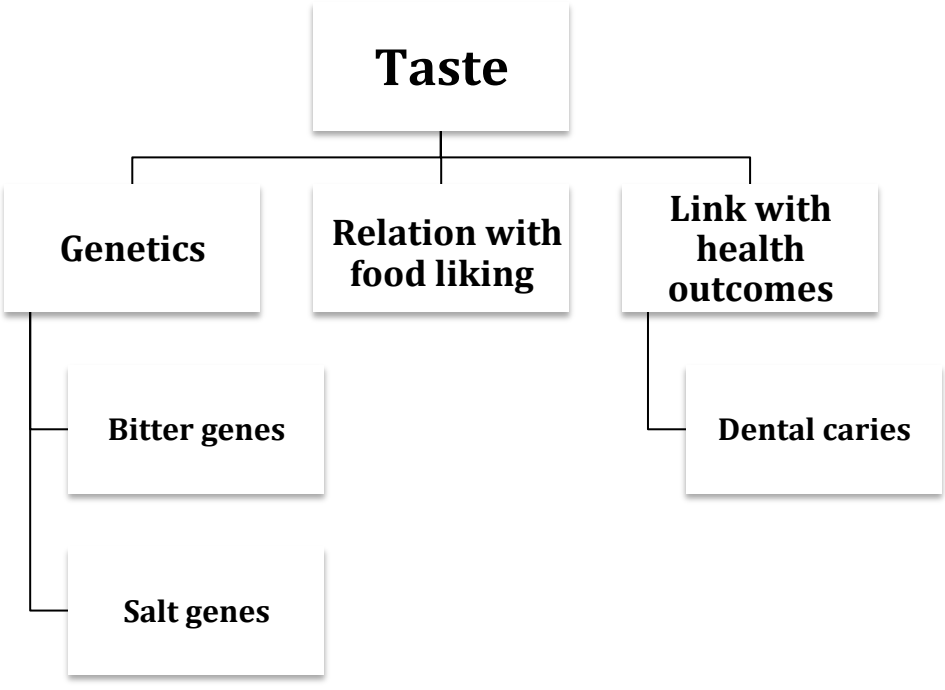

Figure 6. Outline of the objectives of the thesis.

CHAPTER II

Genetic analysis of taste perception

1. BACKGROUND AND AIM

a. Genetics of PTC/PROP bitter perception

Study on the genetics of PTC perception began in 1930 when A.L. Fox found that some individuals, defined “non tasters”, were unable to detect bitterness of this compound, while others, named “tasters”, were much more sensitive (Fox, 1932). Following studies showed that the inability to taste PTC (phenylthiocarbamide), and similar compound like PROP (6-n-propylthiouracil), is transmitted as a simple Mendelian recessive trait (Blakeslee, 1932; Snyder, 1931), while others studies have suggested that incomplete dominance, multiple alleles or multiple genes explain the inheritance of this trait (Kim et al., 2004). The major gene underlies PTC/PROP phenotype is the T2R38, a member of T2R family of bitter taste receptor genes. Three SNPs within this gene lead three amino acid substitutions (A49P, A262V and V291I) that define two most common haplotypes, designed PAV (proline-alanine-valine) and AVI (alanine-valine-isoleucine). While AVI is referred as the major non taster haplotype, PAV is indicated as the major taster haplotype (Kim et al., 2003). Although the *T2R38* gene accounts for a large fraction (50%-80%) of PROP/PTC phenotypic variation (Kim et al., 2003; Drayna et al., 2003), evidences showed that other genes might contribute to the phenotype (Drayna et al., 2003; Reed et al., 2010). A recent work showed that a polymorphism in the gustin gene (CA6), a taste-bud trophic factor which controls the salivary protein carbonic anhydrase VI, alters the functionality of this enzyme and is strongly related to taste responsiveness to PROP (Padiglia et al., 2010). Responsiveness to PROP was also associated with salivary levels of two peptides belonging to the basic proline-rich protein family and both encoded by the *PRB1* gene. These finding suggest that *PRB1* could contribute to individual differences in PROP perception and confirm the hypothesis of the PROP phenotype as a complex genetic trait (Cabras et al., 2012).

b. Genetics of salty perception

Salt intake differs between and within populations, representing a risk factor for the development of cardiovascular diseases and hypertension (Dahl, 2005; Strazzullo et al., 2009; Whelton et al., 2012). Salty perception and genetic variation in taste

receptors may be considered important determinant of differences in salt intake (Hayes, Sullivan, & Duffy, 2010). While genetic variations in taste perception are well known for bitter, sweet and umami taste, to date little is known on the association between genetic polymorphisms in salt receptors and differences in salt taste in human. The epithelial sodium channel amiloride-sensitive (ENaC) is the most well characterized sodium taste receptor. ENaC is stimulated by NaCl at both low (100mM) and high (500mM) concentrations and is amiloride-sensitive and sodium-specific. In mice the lack of ENaC expression in taste cells lead to a complete loss of salt attraction and sodium taste responses, providing evidence for the role of this receptor in salt taste. Furthermore, amiloride, an ENaC blocker, alters sodium currents in taste cells and inhibits taste response to sodium chloride (Chandrashekar et al., 2010). Evidences from rodents have shown that a polymorphism in αENaC gene, which encode for the α subunit of the ENaC taste receptor, is associated with differences in amiloride-sensitive taste responses to sodium chloride (Shigemura et al., 2008). In Drosophila melanogaster, Liu et al. (Liu et al., 2003) also reported that ppk11 (Pickpocket11) and ppk19 (Pickpocket19), genes that code for ENaC channels, are involved in salt taste perception. In addition to ENaC, a genetic variant of TRPV1, a non-selective cation channel, has been identified as possible candidate receptor for salt perception. This receptor responds to a variety of cations including Na+, K+, NH4+, and Ca2+ and is amiloride insensitive. Evidence indicates that TRPV1 mediates in nociceptive neurons thermal pain including the noxious thermal pain produced by vanilloids such as capsaicin and resiniferatoxin (Lyall et al., 2004).

A recent work carried out in humans analysed for the first time the association between genetic variations in ENAC (SCNN1A, SCNN1B, SCNN1G, SCNN1D) and TRPV1 genes with salt taste sensitivity (Dias et al., 2013). Results showed that polymorphisms in the genes that code for TRPV1 cation channel and the β subunit of ENAC channel are responsible of human individual differences in salt taste perception. Despite these recent evidences on specific candidate genes, genetics that underlie salt perception in human is still poorly understood and to date genome-wide association studies aiming to identify new genes have not been conducted.

c. Association analysis for studying genetic bases of taste phenotypes

Differences in taste perception are influenced by both genetic and environmental factors. The study of environmental factors such as dietary habits, culture and experiences, age, gender, olfaction, has raised much attention, while the genetic background is less understood and the most of studies were focused on candidate genes or specific regions of the genome.

Generally, genetic association studies are used to find candidate genes or genome regions that contribute to a specific disease o trait by testing for a correlation between disease status and genetic variation. SNPs are the most widely tested markers in association studies. Technically, a variation must be present in at least 1% of a population to be classified as a polymorphism. A higher frequency of a SNP allele or genotype in a series of individuals affected with a disease can be interpreted as meaning that the tested variant increases the risk of a specific disease or trait. Usually, two different approaches can be used for genetic dissection of complex traits: candidate gene approach and GWAS. Candidate gene studies typically rely on prior scientific knowledge suggesting that the genes have a biological function relevant to the investigated trait (Zhu & Zhao, 2007). Similar to candidate gene approach, GWAS aim to identify associations between SNPs and a trait but involving the characterization of a much larger number of SNPs. However, this type of study proceeds without assumptions or previous knowledge of the relevant genes and the whole genome is scanned for genetic variation, allowing the discovery of new regions or genes of interest (McCarthy et al., 2008).

In the last years, with the help of high-throughput genotyping arrays and genome-wide-association studies it became possible to investigate the genetic contribution to variation in human chemosensory perception.

In the current study I carried out: i) a GWAS for PROP responsiveness aiming to identify new genes in addition to T2R38 contribute; ii) the first GWAS for salty perception exploring variants associated to NaCl responses.

2. MATERIALS AND METHODS

a. Participants

This study includes 2600 participants coming from three different Italian populations: Carlantino, a small village of the South of Italy situated in the extreme northern part of Puglia Region; a population in Northern-Eastern Italy, involves the inhabitants of six different communities of Friuli Venezia Giulia region (San Martino del Carso, Erto/Casso, Clauzetto, Illegio, Sauris and Val di Resia); and finally a population coming from the Val Borbera Valley in Northwest of Italy. Due to geographical, historical, linguistic and/or cultural factors, these populations showed evidences of genetic isolation (Esko et al., 2013). The use of isolated populations, characterized by small effective population size, more inbreeding, more uniform genetic background and largely shared environment, was proved very useful in identification of genetic variants associated to complex traits or diseases (Peltonen, Palotie, & Lange, 2000).

For each participant a questionnaire to obtain socio-demographic information, as well as data on clinical parameters, professional activity, lifestyle, eating habits and family history has been collected.

Subjects gave their written informed consent for participating in the study. The ethical committees of the three different institutions approved the protocol. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

b. NaCl and PROP phenotypes

NACL and PROP taste intensity was determined in all subjects using a filter paper method described in Zhao et al. (Zhao, Kirkmeyer, & Tepper, 2003). Each subject was given two paper disks, the first one was impregnated with 1.0 mol/l NaCl (VWR Scientific, Bridgeport, NJ), and the second disk was impregnated with 50 mmol/l 6-n-2-propylthiouracil (cat. no. P3755; Sigma-Aldrich, St Louis, MO). The subject was asked to rinse the mouth with bottled water, place the paper disk on the tip of the tongue and rate the intensity of the taste using the labelled magnitude scale (LMS). The subjects were also required to rinse with water

between tasting each disk and to wait a minimum 30s before tasting the PROP disk.

The LMS is a quasi-logarithmic 100-mm scale anchored with the labels 'barely taste it', 'weak', 'moderate', 'strong', 'very strong' and 'strongest imaginable' oral sensation (Green et al., 1996). Participants were instructed first to the verbal descriptors of the scale and also to make a mark anywhere on the scale, not only near the descriptors.

In this study the data intensity ratings were used as quantitative phenotype in the association analysis. Given that taste responsiveness was measured on a logistic scale, for the analysis intensity ratings were all transformed using the log10 of the measure (Genick et al., 2011).

c. DNA sampling, genotyping and imputation

DNA for genotyping was extracted from blood of each participant. Genotyping was carried out using the Illumina 370k high density SNP array. Genotype calling was performed with the GenomeStudio software (Illumina). Quality control was conducted independently in each population. Individual call rate, excess of heterozigosity and identity by state (IBS) between each pair of samples were checked. If a pair had IBS > 0.95 the sample with lower call rate was excluded. All SNP with minor allele frequency (MAF) <0.01, Hardy-Weinberg equilibrium (HWE) deviation P-value<1.0E-08 and call rate <0.97 were also removed.

Genotype imputation was conducted using SHAPEIT2 for the phasing step and IMPUTE2 for the imputation using the1000 Genomes phase I v3 reference set (Howie et al., 2012).

d. Association analysis

Association analysis was conducted using the GRAMMAR-Gamma method as implemented in GenABEL package for genotyped SNPs and MixABEL for imputed data (Aulchenko et al., 2007). Association analysis was performed through a mixed model linear regression were the log10 of the PROP or NaCl measure was used as the dependent variable and the SNP the independent variable.

NaCl analysis included sex and age as covariates, while in the PROP analysis sex, age, log10 of NaCl measure and rs10246939 SNP at T2R38 gene were used as covariates. Each subject's phenotype was correct for the T2R38 SNP to adjust

for T2R38 gene, the major responsible of PROP perception, while NaCl was included as covariate to distinguish PROP perception from general taste perception.

In addition, in both analyses the kinship matrix based of on all available genotyped SNPs was used as the random effect. Kinship matrix is a method that allows assessing for relatedness population stratification in samples from homogeneous populations, such as isolated ones. In our study, the genomic kinship was calculated with the ibs function in the GenABEL R package by using shared genotype counts as a measure of genetic distance between individuals.

For the association analysis different genetic models were assumed: additive, recessive and dominant.

Association analysis was conducted separately for each cohort and results have been pooled together through meta-analysis. Meta-analysis was conducted using the inverse variance weighting method.

3. RESULTS

a. GWAS of PROP bitter perception

Meta-analyses have identified some SNPs associated with PROP responsiveness. In table 3 are shown the most significant results obtained using different genetic models (p-value<1.0E-06).

SNP	Chromosome	Position	p-value	Closest Gene	Genetic Model
rs78537477*	3	10296849	3.34E-07	TATDN2, GHRLOS, GHRL	dominant
rs2270454	3	10292140	7.88E-07	"	dominant
rs2005903	3	10299040	9.56E-07	"	dominant
rs2003595	3	10299057	8.64E-07	"	dominant
rs146768860	3	10299656	3.96E-07	"	dominant
rs56284018	3	10300846	9.02E-07	"	dominant
rs2241313	3	10302045	4.78E-07	"	dominant
rs2241314	3	10302056	4.54E-07	"	dominant
rs12200968**	6	52472541	2.19E-07	TMEM14A	additive
rs7746307	6	52473126	6.04E-07	"	additive
rs6458845	6	52473418	7.05E-07	"	additive
rs35936127***	7	141674316	5.66E-08	T2R38	dominant
rs11623995	14	22905725	2.66E-07	NA	additive
rs2331619	14	22910451	3.25E-07	"	additive
rs10137305	14	22914747	5.13E-07	"	additive
rs7144549	14	22921202	6.37E-07	"	additive

Table 3. List of SNPs with p<1.0E−06 associated to PROP responsiveness.
Closest gene refers to closest gene or genes in a region of ±200 Kb upstream and downstream the SNP.
*This snp is associated to the phenotype also using the additive model with p-value=8.46E-07.
**This snp is associated to the phenotype also using the recessive model with p-value=9.11E-07.
***This snp is imputed only in two of the three analyzed populations.

The highest hit was found with a SNP located on chromosome 7 near 5' UTR region of the T2R38 gene, the major gene responsible of PTC and PROP

perception. It is not in linkage disequilibrium with the three SNPs of T2R38 gene already associated to the PROP phenotype, so it could make an independent contribution to PROP perception.

In addition, association was observed for several SNPs located on chromosome 3 in TATDN2 gene and very closed to GHRL and GHRLOS genes.

An additional 3 SNPs, associated with the analyzed phenotype, are located on chromosome 6 closed to TMEM14A gene.

Finally, association between PROP responses and further 4 SNPs on chromosome 14 were found. These SNPs falls into a gene-free region.

Figure 7 shows the regional association plots for the identified top hits.

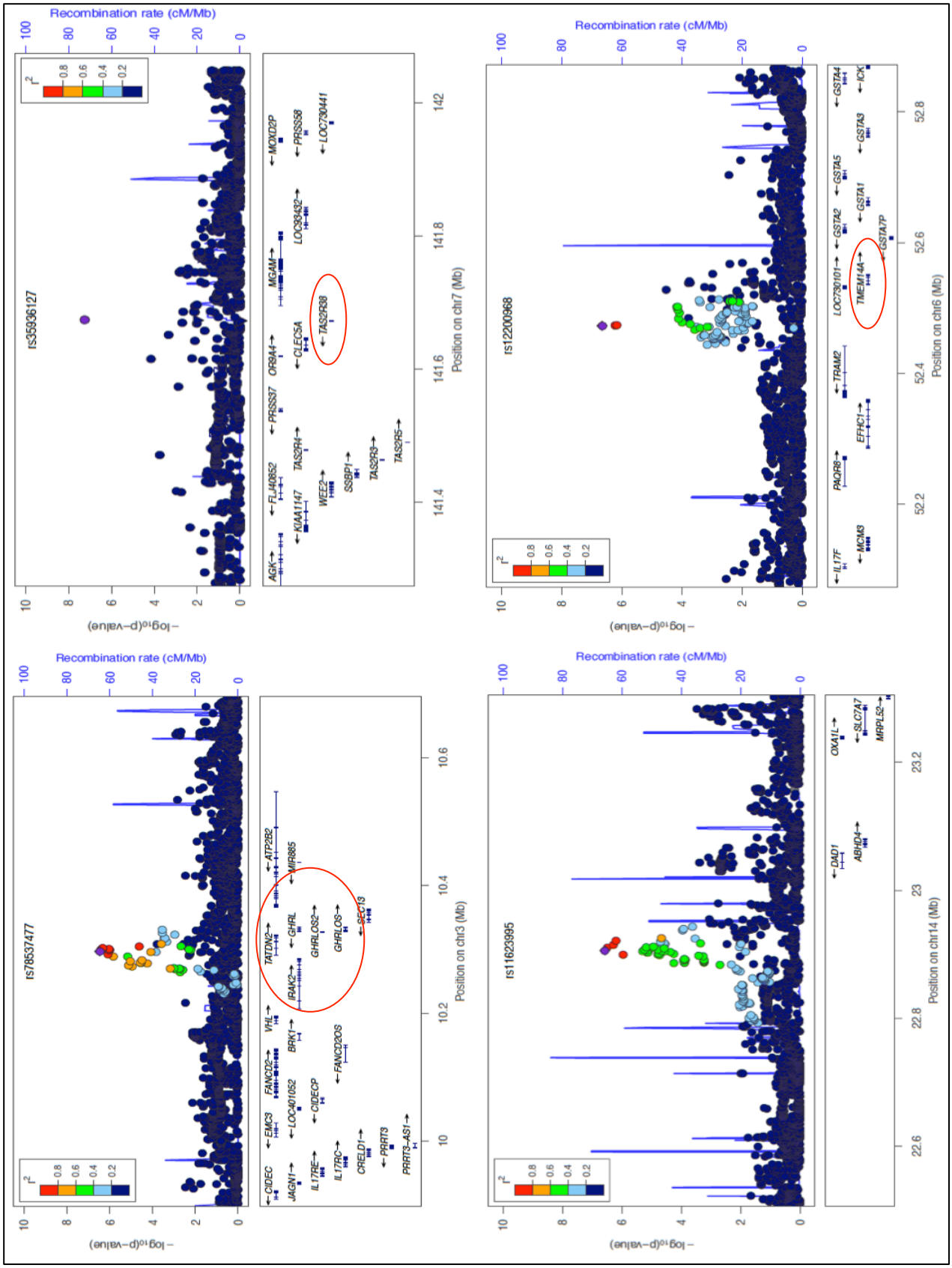

Figure. Regional association plot for the top hits of PROP GWAS.
Plot made using the tool Locus Zoom (https://statgen.sph.umich.edu/locuszoom/). SNPs are plotted with their *P* values (as −log10 values) as a function of genomic position. Estimated recombination rates are plotted to reflect the local LD structure around the associated SNPs.

b. GWAS of salt responsiveness

In the meta-analysis for NaCl responsiveness no significant association results were observed (p<1.0E-06), while looking at each population interesting signals were found. Best hits associated to NaCl responses using different genetic models in each population are reported in table 4.

SNP	Chromosome	Position	p-value	Closest Gene	Genetic model	Population
rs1892700	21	35016137	1.84E-07	ITSN1	additive	CARL
rs10804137	2	205257059	2.23E-07	NA	recessive	CARL
rs12521970	5	135824521	7.89E-07	TRPC7	dominant	CARL
rs547916	12	5324400	5.61E-08	KCNA5	recessive	FVG
rs7983485*	13	111933998	2.76E-07	NA	additive	VB
rs2697696	4	17448293	8.40E-07	NA	recessive	VB

Table 4. Best hits for NaCl responsiveness
Closest gene refers to closest gene or genes in a region of ±200 Kb upstream and downstream the SNP.
In FVG addtitive and dominant models do not show results with p<1.0E-06.
* This SNP is the best hit also using the dominant model (with p-value= 2.7E-08)
(CARL=Carlantino population; FVG=Friuli-Venezia Giulia population; VB=Val Borbera population)

In Val Borbera population two SNPs (rs7983485 and rs2697696) were associated to salt perception, although both SNPs fall in a gene-free region. In Carlantino population 3 different SNPs show association with NaCl responsiveness: rs1892700 in ITSN1 gene, rs10804137 in a gene-free region and rs12521970 closed to TRPC7 gene. Finally, in FVG population only 1 SNP, located close to KCNA5 gene, shows association with the phenotype for all the analyzed genetic models. In particular, TRPC7 and KCNA5 genes are of special interest for their biological role in taste perception.

Figures 8 and 9 show the regional association plot for the KCNA5 gene region and TRPC7 gene region respectively.

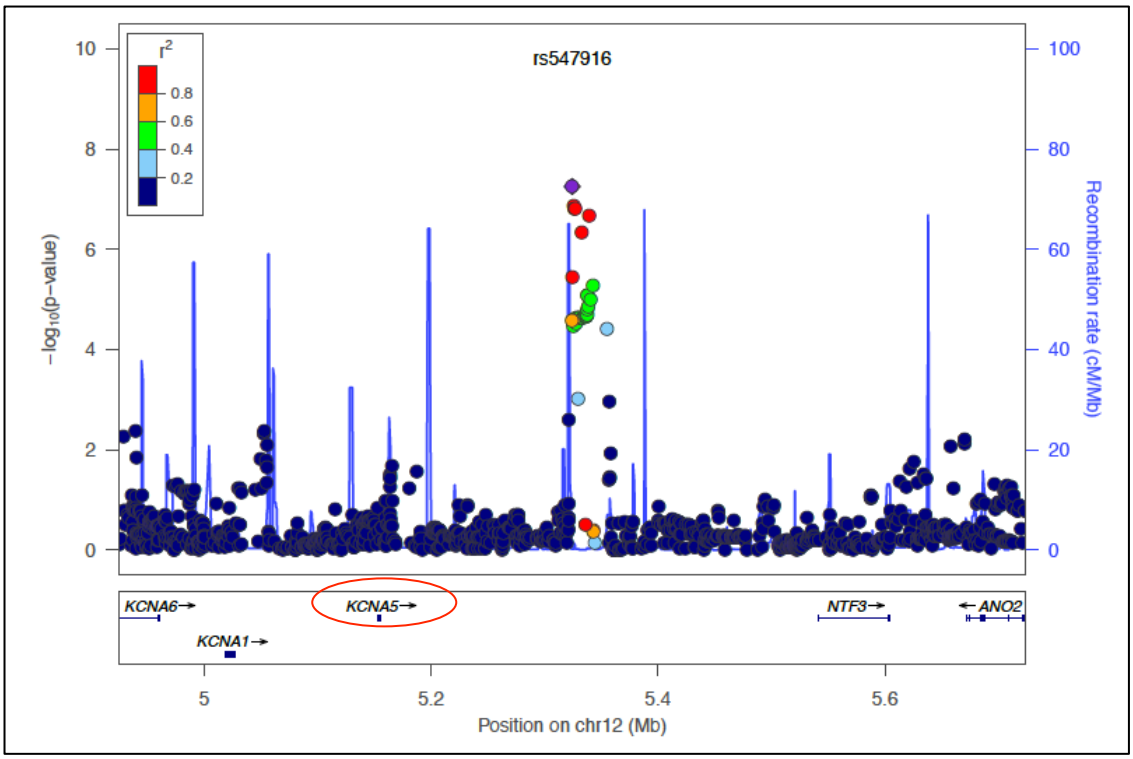

Figure 8. Regional association plot for the top hit of salt GWAS in FVG population.
Plot made using the tool Locus Zoom (https://statgen.sph.umich.edu/locuszoom/). SNPs are plotted with their *P* values (as −log10 values) as a function of genomic position. Estimated recombination rates are plotted to reflect the local LD structure around the associated SNPs.

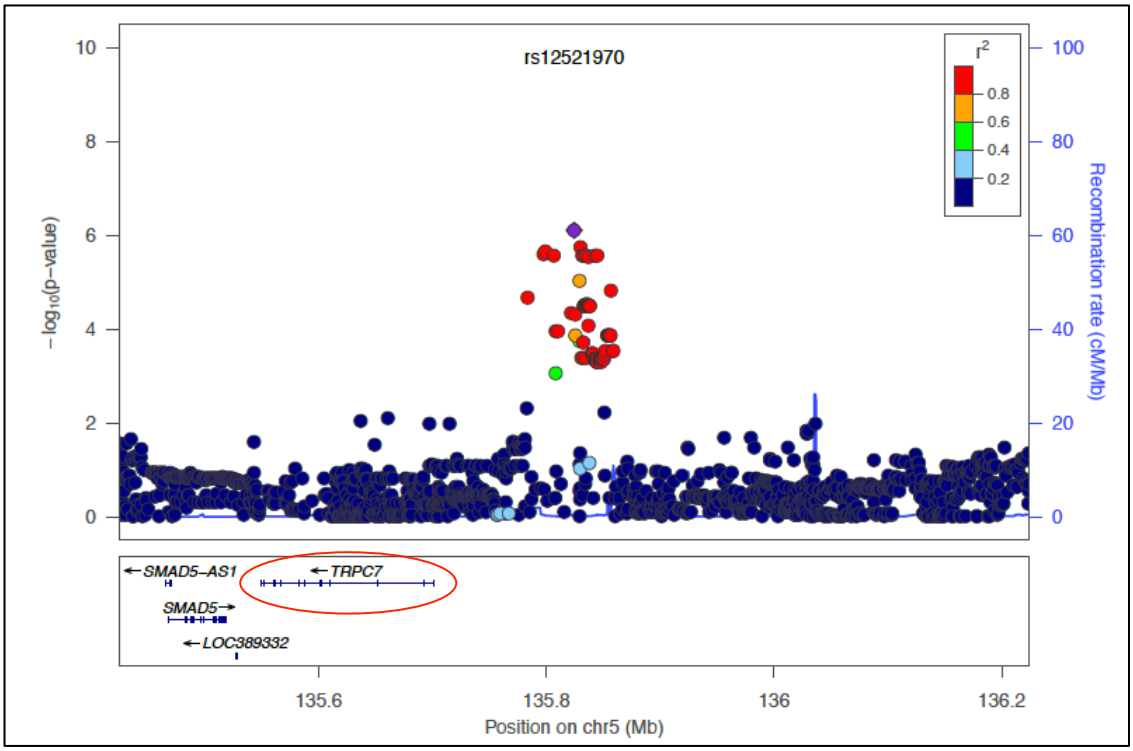

Figure 9. Regional association plot for the top hit of salt GWAS in Carlantino population
Plot made using the tool Locus Zoom (https://statgen.sph.umich.edu/locuszoom/). SNPs are plotted with their p-values (as −log10 values) as a function of genomic position. Estimated recombination rates are plotted to reflect the local LD structure around the associated SNPs.

In addition to further validate the impact of TRPC7 in salt perception, protein–protein interaction network was constructed using STRING v9.1 (Franceschini et al., 2013). The network showed that TRPC7 interacts with others proteins linked to taste function, such as ITPR3 (inositol triphosphate receptor 3) and TAC1 (tachykinin precursor 1) (Figure 10). ITPR3 is involved in bitter, umami and sweet taste transduction (Chaudhari & Roper, 2010), while TAC1 is the precursor of tachykinins and is known that tachykinin receptors are expressed in mouse taste buds (Grant, 2012).

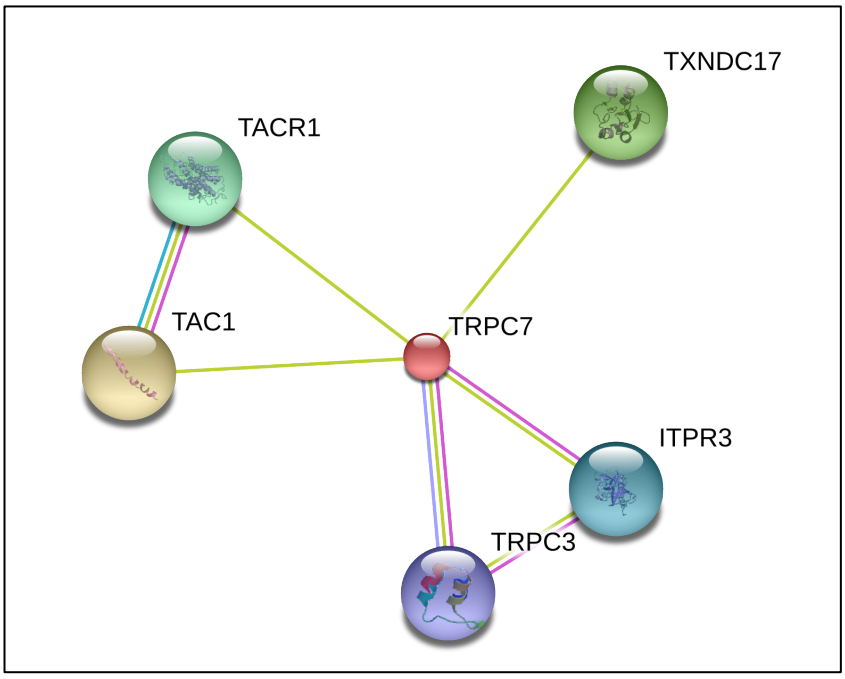

Figure 10. Protein interaction network of TRPC7.
Different line colors represent the types of evidence for the association (green line for previous literature knowledge; pink line for high-throughput experiments, violet line for homology).

4. DISCUSSION

a. Other genetic factors of PROP bitter taste perception

Although a recent GWAS study has failed to detect additional genetic variants that impact PROP perception (Genick et al., 2011), past studies have suggested that additional genetic factors, other than T2R38, may play a role in influencing the variation in PROP bitter taste (Drayna et al., 2003; Reed et al., 2010; Padiglia et al., 2010; Cabras et al., 2012).

Our GWAS study on PROP perception has not showed significant associations. Nevertheless, interesting associations for their biological role were identified such as that with GHRLOS and GHRL genes. GHRL gene encodes ghrelin-obestatin preproprotein, which generates ghrelin and obestatin**.** GHRLOS is the antisense gene of the ghrelin gene. Ghrelin is an extremely important hormone that regulates appetite, food intake, gastric emptying, weight gain and growth hormone secretion. T2Rs bitter receptors are found in some gastrointestinal endocrine cells, including those that secrete the peptide hormones (e.g., ghrelin) in response to stimulation by bitter-tasting compounds. Recent studies on mice have also shown that activation of bitter taste receptors in the gut stimulates ghrelin secretion, with functional effects on food intake and gastric emptying and thus regulating appetite (Janssen et al., 2011). In light of these evidences, the association we found between PROP bitter perception and variants closed to GHRL gene is noteworthy because it suggests that ghrelin could have negative feed back mechanism which regulates bitter perception. However, further studies are needed to clarify the GHRL variants role in PROP perception and if its effect is limited to this particular bitter compound or if it involves bitter perception in general.

Another interesting result is the identification of an additional SNP closed to the 5' UTR region of the T2R38 gene, which might indicate a regulatory region, in agreement with a recent work showing that mRNA expression amounts of the PAV allele of the T2R38 gene correlate with differences in PROP perception (Lipchock et al., 2013). Given that T2R38 gene is the major but not exclusive responsible of differences in PROP perception, even among individuals with the same genotype, our result indicate that both the genotype and the expression levels could modulate PROP bitter perception.

Despite the biological relevance of the genetic variations identified in our study for PROP bitter taste further investigations are needed to replicate present results in an independent cohort and clarify the biological mechanism associated to individual differences in bitter perception.

b. Variants in TPRV7 and KCNA5 genes are linked to salt taste perception

As previously described, despite recent progress very little is known about the genetics bases of salt taste. Our study resulted in the identification of genes that

have a convincing biological role in salt taste perception and that may be considered as good candidates for further investigations. The most relevant gene is KCNA5, encoding a member of a potassium channel voltage-gated, shaker-related subfamily. It belongs to the delayed rectifier K^+ (DRK) class, the function of which could restore the resting membrane potential of cells after depolarization. Interestingly, in the mammalian taste system, DRK channels may play a central roles in specific taste transduction pathways, in which they have been reported to serve as direct or indirect targets for modulation by a variety of taste stimuli, including acids, sweeteners, bitter stimuli and fatty acids (DeSimone et al., 2001; Herness, Sun, & Chen 1997; Zhao, Lu & Herness, 2002; Gilbertson et al., 1997).
Moreover, a study has shown that KCNA5 is the major functional DRK channel expressed in the anterior rat tongue (Liu et al., 2005).
Another noteworthy gene found in our GWAS analysis is TRPC7, a member of the big family of transient receptor potential (TRP) channels. These receptors play a crucial role in many mammalian senses, including touch, smell and taste (Damann, Voets & Nilius, 2008). As regard taste sensation, different TRP genes are expressed in taste receptors cells; for example TRPM5 functions as a downstream component in sweet, umami and bitter taste signal transduction; PKD1L3 and PKD2L1 are both involved in responses to sour stimuli; TRPV1 is the candidate for salt taste perception. Moreover, other members of TRP channels, are involved in eating experience through activation of free nerve ending that innervate tongue, palate and nose. Among them TRPV1 is the receptor for hot compounds responding to capsaicin of chili pepper, TRPM8 is the receptor for cool compounds such as menthol and eucalyptol, TRPA1 is the receptor for pungent compounds such as mustard and cinnamon (Ishimaru & Matsunami, 2009).
In addition, STRING analysis has shown that TRPC7 protein interact with others proteins with a biological link to taste perception. ITPR3 play a role in the taste transduction pathway of bitter, umami and sweet tastes. ITPR3 channels allow the flow of calcium out of the endoplasmic reticulum in response to IP3, resulting in the activation of TRPM5 with leads to a depolarization generation an action potential (Chaudhari & Roper, 2010). TAC1 is the precursor of tachykinins, such as substance P, neurokinin A, neuropeptide K and neuropeptide gamma. Nerve fibers containing substance P and neurokinin A are present in around taste buds

(Nagy et al. 1982; Yoshie et al., 1989) and tachykinin receptors are expressed in mouse taste buds (Grant, 2012). Several studies have also shown that substance P can stimulate or modulate responses in gustatory neurons of the nucleus tractus solitarius and gustatory sensory ganglion (King, Wang & Bradley, 1993; Koga & Bradley, 2000). Interestingly, tachykinin agonists are involved in a decreased salt intake in rats, suggesting that they might modify salt taste sensitivity (Ciccocioppo et al., 1994; Flynn, 2000).

All these evidences support the association we found between salt responses and a variant close to TRPC7 gene, suggesting is involvement in salt taste.

CHAPTER III

The role of PROP bitter perception on food liking

1. BACKGROUND AND AIM

a. PROP bitter taste perception

The sensitivity to bitter taste is a variable trait both within and between human populations, and large individual differences in responsiveness to bitternesshave been well documented (Tepper, 1998). Bitter perception in humans is mediated by a family of 25 T2R taste receptors (Behrens & Meyerhof, 2006). Among them, the most studied is the *T2R38* gene, associated with the ability to taste PTC (phenylthiocarbamide) and PROP (6-n-propylthiouracil) (Bufe et al., 2005).

Approximately 70% of the world's population are considered "tasters", and perceive these substances as moderately to intensely bitter. These compounds are weak or tasteless for the remaining 30% of the population, who are considered "non tasters". Bartoshuk et al. revealed that taster individuals can be divided into two sub-groups: medium tasters, who perceived moderate intensity from PTC/PROP, and super-tasters, who perceived these compounds as extremely bitter. Thus, the population distribution of non tasters, medium tasters and super tasters is approximately 30%, 50% and 20% respectively (Bartoshuk, Duffy & Miller, 1994).

As previously reported (Chapter II), sequence variations in the *T2R38* gene produce three amino acid substitutions: A49P, A262V and V291I that define two common haplotypes, namely PAV and AVI. The AVI haplotype (AVI/AVI homozygous individuals) specifies the non taster phenotype, while it was supposed that the PAV haplotype (PAV/PAV homozygous or PAV/AVI heterozygous individuals) specifies the taster phenotype (Kim et al., 2003). Although supertasting is typically associated with heightened responses to the bitterness elicited by PROP, T2R38 variations cannot explain "general" supertasting more broadly defined as the ability to perceive oral sensations more strongly without regard to PROP status or T2R38 polymorphisms (Hayes, Bartoshuk, Kidd, & Duffy, 2008). Rare haplotypes (AAI, AAV, PAI, and PVI) have also been observed at a frequency of 1–5% (Behrens et al., 2013), but are mainly found in African populations (Campbell et al., 2012).

PTC and PROP are synthetic compounds, not found in nature, but they are chemically similar to isothiocyanates commonly found in broccoli, cabbage and

other bitter-tasting *Brassica* vegetables (Drewnowski & Gomez-Carneros, 2000). The presence of the thiourea group (N-C=S) within these compounds is responsible for their bitter taste. Although the T2R38 receptor is also capable of binding non-thiourea substances (e.g., limonin, ethylpyrazine), compounds with the N-C=S moiety are considered the primary ligands for this receptor (Meyerhof et al., 2010).

b. PROP bitter taste perception and food liking

Taste is considered one of the most important factors influencing food selection. Numerous studies have focused on PROP bitter perception and its relationship with taste preference and even food acceptance. Greater perception of PROP is generally, but not always, associates with dislike and avoidance of *Brassica* vegetables (Tepper, 2008; Dinehart et al., 2006; Keller et al., 2002; Ullrich et al., 2004; Tsuji et al., 2012; Gorovic et al., 2011; Feeney, 2011; Baranowski et al., 2011). There are also numerous reports that supertasters dislike bitter foods that do not contain the thiourea group, as well as other foods that produce strong oral sensations such as sweets, added fats, spicy foods and alcoholic beverages (Hayes & Keast, 2011; Hayes et al., 2011; Hayes & Duffy, 2008). In light of these observations, PROP-tasting has gained attention as general marker for oral sensations and food preferences. This view remains controversial, however, since some studies report no relationship between PROP tasting and general food preferences (Drewnowski, Henderson & Cockroft, 2007; Feeney, 2011) and other markers for oral sensations have emerged (Hayes, Feeney, & Allen, 2013; Hayes et al., 2013).

The present study was designed to address this gap in knowledge. Here, we examined relationships among PROP perception, T2R38 polymorphisms and food liking in different rural communities from the Caucasus region (Georgia, Armenia and Azerbaijan), Central Asia (Uzbekistan and Kazakhstan) and Tajikistan. Data were obtained as part of the scientific expedition Marcopolo 2010 (www.marcopolo2010.it), whose main goals were to analyse individual differences in the human senses (e.g. taste, smell, hearing, vision) across the Silk Road, a major pathway for cultural, commercial, and genetic exchange between individuals from China and Mediterranean countries for almost 3,000 years.

2. MATERIALS AND METHODS

a. Participants

A total of 496 subjects participated in the study (206 males and 290 females), coming from 20 different communities of six countries in the Caucasus and Central Asia: Georgia, Armenia, Azerbaijan, Uzbekistan, Kazakhstan and Tajikistan (Figure 11).

Figure 11. Populations along the Silk Road.
Populations analysed (linked by dashed line), their geographical location and sample size.

All communities belong to the Terra Madre organization (www.terramadre.org). Information, such as age, sex, lifestyle, eating habits, professional activity, smoking and alcohol consumption were collected.

All subjects provided written informed consent before participation. Approval for the research protocol was obtained from the ethical committee of IRCCS-Burlo Garofolo Hospital.

b. DNA sampling and genotyping

Saliva samples were collected from all participants using the Oragene DNA collection kit and DNA was extracted (DNA Genotek, Ontario, Canada). Three

polymorphisms in the T2R38 receptor gene (rs1726866, rs10246939 and rs713598) define the genotype. The first two were genotyped with the Omni Express 700k Illumina Chip. The third one was analysed using TaqMan probe-based assays (Applied Biosystems, Foster City, CA, USA).

c. PROP tasting

PROP taste intensity was determined in all subjects using a filter paper method as previously described (Chapter 2). For this study the LMS was translated in the local language of each community. In addition, translators verbally defined the label descriptors of the scale to each participant and also instructed him/her to make a mark anywhere on the scale, not only near the descriptors.

Using LMS numerical cut-off scores of <15 and >67, the subjects were classified as super tasters and non tasters, respectively. Medium tasters fell between those two limits (> 16 and 67). NaCl ratings were used as a reference standard for classifying subject who gave a borderline rating to PROP. The use of NaCl as a reference standard is based on the observation that super tasters give higher ratings to PROP than NaCl, medium tasters give similar ratings to both, and non tasters give higher ratings to NaCl than to PROP (Tepper, Christensen, & Cao, 2001). These procedures were developed and validated in previous studies (Zhao et al., 2003) and have been used in numerous investigations in English-speaking and non-English speaking populations followed in our previous studies (Tepper et al., 2008; Tepper et al., 2009; Bembich et al., 2010).

d. Food liking questionnaire

Participants completed a 79-item food liking questionnaire that was based on an instrument used in a previous study (Tepper et al., 2009) and supplemented with foods specific to the diets of the communities we studied. The selection of the supplemental foods was based on a survey conducted by collaborators from the Terra Madre organization who carried out a preliminary survey on the local foods consumed by these populations (Pirastu et al., 2012). The questionnaire assessed general food likes and dislikes (e.g. garlic, milk, banana, orange juice). It was administered in the local language of each community by translators who were familiar with the local culture.

Subjects rated their liking of each item on a 5-point scale ranging from “like extremely” (score 5) to “dislike extremely” (score 1). The option “never tasted” was also included.

e. Statistical analysis

The Chi-square test was used to examine the association between *T2R38* genotypes and PROP status for the whole cohort. Chi-square tests were also performed to determine whether the relationship between *T2R38* genotypes and PROP status differed among the populations tested. Correspondence Analysis was also applied to the two-way contingency table of PROP status and participants’ country of residence to obtain a graphical representation of the relationship between the two variables.

Analysis of covariance (ANCOVA) was performed to determine the influence of PROP taster status and *T2R38* genotypes on liking of each food. This analysis was applied to the entire cohort and to each population separately. Sex and age were used as the covariates. Due to the large number of comparisons, statistical significance was set at $p<0.00063$, following Bonferroni correction ($p=0.05/ 79$ foods).

In addition, the foods were grouped (Ullrich et al., 2004) and the same analyses were conducted using food groups. The food groups included fruits (strawberries, lemons, orange juice), vegetables (artichokes, spinach, turnip, cooked carrots, asparagus, fava beans, cabbage), alcohol (red wine, white wine, vodka, brandy, beer), condiments (olives, sardines, onion, garlic, kilka, adgika, chilli pepper), sweets (ice cream, cake, sweet ricotta, biscuits, biscuits with cream, jam, honey, milk chocolate). The mean number of foods within each food group was calculated for each subject and was used for the analyses.

We also sought to determine if variations in food likes and dislikes across populations were related to the distribution of PROP phenotypes or *T2R38* genotypes. To accomplish this task, a series of data matrices were constructed. First, the Kruskal-Wallis test was performed (at $p<0.00063$) comparing the food liking of each population to all others, pairwise. The number of foods that showed statistically significant differences between population pairs were tallied and entered into a distance matrix. Higher values indicated dissimilar patterns (large

distances) in food liking between populations, and lower values indicated similar patterns (small distances) between them. For example, if the pair-wise difference between two populations was high, these two populations had many differences in food liking. On the contrary, if the pair-wise difference was small, the two populations shared similar food liking responses.

In order to describe the phenotypic dissimilarities in bitter perception between populations, we created another distance matrix. Here, we calculated the chi-square statistic (as a distance measure) between phenotypic groups (non taster, medium taster and super taster) for each population, pairwise. Here, higher values represent a large difference (i.e., distance) in PROP bitter responsiveness between population pairs, and lower values represent a small difference in responsiveness between population pairs. The data inputs and procedures for this analysis are similar to those of multiple correspondence analysis (MCA) where data are categorical rather than continuous.

In order to assess possible bias due to the differences in sample size between populations, we performed a bootstrap analysis. We constructed a series of distance matrices by repeatedly (1000 times) sampling 47 individual (the n of the smallest population) from each population. We compared each distance matrix built after bootstrapping with the original one (built using the full dataset) and found a high correlation between them ($r>0.9$), showing that differences in sample size did not affect our results.

Then, we calculated the F_{ST} (Fixation Index) (Reynolds, Weir & Cockerham, 1983) to estimate genetic differences between populations for the SNPs which define T2R38 haplotypes. We also constructed a matrix of F_{ST} values using the whole genome (~356,000 SNPs) to obtain a global estimate of genetic diversity in our sample. Pairwise F_{ST} was performed using the R package Adegenet v1.3-4 (Jombart, 2008).

Finally, the Mantel test (Mantel, 1967) was used to determine the (dis)similarities between distance matrices. The Mantel r statistic is a standardized Pearson correlation coefficient calculated following random rearrangement of the data matrices across multiple permutations. 1000 iterations were used for a critical cut-off value of $p<0.05$.

3. RESULTS

a. PROP phenotypes and haplotypes

All 496 individuals genotyped for *T2R38* were tested for PROP taste intensity. The distribution of PROP status in each population was analysed and is shown in Table 5. In the overall sample 37.0% of individuals were non tasters, 40.0% were medium tasters and 23.0% were super tasters.

	PROP phenotype		
	NT	MT	ST
All (n=496)	37.0%	40.0%	23.0%
Sex			
Males (n=206)	44.2%	41.7%	14.1%
Females (n=290)	32.1 %	39.3%	28.6%
Population			
Georgia (n=116)	50.9%	38.8%	10.3%
Azerbaijan (n=47)	38.3%	46.8%	14.9%
Uzbekistan (n=91)	40.7%	40.7%	18.6%
Kazakhstan (n=57)	31.6%	50.9%	17.5%
Tajikistan (n=80)	36.2%	32.5%	31.3%
Armenia (n=105)	22.0%	39.0%	39.0%

Table 5. Distribution of PROP phenotype by sex and population

Interestingly, the distribution of phenotypes varied among the populations (X-squared=42.1077, p-value=7.1E-06). In particular, the prevalence of non tasters was higher in Georgia (50.9%) as compared to other populations, while the proportion of super tasters was higher in Armenia (39.0%) and Tajikistan (31.3%) relative to the other populations.

Correspondence Analysis revealed the relationships among the populations living in different countries with respect to PROP phenotype. In agreement with the univariate analyses, Georgia was highly associated with the non taster phenotype while Armenia was closely associated with the super taster phenotype. Furthermore, medium tasters were highly represented in the cluster of populations consisting of Azerbaijan, Uzbekistan and Kazakhstan. Tajikistan was distinct from the other groups (having relatively equal frequencies of the three taster

phenotypes), although it was more closely associated with the super taster phenotype, in accordance with the high prevalence of super tasters in this population (Figure 12).

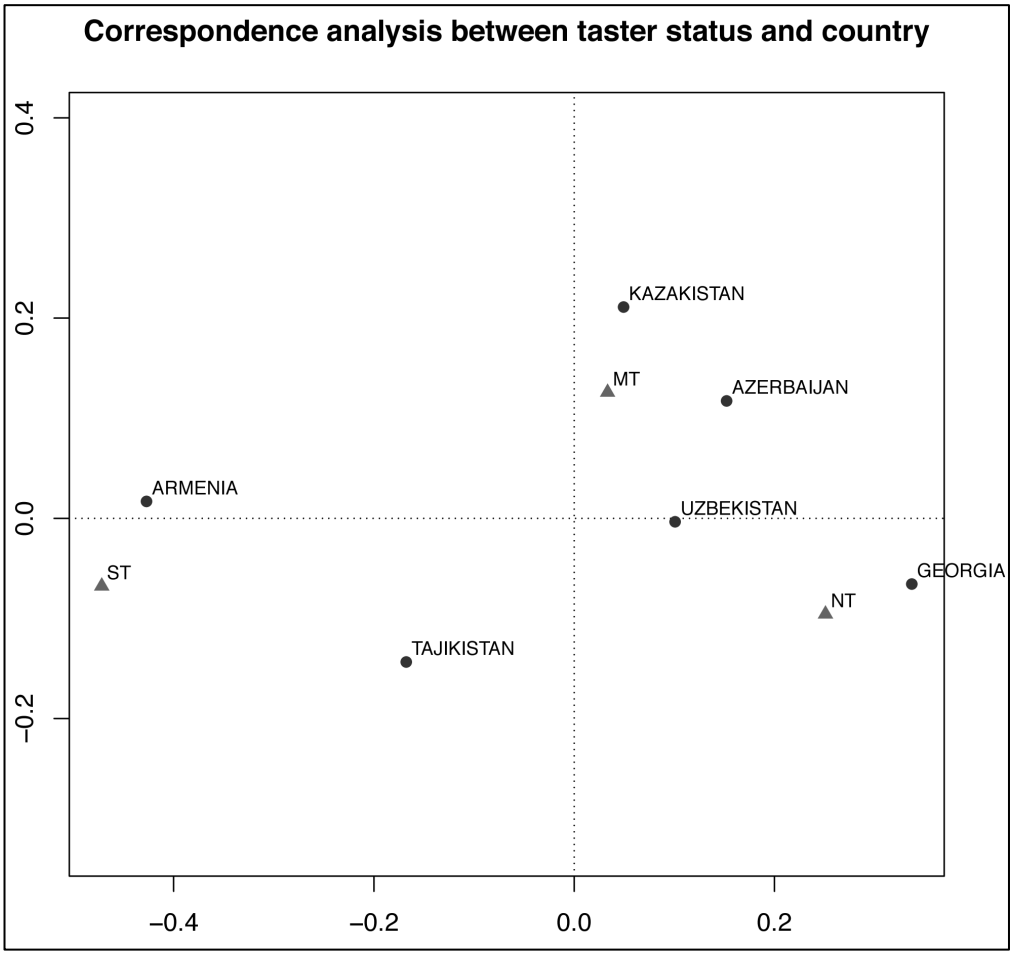

Figure 12. Correspondence analysis between taster status and country.
Correspondence Analysis between taster status and country shows the relationship between them. In particular, super taster status corresponds to Armenia and Tajikistan populations, non taster status to Georgia and medium taster status to Azerbaijan, Kazakhstan and Uzbekistan. Circles and triangles represent the country and the PROP status respectively. NT=non taster, MT=medium taster, ST=super taster. Country accounted for the majority (87.3 %) of variance and taster status accounted for 12.7% of variance in the model.

In contrast to the phenotypic differences observed among populations, we found no differences in *T2R38* haplotypes across populations (X-squared = 8.1822, p-value=0.611) (Table 6). The AVI/AVI, AVI/PAV and PAV/PAV diplotypes accounted for 24.9%, 48.0% and 27.1%, respectively, of the overall sample, in agreement with the allelic frequencies typically reported in Caucasian populations (Kim et al., 2003).

	T2R38 haplotype		
	AVI/AVI	PAV/AVI	PAV/PAV
All (n=496)	24.9%	48.0%	27.1%
Sex			
Males (n=206)	24.8%	48.5%	26.7%
Females (n=290)	25.0 %	47.6%	27.4%
Population			
Georgia (n=116)	33.7 %	42.2%	24.1%
Azerbaijan (n=47)	15.2%	56.5%	28.3%
Uzbekistan (n=91)	22.2%	51.1%	26.7%
Kazakhstan (n=57)	24.6%	49.1%	26.3%
Tajikistan (n=80)	23.8%	47.6%	27.8%
Armenia (n=105)	22.9%	47.6%	29.5%

Table 6. Distribution of T2R38 haplotype by sex and population

As expected, there was a strong association between T2R38 diplotypes and PROP phenotypes (X-squared=151.4019, p-value<2.2E-16). In the entire sample 82.9% of AVI/AVI homozygous individuals were non tasters, compared to 11.4% who were medium tasters and 5.7% who were super tasters. As expected, PAV/PAV homozygous and PAV/AVI heterozygous subjects were mainly medium or super tasters. We observed a similar correspondence between genotypes and phenotypes in each population.

b. PROP phenotype and food liking

The relationship between PROP phenotype and liking for each food on the food liking questionnaire was examined for the entire cohort, and separately for each population, and no associations were found. No relationship was revealed also between PROP status and food preference groups. These same analyses were repeated for *T2R38* haplotypes, and the outcome was the same; no associations were found.

c. Multi-dimensional analyses of food liking

A distance matrix describing the differences in food liking across the populations was constructed, and is graphically presented as a dendrogram in Figure 13.

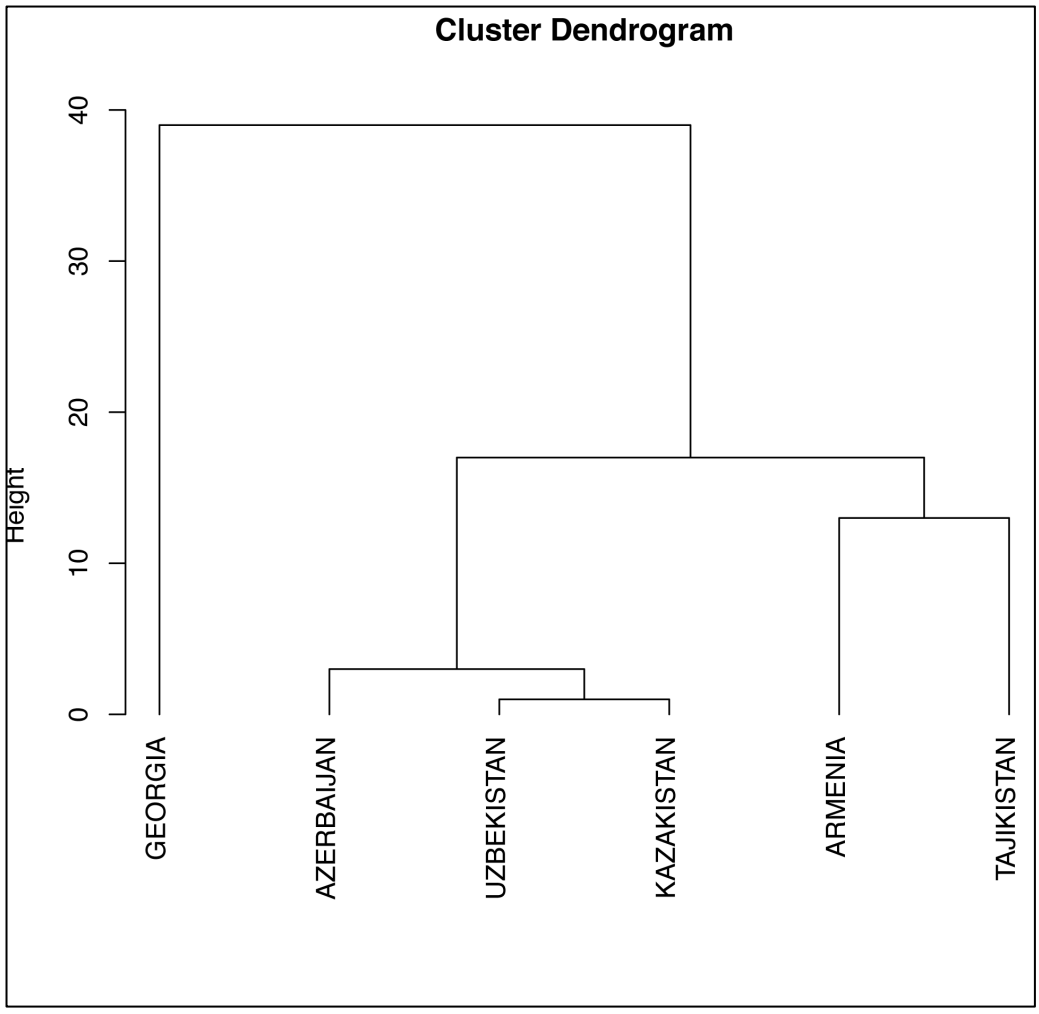

Figure 13. Dendrogram based on differences in food preferences between populations. The dendrogram shows three groups: one composed by Georgia, the second one by Uzbekistan Kazakhstan and Azerbaijan and the third one by Armenia and Tajikistan.

The figure shows three different groups: the first one composed only of Georgia, the second one composed of Uzbekistan, Kazakhstan and Azerbaijan and the third composed of Armenia and Tajikistan. It is clear that countries do not group according to geography, especially in the case of Armenia and Tajikistan. We then determined if the PROP responsiveness phenotypes could explain the observed clustering. Thus, we compared the two distance matrices (the PROP phenotype on one hand and the food liking on the other) and found a strong positive correlation between them (Mantel test: r=0.67, p-value=0.003). The results of the Mantel test between each pair of distance matrices are summarized in Table 7.

	Geography	GenomicFst	T2R38Fst	PROP Status
Genomic Fst	**0.79**			
T2R38 Fst	-0.04	-0.04		
PROP Status	-0.32	-0.37	-0.18	
Food liking	0.20	0.02	-0.30	**0.67**

Table 7. Mantel test results between distance matrices analyzed.

We also tested if the *T2R38* gene was associated with these groupings, and found no evidence of correlation (correlation=0.02, p-value=0.3) between the distance matrix of food liking and the matrix of genetic distance based on *T2R38*. In addition no correlation was found using the distance matrix based on the whole genome using ~356,000 SNPs.

4. DISCUSSION

a. PROP phenotype differences between populations

This study reports, for the first time, data about differences in taste responsiveness to PROP bitterness in populations of rural communities of the Caucasus and Central Asia located along the Silk Road.

Interestingly, differences in the distribution of PROP status between the examined populations were detected. These results do not agree with a simple geographical explanation for the pattern of PROP phenotypes across populations. In particular, the phenotype differences between the populations of Armenia and Georgia were totally unexpected, because these two countries are closely located and have a long standing tradition of cultural and political exchange dating back to the Middle Ages, when the two countries were allied against the Muslim empire (Walker, 1990).

Differences in age, gender and smoking can influence PROP phenotypes (Bartoshuk et al., 1994; Mennella et al., 2010; Mangold et al., 2008). However, these factors did not explain the variability across the populations studied here since our analyses adjusted for these factors. These data support recent findings suggesting that other genetic loci or non-genetic factors contribute to PROP

tasting (Calò et al., 2011; Cabras et al., 2012) and efforts to identify and fully characterize these factors should be an on going goal.

b. PROP phenotype as marker of food selection

The relationship between PROP perception and food liking was dissected using a "population-based approach", in which we exploit phenotypic differences between populations, comparing a distance matrix based on PROP taste responses and a matrix based on food preferences, detecting a strong correlation between the matrices of PROP status and food preferences. These results have two important implications. First, they show that differences in food liking among populations strongly correlate with PROP taster status but not with *T2R38* genotypes. This finding supports the view that polymorphisms in *T2R38* primarily define the ability to taste PROP, but also recognizes that this gene is pleiotropic and influences multiple phenotypic traits such as the perception of non-thiourea, bitter and non-bitter tastes, other oral sensations, food liking, and downstream effects such as dietary behaviour and weight status (Calò et al., 2011; Tepper, 2008; Tepper et al., 2008). Nevertheless, PROP status maybe one of several markers for chemosensory perceptions (Hayes et al., 2013), and multiple markers may be required to fully capture the depth and breath of human chemosensory experiences, and their influence on food selection.

Second, we did not observe any direct relationships between geography and the distribution of *T2R38* haplotypes or between geography and food liking in the populations we studied. Our findings differ from those of Pemberton et al. (Pemberton et al., 2008) who studied *T2R38* haplotypes in Asian Indians born in 15 geographic regions across India. They found that haplotype frequencies varied along a latitudinal cline with more tasters in the northern groups and more non tasters in the southern groups. Although Pemberton et al. did not study food liking, it is intriguing that pungent spices, like chilli pepper are more frequently consumed in southern India (Ferrucci et al., 2010) in the same areas where non tasters predominate. Given the critical role of geography and climate in shaping the genetic features of world populations (Cavalli-Sforza, Menozzi & Piazza, 1994), we can only speculate that the geographical and ecological barriers to genetic and cultural exchanges in the groups residing in India along a north-south gradient

were more formidable than those operating along the Silk Road which has been an east-west corridor for such exchanges for thousands of years.

However, asymmetrical gene flow and the availability of different crops could also be responsible for variability in genetic features across populations (Mitchell-Olds, Willis & Goldstein, 2007).

Therefore future studies involving a deeper analysis of other genes and environmental variables could further elucidate population differences in taste responses and food liking.

In conclusion, we used a population-based approach in which we exploited taste phenotypic differences among populations to reveal differences in food liking patterns across populations that could not be detected using standard methods. This approach, based on comparisons between distance matrices, can be applied to different population groups around the globe to obtain a comprehensive view of the role of PROP tasting in food preferences as well as to explore the role of novel taste-related traits in food choice.

CHAPTER IV

The impact of taste perception and food liking on health status

1. BACKGROUND AND AIM

a. The influence of taste perception on health and disease

As described in chapter I, differences in taste perception can be related to dietary behaviors that increase the risk to develop several disease such as obesity, diabetes, cardiovascular diseases, colorectal cancer, dental caries. Therefore, these differences in taste perception, explaining some of the differences in what we like/dislike to eat, could be used in applying of dietary recommendations that should facilitate the reduction of chronic diseases risk.

In addition, important clinical implications are associated to the presence of taste receptors in the gut and in the airway, with potential effects also for drug development and medical practice. For example, inhaled bitter tastants decrease airway obstruction in a mouse model of asthma (Deshpande et al., 2010), therefore using of synthetic and naturally compounds, which activate bitter taste receptors, could be an efficient therapy in the treatment of obstructive airway diseases such as asthma. In the same way, taste receptors in the gut, playing a key role in digestive behavior and metabolism, could be considered as good targets for the treatment of a number of pathological conditions related to diabetes, obesity, eating and gastrointestinal motility disorders (Depoortere, 2014).

Additionally, given that bitter taste evolved as a warning mechanism against the ingestion of toxic substances, bitter receptors located both in the gut and in the airways may represent a possible defense mechanism toward harmful substances and thus control the inflammatory response in the gut or respiratory infection in the airway, evoked by bacteria (Lee et al., 2012; Depoortere, 2014).

b. The effect of sweet taste perception and liking on dental caries

Dental caries is one of the most prevalent multifactorial diseases, directly influenced by diet and nutrition (Touger-Decker & Mobley, 2003; Moynihan & Petersen, 2004). Evidence from animal and human studies have revealed that sugar is the most important factor in caries development and that dietary intake of sugar is related with increased levels of caries prevalence (Sreebny, 1982; Krasse, 2001). In humans a large number of studies have been conducted in different populations (Wang et al., 1998; Beighton, Adamson & Rugg-Gunn, 1996;

Bruening et al., 1999; Masalin, Murtomaa & Sipilä, 1994), which show that the frequency and amount of intake of sugars are both associated to dental caries. Although many works addressed the association between sugar intake and dental caries, most of them have been conducted on children or on adolescents, a population known to have habitually a higher sugar intake. Similar results were obtained on elders in which frequent sugar consumption was identified among those variables contributing the most to the risk of caries (MacEntee, Clark & Glick, 1993). Despite the attention to this relationship very few studies have been conducted in adults with the exception of two studies showing that increased frequency of sugar consumption is associated with a marked increase in dental caries which halted on withdrawal of sugar from the diet (Gustafson et al., 1954) and that the substitution of sucrose in the diet with a non-cariogenic sweetener results in a robust reduction in dental caries (Scheinin, Mäkinen, & Ylitalo, 1976). Other studies have found that high sugar intake mirrors a greater preference for sweet substances and sweet preferences have been also associated with dental caries (Jamel et al., 1997; Steiner, Sgan-Cohen & Nahas, 1984). Given the influence of diet on caries, several studies have also investigated if the genes involved in taste perception, especially sweet taste, could influence its insurgence. A recent work has identified a significant association between GLUT2 and T1R2 genotypes, both individually and in combination, with caries risk in a young population (Kulkarni et al., 2013). However, to date no studies on adults considering a wide age range have been conducted. Therefore, in the present work we analyze the association of DMFT (Decayed-Missing-Filled Teeth), a measure of the prevalence of dental caries, with: 1) sweet food preferences; 2) sugar intake; 3) polymorphisms in T1R2 and T1R3 (sweet taste receptor genes) and GLUT2 gene (glucose transporter).

2. MATERIALS AND METHODS

a. Dental caries evaluation

The study population consisted of 647 healthy individuals aged 18-65 were came from six different villages of Friuli Venezia Giulia region. Detailed description of the population is reported in Chapter II.

For all the participants an accurate oral evaluation and an additional x-ray examination (panoramic radiography) were collected. Prevalence of dental caries was measured using the DMFT (Decayed-Missing-Filled teeth) index (Larmas, 2010). The DMF Index is applied to the permanent dentition and is expressed as the total number of teeth that are decayed, missing or filled in an individual. A DMFT score (ranges from 0 to 32) was calculated for each subject.

b. Sweet food liking measurement

Food preferences were evaluated using a 45-item food preference questionnaire.

Subjects were invited to rate their food preferences for different sweet foods or beverages using a 9-point scale ranging from “like extremely” (score 9) to “dislike extremely” (score 1). The option “never tried” was also included in the questionnaire. For this study sweet foods or beverages were selected from the questionnaire. In particular: marzipan, panettone, whipped cream, ice cream, milk chocolate, espresso with sugar. For our analyses in order to adjust for scale use and reproducibility differences, the liking scores were modelled taking the residuals from a random effect linear regression model where the individual represented the random effect, as described in Brockhoff & Skovgaard (Brockhoff & Skovgaard, 1994). For each individual a sweet preference was defined as the mean of the corrected liking for each sweet food present in the questionnaire. This score was used in the subsequent statistical analysis.

c. Sugar intake assessment

Simple sugar consumption was assessed on a subsample of 322 people using a dietary history collected by experienced clinical dietitian. The interview evaluates the average daily food intake of a subject, considering its habitual meal patterns and the usual amount and frequency of foods eaten from all food groups, covering

the period of the previous year. To help subjects to define more acutely their food portion sizes visual aids were provided, including a picture atlas of 3 different size portions for each major food category and common household items (tablespoons, teaspoons, cups and glasses of different volumes). Nutrient intake was then assessed trough a software (Win food, 2.7. Medimatica, San Benedetto del Tronto, Italy) based on Italian food composition tables.

d. Genotyping

Details on genotyping were reported in see Chapter II. In this work all the SNP in T1R2, T1R3 and GLUT2 are analyzed.

e. Statistical analyses

The associations between sweet food preferences and consumption with DMFT were tested by fitting a linear model where the DMFT was considered as the as the dependent variable while sweet food preferences or consumption as regressors. Sex and age were used as covariates in the analysis.

Association analysis for the SNPS in sweet genes (T1R2, T1R3, GLUT2) was conducted using linear mixed model regression analysis were DMFT was used as the dependent variable and the each SNP dosage as the independent variable. As random effect the genomic kinship matrix between all subjects estimated with the ibs function in GenABEL was used. Sex and age were also used as covariates. Association analysis was conducted using the GenABEL package for genotyped SNPs and MixABEL package for imputed SNPs (more details in Chapter II).

3. RESULTS

a. Participant characteristics

Main features of participants are shown in Table 8. The mean age of the study sample was 44.9±12.4 (range 18-65 years). 44% (n=285) of the participants were males and 56% (n=362) were females. The mean of DMFT in the overall sample is 15,8±7.3. No differences were detected between males and females.

	All (n=647)	Males (n=285)	Females (n=362)
Age	44.9±12.4	45.7±12.5	44.4±12.2
Teeth (n)	23.2±6.5	22.7±7.1	23.5±6.0
DMFT	15.8±7.3	15.7±7.2	16.0±7.3
Sweet Preference score	0.40±1.26	0.45±1.26	0.38±1.27
Simple Sugar Intake (g/die)	101.8±49.1	107.2±53.8	97.2±44.4

Table 8. Subject characteristics. Mean±sd are reported for all parameters.

b. Association between sweet food liking and DMFT

Using the whole sample we found a strong positive correlation between DMFT and sweet food preferences (r^2=0.26; p=0.0008), with individuals with higher preferences for sweet food that show higher DMFT values.

Using a subsample of 322 for which both preferences and consumption of sweet foods were available we found that, although there was a positive relationship between sweet preferences and simple sugar intake (r^2=0.03, p=0.004), no significant association was found between intake of simple sugars and DMFT while we confirmed the previously observed association with sweet preference (Table 9).

	DMFT Caries prevalence		
	R-squared	Beta estimate	p-value
Simple sugar intake	0.18	0.01	0.094
Sweet preference	**0.24**	**0.80**	**0.005**

Table 9. Regression analysis between DMFT and sweet intake and preferences. Significant results are shown in bold. Sex and age were used as covariates.

c. Association between variations in T1R2 and GLUT2 genes and DMFT

Genetic association analysis detected a significant association with rs3935570, a SNP in the T1R2 gene (p=0.0117). As shown in figure 14, individuals homozygous for the allele G showed higher DMFT compared to both heterozygous G/T and homozygous for the allele T. In addition, we found that rs1499821 in the GLUT2 gene was associated with DMFT (p=0.0273). Individuals homozygous for the allele G showed higher DMFT compared to both heterozygous G/A and homozygous A/A.

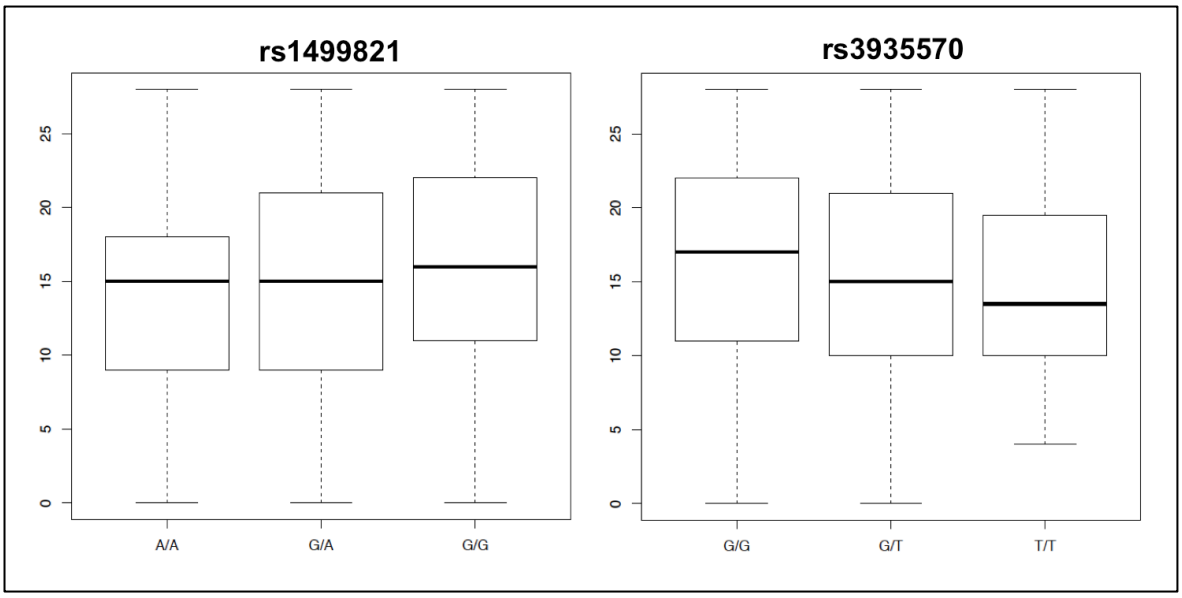

Figure 14. Boxplot comparing DMFT for the genotypes at rs3935570 and rs1499821 SNPs.

Table 3 shows results for the SNPs significantly associated with DMFT.

None of the SNPs in the T1R3 gene were associated with DMFT.

SNP	Chromosome	Position	Beta	MAF	p-value	Gene
rs3935570	1	19167371	-0.937	0.3168	0.0117	T1R2
rs1499821	3	170724729	-1.047	0.1676	0.0273	GLUT2

Table 10. Results for the association analysis of DMFT.
Beta=effect of the effect allele, MAF=minor allele frequency, p=p-value

4. DISCUSSION

a. Sweet liking influences dental caries risk

The present study is the first to examine at the same time the relationship between food preferences and intake on dental caries prevalence in a large adult population. The positive association we found between sweet food liking and dental caries is well supported in the literature. Our findings moreover define that, although sweet liking and intake are related between them, only liking for sweet foods is associated with the prevalence of dental caries.

This result supports past works showing that food preferences are predictive of health outcomes better than food intakes, increasing the ability to found association between diet and risk factors of diseases (Duffy et al., 2007; Duffy et

al., 2009). Duffy and collaborators for the first time have reported that liking of fat foods were better predictors of adiposity and blood pressure than fat intake. In fact, typical intake measures, such as frequency surveys or dietary records, can result difficult to complete and interpret. In addition, cognitive issues, such as memory and dietary restraint, and also under- or over-estimate intakes, can lead to inaccurate conclusions about diet-disease relationships.

Others studies also supported the efficacy of measuring liking for studies of taste. For example, Keskitalo and colleagues (Keskitalo et al., 2007) found that common genetic factor underlie liking for sweet foods, sweet food cravings and use–frequency of sweet foods. In this work authors also suggested that sweet taste preference measures may reveal the most important aspects of the preference and could be used to study the effect of taste preferences on the excess use of sugar.

In this study the importance of food liking as predictor of chronic disease risk factors is highlighted; in fact, as a proxy for reported intakes, the liking measure may represent habitual dietary behaviors.

b. Genetic variations in sweet receptors affect dental caries

Twin studies support the importance of genetic factors in caries, estimating that 40-60% of caries susceptibility is genetically determined (Boraas, Messer, & Till, 1988; Conry et al., 1993; Bretz et al., 2006). To date, only few genes have been associated to human caries, including genes involved in tooth formation, salivary and immunological factors (Slayton, Cooper & Marazita, 2005; Deeley et al., 2008; Peres et al., 2010; Azevedo et al., 2010). Given the influence of dietary habits on dental caries, the effect of taste pathway genes on caries risk was also investigated (Wendell et al., 2010). In the current study the relationship between sweet taste genes and dental caries prevalence was addressed. In agreement with a recent work (Kulkarni et al., 2013), our data revealed that genetic variations in T1R2 and GLUT2 genes are associated to DMFT, an index of the prevalence of dental caries. T1R2 is the receptor responsible of sensitivity to sweet taste, while GLUT2 is a glucose transporter involved in regulation of postprandial glucose levels. Polymorphisms in both these genes are responsible of individual differences in sweet perception and have been already linked to sugar

consumption (Eny et al., 2008; Eny et al., 2010). In the present work we identified additional polymorphisms in T1R2 and GLUT2 genes associated to dental caries risk/protection. We did not observe in our sample differences between identified SNPs and sugar intake or preferences. This lack of association could be related to the method employed to collect sugar intake and sweet preferences or to the sample size.

Overall, these results underline the importance of understanding the role of taste preferences in dental caries risk and the utility of a genetics approach that, contributing to the characterization of genes involved in taste preference and dental caries, may contribute to improve the identification of individuals at risk and to develop targeted preventive strategies before onset of caries.

It is possible that different and individual intervention strategies may prove more helpful for individual subcategories of taste receptor genotypes and thus contribute to early and targeted dental caries prevention. This approach may be effective for all other diseases strongly related to diet and nutrition, such as diabetes or obesity.

Conclusion and future perspectives

The purpose of these three years of PhD was to try to dissect the genetic bases of taste perception and their possible relationships with the health status.
Overall, the results reported in this thesis indicate that:
- GWAS studies have the potential to generate important discoveries in the field of human chemosensory perception. Our GWAS in Italian isolated population identified interesting candidate genes for salt and bitter perception, highlighting the role of genetics on taste perception (Chapter II).
- genetic differences in taste perception may affect food preferences in a complex manner. Our work on different populations located along the Silk Road showed that differences in food liking among populations strongly correlate with PROP taster status but not with *T2R38* genotypes or geography, suggesting that the ability to perceive PROP could be marker for overall perception of taste stimuli (Chapter III).
- differences in taste perception and food liking may impact on health status. In this thesis we showed that genetic differences in sweet taste genes and sweet food liking are both associated to the risk to develop dental caries (Chapter IV).
- food preferences may be better predictors of health outcomes than food consumption, thus may provide a good alternative to assess dietary intake. Therefore the measurement of food preferences may also have further potential in the evaluation of nutrition and intervention programs (Chapter IV).

Future studies are needed to further confirm the findings described in this thesis. In particular, it is necessary to collect larger number individuals for GWAS and replicate our candidate genes in other cohorts.
In addition, to identify causative functional variants (poorly captured by existing arrays used for GWAS) and their role in taste perception, analysis of 250.000 functional variants is underway.
Further analyses on the effect of taste genes and food preferences on obesity, diabetes and hypertension are also planned to further dissect the relationship between taste perception and health status.
Furthermore, the dissection of a field poorly investigated such as that of gene-

environment interactions influencing chemosensory perceptions and food preferences could also provide insight into the biological mechanisms of taste and food preferences and their impact on health outcomes.

In the future, new techniques of genetic analysis, larger data sets and establishing novel links between genes may help to better understand the genetics and lifestyle/environmental factors involved in taste perception, thus contributing to define novel molecular targets for diet-related disease treatment and prevention.

Moreover, in light of the recent evidences on the role of taste receptors in the gut and in the airways, further studies on taste detection may also provide commercial information that could lead to the creation of new products in the food and drug industry, such as functional food or products controlling drug absorption.

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