Genetic Determinants of Cilantro Preference

by

Lilli Katherine Mauer

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Department of Nutritional Sciences
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Lilli Katherine Mauer

Master of Science

Graduate Department of Nutritional Sciences
University of Toronto

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Abstract

Cilantro, the leaf of the *Coriandrum sativum* plant, has been documented as being one of the most polarizing and divisive foods known. It has been proposed that extreme disliking of this herb may be explained by genetic variation. The objectives of this thesis were to quantify the prevalence of cilantro disliking in various ethnocultural groups, to identify genetic polymorphisms that are associated with this trait using genome-wide association studies, and to analyze the associations of these polymorphisms within different ethnocultural groups. Prevalence of cilantro disliking was found to range from 3%, among Middle Eastern subjects, to 21% among East Asians. Two polymorphisms, one in the OR4N5 olfactory receptor gene and the other in the TAS2R1 taste receptor gene, were found to be associated with cilantro preference in the Caucasian subset of the study population. No statistically significant associations were observed within other ethnic groups.

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Chapter 1:

Introduction and Literature Review

1.1 Flavour

Food selection is determined by several factors including, but not limited to, culture, socioeconomic factors, and health concerns. In more affluent populations, availability and cost are less influential, and food selection is based more on an individual's acquired and innate flavour preferences (Rozin, 1990).

Flavour refers to a combination of the taste, aroma, texture, and mouth-feel of a food or beverage (Hollowood, Linforth, & Taylor, 2002). Individual differences in sensory perception therefore contribute to individual differences in food selection and dietary habits.

1.1.1 Taste Perception

Until recently, there were thought to exist five major taste modalities that humans have the ability to perceive: sweet, salty, bitter, sour, and umami (Drayna, 2005), and that taste receptors mediating each of these classes could be mapped to specific regions of the tongue (Chandrashekar, Hoon, Ryba, & Zuker, 2006). Recently, however, it has been suggested that taste perception is much more complex. Taste buds are found on regions of the tongue, palate, larynx, oropharynx, epiglottis, and esophagus. These taste buds are made up of clusters of 50-150 taste receptor cells (TRCs), which are epithelial in origin, and possess neuronal characteristics (Stone, Finger, Tam, & Tan, 1995). The convergence of these TRCs creates a taste pore, into which each cell projects microvilli, where taste receptor proteins are expressed, which then interact with tastant molecules (Bachmanov & Beauchamp, 2007; Chandrashekar et al., 2006). When a compound interacts with a

taste receptor protein, the TRC depolarizes, and an influx of extracellular calcium results from the opening of voltage-gated ion channels. This intracellular calcium causes a signal to be transduced and a message to be sent to the gustatory cortex of the brain (Yoshida, Yasumatsu, Shigemura, & Ninomiya, 2006). This system is not fully understood, though evidence exists for a mechanism known as the 'labeled-line model' (Chandrashekar et al., 2006). This model proposes that recognition of each taste modality – sweet, salty, sour, bitter, and umami – is mediated by a specific subset of cells and receptors, which are dispersed across regions of the tongue. Each TRC is thought to respond specifically to one taste modality, and to be innervated by its own specific nerve fiber. This is thought to be more probable than the 'across-fiber model' (Chandrashekar et al., 2006), for which two distinct hypothesis exist. The first suggests that each TRC responds to a specific taste modality, but one nerve fiber can transmit signals from multiple TRCs, thus these nerve fibers do not show specificity. The second variation of this model suggests that TRCs have the capacity to respond to multiple taste modalities, and consequently each nerve fiber can transmit information about multiple taste modalities (Chandrashekar et al., 2006).

Bitter taste receptors have also recently been detected on the smooth muscle cells of the bronchi in the lungs. These receptors do not appear to be involved in sending signals to the brain, however, very little is known about these receptors as of yet (Deshpande et al., 2010).

Genetic factors are known to explain certain variations in taste perception. The classic example of a single gene influencing taste is the TAS2R38 bitter taste

receptor. Three alleles in this gene contribute to a haplotype, which determines one's ability to detect the compounds phenylthiocarbamide (PTC) and propylthiouracil (PROP)(Bartoshuk, Duffy, & Miller, 1994). Compounds called glucosinolates, which are structurally similar to PTC, are found in cruciferous vegetables such as broccoli, turnip, collard greens and rutabaga (Hartl, 2011), thus it has been suggested that individuals who perceive these compounds as strongly bitter may find cruciferous vegetables to be bitter, and consume them less frequently. However, associations between variation in taste perception, food consumption, and health outcomes are unclear. Aside from certain well-researched examples, it has been hypothesized that many other individual variations in taste perception are likely partly genetically determined (Drayna, 2005). Preference for sweet taste is thought to have evolved as a means of survival for mammals. In nature, sweetness typically indicates a sources of energy, whereas bitterness may indicate a plant contains toxic glycosides or alkaloid compounds (Rozin, 1990). The sweet taste receptor subunit encoded by the TAS1R3 gene is known to influence sweet taste perception, in humans and mice, as well as sweet taste preference in mice. This receptor forms a heterodimer with TAS1R2, in both mice and humans, which is the functional sweet taste receptor. Allelic variation in the TAS1R3 gene is associated with sensitivity to, and perception of sweet tastes (Fushan, Simons, Slack, Manichaikul, & Drayna, 2009). Although, the influence that this has on sweet taste preference is unclear (Reed & Knaapila, 2010). The receptors responsible for salt taste perception are unknown. It has been hypothesized that one or more sodium channel genes are responsible (Chandrashekar et al., 2010; Stähler et al.,

2008), though results are inconclusive as of yet. Furthermore, it is thought that variation in salt taste preference may be more so a result of exposure, rather than genetically determined differences in perception (Reed & Knaapila, 2010). Two candidate genes, the polycystic kidney disease 2-like 1 gene (Pkd2l1) and the polycystic kidney disease 1-like 3 gene (Pkd1l3) have been identified as being involved in sour taste perception in mice. However, it is currently unclear whether these genes play the same role in sour taste perception in humans (Reed & Knaapila, 2010). It has been reported that humans with acquired sour ageusia, or a lack of response to sour taste stimuli, lack expression of these two genes in the taste buds, as well as several acid sensing ion channel (ASIC) genes, ASICs 1α, 1β, 2α, 2β, and 3 (Horio et al., 2011; Huque et al., 2009). Thus, variation in one or more of these genes may explain individual variation with respect to sour taste perception and preference. Umami refers to the taste elicited by L-glutamate, which is sometimes described in English as being savoury or meaty. The umami taste receptor is a heterodimer of the TAS1R1 and TAS1R3. Several polymorphisms have been identified, in both of these genes, which are associated with varying degrees of sensitivity to monosodium glutamate (MSG) (Shigemura, Shirosaki, Sanematsu, Yoshida, & Ninomiya, 2009). It has also been suggested that these variations may play a role in food intake and thus body weight and health. One study found obese women to have lower sensitivity, as well as higher preference for MSG in soups (Pepino, Finkbeiner, Beauchamp, & Mennella, 2010). Recently, is has been suggested that there may exist receptors for more than just the five recognized taste modalities (Eisenstein, 2010). The fatty acid transporter CD36, as well as several G-

proteins, and transient receptor potential channels (such as TRPM5) have been found to be involved in fat taste perception (Cartoni et al., 2010; Laugerette et al., 2005; Liu, Shah, Croasdell, & Gilbertson, 2011). Identifying variants associated with fat taste preference could have extremely significant health implications. Research in this area is still needed, however, as no human evidence has been documented that identifies a strong association between a gene and fat taste sensitivity.

Research looking to identify genetic determinants of food preferences is extremely complex, as a single tastant molecule can present multiple taste modalities, and furthermore, it is rare that an individual ingests a food consisting of only one tastant molecule. This is further compounded by the fact that perception, or sensitivity to a taste, and preference for that taste can be, but are not always strongly associated with one another.

1.1.2 Olfactory Perception

Olfaction is mediated by olfactory receptors (ORs), which are found on olfactory receptor neurons (ORNs) of the olfactory epithelium. It has been estimated that humans have nearly 1000 olfactory receptor genes and pseudogenes, which map to several chromosomes across the genome (Malnic, Godfrey, & Buck, 2004). Approximately 400-500 of these are known functional genes, and at least the same number were likely once functional, but are now nonfunctional pseudogenes. There are also 60 known genes which may be functional or nonfunctional, depending on the inherited variant (Reed & Knaapila, 2010). These are referred to as segregating

pseudogenes, and the functional variant is often rare. These variants tend to appear if a mutation is introduced which reverses the original conversion of gene to pseudogene – the process referred to as pseudogenization (Gerstein & Zheng, 2006). It is currently unknown whether these anomalies contribute significantly to individual variations in olfactory sensation (Gerstein & Zheng, 2006). Over the course of evolution, most mammals have retained many more functional OR genes than humans, as they rely more on olfaction as a means of survival than do humans. Apes have significantly higher numbers of functional OR genes than humans, yet still fewer than rodents such as rats and mice. At least 300 genes which were once functional in humans, but have become pseudogenes, are still functional in rodents and dogs (Gerstein & Zheng, 2006). This gene family has been conserved across vertebrate evolution, even as the size of the family and functioning of specific genes has changed (Malnic et al., 2004).

Each OR shows specificity for a wide range of odorant molecules (Buck, 2004). The dendrites of ORNs face the interior of the nasal cavity, and axons project through the cribiform plate of the ethmoid bone of the skull. Signals are transmitted to the olfactory bulb, and finally to the olfactory cortex of the brain (Buck, 2004). When an odorant interacts with an OR, a signal transduction pathway is initiated, which involves a G-protein cascade (Jones & Reed, 1989). The stimulatory G-protein, G_{olf}, is specific to olfactory signal transduction. G_{olf} is similar in amino acid sequence to the stimulatory G_s protein, and also stimulates adenylate cyclase, thus stimulating downstream phosphorylation cascades. However, its distribution patterns are more limited (Milligan & Kostenis, 2006). It has been hypothesized that this novel

stimulatory G protein was evolved specifically for olfactory signal transduction due to its slightly different kinetic, receptor and effector properties (Jones & Reed, 1989). The specific OR found on an ORN is thought to define the functional identity of that ORN, as well as specifically where the axon of that ORN projects into the olfactory cortex of the brain (Axel, 2005). An ORN only transcribes one OR gene, and thus only expresses one receptor throughout its life. This stability is thought to be necessary to ensure proper odour discrimination (Axel, 2005). This is crucial as the sense of smell is required for functions beyond food selection, including sexual selection, and identifying toxic substances in the environment (Reed & Knaapila, 2010). It has been hypothesized that as our environment has changed, and humans do not need to rely as heavily on olfaction as we once did, some olfactory capacity has been 'traded' for heightened visual acuity. This theory has been demonstrated in monkeys. Old world monkeys, who are more closely related to humans, possess genes that encode a group of retinal proteins that impart trichromatic colour vision, whereas new world monkeys do not (Gilad, Wiebe, Przeworski, Lancet, & P√§√§bo, 2004).

Extensive individual variation exists with respect to olfactory acuity and sensitivity. Some of this variation is the result of variation in genes encoding olfactory receptor proteins (W. S. Cain & Gent, 1991). It was first suggested, in 1918, that individual differences in olfactory perception may be heritable, when researchers documented different reactions to the odour of freesia flowers (Blakeslee, 1918; Glaser, 1918). The concept of a heritable anosmia was later documented in reports of families with a lack of olfactory sensation observed in

multiple generations (Mainland, 1945; Salmon, 1931). Anosmia refers to an inability to detect odour. This can be complete, where an individual has no sense of smell whatsoever (Leopold, Hornung, & Schwob, 1992), or specific to a particular compound. Complete anosmia is more often due to a congenital defect where the olfactory epithelium does not form properly, or is missing (Jafek, Gordon, Moran, & Eller, 1990). Several specific anosmias have been documented, and found to have varying genetic origins and modes of inheritance (Keller, Zhuang, Chi, Vosshall, & Matsunami, 2007; Menashe et al., 2007; Whissell-Buechy & Amoore, 1973; Wysocki & Beauchamp, 1984).

1.1.3 Mouth Feel

Viscosity or texture of food also influences flavour perception. The effect is complex and variable, however, and depends on the taste modality involved and the intensity (Hollowood et al., 2002). Perceived consistency and mouth feel appear to be inherent properties of a given food or food group, and consumer reporting of these properties is somewhat less variable than reported opinions of taste or odour (de Wijk, Engelen, & Prinz, 2003). One study examined the effect of adding varying concentrations of hydroxy propyl methylcellulose (HPMC), a hydrocolloid that increases viscosity, to a solution containing sugar and strawberry flavour volatiles (Hollowood et al., 2002). This study employed a trained sensory panel to rate the various solutions both in terms of sweetness and intensity of strawberry flavour, using magnitude estimation, comparing each to a controlled solution. Researchers also measured release of volatiles in the breath following consumption of each

solution. The results showed that increasing the thickness of a solution decreased perceived flavour intensity and sweetness intensity. It was hypothesized that altering the viscosity of a solution may alter the mobility of water and the conformation of sweeteners in the solution (Hollowood et al., 2002), thus altering sweetness perception. With respect to flavour, it was hypothesized that perception of texture in the mouth may somehow influence perception of tastant molecules in solution (Hollowood et al., 2002), which may be due to altering a tastant's ability to interact with taste receptors. Large differences were also observed between assessors in these experiments, confirming that individual differences exist with respect to flavour perception, which is likely due to a combination of physiological factors and, possibly, genetic differences.

1.1.4 Chemesthesis

A fourth factor which plays a role in the eating experience is chemesthesis, which can be describes as a chemically stimulated feeling of pungency, burning, stinging, or irritation (William S. Cain et al., 2006; Green, Alvarez-Reeves, George, & Akirav, 2005). This distinct sense is mediated through different pathways than either gustation or olfaction. In the nasal and oral cavities, chemesthesis results from stimulation of fibers of the trigeminal nerve and dorsal root ganglia, either by chemical or mechanical stimuli, or extreme temperatures (Silver, Clapp, Stone, & Kinnamon, 2006). Compounds that elicit documented chemesthetic effects include capsaicin and menthol. It has been documented that individual variation exists with respect to the ability to perceive taste via thermal stimulation (Green et al., 2005). A

central neural process involving the three gustatory nerves (glossopharyngeal, greater superficial petrosal, and chorda tympani) is thought to drive these differences. Individuals with a higher level of neural excitability in these three nerves may have a heightened responsiveness to certain odours and tastes (Green et al., 2005). Few studies have examined the association between PTC/PROP taste perception and ability to detect the burn of capsaicin and other chemicals (Karrer & Bartoshuk, 1991; McBurney, Balaban, Popp, & Rosenkranz, 2001; Prescott et al., 2004; J. Prescott & Swain-Campbell, 2000; Tepper & Nurse, 1997). Results have been mixed, and seem to be dependent on conditions, though some associations have been found, suggesting an effect of common higher neural regulation of these senses.

1.2 Cilantro

1.2.1 History and Use

Cilantro is thought to be the oldest herb used by mankind. The coriander plant appears to have existed since biblical times. Exodus 16:31 states "And the house of Israel began to call its name Manna: and it was round like coriander seed...". Coriander seeds were also reportedly recovered from the tomb of Tutankhamen (Zohary & Hopf, 2001). It is unclear when or where the common *Coriandrum sativum* species originated, though it was likely first cultivated for use in southeastern Europe and southern Russia (Diederichsen, 1996). Historically, India has been one of the largest producers and consumers of cilantro, worldwide (Diederichsen, 1996). Cilantro is widely consumed throughout the world, and is a common ingredient in various cooking styles including Thai, Indian, Middle Eastern, Mexican and South American, to name a few (Knowlton, 2009). Cilantro is less frequently used in other regions, though it is grown in most agriculturally viable areas throughout the world. Official production and consumption statistics are scarce as they do not often include cilantro (Diederichsen, 1996).

Fresh cilantro has numerous purported health benefits. It is rich in flavonoid antioxidants, which have been shown to have antibacterial, antiviral, and chemoprotective activity (Sharma, Kansal, & Sharma, 2010). Cilantro has also been shown *in vivo* to chelate and detoxify heavy metals (Kubo, Fujita, Kubo, Nihei, & Ogura, 2004). One compounds thought to be responsible for much of this bioactivity is coriandrin, which is a furoisocoumarin found uniquely in the oil of cilantro leaves.

It is currently unknown however, whether coriandrin, or any of the other bioactive components contribute significantly to the flavour of cilantro.

1.2.2 Flavour

Cilantro is one of the most polarizing and divisive foods known. It has been well documented that descriptions of the flavour of cilantro vary drastically between individuals who like or dislike it (Herz, 2004; McGee, 2010; Rubenstein, 2009). Individuals who like cilantro may describe it as fresh, fragrant, or citrusy, whereas those who dislike cilantro report it tastes like soap, mold, dirt, or bugs, among other descriptors (McGee, 2010; Rubenstein, 2009). Numerous websites and online communities have been created to voice pro- or anti-cilantro opinions. The online community www.IHateCilantro.com has over 3,300 members, who have come together online to discuss “the fight against cilantro”. This unique, highly polarizing nature is not seen with any other common foods, which is why cilantro is of great interest to sensory researchers.

It has been hypothesized that the reason for strong visceral reactions to the flavour of cilantro has to do with odour rather than taste (Herz, 2004), though this has yet to be thoroughly examined. Recent research has aimed to isolate and characterize the ‘offensive’ odorant in cilantro leaves, using techniques such as gas chromatography-olfactometry and mass spectrometry (Eyres, Dufour, Hallifax, Sotheeswaran, & Marriott, 2005). This study established the most abundant compounds present, as well as those that contribute the most odour activity, which is a property of interest when examining flavour perception and preference. While

researchers isolated 81 compounds from the essential oil of fresh cilantro leaves, far fewer were found to present strong or distinctive odours. The compounds that were found to contribute the most to the odour profile of the fresh cilantro leaves were E-2-decenal, Z-2-decenal, E-2-dodecenal, E-2-dodecen-1-ol, 1-dodecanol, b-ionone, and eugenol. Some compounds that were found to be very abundant by mass, such as E-2-Decen-1-ol, did not contribute as significantly to the total odour activity; thus, abundance was not directly correlated with contribution to the odour. Z-2-decenal, the compound which appeared to contribute most to the odour in this analyses, was described by the two trained panelists as aldehylic, pungent, spicy, and coriander-like. E-2-decenal, a stereoisomer of Z-2-decenal, was the third most abundant compound by mass, accounting for 9.1% of the total composition. This compound, when isolated, was described as having an aldehylic, fatty, waxy, pungent odour, which has also been documented elsewhere (Burdock, 2001). Authors hypothesized that this compound is likely the one responsible for the distinctive coriander-like aroma (Eyres et al., 2005). One of the most common adjectives used by cilantro dislikers is soapy, so it is plausible that E-2-decenal may play a role in the flavour, being that alone this compound possesses a waxy odour. E-2-decenal is also found in the emissions of many insects, including several species of stinkbug (El-Sayed, 2011). This is of interest, as a common descriptor used by cilantro dislikers is stinkbug-like ("Ihatecilantro.Com," 2005).

1.2.3 Genetic Basis for Cilantro Preference

It has been well documented that those who like or dislike cilantro seem to perceive the flavour of the herb differently, but it is not well understood why this is

so. Twin studies have suggested that there is strong heritability in cilantro preference (Herz, 2004). When asked to rate the 'pleasantness' of cilantro, over 80% of monozygotic twins gave the same immediate rating as their sibling, whereas only 42% of dizygotic twins gave similar ratings (Herz, 2004). Given that monozygotic twins share 100% of their DNA, this provides evidence to suggest genetic factors are involved. As of yet, no genetic factors have been identified.

As discussed, some variation in sensory perception is known to be due to variation in genes encoding taste and olfactory receptors. Variation in the TAS2R38 bitter taste receptor dictate ones ability to taste PTC. Because of the polarizing nature of cilantro, it has been hypothesized that this same gene may influence preference for or against the flavour of cilantro. One small study was conducted that examined this hypothesis (Noxon & Meyer, 2004), however, it remains unpublished. In this study, approximately 200 individuals were asked whether or not they could detect PTC after tasting PTC strips. They were then asked their preference for or against cilantro after tasting the fresh leaves (Noxon & Meyer, 2004). The results did not show a correlation between these two traits, suggesting that different loci are responsible.

1.2.4 Assessment of Flavour Preferences

Methods used in sensory research depend greatly on the odorant, tastant, food, or food compound of interest. Taste perception is often measured using detection or recognition threshold testing. A detection threshold refers to the lowest concentration at which one is able to detect a tastant, or discriminate between a tastant solution and distilled water (Galindo-Cuspinera et al., 2009). A recognition threshold refers to the lowest concentration at which one is able to correctly characterize a tastant, such as salt (Galindo-Cuspinera et al., 2009). Suprathreshold assessment is the measurement of an individual's perceived bitter taste intensity of a bitter compound above threshold detection levels. Commonly used scales include the Visual Analogue Scale (VAS), and the generalized Linear Magnitude Scale (gLMS), which asks an individual to choose from seven adjectives, ranging from 'nothing' to 'strongest imaginable sensation of any kind' (Bartoshuk et al., 2004).

Sensitivity to a food or food compound is not always associated with preference for that food. Various food preference assessment methods are used to evaluate an individuals liking or disliking of a food. This can be done using a questionnaire, or in a controlled laboratory taste test. Food preference questionnaires often employ a numbered scale, the most widely used being the 9-point scale (H.T. Lawless & Heymann, 2010), which ranges from 'dislike extremely' (1) to 'like extremely' (9), with a neutral midpoint of 'neither like nor dislike (5).

Various methods also exist to assess olfactory acuity, odour identification and discrimination, as well as odour preferences . "Sniffin Sticks" are a recently developed, highly reliable test method used in clinical evaluation of individuals with

olfactory disorders (Kobal et al., 2000). A pen-like odour-dispensing device is used to test olfactory detection threshold, discrimination, and identification. Threshold can be measured using either *n*-butanol or phenylethyl alcohol (PEA). Discrimination and identification are assessed based on 16 different odorants. The sum of these scores give an individual's TDI score (Kobal et al., 2000).

Odour preferences are also assessed using methods similar to those used to assess taste preferences. The 9-point scale is often used (H.T. Lawless & Heymann, 2010). Subjects are asked to rate the pleasantness of an odour from 'extremely unpleasant' (1) to 'extremely pleasant' (9). In both taste and olfactory preference research, this scale is often truncated to either a 7-point or 5-point scale for the purpose of simplicity. This is dangerous however, due to the fact that individuals are often hesitant to select extreme values at either end of a scale, which is referred to as end-use avoidance (H.T. Lawless & Heymann, 2010). Potential limitations of the 9-point scale are, due to it's categorical nature, it does not exhibit ratio properties (Hein, Jaeger, Tom Carr, & Delahunty, 2008). For example, an individual who gives an odorant a rating of 8 ('very pleasant') does not find the odour exactly twice as pleasant as someone who rates 4 ('slightly unpleasant'). Furthermore, interpretation of the adjectives (slightly, moderately, very, extremely) may differ between individuals. Nonetheless, this scale is practical, easy to use, and has proven to be reliable and effective at discriminating between individuals (Harry T. Lawless & Malone, 1986).

1.3 Rationale, Hypotheses, and Objectives

No study has documented whether cilantro preference or consumption differs significantly between different ethnic groups. Therefore, the prevalence of cilantro disliking in any population remains unknown. Furthermore, though it has been suggested that preference for or against the flavour of cilantro may be genetically determined, no candidate genes have yet been identified.

Objectives

1. To determine the prevalence of cilantro disliking among different ethnocultural groups.

2. To identify genetic factors that predict cilantro preference using genome wide scans.

3. To determine whether candidate genetic markers associated with cilantro preference differ between ethnocultural groups.

Hypotheses

1. Prevalence of cilantro disliking will differ across ethnocultural groups.

2. Genetic variants, likely in one or more genes involved in taste or olfactory perception, are responsible for determining an individual's probability of liking or disliking the flavour of cilantro.

3. These genetic factors will show varying effects across ethnocultural groups, as genetic and cultural factors are both very influential in determining individual food preferences.

Significance and Implications

The proposed research will further our knowledge of the genetic factors that influence flavour perception, food preferences, and human eating behaviours. Identifying genetic factors responsible for cilantro disliking is of particular interest as it is such a divisive, polarizing food. In addition, genes that predispose individuals to dislike cilantro may be responsible for other taste aversions. The extent to which one can taste phenylthiocarbamide (PTC) is a genetic trait that we know can explain certain dietary habits. For example, because cruciferous vegetables contain a natural compound very similar to PTC, PTC tasters and supertasters are more likely to dislike these vegetables (Dinehart, Hayes, Bartoshuk, Lanier, & Duffy, 2006). This knowledge is important for predicting long-term health and risk of chronic disease. If the proposed research uncovers strong genetic associations between genotype and cilantro preference, cilantro may have future use as a PTC-like marker for other food choices, and perhaps other diet-related health outcomes.

Chapter 2

Prevalence of Cilantro Disliking Across Ethnocultural Groups

2.1 Abstract

Cilantro, the leaf of the *Coriandrum sativum* plant, is an herb that is widely consumed globally, and has purported health benefits ranging from antibacterial to anticancer activities. Some individuals report an extreme disliking for cilantro, and this may explain the different cilantro consumption habits between populations. However, the prevalence of cilantro disliking has not previously been reported in any population. The objective of this study was to determine the prevalence of cilantro disliking among different ethnocultural groups from a population of young adults living in Canada. Subjects (n=1,639) between the ages of 20-29 years were participants of the Toronto Nutrigenomics and Health Study. Individuals rated their preference for cilantro on a 9-point scale from 'dislike extremely' to 'like extremely'. Subjects also had the option to select 'have not tried' or 'would not try'. Subjects who selected 1-4 were classified as disliking cilantro. The prevalence of disliking ranged from 3-21%. The proportion of subjects classified as disliking cilantro was 21% for East Asians, 17% for Caucasians, 14% for those of African Descent, 7% for South Asians, 4% for Hispanics, and 3% for Middle Eastern subjects. These findings show that the prevalence of cilantro disliking differs widely between various ethnocultural groups.

2.2 Introduction

Numerous factors influence food selection such as socio-cultural and economic factors. Familiarity with, and exposure to certain foods can also influence our preferences (Birch, 1999). Flavour perception, which is partly genetically determined, is one of the most important factors influencing our innate likes and dislikes (Birch, 1999; Garcia-Bailo, Toguri, Eny, & El-Sohemy, 2009).

It is currently unknown whether strong reactions to the flavour of cilantro are a result of differential perception the odour or the taste. Whereas some research has investigated odorants (Eyres et al., 2005), taste mechanisms have yet to be examined, although anecdotal evidence indicates that those who find cilantro offensive dislike the taste as well as the smell.

Anecdotally, the polarizing nature of cilantro has been well documented (Herz, 2004; Rubenstein, 2009), however, the prevalence of cilantro disliking remains unknown. While cilantro is consumed throughout the world, consumption differs between regions. Cilantro is more frequently used in traditional cuisine in most parts of Asia, South and Central America, Mexico, some parts of Africa, and the Middle East. It is and is less commonly used, although still produced and consumed in North America, northern Europe and Australia (Diederichsen, 1996).

This observational study aimed to determine the prevalence of cilantro disliking in different ethnocultural groups from a population of young adults.

2.3 Methods

2.3.1 Subjects

Participants (n = 1,639; 1,117 women and 522 men) were enrolled in the Toronto Nutrigenomics and Health Study, which is a cross-sectional study investigating gene-diet interactions and biomarkers of chronic disease, as well as genetic determinants of eating behaviors. Subjects were between 20 and 29 years of age at the time of screening, and were excluded if they were pregnant or breastfeeding, due to metabolic and dietary changes that take place during this period. Subjects who could not communicate in English, or who did not provide a 12-hour fasting venous blood sample were also excluded. Smokers (n = 105) were excluded from the present analysis because of the known effects of smoking on taste and odour perception (Sato, Endo, & Tomita, 2002). Subjects with any missing data were also excluded (n = 10). At the time of screening, subjects identified the ethnocultural group(s) they identify with. Subjects who listed more than one ethnicity (n=143) or any group with fewer than 20 subjects were excluded from the current analyses, and the remaining individuals were classified into one of six groups (Caucasian, n = 581; East Asian, n = 540; South Asian, n = 165; Middle Eastern, n = 36; African descent, n = 32; and Hispanic, n = 27). After exclusions, the final sample population consisted of 1,381 subjects (962 women and 419 men). All subjects provided written informed consent, and the University of Toronto Research Ethics Board approved the study protocol.

2.3.2 Cilantro Preference Data Collection

Subjects completed a 63-item food preference checklist, which included a range of common foods and beverages, as well as food garnishes and condiments. Participants gave each item a rating from 1 (dislike extremely) to 9 (like extremely). Alternatively, subjects had the option of selecting 'never tried' or 'would not try'.

2.3.3 Statistical Analysis

All statistical analyses were conducted using Statistical Analysis Systems software (SAS version 9.2; SAS Institute, Cary, NC). The frequency procedure was used to compare preference responses between ethnocultural groups, and χ^2 tests were used to examine differences between preference distributions. *Dislikers* were defined as those reporting 1, 2, 3, or 4 (dislike extremely, dislike very much, dislike moderately, dislike slightly) on the 9-point scale. Those selecting 5 (neither like nor dislike) were classified as *neutral*, and those selecting 6, 7, 8, or 9 (like slightly, like moderately, like very much, like extremely) were classified as *likers*. The mean and median ratings fell to the right of the arithmetic center of the scale (6.08 and 6, respectively), suggesting a slightly skewed distribution, which was confirmed using a Shapiro-Wilk test for normality. Those selecting 'never tried' were included in analyses, in order to examine the ethnocultural breakdown of this group. Those selecting 'would not try' were also included in analyses, since some of these individuals may dislike the odour so strongly that they would never consume cilantro. For comparison, leaf lettuce preference distributions were examined using the same

methods. Leaf lettuce is a food commonly used as a garnish, but is not known to elicit the same polarizing responses as cilantro.

2.4 Results

Table 1 displays the characteristics of the 1,381 subjects (419 male and 962 female) for which complete data was collected on all variables of interest. 43% of females were Caucasian, which was significantly higher than the 40% of males who were Caucasian ($p<0.0001$). 41% of females were East Asian, which was significantly higher than the 35% of males who were East Asian ($p<0.0001$). Among South Asian subjects, the reverse gender representation was observed with 15% of males being South Asian compared to 11% of females who were South Asian ($p=0.002$). No other ethnocultural groups had significantly different proportions of men and women.

Table 2-1 Subject Characteristics[a]

Characteristic	Males (n=419)	Females (n=962)
Age, years	22.9 ± 2.5	22.6 ± 2.4
Ethnicity		
Caucasian	210 (40)	452 (43)
East Asian	161 (35)	404 (41)
South Asian	69 (15)	105 (11)
Middle Eastern	19 (4)	21 (2)
Hispanic	13 (3)	16 (2)
African Descent	13 (3)	21 (2)

[a] Values are mean ± standard deviation (SD) for continuous variables, and number (%) for categorical variables.

Distribution of cilantro preference ratings in the population is shown in table 2. The proportions of dislikers were not significantly different between men and women ($p=0.15$), with 14% of females and 10% of males being dislikers. No significant differences in the proportions of dislikers were observed between men and women in any ethnocultural group. However, the overall response distributions differed significantly between men and women when examining either the Caucasian or East Asian groups individually ($p=0.02$, $p=0.01$). This was not the case with any other group, or in the population as a whole. The response distributions differed significantly between the ethnocultural groups ($p<0.0001$) with the Middle Eastern, Hispanic, and South Asian groups having the lowest proportions of dislikers (3%, 4%, and 7%, respectively). The Hispanic and South Asian groups both also had significantly higher proportions of likers than any other groups (92% and 75%, respectively; $p < 0.001$). A high proportion of East Asians, Caucasians and individuals of African descent had never tried cilantro (27%, 16% and 31%, respectively); these groups also had the highest prevalence of dislikers. The proportion of individuals who would not try cilantro was highest among East Asians at 1.1%.

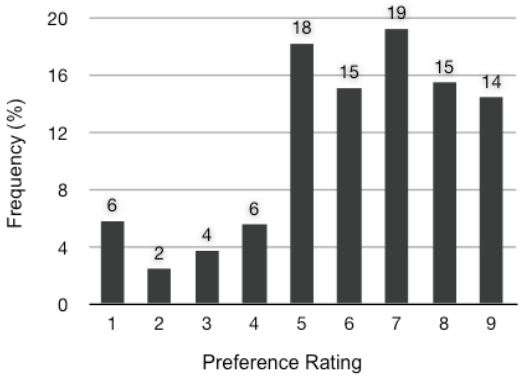
Table 2-2 Cilantro preference distributions between ethnocultural groups

	Preference category[a]				
	Have tried[b]			Have not tried[c]	
	Like	Neutral	Dislike	Never tried	Would not try
Caucasian (n=662)	311 (64)	88 (18)	85 (17)	96 (16)	1 (0.2)
East Asian (n=565)	207 (53)	102 (26)	81 (21)	144 (27)	6 (1.1)
South Asian (n=174)	119 (75)	27 (17)	11 (7)	8 (5)	0
Middle Eastern (n=40)	8 (69)	20 (28)	1 (3)	7 (19)	0
Hispanic (n=29)	24 (92)	1 (4)	1 (4)	1 (4)	0
African Descent (n=34)	13 (59)	6 (27)	3 (14)	10 (31)	0

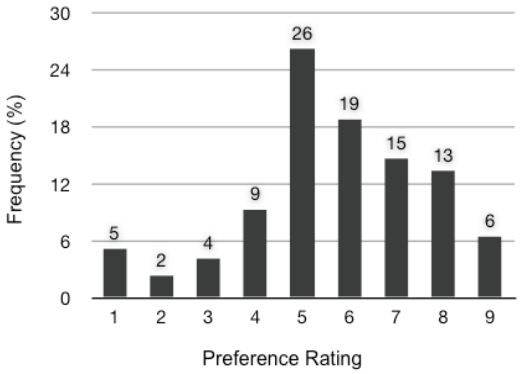
[a] Subjects selecting 1-4 are classified as *dislikers*, 5 are *neutral*, 6-9 are *likers*;
[b] Values are n(% of subjects who *have* tried cilantro)
[c] Values are n(% of ethnocultural group)

Figure 2-1 shows the distribution of cilantro preference ratings on the 9-point scale for the 3 major ethnocultural groups: Caucasians, East Asians, and South Asians. These histograms show the specific breakdown of *liker*, *neutral*, and *disliker* categories.

A.



B.



C.

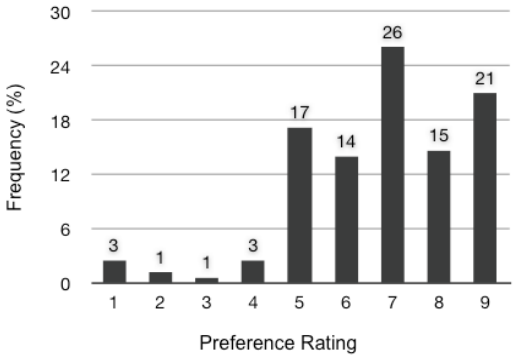


Figure 2-1 Cilantro preference distribution histograms for Caucasians (A), East Asians (B), and South Asians (C). Scale ranges from *dislike extremely* (1) to *like extremely* (9).

Table 2-3 shows the distribution of leaf lettuce preferences amongst the ethnocultural groups. This demonstrates a typical preference distribution for a food that is considered non-polarizing. The most frequently selected preference response for this food was 7 (like moderately) within each ethnocultural group. The prevalence of dislikers ranged from 0-6%. A significantly lower proportion of individuals within each ethnocultural group reported disliking leaf lettuce as compared to cilantro (Caucasian: $p<0.0001$, East Asian: $p<0.0001$, South Asian: $p=0.02$).

Table 2-3 Leaf lettuce preference distributions between ethnocultural groups

	Preference category[a]				
	Have tried[b]			Have not tried[c]	
	Like	Neutral	Dislike	Never tried	Would not try
Caucasian (n=581)	518 (89)	50 (9)	11 (2)	2 (0.3)	0
East Asian (n=540)	441 (82)	78 (15)	17 (3)	4 (0.7)	0
South Asian (n=165)	133 (84)	22 (14)	3 (2)	7 (4.2)	0
Middle Eastern (n=36)	36 (100)	0	0	0	0
Hispanic (n=27)	26 (96)	0	1 (4)	0	0
African Descent (n=32)	23 (74)	6 (19)	2 (6)	1 (3)	0

[a] Subjects selecting 1-4 are classified as *dislikers*, 5 are *neutral*, 6-9 are *likers*;

[b] Values are n(% of subjects who *have* tried leaf lettuce)

[c] Values are n(% of ethnocultural group)

2.5 Discussion

Despite the well-recognized extreme differences in cilantro preference between individuals (Herz, 2004), no study has previously reported the prevalence of this trait in any population. In the present study, we examined the prevalence of cilantro disliking in different ethnocultural groups from a population of young adults living in Canada. We observed a difference in the distribution of preferences between the different ethnocultural groups as well as between men and women among certain ethnocultural groups, which may be attributed to both biological and social factors.

The Middle Eastern, Hispanic and South Asian groups had the lowest proportions of cilantro dislikers. This may be due to frequency of exposure, as cilantro is most popular in these styles of cuisine (Wong & Kitts, 2006), and culture does modify food-related behaviors (Axelson, 1986). The lower prevalence of cilantro disliking among these groups could also be due to genetic differences influencing cilantro flavour perception. East Asians and Caucasians had the highest prevalence of cilantro dislikers. One limitation of our study was that the East Asian group included individuals of Thai, Korean, Japanese, Vietnamese and Chinese descent. Cilantro may be more widely used in certain East Asian cuisines, such as Thai and Vietnamese (Cadwallader Keith, Benitez, Pojjanapimol, Suriyaphan, & Singh, 2005), and less so in others, which may have influenced our estimated proportions of East Asians who dislike or have never tried cilantro (21% and 27%, respectively). Furthermore, the Caucasian group also consisted of individuals from a wide variety of European countries. Dietary patterns vary greatly between the

different regions of Europe and it was not possible to distinguish whether regional differences may have influenced cilantro preference responses in our large, heterogeneous Caucasian group. Nonetheless, differences were observed between ethnicities. It has been suggested that genetic factors may be responsible for differential perception of the flavour of cilantro (Herz, 2004). Genetic heterogeneity between ethnocultural groups may thus contribute to the different preference distributions.

Table 3 shows the preference distribution of leaf lettuce, an example of a common food that is considered to be non-polarizing. Among each ethnocultural group, the response distribution curves were normal, with peaks at 7 (like moderately). Similar findings would be the expected when examining most common foods. While leaf lettuce likers and dislikers seem to exist, reactions are not extreme. This emphasizes the unusual, divisive nature of cilantro.

Because qualitative descriptions of the flavour of cilantro differ considerably between those who like and dislike it, differences in perception of the flavour are likely driving the observed differences in preference. Whether this is due to differential perception of an odorant or tastant molecule is currently unknown, however, it has been suggested, by some, that odour is likely responsible (Herz, 2004; Rubenstein, 2009). It may be that individuals who dislike cilantro are anosmic to one or more of the pleasant smelling compounds found in cilantro. Alternatively, those who like cilantro may be anosmic to an unpleasant smelling compound - perhaps an aldehyde that, alone, smells of soap (Rubenstein, 2009). E-(2)-Decenal has been proposed as a candidate compound, as it is emitted by stink bugs and

other insects in defensive secretions (Borges & Aldrich, 1992; Potter, 1996). It has been inferred that this may be one of the compounds in cilantro that individuals find unpleasant. Because of the complex chemical composition of the oil of cilantro leaves, there are many potential candidates. Because there exist over 600 olfactory receptor genes and pseudogenes (Malnic et al., 2004), there are many potential candidates that could explain interindividual differences in cilantro preference. As it has not been elucidated whether it is taste, olfaction, or a combination of both that is responsible for strong reactions to cilantro, the pool of potential candidates is immense.

In summary, we report that cilantro disliking varies from 3% to 21% depending on the ethnocultural group. The contribution of individual genetic differences to this trait remains to be determined.

Chapter 3

Identification of Genetic Variants Associated with Cilantro Preference

3.1 Abstract

Cilantro consumption differs between populations and descriptions of its taste and odour differ greatly between those who either like or dislike the herb. Although genetic factors have been proposed to explain the difference in cilantro preference, no genes have yet been identified. The objective of this work was to identify genetic polymorphisms that influence cilantro preference. Caucasian female subjects (n=394) between the ages of 20-29 years rated their liking/ disliking of cilantro on a 9-point scale. Subjects reporting 'have not tried' or 'would not try' (n=76) were excluded from analyses. Preference ratings of 1-4 were classified as 'disliking', 5 was deemed 'neutral', and 6-9 was deemed 'liking'. DNA was isolated from whole blood, and genome-wide scans were performed using an Affymetrix 6.0 chip. A total of 16 SNPs reached GWAS significance ($p<5.5x10^{-6}$). Each SNP was examined for biological plausibility, and two relevant candidates were identified. The first SNP was identified on chromosome 14, in a region close to both the OR4N5 and OR11G2 olfactory receptor genes. Frequency of disliking was highest among individuals homozygous for the minor allele, as compared with heterozygotes or those homozygous for the major allele (56%, 21%, and 13%, respectively). A second SNP was identified on chromosome 5 near the taste TAS2R1 bitter taste receptor gene. Frequency of disliking was highest among individuals homozygous for the minor allele, followed by heterozygotes, and those homozygous for the major allele (33%, 19%, and 9%, respectively). When combined genotypes were analyzed, 75% of individuals homozygous for the minor allele of both SNPs reported disliking, whereas 0% of subjects homozygous for the major allele of both SNPs reported disliking.

3.2 Introduction

Development of specific food preferences is complex, and numerous factors contribute. Culture is an important factor determining an individual's dietary preferences, it is clear that within-culture variation in exists. It was demonstrated in the previous chapter that the prevalence of cilantro disliking varied significantly across ethnocultural groups. This suggests that other factors, such as genetics, play a role as well. Genetic factors are known to influence perception of certain odours and tastes (Reed & Knaapila, 2010).

Twin studies have suggested strong heritability in cilantro preference, however, no genetic factors have yet been identified. *Wysocki et al*. asked numerous sets of monozygotic and dizygotic twins to rate the 'pleasantness' of cilantro on a scale ranging from -11 to +11. What they found was that approximately 80% of monozygotic twins gave similar ratings to their sibling, as compared with approximately 40% of dizygotic twins (Herz, 2004; Rubenstein, 2009).

Chapter 2 reported the prevalence of cilantro disliking across six ethno-cultural groups. The present study aimed to identify genetic variants that influence cilantro preference, and predispose individuals to either like or dislike the flavour of cilantro.

3.3 Methods

3.3.1 Subjects

Subjects came from the Toronto Nutrigenomics and Health Study (TNH) population. Refer to Section 2.3.1. The genome-wide association scan (GWAS) was conducted on a subset of these subjects, which consisted of 543 Caucasian subjects. In the previous study, cilantro preference distributions were stratified by sex within each ethnocultural group, and the distributions differed significantly between males and females in the largest groups (Caucasians and East Asians). Among female subjects, the distribution appeared to more accurately reflect the polarizing nature of cilantro that is often documented. Among male subjects, however, a significantly higher proportion reported neutral preferences (ratings of 5 on the 9-point scale). For this reason, GWAS analyses were conducted using female subjects only.

3.3.2 Cilantro Preference Data Collection

Refer to Section 2.3.2.

3.3.3 Genome-Wide Association Study – Quality Control

Quality control testing was conducted at the individual level, and with respect to quality of SNPs before performing GWAS analyses. QC was performed using Golden Helix analysis software. Filtering of SNPs was completed before filtering of subjects, as this order may prevent a number of individuals from being excluded

(Weale, 2010), due to poor call rates, and thus, when sample size is a central concern, less data is lost.

At the SNP level, markers eliminated first were those with call rates less than 95% (16,781 SNPs). Hardy-Weinberg equilibrium (HWE) was then assessed, and SNPs (30,711) with HWE P values less than 1×10^{-8} were excluded, as this suggests that they are not in HWE in this population. Next, a linear regression was calculated, using sex as the dependent variable to eliminate markers (81) associated with sex ($P<1\times10^{-8}$). Golden Helix automatically excluded SNPs (36,858) if a high proportion of values were missing. After excluding a total of 84,431 SNPs, a total of 822,170 remained.

At the individual level, DNA samples were filtered based on call rate, or the percentage of SNPs for which genotypes were successfully obtained. Those with contrast call rates less than 85% were excluded (n=3). Samples were then checked for autosomal heterozygosity, which was done to identify any potential DNA sample contamination. If an individual is found to be heterozygous at an abnormally high number of loci in the genome, it indicates there may have been sample contamination. Subjects (n=5) with a percent heterozygosity greater than 5 standard deviations from the mean percent heterozygosity were excluded. A sex check was performed to eliminate any gender misidentification. This test calculates an individual's X-chromosome heterozygosity. Subjects (n=3) were excluded if chromosomal sex and self-reported sex did not match. A principal components analysis (PCA) was completed to examine the sub-structure of the population. The first step was to select a subset of autosomal markers that were not in linkage

disequilibrium with one another. This was done using the LD pruning method in Golden Helix, which eliminated any SNPs with r^2 values >0.1. These parameters are consistent with the literature (Wang et al., 2009; Weale, 2010). Next, the PCA was run, and 7 patterns were observed within the population. An outlier for a particular component was defined as an individual whose loading score for that component fell greater than five standard deviations from the mean score. An overall PCA outlier was defined as an individual who was an outlier for each of the seven principal components (Laurie et al., 2010). No subjects were excluded on these grounds. After exclusions, a total of 532 subjects remained for analysis. Figure 3-1 provides a summary of these exclusions.

3.3.4 Genome-Wide Association Study

Individuals were excluded from this particular analysis if they were male (161), or had reported multiple ethnicities (4). While all individuals included in the GWAS study had reported their primary ethnicity as Caucasian, a small number also reported being of Hispanic or Middle Eastern ancestry. These individuals may be genetically very similar to Caucasians; however, the trait in question is likely influenced by genetic as well as cultural factors. For this reason, these individuals were excluded. Subjects who had never tried cilantro (60), or would never try cilantro (2) were also excluded. A total of 316 female subjects were included in this GWAS analysis. Golden Helix software was used to calculate linear regressions for each SNP using cilantro preference as the dependant variable. Bonferroni correction was applied, and statistical significance threshold was set at $P<1\times10^{-8}$.

Results were examined and prioritized based on relevance to, or involvement in, taste or olfactory perception. Minor allele frequencies of each SNP in this population were also examined. This is standard practice in the GWAS literature, as a means of eliminating spurious results (Miyagawa et al., 2008). Linkage patterns were also examined using Haploview. Bioinformatics tools exist which utilize calculations to integrate these factors, along with factors such as protein structure and RNA splicing (Mooney, Krishnan, & Evani, 2010; Saccone et al., 2010; Yuan et al., 2006). Based on a priori knowledge of the physiology of taste and olfaction, it was hypothesized that a taste or olfactory receptor would be identified. Each top result was examined using the NCBI Gene database, and fine mapping was conducted using the Map Viewer function ("The ncbi handbook," 2002). Candidate genes identified were then examined further within the GWAS population. The frequency of disliking cilantro (selecting 1-4 on the 9-point preference scale) was calculated across genotypes.

3.3.5 Replication in Toronto Nutrigenomics and Health (TNH) Study Cohort

All subjects from the TNH cohort (n=1,639; 1,117 women and 522 men) were genotyped for candidate polymorphisms identified in the GWAS using the Sequenom MassArray analyzer. This multiplexed SNP genotyping platform allows for simultaneous analysis of close to 30 SNPs in a single reaction, using dideoxynucleotide triphosphates (ddNTPs) as chain terminators and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to detect markers. This method is highly accurate, efficient, and cost-

3.3.6 Statistical Analysis

Statistical analyses were completed using Statistical Analysis Systems software (SAS version 9.2; SAS Institute, Cary, NC). Proportions of dislikers were examined across ethnicities, and across genotypes and combined genotypes within each ethnicities. Pearson χ^2 tests were conducted to identify statistical differences in preferences across groups.

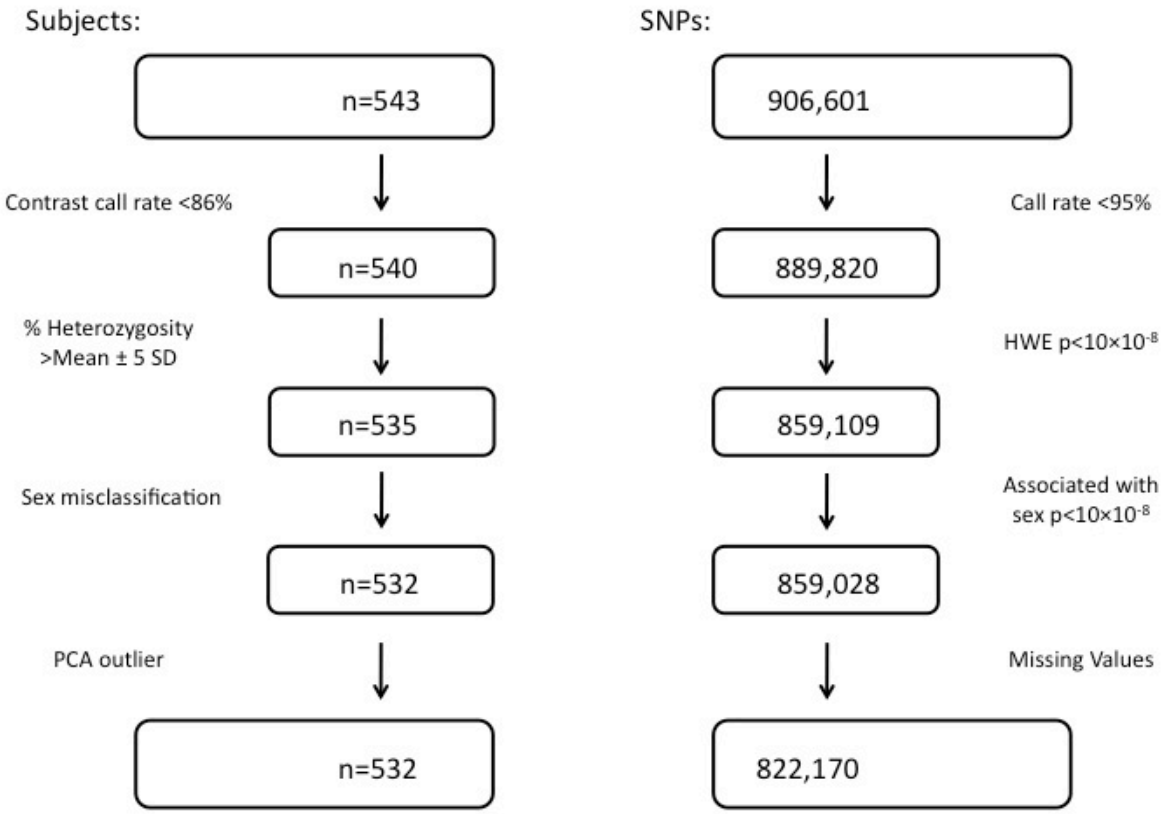


Figure 3-1 Summary of GWAS Quality Control

3.4 Results

3.4.1 Genome-wide association scan

Table 3-1 shows the top results from the GWAS regression analysis. Several of the genes identified in this GWAS, have not been significantly researched, and thus, bioinformatics tools and databases did not provide a conclusive indication of which should be pursued further. Based on quality control methods described, *a priori* knowledge of the physiology of taste and olfactory perception, and post-hoc examination and cleaning of the results, two biologically plausible candidate SNPs were identified. The first was found in the TAS2R1 bitter taste receptor gene, and the second in the OR4N5 olfactory receptor gene. Figure 3-2 is a Manhattan plot depicting the chromosomal distribution of the results of the GWAS analysis. Each chromosome is represented on the X-axis by a different colour, and the Y-axis represents the -log10(p value) for the association between cilantro preference and genotype for a given SNP. This figure shows that there appeared to be statistically significant results scattered across several chromosomes.

Table 3-1 Genome-Wide Association Scan Results

SNP	Chromosome	Gene	Position	Full Model P-Value	Minor Allele Frequency
rs2334911	3	CLSTN2	140146464	5.53E-07	0.11
rs2839627	21	PKNOX1	44448718	1.17E-06	0.12
rs427871	5	TAS2R1	10000817	5.49E-06	0.49
rs4975117	4	FRAS1	79376794	7.74E-06	0.35
rs10509376	10	C10orf11	78198607	7.90E-06	0.06
rs16830177	2	NEB	152392787	9.74E-06	0.01
rs7916911	10	GATA3	8722944	1.08E-05	0.29
rs12253861	10	MIR1303	101190507	1.24E-05	0.10
rs10206129	2	CTNNA2	82276197	1.71E-05	0.47
rs7878438	X	SPANXA2	140388078	2.00E-05	0.32
rs4605077	14	PRKCH	61885440	2.17E-05	0.21
rs11627158	14	PRKCH	61885857	2.17E-05	0.21
rs35732053	4	ANP32C	165579600	2.21E-05	0.03
rs311202	6	C6orf203	107359734	2.29E-05	0.03
rs1619276	6	C6orf203	107355897	2.29E-05	0.03
rs7155214	14	PRKCH	61886406	2.68E-05	0.21
rs17277172	14	OR4N5	20659073	2.82E-05	0.31
rs17080793	4	ARAP2	32003499	2.85E-05	0.04
rs827392	10	GATA3	8700934	3.26E-05	0.26
rs13146451	4	FRAS1	79389132	3.57E-05	0.35

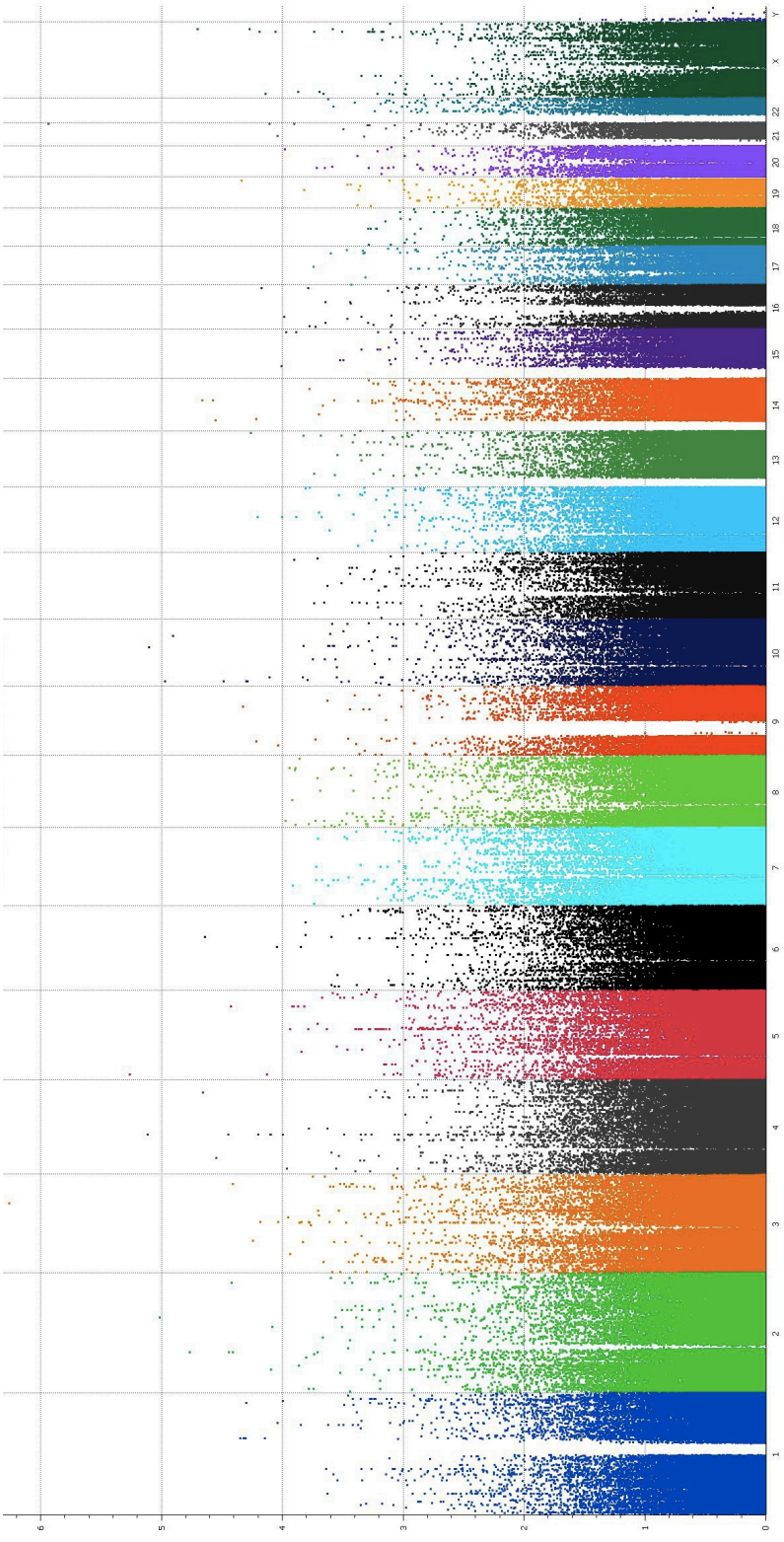


Figure 3-2 Manhattan Plot of GWAS Results

Table 3-2 shows the genotypic frequency and frequency of disliking across genotypes for the rs427871 polymorphism, in the TAS2R1 taste receptor gene. Individuals homozygous for the minor allele of this SNP had the lowest mean cilantro preference. Figure 3-3 shows the proportion of dislikers across genotypes this polymorphism. Frequency of disliking was highest among individuals homozygous for the minor allele, as compared with heterozygotes or those homozygous for the major allele (33%, 19%, and 9%, respectively).

Table 3-3 shows the genotypic frequency and frequency of disliking across genotypes for the rs17277172 polymorphism, in the OR4N5 olfactory receptor gene. As in the case of the previous SNP, individuals homozygous for the minor allele of the rs17277172 polymorphism had the lowest mean cilantro preference. Figure 3-4 shows the proportion of dislikers across genotypes for this SNP. Frequency of disliking was, again, highest among individuals homozygous for the minor allele (56%, 21%, and 13%, respectively).

Table 3-2 Mean Cilantro Preference Across Genotypes for the rs427871 SNP

Genotype	Frequency	Cilantro Preference (Mean ± SD)
CC	27%	5.3 ± 2.5
CA	47%	6.3 ± 2.2
AA	26%	6.9 ± 1.9

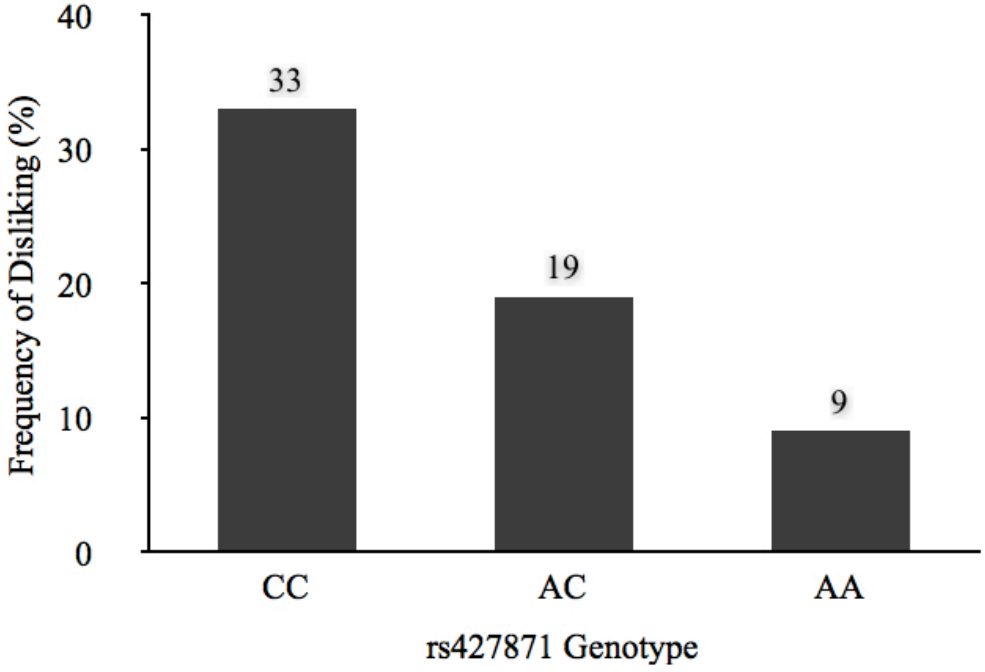


Figure 3-3 Frequency of Disliking Across Genotypes for the rs427871 SNP

Table 3-3 Mean Cilantro Preference Across Genotypes for the rs17277172 SNP

Genotype	Frequency	Cilantro Preference (Mean ± SD)
TT	9%	4.1 ± 2.2
CT	46%	6.2 ± 2.4
CC	45%	6.5 ± 1.9

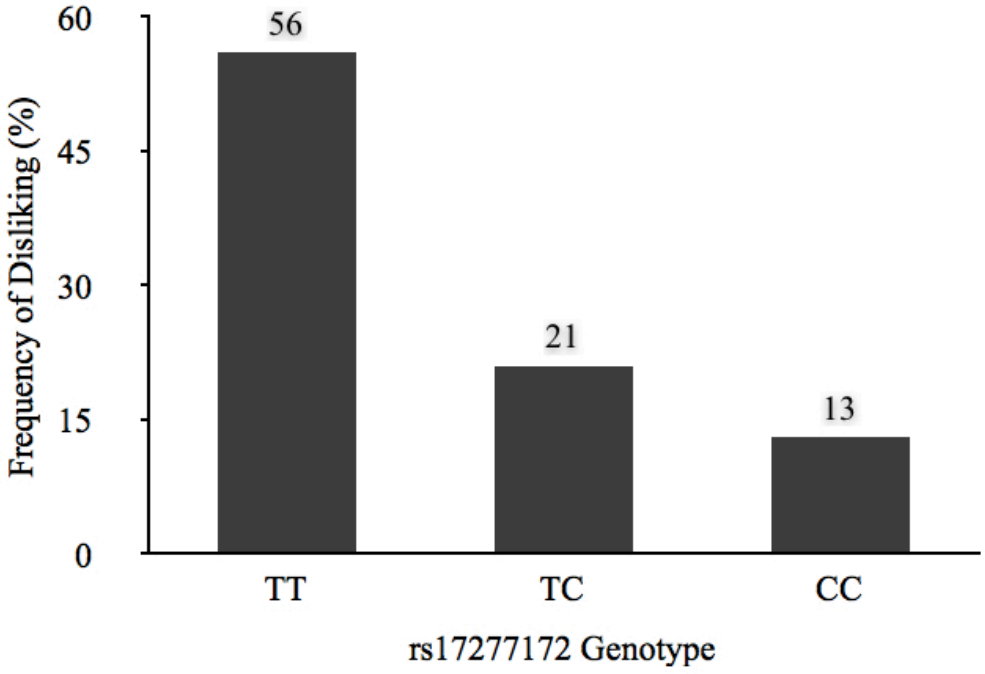


Figure 3-4 Frequency of Disliking Across Genotypes for the rs17277172 SNP

We then examined the frequency of disliking across genotypes based on subjects combined genotypes. Table 3-4 shows the genotypic frequencies of each combined genotype. Figure 3-4 shows the frequency of disliking based on combined genotype. Among those homozygous for the minor allele of both SNPs, 75% reported disliking cilantro, whereas 0% of subjects homozygous for the major allele of both SNPs reported disliking.

Table 3-4 Genotypic Frequencies for Each Combined Genotype

Combined Genotype (rs427871 + rs17277172)	Frequency
TT+CC	2%
TT+AC	4%
TT+AA	3%
TC+CC	11%
TC+AC	23%
TC+AA	12%
CC+CC	11%
CC+AC	22%
CC+AA	11%

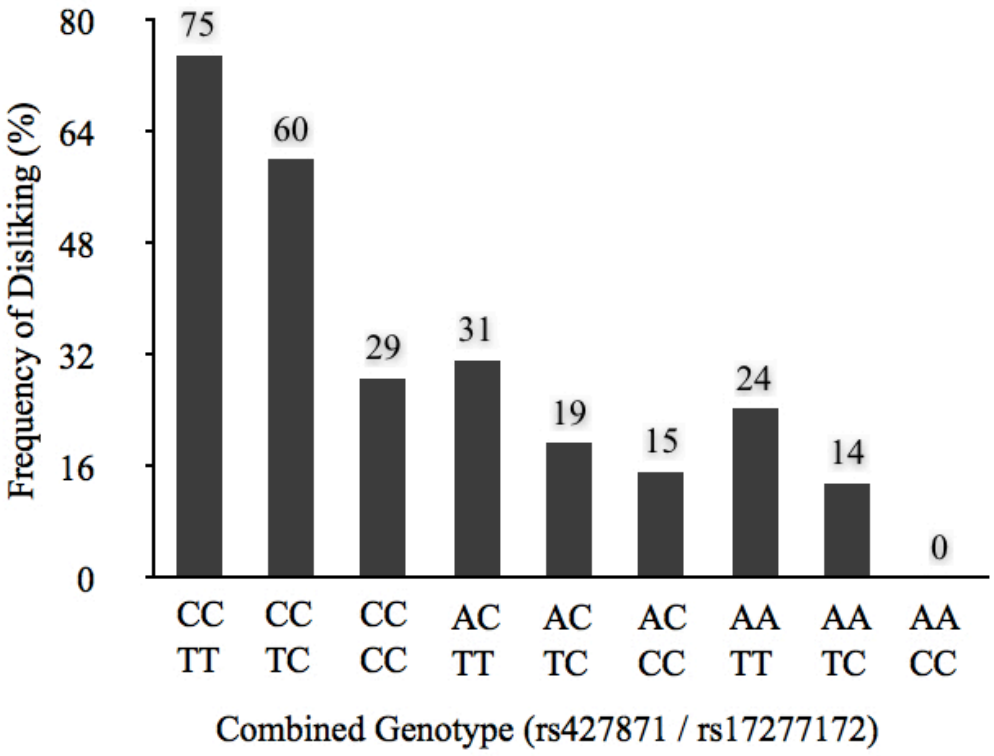


Figure 3-5 Frequency of Disliking Across Combined Genotypes

3.4.2 Replication in Toronto Nutrigenomics and Healthy Study Cohort

Table 2-1 (Chapter 2) shows the ethnocultural distributions of the male and female participants enrolled in the Toronto Nutrigenomics and Health (TNH) study.

Table 3-5 shows the genotypic frequencies and frequency of disliking across genotypes for the rs427871 and rs17277172 polymorphisms, among females within each ethno-cultural group represented in the TNH cohort. Based on the reasoning explained previously, the present analyses focused on females due to apparent differences in the accuracy of food preference reporting between males and females. The patterns observed in the GWAS analyses were seen only in the Caucasian subset of the TNH cohort. In the South Asian subset of the population, the rs427871 SNP, in the TAS2R1 gene, appeared to be associated with frequency of disliking, though the sample size was not sufficient to draw statistical conclusions. In Middle Eastern individuals, the Frequency of disliking was also highest among individuals heterozygous for the minor allele of either the rs427871 or rs17277172 polymorphism. Again, however, the sample size did not allow for statistical conclusions to be drawn.

Table 3-5 Genotypic Frequency and Frequency of Disliking Across Genotypes

	rs427871 (TAS2R1)			rs17277172 (OR4N5)		
	Genotype	Genotypic Frequency (%)	Frequency of disliking (%)	Genotype	Genotypic Frequency (%)	Frequency of disliking (%)
Caucasian (n=452)	CC	28	33	TT	10	49
	CA	47	19	TC	46	21
	AA	26	11	CC	44	15
East Asian (n=404)	CC	12	19	TT	15	18
	CA	37	20	TC	30	22
	AA	51	25	CC	55	23
South Asian (n=105)	CC	19	10	TT	14	0
	CA	42	9	TC	39	10
	AA	38	2	CC	46	6
African Descent (n=21)	CC	6	0	TT	0	-
	CA	12	0	TC	29	0
	AA	82	7	CC	71	8
Middle Eastern (n=21)	CC	26	20	TT	21	25
	CA	42	0	TC	42	0
	AA	32	0	CC	37	0
Hispanic (n=16)	CC	0	-	TT	0	-
	CA	56	0	TC	31	0
	AA	44	0	CC	69	0

3.5 Discussion

The GWAS analysis identified two single nucleotide polymorphisms that appear to be strongly associated with self-reported cilantro preference. These associations were observed in the Caucasian cohort, consisting of 316 females; the larger Caucasian cohort of the TNH study, consisting of 452 females; and also seemed to persist in the smaller South Asian and Middle Eastern groups, despite very small sample sizes. These results are limited by of the power of this study, as the certain ethnocultural groups were less represented than others in this study population, and it was this difficult to study these small groups. The self-report method of phenotype assessment may have also been a limitation, as responses were from memory, and no sample or visual cue was offered that would ensure the subjects knew exactly what cilantro was. As discussed in section 2.5, the heterogeneity within ethnic subgroups, such as the East Asian group was a concern with this study, which, in combination with other factors, may explain the unexpected results observed within this group. The ability to draw conclusions based on GWAS work is limited by the representation of relevant genes on the chip, which is an inherent limitation of GWAS studies. One or both of the identified SNPs may be acting as markers for other variants either in the same or different genes. The TAS2R1 gene is located on chromosome 5, in a region that contains a high degree of linkage, although, the linked genes do not appear to be biologically related, or involved in taste or olfactory perception. Olfactory receptors represent the largest gene family in the human genome. These genes tend to be found clustered and on chromosomes, and often in tight linkage with one another. Figure 3-6 shows the

chromosomal region, on chromosome 14, where the OR4N5 gene is located. The high degree of linkage present is represented by the overlapping gene names. This region also has significant copy number variation, which could not be accounted for by the present analyses. The large number of olfactory receptors in the region provides compelling evidence to further pursue this result, because of the previously discussed hypothesis that variation in olfactory perception is driving the phenotype in question. Whether or not the rs17277172 is the variant responsible, there is likely an association with one or more genes in this region. Though the ability to draw conclusions based on GWAS work is limited, replication in additional populations, using sensory evaluation studies as a mean of collecting phenotypes, will elucidate the role of the two polymorphisms identified in the present study.

Figure 3-6 Olfactory Receptor Representation on Chromosome 14 ("The ncbi handbook," 2002)

Chapter 4

Discussion

4.1 Overview of Conclusions

Research examining genetic determinants of food preferences and dietary selection is currently fairly limited. Numerous factors are known to influence the diet, and it is the specific contributions of various factors are difficult to examine individually. The prevalence of cilantro disliking, and the distribution of cilantro preferences have not been studied or documented in any population, though differences in consumption do exist between regions and cultures (Diederichsen, 1996). It has been hypothesized that genetic factors are responsible for apparent differences in preference, though no genes have been identified that accurately predict a predisposition to cilantro liking or disliking.

Objective 1: To determine the prevalence of cilantro disliking among different ethnocultural groups.

Results: It was demonstrated that prevalence of cilantro disliking varied significantly between ethnocultural groups, ranging from 3% among Middle Eastern individuals, to 21% among East Asian individuals.

Objective 2: To identify genetic factors that predict cilantro preference using genome wide scans.

Results: Two single nucleotide polymorphisms were identified that are significantly associated with cilantro preference: the rs427871 SNP in the TAS2R1 gene, and the rs17277172 SNP in the OR4N5 gene.

Objective 3: To determine whether candidate genetic markers associated with cilantro preference differ between ethnocultural groups.

Results: The two SNPs identified in the GWAS analysis were associated with cilantro preference within the Caucasian group, and may be associated with cilantro preference among Middle Eastern and South Asian individuals.

These findings demonstrate that cultural factors and exposure do play a role in influencing flavour preferences, as demonstrated in chapter 2. It is clear, however, that genetic factors also contribute, as demonstrated in chapter 3. Cilantro is more commonly used in certain styles of cuisine, such as those of Thailand, India, Mexico, and the Middle East, which may explain the differences in cilantro preference distributions observed. Differences in the effect of these genetic variants were not seen with the small number of individuals of each ethnicity in this study, though the results observed in the Caucasian subset do suggest an association worthy of examining further.

4.2 Limitations

Cilantro preference data was collected using a self-report method, which may have introduced some error in this study. Subjects who did not speak perfect English, or who were not exactly sure what cilantro was, may have simply selected an arbitrary response. As cilantro has been described, anecdotally as being extremely polarizing flavour, it would be expected that preference distributions would reflect this, with two peaks, and few neutral individuals. As described, however, food preferences are complex and, through repeated exposure, individuals can acquire a tolerance to certain foods if they are frequently consumed in that individual's home, or their culture.

The 9-point scale used to assess cilantro preference also did not allow subjects to go into detail about the reason for their liking or disliking. Because both an olfactory receptor and a taste receptor were identified as candidate genes, knowledge about what an individual likes or dislikes about the flavour (whether it is the taste or the smell) would be useful.

Sample size was one major limitation of this study, particularly with respect to the third objective. Associations that may have been detected across ethnocultural groups were not observed due to very low numbers of subjects in certain groups.

4.3 Future Directions

These results provide a framework upon which to build many future studies. Replication of these findings is a crucial step, to observe whether the rs427871 and rs17277172 polymorphisms show the same association with cilantro preference in other populations.

In order to study in more detail the effect of each of the identified SNPs, a follow-up questionnaire is being developed, which will be administered to TNH study participants. This questionnaire will ask subjects' to rate their liking or disliking of the odour and taste of cilantro separately. In order to ascertain which of the many compounds in cilantro may be responsible for strong reactions to the flavour, this questionnaire will include two lists of descriptors, one for taste, and the other for odour. Subjects will be asked to check off all of the adjectives they would use to describe the odour and the taste individually. These lists will be populated based on other odour lexicons used in olfactory research, as well as numerous common descriptors used by cilantro likers and dislikers, from various sources ("Ihatecilantro.Com," 2005; Talavera-Bianchi, Chambers Iv, & Chambers, 2010). According to the online community www.ihatecilantro.com, descriptions of the flavour of cilantro vary greatly between individuals, and include soapy, rancid, metallic, moldy, plastic-like, urine-like, and numerous others ("Ihatecilantro.Com," 2005"Ihatecilantro.Com," 2005). With more precise descriptions of how individuals actually perceive the flavour of cilantro, it will be easier to elucidate what specific aspects of sensory perception the genetic variants in question are altering. The TAS2R1 gene is classified as a bitter taste receptor gene, but further research may

identify a novel role for this receptor.

Future work should also involve more precise phenotyping methods. The ideal would be to conduct a controlled sensory evaluation study, where subjects would smell or taste cilantro leaves before selecting their preference rating. This will eliminate some of the issues discussed. In order to further understand what compounds may be responsible, after assessing participants liking or disliking, they should be asked to smell and/or taste a variety of the compounds found in cilantro, and then describe and rate their liking of each compound.

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Research Consent Information

Title of Research Study:	**Nutrition, genetics and health**
Principal Investigator:	Dr. Ahmed El-Sohemy
	Dept. of Nutritional Sciences, University of Toronto
	Phone: 416-946-5776
Study Co-ordinator:	Daiva Nielsen, BSc.
	Dept. of Nutritional Sciences, University of Toronto
	Phone: 416-978-6461
Study Sponsor:	This study is being funded by the Networks of Centres of Excellence (Advanced Foods and Materials Network).
Conflict of Interest:	None

This form provides all the information we think you need to decide if you want to take part in this study. If you have any questions after you read this form, please call our study office. You should not sign this form until you are sure that you understand everything on it.

Purpose of the Research

The goal of this project is to develop an extensive database of diet, genetic markers and biomarkers of chronic disease. Biomarkers are characteristics (e.g., cholesterol levels in the blood) that are measured and evaluated as indicators of normal biological processes or disease processes. The blood will be used to analyze these markers related to bone turnover, blood lipids, inflammation, and nutrient status. Genes that will be measured are those which determine food preferences, affect nutrient metabolism and modify risk for cancer, heart disease, diabetes and osteoporosis.

The study we are doing is to add to our knowledge of how genes affect food choices and markers of health. We are gathering this information by studying a group of people and the study is not meant to test your personal medical status. For these reasons, we will not give you the results of our research on your sample.

Description of the Research

We will ask approximately 2,000 young adults (aged 20-29) to take part in this study. We are studying young adults because at this age genetic effects are often more evident than in older adults as environmental factors have not had the time to significantly modify phenotype (visible characteristics and/or behaviour that result from the interaction of one's genes with the environment).

The study office is at the University of Toronto in the Department of Nutritional Sciences

To be eligible for this study, you must be between 20 and 29 years old, be able to provide a venous blood sample, and not be pregnant or breastfeeding. Women who are pregnant and breastfeeding will not be included as their metabolism and food intake are altered during these conditions.

If you agree to participate, we will take your blood pressure, weight, height, and waist measurements. We will ask you to taste a few pieces of filter paper and rate them for bitterness. Some of the pieces of filter paper contain a bitter compound commonly found in certain foods. We will ask you to complete three questionnaires about your general health and lifestyle, eating habits, and food preferences. We will ask you to complete the general questionnaire in our study office, but you may take the other two questionnaires with you to complete at your convenience. We will give you a requisition and ask you to go to a LifeLabs Lab sometime within the next week between 8 am and 10:30 am to have your blood drawn after fasting overnight for 12 hours. The amount of blood that is required for the study is about 44 mL (or about 3 tablespoons) and will be collected into 8 tubes. All the questionnaires may be returned to the office either in person or by mail in an envelope we can provide you with postage affixed. It is estimated that the initial visit will take about half an hour, the blood draw visit about 20 minutes, and the completion of the questionnaires approximately 45 minutes to 1 hour. The entire time allocated to this study is not expected to exceed 2 hours.

Right to refuse or withdraw

Your participation in this study is voluntary. You may withdraw from the study at any time. You may decline to answer any question or to complete any part of the procedures/tasks. You may also request to have your sample destroyed at any time. If you wish to do this, please make this request in writing to Dr. Ahmed El-Sohemy, Dept. of Nutritional Sciences, 150 College Street, University of Toronto, Toronto, ON, M5S 3E2.

Risks and Benefits

While there is little direct benefit to you from the study, the knowledge gained will help to determine the effect of genes and dietary choices on markers of health.

Having blood drawn from a vein may be mildly painful, but it involves very little risk, other than a slight risk of bruising in the area of the needle prick.

The kind of genetic information from the blood sample we will look for is not likely to tell you anything specific about your personal health. Even so, there is a risk that if people other than the researchers got your genetic information they would misuse them. We think the chance of this ever happening to you is very small. We will protect the confidentiality of your sample by assigning it a specific code. When you go to the LifeLabs lab the only information provided to them will be your sex, date of birth, and your unique code number. They do not need your name or OHIP card and if asked you may refuse this information. We will not keep your name and address with the sample, only the code number. Only the principal researcher or an individual he authorizes will be able to tell which is your sample. Your sample will be stored in a locked freezer for up to 7 years after the completion of the

study. The DNA will be stored anonymously so that as new genes are discovered it is possible for research in this area to continue.

Confidentiality and Privacy

The information that we collect will be used for research purposes only. Your name will not be attached to any of the information we collect, or to the blood sample. You will not be identified by name when the data are analyzed, or in any publication that arises from the study. All personal information that can be identified with your name for this study will be held securely at the study office at the University of Toronto in locked cabinets in locked rooms. Your name and address are linked to your study number, for future follow-up purposes, in a database that is protected by passwords and kept in locked offices with controlled access, only available to the study staff of this research team. All analyses and reports will use groups of data, so that no one individual can be identified.

Publication of Results

The results of this study may be presented at scientific conferences, seminars or other public forums and they may be published, but you will not be identified.

Future Follow-up

On one of the questionnaires we will ask the following question: "Would you be interested in being contacted for a follow-up study?" Any follow-up would be done within 5 years of the start of the study. We ask you this now so that if you are not interested in doing further questionnaires then we would not contact you. Any contact we would have with you would be to clarify any answers in your present set of questionnaires.

Reimbursement

Upon completion of the blood draw and questionnaires, you will be given an honorarium of $20.00.

Compensation for Injury

In no way does signing this form waive your legal rights nor relieve the investigator, sponsors or involved institutions from their legal and professional responsibility.

Request for more information

If you have any questions or concerns regarding the research or your participation in it, now or at any time in the future, please feel free to contact the Study Coordinator, Daiva Nielsen, at the University of Toronto, telephone 416-978-6461. She will answer any questions you have. You may also talk to someone who is not involved in the study at all but who can advise you on your rights as a subject. You may call: University of Toronto Research Ethics Board 416-978-5585.

NUTRITION, GENETICS AND HEALTH

Consent Form

The research study described above has been explained to me and a copy of the Information Sheet / Consent Form has been provided for me to keep. Any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw at any time. This includes the destruction of the blood or DNA sample if I request it. The potential risks, harms and discomforts have been explained to me and I also understand the benefits of participating in the research study.

I understand that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional duties. I know that I may ask now, or in the future, any questions that I have about the study or the research procedures. I have been assured that records relating to me will be kept confidential and that no information will be released or printed that would disclose my identity without my permission unless required by law. I have been given sufficient time to read and understand the above information.

I further understand that:

(1) I am being asked to complete three questionnaires, concerning my general health and lifestyle, my dietary habits, and my food preferences.
(2) I am being asked to have my blood pressure, height, weight, and waist measured.
(3) I am being asked to taste filter papers and rate their bitterness.
(4) I am being asked to give a venous blood sample after fasting overnight for 12 hours.

I have read the Information Sheet that describes the study, and I agree to participate.

_____	_____	_____
Participant's Name (please print)	**Participant's Signature**	**Date**
_____	_____	_____
Witness' Name (please print)	**Witness' Signature**	**Date**

Food Preference Checklist

We would like to know how much you like the following foods. Please put a checkmark (✓) in the space below the number, which best describes how much you like the food. If you have never tried the food, or would not try it, please tick the appropriate box at the end of the row.

	FRUITS									
Apples	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Apricots	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Bananas	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Cantaloupe	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Grapes	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Grapefruit	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would

	_____	_____	_____	_____	_____	_____	_____	_____	_____	not try
Grapefruit juice	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Oranges	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Orange juice	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Nectarines	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Strawberries	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Watermelon	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
	VEGETABLES									
Asparagus	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never

					Dislike					Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Broccoli	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Brussels sprouts	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Cabbage (raw)	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Cauliflower	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Coriander (cilantro)	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Endive	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Kale	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried

	_____	_____	_____	_____	_____	_____	_____	_____	_____	❑ Would not try
Leaf lettuce	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Onions (raw)	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Onions (cooked)	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Parsley	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Potato – baked	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Potato – French fried	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Potato – sweet	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑

	_____	_____	_____	_____	_____	_____	_____	_____	_____	Would not try
Radicchio	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Radish	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Rapini	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Romaine lettuce	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Spinach	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Turnip	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
	BEVERAGES									
Coffee	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never

					Dislike					Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Cola	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Diet Cola	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Iced Tea	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Green Tea	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Black Tea (orange pekoe)	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Red wine	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
White wine	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried

	_____	_____	_____	_____	_____	_____	_____	_____	_____	❑ Would not try
Beer	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Milk	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Soy milk	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
	OTHER									
Miso	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Sushi	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Tofu	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would not try
	_____	_____	_____	_____	_____	_____	_____	_____	_____	
Yogourt	1=Dislike	2=Dislike	3=Dislike	4= Dislike	5=Neither	6=Like	7=Like	8=Like	9=Like	❑

	Extremely _____	Very Much _____	Moderately _____	Slightly _____	Like nor Dislike _____	Slightly _____	Moderately _____	Very Much _____	Extremely _____	Never Tried ❑ Would not try
	BREADS									
White bread	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Whole-wheat bread	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Pumper-nickel bread	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Rye bread	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
	MISCELLANEOUS									
Mustard	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Hot peppers	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would

	_____	_____	_____	_____	_____	_____	_____	_____	_____	not try
Ginger	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Horseradish	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Wasabi	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Garlic	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Salt (added to food)	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Chocolate (semi-sweet or bittersweet)	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Chocolate (dark, sweet)	1=Dislike Extremely	2=Dislike Very Much	3=Dislike Moderately	4= Dislike Slightly	5=Neither Like nor Dislike	6=Like Slightly	7=Like Moderately	8=Like Very Much	9=Like Extremely	❑ Never Tried ❑ Would

	_____	_____	_____	_____	_____	_____	_____	_____	_____	not try
Chocolate (milk)	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try
Chocolate (white)	1=Dislike Extremely _____	2=Dislike Very Much _____	3=Dislike Moderately _____	4= Dislike Slightly _____	5=Neither Like nor Dislike _____	6=Like Slightly _____	7=Like Moderately _____	8=Like Very Much _____	9=Like Extremely _____	❑ Never Tried ❑ Would not try