Genetic Variation in Salt Taste Receptors Impact Salt Intake, Blood Pressure and Cardiovascular Disease Risk Factors in the Guelph Family Health Study

by

Fatima Chleilat

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ABSTRACT

GENETIC VARIATION IN SALT TASTE RECEPTORS IMPACT SODIUM INTAKE, BLOOD PRESSURE AND CARDIOVASCULAR DISEASE RISK FACTORS IN THE GUELPH FAMILY HEALTH STUDY

Fatima Chleilat
Advisor:
University of Guelph, 2016
Dr. David W.L. Ma

The objective of this thesis was to investigate polymorphisms found in salt taste receptors on dietary sodium intake, blood pressure and cardiovascular disease (CVD) risk factors in children and parents of the Guelph Family Health Study (GFHS). The study was a cross-sectional analysis of 70 families (children, n=95 and parents, n=117). A primary finding showed that children, T allele carriers of the rs239345 (A>T) polymorphism consumed 34% more sodium than the AA genotype. Child and female adult carriers of the T allele of the rs8065080 (C>T) polymorphism consumed 21% and 41% lower amounts of sodium respectively. Findings show that genetic polymorphisms in genes SCNN1b and TRPV1 may influence sodium consumption and CVD risk in adults and children.
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LIST OF ABBREVIATIONS

BMI: Body Mass Index
BP: Blood Pressure
CEU: Caucasian
CVD: Cardiovascular Disease
DBP: Diastolic Blood Pressure
DNA: Deoxyribonucleic Acid
ENaC: Epithelial Sodium Channel
eGC: Endothelial Glycocalyx
HDL: High Density Lipoprotein
LDL: Low Density Lipoprotein
RT-PCR: Real-time Polymerase Chain Reaction
SBP: Systolic Blood Pressure
SCNN1B: Sodium Channel Epithelial 1 Beta Subunit
SNP: Single Nucleotide Polymorphisms
TRPV1: Transient Receptor Potential Cation Channel Subfamily V Member 1
Chapter One

INTRODUCTION
1.0 INTRODUCTION

According to the World Health Organization (WHO), cardiovascular disease (CVD) is the 2nd leading cause of death in both Canada and the world (WHO 2014). Globally, approximately 1.7 million CVD-related deaths annually are linked to high dietary sodium consumption and high blood pressure (BP) (He and MacGregor 2009, Mozaffarian et al. 2014), accounting for 10% of all CVD deaths (Mozaffarian et al. 2014). Sodium intake increases blood pressure increases sodium in the bloodstream, consequently resulting in an increase in water. Excess water, reduces the kidney’s ability to excrete it, and elevates blood pressure. This results in an increase in cardiac output, because of an increase in preload of the left ventricle following sodium consumption (Schmeider et al. 1988). These findings have been supported by numerous epidemiological- and experimental-based studies (Strazzullo et al. 2009, Cook et al. 2007, Liu et al. 1983, Elliott et al. 1996). Both individual- and population-based studies have shown that genetic and environmental factors significantly influence sodium consumption and consequently, BP. Other contributors linked to elevated BP include, body composition, blood biomarkers (such as serum cholesterol and triglycerides), and a poor overall diet (Elliott et al. 1996; Alderman et al. 1998; Eny 2010).

Globally, average dietary consumption of sodium significantly exceeds the 1500 mg/day required to maintain normal physiological function (Appel et al. 2011; Garriguet 2007). Based on the prominent role sodium has on BP and CVD risk, population-wide reductions in sodium intake are recommended to lower the incidence of CVD. In normotensive and hypertensive individuals, randomized controlled clinical trials and population-based intervention studies have found that it is possible to reduce BP with reductions in dietary sodium intake (Midgley et al. 1996).
Sodium ingestion is largely modulated by the sense of taste (Hill and Mistretta 1990). Two cation channels expressed on the apical membrane of salt taste receptor cells have been identified as likely salt taste receptors: the amiloride-sensitive and sodium-specific epithelial sodium channel (ENaC), and the Transient Receptor Potential Cation Channel Subfamily V Member 1 (TRPV1) (DeSimone and Lyall 2006). ENaC is the primary mediator of salt taste in multiple herbivores and is believed to be responsible for the appetitive behavioural responses, or a response that yields satisfaction, produced by salt taste (DeSimone and Lyall 2006; Chandrashekar et al. 2010). In humans, ENaC is thought to be responsible for 5-40% of sodium attraction and salt taste responses (Feldman et al. 2013). This indicates that other cation channels may also be responsible for salt taste in humans, including the amiloride-insensitive TRPV1 channel (Stewart et al. 1997). This receptor responds to vanilloids like resiniferatoxin and capsaicin. Both are known to alter amiloride-insensitive nerve responses resultant of a salt stimulus (Lyall et al. 2004). It is believed that disturbing both ENaC and TRPV1 channels simultaneously will fully abolish chorda tympani nerve responses mediated by salt taste and consequently lower intake (Lyall et al. 2004). Chorda tympani is a nerve that originates from a branch of the facial nerve that derives taste messages from taste buds and delivers them to the brain.

Rodent models have demonstrated that heritability affects the inter-strain variability in sodium intake and preference (Bachmanov et al. 2002; Todoff et al. 2007). The candidate gene approach, examining known genes has explained some of the interstrain variability in salt taste perception in mice (Shigemura et al. 2008). They found that inter-individual salt taste perception may be influenced by genetic variation in salt taste receptors, notably ENaC salt taste receptors (Shigemura et al. 2008). No murine models have examined variations in the TRPV1 gene. In
humans, one study has examined salt taste acuity and genetic variation demonstrating an association between polymorphisms in the beta subunit of ENaC and the \textit{TRPV1} gene as modifiers of salt taste perception. These novel observations form the rationale of this thesis to investigate the potential link between single nucleotide polymorphisms (SNPs) in the salt taste receptor genes \textit{SCNN1B} and \textit{TRPV1} with dietary salt intake, BP and CVD blood biomarkers in young children and parents of the Guelph Family Health Study (GFHS).
Chapter Two

LITERATURE REVIEW

DIETARY SODIUM, CARDIOVASCULAR DISEASE AND GENETICS
2.0 LITERATURE REVIEW ON DIETARY SODIUM, CARDIOVASCULAR DISEASE AND GENETICS

2.1 Cardiovascular Disease

Cardiovascular disease (CVD) is the leading cause of death among individuals 60 years and older and the second leading cause among those 15-59 years. The World Health Organization has attributed 62% of the global incidence of stroke and 49% of coronary heart disease to elevated blood pressure (BP) (WHO 2012).

2.1.1 Hypertension and Sodium

Ten percent of CVD-related deaths are attributed to sodium intake. This pathology involves an increase in cardiac output resultant of elevated BP from an excess of water in the blood, following elevated dietary sodium consumption. This elevated BP causes an increase in preload of the left ventricle (Schmeider et al. 1988). A significant positive correlation between 24-hour sodium excretion and systolic BP was established within individuals across various populations (Intersalt 1988). Furthermore, a cross-sectional analysis derived from global longitudinal studies found that every population and group (excluding remote populations), exhibited higher BP values with increased sodium intake (Stamler 1967; Paffenbarger 1968; Stamler 1979).

There has been some conflicting research regarding the association between dietary sodium intake and BP. Some have argued that increased dietary sodium consumption has a direct causal link with elevated BP and CVD through increasing water retention and subsequently increasing BP (Mozzafarian et al. 2014; WHO 2012; Frieden and Berwick 2011; Whelton et al. 2012). Population-wide reductions in dietary sodium intake have shown considerable benefit, estimating a decrease in the incidence of 1.25 million deaths from stroke and over 3 million CVD-related
deaths worldwide (Strazzullo et al. 2009). On the other hand, three major meta-analyses have argued that sodium reductions in the diet had little effect on BP in normotensive persons (Law et al. 1991; Graudal et al. 1998; Mente et al. 2016). The most recent meta-analysis pool analyzed 133,000 individuals, with or without hypertension (Mente et al. 2016). They found that compared to moderate sodium intake (<150mmol), high sodium intake (>150mmol) was associated with higher CVD risk and deaths in hypertensive individuals (Mente et al. 2016). No association between sodium intake between normotensive individuals was observed (Mente et al. 2016). Conversely, hypertensive and normotensive individuals on a low sodium diet (<3000 mg/day) were found to have increased risk of CVD events and death and potential adverse effects on blood lipids, including cholesterol and triglycerides (Graudal et al. 1998). The cumulative analysis derived from a meta-analysis of 59 randomized controlled trials found that these adverse effects on blood lipids was attributed with reduced blood volume owing to lowered sodium intake, thus activating the renin-angiotensin-aldosterone and sympathetic nervous system (Graudal et al. 1998). Further, sodium reduction strategies, without a simultaneous reduction in dietary lipids could result in elevated concentrations of blood lipids (Graudal et al. 1998). These findings suggest that population-wide sodium reductions should be targeted solely to hypertensive patients (Mente et al. 2016). No studies have examined the effect of reduced sodium on blood lipids in children.

Elevated BP is linked to an increased risk of CVD as well as some adverse effects on blood lipids. In adults, hypertension is diagnosed when 2 or more systolic BP measurements exceed 140 mmHg and the average of 2 or more diastolic BP measurements are >90 mmHg. Twenty percent of adult Canadians have hypertension or utilize antihypertensive medication and these rates rise by age and ethnicity (Go et al. 2014). For example, 52% of individuals between the
ages of 60 to 79 years are hypertensive (Go et al. 2014). It is important to note that age-related hypertension, is generally due to systolic blood pressure elevations.

BP reduction may be achieved with decreased sodium consumption- suggesting a possible dose-dependent relationship in normo- and hyper-tensive individuals (He and MacGregor 2009). The Intersalt study, a large epidemiological-based study, estimated that decreases in dietary sodium by 100 mmol/day at the individual and population level could lower systolic pressure by ~10 mmHg and diastolic blood pressure by ~6 mmHg. In doing so, this could substantially mitigate the rates of CVD (Elliott 1996).

In children between the ages of 2-15 years, nine randomized controlled trials examined the effect of sodium reduction on blood pressure (He and MacGregor 2006). One examined this effect over a seven-year period (Wilson et al. 1998). The controlled trials collectively found that low resting systolic and diastolic blood pressure was associated with low consumption of dietary sodium (He and MacGregor 2006). The 7-year cohort study in children reported an even greater increase in blood pressure in individuals who had the highest intake of dietary sodium compared to the lowest amount (Wilson et al. 1998). The effect of dietary sodium and blood pressure on increased risk of CVD events later in life have been estimated by concurrently extrapolating incidences of stroke, heart failure, coronary heart disease and death from longitudinal and observational-based studies in adults. This has been considered acceptable in inferring the pathological effect of sodium intake and blood pressure on CVD risk later in life because renal function is fully developed in early childhood (Daniels et al. 1998).
2.2.2 Salt sensitivity and BP

Salt sensitivity is a measure of how BP responds to salt-loading, or sodium intake (Oberleithner 2012). The most reliable measure of salt-sensitivity involves the measurement of blood pressure variation over a 7-day period of normal (109mmol/d), low (10mmol/d) and then high (250mmol/d) intake of sodium to determine individual salt sensitivity (Sanada et al. 2011). Individuals who exhibited a minimum 10% increase in mean arterial pressure between the low and high salt loads may be considered salt sensitive (Weinberger 1996). Other diagnostic tools for salt sensitive hypertension include measures of mean arterial pressure (MAP) where MAP increases at least 4 mmHg (i.e. 24-h ambulatory blood pressure monitoring) with increases in dietary sodium intake (De la Sierra et al. 2002). Another measure of salt sensitivity includes a minimum 5% difference between MAP on a low-sodium diet and high-sodium diet, divided by the MAP on a low sodium diet (Yatabe et al. 2010). Alternative quantifications of salt sensitivity involve an increase of a minimum of 10 mmHg following the infusion of 21 normal saline concentrations over 4-hours and compared to measurements of MAP a day after a low sodium diet (<10mmol) (Weinberger et al. 2001). Approximately 30% of the global population is salt sensitive, which is a major implicator of arterial hypertension in late adulthood (Oberleithner 2012).

Growing evidence suggests that the endothelium has a regulatory role in sodium intake and excretion (Lang 2011). It has been suggested that the vascular endothelium is crucial to salt buffering because of its capacity to maintain vascular tone and prevent blocking nitric oxide (Cannon 1998). This is crucial because nitric oxide is a soluble gas that is constantly synthesized by the endothelium and functions to maintain vascular homeostasis (Cannon 1998). This
includes modulation of vascular dilator tone (Cannon 1998). Reduced release of nitric oxide has been associated with hypertension and other atherosclerotic conditions (Cannon 1998).

The endothelium consists of two salt-sensitive barriers in series and the endothelial glycocalyx (eGC), which function to buffer sodium and the endothelial cell membrane containing sodium channels (Oberleithner 2012). The endothelium and the eGC act in opposition (Oberleithner 2012). Research has shown that high plasma sodium concentrations result in Stiff Endothelial Cell Syndrome, which effects nitric oxide release (Bragululat et al. 2001). As a consequence, over time, vascular smooth muscle tone increases, resulting in an elevation of BP. Thus, it is speculated that the endothelium may serve as a salt sensor (Oberleithner et al. 2007). These findings illustrate that an intact eGC is an essential buffering system for sodium ions, with a sodium-buffering capacity of about 35mmol, which is equivalent to the amount of sodium found in a salty snack (Oberleithner 2012a). There are two opposing examples illustrating what occurs after the ingestion of this concentration of sodium. Firstly, individuals consuming a low sodium diet, with an intact eGC and low expression of sodium channels in the endothelial cell membrane will experience a rise in plasma sodium for a transient period (Oberleithner 2012a). This is resultant of eGC’s functional buffering capacity and the comparatively low permeability of endothelial cells (Oberleithner 2012a). Following this, sodium is eliminated by the kidneys. Meanwhile, individuals consuming a high sodium diet or have a genetic polymorphism to consume more sodium they will have lower a functional eGC and an increase endothelial sodium channels expression (Oberleithner 2012a). This will yield opposing responses to example 1, where the deteriorated eGC will facilitate sodium entering the endothelial cells because of insufficient buffering in the eGC (Oberleithner 2012a). Further, it is possible that lowering dietary sodium may adversely affect the vascular endothelium by
stimulating the renin-angiotensin system and contributing to the pathogenesis of salt-sensitive hypertension (Alderman et al. 1997). Further, sodium lowering may also have adverse effects on serum total and low-density lipoprotein cholesterol (Graudal et al. 1998). Differences in salt sensitivity have made the effects of salt restriction much less clear. In varying cohort studies, hypertensive individuals on a low salt diet have been linked with increases in CVD and populations have been associated with increases in all-cause mortality (Alderman et al. 1995; Alderman et al. 1998; Tunstall-Pudoe et al. 1997). On the other hand, obese individuals on a low salt diet have been linked with reductions in CVD risk (He et al. 1999).

2.2.3 Sodium consumption on body mass

Weight and sodium have been linked to increased risk of hypertension (Strazzullo et al. 2009). A BMI exceeding 25 is often linked with elevated blood pressure and directly involved in hypertension development. Nine of 13 studies in a meta-analysis conducted by Strazzullo et al. found that habitual sodium consumption, in combination with a BMI exceeding 25, exacerbated the incidence of hypertension, risk of stroke and other CVD events (He et al. 1999; Nagata et al. 2004; Cohen et al. 2006; Geleijnse et al. 2007; Cook et al. 2007; Larsson et al. 2008; Umesawa et al. 2008; Cohen et al. 2008; Downs et al. 1998; Strazzullo et al. 2009). This may be attributed to findings in obese participants who were especially salt sensitive to high sodium intake because of imbalances in central sympathetic inhibition, resulting in impaired renal tubular sodium handling (Strazzullo et al. 2001). Although a high sodium diet has been linked with increased CVD risk, secondary to elevations in BP, it is important to note additional factors linked with adiposity (i.e. insulin resistance) can increase CVD risk.
2.2 Global and Canadian sodium consumption

Presently, global consumption of sodium is greater than 1500 mg/day, with a mean sodium intake of 3950 mg/day (WHO 2014). In Canada, the average sodium intake is 3400 mg, however, basal sodium requirements of sodium for development and physiological requirements are estimated to be 1500 mg/day for adults and children (Appel et al. 2011; Garriguet 2007). In children ages 1-3 years and 4-8 years, the WHO recommends less than 1500 mg/day and 1900 mg/day of sodium respectively. The majority of dietary sodium comes from processed foods while discretionary addition of salt to foods accounts for 20-25% dietary sodium (Liem et al. 2011). Owing to the increased palatability of salt-enriched foods, there is a greater motivation to consume more sodium globally (Strazzullo et al. 2009). In industrialized nations, about 75% of sodium in the diet is used to increase flavor, palatability, preservation of foods and microbial safety (Liem et al. 2011). Regarding palatability and flavor, sodium is known to improve saltiness, reduce bitter taste and enhance sweet taste modalities (Liem et al. 2011). Regarding preservation and microbial safety, sodium functions to reduce water activity in foods, thus preventing the growth of pathogens (Doyle and Glass 2010). As an example, processed meats and cheeses utilize sodium chloride to limit byproduct toxins produced by Clostridium botulinum (Taormina 2010). Further, cold-cut meats require sodium diacetate and sodium lactate to inhibit the growth of Listeria monocytogenes and lactic acid increasing product shelf life (Liem et al. 2011). It has been suggested that sodium reduction consequently may increase the incidence of global food outbreaks, and shorten the shelf life of products (Liem et al. 2011).
2.3 Sodium and physiological function

The kidneys play a central role in sodium homeostasis and BP modulation (Sparks et al. 2014). Sodium is a critical cation found predominantly outside the cell. The human body possesses strong sodium retention mechanisms, capable of maintaining sodium balance even when sodium intake is low (Sparks et al. 2014). Superfluous sodium is excreted from the body because excess sodium, decreases renin-angiotensin-aldosterone system activity, which will result in sodium loss from the body via the kidneys, sweat and feces (Sparks et al. 2014).

Low renal perfusion pressure stimulates the release of renin, which influences the production of substrate molecule angiotensinogen (Agt), a ubiquitous serum globulin synthesized in the liver (Sparks et al. 2014). A sequential enzymatic cascade produces angiotensin I via renin from the kidney cells (Sparks et al. 2014). This is further cleaved into angiotensin II by angiotensin-converting enzyme (ACE), which is found in the endothelium and plasma in pulmonary blood (Sparks et al. 2014). Angiotensin II normalizes the low perfusion pressure by causing blood vessels to constrict and increase sodium retention by directly acting on the proximal renal tubule by an effect modulated by aldosterone, a hormone that gets secreted in response to Angiotensin II (Sparks et al. 2014). Aldosterone is secreted by the adrenal cortex. The primary adrenal hormone, aldosterone, increases during sodium depletion as well as during sodium excess (Sparks et al. 2014). Angiotensin II is the primary physiological modulator of aldosterone secretion. High plasma potassium also increases aldosterone secretion, owing to aldosterone’s dual capability in retaining sodium and instigating potassium loss by the kidney (Sparks et al. 2014).

Further, other factors in addition to aldosterone and angiotensin II influence sodium excretion (Sparks et al. 2014). Atrial peptide, secreted by the heart is also responsible for sodium loss by
the kidneys during high sodium states, owing to excess or cardiac ailment (Sparks et al. 2014). Elevated BP will also stimulate loss of sodium and low BP will typically result in sodium retention (Sparks et al. 2014).

2.4 Determinants of sodium intake

2.4.1 Development of taste

All human senses develop during the embryonic phase and early foetal phase in weeks 1-8 of gestation and mature at different rates (Menella and Beauchamp 1996). This development and maturation of sensory organs are associated with the development and maturation of the central nervous system (Menella and Beauchamp 1996). Taste is one aspect of this early maturation, with taste buds first developing at 8 weeks of gestation (Menella and Beauchamp 1996). During fetal development, maternal diet reaches the amniotic fluid allowing the fetus to familiarize itself with cultural taste patterns prior to birth. This familiarization contributes to taste preference and acceptance which contributes largely to food intake.

2.4.2 Development of salt taste

Salt taste preference is the appeal of sodium-rich foods, absent of need (Desor et al. 1975). Factors influencing inter-individual variability in salty taste preference are poorly understood. It is believed that environmental influences are major contributors of salty taste preference, including, concentration of sodium in foods, overall diet and the mere exposure effect, resultant of cultural environment or familial influences. Prenatal factors may have an effect on offspring preference or aversion of salt taste (Crystal and Bernstein 1995). Severe maternal emesis will have lasting effects on salty taste in their offspring (Crystal and Bernstein
1995; Leshem 1998). This is consistent with animal studies observing an increased preference for salt in the offspring if the mother experienced transient periods of salt depletion or dehydration during pregnancy. This illustrates evidence of developmental plasticity, whereby the fetus adapts to an environment low in electrolytes through the mere exposure effect in a familial setting (Godfrey and Barker 2000; Law et al. 2002). Thus, despite relatively stable taste preferences towards salty taste, consistent exposure and experience may yield the strongest effect on sodium intake.

Newborns do not possess the capacity to differentiate between salt and water solutions until 4 months of age (Beauchamp et al. 1986). Rat and sheep studies have identified a maturational lag in salt taste detection owing to delayed peripheral innervation patterns and the development of sodium ion channels in taste cells (Hill and Mistretta 1990). Similar patterns have been observed in humans, due to a proposed parallels between taste maturation of neural and receptor transduction mechanisms (Beauchamp et al. 1986). Saline solutions, compared to distilled water, are preferred by children ages 4-24 months, but from 21-60 months preference resembles that of adults, rejecting saline solutions, the cause of which is unknown. Some studies have speculated that adolescents prefer much higher concentrations of sodium compared to adults (Cowart and Beauchamp 1986; Desor et al. 1975). Different ethnicities also differ in sodium preference. For example, African Americans have been shown to prefer greater concentrations of sodium compared to Caucasian populations (Desor et al. 1975).

Quantitatively, salt taste preferences translate into neural impulses, which are transmitted to neuronal clusters in the ventral pallidum in the basal ganglia, eliciting signals of hedonic preference of sodium at low or moderate concentrations (Berridge 2009). The same neurons will not fire at high concentrations of sodium because it perceives it as aversive (Berridge 2009).
Therefore, a preference for sodium-rich foods is a strong indicator of food selection and may result in a higher intake of dietary sodium (Birch and Fisher 1995).

In mice, researchers have identified distinct physiological and behavioural responses using novel functional imaging technology of taste receptor cells in response to a salt stimulus (Chandrashekar et al. 2010). These behavioural responses were further substantiated in a human study, which investigated the link between salt taste receptor SNPs, salt detection thresholds and suprathreshold sensitivity to salt. They utilized recognition threshold tests to identify preferred thresholds and aversive suprathresholds (Dias et al. 2012). These murine neurobiological responses correspond to qualitative ratings in human subjects who were given saline solutions at low or moderate concentrations perceived to be pleasant or preferential and high saline concentrations which are perceived as aversive (Beauchamp and Cowart 1985; Bertino et al. 1983).

2.4.4 Biology of salt taste

Salty taste perception originates in all regions of the oral cavity where taste receptors are found (Lindemann 2001). Taste signals from ingested food chemicals activate these taste receptors from taste buds located within papillae on the tongue (Lindemann 2001). The fungiform papillae transmit electrical signals to neurons in the geniculate ganglion by means of the chorda tympani as well as the superficial petrosal nerve (Lindemann 2001). The foliate and circumvallate papillae are innervated namely by the glossopharyngeal nerve (Lindemann 2001).

Individual cell types expressing distinct receptors perceive specific taste modalities following the physical interaction of a tastant molecule, like salty taste (Hoon et al. 1999). The functional substrates of taste perception are taste-receptor cells (TRCs), concentrated on the surface of the
tongue and categorized into 3 groups based on morphology, physiology and biochemical properties, Type I, II and III cells.

About 50-150 taste receptor cells (TRCs) are amassed into taste buds that are distributed across papillae on the tongue (Hoon et al. 1999). There are 3 different types of papillae in humans, circumvallate papillae located at the base of the tongue, which harbor thousands of taste buds; foliate papillae contain hundreds of taste buds and they are located at the posterior lateral borders of the tongue; fungiform papillae contain a mere few taste buds and are located across the anterior two-thirds of the tongue (Adler et al. 2000). Different cells modulated by specialized receptors recognize the salt taste modality. These specialized receptors translate taste qualities into neural signals by interacting with tastants at the taste pore (Adler et al. 2000). This taste pore protrudes at the apical surface of the taste bud. Two hypotheses exist regarding the encoding of taste modalities at the periphery (Chandrashekar et al. 2006). The labeled-line model suggests receptor cells are regulated to detect a single taste quality, like salty taste, and innervated by distinct cells and fibers that do not overlap (Chandrashekar et al. 2006). These distinct cells and fibers specifically transmit neural impulses to neuronal clusters in the brain to elicit this salty taste. The Across-fibre model proposes that single TRCs detect any of the different taste qualities, further suggesting that individual afferent fibers delivers information for multiple taste qualities (Chandrashekar et al. 2006). The research, however, cumulatively leans more strongly towards the labeled-line model.

Salt in human and rodent models elicit a preferential, aversive or neutral taste response. This variability depends on numerous factors including sodium concentration ingested and genetic composition (Bachmanov et al. 2002). The primary mechanism of transduction for the salt modality involves specific ion channels found on the apical membrane of receptor cells that
allow passage of sodium and lithium (lithium also elicits salty taste) cations. This sodium transduction is regulated by two mechanisms: 1) Amiloride-sensitive mechanisms and 2) Amiloride-insensitive mechanisms.

Amiloride-sensitive mechanisms involve direct taste cell depolarization by Na⁺ permeation of ENaC (Vandenbeuch et al. 2008). All taste cells expressing functional amiloride sensitive channels, appear to contain sizeable voltage-gated sodium currents, which stimulate the production of action potentials (Bigiani and Cuoghi 2007). Amiloride-insensitive pathways involve a paracellular shunt pathway, encompassing the transient receptor potential cation channel subfamily V member 1 (TRPV1) receptor whereby Na⁺ diffuses through tight junctions on the epithelium to interact with basolateral channels to depolarize the cells. In humans, it has been proposed that both of these receptors play a role in salt taste (Elliott and Simon 1990).

Taste perception is the most crucial determinant of food preference and is influenced by genetics, cultural factors, exposure to the tastant and age (Glanz et al. 1998; El-Sohemy et al. 2007; Garcia-Bailo et al. 2009). Taste responses have been documented based on facial expressions of newborns to salty and sweet solutions (Berridge 1996), signifying an innate genetic link to taste perception. Understanding taste perception in humans has been refined recently in a study employing gene microarray and 3-alternative forced-choice staircase models (Dias et al. 2012) leading to the genotyping of three SNPs on two salt taste receptors, which may alter salt taste perception in humans (Dias et al. 2012). These salt taste receptor channels include the lingually expressed cation channels, 1) ENaC encoded by SCNN1b and 2) the TRPV1 channel encoded by TRPV1.

ENaC has been identified as a key sodium transduction channel in mammals of the salt taste modality. For a long time, this channel has been focused on in the renal collecting ducts of the
kidneys, as the major regulators of systemic sodium concentrations. However, this channel is
also expressed on the apical membrane of countless epithelial cells and is responsible for the
direct transport of Na+ across apical membranes (Garty and Palmer 1997). These channels
materialize to be functionally expressed in taste cells that do not possess voltage-dependent
inward currents, and further believed to encompass Type I taste cells (Vandenbeuch et al. 2008).
They are also situated in fungiform papillae taste cells (Vandenbeuch et al. 2008).

Further, ENaC activity is modulated by the salt and water balance-regulating hormone,
aldosterone. Aldosterone functions to maintain body volume and BP. It does this by triggering
neuronal responses after it’s secretion, which send signals to the kidneys to increase the amount
of sodium in circulation, excrete sodium and facilitate the reabsorption of water in circulation
with sodium to elevate blood volume (Sparks et al. 2014). Genetic polymorphisms or over-
activity in ENaC results in a significant increase in hypertension risk (Bubien 2010). There is
speculation that ENaC and other extrarenal tissues, such as endothelial cells, impact the
pathophysiology of hypertension (Briett and Schiffrin 2010), which may be the mechanism
responsible for the elevation in BP seen with high salt sensitivity. Nanotechniques have recently
elucidated that sodium ions may modify the mechanical properties of endothelial cells, resulting
in compromised endothelial function (Lang 2011). The endothelial glycocalyx (eGC) possesses
the capacity to briefly harbor sodium ions that are bound to the negative charges of
proteoglycans and bar sodium ion permeation through the apical membrane (Oberleithner 2012).
In doing so, it acts as a transient shield against the rapid uptake of cellular sodium via endothelial
ENaCs, which decelerates sodium ion flux from the blood into the interstitum (Oberleithner
2012a). Superfluous plasma sodium entering endothelial cells by means of the ENaCs occurs
when the eGC is compromised, either genetically or via elevated sodium consumption, thus
resulting in elevated water retention, consequently increasing BP in salt sensitive individuals. This mechanism may commence on lingual surfaces, where ENaC has been also identified as a chief mediator of sodium transduction in rodents, and is believed to be linked with appetitive behavioural responses resultant of salt taste (Chandrashekar et al. 2010).

2.4.5 Genetics of salt taste

The candidate gene approach (Shigemura et al. 2008) has been largely advantageous in finding genes involved in ingestive behaviours, like food smell, taste preference and taste aversion (Chandrashekar et al. 2006; Dias et al. 2010). Understanding the genetic determinants of food intake will provide some information regarding the 4 stages of ingestive behaviour, which include: 1) Initiation [smell and sight- the incentive value of food]; 2) Procurement [learning and memory]; 3) Consummatory [brain signals to gastrointestinal stages which form memories of either reward or aversion] and 4) Termination [involves kidney absorption of nutrients and hormone regulation, i.e. satiety hormone-ghrelin] (Watts 2000; Berthoud 2002). Biological determinants of ingestive behaviour may be divided into sensory, energy homeostatic and reward aspects of food intake. Exploring genetic variation in taste receptors has the potential of aiding clinicians in comprehending food intake behaviour and personalize care to prevent metabolic syndrome, including hypertension, owing to differences in food preferences. Polymorphisms in ENaC and TRPV1 channel have been previously linked with salt taste and cardiovascular disease risk.

The activity and function of ENaC is modulated by three essential subunits, α, β, γ. These 3 homologous subunits of ENaC are found on the kidney and have been reported to occur three times more frequently in individuals with hypertension compared to normotensive males. Salt-
sensitive hypertension diseases have been identified resultant of polymorphisms on the carboxyl terminus of βENaC and γENaC, located on chromosome 16p (Shimkets et al. 1994). Inactivity in βENaC and γENaC regions have been associated with severe hypotension and salt wasting (Chang et al. 1996). These early studies were the first to suggest that ENaC is linked to BP by regulating renal sodium balance. In 2000, a Spanish cohort examined variations in γENaC, however, they did not find differences between genotypes and SBP. Further they also found no differences among γENaC genotypes and diastolic blood pressure, mean blood pressure, plasma renin activity and plasma aldosterone stimulated by a high sodium diet (Pock et al. 2000). This was consistent with a nutrigenomics study in 2012 where subunits α and γ did not show any significant associations with salt taste sensitivity (Dias et al. 2012). However, subunit β, or SCNN1B had been associated with BP and hypertension in Caucasian individuals (rs239345) (Hannilla-Handelberg et al. 2005), South Americans (GT short tandem repeat located in intron 8) (Gonzalez et al. 2007), and Asians (rs7205273 and rs8044970) (Jin et al. 2010). In Caucasians, the polymorphism (rs239345) associated with BP and hypertension was also associated with salt taste sensitivity (Dias et al. 2012).

TRPV1 channel has been identified as another mediator of salt intake in humans linked with aversive behavioural responses resultant of salt taste (Chandrashekar et al. 2010) by altering an individual’s suprathreshold sensitivity (Dias et al. 2012).

TRPV1 channel has been studied vastly and it has been found to be largely associated with nociception where the sensory nervous system responds to a wide range of harmful or potentially harmful stimuli, including pH, capsaicin, or temperature and leading to its activation (Hayes et al. 2000). Further, these associations in pain sensitivity were found among different genotypes. They found lowered pain sensitivity in individuals homozygous for the C allele in SNP
rs8065080, compared to carriers of the T allele (Kim et al. 2004; Lotsch et al. 2009; Valdes et al. 2011). Another study found similar findings between genotypes as they relate to another noxious compound at high concentrations, salt. They found that individuals homozygous for the C allele were less sensitive (meaning they perceived it less strongly) to salt stimuli than carriers of the T allele (Dias et al. 2012). These findings suggested that CC genotype either does alter protein function corresponding to decreased response or it lowers sensitivity to the salt stimulus.
Chapter Three

HYPOTHESIS AND OBJECTIVES
3.1 RATIONALE

In mammals, two opposing behavioural responses have been identified with sodium intake (Dias et al. 2012, Beauchamp et al. 2002; Hettinger and Frank 1990). Low concentrations of sodium (10-150mmol) are typically preferred, while high concentrations of sodium solutions (>150 mmol/L) trigger a behavioural aversion (Hettinger and Frank 1990). Emerging evidence suggests that the variability in habitual sodium intake may be explained by genetic differences in putative salt taste receptors (Dias et al. 2012).

To date, no research has examined how genetic variation in salt taste receptors affects food intake in children and adults. This research is relevant given the role high sodium intake has on hypertension and CVD risk (WHO 2014).

The overall objective was to examine the link between SNPs in salt taste receptors, dietary sodium intake and cardiovascular disease risk factors.
3.2. HYPOTHESES

It is hypothesized that genetic variation found in salt taste receptor genes are differentially linked with dietary intake of sodium, resting blood pressure, and blood lipid biomarkers related to cardiovascular disease risk.

3.3 OBJECTIVES

The objectives are to:

1. determine the link between SNP variation in salt receptor genes, (SCNN1b and \textit{TRPV1}) and dietary salt intake using 3-day food records and Elizabeth Stewart Hands and Associates (ESHA) software in children as well as the Automated Self-Administered 24 hour (ASA24®) recall system in adults.

2. determine the link between SNP variation in salt taste receptor genes, \textit{SCNNB1} and \textit{TRPV1}) and measures of blood pressure using an automated oscillometric device in children and adults.

3. determine the link between SNP variation in salt receptor genes, \textit{SCNN1b} and \textit{TRPV1}) and CVD-related blood biomarkers, including plasma triglycerides (TG) in plasma, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c).
Chapter Four

Genetic variation in salt taste receptors impact sodium intake, blood pressure and cardiovascular disease risk factors in pre-school children
4.0 Genetic variation in salt taste receptors impact sodium intake, blood pressure and cardiovascular disease risk factors in pre-school children

4.1 Abstract

Background. High blood pressure in children has been shown to increase hypertension risk in adulthood as well as early development of cardiovascular disease and mortality. High sodium intake has been identified as a key risk factor for hypertension development in children. Whether this link is modified through the genetic of taste is unknown. Therefore, the objective of this study was to determine the link between single nucleotide polymorphisms (SNP) in salt receptor genes on dietary salt intake, blood pressure and CVD blood biomarkers in pre-school children.

Methods. The study included children between the ages of 1.5-5 years (n= 95) residing in the Guelph-Wellington area. DNA was extracted from saliva and genotyped for SNPs found in the sodium channel epithelial 1 beta subunit (SCNN1B, rs239345) and transient receptor potential cation channel subfamily V member (TRPV1, rs8065080) by real-time polymerase chain reaction. Sodium intake was analyzed using 3-day food records.

Results. T allele carriers of rs239345 (A>T) were observed to consume 34% more sodium, compared to non-carriers (p<0.05). Individuals with SNP genotypes linked with sodium preference were found to have elevated levels of various risk factors for CVD. These included elevated systolic and diastolic blood pressure (rs239345: SBP, TT/ AT: 107±2 vs AA: 97±2, p<0.05; DBP, TT/AT: 67±3 vs. AA: 56 ±2, p<0.05). Individuals with SNP genotypes linked with sodium aversion were found to have lower levels of LDL (rs8065080: CC: 2.9±0.2 vs. CT/TT: 2.1±0.1, p<0.05) and total cholesterol (rs8065080: CC: 4.5±0.2 vs. CT/TT: 3.8±0.2, p<0.05).
Conclusions. Our findings demonstrate a genetic basic of salt intake in young children with links to established CVD risk factors.

4.2 Introduction

Findings from the Canadian Community Health Survey (CCHS) in 2004 showed that 77% of children between the ages of 1 to 3 years and 93% of children between the ages of 4-8 consumed quantities of sodium beyond that of the upper tolerable limit outlined by Health Canada (Health Canada 2016). Twenty observational studies on sodium consumption and blood pressure in children predominately showed positive associations (He and Macgregor 2008; He and Macgregor 2010). This may have implications for future risk of hypertension and CVD (Strazzullo et al. 2009, Cook et al. 2007, Liu et al. 1983, Elliott et al. 1996). A recent meta-analysis involving 10 randomized controlled trials found that even a modest reduction in sodium consumption among children was associated with a small, but significant reduction in BP (He and Macgregor 2006). Assessing risk factors for hypertension among children consuming sodium in excess is critical for developing effective intervention strategies that could mitigate the incidence of hypertension and cardiovascular disease later in life. Sodium consumption is related to taste perception, which may be influenced by genetic variation (Eny et al. 2010; Fushan et al. 2010).

In humans, salt taste is mediated by a cluster of taste receptor cells (TRCs), which express the Epithelial Sodium Channel (ENaC) and the transient receptor potential cation subfamily V member 1 (Trpv1) channel. The receptors mediate two different responses to sodium, a behavioural preference at low concentrations of sodium (10-150mmol) or a behavioural aversion at high concentrations of sodium (>150 mmol/L) (Chandrashekar et al. 2010).
2010; Hettinger and Frank 1990; Heck et al. 1984). To our knowledge only two studies demonstrated that some of the genetic variability in sodium intake and perception may be attributed to heritability (Shigemura et al. 2008; Dias et al. 2012). The first study in rodents found that SNPs in the alpha subunit of ENaC explain interstrain variation related to salt taste perception (Shigemura et al. 2008). They utilized amiloride, a lingual diuretic drug known to inhibit sodium chloride responses in the chorda tympani gustatory nerve (Shigemura et al. 2008). They found that genetic sequence variability, primarily in the alpha subunit of ENaC is responsible for amiloride-sensitive salt taste responses in mice (Shigemura et al. 2008). Supporting these results, were early findings regarding behavioural responses to varying degrees of sodium intake. It has been established that mice will preferentially consume sodium at low concentrations of (10-150mmol) and this behaviour may be attenuated by orally administering the diuretic drug amiloride (Bachmanov et al. 2002). Conversely, at high concentrations of sodium, mice exhibit an innate aversive response, which remain unchanged by amiloride.

Previous research has identified the TRPV1 receptor as a key determinant in behavioural aversion to salt taste (Chandrashekar et al. 2010). This aversive pathway has been recently identified to detect and transduce sodium as well as a multitude of different salts via a taste variant of the vanilloid receptor-1 non-selective cation channel, TRPV1 channel (DeSimone and Lyall 2006). The second study examined associations between SNPs in the ENaC genes (SCNN1A, SCNN1B, SCNN1G, SCNN1D) and TRPV1 gene (TRPV1) and taste sensitivity (Dias et al. 2012). They found no links between salt sensitivity and polymorphisms in ENaC genes SCNN1A, SCNN1G and SCNN1D. However, they found significant differences between salt taste sensitivity and SNPs rs239345, of the SCNN1B gene and rs8065080 of the TRPV1 gene.
The present study is the first to examine links between genetic variation in salt taste receptor genes, previously identified to modify salt taste (Dias et al. 2012), on sodium intake and cardiovascular disease risks, including blood pressure, blood lipids and body composition in pre-school age children. It is hypothesized that SNPs found in salt taste receptor genes will be linked differentially with dietary intake of sodium; resting blood pressure, and blood biomarkers related to cardiovascular disease risk in children.

4.3 Methods

4.3.1 Subjects

Subjects were recruited from the Guelph Family Health Study (GFHS), a longitudinal family cohort study conducted in the city of Guelph and surrounding areas. The GFHS aims to test strategies, which promote healthful lifestyle changes in families as well as prevent obesity and chronic disease. The majority of participants (86%) were Caucasian. The only exclusion criteria was that subjects needed to be between the ages of 1.5 to 5 years and reside within the Guelph-Wellington area (Appendix A). The study was approved by the University of Guelph Research Ethics Board (RCT 02223234). Written informed consent was obtained from all parents of the participants and assent was obtained from each child using a standardized script for all measures (Appendix B).

4.3.2 General protocol

The present study assessed the genetic determinants of sodium intake and biomarkers of cardiovascular disease risk in children. A home visit was arranged to explain the study and parents of the participants completed a detailed food record for their children (Appendix C).
Following this, subjects were asked to attend two morning visits within 2 weeks. The first visit was located at the Body Composition and Metabolism Laboratory at the University of Guelph and entailed a comprehensive health assessment. Participants were asked to refrain from vigorous physical activity for a minimum of two hours prior to the health assessment visit where measures of anthropometrics, body composition blood pressure and saliva samples were collected. The second visit was located at a nearby Lifelabs clinic to provide blood samples. Blood was analyzed for high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol and triglycerides (Figure 4.1).

**Figure 4.1.** Flow diagram of the recruitment of the participants for the Guelph Family Health Study

### 4.3.3. Health assessment

*Anthropometrics.* Height and weight were obtained in triplicate and averaged to the nearest 0.1 cm and 0.1 kg, respectively. Body Mass Index (BMI) was then calculated using the equation $\text{BMI} = \frac{\text{mass (kg)}}{\text{height}^2 (\text{m})}$. 
Blood Pressure. Blood pressure was measured from the right brachial artery using an automated oscillometric device (HBP-1300 OMRON, Mississauga, Ontario). Cuff size was determined based on arm circumference. Three systolic and diastolic blood pressure measurements were obtained via an automatic reading while participants were seated in an upright position. The first measurement was omitted and the last two BP measurements were averaged.

4.3.4 Blood Measurements

Approximately 20 mL of blood was collected intravenously after a 12 hour fast. Measurements of blood chemistry were determined by Lifelabs, including glucose (mM) from whole blood, triglycerides (TG) (mM) in plasma, total cholesterol (TC) (mM), high-density lipoprotein cholesterol (HDL-c) (mM) and low-density lipoprotein cholesterol (LDL-c) (mM) in plasma.

4.3.5. DNA extraction

DNA Samples. DNA was collected from saliva samples using the Oragene DNA collection kit (OG-575, DNA Genotek. Inc., Ontario, Canada). This is a non-invasive method used for the collection of high quantity and high quality DNA from children, using absorbent sponges. The sponge is rubbed against the child’s gum and inner cheek multiple times until the sponge is inundated with saliva. This sponge is subsequently inserted into the v-notch of the collection vial and twisted to facilitate saliva extraction from the sponge. This technique is repeated until 0.75 mL of saliva is attained. According to the manufacturer, this method yields a median DNA yield of 17.3 ug. Participants were fasted for a minimum of 30 minutes before the
saliva sample was provided. The collection kits were incubated overnight at 50°C in a water incubator then transferred to a larger tube where a lysing reagent (PT-L2P; DNA Genotek) was added to the saliva. Subsequently, samples were incubated on ice for 10 minutes and centrifuged at room temperature for 10 minutes at 3500×g to pellet impurities. The DNA in the supernatant was precipitated with 95% ethanol and pelleted by centrifugation for 10 minutes at 3500×g. The resultant DNA pellet was washed with 70% ethanol, dried, and rehydrated in 1X Tris-EDTA pH 8.0 buffer. The DNA solution underwent an RNase digestion with ribonuclease A and ribonuclease T1. DNA yield was quantified using NanoDrop Spectrophotometer.

4.3.6. Genotyping

SNPs rs239345 and rs8065080 (Life Technologies, Burlington, Ontario, Cat# 4351379, Assay ID: C__2387896_30 and C__11679656_10 respectively) were genotyped by real-time polymerase chain reaction (RT-PCR) using the BioRad CFX96 RT-PCR detection system. Primers flanking these SNPs, Taqman oligonucleotide probes and SNP genotyping mastermix were obtained from Thermo Fisher Scientific (Burlington, Ontario, Cat# 4371353).

Carriers of the T allele (TT and AT) for SNP rs239345 have been reported previously to have a dominant mode of inheritance. Therefore TT and AT genotypes were grouped together to investigate the link with CVD risk factors (Dias et al. 2012). Further, carriers of the T allele (CT and TT) for SNP rs8065080 have been previously found to distinguish salt solutions more effectively, likely due to an aversive salt taste mechanism (Dias et al. 2012). As a result, T allele carriers were grouped together.
4.3.7. Dietary Intake of Sodium

Nutrient intake of participants was obtained using 3-day food records completed by the child’s parents. Specific instructions were provided to parents including portion measurements, and food preparation methods to accurately report quantity and quality of ingested food of children. Food records included three consecutive days (two week days and one weekend day). Each food record was reviewed by 2 independent assessors prior to analysis using the Elizabeth Stewart Hands and Associates (ESHA) software (Food Processor 8.1, Salem, OR). If clarification was needed, parents were contacted via telephone.

4.3.8. Statistical Analysis

Data are shown as mean ± SEM in unadjusted and adjusted models. The adjusted model controlled for BMI, gender, age, energy (kcal), water intake, fat intake, and saturated fat intake covariates that could be considered potential confounders of blood pressure and biomarkers of CVD risk. Generalized estimating equations were used in these analyses to account for cluster randomization, whereby siblings were considered clusters of individuals (Liang and Zeger 1986). Outcomes of individuals in different clusters were independent, whereas outcomes of individuals within a cluster (siblings) were correlated. Data analysis was performed using SAS version 9.3 and significance was set at p<0.05.

4.4. Results

Baseline descriptive characteristics are reported in Table 4.1. There were 95 participants between the ages of 18 months to 5 years with a mean age of 4.0±1.3 years. The number of participants who provided saliva for DNA analysis were n=89. The distribution of participants across genotypes were, TT/AT (n=61) and AA (n=28) for rs239345; and CC (n=28) and CT/TT
(n=61) for rs8065080. Distribution of participants across combinations of genotypes from SNPs rs239345 and rs8065080 were, TT/AT| TT/CT (n=43), TT/AT|CC (n=18), AA|TT/CT (n=10), AA|CC (n=18).

Table 4.1 Descriptive statistics of GFHS children

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children</th>
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<tbody>
<tr>
<td>Number of participants (n)</td>
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</tr>
<tr>
<td>Sex (M/F)</td>
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</tr>
<tr>
<td>Age (y) Mean</td>
<td>4.0±1.3</td>
</tr>
<tr>
<td>Ethnicity (%Caucasian)</td>
<td>86</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>103</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>63</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>76</td>
</tr>
<tr>
<td>BMI (kg/m2) Mean</td>
<td>16</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* BMI z-score denotes standard deviation for mean BMI based on age-relevant percentile

Carriers of the rs239345 T allele were found to consume more sodium than individuals homozygous for the A allele (p=0.04; Figure 4.2). No significant differences were observed between genotypes of the rs8065080 SNP and sodium intake (Figure 4.3). Table 4.2 shows the unadjusted and adjusted links between rs239345 of SCNN1B and CVD risk factors. Carriers of the T allele of rs239345 were found to have lower triglyceride concentrations than individuals homozygous for the A allele (p<0.01; Table 4.2).

Table 4.3 shows the unadjusted and adjusted links between rs8065080 and SBP, DBP and resting heart rate. None of the outcomes were associated with genotypes of this SNP, except LDL and total cholesterol. Individuals homozygous for the C allele had significantly higher LDL cholesterol (p=0.0002). Similarly, individuals homozygous for the C allele had significantly higher total cholesterol concentrations in the blood (p=0.0009) (Figure 4.5).
**Figure 4.2.** Dietary intake of sodium and rs239345 of the SCNN1B gene. TT/AT, n=50; AA, n=23. Statistical differences were determined using Proc GENMOD with GEE, Mean±SEM. *p<0.05.

**Figure 4.3.** Dietary intake of sodium and rs8065080 of the TRPV1 gene. TT/AT, n=41; AA, n=32. Statistical differences were determined using Proc GENMOD with GEE, Mean±SEM.
Figure 4.4 Blood pressure and rs239345 of the SCNN1B gene. TT/AT, n=50, AA=23. Statistical differences were determined using Proc GENMOD with GEE, Mean±SEM. *p<0.05
### Table 4.2

Metabolic markers of cardiovascular disease risk and rs239345 of the *SCNN1B* gene. Unadjusted genotypes do not include covariates. Adjusted model includes covariates: BMI, gender, age, energy (kcal), water intake, fat intake and saturated fat intake. Lipid panel, TT/AT, n=21, AA=38. Statistical differences were determined using Proc GENMOD with GEE, Mean±SEM. *p<0.05

<table>
<thead>
<tr>
<th>Metabolic Outcomes</th>
<th>Unadjusted</th>
<th></th>
<th>Adjusted</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT/AT</td>
<td>AA</td>
<td>P-value</td>
<td>TT/AT</td>
</tr>
<tr>
<td>Lipid Panel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3±0.1</td>
<td>1.2±0.1</td>
<td>0.5</td>
<td>1.2±0.05</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.4±0.1</td>
<td>2.6±0.2</td>
<td>0.5</td>
<td>2.5±0.1</td>
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<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.0±0.1</td>
<td>4.3±0.3</td>
<td>0.4</td>
<td>4±0.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.8±0.</td>
<td>1.1±0.2</td>
<td>0.09</td>
<td>0.8±0.07</td>
</tr>
</tbody>
</table>
Table 4.3 Metabolic markers of cardiovascular disease risk and rs8065080 of the TRPV1 gene.
Unadjusted genotypes do not include covariates. Adjusted model includes covariates: BMI, gender, age, energy (kcal), water intake, fat intake and saturated fat intake. Blood Pressure, CC, n=41, CT/TT, n=48. Lipid panel, CC, n=15, CT/TT=14. Statistical differences were determined using Proc GENMOD with GEE, Mean±SEM. *p<0.05

<table>
<thead>
<tr>
<th>Metabolic Outcomes</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT/TT</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>103.4±3.2</td>
<td>102.4±1.6</td>
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<tr>
<td>Diastolic (mmHg)</td>
<td>64.0±3.6</td>
<td>62.3±2.4</td>
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<tr>
<td>Lipid Panel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.2±0.1</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.7±0.2</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.3±0.2</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.9±0.1</td>
<td>0.9±0.1</td>
</tr>
</tbody>
</table>
Figure 4.5 Distribution of participants with rs230345|rs8065080 genotype combinations.
Figure 4.6 Mean sodium intake and genotype combinations from SNPs rs239345 and rs8065080. TT/AT| TT/CT: sodium preferring| aversive to sodium. TT/AT| CC: sodium preferring| neutral to an aversion. AA|CC: neutral to a preference| neutral to an aversion. AA|TT/CT: neutral to a preference| sodium aversive. Statistical differences were determined using Proc GENMOD with GEE. Post hoc analysis was done to analyze differences between...
Genotype combinations of rs239345 and rs8065080 are presented in Figure 4.6. Carriers of the T allele (major) of rs239345 in combination with carriers of the T allele (minor) of rs8065080 occurred most frequently in pre-schoolers (43 / 89, or ~48%). The major genotypes of rs239345 (TT/AT) in combination with the major allele of rs8065080 SNP (CC) accounted for 18 out of 89 participants or ~20%. The minor genotype of rs230345 SNP (AA) in combination with the minor genotype of rs8065080 SNP (TT/CT) accounted for 18 out of 89 participants or ~20%. Carriers with a combination genotype exhibiting no particular sodium preference accounted for approximately 10 out of 89 genotyped participants or ~10%. Sodium intake in these genotype combinations differed significantly (p<0.05). Individuals who preferred sodium (rs239345, TT/AT) and neutral to high concentrations (rs8065080, CC) consumed the most sodium. (Figure 4.7).

4.5. Discussion

The findings of this study suggest a novel genetic link between SNPs in salt taste receptors, dietary sodium intake and BP in preschool age children. Our findings have found significant links between genetic polymorphisms in SCNN1B and sodium intake in children (Figure 4.2). The research regarding sodium intake and genetics is limited. To our knowledge, only one study in adults has assessed the link between genetics and salt taste (Dias et al. 2012). They found no difference between rs239345 SCNN1B genotypes and determined that environmental influences play a much greater role than genetics in adults. Since children have very minimal environmental influences, it is possible that genetics will play a greater role influencing dietary sodium intake.
It is well established that sodium consumption is associated with elevated blood pressure across multiple populations and age groups (Mozaffarian et al. 2014; WHO 2012; Frieden and Berwick 2011; Whelton et al. 2012). In the present study, higher systolic and diastolic blood pressure values were observed in carriers of the T allele for rs239345, with a genetic predisposition to prefer the taste of sodium (Figure 4.4). Regression analysis showed that sodium intake was positively correlated with systolic and diastolic blood pressure (SBP, p=0.07; DBP, p=0.06) (Data not shown). Sodium intake and blood pressure were also independently linked with rs239345 (Sodium, p= 0.04, SBP, p=0.004; DBP, p=0.004).

We grouped individuals according to the four possible genotype combinations related to sodium preference or aversion. The majority of the population (48%) were carriers of both TT/AT genotype, eliciting a preference for sodium at low concentrations (Chandreshekar et al. 2010), as well as CT/AT genotype, eliciting an aversion towards sodium at high concentrations (Dias et al. 2012). This suggests a protective effect whereby individuals prefer sodium consumption at low concentrations to maintain daily sodium requirements for proper physiological function, and reject high concentrations of sodium based on an innate predisposition of aversion. We speculate that carriers of T allele, known to elicit a preferential behavioural response to sodium at low concentrations blocks or limits the activation of the TRPV1 channel by eliciting an aversive behavioural response once sodium concentrations increase. This is consistent with findings assessing risk of or sensitivity to pain in rs8065080 (Kim et al. 2004; Lotsch et al. 2009; Valdes et al. 2011). They found that individuals homozygous for the C allele experienced lower sensitivity to pain compared to carriers of the T allele (Kim et al. 2004; Lotsch et al. 2009; Valdes et al. 2011). Increases in BP due to sodium consumption may be mediated by the combination of genetic predisposition to prefer sodium in
and a neutral response for sodium aversion. It is possible that individuals with the genetic predisposition to prefer sodium at low concentrations do not elicit an aversive taste response once sodium concentrations reach a high level. This ultimately causes sodium to be consumed in excess, which in the long term will result in pathological consequences like hypertension or any number of cardiovascular disease events (Alderman et al. 1995; Appel et al. 2011; Berenson et al. 2002; Cohen et al. 2008). The prevalence of this combination is about 1 in 5 children (Figure 4.5). This is consistent with the prevalence of salt sensitivity in Caucasian cohorts (Oberleithner 2012). To test these mechanisms, sodium channel blockers may be used in the future because, it has been suggested that vascular salt-sensitivity is dependent on, a well-developed eGC and the abundance of sodium channels, specifically focusing on sodium channel, ENaC (Oberleithner 2012). Genetic polymorphisms or acquired over-activity of the ENaC is accompanied with arterial hypertension (Bubien 2010). Research on the alpha subunit of ENaC suggests a potential implication on blood pressure regulation (Shigemura et al. 2008). It is possible that rs239345 on the beta subunit in ENaC may be associated with another SNP, perhaps, the same one that implicates blood pressure in the alpha subunit in ENaC (Wong et al. 2002).

Similar to elevated intakes of sodium, high low-density lipoprotein (LDL) and total cholesterol have deleterious effects on circulatory health (Chiu et al. 2016). Increases in LDL and total cholesterol contribute to vascular plaque formation, forcing the heart to increase cardiac output and increase CVD risk (Chiu et al. 2016). Foods high in saturated fats are often accompanied with high concentrations of sodium, which may exacerbate an individual’s risk of CVD (Chiu et al. 2016). The present study found significantly higher concentrations of LDL and total cholesterol (p<0.05) in individuals who have a lowered sensitivity to an aversive sodium stimulus (CC genotype, rs8065080) (Table 4.5), even after controlling for confounders like,
BMI, total fat intake and saturated fat intake. This sensitivity denotes little to no activation of neuronal signals in the brain evoking an aversive response. Therefore, although LDL and total cholesterol do not have a direct link to SNP genotypes in salt taste receptors, they may act in tandem with sodium intake to exacerbate CVD risk later in life. It is possible that these significant differences in rs8065080 genotypes may solely be linked to the limited ethnic variability within this study. Previously, a cross-sectional study assessed lipid profiles between ethnicities. They found that individuals of European descent had higher serum total cholesterol and LDL compared to individuals of Asian descent (Meshkini et al. 2016). This may be resultant of the predominantly Caucasian cohort in this study in combination with genotype (86%, Table 1). Individuals whom are less sensitive to salt taste aversion (CC) (or neutral to salt taste aversion) consume higher concentrations of sodium compared to salt aversive individuals (TT/CT), which may explain a greater preference for fat taste. Future research combining SNPs from the fat taste modality with SNP rs8065080 may indicate a significant co-mediator effect, explaining an elevated draw to salt- and fat-rich foods as well as elevated blood pressure.

There were some limitations of the present study. Our study was limited in ethnic diversity to assess rare variants and all SNPs with a minor allele frequency less than 15% (Hapmap 2016). Further, despite our best efforts, we could not be certain that salt preference or aversion impacted salt intake in children. Parents were asked to report quantities and types of foods their children ingested, specifying what their child asked for and what their child left on their plate after a meal (Appendix C). However, often parents cook meals based on their own preference or aversion and children are required to finish their meals. Therefore it is possible our findings are not truly representative of preference or aversion towards sodium in children.
Nevertheless, our data did report a correlation between sodium and blood pressure. Future research is required to replicate these findings in adults or an older child cohort.

In conclusion, this study has shown that genetic differences in \textit{SCNN1B} are significantly associated with sodium intake in children. Individuals with a genetic predisposition to prefer sodium were also shown to have significantly higher systolic and diastolic blood pressure values. These findings produce evidence of a strong links between genetic influences on sodium intake, blood pressure, and other CVD risk factors in children.
Chapter Five

Genetic variation in salt taste receptors impacts sodium intake, blood pressure and cardiovascular disease risk factors in adults
5.0 GENETIC VARIATION IN SALT TASTE RECEPTORS IMPACTS SODIUM INTAKE, BLOOD PRESSURE AND CARDIOVASCULAR DISEASE RISK FACTORS IN ADULTS

5.1 Abstract

Background. Increased sodium intake is a risk factor for elevated resting blood pressure and cardiovascular disease (CVD). No research has looked at the genetic basis of salt taste preference and aversion, eating behaviour and cardiovascular disease (CVD) risk in adults. The objective of this study was to determine the link between single nucleotide polymorphisms (SNP) in salt receptor genes of Sodium Channel Epithelial 1 beta subunit (SCNN1B) and transient receptor potential cation channel subfamily V member 1 (TRPV1) on dietary salt intake, blood pressure and CVD blood biomarkers in adults.

Methods. The study is a cross-sectional study of female and male adults (n= 118; male: n=53; female: n=65) between the ages of 26 and 45 years. DNA was extracted and amplified from saliva samples using RT-PCR (SCNN1B rs239345, TRPV1 rs8065080). Sodium intake was analyzed using ASA-24. Blood Pressure and fasted blood samples of triglycerides, total cholesterol, high-density lipoprotein and low-density lipoprotein were assessed.

Results. Female carriers of the T allele of rs8065080 (C>T), reflective of sodium aversion, were found to consume 41% (p<0.05) lower amounts of sodium. Individuals with genetic predispositions to prefer sodium were found to have elevated levels of LDL in circulation (rs239345: TT/AT 2.9±0.1 versus AA, 2.5±0.2, p<0.05).
Conclusion. Our findings show that genetic variation may influence sodium consumption and cardiovascular disease risk factors in adults.

5.2 Introduction

Cardiovascular disease is the 2\textsuperscript{nd} leading cause of death worldwide. Hypertension is considered a major risk for both heart attack and stroke. Elevated systolic blood pressure has been estimated to contribute to 49\% of coronary events and 62\% of all major stroke (Mackay and Mensah 2004). Thus the burden of morbidity and mortality from hypertension is a leading health problem worldwide. Experimental, epidemiological, intervention and migration-based studies have suggested that population-based reductions in sodium intake may significantly decrease blood pressure and cardiovascular disease risk.

Sodium is the primary cation of the extracellular fluid and plays a critical role in the maintenance of extracellular fluid volume, and the generation of the membrane potential of cells (Dahl 2005). However, while sodium is an essential nutrient, it is considered to be consumed in excess globally, in varying amounts (Dahl 2005). One of the leading causes of food intake is taste variability, which may expound inter-individual variation in sodium perception (El-sohemy et al. 2007). Prior research involving sweet and bitter taste modalities demonstrated that genetic variants in taste receptors may alter an individual’s taste function (Eny et al. 2010; Fushan et al. 2010). To our knowledge, only one study has looked at the genetic determinants of salt in adults identifying salt perception and polymorphisms that may alter taste function (Dias et al. 2012). This taste perception is generally attributed to 1) threshold perception at low concentrations of sodium and attributed to salt taste preference or 2) suprathreshold perception at high, potentially
aversive concentrations.

The two lingually expressed cation channels involved in salt taste transduction are epithelial sodium channel (ENaC) and transient receptor potential cation subfamily V member 1 (TRPV1) channel (DeSimone and Lyall 2006). No research has tested the genetic composition of the sodium consumption phenotypes as they relate to habitual sodium intake in adults. This may lend critical use to clinicians in proactively addressing the sodium intake issue resulting in salt sensitive hypertension and cardiovascular disease risk. The present study aimed to assess genetic variation in salt taste receptors and their link with cardiovascular disease risk factors in adults by looking at how genotypes from two distinct SNPs may differentially influence sodium intake, blood pressure and other cardiovascular disease risk factors. We hypothesized that SNPs found in salt taste receptor genes associated with a behavioural preference or aversion to sodium will be differentially linked with dietary intake of sodium, resting blood pressure, and blood biomarkers related to cardiovascular disease risk.

5.2 Materials and Methods

5.2.1 Subjects

Subjects were recruited from the Guelph Family Health Study (GFHS), a longitudinal family cohort study conducted in the city of Guelph and surrounding areas. The GFHS aims to test strategies, which promote healthful lifestyle changes in families as well as prevent obesity and chronic disease. Number of subjects included n=117 from 70 families. The majority of participants (88%) were Caucasian. Eligible participants were not pregnant or breastfeeding. The study was approved by the University of Guelph Research
Ethics Board (RCT 02223234). Written informed consent was obtained from all participants.

5.2.2 General protocol

The present work was a cross-sectional study, assessing the genetic determinants of sodium intake and biomarkers of cardiovascular disease risk in adults. Subjects were asked to attend two independent morning visits within 2 weeks. The first visit was located at the Body Composition and Metabolism Laboratory at the University of Guelph and it entailed a comprehensive health assessment. Subjects were asked to refrain from any vigorous physical activity for a minimum of two hours prior to the baseline visits where measures of anthropometrics, body composition blood pressure and saliva samples were collected. The second visit was located at a nearby Lifelabs clinic to obtain blood samples. Blood was analyzed for HDL cholesterol, LDL cholesterol, total cholesterol and triglycerides. After the second visit, participants completed a detailed Automated Self-Administered 24 hour (ASA24®) recall system, validated to account for sodium quantities in the diet (ASA 2016).

5.2.3. Health assessment

*Anthropometrics.* Measurements of height were obtained in triplicate and averaged to the nearest 0.1cm and 0.1 kg respectively. Body Mass Index (BMI) was then calculated using the equation \(\text{BMI} = \frac{\text{mass (kg)}}{\text{height}^2 (m)}\).

*Blood Pressure.* Blood pressure was measured from the right brachial artery using an automated oscillometric device (HBP-1300 OMRON, Mississauga, Ontario). Cuff size
was determined based on arm circumference. Three systolic and diastolic blood pressure measurements were obtained via an automatic reading while participants were seated in an upright position. The first measurement was omitted and the last two BP measurements were retained.

4.2.4 Blood Measurements

Blood collection was performed at LifeLabs laboratories by trained and experienced phlebotomists. Approximately 20 mL of blood was collected intravenously after a 12 hour fast. Measurements of blood chemistry were determined by Lifelabs, including glucose (mM) from whole blood, triglycerides (TG) (mM) in plasma, total cholesterol (TC) (mM), high-density lipoprotein cholesterol (HDL-c) (mM), low-density lipoprotein cholesterol (LDL-c) (mM) in plasma.

5.2.5. DNA extraction

DNA Samples. Saliva samples for genotyping SNPs were collected using the Oragene DNA collection kit OG-500 (DNA Genotek. Inc., Ontario, Canada). This is a non-invasive collection method that mediates the clustering of high quantity and quality DNA from saliva from adults. This technique is repeated until 2 mL of saliva is attained. According to the manufacturer, this method yields a median DNA yield of 110 ug. Participants were fasted for a minimum of 30 minutes before the saliva sample was provided. The collection kits were incubated overnight at 50°C in a water incubator then transferred to a larger tube where a lysing reagent (PT-L2P; DNA Genotek) was added to the saliva. Subsequently, the samples were incubated on ice for 10 minutes and centrifuged
at room temperature for 10 minutes at 3500 \( \times \) g to pellet impurities. The DNA in the supernatant was precipitated with 95% ethanol and pelleted by centrifugation for 10 minutes at 3500 \( \times \) g. The resultant DNA pellet was washed with 70% ethanol, dried, and rehydrated in 1X Tris-EDTA pH 8.0 buffer. The DNA solution underwent an RNase digestion with ribonuclease A and ribonuclease T1. To verify DNA yield, quantification was conducted by absorbance method (NanoDrop Spectrophotometer) after full DNA extraction from saliva samples.

5.2.6. Genotyping

SNPs rs239345 and rs8065080 (Life Technologies, Burlington, Ontario, Cat# 4351379, Assay ID: C___2387896_30 and C__11679656_10 respectively) were genotyped by real-time polymerase chain reaction (RT-PCR) using the BioRad CFX96 RT-PCR detection system. Primers flanking these SNPs, Taqman oligonucleotide probes and SNP genotyping mastermix were obtained from Thermo Fisher Scientific (Burlington, Ontario, Cat# 4371353).

5.2.7. Dietary Intake of Sodium

Nutrient intake data of a small subset of individuals completed the Automated Self-Administered 24-hour (ASA24®) Dietary Recall System (n=10). Specific questions, including, portion measurements, recipes, and food preparation methods were prompted from participants in order to accurately fill the ASA24®.
5.2.8. Statistical Analysis

Data are shown as Mean ± SEM. A comparative setting was used to estimate the relationship between unadjusted and adjusted models. The adjusted model controlled for gender, BMI and age covariates that could be considered potential confounders. Generalized estimating equations were used in these analyses to account for cluster randomization, whereby families were considered clusters of individuals (Liang and Zeger 1986). Outcomes of individuals in different clusters were independent, whereas outcomes of individuals within a cluster (families) were correlated. Data analysis was performed using SAS version 9.3 and a significance was set at p<0.05.

5.4. Results

Descriptive characteristics at baseline are reported in Table 5.1. There were 117 participants with a mean age of 36±8.4. Average blood pressure values were within a normotensive range, however, the majority of participants were either overweight or obese (BMI>25). Eighty-eight percent of participants were Caucasian, justifying our use of SNPs, rs239345 and rs8065080 with minor allele frequencies of 28% (A allele) and 36% (C allele) among Caucasians (International HapMap Project).
Table 5.1. Descriptive statistics of GFHS adults

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Adults n=117</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants (n)</td>
<td>118</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>53/65</td>
</tr>
<tr>
<td>Age (y) Mean</td>
<td>36.0±8.4</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>88</td>
</tr>
<tr>
<td>Annual Income &lt;39,000 (CAD)</td>
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</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>121±14</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>76±13</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>91±15</td>
</tr>
<tr>
<td>BMI (kg/m2) Mean ±SD</td>
<td>28.2 ±4.7</td>
</tr>
<tr>
<td>BMI ≤ 25</td>
<td>44%</td>
</tr>
<tr>
<td>BMI ≥ 25</td>
<td>27%</td>
</tr>
<tr>
<td>BMI ≥30</td>
<td>36%</td>
</tr>
</tbody>
</table>

No difference was observed between genotypes of rs239345 owing to the small subset of individuals that completed an ASA24 ®. No data regarding food intake was derived from individuals homozygous for the A allele. Figure 5.1 shows the link between SNP rs8065080 of the TRPV1 gene and sodium intake. Carriers of the T allele (TT and AT) have been previously reported to have an apparent dominant mode of inheritance (Dias et al. 2012), so they were grouped together and the link between genotype and cardiovascular disease risks were reported. Female carriers of the T allele, known to elicit a non-aversive response to a salt stimuli at high concentrations of sodium were observed to consume significantly higher amounts of sodium than individuals homozygous for the C allele (p=0.02). No difference was found among males. This may be attributed to the small subset of males who provided food intake data.

Table 5.2 shows the unadjusted and adjusted link between rs239345 of the SCNN1B gene and CVD risk factors. SNP rs239345 was not associated with blood pressure or
measures of blood lipids except LDL. The adjusted model showed that female carriers of the T allele had significantly higher LDL (p=0.04).

Table 5.3 shows the unadjusted and adjusted link between SNP rs8065080 of the \textit{TRPV1} gene and CVD risk outcomes. None of the outcomes were associated with genotypes of this SNP.

Genotype combinations of rs239345 and rs8065080 are presented in Figure 5.2 and further stratified by sex in Figure 5.3a and 5.3b. Carriers of the T allele (major) of the rs239345 SNP in combination with carriers of the T allele (minor) of the rs8065080 SNP occurred most frequently in adults (42.7% overall population and 44.6% and 40.4% in females and males respectively). The major allele of the rs239345 SNP (TT/AT, salt-likers) in combination with the major allele of the rs8065080 SNP (CC, non-salt-dislikers) frequented 23.1%. The minor allele of the rs230345 SNP (AA, non- salt-likers) in combination with the minor allele of the rs8065080 SNP (TT/CT, salt-dislikers) frequented 23.1%. Carriers with a combination genotype exhibiting no particular sodium preference frequented approximately 11.1% of the time.
**Figure 5.1** Dietary sodium intake and rs8065080 genotype in adult females. CC n=4; CT/TT n=4. Statistical differences were determined using Proc GENMOD with GEE. Data shown as Mean± SEM. Considered significant when p<0.05.
<table>
<thead>
<tr>
<th>Metabolic Outcomes</th>
<th>Unadjusted</th>
<th></th>
<th>Adjusted</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT/AT</td>
<td>AA</td>
<td>p-value</td>
<td>TT/AT</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>121±1</td>
<td>122±2</td>
<td>0.3</td>
<td>121±1</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>77±1</td>
<td>76±2</td>
<td>0.7</td>
<td>72±1</td>
</tr>
<tr>
<td>Lipid Panel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3±0.1</td>
<td>1.4±0.1</td>
<td>0.2</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.9±0.1</td>
<td>2.5±0.2</td>
<td>0.1</td>
<td>2.9±0.1</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.7±0.1</td>
<td>4.3±0.2</td>
<td>0.2</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.3±0.1</td>
<td>1.0±0.2</td>
<td>0.2</td>
<td>0.9±0.1</td>
</tr>
</tbody>
</table>

Table 5.2 Metabolic markers of cardiovascular disease risk and rs239345 of SCNN1B in adults. Blood pressure, TT/AT n=67; AA n=46. Lipid panel, TT/AT n=37, AA n=21. Statistical differences were determined using Proc GENMOD with GEE. Data shown as Mean± SEM. Considered significant when p<0.05.
<table>
<thead>
<tr>
<th>Metabolic Outcomes</th>
<th>Unadjusted</th>
<th>Adjusted</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC Females</td>
<td>CT/TT Males</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Blood Pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>122±2</td>
<td>122±1</td>
<td>0.67</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>76±2</td>
<td>82±1</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Lipid Panel</strong></td>
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<td></td>
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</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3±0.1</td>
<td>1.4±0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.7±0.1</td>
<td>2.8±0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
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<td>4.7±0.2</td>
<td>0.38</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.9±0.2</td>
<td>1.3±0.1</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**Table 5.3** Metabolic markers of cardiovascular disease risk and rs8065080 of *TRPV1* in adults. Blood pressure, CC n=59; CT/TT n=57. Lipid panel, CC n=29, CT/TT n=29. Statistical differences were determined using Proc GENMOD with GEE. Data shown as Mean±SEM. Considered significant when p<0.05.
Figure 5.2 Genotype combinations from SNPs rs239345 and rs8065080 in adults in the Guelph Family Health Study. TT/AT | TT/CT: sodium preferring | aversive to sodium. TT/AT | CC: sodium preferring | neutral to high concentrations of sodium. AA|TT/CT: neutral to low concentrations of sodium | sodium aversive. AA|CC: neutral to low concentrations of sodium | neutral to high concentrations of sodium.
Figure 5.3 Genotype combinations from SNPs rs239345 and rs8065080 in adults in the Guelph Family Health Study. 5.3a represent the adult female population and 5.3b represent the adult male population. TT/AT| TT/CT: sodium preferring| aversive to sodium. TT/AT| CC: sodium preferring| non-sodium aversive. AA|CC: non-sodium preferring| non-sodium aversive. AA| TT/CT: non-sodium preferring| sodium aversive.
5.6. Discussion

The present study is the first to assess the link between polymorphisms in genes in salt taste receptors, previously found to be linked with salt preference (Chandrashekar et al. 2010; Dias et al. 2012), sodium intake and CVD risk factors in adult humans. Genetics and environmental factors are major contributors of blood pressure (BP) regulation (Sparks et al. 2014). In particular, dietary sodium has been identified as one of the most prominent environmental risk factors for elevated BP (Campese 1994; Luft 2001; Mozaffarian et al. 2014; Frieden and Berwick 2011; Whelton et al. 2012).

BP differences resultant of sodium intake has varied tremendously among individuals, this is known as salt sensitivity. Salt sensitivity is a measure of how individual BP responds to salt-loading or ingested sodium (Oberleithner 2012). Salt sensitivity of BP has been linked with increased hypertension, cardiovascular disease and mortality (Mozaffarian et al. 2014; Frieden and Berwick 2011). Prior research has suggested that personalized genetic profiles may contribute to their BP response to sodium intake (Miller et al. 1987; Zhao et al. 2010).

In humans, amiloride inhibits salt taste by 20-60%, suggesting that human salt taste is in part mediated by ENaC (Feldman et al. 2003). Appetitive salt is believed to be facilitated by ENaC, which is triggered at low concentrations (Chandraseshekar et al. 2010). In the present study we did not find any significant differences between carriers of the salt preferring genotype and sodium intake because a limited subset of participants provided information regarding their dietary intake using the ASA24®. However, the TRPV1 gene’s rs8065080 (C>T) SNP was associated with sodium intake in females. Carriers of the T allele, known to elicit a behavioural aversive response to sodium
consumed significantly lower amounts of sodium compared to those homozygous for the C allele. This is consistent with former research, which suggests that the CC genotype for rs8065080 is less sensitive to a salt stimuli compared to carriers of the T allele (Dias et al. 2012). This research further substantiated findings that functional variation in the TRPV1 gene alters suprathreshold sensitivity, suggesting that TRPV1 is linked with salt perception at higher, potentially aversive concentrations (Dias et al. 2012).

Low-density lipoprotein in serum was shown to be significantly higher in sodium-preferring genotypes (rs239345, Table 5.2). Owing to the limited information available regarding dietary intake, this study was unable to control for dietary intake of saturated and total fats. High fat diets are also high in sodium (Harsha et al. 2004). Therefore it is speculated that individuals consuming higher levels of salt due to their genetic predisposition (CC genotype, rs8065080) also consume high fat. In a study comparing the typical high fat American diet to the Dietary Approaches to Stop Hypertension (DASH) diet, it was found that sodium intake did not directly influence blood lipids, however high fat foods were often accompanied with higher sodium concentrations, thus explaining lower concentrations of LDL, HDL and triglycerides in the DASH diet group compared to the American diet (Harsha et al. 2004).

There were some limitations of the present study. The majority of our population (88%) within the Guelph Family Health study were Caucasian, therefore we were unable to consider rare polymorphisms that may be linked to a sodium intake or cardiovascular disease risk phenotype. No SNPs were utilized that had a Minor Allele Frequency <15% in individuals of European ancestry (Hapmap 2016). Furthermore, owing to the
multifactorial nature of essential hypertension due to high blood pressure (Hannila-Handelberg et al. 2005), it is difficult to assess singularly the genetic factors involved in the pathology of the disease. Future research is needed to substantiate these findings and assess contributing environmental factors that influence intermediary pathway like the renin-angiotensin aldosterone system (Sparks et al. 2014), sympathetic nerve activity (Strazzullo et al. 2001), sodium excretion (Elliott et al. 1996), cardiac contractility (Schmeider et al. 1988) among many others- for example, how polymorphisms attributed with sodium preference or aversion may implicate all of the above pathways, culminating in an increased risk for CVD.

In conclusion, this study assessed the link between variation in the SCNN1b and TRPV1 gene on some dietary sodium intake data. We found significant differences in behavioural aversive genotypes and sodium intake in adult females. Significant differences were also found in LDL and genotypes linked with a behavioural preference of sodium intake, suggesting a possible indicator of CVD risk.
Chapter Six

General Discussion, Future Direction and Conclusion
6.1 General Discussion

Hypertension is a complex, multifactorial condition affected by environmental and genetic factors. Sensitivity of blood pressure to differences in dietary sodium is a common condition in hypertensive individuals and is often attributed with genetic mutations in the beta or gamma subunits (SCNN1B and SCNN1G respectively) of ENaC (Hannson et al. 1995; Wong et al. 1999). Genetic polymorphisms in either gene of the ENaC will culminate in the loss of the normal cytoplasmic c-terminus of the encoded subunit, which will result in channel over-activity, culminating in increased sodium reabsorption, plasma volume expansion and hypertension (Hannson et al. 1995; Wong et al. 1999). Often these genetic differences are not distinguishable phenotypically, but there is evidence that different polymorphisms might exhibit subtly different phenotypic effects, resulting in problematic conditions later in life (Wong et al. 1999). This observed polygenic nature of BP control in combination with rs239345, (on subunit SCNN1B) may have relevant contributions to dietary sodium differences in the general population. Our findings highlighting a genetic link between carriers of the T allele for rs239345 and sodium intake may have application regarding the pathophysiology and prevention of high BP.

It has been demonstrated that BP in children follows a tracking pattern that continues into the third and fourth decades of life (He and MacGregor 2006). This suggests that BP in early life may be an indicator of the risk for adult hypertension and early intervention may lead to reduction of high incidence of hypertension. Our results found a significant genetic link between systolic and diastolic blood pressure and rs239345 for SCNN1b. This is consistent with findings from a genetic linkage study of
beta and gamma subunits of ENaC (Wong et al. 1999). They utilized four highly polymorphic microsatellite markers to genotype families (Wong et al. 1999). Their findings illustrated significant linkage between systolic blood pressure and chromosome 16p12, which is where the SCNN1B and SCNN1G genes map to (Wong et al. 1999). Other genetic variants in or around these genes may contribute to differences in BP and the risk of cardiovascular disease at the population level (Luft and Weinberger 1997). This has tremendous application for diagnosis, prevention and treatment of cardiovascular disease. Our findings from rs8065080 for TRPV1 yielded no substantive evidence in adults because the sample size was too small.

6.2 Limitations

While there were many strengths to our study, this research was not without limitations. First, given the data derived for this thesis were from both the pilot and the formal Guelph Family Health Study, the protocol for children and adults differed. For example, we only collected food record data from children, not adults. During the six-month follow-up for the formal GFHS study, ASA-24 data was collected. During this time, only 10 ASA-24 food record data were collected, thus insufficient to make any conclusions.

A second limitation was the use of a dietary-recall system in a young cohort. Parents were reporting their children’s food intake, which may result in an inaccurate assessment of food preference and aversion. We attempted to mitigate this bias by training parents to quantify foods that were consumed and those that were left on the plate. However, ultimately children for the most part are required to consume foods that
their parents prepare or prefer. Therefore it is possible children are consuming foods based on their parent’s dietary preferences.

A third limitation was the minimal ethnic variation found within our cohort (>80% Caucasian). Ethnic variances are critical in SNP, blood pressure and CVD risk research, since different ethnicities are often more susceptible to carrying a specific polymorphism or their more at risk of hypo- or hyper-tension. Further, no SNPs were utilized that had a Minor Allele Frequency < 15% in individuals of European ancestry (CEU), therefore limiting the examination of rare variants and sodium intake within the GFHS. Also, owing to the multifactorial nature of hypertension, it is difficult to singularly assess the genetic factors involved in the pathology of the disease.

6.3 Future Direction

This research served to fill in the gaps regarding genetic variability, sodium intake and CVD risk in humans. Future research may utilize these findings to implement a nutritional intervention strategy to decrease population-wide sodium intake. Primary prevention through blood pressure control has and will continue to focus on lowering sodium consumption at the population level (WHO 2007). It is important to determine what degree genetics or environmental factors may influence on sodium intake and population-based susceptibility of salt-sensitive hypertension and CVD risk.

It is also critical to understand the development of ENaC or TRPV1 channels with modified temperature profiles, and assessing the behavioural and physiological influence of changes on taste responses. This was an idea first developed by Talavera and colleagues on the Transient receptor potential cation channel subfamily M member
5 (TRPM5) channel as it relates to the sensitivity of sweet food (Talavera et al. 2005). It is speculated that a positive responsiveness to the salty taste quality of relative concentrations of NaCl materializes within the first few months of birth. This is likely in parallel with the maturation of neural and receptor transduction mechanisms, but has not been confirmed (Beauchamp et al. 1986; 1994). The variability in transduction mechanism in addition to maturation and development may be owing to temperature and taste detection, and ultimately perception.

Further, a more refined understanding of taste variability may lead to better comprehension of connectivity pathways linked with each taste modality separately and provide insight related to innate eating behaviours.

6.4 Conclusions

In summary, this thesis assessed genetic polymorphisms in genes SCNN1B and TRPV1 and their link with sodium intake and CVD risk. This was a cross-sectional analysis that was completed using data analyzed from 70 families participating in the Guelph Family Health Study. The study found significant differences in rs239345 genotypes, sodium intake and systolic and diastolic blood pressure in pre-school aged children. Significant links were also found in rs8065080 genotypes and sodium in adult females, however further research is required to substantiate these findings since the sample size was very small (n=8). Overall, the findings from this study suggest a link between the genomics of salt taste, salt intake and CVD risk biomarkers.
Chapter Seven

References


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Chapter Eight

Appendices
8.0 Appendices

Appendix A- Eligibility Questionnaire

Guelph Family Health Study
Eligibility Screening Questionnaire

1. Do you have at least one child who is between the ages of 18 months and 5 years?
   □ 1  Yes
   □ 2  No

2. Do you live the Guelph area (i.e, Wellington County- includes Guelph, Rockwood, Fergus, Elora, Mount Forest, Puslinch)?
   □ 1  Yes
   □ 2  No

3. Are you planning to move from the Guelph area in the next year?
   □ 1  Yes
   □ 2  No

Thank you for completing this eligibility questionnaire!
Appendix B- Child Assent Script

Guelph Family Health Study
Child Assent Scripts
(Children are aged 3-5 years)

Height and Weight
We want to find how much you grow. We want to measure how tall you are and how much you weigh. Later we will measure you again and see how much you’ve grown. Your mom/dad is going to be with us the whole time. You don’t have to if you don’t want to. No one will be upset with you.
Do you want to try? Great.

If you stand on this height board (show the child the height board) we can see how tall you are. Are you ready to try?

Now, if you stand on this scale (show the child the scale) we can see how much you weigh.
Are you ready for us to find out how much you weigh?

Body Composition
Now we want to find out how much muscle you have. This is the “muscle measuring machine” (show them the BIA machine).

You lie down and we put a patch on your hand and on the top of your foot. You will have to lie fairly still for 5 minutes, but I’ll be reading this book to you while you wait.

It doesn’t hurt but if you don’t like it, we can stop. Do you want to try?

Blood
Have you ever seen the blood from inside your body? Do you know what it looks like? It takes food to all the different parts of your body. We want to find out what it’s carrying today. We want to take some blood from your arm.

This is the chair for you to sit in. Now we need to clean a small spot on your arm. Your arm will feel a little cool after it has been wiped clean.

Next we will put a small needle into this spot to take the blood out. You will feel a prick or stinging for a moment. It will hurt, but it will be very quick and won’t hurt for a long time the way it might when you fall. You can watch blood going into the tube if you like.

You get to decide if you want to do this or not. Your mom/dad is going to do it, and you can watch them first if you want. They’ll be with you the whole time. If you decide not to do it, no one will be upset with you. Are you ready for us to find out what is inside you?
Appendix C: 3 Day Food Record Example

Guelph Family Health Study
Day One Date __________________________

<table>
<thead>
<tr>
<th>Time</th>
<th>Eating Occasion</th>
<th>Food Description and/or preparation method *** Please include as many details as possible</th>
<th>Time</th>
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