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Assessing the Relationship between a Single Nucleotide Polymorphism in PKD2L1, Body Composition, and Dietary Intake in Young Adults in Mississippi

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Assessing the relationship between a single nucleotide polymorphism in *PKD2L1*, body composition, and dietary intake in young adults in Mississippi

By

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Single nucleotide polymorphisms (SNPs) in various taste receptor genes have previously been linked to outcomes such as differences in taste thresholds, food liking, and body mass index, but no studies of this sort have examined sour taste. This study genotyped 501 young adults for *PKD2L1* rs603424 and administered a Food Frequency Questionnaire and Tanita body composition testing to look for associations between the noted SNP, dietary intake, and body composition. Intake of citrus fruit, vitamin C, caffeine, and alcohol were significantly associated with genotype in two-way ANOVA analyses looking at the effect of genotype and race or sex on dietary intake. Regarding body composition, genotype was significantly associated with BMI, but not body fat percentage or fat free mass. These findings suggest that rs603424 may influence intake of certain sour and bitter dietary components; however, further research will be needed to confirm these findings.

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CHAPTER I

INTRODUCTION

Nutrition related chronic diseases such as obesity, cardiovascular disease, and hypertension are examples of common health concerns where gene-environment interactions are crucial to study. Nutrition is an environmental factor that plays a major role in the development of these chronic diseases, but nutrient-gene interactions and genetic variations between individuals also influence disease risk status.

Since the completion of sequencing of the human genome in 2003, genome wide association studies have become possible which provide the opportunity to find single nucleotide polymorphisms (SNPs) that may influence health risk status. SNPs are a common source of genetic variation between individuals, with SNP mutations occurring every 300 base pairs on average. They are a difference of only one single nucleotide (A, T, C, or G) that is common enough to occur in at least one percent of the population and may or may not affect protein function. SNPs in taste receptor genes are currently of interest to test the hypothesis of whether any of them may influence taste perception, food choice, and/or potentially also obesity development long term.

The five basic human tastes are sweet, salty, sour, bitter, and umami. Nutrients dissolved in saliva interact with taste receptors, which are distributed in taste bud cells across the tongue. Taste bud cells are classified as being type I, II, III, or IV. Type I cells contain ion channels that are responsible for the salty taste of sodium chloride

(Chandrashekar et al., 2010). Type II cells are also known as receptor cells and contain G protein coupled receptors needed for umami, sweet, and bitter taste perception (Adler et al., 2000). Type III cells are also known as presynaptic cells and are involved in detecting sour taste (Huang et al., 2006). Lastly, type IV cells are basal cells that are thought to be able to differentiate into other taste cell types (Sullivan et al., 2010). Each type of taste cell can be found spread throughout the tongue. They are not localized to specific regions as originally proposed with the tongue taste "map," however, there may be regions of the tongue that are more or less sensitive to certain tastes (Huang et al., 2006, Feeney & Hayes, 2014).

Humans have individual differences in perceived taste intensities, which can be attributed in part to variation in taste receptor genes. SNPs in the genes for bitter, fat, sweet, umami, and salty taste receptors have previously been associated with outcomes ranging from differences in taste threshold levels to longevity. One of the most extensively studied taste genes is taste 2 receptor member 38 (*TAS2R38*) which is responsible for sensing (or not sensing) the bitter compounds phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP). It is estimated that about 70% of people can detect these compounds, and the remaining 30% are non-tasters (Bartoshuk et al., 1994).

The phenomenon of PTC tasters/non-tasters was first discovered in 1931 (Fox, 1931), and years later we are still discovering the breadth of its significance. A 2012 study found five polymorphisms from the taste 2 receptor member 16 (*TAS2R16*), taste 2 receptor member 4 (*TAS2R4*), and taste 2 receptor member 5 (*TAS2R5*) bitter taste receptor genes to be present at higher frequencies in centenarians, which could be in part due to their influence on dietary intake of various beneficial compounds found in bitter

vegetables (Campa et al., 2012). PROP tasting status has previously been associated with self-reported taste perception of bitter foods such as coffee or dark chocolate, but not with reported food liking or food acceptability (Tepper et al., 2009). PTC tasting status may be associated with risk of tobacco use, as the genetic haplotype for tasting PTC is seen less often in individuals who report being cigarette smokers than in individuals who are non-smokers (Risso et al., 2016). In addition, individuals who are less sensitive to PROP report greater preference for alcoholic beverages which may indirectly influence alcohol intake (Lanier et al., 2005).

Cluster determinant 36 (*CD36*) has recently been proposed as a fat taste receptor, however, this is still undergoing further study to identify which types of taste cells house CD36, and whether fat taste perception is indeed receptor dependent or if it is texture dependent. A study of SNP rs1761667 in the proposed *CD36* fat taster gene demonstrated an association between the AA genotype and a higher taste detection threshold level for long chain fatty acids. The same study also demonstrated an association between a higher taste detection threshold for long chain fatty acids and a higher BMI (Karmous et al., 2017). Another *CD36* SNP, rs1527483, was studied in an African American population and was associated with differences in fat preference and obesity as well as fat taste perception (Keller et al., 2012).

Sweet taste is sensed by the taste receptor type 1 member 2 and taste receptor type 1 member 3 (T1R2/T1R3) heterodimer. This heterodimer is responsible for sensing a variety of sweet substances such as glucose, fructose, and sucrose. *T1R2* is in the top 10% for number of polymorphisms present in a human gene (Kim et al., 2006). *T1R2* SNP rs35874116 has been associated with consumption of sugars in an overweight population,

and two SNPs in the *T1R3* promoter region (rs307355 and rs35744813) were discovered to alter sucrose taste sensitivity by altering transcription levels of *T1R3* (Fushan et al., 2009).

T1R3 has shared responsibility for both sweet and umami taste. Umami taste is determined by another heterodimer made up of T1R3 and T1R1, which responds to the presence of amino acids, particularly monosodium glutamate (MSG). SNPs in the genes for the savory, umami taste receptors *T1R1* and *T1R3* have also been identified as causing an increase or decrease in umami taste detection, with some individuals potentially being umami non-tasters as well (Lugaz et al., 2002).

Regarding salty taste, SNPs in the genes for two putative salt taste receptors transient receptor potential cation channel subfamily V member 1 (*TRPV1*) and epithelial sodium channel (*ENaC*) have both been linked to differences in salt taste perception, showing that despite the established significant role environment plays in salt perception, genetics is still a factor (Dias et al., 2013 and Wise et al., 2007). Further studying the relationship between genetic variation in taste receptor genes, taste perception, dietary preference, and health status can potentially further efforts to understand if and how individual variation in food preference effects obesity development and chronic disease risk.

CHAPTER II

LITERATURE REVIEW

Compared to bitter, fat, sweet, umami, and salt taste sensing, much less is known about sour taste and any possible roles it may have in diet and health because the mechanism behind sour taste transduction is still not fully understood. Sour taste receptor cells belong to the type III presynaptic group of taste cells. Type III cells become depolarized upon an influx of protons through an ion channel, which in turn causes an influx of extracellular Ca^{2+}. Finally, this causes serotonin to be released which forms a synaptic connection with nerve fibers in the taste bud (Huang YA et al., 2008). Two different stimuli may cause type III cells to become activated and recognize the presence of sour: intracellular protons and intracellular protonated organic acids (Ishimaru Y, 2015). A number of different potential sour taste receptors have been proposed to date, including the acid sensing ion channels (ASICs), Carbonic anhydrase-4 (CAR4), the polycystic kidney disease 2-like 1 protein (PKD2L1) and the polycystic kidney disease 1-like 3 protein (PKD1L3), and Otopetrin 1 (OTOP1). None of these however has appeared to act as a "master" sour taste sensor in the way our other taste senses have a "master" sensor.

Proposed Mechanisms for Sour Taste Perception

Acid sensing ion channel 2 (ASIC2) was initially proposed as a mammalian sour taste sensor after a study using rats demonstrated its presence in sour tasting cells, and its

necessity for sour sensing in rats (Ugawa S et al., 1998; Liu L & Simon SA, 2001). However, since ASIC2 is not expressed in mouse sour taste receptor cells, and a knock out study of ASIC2 in mice showed ASIC2 has no influence on sour tasting in mice, it could thus be concluded that ASIC2 is not a "universal" or "master" sour taste receptor for mammals (Richter TA et al., 2004).

The relationship between *Car4* and sour tasting is a more indirect one, but also a more understood one. In mice, *Car4* responds to the presence of aqueous and gaseous carbon dioxide (CO_2) and is expressed only in type III sour sensing taste receptor cells—not in other taste receptor cells (Chandrashekar J et al., 2009). *Car4* knock out mice have a loss of nerve response to CO_2, but still have normal responses to citric acid. It is believed that the taste response to carbonation is similar to the taste response to sour substances due to CO_2 being converted into bicarbonate and free protons, and the free protons stimulating type III taste cells (Superan CT, 2008). While it has not been confirmed whether *Car4* senses CO_2 the same way in humans, the mechanism is believed to be the same based on anecdotal evidence that mountain climbers who take carbonic anhydrase inhibitors to combat altitude sickness are unable to register the presence of carbonation in a carbonated beverage while the drug is in their system (Zuker, 2009).

The transient receptor potential (TRP) channels PKD2L1 and PKD1L3 are large transmembrane proteins that are exclusively expressed in type III taste receptor cells (Lopez-Jiminez et al., 2006). Mice that have *Pkd2l1* expressing cells ablated completely lose all sour sensing ability which has led researchers to treat PKD2L1 as a marker of sour taste receptor cells in subsequent studies (Huang et al., 2006). The tongue contains three different types of taste papillae, but PKD2L1 and PKD1L3 are only co-expressed in

the circumvallate and foliate papillae. In the fungiform papillae, only PKD2L1 is expressed (Ishimaru Y et al., 2010). This is significant because PKD2L1 and PKD1L3 interact with each other through their C-terminal cytoplasmic tails, and both proteins are necessary for a functional receptor/channel on the cell surface (Ishimaru Y et al., 2006). Since they are co-expressed only in the circumvallate (toward the back of the tongue), and foliate (sides of tongue), one would think that PKD2L1/PKD1L3 would have a role in sour sensing in those two papillae only, and not the fungiform papillae, but this remains unclear. There is evidence that PKD2L1 has at least a small to moderate role in sour sensing in fungiform papillae because *PKD2L1* knock out mice lose 25-45% of their sour taste response compared to wild type mice as measured by responses seen in fungiform papillae cells (Horio et al., 2011). In the circumvallate papillae where PKD2L1 and PKD1L3 are co-expressed, an "off response" has been observed where the PKD2L1/PKD1L3 ion channel becomes activated in the presence of an acid, but the channel doesn't open until the sour stimuli is removed (Inada et al., 2008). As can be expected, this response was not observed in fungiform papillae where only PKD2L1 is expressed. Altogether, the current evidence suggests that while *PKD2L1* and *PKD1L3* are likely at least partially required for sour tasting, they are not likely the primary "master" or "universal" sour taste receptor (that if such a thing does exist, has not been discovered yet).

OTOP1 is the most recent gene/protein proposed to be involved in sour tasting. OTOP1 is an ion channel that is exclusively expressed in PKD2L1 containing taste cells in the tongue. Upon expression of *Otop1* in human embryonic kidney 293 cells and *Xenopus laevis* oocytes, protons will rapidly permeate the cell membrane when the

extracellular pH is lowered (Tu et al., 2018). Thus far Otop1 has only been studied in the two previously mentioned cell lines though, and further testing still needs to be carried out to understand the significance of this to sour taste transduction.

Non-Genetic Influencers of Sour Taste Perception

Sour taste sensitivity is affected by several factors including mood, age, environment, and genetics. Altered noradrenaline levels, such as that occurring in states of anxiety and depression, may increase sour taste thresholds, even at mild subclinical levels (Heath et al., 2006). Another study demonstrated that showing individuals a video which would put them in a more positive or negative mood caused them to perceive a citric acid solution as more intense than at their baseline mood (Platte et al., 2013). Age is also important to consider when studying sour taste because children tend to be more accepting and welcoming of sour tastes than adults, and children who prefer more intensely sour tastes are less likely to be picky eaters and more likely to consume a wide variety of fruits and vegetables (Liem & Mennella, 2003).

Regarding the effect of environment and genetics on sour taste, a twin study by Wise et al. (2007) revealed that the degree to which a person can detect low concentrations of a sour solution is more heavily influenced by genetics than environment, and that the level of heritability for sour tasting is high—comparable to that of sensitivity to PROP and PTC. The authors also noted that genetics plays a much larger role in sour sensitivity than it does for salt sensitivity (which is more largely influenced by environment). So, while the putative sour taste receptor genes have been minimally studied thus far regarding impact on diet and weight status, perhaps the strong genetic

influence on sour sensitivity means they may be one of the more important areas of taste genetics to study.

Another consideration is the interaction between sour taste stimulants with other taste stimulants and how that may influence taste perception. Taste-taste interactions are common and of particular interest to sensory food scientists as nearly all foods humans consume are composed of multiple taste components. One such study demonstrated that sour taste masks sweet tastes, which brings up the idea that perhaps those who are genetically more sensitive to sour may consume more sugar in order to perceive the same level of sweet (Di Salle et al., 2013). Capsaicin, the compound responsible for the "heat" in chili peppers may also cause a taste-taste interaction with sour by inhibiting the action of PKD2L1/PKD1L3, as demonstrated in mice (Ishii et al., 2012). As the mechanism(s) behind sour tasting are further uncovered more discoveries on taste-taste interactions involving sour receptors will likely be uncovered.

When studying taste related research questions, whether sour or another taste, another factor that must be kept in mind is the extent to which not only taste sensitivity may vary between individuals, but the extent to which liking of a taste at a certain perceived intensity may vary. Previous research has shown that differences in perceived intensities of various tastes may not correlate to reported liking of the same tastes. For example, in a study on PROP sensitivity, bitter supertasters reported experiencing a heightened bitter sensation from coffee and dark chocolate, but there was still no significant difference in reported liking of these foods between them and PROP non-tasters (Tepper et al., 2009). Research regarding taste sensitivity and dietary intake has also had mixed results, perhaps because even when a SNP that increases or decreases

sensitivity to a particular taste sensation is present, its effect may be overpowered by the influence of other non-genetic factors (Mattes, 2004). There is a need for more studies in taste research that account for personal characteristics other than just taste sensitivity to fully understand what drives food choice.

While it is believed that the ability to taste "sour" evolutionarily provided protection from consuming spoiled foods, there are also many healthful foods with a prominent sour taste. These include citrus fruits, some vegetables, and fermented foods (sauerkraut, kombucha, kimchi, miso, tempeh, yogurt, kefir, etc.). The sour taste of fermented foods comes from the byproducts of fermentation including acetic acid, citric acid, lactic acid, and tartaric acid. These tastes may have been a warning of spoiled food in the past, but today they are sought after by many. Another possible theory regarding the evolutionary importance of sour taste is that it could have been a mechanism to help identify food sources of vitamin C (such as fruit) since humans and other primates are not able to synthesize vitamin C due to a mutation in the gene for gluconolactone oxidase (Breslin, 2013). Whatever the reason was evolutionarily, there is still much to learn about the role of sour taste for humans today. This study aims to expand on the relatively small existing body of literature on sour taste to see if a SNP in *PKD2L1* may correlate with self-reported dietary intake and body composition.

CHAPTER III

METHODS

Study Design

This study was cross sectional in nature and designed to answer the following research questions:

1. Is there an association between SNP rs603424 and self-reported dietary intake?
2. Is there an association between SNP rs603424 and body fat percentage or percent fat free mass?

SNP Selection

This study focuses on one SNP (rs603424) present in *PKD2L1*. This SNP is in the second intron of *PKD2L1* and is overlapped with an enhancer element with H3K4me1, H3K27ac, and H3K9ac histone modifications (Hu et al., 2017). This gene and SNP were chosen based on the current body of evidence regarding potential genes/proteins that are involved in sour taste transduction. At this time the PKD2L1/PKD1L3 heterodimer has been the most heavily studied proposed mechanism and has been suggested to account for 25-45% of sour sensing. No studies, however, have examined genetic variation in *PKD2L1* and *PKD1L3* in relation to possible interindividual differences in sour taste threshold, food liking and dietary intake, or body composition, as has been done for sweet, bitter, umami, salt, and fat taste receptor genes.

Three recent genome wide association studies have found that SNPs in *PKD2L1* and *PKD1L3* are significantly associated with total cholesterol, LDL cholesterol, palmitoleic acid (16:1n-7), and lysophosphatidylcholine 16:1 (LPC 16:1) blood serum levels (Tang et al., 2015; Wu et al., 2013; Demirkan et al., 2012). While marginally significant (p=0.058), Tang et al. (2015) also found a decreased risk of coronary artery disease in individuals possessing the minor allele of *PKD1L3* rs7185272. The association between rs603424 and circulating palmitoleic acid levels may be further explained by its association with stearoyl-CoA desaturase. SNP rs603424 is located 31 kb away from the Stearoyl-CoA desaturase (*SCA*) gene and is associated with stearoyl-CoA desaturase activity in adipose tissue (Marklund et al., 2018). Stearoyl-CoA desaturase is an enzyme involved in fatty acid metabolism and catalyzes the formation of monounsaturated fatty acids (such as palmitoleic acid) from stearic acid (a saturated fatty acid). These previous studies examining outcomes associated with *PKD2L1* and *PKD1L3* SNPs, however, only looked for associations between various SNPs in relation to blood lipid levels. As mentioned previously, there have been no studies looking at *PKD2L1* or *PKD1L3* SNPs in relation to dietary intake or food liking to date.

Subjects

Self-reported healthy students were recruited from Mississippi State University via classroom announcements and email. Subjects were required to make one visit to the Nutritional Performance Assessment Composition Testing (NPACT) laboratory located on the university campus to complete the study. Subjects had to be at least 18 years of age, able to read and write in English, and be willing to complete all parts of the study. All subjects gave informed and written consent before participating. This thesis project

was declared exempt by the Institutional Review Board as it utilized data previously collected from the study of Dr. Terezie Mosby, "Identifying polymorphisms of taste receptors as biomarkers (or risk factors) for obesity." Letter of exemption and IRB approval (IRB-18-036) can be found in Appendix A.

Body Composition

Body composition was measured using a single frequency (50-kHz) bioelectrical impedance analyzer (TBF-300A, Tanita Corp, Tokyo, Japan) and a stadiometer for height (235 Heightronic Digital). Tanita output included weight, BMI, body fat percentage, basal metabolic rate, impedance, free fat mass, total body water, desirable range of body fat percentage, and fat mass. Subjects were asked to avoid eating or drinking for four hours prior to the visit, and were asked to remove shoes, socks, and jackets prior to body composition measurements being taken. Two to three additional pounds of weight was entered into the Tanita scale to account for clothing, depending on how the participant was dressed (e.g. light t-shirt and shorts in the summer, or jeans and a sweater in the winter). Participants were given the opportunity to view their body composition results at the time measurements were taken.

Dietary Intake

The web-based NIH Diet History Questionnaire II (DHQ II) was administered to all participants to obtain data on dietary intake (National Cancer Institute, 2010). All participants received a link to complete the online survey via email prior to their scheduled appointment, although all were also offered the option to complete the survey in our lab at the time of their appointment if they desired. The DHQ II is a validated food

frequency questionnaire that asks about the types and portion sizes of foods and beverages consumed over the past 12 months, with 134 food items included and eight questions on dietary supplements. Estimated completion time for the DHQ II is about one hour. The corresponding DHQ Nutrient Database and Diet*Calc software was used to estimate food group and nutrient intakes based on subjects' responses (National Cancer Institute, 2012).

Saliva Collection

Two, 2-ml vials of whole saliva were collected from each participant using the passive drool collection method. Participants were asked to rinse their mouth with water prior to providing the saliva sample to reduce the likelihood of food particles or other contaminants being present. To obtain each vial of saliva a Saliva Collection Aid (Salimetrics, State College, PA) was screwed onto the top of a labeled cryovial collection tube. Next, the participant placed the tip of the Saliva Collection Aid into their mouth and was instructed to tilt their head slightly forward, let saliva pool in their mouth, and gently guide the saliva into the tube. Once two ml of saliva had been obtained in tube one, the Saliva Collection Aid was removed and placed onto the top of the second tube to repeat the process. Both tubes were then capped and stored in a freezer at -80℃.

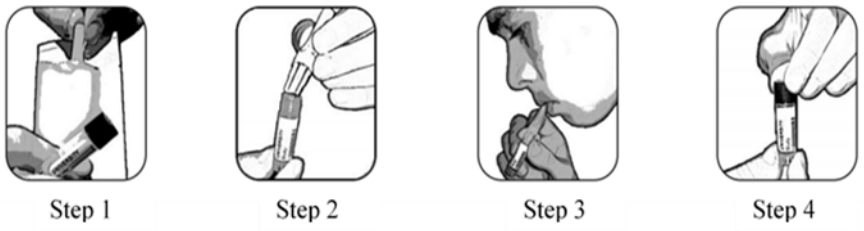

Figure 3.1 Diagram of Saliva Collection Method, Copyright Salimetrics, 2019

DNA Extraction

Saliva from each subject was blotted onto filter paper using a disposable pipet and allowed to dry. Once dry, a portion of the filter paper, about 2 cm in diameter was cut out. This 2 cm in diameter circle of dried filter paper was further cut into 3 mm x 3 mm pieces and these pieces were deposited into a 1.5 ml microcentrifuge tube. DNA was extracted from the dried and cut filter paper using Applied Biosystems' DNA Extract All Reagents kit which contains a lysis and a stabilizing solution. 50 µl of lysis solution was added to each microcentrifuge tube of cut filter paper. Each tube was then incubated at 95° C for three minutes. Following incubation, 50 µl of stabilizing solution was added to each tube. The DNA lysate solution, minus the filter paper, was then transferred to a 0.5 ml microcentrifuge tube for long term storage at -20°C.

Genotyping

Genotyping was carried out as follows using the Taqman method for real-time quantitative PCR (qPCR) with a QuantStudio 5 instrument.

1. Clean workspace with ethanol and water.
2. Calculate the quantity of genotyping assay, Master Mix, and RNase/DNase free water that will be needed, based on Table 3.1.
    a. Each subject being genotyped will be allotted three wells of a 96-well PCR plate and three wells of each plate run will be designated for a negative control.

3. Create a PCR reaction mix by pipetting the calculated quantities of each of the components in Table 3.1 into one 1.5 ml microcentrifuge tube. Mix and centrifuge the tube before continuing on to the next step.
4. Pipet 16 µl of the prepared solution into each well of the PCR plate.
5. Pipet 4 µl of previously prepared DNA lysate solution into each well, being careful to change pipet tips each time to avoid cross contaminating the DNA sample or wells for other subjects.
    a. Subjects were genotyped in triplicates for quality control, so 4.0 µl of DNA lysate was pipeted into each of three wells for every subject.
    b. Three wells in every plate were designated as negative controls and received 4.0 µl of RNase free water in place of DNA lysate.
6. Cover plate with adhesive film and centrifuge for 30 seconds.
7. Insert plate into QuantStudio5 and run fast qPCR with the PCR step programmed for conditions of 60℃ for 40 cycles.
8. After plate has finished running analyze results for genotype using Thermofisher Connect web-based software for real-time qPCR.

Table 3.2 Components in PCR Reaction Mix

Component	**Volume per well for a 20 µl reaction**
Taqman SNP Genotyping Assay, 20x (C__1345774_10, Applied Biosystems)	1.0 µl
GTXpress Master Mix (Applied Biosystems)	10.0 µl
RNase/DNase free water	5.0 µl
Total	16.0 µl

Data Analysis

Genotyping results were coded as 1=AA, 2=AG, 3=GG and entered into the database. Basic descriptive statistics for participants' data are expressed as means ± standard deviations. Two-way ANOVA analyses were carried out to determine the effect of race and genotype on body fat percentage and the various measures of dietary intake. Variables that failed Levene's test for homogeneity of variance were log transformed prior to ANOVA analysis. Tests for simple main effects were performed in the presence of a s significant interaction effect between genotype and race. Tukey's HSD post hoc tests were performed when indicated. An additional analysis was carried out with two-way ANOVA looking at the effect of genotype and sex on dietary intake. Statistical analysis of data was conducted using IBM SPSS statistical software version 24.0 (SPSS, Chicago, IL, USA). All reported p-values were two-tailed and p-values less than 0.05 were considered statistically significant.

CHAPTER IV

RESULTS

Characteristics of the Study Population

A total of 501 subjects were genotyped for PKD2L1 rs603424. Subjects were primarily Caucasian (n=346, 71.9%) and African American (n=117, 24.3%). Subjects were 82.8% female (n=414) and had a mean age of 20.46±2.92 years. Subjects had a mean BMI of 24.57±5.90 and a mean body fat percentage of 26.74±9.55%. Genotype frequencies for this study sample were 13.8% AA (n=69), 30.5% AG (n=153), and 55.7% GG (n=279). See Table 4.1 and 4.2.

Table 4.1 Characteristics of the Study Population

Variable	Total (n=501)	Caucasian (n=346)[a]	African American (n=117)[a]
Age	20.46±2.92	20.30±2.70	20.11±1.62
Sex	82.8% female (n=414)	84.97% female (n=294)	79.49% female (n=93)
Weight (lbs)	150.73±40.44	145.77±36.43	166.15±49.23
BMI (kg/m^2)	24.57±5.90	23.78± 5.03	26.97±7.57
Body Fat Percentage	26.74±9.55	25.86±8.63	29.98±11.08

[a]38 participants excluded from final on the basis of missing data for race (n=20), inadequate information on race (n=6 declared "other"), or too small of a sample size for given race (n=9 Asian, n=3 Hispanic)

Table 4.2 *PKD2L1* rs603424 Genotype Frequencies by Race

	Caucasian	African American	Asian	Hispanic	Other	Total
AA	18	46	0	0	1	65
AG	86	54	5	0	3	148
GG	242	17	4	3	2	268
Total	346	117	9	3	6	481[a]

[a] Missing data on race for 20 subjects

Selection of Dietary Variables and Removal of Outliers

Dietary variables analyzed for association with rs603424 genotype were limited by the parameters of the DHQ II food frequency questionnaire. Items selected for analysis were based on them having a sour taste component (Vitamin C, citrus fruit, yogurt), another distinct flavor component that could have flavor-flavor interactions with sour (sugar, sodium, caffeine), or being a single food component, or close to it, rather than a large food or nutrient group that would be difficult to draw meaningful conclusions from (such as analyzing "whole grains" instead of "total carbohydrate intake").

Subjects that reported daily caloric intakes that fell outside of the range of 600-4,400 kcal/day were considered outliers and excluded from data analysis. In total, 58 subjects (11.58%) were excluded from the genotype and dietary intake analysis based on this criterion. The caloric range of 600-4,400 kcal/day is based on the 5th and 95th percentiles of energy intake from NHANES data for adult women greater than or equal to 12 years of age and is used by the National Cancer Institute's Automated Self-administered 24-hour dietary assessment tool for exclusion of implausible energy intakes (National Cancer Institute, 2017). The caloric range for women was used on the basis of it being more conservative than the range provided for men, and our study sample being over 80% female. There is no standardized recommendation for excluding outliers by

implausible energy intake for the DHQ II. Another 18 subjects who identified as Asian (n=9), Hispanic (n=3), or Other (n=6) for race were also excluded from analysis on the basis of the sample size of these groups being too small (Asian and Hispanic) or the category not providing meaningful data to draw conclusions from ("Other").

Genotype, Race, and Dietary Intake

After removing of outliers for implausible energy intake and selecting for Caucasian and African American subjects, a total of 425 subjects were included in this analysis. Whole grain intake was significantly influenced by both race ($p=0.005$) and genotype ($p=0.047$) (Figure 4.1). For nut intake there was a significant main effect for race ($p\leq0.001$), but not genotype, with Caucasian subjects having a greater mean intake of nuts than African American subjects (1.16 ± 1.79 vs 0.34 ± 0.60 servings/day). Meat intake and fatty fish intake were not significantly associated with race or genotype; however, poultry intake was significantly associated with race ($p=0.004$) and genotype ($p=0.031$) (Figure 4.2). There was no significant association with race or genotype for total vegetable intake, tomato intake, milk, eggs, and sodium intake.

Ten of the twenty dietary variables assessed had a significant interaction effect between race and genotype (Table 4.3). For these variables tests for simple main effects were carried out to look at the effect of genotype on dietary intake by each race separately (Table 4.4). Total fruit, citrus fruit, and vitamin C were significantly associated with genotype for Caucasian subjects, but not African American subjects. For each of these variables the GG genotype was associated with a significantly greater intake than the AA genotype. Total Dairy intake was significantly associated with genotype (AA vs AG) for African American subjects only (Figure 4.6), whereas yogurt intake was

significant for genotype for Caucasian subjects only (AA vs GG, Figure 4.7). For total sugar intake there was a significant difference in intake between the AG and GG genotypes for the Caucasian and African American groups (Figure 4.8). Added sugars intake was also significantly different between the AG and GG genotypes, but only for the African American group (Figure 4.9). The effect of genotype on caffeine intake was significant for the African American group only with a significant difference between the AG and GG genotypes (Figure 4.10). Simple main effects for genotype on alcohol intake and intake of non-whole grains were not significant for the Caucasian or African American groups.

Table 4.3 Two-way ANOVA Results Testing Effect of Genotype and Race on Dietary Intake

		Genotype		Race		Genotype x Race	
Dietary Variable	**n**	**F**	**p**	**F**	**p**	**F**	**p**
Total Fruit[c]	425	0.204	0.816	10.28	0.001	4.07	0.018
Citrus Fruit[c]	421	0.988	0.373	22.16	<0.001	6.15	0.002
Vitamin C[c]	425	0.816	0.443	25.81	<0.001	7.03	<0.001
Total Vegetables	425	1.206	0.300	0.920	0.338	2.554	0.079
Tomatoes	425	0.699	0.498	2.467	0.117	0.733	0.481
Total Dairy[c]	425	0.970	0.380	1.259	0.262	3.224	0.041
Milk	422	1.646	0.194	3.537	0.061	2.096	0.124
Yogurt[c]	272	0.271	0.763	1.763	0.185	3.497	0.032
Eggs	423	0.897	0.409	2.676	0.103	0.145	0.865
Meat	423	0.076	0.927	0.042	0.837	0.878	0.417
Fatty Fish	307	1.353	0.260	0.431	0.512	1.227	0.295
Poultry[a,b]	422	3.498	0.031	8.168	0.004	2.813	0.061
Nuts[b]	412	1.967	0.141	54.458	<0.001	0.060	0.942
Whole Grains[a,b]	422	3.090	0.047	7.954	0.005	0.328	0.721
Non-Whole Grains[c]	425	0.110	0.896	0.524	0.469	3.185	0.042
Total Sugars[c]	425	2.236	0.108	32.361	<0.001	6.99	0.001
Added Sugars[c]	425	2.488	0.084	28.446	<0.001	4.842	0.008
Sodium	425	0.872	0.419	0.621	0.431	2.543	0.080
Caffeine[c]	424	4.598	0.011	13.000	<0.001	6.841	0.001
Alcohol[c]	371	3.065	0.048	0.014	0.906	4.855	0.008

[a] Significant effect of genotype, [b] Significant effect of race, [c] Significant interaction effect

Table 4.4 Simple Main Effects by Race for Dietary Variables with a Significant Genotype x Race Interaction Effect

	Caucasian			African American		
Dietary Variable	**n**	**F**	**p**	**n**	**F**	**p**
Total Fruit[a]	329	6.54	0.002	96	1.18	0.312
Citrus Fruit[a]	325	6.50	0.002	96	3.06	0.052
Vitamin C[a]	329	6.22	0.002	96	2.93	0.058
Total Dairy[b]	329	0.81	0.445	96	3.86	0.025
Yogurt[a]	226	3.33	0.037	46	1.19	0.314
Non-Whole Grains	329	2.81	0.061	96	1.40	0.252
Total Sugars[a,b]	329	3.08	0.047	96	3.67	0.029
Added Sugars[b]	329	1.57	0.209	96	3.88	0.024
Caffeine[b]	328	0.422	0.656	96	6.05	0.003
Alcohol	292	2.417	0.091	79	2.96	0.058

[a] Significant effect of genotype on dietary variable for Caucasian group [b] Significant effect of genotype on dietary variable for African American group

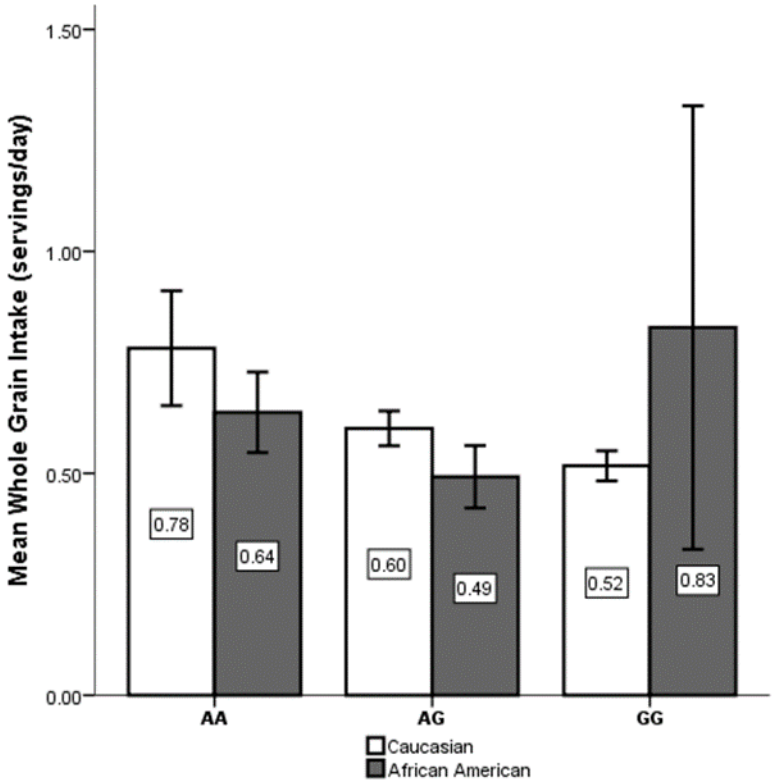

Figure 4.1 Whole Grain Intake (servings/day) by Genotype and Race

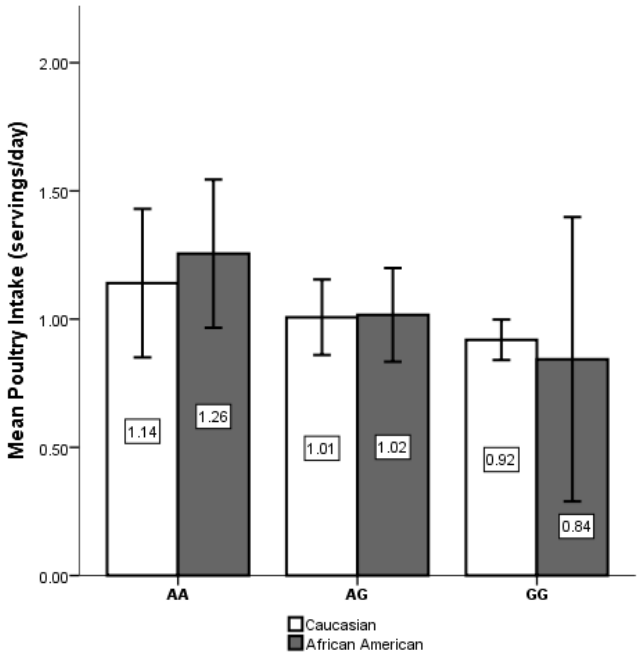

Figure 4.2 Poultry Intake (servings/day) by Genotype and Race

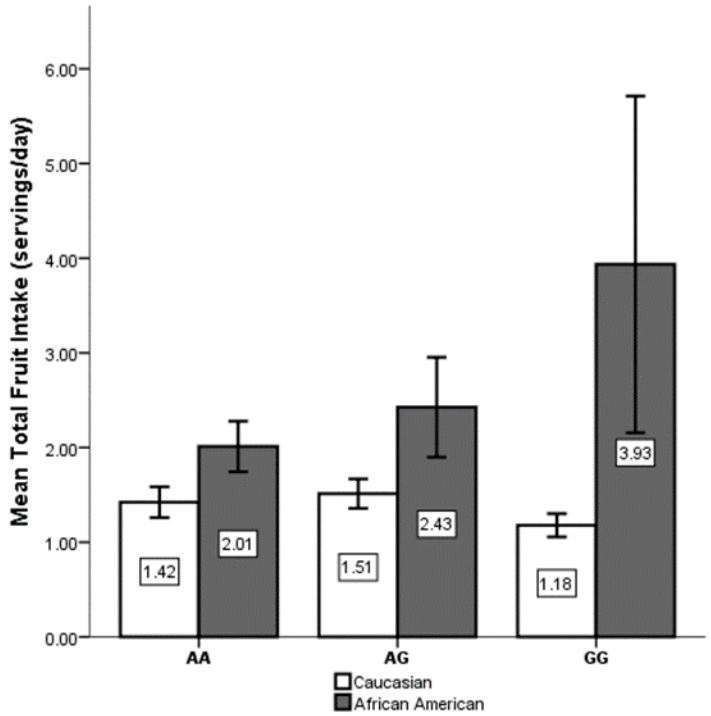

Figure 4.3 Total Fruit Intake (servings/day) by Genotype and Race

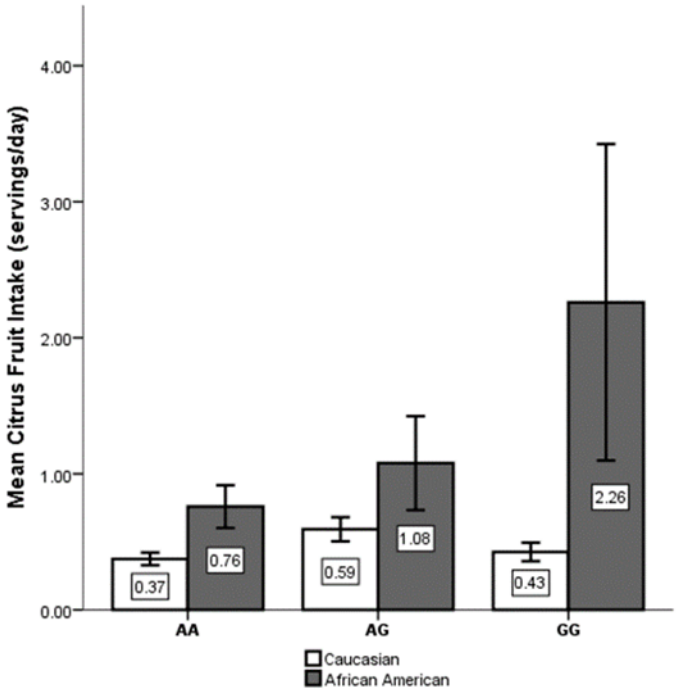

Figure 4.4 Citrus Fruit Intake (servings/day) by Genotype and Race

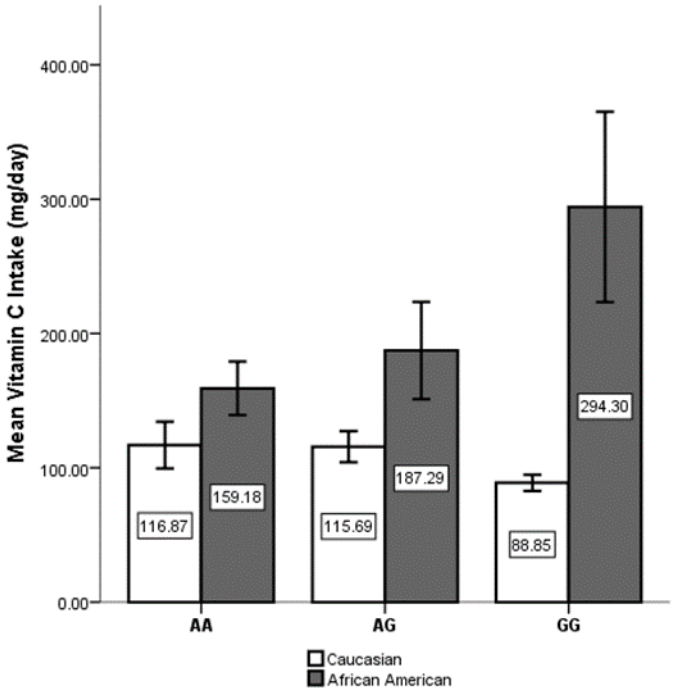

Figure 4.5 Vitamin C Intake (mg/day) by Genotype and Race

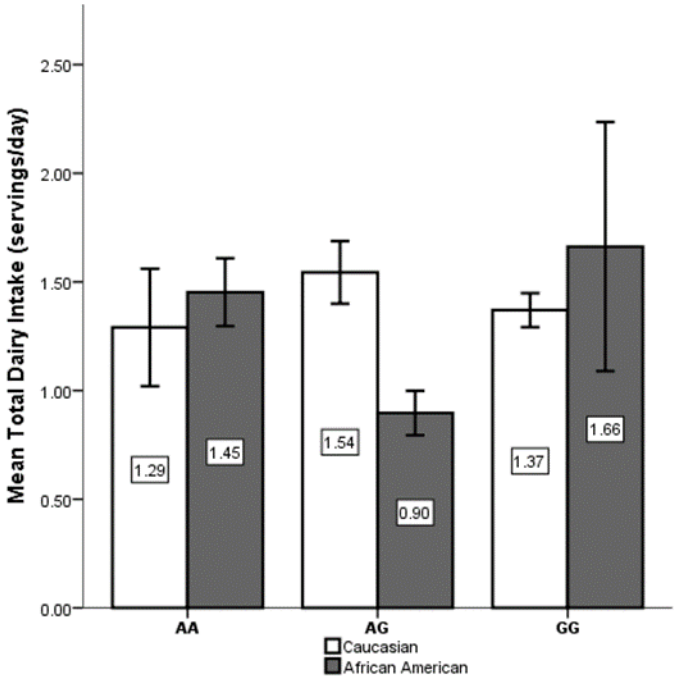

Figure 4.6 Dairy Intake (servings/day) by Genotype and Race

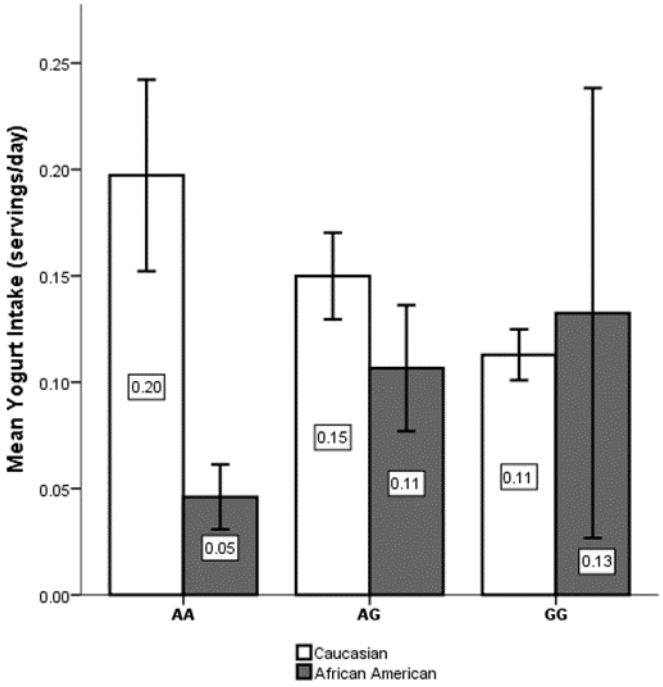

Figure 4.7 Yogurt Intake (servings/day) by Genotype and Race

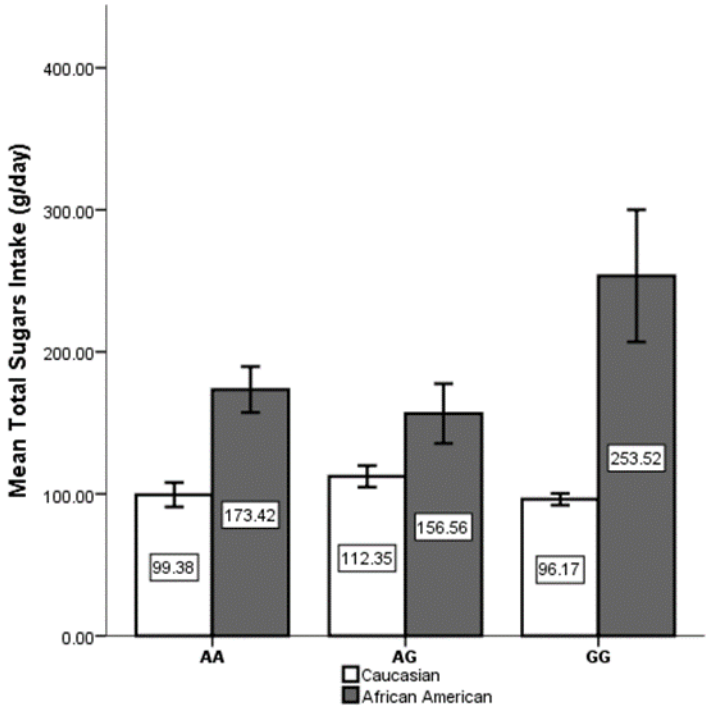

Figure 4.8 Total Sugars Intake (g/day) by Genotype and Race

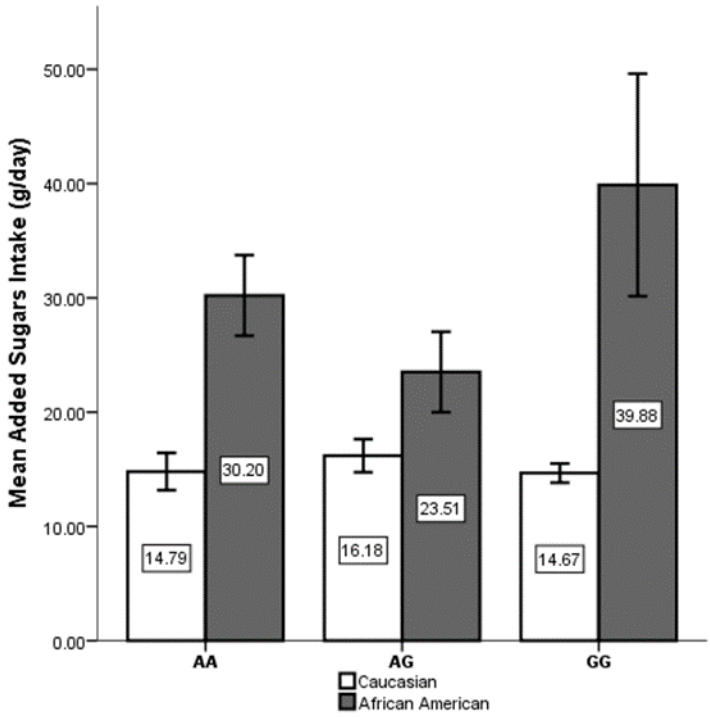

Figure 4.9 Added Sugars Intake (g/day) by Genotype and Race

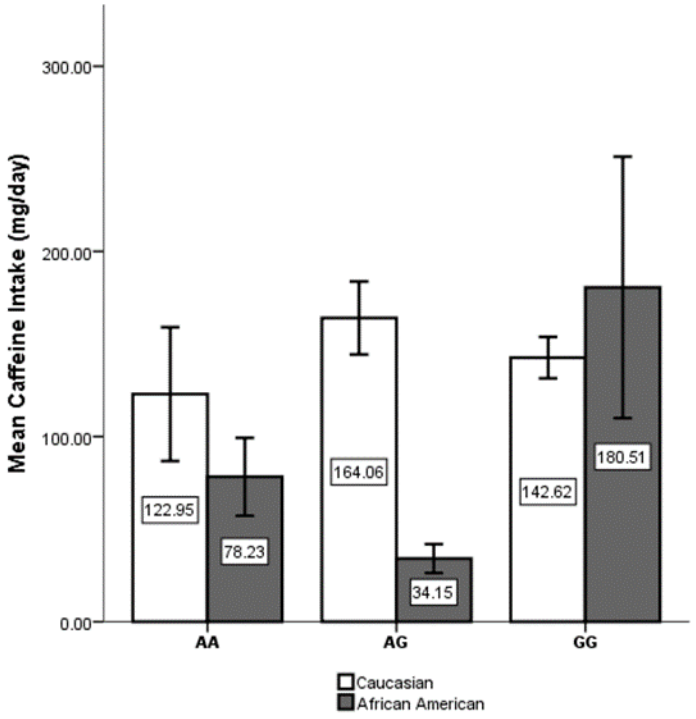

Figure 4.10 Caffeine Intake (mg/day) by Genotype and race

Genotype, Sex, and Dietary Intake

Another two-way ANOVA analysis was run to analyze the effect of genotype on dietary intake while accounting for sex rather than race (Table 4.5). When run this way citrus fruit, vitamin C, alcohol, caffeine, and total sugar intake were each significantly associated with genotype, but not sex, with no interaction effects. Added sugars were significant for sex as well as genotype. The dietary variables eggs, meat, fatty fish, and sodium were significantly different between sexes, but not by genotype.

Table 4.5 Two-way ANOVA Results Testing Effect of Genotype and Sex on Dietary Intake

		Genotype		Sex		Genotype x Sex	
Dietary Variable	**n**	**F**	**p**	**F**	**p**	**F**	**p**
Total Fruit[c]	393	5.38	0.005	0.59	0.443	5.09	0.007
Citrus Fruit[a]	389	3.26	0.039	2.35	0.126	1.471	0.231
Vitamin C[a]	393	4.34	0.014	1.66	0.198	1.117	0.328
Total Vegetables	393	0.53	0.59	0.13	0.716	0.609	0.544
Tomatoes	393	0.79	0.46	0.37	0.542	0.404	0.668
Total Dairy	393	0.18	0.837	0.972	0.325	0.487	0.615
Milk	391	0.46	0.631	1.771	0.184	1.557	0.212
Yogurt	246	0.05	0.948	0.043	0.836	0.017	0.983
Eggs[b]	391	1.74	0.177	6.96	0.009	2.818	0.061
Meat[b]	391	0.051	0.950	27.55	<0.001	0.271	0.762
Fatty Fish[b]	279	0.465	0.629	5.846	0.016	0.375	0.687
Poultry	390	0.68	0.506	0.394	0.531	0.72	0.487
Nuts	381	0.727	0.484	2.74	0.099	0.279	0.757
Whole Grains	393	0.963	0.383	0.275	0.601	1.539	0.216
Non-Whole Grains	393	0.167	0.846	1.28	0.258	0.088	0.916
Total Sugars[a]	393	3.57	0.029	3.754	0.053	0.858	0.425
Added Sugars[a,b]	393	4.729	0.009	7.331	0.007	0.209	0.812
Sodium[b]	393	0.900	0.407	17.310	<0.001	0.108	0.89833
Caffeine[a]	392	3.85	0.022	1.454	0.229	0.017	0.983
Alcohol[a]	339	3.87	0.022	0.625	0.430	1.876	0.155

[a] Significant effect of genotype, [b] Significant effect of sex, [c] Significant interaction effect

Genotype, Race, and Body Composition

PKD2L1 rs603424 genotype was significantly associated with BMI, but not body fat percentage or fat free mass (Table 4.4). There was, however, a significant association between body fat percentage and race (p=0.021). African American subjects had a higher mean body fat percentage than Caucasian subjects with the AA and AG genotype, but mean body fat percentages were similar for each race at the GG genotype (Figure 4.1).

Table 4.6 Two-way ANOVA Results Testing Effect of Genotype and Race on Body Composition

		Genotype		Race		Genotype x Race	
Body Composition Variable	**n**	**F**	**p**	**F**	**p**	**F**	**p**
Body Fat Percentage[b]	460	2.230	0.109	5.403	0.021	1.809	0.165
BMI[a,b]	460	4.680	0.010	5.767	0.017	2.780	0.063
Fat Free Mass (lbs)	460	1.485	0.228	1.849	0.175	1.911	0.149

[a] Significant effect of genotype, [b] Significant effect of race, [c] Significant interaction effect

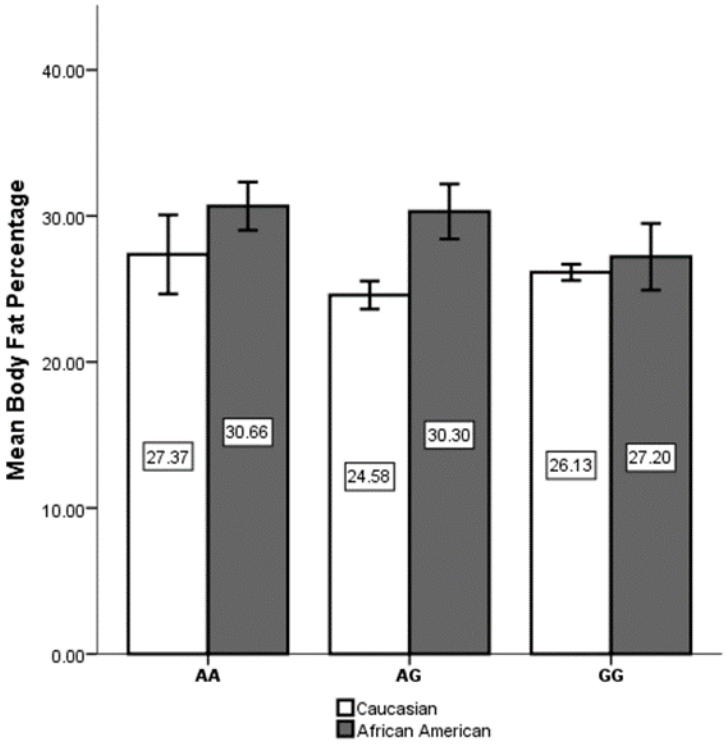

Figure 4.11 Body Fat Percentage by Race and *PKD2L1* rs603424 Genotype

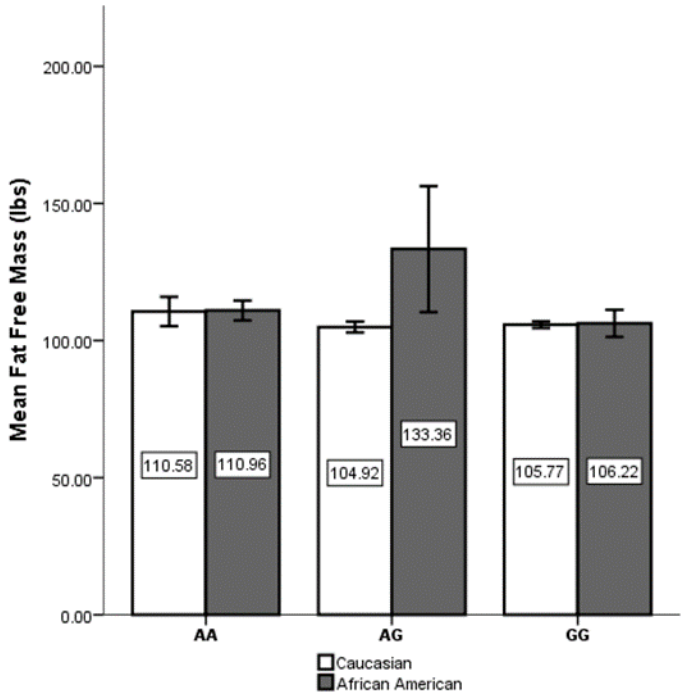

Figure 4.12 Fat Free Mass by Race and *PKD2L1* rs603424 Genotype

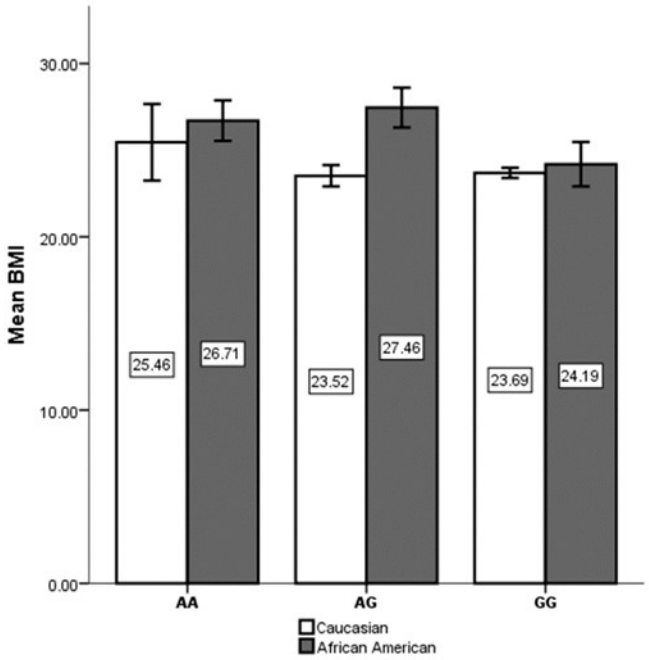

Figure 4.13 BMI by Race and *PKD2L1* rs603424 Genotype

CHAPTER V

DISCUSSION AND CONCLUSION

In this study 20 dietary variables were assessed for any relationship between their self-reported intake and *PKD2L1* rs603424 genotype in a group of young adult college students in Mississippi. The dietary variables most closely associated with sour taste (total fruit, citrus fruit, vitamin C, and yogurt) had mixed results. Total fruit, citrus fruit, yogurt, and vitamin C intake all had significant interaction effects between race and genotype. When looking further at the simple main effects, genotype was only significantly associated with their intake for Caucasian subjects, and it was only the AG and GG genotype groups that significantly differed. In a separate analysis looking at genotype and sex rather than genotype and race, citrus fruit and vitamin C intake were found to be significantly associated with genotype, but total fruit and yogurt no longer had any association with genotype. Part of this discrepancy is likely due to some tests for race and gender being underpowered due to a low number of male subjects, subjects of African American race, and subjects with the minor allele. For example, while there was only a significant difference in citrus fruit and vitamin C intake for the AG and GG groups for Caucasian subjects, it's possible that the sample size of the AA group (n=18 AA Caucasians) was simply too small to detect a statistical difference. For caffeine and alcohol, two substances with a bitter taste component that are commonly found in beverages, there were mixed results as well. Caffeine was only significantly associated

with genotype for African American subjects in the first ANOVA analysis, and post hoc testing revealed the only groups to be significantly different from each other to be the AG and GG genotypes for African Americans. In the second ANOVA analysis run by sex and genotype caffeine was significantly associated with genotype, but not sex, with no interaction effects. For alcohol intake by race and genotype simple main effects needed to be looked at as well. After looking at simple main effects there was no association with genotype for the Caucasian or African American group; however, when two-way ANOVA was run by sex and genotype alcohol intake was significantly associated with genotype. Total sugars intake, added sugars intake, total dairy, whole grains, and poultry intake also had varying degrees of mixed results when accounting for race or gender which as stated previously, is likely due to the vast majority of subjects being Caucasian females with the GG genotype, thus making it difficult to statistically detect differences in the other groups.

Regarding body composition, genotype was not significantly associated with body fat percentage or fat free mass; however, body fat percentage did vary significantly by race. Even though body fat percentage and fat free mass were not significantly associated with genotype, there was a trend of minor allele carriers having a higher body fat percentage and higher fat free mass than GG homozygotes. Since BMI does not discriminate between fat mass and fat free mass, there was likely a significant effect for genotype on BMI due to the additive effect of A carriers having a slightly higher fat mass and fat free mass.

An unexpected finding was that many dietary variables varied significantly by race or gender, regardless of genotype of the subject. Males consumed significantly more

sodium, fatty fish, meat, and eggs than females, and African American subjects had mean greater intakes of whole grains and poultry compared to Caucasian subjects. Overall, these results suggest that rs603424, a SNP in a gene proposed to be responsible for some degree of sour sensing, may influence dietary intake of citrus fruit, vitamin C, caffeine, and alcohol. The significance of these findings will remain somewhat uncertain until taste threshold studies and studies on perceived intensities of various taste sensations are done with this SNP.

Alcohol and caffeine both have very distinct bitter flavor components that contain a large degree of variation in interindividual liking. It is possible that there is some interaction between bitter and sour taste perception with a phenomenon described in the literature as "sour-bitter confusion." Many people, upon being given citric acid and caffeine solutions, will not be able to correctly label the citric acid as "sour" and the caffeine as "bitter." There is some debate over whether this could be due to lack of familiarity with the terms or with tasting these components in a pure form, or if it could be due to an underlying physiological difference. There is some evidence that it could be due to an underlying physiological difference. In one study, researchers took the subjects who misidentified sour as bitter or bitter as sour and attempted to train them on the different tastes and how to differentiate between them. The training resulted in some subjects correctly labeling the solutions in a future trial, but 35% of the subjects continued to make the same errors (Meiselman et al., 1967). In another study, it was discovered that the subjects with sour-bitter confusion tended to be PTC non-tasters, current smokers, or previous smokers (Doty et al., 2017). This points to the potential for the confusion to be not due to not understanding the sensory characteristics associated

with bitter and sour, but rather to be due to a true physiologic inability to differentiate between the two.

Future research will be needed to confirm the findings of this study as there were several confounding factors not accounted for here. Dietary intake is influenced by factors other than taste perception such as socioeconomic status, the food environment and convenience of foods, different cultural/regional eating patterns, cooking skills, nutrition knowledge level, and medically necessary diet adjustments such as for food allergies or intolerances. Most subjects in this study were students recruited from Mississippi State University dietetics courses. College students are a group gaining attention for being at an increased risk of food insecurity, and it is possible that degree of food security or food insecurity could be a factor in what students reported they were consuming over the past year (Henry L, 2017). Additionally, students enrolled in a college level nutrition course can be assumed to have a higher level of food and nutrition knowledge than the general population. Since most students in these classes elected to be there, it is also possible that this group has a greater degree of motivation to eat healthy than the general population does.

Another consideration when interpreting the results presented in this study is that the minor allele frequency for this SNP varies greatly by race. The minor allele for rs603424 is "A." In European populations the minor allele frequency for this SNP is 0.20; however, in African American populations the minor allele frequency is 0.61. (NCBI, 2018). These numbers appear consistent with the genotyping results of this study where 90% of subjects with the GG genotype were Caucasian and 70% of subjects with the AA genotype were African American. Additionally, Mississippi has a large racial disparity in

obesity rates, and this disparity could be seen in our study with 26.53% of Caucasian subjects being classified as obese by body fat percentage, and 46.15% of African American subjects being classified as obese by body fat percentage. When looking only at distribution of obesity status by genotype, without regard to race, one may initially be led to believe that this SNP is associated with obesity, when in fact this is more likely related to AA homozygotes being represented by the African American population (which has a great degree of health disparity in Mississippi), and the GG homozygotes being represented primarily by Caucasian subjects (who presented with a significantly lower rate of obesity than African American subjects).

With GG homozygotes consisting of mostly healthy weight Caucasian subjects and AA homozygotes consisting of mostly overweight or obese African American subjects, it is not unreasonable to assume that some differences in dietary habits by "genotype" are actually more likely attributed to differences in dietary pattern by obesity status. Studying the association between taste perception, food liking, and food intake is uniquely difficult in obese populations because while these factors may possibly influence obesity development, obesity also effects taste. The results of a recently published mice study suggest that taste loss is a metabolic consequence of obesity ultimately caused by obesity related chronic inflammation leading to decreased taste bud cell turnover (Kaufman et al., 2018). This is in line with previous studies in humans that have demonstrated a negative association between obesity status and taste sensitivity, and studies that have demonstrated the return of taste sensitivity to normal levels following weight loss surgery in obese individuals (Bartoshuk et al., 2006; Skrandies et al., 2015, Burge et al., 1995, Pepino et al., 2014).

In addition, as the subjects in this study were nearly all female (83%), it cannot be assumed that these results are generalizable to males as well. Eating patterns and habits can differ between males and females, and there may be differences in taste responsiveness by sex as well. A recent study done on a similarly aged population to this current study found that in college aged males, even a 1% increase in body weight resulted in taste responsiveness to sweet and salty decreasing. On the other hand, for college-aged females in the same study, taste responsiveness did not decrease with a similar amount of weight gain, and even increased by 6.5% for sour taste (Noel et al., 2017). Considering these results, perhaps in this current study (in which the vast majority of subjects are female) our overweight and obese female subjects may perceive sour stimuli as more intense than their healthy weight female counterparts, and more intense than their male counterparts.

Conclusion

There are many factors that contribute to the difficulty in finding and attributing differences in dietary intake and body fat percentage to genotype in this study sample. Future studies attempting to answer these questions may consider gender, race, and weight status matched participants. Future studies may also consider other methods of measuring food choice and dietary intake than the DHQ II. Food frequency questionnaires such as the DHQ II are commonly used in large, epidemiological studies in nutrition because they are inexpensive and pose a relatively low burden on the researcher to administer and the respondent to answer. Their strength lies in their ability to capture the general dietary habits of a large population. It is possible that if this SNP did indeed cause interindividual variation in sour taste perception, the effect on food

choice may be too small to be captured in a dietary assessment method meant for capturing broad differences in macronutrient and micronutrient intake across large populations. In addition, as the mechanism behind sour tasting at the molecular level becomes more understood, new avenues for studying variation in sour tasting genotype and phenotype will likely emerge.

In the meantime, there continues to be a significant gap in the literature regarding interindividual differences in sour taste perception, liking of sour foods, and how sensitivity to sour may change with body weight changes. Sour taste has historically lagged behind the other taste sensations in this field of research, but perhaps with the newfound interest in the topic caused by the discovery of Otop1 we will see answers to these questions soon. The results of this study noted significant differences in rs603424 genotype by race, and significant differences in dietary intake of caffeine, alcohol, citrus fruit, and vitamin C by genotype. Future studies should consider genetic variation in taste receptors between different racial and ethnic groups, as this could be a factor contributing to racial disparities in obesity development and chronic disease risk, as well as differences in general dietary patterns between ethnic groups. Health disparity is a complex issue influenced heavily by socioeconomic status, but if genetic variation in taste receptors by race contributed to even a small portion of this disparity it would still be of great importance to know and understand.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL LETTER

Office of Research Compliance

Institutional Review Board for the Protection of Human Subjects in Research
P.O. Box 6223
53 Morgan Avenue
Mississippi State, MS 39762
P. 662.325.3294

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NOTICE OF DETERMINATION FROM THE HUMAN RESEARCH PROTECTION PROGRAM

DATE:	May 07, 2018
TO:	Terezie Mosby, Food Sci Nutrition Hlth Promo, Qian Zhou;Wen-Hsing Cheng
PROTOCOL TITLE:	The influence of sour taste receptor genes on body composition and diet.
PROTOCOL NUMBER:	IRB-18-036
	Approval Date: May 07, 2018 Expiration Date: May 06, 2023

EXEMPTION DETERMINATION

The review of your research study referenced above has been completed. The HRPP had made an Exemption Determination as defined by 45 CFR 46.101(b)4. Based on this determination, and in accordance with Federal Regulations, your research does not require further oversight by the HRPP.

Employing best practices for Exempt studies are strongly encouraged such as adherence to the ethical principles articulated in the Belmont Report, found at www.hhs.gov/ohrp/regulations-and-policy/belmont-report/# as well as the MSU HRPP Operations Manual, found at www.orc.msstate.edu/humansubjects. Additionally, to protect the confidentiality of research participants, we encourage you to destroy private information which can be linked to the identities of individuals as soon as it is reasonable to do so.

Based on this determination, this study has been inactivated in our system. This means that recruitment, enrollment, data collection, and/or data analysis CAN continue, yet personnel and procedural amendments to this study are no longer required. **If at any point, however, the risk to participants increases, you must contact the HRPP immediately. If you are unsure if your proposed change would increase the risk, please call the HRPP office and they can guide you.**

If this research is for a thesis or dissertation, this notification is your official documentation that the HRPP has made this determination.

If you have any questions relating to the protection of human research participants, please contact the HRPP Office at irb@research.msstate.edu. We wish you success in carrying out your research project.

Review Type:	EXEMPT
IRB Number:	IORG0000467

Figure A.1 IRB Approval Letter

APPENDIX B

ABBREVIATIONS USED IN THESIS

Abbreviations Used in Thesis

ASIC: Acid sensing ion channel

CAR4: Carbonic anhydrase-4

CD36: Cluster determinant 36

DHQ II: Diet history questionnaire II

ENaC: Epithelial sodium channel

H3K4m1: Monomethylated histone H3, lysine 4

H3K27ac: Acetylated histone H3, lysine 9

H3K9ac: Acetylated histone H3, lysine 9

OTOP1: Otopetrin 1

PCR: Polymerase chain reaction

PKD1L3: Polycystic kidney disease 1 like 3

PKD2L1: Polycystic kidney disease 2 like 1

PROP: 6-n-propylthiouracil

PTC: Phenylthiocarbamide

SNP: Single nucleotide polymorphism

TAS2R4: Taste 2 receptor member 4

TAS2R5: Taste 2 receptor member 5

TAS2R16: Taste 2 receptor member 16

TAS2R38: Taste 2 receptor member 38

T1R2/T1R3: Taste receptor type 1 member 2/taste receptor type 1 member 3 heterodimer

T1R1/T1R3: Taste receptor type 1 member 1/taste receptor type 1 member 3 heterodimer

TRPV1: Transient receptor potential cation channel subfamily V member 1

APPENDIX C

SAMPLE EXCERPT FROM WEB-BASED DIET HISTORY QUESTIONNAIRE II

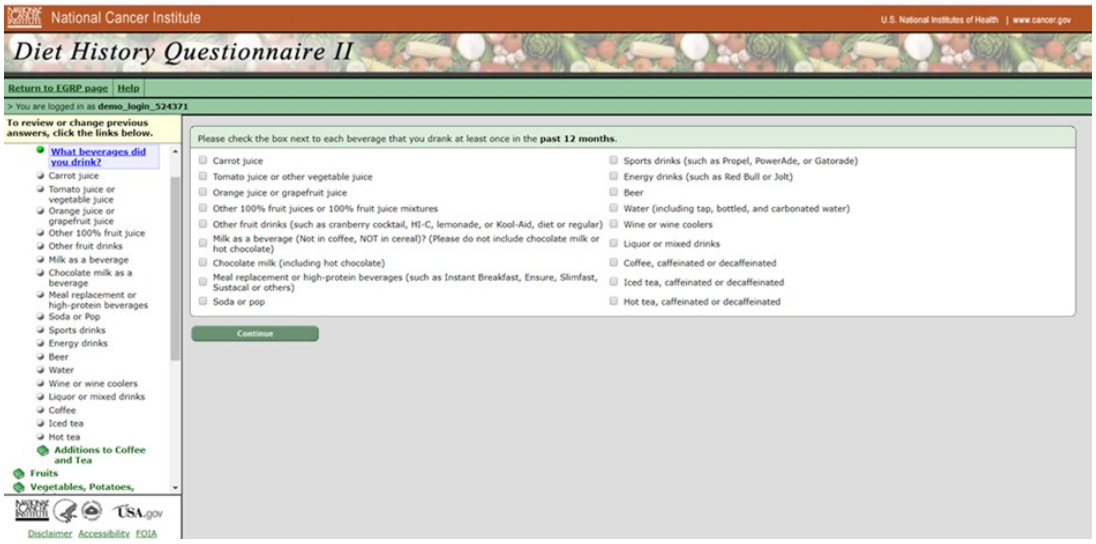

Figure C.1 Sample excerpt of question on beverages consumed over the past year

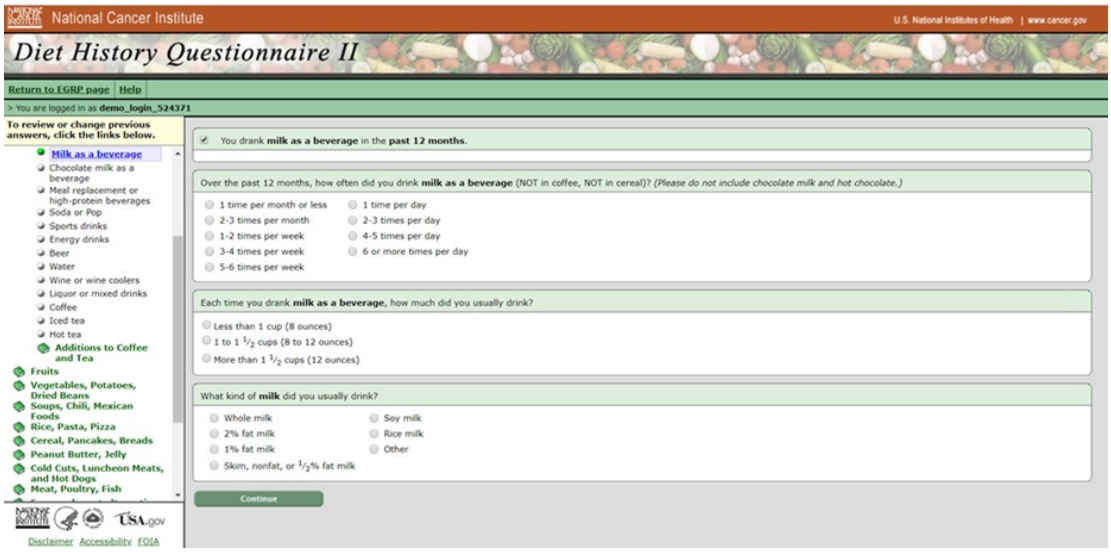

Figure C.2 Sample excerpt of portion sizes of beverages consumed