May 2017

Association of Genetic Factors with Chronic Kidney Disease in US Young Adults

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ASSOCIATION OF GENETIC FACTORS WITH CHRONIC KIDNEY DISEASE IN US YOUNG ADULTS

By

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Bachelor of Science- Health, Physical Education and Exercise Science
Virginia Commonwealth University
2014

A thesis submitted in partial fulfillment of the requirements for the

Master of Public Health

Department of Environmental and Occupational Health
School of Community Health Sciences
Division of Health Sciences
The Graduate College

University of Nevada, Las Vegas
May 2017

UNLV | GRADUATE COLLEGE

Thesis Approval

The Graduate College
The University of Nevada, Las Vegas

April 6, 2017

This thesis prepared by

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entitled

Association of Genetic Factors with Chronic Kidney Disease in US Young Adults

is approved in partial fulfillment of the requirements for the degree of

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Abstract

Albumin, a protein that when found in urine, is an early indicator of Chronic Kidney Disease (CKD). Genetic contributions have been shown to illustrate the gene-environment interactions that may lead to CKD in an individual; however, few studies have highlighted the interaction between responses of gene variations associated with the risk of increased albumin in young adults. This study's purpose is to evaluate the hypothesis that candidate genes are related to microalbuminuria in young adults of the third wave of the National Longitudinal Study of Adolescent Health (Add Health Wave III). This longitudinal study includes an assessment of various candidate genes typically reported to be associated with behavioral differences in individuals and urinalysis results collected from a large population of young adults. The data set also contains a twin & sibling sample that is analyzed to find an association between various genes while controlling for family relativity. Findings include an association found within the sibling sample between women and single nucleotide polymorphisms (SNPs), rs892413 & rs4950. A better understanding of genetic factors associated with kidney functioning in this age group could improve our knowledge of the complex genetics of kidney function as well as guide prevention and intervention methods.

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Chapter 1: Introduction

"Chronic Kidney Disease (CKD) is a condition in which your kidneys are damaged and cannot filter blood as well as healthy kidneys" (Center for Disease Control and Prevention, 2014). When kidneys are unable to filter waste, waste from blood remains in the body which can initiate many health problems. People living with early CKD rarely notice symptoms or feel ill and CKD can only be diagnosed through specific blood and urine testing (Center for Disease Control and Prevention, 2014). The progression of kidney disease can be slowed down or prevented through early detection and treatment, but many adults are not screened for kidney disease until it is in its advanced stages (National Kidney Foundation, 2016). Complications associated with Chronic Kidney Disease include high blood pressure, anemia, weak bones, poor nutritional health and nerve damage (National Kidney Foundation, 2016). Nephrologists, or Kidney Specialists, classify Chronic Kidney Disease by five stages according to the rate of Glomerular Filtration (GFR) ("Chronic Kidney Disease," n.d.). GFR is the rate at which kidneys filter waste and decreases in an individual as CKD progresses ("Chronic Kidney Disease," n.d.). Stage 5 CKD ultimately leads to necessary dialysis or kidney transplant in order to avoid death (National Kidney Foundation, 2016). Those at higher risk of CKD include adults over 60, with diabetes, high blood pressure, cardiovascular disease or family history of kidney failure, and African American and Hispanic ethnicities (National Kidney Foundation, 2016). According to the National Kidney Foundation (2016), 26 million adults in the United States have CKD and 1 in 3 American adults are currently at risk for developing kidney disease.

As the prevalence of diseases associated with overweight and obesity increase in adolescents, early Chronic Kidney Disease (CKD) prevalence has increased in adolescents in the past 20 years (Ferris et al, 2007). In the past 30 years in the United States, obesity has "doubled

in children and quadrupled in adolescents." (Centers for Disease Control & Prevention, 2015). From 1999-2010, National Health and Nutrition and Examination Survey (NHANES) reported that among children and adolescents in the U.S population, one-third were overweight or obese. Of U.S residents aged 2-19, adolescents (ages 15-19) were more likely than younger children (ages 2-4) to be overweight or obese ("Chronic Kidney Disease Surveillance System," n.d.). Factors associated with being overweight or obese increase the rates of hypertension, diabetes and other metabolic disease in adolescents (Ferris et al, 2007). According to the CDC, between 2011 and 2013 adolescents (ages 18-21) had the highest adjusted incidence of treated End-Stage Renal Disease (ESRD) of those under 22 years of age at 30 cases per million persons. ("Chronic Kidney Disease Surveillance System," n.d.).

Chapter 2: Background

While the risk of chronic kidney disease is increasing in the population due to higher rates of metabolic diseases, the understanding of the underlying risk factors associated with the progression of renal disease is still complex. The development of CKD varies in individuals based on ethnic background, lifestyle factors, and genetic factors (Kottgen et al, 2010). Risk factors such as hypertension or diabetes that may contribute to the progression of kidney disease can be prevented by moderate exercise, a healthier diet, and weight reduction (Office of Disease Prevention and Health Promotion, 2017). Behavior modifications to lifestyle choices can significantly reduce the progression of CKD, but some populations, such as Hispanics or African Americans, are disproportionately disadvantaged (Office of Disease Prevention and Health Promotion, 2017). Modifiable factors within an individual's immediate environment have a significant influence on the progression of CKD, but genetic determinants also have a large influence on the development of CKD (Office of Disease Prevention and Health Promotion, 2017). While it is difficult to indicate one specific gene x environment interaction with the risk of CKD, many studies have characterized chronic kidney disease as having multiple environmental exposures that could influence the many biological processes involved with the progression of CKD (Bruce et al., 2009). Exposure to environmental, social or behavioral factors, such as depression, anxiety, stress, economic deprivation, discrimination, smoking, and drug use can adversely affect the etiology of CKD and initiate responses in the nervous or vascular system that may increase the risk for progression of CKD (Bruce et.al, 2009). Responses that effect blood pressure, heart rate, and vascular reactivity are thought to be associated with alterations in the sympathetic/autonomic nervous system and may provide an

etiological link between stress, hypertension and CKD, particularly in high risk populations (Bruce et al., 2009)

Identifying gene variants associated with the risk of common diseases has been difficult, but recent genome-wide association studies (GWAS) evaluation of common diseases have assessed common diseases and improved our overall knowledge of genetic influences (Melzer et al, 2008). Rather than associating genetic factors to the disease, many studies have begun to approach disease etiology by studying the association of gene variation and gene expression (Melzer et al, 2008). Identifying genetic variations that influence serum or plasma protein levels of common disease are more likely to be more associated directly to the progression of a specific disease (Melzer et al, 2008). Although there are multiple factors that influence serum and plasma protein levels, the role that genetic variation has on differences in mRNA transcription and the MRNA translation to protein that follows mRNA transcription could be more implicative of an association of a genetic variation and the resulting expression of a disease (Melzer et al, 2008). It is often unknown whether protein levels are involved in the disease progress or a result of the disease process and identification of gene variants associated with serum and plasma proteins could improve the knowledge on these relationships (Melzer et al, 2008).

Multiple GWAS have already demonstrated the relationship between some specific genetic variants, estimated glomerular filtration rate (eGFR), CKD and End Stage Renal Disease (ESRD). Markers of Chronic Kidney Disease, such as serum creatinine (SCr), eGFR, and/or albumin, have been found to account for 29% to 33% of heritability estimates (Gudbjartsson et al, 2010). Recent GWAS have found significant associations between estimated glomerular filtration rate based on serum creatinine (eGFRcrea) and genes at three loci: UMOD, SHROOM3, and GATM-SPATA5L1. Gudbjartsson and colleagues (2010) also found significant

associations in an Icelandic adult population by analyzing eGFR based on cystatin C (eGFRcys), another measure of kidney function, and genes at two loci: CST9 and STC1 (Gudbjartsson et al, 2010). Additional genes found associated with renal function and Chronic Kidney Disease include: LASS2, GCKR, ALMS1, TFDP2, DAB2, SLC34A1, VEGFKA, PRKAG2, PIP5K1B, ATXN2, DACH1, UBE2Q2, and SLC7A9 (Kottgen et al, 2010). Kottgen and collegues (2010) also found 7 genes suspected to affect production and secretion of the creatinine protein-CPS1, SLC22A2, TMEM60, WDR37, SLC6A13, WDR72, and BCAS3. Other significant associations have also been shown in African Americans and genes associated with albuminuria located at two loci: MYH9 & APOL1 (Freedman et al, 2009). Risk alleles common in African Americans demonstrate the genetic and environment differences between individuals that are deserving of additional study (Friedman et al, 2011). Other loci found to be considered associated with genetic kidney diseases are ELMO1, NPHS1, NPHS2, INF2, LAMB2, PLCE1, ACTN4, TRPC6, WT1, PKHD1, and NPHP1-NPHP9 (Hildebrandt, 2010).

Genetic technology has increased knowledge of diagnosis, classification, pathogenesis, and personalized therapy surrounding kidney disease (‘Inherited Kidney Diseases’, 2014). Increased susceptibility to CKD can be caused by a combination of environmental factors, genes, and their interactions (Satko et al, 2005). Even with the most common causes of kidney disease being diabetes and high blood pressure, genetic factors increase the varying degrees of progression of chronic kidney disease in individuals (Eikmans et al, 2006). Genetic variants found in association with the risk of CKD can be identified in genes for heart and vascular diseases, glomerulonephritis, diabetes, inherited renal diseases or genes that maintain ionic balance (Rosas, 2012). According to Eikmans and colleagues (2006), genetic components of kidney disease could be initiated from a single gene mutation which could result in interrupting

the function of a corresponding protein; or several genetic factors could mutate only in the presence of other systemic diseases, such as hypertension or diabetes. The etiology surrounding many kidney diseases has been revealed to be due to single-gene defects and are associated with risk alleles (variants of genetic material related to an increased risk of developing a certain disease) ('Inherited Kidney Diseases', 2014). Single- gene disorders, or monogenic diseases, occur when a mutation of a single gene (out of more than 25,000 genes) is the cause of a disease. A polygenic disorder occurs when multiple genes mutate simultaneously and result in a disease (Hildebrandt, 2010). According to Hildebrandt (2010), a mutation within a monogenic recessive gene has an increased power to "fully penetrate" or manifest into CKD during childhood or adolescence and leave little room for environmental influences. When compared to monogenic recessive genes, monogenic dominant genes may not fully manifest into the outcome of CKD until adulthood and may vary according to generation or organ involvement (Hildebrandt, 2010). In contrast, multiple risk alleles associated with polygenic diseases typically manifest during adulthood and they leave more room for environmental influences since they show less heritability within an individual (Hildebrandt, 2010). Regardless of where the gene is expressed, the relationship between progression of renal disease and genetic expression is obvious, and identifying additional genetic variants that could impact the progression of renal function may increase the understanding of the etiology of CKD in adolescents (Eikmans et al, 2006).

Detection of CKD early can prevent the progression of the disease, but early detection can be difficult due to signs and symptoms of CKD being overlooked until later stages of CKD ("Chronic Kidney Disease," n.d.). Albumin, a protein sometimes found in the urine, has well documented cases of understanding kidney disease in the older population, including being a marker for early diagnosis of Cardiovascular Disease (CVD) in the short term and End-Stage

Renal Disease (ESRD) in the long term (National Kidney Foundation, 2016). In individuals with or without diabetes, testing for albumin in the blood serum may be the first step to detecting undiagnosed CKD (Jong et al, 2006). Albumin is present normally in the urine but when the level is elevated to between 30-300mg/dL, it is an early indicator of a progressive loss of renal function. Elevation of albumin above 30mg/dL, or microalbuminuria, is an early sign of progressive cardiovascular and renal disease and albumin levels above 300mg/dL usually indicated that the person is in stage 3 or 4 CKD (Jong et al, 2006).

The interaction of specific genetic alleles with albumin expression can be important in understanding additional gene and environment interactions. The purpose of this study is to assess the association between candidate genes and urinalysis results collected from a large cohort. We will evaluate the overall association of various candidate genes and urinalysis results, the association of candidate genes and urinalysis results between genders, and the association of candidate genes and urinalysis results when controlling for other risk factors related to Chronic Kidney Disease.

A better understanding of additional genetic variations associated with kidney functioning in adolescents could improve our knowledge of the complex biological pathways effecting the decrease in kidney function as well as guide prevention and intervention methods.

Research Questions

The research questions that will be evaluated in this study are as follows:

1. Is there a significant association between candidate genes related to psychosocial & behavioral traits and elevated albumin in adolescents?
2. Is there a significant association between candidate genes related to psychosocial & behavioral traits and elevated albumin when separated by gender?

3. Are the various candidate genes related to psychosocial & behavioral traits significant predictors of elevated albumin when controlling for additional risk factors of Chronic Kidney Disease?

Hypotheses

The hypotheses for research question #1 to be evaluated in this study are:

H1o: There is no significant association between candidate genes related to psychosocial & behavioral traits and elevated albumin found in adolescents ($\rho=0$)

H1a: There is a significant association between candidate genes and elevated albumin found in adolescents ($\rho\neq0$)

The hypothesis for research question #2 to be evaluated in this study are:

H2o: There is no significant association between candidate genes and elevated albumin when separated by gender ($\rho M=0$)

H2a: There is a significant association between candidate genes and elevated albumin when separated by gender ($\rho\neq0$)

The hypothesis for research question #3 to be evaluated in this study are:

H3o: Each candidate gene is not a significant predictor of elevated albumin when controlling for additional risk factors ($\rho=0$)

H3a: Each candidate genes is a significant predictor of elevated albumin when controlling for additional risk factors ($\rho\neq0$)

Chapter 3: Methods

Study population

The purpose of this retrospective cohort study is to assess the hypothesis that candidate genetic variants are associated with albuminuria in young adults who participated in the third wave of the National Longitudinal Study of Adolescent Health (Add Health Wave III). This study will include an assessment of urinalysis and DNA samples collected from a large population of young adults, which is not commonly studied concerning progressive CKD.

The National Longitudinal Study of Adolescent Health, lasting over 10 years, is the largest nationally representative data set to also include a urinalysis for most of its participants. The study, initiated in 1994, consists of in school or in home interviews between 4 different waves. The participants of the study are part of a cohort or young adults aged 18 to 26 years. The study used multistage, stratified, clustered sampling within schools to ensure the population was representative of U.S. adolescents. After randomization, students were stratified by grade and sex, and selected out of 200 from each school pair. In Wave 3, conducted in 2001-2002, there was 77.4% response rate of participants from Wave 1. Wave 3 consisted of 15,197 participants from Wave 1, many of which were in between the ages of 18-26 at the time of Wave 3. During Wave 3, 1,507 romantic partners were also interviewed. Biomarkers collected during this wave included: height and weight used to determine BMI, urine sample collection in order to determine STI status, and genetic buccal cell DNA to compare twin and sibling data.

Urinalysis data and specimen collection

Respondents completed a survey including responses on demographics, education, socioeconomic status, height, weight, medical conditions, and smoking history. Height and

weight was measured by field workers and BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). 12, 566 urine samples were initially available. Samples were discarded if they were improperly shipped, not kept at the correct temperature, or insufficient (n=390). All other samples (n=12,176) underwent urinalysis. Urine samples from romantic partners were excluded, leaving the total sample for urinalysis at 10,867 respondents.

Urinalysis results were defined as positive for albumin when >30 mg/dl (0.30 g/L). Macroalbuminuria and microalbuminuria measurements were collected in a subset of respondents, chosen by BMI to maximize statistical power. Of the 10,868 urinalysis results from Wave I participants (romantic partners excluded), 2000 samples were randomly selected from three BMI groups (n=6000): ≤ 28, 28.01 to 29.99, ≥ 30 kg/m (Smolen & Hewitt, 2009). To limit false-positive albuminuria values, exclusions were applied based on the PREVEND study by excluding pregnant (n=219) women and those with >75 WBC/ul (n=1,026) or >50 RBC/ul (n=599) (Verhave et al, 2004). "Exclusion criteria was applied to rule out microalbuminuria associated with pregnancy, urinary tract infections or any blood disorders not related to kidney disease; therefore they differ from definitions that typically describe urinalysis findings, with the exception of glycosuria." (Verhave et al, 2004). After applying exclusion criteria, urine results were merged with the genetic sample yielding 1,210 respondents that were included in the analysis for albuminuria.

Additional measures in this study include race, age, gender, education and having smoked at least one cigarette within the past 30d. History of hypertension and diabetes were self-reported.

DNA data and specimen collection

Respondents who were selected for DNA sample were asked to insert one sterile cytology brush into the mouth and rub the cheeks and gums for 20 seconds to collect buccal cells. Test tubes were labeled for each respondent and packaged for shipment with ice packs to maintain a temperature of 4°C until received at the University of Arizona laboratory for DNA extraction. Specimens were later transferred to the University of Colorado for genetic typing and analysis (Smolen & Hewitt, 2009).

To genotype DNA for five candidate polymorphisms, saliva samples were collected from full siblings or twins. "These candidate genes have been reported to be associated with individual differences in behavior related to mental health; are reported to be functional, exonic, in promoter regions, or affect gene expression; are expressed in the brain; and have prima facie involvement in neurotransmission" (Smolen & Hewitt, 2009). The candidates are the dopamine transporter (DAT1), the dopamine D4 receptor (DRD4), the serotonin transporter (5HTT), monoamine oxidase A (MAOA), and the dopamine D2 receptor (DRD2). The following additional genotypes were also available for Wave III respondents: CYP2A6, rs2304297, rs892413, rs4950, and rs13280604. The candidate genes evaluated in this study are introduced in detail below:

Dopamine Transporter (locus symbol: SLC6A3) - Variable Name: DAT1 A & B

The dopamine active transporter 1 gene, mediates the reuptake of dopamine from the synapse and is shown to be associated with expression of the DAT protein in lymphocytes and in human striatum ("DAT1 Gene," n.d.). DAT1 contains variable number tandem repeats (VNTRs), where specific locations in the gene can have multiple numbers of repeats and can cause individual differences in expression of protein levels. VNTRs in the DAT1 gene have been

reported to be associated with attention deficit/hyperactivity disorder (ADHD), bipolar disorder, and antidepressant use (“DAT1 Gene,” n.d.). Allelic sequences within this study to be evaluated per the DAT1 gene include 11R 10R, 7R, 8R, and 9R as reported individually.

Dopamine D4 Receptor (DRD4) - Variable Name: DRD4 A & B

The dopamine D4 receptor gene, encodes for a D4 subtype which is a G-protein couple receptor that inhibits adenylyl cyclase (“DRD4 Gene-Protein Coding,”n.d.). The dopamine D4 receptor “is a target for drugs that treat schizophrenia and Parkinson disease and mutations within this gene have been related to many behavioral expressions such as autonomic nervous system dysfunction, ADHD, and the personality trait of novelty seeking” (“DRD4 Gene-Protein Coding,”n.d.). Allelic sequences within this study to be evaluated per the DRD4 gene include 10R, 9R, 8R, 7R, 6R, 5R, 4R, 3R, and 2R as reported individually.

Serotonin Transporter (5-HTT, locus symbol: SLC6A4) – Variable Name: HTTLPR A & B

The serotonin transporter encodes for a protein which is responsible for the reuptake of serotonin into the presynaptic cell after being released into the synaptic cleft to signal the adjacent neuron (“5-HTT:The Depression Gene,"n.d.). Alleles of the 5-HTT gene are either short (S) or long (L) , and encode for less or more of a protein according to the length of the allele. Variations of the serotonin transporter has shown to be associated with anxiety related personality traits, risk of developing depression, alcoholism or suicidal behavior (“5-HTT:The Depression Gene,"n.d.). Recent studies of Argentinean adolescents have found an association between the polymorphism of the serotonin transporter and being overweight (Fuemmeler, 2009).

Monoamine Oxidase- A promoter (MAOA-uVNTR) – Variable Name: MAOA_VA & MAOA_VB

The product of gene Monoamine Oxidase-A plays an important role in the degradation of neurotransmitters such as serotonin, norepinephrine, and dopamine (Brummett et al, 2007). MAOA expression has been related to psychological and physical measures and individuals with less active MAOA-uVNTR alleles have found to be at increased risk for depressive symptoms and poor sleep (Brummett et al, 2007). Allelic sequences within this study to be evaluated per the MAOA-uVNTR gene include 5R, 4R, 3.5R, 3R and 2R as reported individually.

Dopamine D2 Receptor (Taq1A) – Variable Name: DRD2

The dopamine D2 receptor gene contains multiple variants which include single nucleotide polymorphisms (SNPs), di-nucleotide repeats, and restriction endonuclease sites in coding and non-coding regions (Pohjalainen et al, 1998). Alleles associated with the dopamine D2 receptor gene are found to be associated with a reduced number of dopamine binding sites, which can play a role in addictive behaviors such as alcoholism, smoking, and certain neuropsychiatric disorders (Pohjalainen et al, 1998). DRD2 expression has also been found to be associated with high blood pressure and protective effects in human renal proximal tubule cells (Pohjalainen et al, 1998). This association suggest that carriers of this SNP may be prone to chronic renal disease and high blood pressure (Pohjalainen et al, 1998).

Cytochrome P450 (CYP2A6) - Variable Name: CYP2A6B

The cytochrome P450 2A6 is the main catalyst of the oxidation of nicotine and continine (“CYP2A6 Gene-Protein Coding,”n.d.). CYP2A6 encodes for a member of the cytochrome P450 superfamily of enzymes (“CYP2A6 Gene-Protein Coding,”n.d.). Cyctochrome proteins are found to be associated with the metabolism and synthesis of various drugs, cholesterols, steroids,

and other lipids (“CYP2A6 Gene-Protein Coding,”n.d.). Individuals containing a certain allelic variant of CYP2A6 have shown to have a poorly metabolize coumarin or nicotine and is associated with reduced number of cigarettes smoked (“CYP2A6 Gene-Protein Coding,”n.d.).

Neuronal nicotinic cholinergic Receptors (nAChRs)

Four receptors have been identified within the CHRNA6 & CHRNB3 genes of the nAChRs family. They are expressed in cell bodies and nerve terminal within the brain where they regulate the release of neurotransmitters such as glutamate, GABA, serotonin, norepinephrine, and dopamine (”CHRNB3 Gene-Protein Coding,”n.d.). nAChRs are typically active by acetycholine, but nicotine and other compounds have been found to affect the function of these specific receptors, at low concentrations (”CHRNB3 Gene-Protein Coding,”n.d.). Genome wide studies have shown that variations of these receptors are related to the likelihood and severity of nicotine dependence (”CHRNB3 Gene-Protein Coding,”n.d.). A total of two single nucleotide polymorphisms (SNPs) has been genotyped for each cholinergic receptor, CHRNA6 & CHRNB3, respectively (”CHRNB3 Gene-Protein Coding,”n.d.). For subunit nAChR alpha-6 (CHRNA6), SNPs include rs2304297 (variable name=s000001) and rs892413 (variable name=s000002). For subunit nAChR beta-3 (CHRNB3), SNPs include rs4950 (variable name=s000003) and rs13280604 (variable name=s000004).

Hypothesis 1 Analytical Methods

Frequencies, percentages, or means with standard errors (SE) were included as descriptive statistics for demographics, health characteristics, and urinalysis results. For independent samples, a Pearson Chi-squared test will be calculated for each candidate gene to test the hypothesis that various gene types are associated with microalbuminuria (albumin >30

mg/dl). The Pearson Chi-Squared test is calculated by using frequencies observed within categorical variables and frequencies expected within those categorical variables (Fields, p.690). Fisher's exact test will also be used to compute the exact probability of the chi-squared statistic and its accuracy when sample sizes are too small (Fields, p.688). Because the genetic sample includes families that consist of twins and full or half siblings, a repeated measures model will be used to control for the association of family relativity. Therefore, to effectively test the association between the gene variant and albuminuria rather than the association within the family, a test statistic from the repeated measurement model will be used to accurately analyze the association. Associations will be considered significant if the p-value is less than 0.05. If the p-value is less than 0.05, the null hypothesis will be rejected. Rejecting the null hypothesis will indicate that the candidate gene is associated with microalbuminuria. Frequencies and regression models will be performed by using SPSS 23 and SAS 9.4.

Hypothesis 2 Analytical Methods

The Pearson Chi-squared test will be calculated within the independent samples population for each gender along with each candidate gene to test the hypothesis that various gene types are associated with microalbuminuria (albumin >30 mg/dl). Fisher's exact test will be used to compute a stronger more accurate probability of the chi-squared statistic when the sample size is too small. The repeated measures model will be used in the sibling & twin sample to test associations while controlling for family relativity. Associations will be considered significant if the p-value is less than 0.05. If the p-value is less than 0.05, the null hypothesis will be rejected. Rejecting the null hypothesis will indicate that candidate genes within genders are

associated with microalbuminuria. The Pearson Chi-Squared test and repeated measures models will be performed using SPSS 23 and SAS 9.4.

Hypothesis 3 Analytical Methods

Gene variants found to be significantly associated from the Pearson Chi-Squared test analysis will be used for evaluation in the final multilinear model. Logistic regression will be used to assess the association of significant genetic variations with albuminuria as well as race, sex, Wave III BMI, diabetes, hypertension, and smoking. The repeated measures model will be used in the sibling & twin sample to test for associations while controlling for family relativity. Variables associated with albuminuria in univariate analysis or that influenced the association between DNA types and albuminuria will be considered significant if the p-value is less than 0.05. Rejecting the null hypothesis will indicate that various candidate genes are associated with microalbuminuria when controlling for other risk factors. Multiplicative interactions of BMI with sex and race will be explored using multilinear regression. Logistic models will be analyzed using SPSS 23 and SAS 9.4.

Limitations

There are several limitations in this study due to data availability and data collection. "Kidney Disease cannot be assessed using a single urine specimen, and could be subject to random variation in measures of proteinuria due to prolonged standing or exercise" (Ferris et al, 2007). This study was not able to use urinalysis samples collected over a time-period to show steady albumin levels within respondents. This study also lacked the use of an albumin-to-creatinine measurement which is clinically preferred for diagnosis of kidney disease; however,

"single-measure albuminuria does reflect a chronic condition in 63% of the general population" (Ferris et al, 2007). Collection of urine for urinalysis samples was collected by respondents at any time of the day, whereas first-morning midstream urine is more specific for albuminuria and urinalysis in clinical settings (Ferris et al, 2007). This study utilized self-reported hypertension and diabetes which could indicate undiagnosed or untreated diabetes but did not include clinical factors such as measured blood pressure, serum creatinine, or medication use (Ferris et al, 2007).

Chapter 4: Results

The Add health respondents for whom urinalysis (n=10,226) and macroalbumin and microalbumin (n=6,306) were analyzed are similar to the overall Add Health Wave III respondents (n=15,197) with respect to age, education, diabetes, hypertension, and smoking (see Table 1).

In Add health respondents for whom genetic samples were analyzed along with macroalbumin and microalbumin (n=1,210), the measured mean for macroalbumin was 12.78 mg/dL (range 0.1-5567.20) and 9.87 mg/dL (range 0.001-7641.05) for microalbumin. Microalbuminuria was prevalent among 4% or respondents with a macroalbumin measurement (n=48), while microalbumin was present among 6.3% of respondents with a microalbumin measurement (n=76). With the exception of BMI, those with or without macroalbumin or microalbumin measurements were not different among many characteristics, although those with microalbuminuria had consistently higher representations of hypertension and smoking. BMI was higher among those with microalbumin in the highest BMI category (>35), which is consistent with recent findings (See Tables 2 & 3).

The respondents included in the independent sample (n=578) were analyzed using a Pearson Chi-Squared test and a Fisher's Exact test when applicable, while the sibling & twin sample (n=1,210) were analyzed using a repeated measurements model.

Hypothesis 1 Results

Gene variants to be found significantly associated ($p>0.05$) with microalbuminuria in the independent sample included Cytochrome P450 2A6-allele B (variable name: CYP2A6B), rs2304297- allele A (variable name: S000001A), rs2304297- allele B (variable name:

S000001B), rs892413- allele A (variable name: S000002A), rs892413- allele B (variable name: S000002B), rs4950- allele B (variable name: S000003B), and rs13280604- allele B (variable name: S000004B). No gene variants were found to be significantly associated with microalbuminuria in the sibling & twin sample (See Table 4). No gene variants were found to be significantly associated with microalbuminuria in the independent sample or the sibling & twin sample. Although there was no significant association found between microalbuminuria and gene variants, dopamine transporter-DAT1-allele B (variable name: DAT1B) & rs892413 - allele B (variable name: S000002B) were approaching significance (See Table 4).

Hypothesis 2 Results

Significance in the association between genetic variations and albumin differed between male and female respondents. For women within the independent sample population, rs2304297 - allele A (variable name: S000001A), rs2304297 - allele B (variable name: S000001B), rs892413 - allele B (variable name: S000002B), rs4950 - allele A (variable name: S000003A), rs4950 - allele B (variable name: S000003B), and rs13280604 - allele A (variable name: S000004A) were significantly associated with macroalbumin when compared to male respondents. For women in the sibling & twin sample, rs892413 - allele B (variable name: S000002B) was found to be significantly associated with macroalbuminuria at p=0.03 (See Table 5). For microalbumin in the independent sample population, rs2304297 - allele B (variable name: S000001B), rs892413 - allele B (variable name: S000002B), and rs4950 - allele A (variable name: S000003A) were significantly associated for both male and female respondents, while Monoamine Oxidase A-uVNTR-allele A (variable name: MAOA_A), Monoamine Oxidase A-uVNTR-alB (variable name: MAOA_B), & rs13280604 - allele A (variable name: S000004A) were only significantly associated for men. In both samples collectively, female gender was

found to be significantly associated with rs892413 - allele B (variable name: S000002B) and rs4950 - allele A (variable name: S000003A) (See Table 6).

Hypothesis 3 Results

A logistic model was used to control for variables such as gender, race, BMI, hypertension, diabetes, and age. Genetic variants that were found to be significantly associated with macroalbumin within the independent sample when controlling for these factors include: rs2304297 - allele A (variable name: S000001A) (p=0.005), rs892413 - allele A (variable name: S000002A) (p=0.029), and rs4950 - allele B (variable name: S000003B) (p=0.004). No genetic variants were found to be significantly associated with macroalbumin in the sibling & twin sample when controlling for gender, race, BMI, hypertension, diabetes, and age. For microalbumin, Dopamine Transporter-DAT1-allele B (variable name: DAT1B) was found to be significantly associated with microalbumin the independent sample group with p=0.007 on loci 9R (See Table 7). Multicollinearity was assessed between variables, but no multicollinearity was found.

Chapter 5: Discussion

The above results suggest that there may be an association between levels of urine albumin in adolescents and genetic variants such as Cytochrome P450 2A6-allele B, rs2304297 - allele A, rs2304297 - allele B, rs892413 - allele A, rs892413 - allele B, rs4950 - allele A, rs4950 - allele B, rs13280604 - allele A, rs13280604 - allele B, Monoamine Oxidase A-uVNTR-allele A and Monoamine Oxidase A-uVNTR-allele B. Association in were still significant in rs2304297 - allele A, rs892413 - allele A & rs4950 - allele B even after controlling for gender, age, hypertension, diabetes, BMI and race in the logistic model. For this study, we reject the null hypothesis for hypothesis 1, 2, and 3. These results suggest that there is an association between some of the genetic variants analyzed and albuminuria in adolescents. These results also suggest there is a significant association between some of the genetic variants when compared by gender and control for factors such as race, age, or disease status. While the differences observed among this population of respondents was statistically significant, it is important to note that these differences are not clinically significant. Those adolescents with higher levels of urine albumin should ideally be followed up, within a specific amount of time, with serial albuminuria level measurements for definitive diagnosis. For future study, inclusion of race and ethnicity would also be important to include and control for in the final model.

Although there are many limitations to a genetic association study, there are also many strengths. For starters, the population used within this study from the National Longitudinal Adolescent Health Wave III is large and comprehensively covers people of many different demographics. A study that is able to use such a cohort is valid for many populations. Not only was it a large population but a large group of adolescents has rarely been studied regarding an "old-age" disease such as Chronic Kidney Disease. Another strength of this study includes the

analysis of the association of genetic variants with albuminuria in a sample that had similar genetic traits because of known familial relationships. This study was able to use a repeated measures model in order to efficiently control for familial similarities when analyzing the association between genetic variants and albuminuria. This study included two albumin measurements, titled macroalbumin and microalbumin, which were collected from the Add Health Data committee during Wave III. For replication of this study, it is advised to only use one measurement. Both measurements were used to ensure inclusiveness of all the respondents.

For future recommendations, results from this study are important in order to increase the amount of research being done on Chronic Kidney Disease in younger adults. With the increasing rates of childhood obesity, more children are being diagnosed at an earlier age with Chronic Kidney Disease. Having a better understanding of the mechanisms of Chronic Kidney Disease can help lead early diagnostic & prognostic efforts at a molecular level, as well as provide specific therapies and targeted drugs. Melzer & colleagues (2008) say that the direction of the relationship between genetic variations and proteins is unknown in many disease processes, which may indicate that there may be more pathways involved in CKD than we currently know. In order to effectively decrease the risk of chronic kidney disease in adolescents, it is imperative for researchers to understand if environmental effects on gene variants that may alter protein levels are involved with the disease progression or a result of the disease progression (Melzer et al, 2008).

For future research, replication of this study could increase knowledge of the interaction between several pathways and mechanisms involved in CKD. Future research should follow up on the association between albuminuria and the penetrance and frequency of associated alleles found in this study. The interaction of genes and their environment will make it easier to guide

the implementation of genetic counseling and molecular pharmaceutical design pertaining to Chronic Kidney Disease in adolescents.

Table 1. Demographic and health characteristics of the Add Health Wave III Study respondents

Characteristics	All Add Health Respondents (n=15,197)	Sample with Urinalysis Evaluation (n=10,226)	Sample with Macroalbumin measurement (n=6306)	Sample with Microalbuminuria Measurement (n=6306)
Age (yr: mean±SD)	21.9 ± 1.77	21.9 ± 1.77	21.83 ± 1.742	21.83 ± 1.742
Male (n [%])	7167 (47.2)	5266 (51.5)	3002 (47.6)	3002 (47.6)
BMI (kg/m²; mean ±SD)[1]	27.60 ± 9.79	27.76 ± 10.06	29.47 ± 10.59	29.47 ± 10.59
BMI category (kg/m²; n [%])				
<25	5301 (42.8)	3519 (41.8)	1623 (30.2)	1623 (30.2)
25 to <30	3547 (28.7)	2457 (29.2)	1665 (31.0)	1665 (31.0)
30 to <35	1730 (14)	1182 (14.1)	979 (18.2)	979 (18.2)
≥35	1795 (14.5)	1252 (14.9)	1109 (20.6)	1109 (20.6)
Self-Reported Conditions (n [%])				
Diabetes	152 (1.0)	103 (1.0)	81 (1.3)	81 (1.3)
Hypertension	846 (5.6)	590 (5.8)	406 (6.4)	406 (6.4)
Smoked within 30 d	4913 (32)	3400 (33.3)	2014 (31.9)	2014 (31.9)

Table 2. Characteristics in respondents with macroalbumin measurement within the genetic sample of Add Health Wave III respondents (n=1197*)[1]

Characteristics	**Sample with Macroalbumin measurement (n=76)**	**No Macroalbumin (n=1121)**
Age (mean ± SD)	21.83 ± 1.872	21.91 ± 1.704
Male (n [%]) **Female (n [%])**	28 (36.8) 48 (63.2)	565 (50.4) 556 (49.6)
BMI (kg/m²; mean ±SD)[1]	30.45 ± 8.7	29.14 ± 11.02
BMI category (kg/m²; n [%])		
<25	23 (33.3)	304 (27.1)
25 to 29.99	17 (22.4)	303 (31.6)
30 to 34.99	6 (7.9)	178 (18.6)
≥35	23 (30.3)	173 (18.1)
Self- Reported Conditions (n [%])		
Diabetes	2 (2.6)	10 (0.9)
Hypertension	6 (7.9)	74 (6.6)
Smoked within 30 days	23 (30.3)	344 (30.7)

***Missing 13 respondents**

[1]Excluding pregnant women, ≥75WBC, <50 RBC

Table 3. Characteristics in respondents with microalbumin measurement within the genetic sample of Add Health Wave III respondents (n=1210)[1]

Characteristics	**Sample with Microalbumin measurement (n=48)**	**No Microalbumin (n=1162)**
Age (mean ± SD)	21.92 ± 1.966	21.89 ± 1.707
Male (n [%]) **Female (n [%])**	16 (33.3) 32 (66.7)	580 (49.9) 582 (50.1)
BMI (kg/m²; mean ±SD)[1]	30.77 ± 8.83	29.13 ± 10.9
BMI category (kg/m²; n [%])		
<25	12 (27.3)	321 (32.2)
25 to 29.99	13 (29.5)	310 (31.2)
30 to 34.99	6 (13.6)	179 (18)
≥35	13 (27.1)	184 (18.5)
Self-Reported Conditions (n [%])		
Diabetes	2 (4.2)	10 (0.9)
Hypertension	3 (6.3)	78 (6.7)
Smoked within 30 d	13 (27.1)	360 (31)

[1]Excluding pregnant women, ≥75WBC, <50 RBC

Table 4. Association between genetic variants and respondents with macroalbumin and/or microalbumin measurements

Macroalbumin				Microalbumin			
	Independent Sample		Sibling &Twin Sample		Independent Sample		Sibling & Twin Sample
Gene Variant	**Pearson Chi-Square**	**Fisher's Exact Test (if applicable)**	**Repeated Measurements P-value**	**Gene Variant**	**Pearson Chi-Square**	**Fisher's Exact Test (if applicable)**	**Repeated Measurement model p-value**
DAT1A	0.949	-	0.9189	**DAT1A**	0.634	-	0.9360
DAT1B	0.792	-	0.9422	**DAT1B**	0.059	-	0.3491
DRD4A	0.683	-	0.6346	**DRD4A**	0.686	-	0.8199
DRD4B	0.270	-	0.4252	**DRD4B**	0.618	-	0.8990
HTTLPRA	0.897	1.000	0.6100	**HTTLPRA**	0.450	0.499	0.4229
HTTLPRB	0.514	0.656	0.2471	**HTTLPRB**	0.838	0.772	0.5280
MAOA_VA	0.515	-	0.4428	**MAOA_VA**	0.473	-	0.7811
MAOA_VB	0.393	-	0.6300	**MAOA_VB**	0.168	-	0.6250
CYP2A6B	0.054	0.075	0.0846	**CYP2A6B**	0.956	1.000	0.8501
DRD2A	0.613	1.000	0.7257	**DRD2A**	0.648	1.000	0.5376
DRD2B	0.631	0.737	0.6628	**DRD2B**	0.556	0.657	0.8565
S000001A	0.003	0.010	0.1412	**S000001A**	0.153	0.182	0.7545
S000001B	0.058	0.083	0.1998	**S000001B**	0.742	0.822	0.5143
S000002A	0.020	0.033	0.1267	**S000002A**	0.741	1.000	0.8194
S000002B	0.031	0.040	0.0675	**S000002B**	0.264	0.362	0.0595
S000003A	0.095	0.125	0.5338	**S000003A**	0.741	0.818	0.2925
S000003B	0.004	0.011	0.1459	**S000003B**	0.371	0.325	0.8839
S000004A	0.135	0.162	0.4644	**S000004A**	0.860	1.000	0.2991
S000004B	0.048	0.059	0.2465	**S000004B**	0.252	0.341	0.6002
DNA2	0.152	0.235	0.5882	**DNA2**	0.188	0.313	0.6928
CONCORD	0.996	-	-	**CONCORD**	0.994	-	-
ZYGCHG	0.252	0.598	0.4425	**ZYGCHG**	0.542	1.000	0.4976

Table 5. Association of genetic variants for each gender in those respondents with macroalbumin measurements

Men				**Women**			
	Independent Sample		**Sibling & Twins Sample**		**Independent Sample**		**Sibling & Twin Sample**
Gene Variant	**Pearson Chi-Square**	**Fisher's Exact Test (if applicable)**	**Repeated Measurements p-value**	**Gene Variant**	**Pearson Chi-Square**	**Fisher's Exact Test (if applicable)**	**Repeated Measurements p-value**
DAT1A	0.661	-	0.6601	**DAT1A**	0.862	-	0.9306
DAT1B	0.673	-	0.6555	**DAT1B**	0.657	-	-
DRD4A	0.820	-	0.6760	**DRD4A**	0.675	-	0.7573
DRD4B	0.994	-	0.9405	**DRD4B**	0.068	-	0.1815
HTTLPRA	0.406	0.510	0.9008	**HTTLPRA**	0.940	1.000	0.3714
HTTLPRB	0.959	1.000	0.6244	**HTTLPRB**	0.611	0.778	0.2412
MAOA_VA	0.979	-	0.9148	**MAOA_VA**	0.268	-	0.3666
MAOA_VB	0.979	-	0.9148	**MAOA_VB**	0.181	-	0.5133
CYP2A6B	0.265	0.309	0.5139	**CYP2A6B**	0.156	0.162	0.2127
DRD2A	0.358	1.000	0.8798	**DRD2A**	1.000	1.000	0.8221
DRD2B	0.844	1.000	0.6173	**DRD2B**	0.621	0.692	0.9051
S000001A	0.541	0.629	0.3152	**S000001A**	0.002	0.005	0.4535
S000001B	0.560	0.750	0.8290	**S000001B**	0.012	0.013	0.1807
S000002A	0.099	0.124	0.1389	**S000002A**	0.072	0.106	0.5373
S000002B	0.683	0.756	0.7721	**S000002B**	0.006	0.008	0.0366
S000003A	0.551	0.749	0.3047	**S000003A**	0.017	0.025	0.1164
S000003B	0.486	0.621	0.6203	**S000003B**	0.005	0.010	0.2242
S000004A	0.602	0.751	0.5674	**S000004A**	0.047	0.058	0.2167
S000004B	0.594	0.638	0.8042	**S000004B**	0.076	0.103	0.3235
DNA2	0.296	0.540	0.0573	**DNA2**	-	-	0.0518
CONCORD	0.957		0.9260	**CONCORD**	-	-	-
ZYGCHG	0.658	1.000	0.5022	**ZYGCHG**	-	-	0.6617

Table 6. Association of genetic variants for each gender in respondents with microalbumin measurement

Men				**Women**			
	Independent Sample		**Sibling & Twin Sample**		**Independent Sample**		**Sibling & Twin Sample**
Gene Variant	**Pearson Chi-Square**	**Fisher's Exact Test (if applicable)**	**Repeated Measurement model p-value**	**Gene Variant**	**Pearson Chi-Square**	**Fisher's Exact Test (if applicable)**	**Repeated Measurement model p-value**
DAT1A	0.990	-	0.9714	**DAT1A**	0.446	-	0.8461
DAT1B	0.792	-	0.7703	**DAT1B**	0.008	-	-
DRD4A	0.608	-	0.7410	**DRD4A**	0.681	-	0.7610
DRD4B	0.477	-	0.8897	**DRD4B**	0.756	-	0.7113
HTTLPRA	0.409	0.668	0.5703	**HTTLPRA**	0.574	0.788	0.7410
HTTLPRB	0.811	1.000	0.6830	**HTTLPRB**	0.520	0.459	0.7502
MAOA_VA	0.000	-	0.2890	**MAOA_VA**	0.623	-	0.5737
MAOA_VB	0.000	-	0.2890	**MAOA_VB**	0.312	-	0.8938
CYP2A6B	0.617	1.000	0.5553	**CYP2A6B**	0.775	0.552	0.8534
DRD2A	0.481	1.000	0.5215	**DRD2A**	0.944	1.000	0.8803
DRD2B	0.135	0.220	0.2314	**DRD2B**	0.090	0.112	0.5208
S000001A	0.328	1.000	0.9300	**S000001A**	0.023	0.039	0.8775
S000001B	0.015	0.030	0.4452	**S000001B**	0.056	0.096	0.1848
S000002A	0.342	1.000	0.9076	**S000002A**	0.784	0.678	0.8902
S000002B	0.022	0.032	0.5648	**S000002B**	0.005	0.006	0.0090
S000003A	0.015	0.030	0.1972	**S000003A**	0.037	0.047	0.0334
S000003B	0.344	1.000	0.5634	**S000003B**	0.113	0.120	0.8969
S000004A	0.018	0.030	0.3354	**S000004A**	0.080	0.094	0.0745
S000004B	0.314	0.599	0.4286	**S000004B**	0.068	0.079	0.3506
DNA2			0.1073	**DNA2**	0.296	0.540	0.5835
CONCORD			0.8283	**CONCORD**	0.957		-
ZYGCHG			0.5912	**ZYGCHG**	0.658	1.000	0.2409

Table 7. Logistic Regression Model

Macroalbumin			**Microalbumin**		
	Independent Sample	**Sibling & Twins Sample**		**Independent Sample**	**Sibling & Twins Sample**
Gene Variant	**Logistic Regression**	**Repeated Measurements p-value**	**Gene Variant**	**Logistic Regression**	**Repeated Measurements p-value**
DAT1A	0.828	0.8217	**DAT1A**	0.087	0.8688
DAT1B	0.241	0.9361	**DAT1B**	0.007	0.3449
DRD4A	0.226	0.5737	**DRD4A**	0.163	0.9371
DRD4B	1.000	0.5915	**DRD4B**	1.000	0.9863
HTTLPRA	0.735	0.7368	**HTTLPRA**	0.488	0.4508
HTTLPRB	0.218	0.2697	**HTTLPRB**	0.968	0.5074
MAOA_VA	0.787	0.4835	**MAOA_VA**	0.644	0.5512
MAOA_VB	0.782	0.5772	**MAOA_VB**	0.231	0.5844
CYP2A6B	0.054	0.0888	**CYP2A6B**	0.838	0.8343
DRD2A	0.579	0.5348	**DRD2A**	0.597	0.3567
DRD2B	0.913	0.4492	**DRD2B**	0.752	0.7191
S000001A	0.005	0.7965	**S000001A**	0.113	0.6826
S000001B	0.099	0.7933	**S000001B**	0.782	0.8451
S000002A	0.029	0.7473	**S000002A**	0.731	0.5271
S000002B	0.089	0.3815	**S000002B**	0.239	0.1462
S000003A	0.110	0.8421	**S000003A**	0.625	0.3397
S000003B	0.004	0.5191	**S000003B**	0.487	0.7256
S000004A	0.170	0.8029	**S000004A**	0.744	0.5975
S000004B	0.056	0.7327	**S000004B**	0.329	0.9347
DNA2	0.175	0.3206	**DNA2**	0.983	0.9801
CONCORD	1.000	-	**CONCORD**	1.000	-
ZYGCHG	0.999	0.6816	**ZYGCHG**	0.998	0.3041

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Curriculum Vitae

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[B.S. in Health, Physical Education and Exercise Science] **May 2014**
[Area of Concentration: Community Health Education]

PROFESSIONAL EXPERIENCE

[Southern Nevada Health District, Las Vegas, NV]
[Intern]
[Developed syllabus and overall course structure, and administered all grades.]

CERTIFICATIONS

[Certified Health Education Specialist]
[National Commission for Health Education Credentialing, Inc.] 2016

MEMBERSHIPS

[Nevada Public Health Association]