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Genetic Predictors Of Metabolic Side Effects Of Diuretic Therapy

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GENETIC PREDICTORS OF METABOLIC SIDE EFFECTS OF DIURETIC THERAPY

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A

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ABSTRACT

Thiazide diuretics are a recommended first-line monotherapy for hypertension (i.e.SBP>140 mmHg or DBP>90 mmHg). Even so, diuretics are associated with adverse metabolic side effects, such as hyperlipidemia, hyperglycemia and hypokalemia which increase the risk of developing type II diabetes. This thesis used three analytical strategies to identify and quantify genetic factors that contribute to the development of adverse metabolic effects due to thiazide diuretic treatment. I performed a genome-wide association study (GWAS) and meta-analysis of the change in fasting plasma glucose and triglycerides in response to HCTZ from two different clinical trials: the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) and the Genetic Epidemiology of Responses to Antihypertensive (GERA) studies. Two SNPs (rs12279250 and rs4319515 (r2=0.73)), located at 11p15.1 in the NELL1 gene, achieved genome-wide significance for association with change in fasting plasma triglycerides in African Americans, whereby each variant allele was associated with a 28 mg/dl increase in the change in triglycerides. NELL1 encodes a cytoplasmic protein that contains epidermal growth factor (EGF)-like repeats and has been shown to represses adipogenic differentiation. No statistical significant association was found in the case of change in glucose or change in triglycerides in European-Americans in this study.

In order to increase the sample size and signal for the change in glucose, I performed a GWAS of longitudinal data and meta-analysis from 14 cohorts which are part of the CHARGE consortium. No statistically significant association was found. The lack of positive results in this analysis suggested that it is unlikely that there is a single common SNP with a large effect on the adverse reaction to the diuretic use. Therefore, we can speculate about the possible interaction of multiple variants each with modest effect sizes or the fact that rare variants are playing a greater part in this particular phenotype.

Finally, I performed a genome-wide association study and a Multi-Ethnic Meta-Analysis of change in blood potassium levels 718 European- and African-American

hypertensive participants. SNPs rs10845697 (Bayes Factor=5.560) on chromosome 12, near to the HEME binding protein 1 gene, and rs11135740 (Bayes Factor= 5.258) on chromosome 8 near the Mitoferrin-1 gene reached GWAS significance (Bayes Factor > 5). These results, if replicated, suggested a novel mechanism involving effects of genes in the HEME pathway influencing hydrochlorothiazide-induced renal potassium loss.

The main goal of this research was to explore first steps in developing hydrochlorothiazide personalize medicine in order to provide a lasting and positive impact on public health.

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CHAPTER 1: BACKGROUND AND SIGNIFICANCE

1.1 Definition of BP and hypertension

The beating heart is the most important organ in the circulatory system which is responsible for delivering nutrients and oxygen to all cells in the body as well as removing waste that the cells produce as a result of normal respiration. To accomplish this function, the heart needs to repeatedly contract (i.e. systole), and relax (i.e. diastole). The heart consists of 4 chambers, two atria in the upper part and two ventricles in the lower part. This pumping action generates a blood pressure (BP) within the circulatory system. Contraction or systole of the left ventricle opens the aortic valve and pushes oxygenated blood into the aorta. This phenomenon causes the artery's wall to stretch and the pressure to rise to a maximum that is called the systolic blood pressure (SBP). The pressure in the aorta falls during relaxation or diastole of the left ventricle allowing the closing of the aortic valve. Before the left atrium contracts again, the pressure in the aorta reaches a minimum that is called the diastolic blood pressure (DBP)[1].

Average values of SBP and DBP at birth are approximately 70 and 50mmHg, respectively, but increase to 120 and 80mmHg during adulthood[2]. In westernized populations, SBP continues to rise throughout life reaching an average value of 140mmHg by the seventh decade[3]. In the case of DBP, it reaches an average value of 80mmHg by the fifth decade where it tends to be flat or even decline[3]. Isolated non-westernized populations do not show a significant age-related increase in BP in adulthood after reaching the "normal BP values" from normal growth[4]. Interestingly, this pattern changes when individuals from these isolated population migrate to western cultures[5].

Prominent factors that influence the age-related increase in blood pressure are gender, ethnicity, earlier blood pressure levels, obesity and excess salt intake. When individuals reach adolescence, BP levels in men are on average higher than in women. This difference disappears in later stages of life and in fact is reversed at older ages, perhaps due to higher mortality in hypertensive men[3]. Considering ethnicity, Hispanics have lower BP levels than Caucasians and Asians[6]. The BP of African-Americans increases more sharply during their second decade than Whites[6]. In the case of previous BP levels, it is well known that BP values in a population are distributed as a near Gaussian distribution[4] and that members of the population tend to remain in the same relative position of the BP distribution later in life[7, 8]. This phenomenon is known as "tracking". The US Health and Nutrition Education Survey

shows the tendency that individuals in the upper percentiles of the BP distribution have a greater age-related increase in BP than the rest of the population[9]. In reference to obesity, the Framingham study reported a clear relationship between the excess adipose cells, which is a main characteristic of obese individuals, and an increase of SBP[10]. The trend was 4.5mmHg average increase of SBP for every 4.5 kg of weight gain. Finally in the case of salt intake, an early experiment was conducted in 500 newborn infants for 6 month, in which the sodium intake was reduced by half in 250 newborn[11]. This study showed that by reducing the sodium intake, SBP was reduced by 2.1mmHg in comparison a newborn with normal sodium intake. Later on, two studies showed that patients (with or without hypertension) who restrict their sodium intake for 36 month[12] to 5 years[13] had reduced BP levels and decrease incidence of hypertension compared to patients without a reduction in sodium intake.

Inter-individual differences in BP values may be influenced by genetic differences among individuals, shared environmental factors such as a household or school effects, and factors that are unique to an individual, including measurement error. All of these factors that influence inter-individual BP variability can be investigated in family studies where familial aggregation of BP values occur[4]. The Montreal Adoption[14, 15]study showed strong correlation coefficients for SBP and DBP between related individuals that share genetic factors as well as environmental factors[16]. On the other hand, the correlation coefficients for SBP and DBP in unrelated individuals who only share environmental factors are lower (Table 1.). Taken together, I conclude that there are clear genetic and environmental factors that influence interindividual BP variability.

In December 2003, the Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC) VII report was published[2] and four classifications for blood pressure were introduced (Table 1.). According to this report, hypertension is defined as high SBP and DBP values (more than 140mmHg or 90mmHg respectively). When the causes of elevated SBP and/or DBP are unknown, the term “essential hypertension” is used. On the other hand, the term “secondary hypertension” is given to cases in which the reasons for hypertension are well known such as renal artery stenosis, bilateral renal parenchymal disease, primary aldosteronism, pheochromocytoma, Cushing’s syndrome and oral contraceptive agents. Over 95% of adult hypertension is essential hypertension[17].

*total pairs of relative compared; +p=0.001; &p=0.01

Relationship	N*	SBP	DBP
Spouses	521	0.146+	0.175+
Father-Natural Child	198	0.237+	0.205&
Mother-Natural Child	198	0.271+	0.260+
Natural Siblings	94	0.382+	0.525+
Father-Adopted Child	442	0.089	0.134&
Mother- Adopted Child	442	0.078	0.099
Adopted Siblings	154	0.164	0.285+
Natural child- Adopted child	119	0.186	0.269+

Table 1.1: Estimated SBP and DBP correlation between household members[14, 15]

*based on blood pressure values alone

Blood Pressure Classification	SBP mmHg	DBP mmHg	Lifestyle Modification	Drug Therapy*
Normal	<120	and <80	No	No
Prehypertension	120–139	or 80–89	Encourage	No
Hypertension Stage 1	140–159	or 90–99	Yes	Yes
Hypertension Stage 2	>160	or >100	Yes	Yes

Table 1.2 Classification of blood pressure for adults

1.2 Hypertension as Cardiovascular Risk Factor

Multiple studies have showed a consistent and continuous relationship between hypertension and risk of cardiovascular disease (CVD) events (atherosclerosis[18], hypertension retinopathy[19], coronary artery disease, stroke, myocardial infarction and kidney disease[2, 20, 21]). Lewington et al[22] performed meta-analysis of 61 prospective studies that included 1 million individuals; this analysis indicated that stroke and ischemic heart disease (IHD) increase linearly with BP levels. The Framingham Heart Study[23] reported that individuals, with BP classified as prehypertensive or stage 1 hypertension, have a two-fold increased risk to develop CVD compared to individuals with normal BP. The National High Blood Pressure Education Program and other public health programs aim to increase the control of hypertension and its treatment to reduce morbidity and mortality in the American population[2, 24]. However, the prevalence of hypertension between 1999 and 2006 did not show a significant decline (28% to 30% respectively). In addition, among those with hypertension, only 78% are aware of their condition, and of them, only 68% are using antihypertensive medication, and of them, only 64% are able to control their BP below the recommended levels (BP less than 140/90mmHg)[25]. In general, hypertension affects 28% of the adult population in America; and continues to be a significant contributor to morbidity and mortality in the United States.

1.3 Treatment of Hypertension

The primary guidelines for the prevention and treatment of hypertension in the United State are published by the U. S. Department of Health and Human Services, and its most recent guidelines are given in “The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report” (JNC VII)[2]. According to JNC VII, the main objective of antihypertensive therapy is to reduce SBP levels below 140mmHg; and DBP rates to 90mmHg in order to decrease CVD complications[26]. However, in patients with diabetes or renal disease the goals are below 130mmHg for SBP and below 80mmHg for DBP[27].

To reach these goals, lifestyle modification [28,29,30,31, 32] and pharmacological treatment are needed. With respect to pharmacologic treatment, there are diverse drug classes that can be used to control hypertension such as: thiazide diuretics (TZDs), beta blockers (BBs), angiotensin II antagonist, aldosterone receptors blockers (ARB), angiotensin converting enzyme inhibitors (ACEI), and calcium channels blockers (CCB).

Most hypertensive patients need a combination of drug classes to control their hypertension[33, 34]. As an example, in the ALLHAT study, 60 % of patients on a two drugs class treatment were able to reduce their BP to less than 140/90mmHg in comparison with 30% that achieved the same goal with only one drug treatment[35]. The JNC VII report cites the findings from the ALLHAT study, which showed[35] that the primary coronary heart disease(CHD) outcome and mortality among patients using chlorthalidone (thiazide diuretic-like), or lisinopril (ACEI) or amlodipine (CCB) were not different, suggesting the use of thiazide diuretics, due to their cheaper price, as the main drug (either alone or in combination with other drugs) to control hypertension.

Thiazide diuretics act mainly in the cortical portion of the ascending loop of Henlé (ALH) and the distant convoluted tubule (DCT) after being excreted into the lumen by OCT1, coded for by the SLC22A6 gene, and OCT3 coded for by the SLC22A8 gene[36]. The main target of thiazide diuretics is the electro-neutral Na^+-Cl^- symporter (NCC). The NCC reabsorbs Na^+ and Cl^- from the lumen of the tubule into the tubular cells due to the higher concentration of sodium in the ALH and DCT. This sodium gradient is produced by the depletion of sodium and gain of potassium from the tubular cell due to the Na^+-K^+-ATPase antiport. This antiport is located in the basolateral membrane in the tubular cells. The NCC binds sodium first which then increases the affinity for chloride at its binding site on the symporter. When both ions are bound, the symporter transfers both Na^+ and Cl^- into the tubular cells. TZDs inhibit the reabsorption of sodium and chloride by the symporter, but the detailed way that the inhibition occurs is still unknown. It has been speculated that TZDs compete with chloride for the chloride binding site[37]. TZDs also increase the excretion of potassium and hydrogen ion for sodium at the DCT. It is not surprising then, that some mutations in the NCC lead to the inherited hypokalemic alkalosis called Gitelman syndrome[38-40]

The Veterans Administration Cooperative Study on Antihypertensive Agents[41], trial showed the importance of TZDs not only as antihypertensive drug therapy, but also as a way to reduce CVD events (such Strokes, CHD and heart failure (HF)). Antihypertensive drugs like aldosterone receptors blockers, angiotensin converting enzyme inhibitors, beta blockers and calcium channel blockers have been used over the years in multiple clinical trials[33, 34, 42-46] with similar as in the case of TZDs. However, the ALLHAT clinical trial with

more than 40000 hypertensive individuals, showed that the incidence of HF was greater in those randomized to the CCB arm compared to those to the TZDs.

1.4 Hydrochlorothiazide Treatment and its adverse effects

Hydrochlorothiazide (HCTZ), amongthethiazide diuretics, is one of the most common prescribed drugs for BP control[2], but some adverse effects have been reported. These adverse effects can be classified into three main groups: physiological problems[47, 48], electrolyte defects (hyperuricemia, hyponatremia, and hypokalemia), and metabolic abnormalities (hyperlipidemia and hyperglycemia)[49]. In the paragraphs below, I review each of these adverse effects individually.

Hyperuricemia is defined as a serum uric acid concentration greater than 0.42mmol/L[50]. It has been observed that patients treated with HCZT showed average levels of serum uric acid of 0.54mmol/L[51]. When HCZT is taken, it competes with the uric acid for transport into the cell[52]. This process results in hyperuricemia in HCZT-treated hypertensive patients.

Hyponatremia is defined as serum sodium level less than 134mmol/L and approximately 11% of geriatric patients treated with HCZT to control their blood pressure develop hyponatremia[53]. Due to the activity of HCZT in the distal tubule, the activity of the antidiuretic hormone (ADH) is enhanced so that water re-absorption occurs. As a result, the concentration of Na in urine is higher than the concentration in plasma[54, 55].

Hypokalemia is defined as serum K^+ concentration less than 3.5mmol/L. Approximately 50% of patients treated with HCZT to control their blood pressure develop hypokalemia[56]. The physiological mechanism responsible for the increase of K^+ excretion in the urine is thought to be inhibition of the renal sodium chloride co-transporter (symport) by HCZT[57]. This inhibition increases the amount of sodium and water in the distal convolute tubule and consequently the flow rate of urine. The increase in the flow rate stimulates distal potassium secretion due to the wash-out of K from the lumen[58].

Dyslipidemia is defined as the alteration of the amount of lipids in the blood. It has been shown that patients using HZCT treatment for a short-term have modest increases in the amount of LDL-cholesterol and triglycerides levels but HDL-cholesterol level does not show any significant change[49, 59, 60]. As in the case of hyperglycemia, there is no agreed-upon mechanism for how HZCT influences lipid concentration in the blood. The main hypothesis involves increased insulin sensitivity in patients as well as increased activity in the RAA system[49].

Hyperglycemia is defined as high blood sugar. Two different epidemiologic studies[61, 62] have reported an association between new-onset diabetes or hyperglycemia with HZCT treatment for hypertension. The precise mechanisms of how HZCT influences hyperglycemia are not well understood but there are two contemporary hypotheses. The first one suggests that due to the block of the renal sodium chloride channel by HZCT, the amount of excreted sodium increases, causing excretion of potassium in the urine[63]. The second hypothesis states that due to the blood volume depletion resulting from HZCT, the RAA system is activated, allowing aldosterone secretion which leads to increased potassium secretion[63]. In either scenario, the hypotheses conclude that hypokalemia induces higher proinsulin secretion over insulin. Proinsulin is less active than insulin. Therefore the concentration of glucose in the blood increases[56]. It is my hope that additional insight into the underlying mechanism of the metabolic side effects of diuretics will be obtained by identifying genes contributing to the glucose-related response to diuretic therapy (Chapter 2 and 4).

In recent years, it has been theorized that HCTZ may promote kidney damage[47, 48]. Although the main mechanisms remains unclear, animal models have shown that the use of thiazide chronically induces the wrinkling and thickening of the glomerular basement[64] as well as apoptosis in distal tubule cells[65, 66].

1.5 Role of potassium

On average, the total amount of potassium in the human body is 53.8mmol/kg body weight; 2% is found in the extracellular spaces and 98% is intracellular[67, 68]. The plasma concentration of potassium is kept between 3.5mEq/L to 5.0mEq/L. The balance of potassium concentration and distribution is regulated by four main mechanisms: 1) the gastrointestinal tract, 2) the Na^+-K^+-ATPase in the plasma membrane which is controlled by several hormones, 3) activity of the sympathetic nervous system, and 4) the kidneys. Each mechanism is discussed below:

1) The gastrointestinal tract absorbs most of the dietary intake of potassium, which on average is 100mmol/day (80 to 120mmol/day)[67, 69]. However, 5% to 10% of the dietary potassium is excreted through the feces. The colon is incapable of increasing potassium secretion by itself, but during an episode of diarrhea[68], the loss of potassium could reach 30% to 60%.

2) The Na^+-K^+-ATPase pump keeps the transmembrane gradients of Na^+ and K^+[70] in equilibrium. When sodium enters the cytosol, it produces a change in the membrane potential (also known as hyperpolarization) due to the unequal exit of potassium (three of Na^+ for two of K^+) to the lumen. Hyperpolarization is a phenomenon that occurs before muscular movement.

3) Due to the interaction between the neurohumoral mediators (acetylcholine, substance P) and their receptors in endothelial cells, the concentration of calcium increases, the first step for the endothelium-derived hyperpolarizing factor or EDHF-mediated response. The increase of intracellular calcium activates two different potassium channels, known as the calcium-activated potassium channels of small and intermediate conductance (SK_{Ca}, and IK_{Ca} respectively)[70, 71]. These channels release potassium to the lumen of the blood vessel as well as to the intercellular space between endothelial and smooth muscle cells. Endothelial cells take advantage of the hyperpolarization of vascular smooth cells to regulate the diameter of blood vessels by the EDHF[70, 71] in which potassium plays an important role.

4) The last mechanism in the regulation of potassium concentration occurs in the kidneys, where potassium is excreted by a combination of filtration, reabsorption and secretion. All of these steps occur primarily in nephrons, which are formed by eight parts (Figure 1.5.1): 1) the glomerulus, 2) the proximal tubule, divided in two parts: the convoluted tubule (PCT) and the proximal straight tubule (PST), 3)Henle's loop which consists of thin descending limb of Henle's loop (tDHL), thin ascending limb of Henle's loop (tALH) and thick ascending limb of Henle's loop (TAL), 4) the distal convoluted tubule (DCT), 5) the connecting tubule (CNT), 6) the initial convoluted tubule (ICT), 7) the cortical collecting tubule (CCT), and 8) the medullary collecting tubule (MCD) which consists of the outer medullary collecting duct (OMCD) and the inner medullary collecting tubule (IMCD). Around ~800mmol of potassium is filtered in the glomerulus every day. Most of it, approximately 80%, is reabsorbed by the PCT and 10% at the loop of

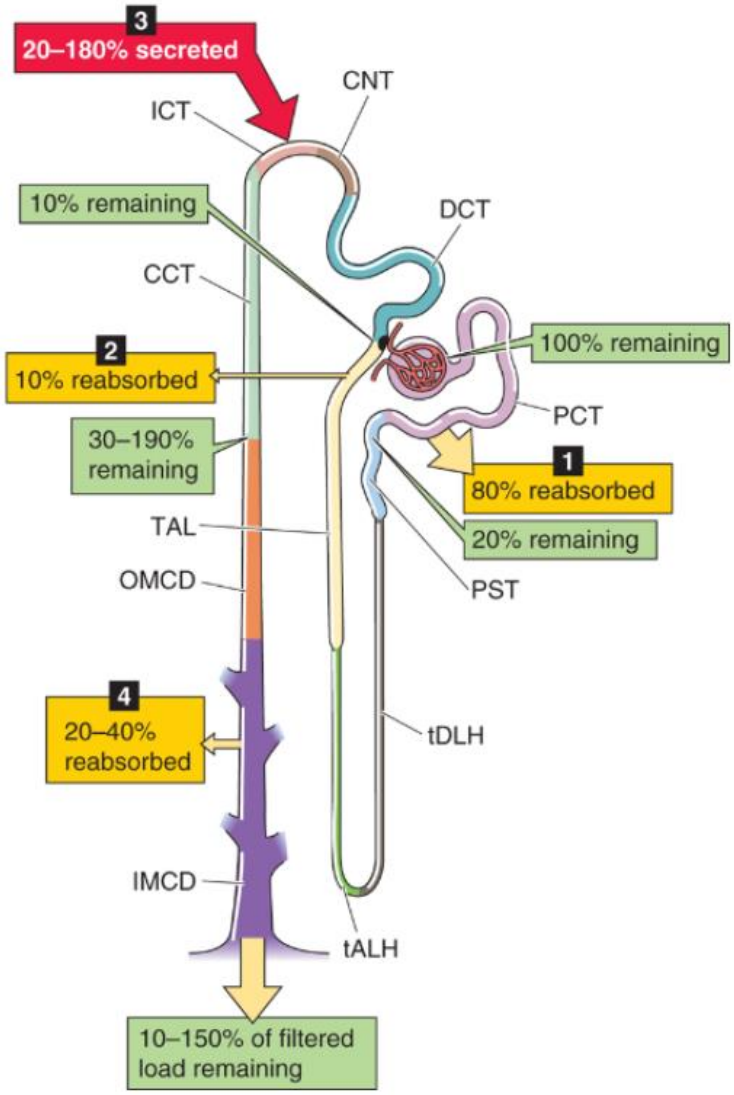

Figure 1.5.1: Potassium handling in superficial nephrons. Schematic was taken from Medical Physiology by Boron and Boulpaep[67]. PCT(proximal convoluted tubule), PST(proximal straight tubule), tDHL(thin descending limb of Henle's loop), tALH(thin ascending limb of Henle's loop), TAL(thick ascending limb of Henle's loop), DCT(distal convoluted tubule), CNT(connecting tubule), ICT(initial convoluted tubule), CCT(cortical collecting tubule), OMCD(outer medullary collecting duct), IMCD(inner medullary collecting tubule)

Henle. Only 10% of potassium reaches the DCT and finally the MCD reabsorbs around 20% to 40% (of the remaining 10%) the rest is excreted in the urine[67, 68]. Low dietary potassium intake stimulates its absorption which occurs in the classic distal tubule (DCT, CNT, and ICT) and CCT[67] and decreases the activity of Big-potassium (BK) and renal outer medullary potassium (ROMK) channels. On the other hand, in high dietary potassium intake, nephrons will secrete potassium to the tubule lumen mainly from the distal K^+ secretory system (CNT, ICT, CCT and OMCD)[67]. The secretion of potassium is mediated by aldosterone-dependent and aldosterone-independent mechanisms[72]. The release of aldosterone increases ROMK and BK channels in CNT and CCT.

Thiazide diuretics reduce blood pressure by reducing plasma volume due to diuresis. HCTZ diuretics block the renal sodium chloride channel which increases the amount of sodium, chloride and water that is excreted[5]. The high sodium load-in the distal tubule-produces, an increase in the excretion of potassium due to the high activity of the Na^+-K^+-ATPase pump. Furthermore, the increase in potassium in the collecting tubes facilitates the exchange of K^+ for H^+ by H^+-K^+-exchangers in the intercalated alpha cells producing alkalosis. Diuretic hypovolemia, also, activates the renin-angiotensin-aldosterone system (RAAS)[63] producing more aldosterone which stimulates the Na^+-K^+-ATPase pump, resulting in more loss of potassium.

1.6 Pharmacogenetics and Pharmacogenomics

Response to any pharmacological treatment is remarkably variable among individuals. Some patients respond favorably to a drug while others may have no (or even adverse) response to the same drug. Reasons for this variability in drug response can be explained by drug interaction, patients' age, nutritional status, liver or renal activity and the pathogenesis of the treated disease. Early investigators[73, 74] showed evidence of variation in genes that code for drug receptors, in genes that code for proteins that metabolize the drugs and influence drug response genes that code drug transporters. As a result, pharmacogenetics appeared as the science that studies the relationship between drug response and genetic variation.

Most previous pharmacogenetic studies have been based on a candidate gene approach, in which genes that influence pharmacological response (drug transport, drug metabolism and drug targets) are targeted to see how their variation influences drug

response[66]. In most cases, multiple genes are acting in concert to influence drug response[75]. To consider all possible genes, pharmacogenetics morphed its name to pharmacogenomics, where a key approach is the genome-wide association (GWA) study, in which we do not need to invoke *a priori* candidate genes but we can identify and estimate possible associations among all genes and drug response.

Relevant to pharmacogenomics, there are two main problems that all clinicians face. First, not everybody equally metabolizes a drug[76]. In real life, clinicians are forced to address "trial and error" approach in order to administrate the right drug dose that can help every patient. The second problem is Adverse Drug Reactions (ADR) that result from many drugs treatments. These ADRs produce illness and in some cases even death. In a typical hospital, 3 to 11% of its ER admissions are related ADRs[77], which leads to 2 million admissions in a year and 110,000 deaths in the same period of time due to ADRs. These estimates put ADRs between the 4th to 6th leading causes of death in the United States[77], with an estimated cost of more than $4 billion[78]. A goal of pharmacogenomics is to tailor therapy selection to improve drug treatment as well as to reduce ADRs. The warfarin drug case is a good example of what pharmacogenetics can accomplish.

Warfarin is a common drug that is used as an oral anticoagulant[79]. It targets the vitamin K cycle which is important in the carboxylation of the glutamic acid residues that are found in clotting factors. Warfarin is metabolized by CYP2C9; there are two significant alternative functional versions of the enzyme that are common in populations of European ancestry, the CYP2C9*2 (Arg144Cys) and CYP2C9*3(Ile359Leu)[79]. If a patient is at risk for thrombosis and has the CYP2C9*3/*3 variant, normal doses of warfarin will not be effective, and a lower concentration of the drug will be needed. On the other hand, if the patient has the CYP2C9*1/*1 variant of the enzyme, a normal dose of warfarin will be effective. Therefore, knowing in advance the genotype of CYP2C9 enzyme in each patient will facilitate safe initiation of warfarin therapy. This approach was the basis of a clinical trial reported by Pirmohamed et al[80], but contradicted by Kimmel at el[81]. It is the case that events measured in both clinical trials, such as rates of bleeding or thromboembolic events did not differ between groups, but these trials were not powered to calculate these outcomes.

In the case of Thiazide diuretics, there is not yet a clear result ready to make the transition from the "bench to bedside". However there are interesting polymorphisms that have been reported using pharmacogenetics and pharmacogenomics during the past years. Turner et al[82] showed mean declines in SBP and DBP (6±2mmHg and 5±1mmHg) in TT than in CC homozygotes in the C825T polymorphism of the gene G-protein β3-subunits

(GNB3) in association with HCTZ. A difference in gender response was also found between ACE insertion/deletion (I/D) polymorphism and HCTZ by Schwartz et al[83]. Women with the II genotype have greater mean declines in SBP and DBP than DD homozygotes meanwhile in the case of men, DD were the ones with greater response than II homozygotes. The Glu298Asp polymorphism of the endothelial nitric oxide synthase (NOS3), related to smooth muscle relaxation, was associated between African-Americans and European-Americans DBP (GG=-8.6±0.4 vs GT+TT=-7.1±0.6) response and HCTZ[84]. This polymorphism explains 1% of interindividual variation response to HCTZ. Matayoshi et al[85] found a difference between responders and non-responders to HCTZ in Japanese individuals with a polymorphism in the Na^+-Cl^- cotransporter SLC12A3 C1784T and in the β3-adrenonergic receptor (ADRB3) T727C. The odds ration of the former was 3.81 (C allele vs T allele) and of, the latter was 4.59 (T allele vs C allele). The gene WNK lysine deficient protein kinase 1 (WNK1), has three SNPs[86] that have been shown to be associated with ambulatory BP and they predict 4% of variation in SBP and DBP responses to HCTZ. In Chinese individuals, Lou et al[87] found that rs4149601(G/A) in neural precursor cell-expressed developmentally downregulated 4-like(NEDD4L) was associated to response in BP due to HCTZ. The A carriers had a greater reduction in BP than GG homozygotes (SBP: 6.1mmHg, DBP: 2.7mmHg).

In one of the first pharmacogenomic studies of its kind, Turner et al[88] identified novel genes associated with DBP response to HCTZ in a region on chromosome 12. The following genes were located in this region: lysozyme (LYS), YEAST domain-containing 4 (YEAST4) and fibroblast growth receptor substrate 2 (FRS2). This association was replicated by Duarte et al[89] in an independent data set of 746 European-Americans and African-Americans.

1.7 Specific Aims

My dissertation research investigated the association between genome-wide common single nucleotide polymorphism (SNP) variation and the onset of thiazide-induced ADRs in multiple samples of primary hypertensive patients. Following this introductory chapter, this project dissertation consists in the following aims and chapters:

Aim 1 and Chapter 2: *Use a genome wide association approach in the GERA and PEAR studies to identify loci significantly associated with glucose and triglycerides response.*

Linear regression and meta-analysis were used to evaluate the association between genome-wide SNP data and HCTZ-induced glucose and triglycerides response in unrelated primary hypertensive individuals.

Aim 2 and Chapter 3: *Use a genome-wide association approach in the CHARGE consortium to identify loci influencing the change in fasting glucose and fasting insulin levels after initiating diuretic treatment.*

Longitudinal regression and meta-analysis were used to evaluate the influence single nucleotide polymorphism on thiazide-associated changes in fasting glucose and fasting insulin levels.

Aim 3 and Chapter 4: *Use a genome wide association approach in the GERA and PEAR studies to identify loci significantly associated with the change in blood potassium levels following HCTZ therapy*.

Linear regression and trans-ethnic meta-analysis were used to evaluate the association between the genome data and change in potassium levels due to HCTZ in unrelated primary hypertensive individuals.

The findings from studies are summarized and synthesized in a concluding Chapter 5 that contains thought regarding the pharmacogenomics antihypertensive field as well as future research directions.

CHAPTER 2: GENOME-WIDE ASSOCIATION ANALYSES SUGGEST NELL1 INFLUENCES ADVERSE METABOLIC RESPONSE TO HCTZ IN AFRICAN-AMERICANS

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2.1 INTRODUCTION

Hydrochlorothiazide (HCTZ) is a prescribed drug for treatment of hypertension[90] but its ability to produce a variety of adverse drug reactions (ADR), such as hyperglycemia [61-63] and dyslipidemia [49, 59, 60, 63], is well-known. However, the mechanisms of these thiazide-induced ADRs are not well understood.

There are two hypotheses about how HCTZ influence the change in concentration plasma glucose. The first one states that a relationship between HCTZ-induced hypokalemia [49, 91] and impairing insulin secretion is the main cause of the change in glucose. The second hypothesis suggested that HCTZ increases visceral and hepatic fat accumulation, which could promote insulin resistance [10].

Other two hypotheses try to explain the of HCTZ-induced dyslipidemia. The first hypothesis, which is contradictory [92, 93], suggests that hemoconcentration, caused by the volume depletion due to HCTZ, stimulates the renin-angiotensin-aldosterone system (RAAS) thereby stimulating catecholamine release and subsequent adipose tissue lipolysis. The second hypothesis is an incremental reduction of lipoprotein lipase activity due to interference in the production, release or action of insulin by HCTZ [12].

This chapter tries to identify the possible molecular mechanisms of these ADR by identifying genetic variations that are predictive of inter-individual variation in ADR after HCTZ treatment using a genome-wide association study (GWAS).

2.2 MATERIALS AND METHODS

2.2.1 Study population

Phenotype and genotype data were collected from The Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR-clinicaltrials.gov identifier NCT00246519) study and the Genetic Epidemiology of Responses to Antihypertensive (GERA-clinicaltrials.gov identifier NCT00005520) study. In both studies, participants had mild to moderate primary hypertension without a history of heart disease or diabetes mellitus. Primary hypertension was defined as blood pressure levels greater than 140/90 mmHg or current use of prescription antihypertensive medications in the absence of a known cause for elevated BP. If at any time during the study protocols described below the average diastolic blood pressure (DBP) rose to greater than 110 mmHg or the systolic blood pressure (SBP) to greater than 180mmHg, participants were withdrawn from further study

participation and prescribed effective antihypertensive drug therapy. Details about the study designs and exclusion and inclusion criteria have been described previously. Briefly, in the PEAR study, individuals of any race-ethnicity and gender combination from age 17 to 65 years old with mild to moderate primary hypertension were recruited. Participants were enrolled in Gainesville, Fl; Atlanta, GA; and Rochester, MN. All participants were newly diagnosed hypertensives, untreated hypertensives or treated hypertensives taking less than three antihypertensive drugs. The protocol of the study was as follows: A wash out period of approximately 4 weeks was done in order to remove the effects of previous blood pressure medication (if any) from the participants. If at the end of the wash out period the average seated home DBP was > 85mmHg, office DBP was > 90 mmHg and the home and office SBP was < 180mmHg the individuals were enrolled into the randomized phase. All biological samples (blood and urine) were collected in the fasting state. As soon as the baseline evaluations were completed, the individuals were randomized to HCTZ (thiazide diuretic 12.5mg orally once daily) or atenolol (β-blocker 50mg orally once daily) for three weeks, with dose doubling (25 mg) for those with BP > 120/70 mmHg for an additional 6 weeks. More than 90% of PEAR participants received the higher 25 mg dose of HCTZ. For this AME GWAS analysis, we used only the patients randomized to the HCTZ from the PEAR study, referred to hereafter as PEAR HCTZ monotherapy.

In the GERA study, African-Americans and European Americans with primary hypertension were recruited at Emory University in Atlanta, GA and at the Mayo Clinic in Rochester, MN, respectively. The protocol for the GERA study was as follows: a wash-out period of at least 4 weeks was done in order to remove the effects of previous blood pressure medication. If at the end of the wash out period the average office DBP was> 90 mmHg, qualifying individuals were treated with HCTZ (25 mg orally once daily) for 4 weeks. Blood pressure was measured in the seated position using a mercury sphygmomanometer and blood samples were obtained for baseline biochemical measurements. At the end of the 4 week diuretic treatment period, blood pressure was measured and blood samples were again obtained for biochemical measurements. All blood collections were done in the morning after 8 hours of fasting.

All patients enrolled in PEAR and GERA provided written informed consent, and the institutional review boards of participating study centers approved the study protocols.[94]

2.2.2 Phenotype and Genotype data

All biomedical measurements were made in a central laboratory at the Mayo Clinic. In the PEAR study, these methods were implemented on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN, USA). In the GERA study, plasma glucose concentrations were determined by automated spectrophotometric methods implemented on an IL Monarch Chemistry system 760 (Instrumentation Laboratories, Lexington, MA, USA). Triglyceride concentrations were determined spectrophotometrically using Roche reagents on a Cobas Mira analyzer. In both studies, plasma glucose and triglyceride concentrations were measured at the end of the wash-out period (baseline) and at the end of the HCTZ period (final). Glucose and triglyceride responses to HCTZ were defined as the difference between the levels at the final and the baseline visits. Individuals with response values under or over 3 standard errors from the mean response were removed from the analysis. Plasma insulin, which was used as a covariate during statistical analyses, was measured using the Access Ultrasensitive Insulin immunoassay system.

In the PEAR study, individuals were genotyped on the Illumina HumanOmni1-Quad (Illumina, San Diego, California, USA). GERA participants from the opposite extremes of the DBP response distribution were genotyped using the GeneChip Human Mapping 500k Array, (Affymetrics, Santa Clara California, USA) using standard procedures. As part of routine quality control steps, single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) <1%, call rates <95%, Hardy-Weinberg equilibrium p-values $\geq 10^{-5}$ and individuals with more than 10% missing genotypes were removed from the analysis. The software MACH[95] (www.sph.umich.edu/csg/abecasis/MACH/download) was used to impute the approximate 2.5 million HapMap SNPs using the Phase II CEU as the reference panel for European-Americans and a cosmopolitan sample of CEU and YRI for African-Americans. Quality control for the imputed results was done using standard procedures (RSQ_HAT <0.3 and MAF ≤ 0.05). MACH generated a file with the highest posterior probabilities for each imputed SNP which was used in the analysis. After quality control and imputation, there were more than 2 million SNPs used for the genotype-phenotype association studies in each race-study group.[94]

2.2.3 Statistical analysis

Analyses were done by self-reported race within each study and later the results were combined across studies within races using meta-analysis using a fixed effect model weighted by the inverse of the variance of the race and study-specific β-estimates. Linear

regression assuming an additive model was performed using ProbABEL to evaluate the association between each SNP and metabolic responses to HCTZ. The variables sex, age, waist circumference, baseline glucose or triglycerides, and baseline insulin were used as covariates. The inclusion of the baseline value as covariate has both detractor and supporters, and our goal was to identify loci that influence change in glucose and triglycerides independent of any baseline effects.

To avoid the possibility of spurious association as the result of population substructure, the two first principal component scores (PCS) were used as covariates in the analysis. These PCS were obtained using the EIGENSTRAT software (genepath.med.harvard.edu/~reich/EIGENSTRAT.htm). After meta-analysis, the definition of statistical significance was $p<5x10^{-8}$ and the same direction of effects (i.e. the sign of β) in each study. A statistically suggestive p-value was defined as $p<1x10^{-5}$ and the same direction of effects and was used in order to avoid discounting true positive associations.[94]

2.3 RESULTS

Baseline characteristics of the European-American and African-American study participants are shown in Table 2.3.1 (A and B), which shows the relative similarity in participant characteristics between the two studies. In both PEAR and GERA, there were a higher percentage of European-American male participants, while for African Americans there were a higher percentage of females. Average age and BMI were nearly identical in PEAR and GERA. Baseline triglycerides were higher in European Americans from GERA compared to the other groups. Response to HCTZ treatment are shown in Table 2.3.2, and document that HCTZ significantly increased glucose and triglyceride in both race groups in both studies.[94]

(A)

Variables	PEAR: European Americans	GERA: European Americans
Total	229	196
Gender(%men)	59.8	57.1
Age(y)	49.9±9.4	48.5±7.3
Waist Circumference (cm)	94.8±13.6	103.7±14.7
Insulin ((μIU/ml)	9.4±8.7	8.5±4.9
Plasma Glucose(mg/dL)	92.9±12.2	92.4±12.5
Plasma triglyceride(mg/dL)	149.6±100.9	184.3±97.6
Systolic blood pressure(mmHg)	151.8±13.5	142.6±12.6
Diastolic blood pressure(mmHg)	97.9±5.8	95±5.7

(B)

Variables	PEAR: African Americans	GERA: African-Americans
Total	148	194
Gender(%men)	37.84	48.5
Age(y)	47.4±8.9	47.4±6.3
Waist Circumference (cm)	97.3±13.5	96.8±14.4
Insulin ((μIU/ml)	9.9±9.6	12.9±10.1
Plasma Glucose(mg/dL)	90.8±12.7	97.4±14.4
Plasma triglyceride(mg/dL)	105.84±93.98	122.4±57.5
Systolic blood pressure(mmHg)	151.8±13.14	150.8±14.9
Diastolic blood pressure(mmHg)	97.9±5.8	97.2±5.5

Table 2.3.1 PEAR and GERA sample characteristics measured at baseline[94]

(A)

Variables	PEAR: European Americans	GERA: European Americans
Plasma Glucose response (mg/dL)	1.44±8.9 (p =0.01)	3.03±7.6 (p<0.0001)
Plasma triglyceride response (mg/dL)	8.21±58.7 (p = 0.2)	14.57±59.9 (p<0.0001)

(B)

Variables	PEAR: African Americans	GERA: African Americans
Plasma Glucose response (mg/dL)	2.55±10.8 (p <0.005)	4.3±9.5 (p <0.0001)
Plasma triglyceride response (mg/dL)	7.95±40.6 (p = 0.02)	9.56±40.3 (p <0.0001)

Table 2.3.2 HCTZ treatment response in PEAR and GERA sample[94]

2.3.1 GWAS of adverse effect of HCTZ on glucose:

The meta-analysis of glucose change during HCTZ treatment revealed no SNPs that achieved the *a priori* definition of genome-wide significance in either European Americans or African Americans. The Manhattan plots and QQ plots for these analyses are shown in Supplement Figure 2.5.1 and Figure 2.5.3 (supplementary information pp. 28-48) However, in European Americans 48 SNPs from 8 genomic regions achieved our definition of suggestive significance with $p < 1x10^{-5}$ having effects in the same direction in both studies. Likewise, in African Americans 61 SNPs in 12 genomic regions met these criteria. Each of these SNPs is summarized in Supplemental Table 2.5.1, Table 2.5.2 and Table 2.5.5, Table 2.5.6 (supplementary information pp. 28-48).[94]

2.3.2 GWAS of adverse effect of HCTZ on triglycerides:

Among African Americans, two SNPs (rs12279250 and rs4319515 (r^2=0.73)) on chromosome 11, reached genome-wide significance (β=28 mg/dl, $p=6.6x10^{-9}$and β=27 mg/dl, $p=4.05x10^{-8}$ respectively). The Manhattan plot is shown in Figure 2.3.1, and Figure 2.3.3 displays the triglyceride response by genotype in both PEAR and GERA. These SNPs

are in the NELL1 gene, whose encoded protein is involved in adipose cell differentiation. No SNPs achieved genome-wide significance for triglyceride response among European Americans(supplementary Figure 2.5.2) .

As was the case with the HCTZ-induced change in glucose, the triglyceride response also had many SNPs that met our suggestive significance threshold. In European Americans 25 SNPs in 10 genomic regions met the threshold of $p < 1x10^{-5}$ with a same direction effect, with 77 SNPs in 27 genomic regions meeting this threshold in African Americans. Many of these SNPs were genes previously associated with metabolic syndrome, diabetes, insulin and other metabolic traits. These SNPs are described in detail in Supplemental Table 2.5.3, Table 2.5.4 and Table 2.5.7, Table 2.5.8 (supplementary information pp 28-48) [94]

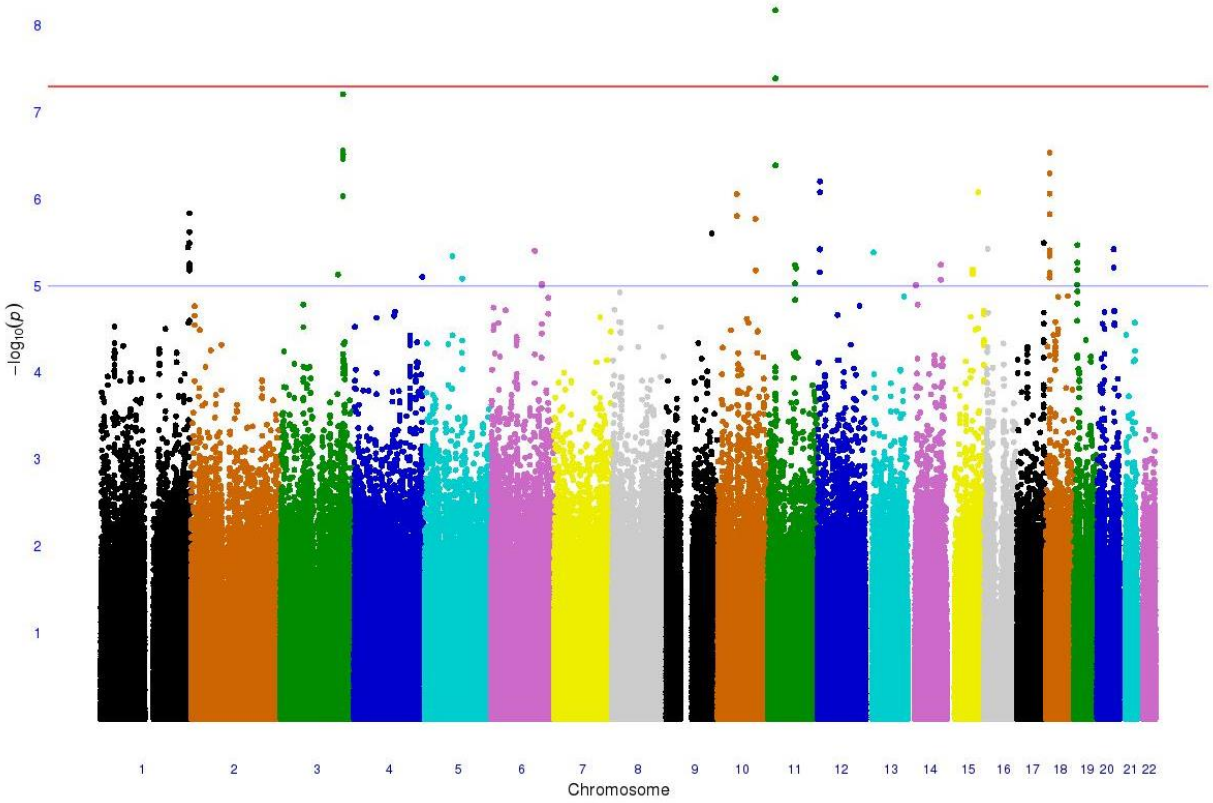
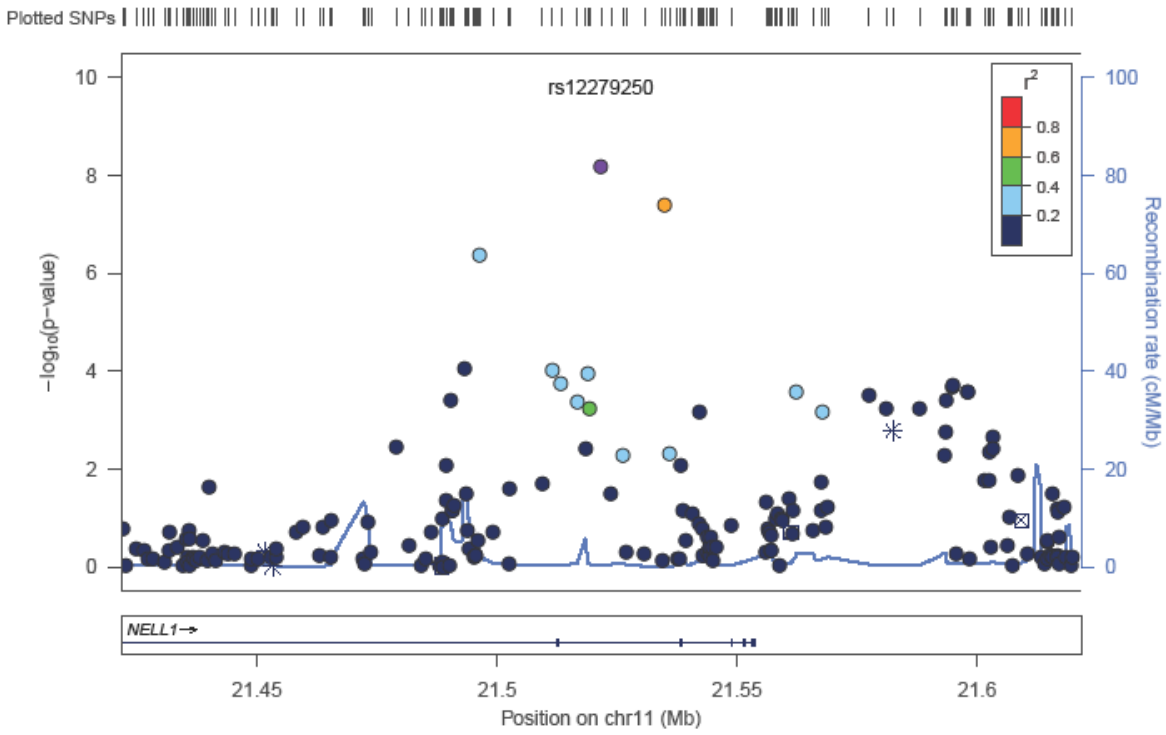


Figure 2.3.1 GWAS meta-analysis Manhattan plot showing the association of triglyceride response to HCTZ treatment in African Americans in the PEAR and GERA studies. Two SNP on chromosome 11 showed genome-wide significance ($p=5 \times 10^{-8}$). Eighteen loci showed p-values less than the suggestive threshold ($p=1 \times 10^{-5}$).[94]



NELL1	New function of Nell-1 protein in repressing adipogenic differentiation (PMID: 21723263)	**Direction**	**β**	**P.value**
		--	-28.20	6.60E-09

Figure 2.3.2: Regional Plot oon Chromosome 11 showing the position of the top signal associated of triglyceride response to HCTZ treatment in African Americans in the PEAR and GERA studies

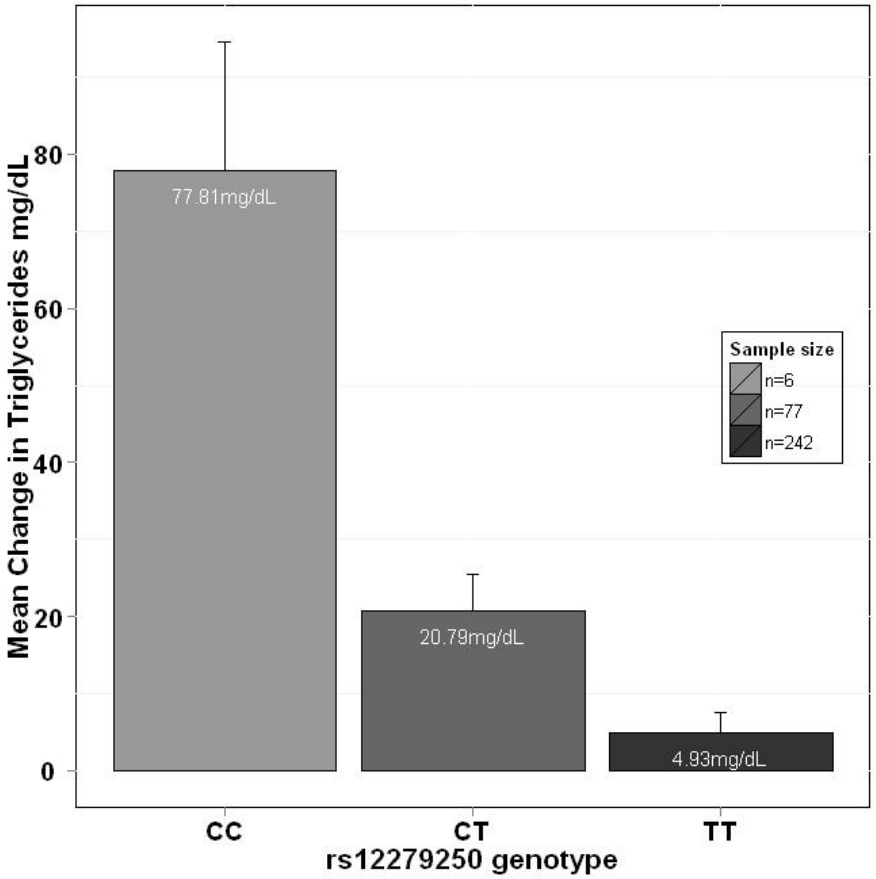


Figure 2.3.3: Means change in triglycerides adjusted for sex, age, waist circumference, base line insulin, base line triglycerides, and the two principle components depending on rs12279250 genotype (CC, CT, TT) for PEAR and GERA studies. Error bars represent standard error of the mean.[94]

2.4 DISCUSSION

In this chapter, I performed meta-analyses of GWAS result for the change in plasma glucose and triglyceride levels following HCTZ treatment from two biracial pharmacogenetic studies consisting of over 425 and 342 individuals, respectively.

In European Americans, I was not able to identify any locus that met the *a prior* definition of genome-wide significance for the change in glucose and triglycerides levels in response to HCZT treatment. However, 73 SNPs (48 SNPs in 8 regions for glucose and 25 SNPs in 10 regions for triglycerides) reached our criteria of suggestive association.

In African Americans, change in glucose levels did not met the *a prior* defined genome-wide significance level for a GWAS, however 61 SNPs (13 different regions) reached our criteria of suggestive association. In contrast to the above, and in addition to 75 SNPs in 27 regions that reached our criteria of suggestive association, I was able to identify one region marked by two SNPs (rs12279250, p=6.6e-9; and rs4319515, p=4.0e-8) that was significantly associated with the change in plasma triglyceride levels in response to HCZT treatment. These two SNPs are in high LD and are near the NELL1 gene on chromosome 11. The NELL-1 protein has osteoinductive properties [96] and has been found to repress adipogenic differentiation in both unipotent preadipocytes and imultipotent adipose-derived stromal cells[97]. The regulation of adipose stores through differentiation is tightly controlled, with adverse metabolic consequences of disordered fat storage occurring in the setting of either too much (obesity) or too little fat (lipodystrophy)[98]. Therefore, we conjecture that HCTZ could be modulating adipocyte differentiation through NELL1 leading to accumulation of plasma triglycerides in susceptible patients. Although an interesting finding, this result needs to be confirmed in independent studies of hypertensive patients treated with HCTZ.

For glucose response, It has been hypothesized that the mechanism by which HCTZ leads to hyperglycemia involves increased potassium excretion and hypokalemia leading to defects in insulin secretion[99] However, this hypothesis has been controversial given that many genetic causes of chronic hypokalemia (eg Gitelman's Syndrome) are not associated with hyperglycemia or increased risk of diabetes[48]. Therefore, it is interesting to note that none of the genes identified to be suggestively associated with change in glucose were obviously related to potassium transport or excretion[49]. As such, our results are more consistent with a mechanism involving-insulin resistance, perhaps secondary to visceral fat redistribution and hepatic fat accumulation as suggested by Eriksson et al.[100]

The strengths of this study include the population based design, the high quality of genotyping and phenotyping, the wash out period between drugs as well as the detailed follow-up of study participants. At the same time, we are aware of the limitation posed by the small sample size. While under-powered, it is interesting to note that some of the suggestive regions described in the Supplementary results were related to previous GWAS signals or candidate genes for traits related to type 2 diabetes, obesity, fatty acid metabolism, lipid metabolism, glucose metabolism or BMI.

2.5 SUPPLEMENTARY INFORMATION

2.5.1 Top SNPs associated with glucose response in European Americans

Forty-eight SNPs reached the suggestive p-value of 1 X 10^{-5} for glucose response to HCTZ in Non-Hispanic whites. These SNPs were located in 8 different chromosomes or regions (Table 2.5.1). The SNPs with the lowest p-values of association in each region were selected as index SNPs. These index SNPs were also in high LD with the rest of the SNPs in the same region. Table 2.5.2 shows detailed information for index SNPs. Chromosome 17 contains the SNP rs11077614, which the lowest p-value (p=2.83e-6) for this group. It has a β value indicating that with the presence of each minor allele (G) the change in glucose levels are decreased by 3.25 mg/dL. The closest gene to this SNP is the solute carrier family 39, member 11 (SLC39A11) which is a metal ion transporter. The index SNPs rs6870564, rs890749 and rs6859974 were located on chromosome 5 but in different loci. Three other lead SNPs (rs1974942, rs1511453 and rs17644018) were located on chromosome 4 but also in different loci. Among the SNPs on chromosome 5, the SNP rs6859974 is closed to mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase gene (MGAT1) which is related to fatty acid metabolism[101]. MGAT1 was previously associated with obesity in linkage and GWAS. The SNP rs7762018 is located on chromosome 6 in the region known as insulin-dependent diabetes mellitus 8 (IDDM8). This region was associated with type 1 diabetes in an affected-sib-pair analysis, and confirmed later in a larger family study and later in a GWAs studies. Other genes in these regions have been previously associated with overweight and obesity include neuromedin U receptor (NMUR1) and 5-hydroxytryptamine (serotonin) receptor 2B (HTR2B). Both genes are closed to rs1669070 (p=4.26e-6) on chromosome 2. A previous meta-analysis and candidate gene study have shown a significantly associated risk of Type 2 diabetes and the polymorphism Thr394Thr(G/A) in the gene peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGC1A) in Indians and Asians which is close to rs1511453 (p= 4.71e-6) in chromosome 4 in our study. The Thr394Thr(G/A) polymorphism is equivalent to rs2970847, which as a p-value of 0.93 in these data.[94]

2.5.2 Top SNPs associated with triglyceride response in European Americans

Twenty five SNP in 10 different chromosomes reached suggestive p-values for the change in plasma triglycerides in non-Hispanics whites (Table 2.5.3). The SNP rs17560407

in chromosome 5 had the lowest p-value (p=5.41e-7) for this phenotype. This SNP is close to the myocyte enhancer factor 2C gene (MEF2C) which is reported to be required for the expression of GLUT4 gene as well as to be related to type 2 diabetes. Another gene related to GLUT4 translocation in adipocytes is the pleckstrin homology-like domain, family B, member 1(PHLDB1) located in chromosome 11 (rs11216831, p= 4.11e-6). The list of lead SNPs are shown in Table S3.4. rs4722750 (p= 8.51E-6) on chromosome 7 is closed to the JAZF zinc finger 1 gene (JAZF1) which was previously identified in a GWAS o type 2 diabetes. The gene cytochrome c oxidase subunit IV isoform 1 (COX4I1), close to rs11648716 (p=7.05e-6) on chromosome 16, was identified as a potential factor in the development of type 2 diabetes in a quantitative proteomics approach using mice as an animal model. On chromosome 12, the SNP rs12303914 (p= 6.83e-6) is next to the killer cell lectin-like receptor subfamily A1 gene (KLRA1), which was identified in a GWAS of end-stage renal disease (ESRD). The genes cytochrome P450, family 7, subfamily B, polypeptide 1 (CYP7B1) on chromosome 8 (rs2980003, p= 4.80e-6) and glutamate-cysteine ligase, modifier subunit (GCLM) on chromosome 1 (rs22744788, p= 1.08e-6) have been previously related to lipid metabolism.[94]

2.5.3 Top SNPs associated with glucose response in African-Americans

Sixty-one SNPs in 12 regions were suggested to be associated with change in plasma glucose after HCTZ treatment in African-Americans (Table 2.5.5). Table 2.5.6 provides details for the lead SNPs within each region. The SNP rs225675, located on chromosome 6, had the lowest p-value (p= 1.09e-7) for this phenotype. Among the closest genes to this SNP, the G protein-coupled receptor 126 gene (GPR126) was associated with BMI in a previous GWAS. Two genes, major facilitator superfamily domain containing 9 (MFSD9) and branched chain amino-acid transaminase 1 (BCAT1), located on chromosome 2 near to rs13402330 (p= 5.58e-6) and chromosome 12 near to rs7965364 (p= 6.00e-6), respectively, were previously reported in other GWAS of T2D. In the same region on chromosome 2 near rs13402330 resides *SCL9A2*, a known sodium/hydrogen exchanger associated with hypertension. The SNP rs11599315 (p= 5.03e-6) is close to the WD repeat domain 37 (WDR37), which was reported to be associated with estimated glomerular filtration rate (eGFR). In another region on chromosome 12, the SNP rs7964748 (p=2.45e-6) is next to the insulin-like growth factor 1 gene (IGF1) which is associated to fasting insulin and insulin resistance (HOMA-IR).[94]

2.5.4 Top SNPs associated with triglyceride response in African-Americans

There were 77 SNPs in 27 regions that reached the suggestive p-value threshold for triglyceride response in African-Americans (Table 2.5.7). Table 2.5.8 shows detailed information for the lead SNP in each region. On chromosome 1 and close to rs11810574 (p= 3.60e-6) is the gene cholinergic receptor muscarinic 3 (CHRM3), which has been associated with insulin secretion. The genes ER lipid raft associated 1 (ERLIN1) on chromosome 10 (rs9420790, p= 1.68e-6) and IQ motif containing GTPase activating protein 2 (IQGAP2) on chromosome 5 (rs2460504, p=4.53e-6) have been previously related to liver function. The former has been reported to associated with plasma levels of liver enzymes and the latter with enhanced hepatic insulin sensitivity. Cholesterol levels have been reported to be associated with Calpain 5 gene (CAPN5) located on chromosome 11 (rs10899383, p=6.27e-6). Genes that have been related to the metabolic syndrome include angiopoietin-like 6 (ANGPTL6) on chromosome 19 (rs2116940, p=3.39e-6), growth differentiation factor 3 (GDF3) on chromosome 12 (rs12307997, p=6.24e-7) and ubiquitin-like 5[102] (UBL5) on chromosome 19 (rs2116940, p=3.39e-6).[94]

Region		Name	CHR	Allele1	Allele2	Position	Freq Allele1	Direction	B	P.value
Region I		rs11077614	17	A	G	68106224	0.5066	--	**-3.249**	**2.83E-06**
Region II	**A**	rs6870564	5	A	G	17056085	0.1295	++	**3.841**	**3.57E-06**
		rs11959796	5	A	T	17059405	0.1293	++	3.836	3.68E-06
		rs11953330	5	T	C	17059372	0.8707	++	3.836	3.68E-06
	B	rs890749	5	T	C	121464495	0.8094	--	**-3.246**	**7.93E-06**
		rs1834915	5	A	T	121465418	0.1907	--	-3.245	7.93E-06
		rs2560322	5	C	G	121467234	0.809	++	3.242	7.97E-06
		rs890750	5	T	G	121463478	0.8101	--	-3.245	8.10E-06
		rs970041	5	A	C	121467568	0.1921	--	-3.212	8.79E-06
		rs1427943	5	A	T	121471930	0.1956	--	-3.151	9.94E-06
	C	rs6859974	5	T	C	180138544	0.1431	++	**3.674**	**8.37E-06**
		rs1021001	5	C	G	180136710	0.143	--	-3.676	8.46E-06
		rs7702121	5	A	C	180131859	0.1424	--	-3.708	8.55E-06
Region III		rs427576	16	A	C	72386771	0.5609	--	**-2.618**	**3.88E-06**
		rs417278	16	T	C	72387977	0.5803	++	2.607	5.30E-06
		rs379401	16	C	G	72387865	0.5863	--	-2.596	5.56E-06
Region IV		rs1669070	2	T	C	231900549	0.403	--	**-3.021**	**4.26E-06**
Region V	**A**	rs1511453	4	A	G	23197560	0.0762	++	**4.948**	**4.71E-06**
		rs1397828	4	T	G	23205817	0.0717	--	-4.938	4.53E-06
		rs13112409	4	T	C	23220756	0.9222	++	4.814	6.93E-06
		rs13121520	4	A	G	23231629	0.0723	++	4.833	5.78E-06
		rs13118539	4	A	G	23240961	0.0713	++	4.843	6.06E-06
		rs306063	4	A	G	23258524	0.0709	++	4.913	5.11E-06
		rs3112978	4	T	C	23266700	0.0707	--	-4.943	4.75E-06
		rs306054	4	T	G	23272551	0.9306	++	5.045	3.62E-06
		rs306050	4	A	C	23278321	0.0704	++	4.960	4.47E-06

		rs306051	4	A	G	23282627	0.9296	--	-4.962	4.46E-06
		rs1948355	4	A	G	23306856	0.9307	--	-5.05	5.07E-06
		rs6830932	4	C	G	23307048	0.9307	--	-5.051	5.09E-06
	B	rs1974942	4	A	G	129915671	0.1046	--	**-5.296**	**4.09E-06**
	C	rs17644018	4	T	C	70966894	0.7003	++	**2.704**	**1.00E-05**
Region VI		rs10989824	9	T	C	103907659	0.0588	--	**-5.850**	**6.82E-06**
		rs7034610	9	A	G	103917056	0.0602	++	5.724	6.99E-06
		rs10820064	9	A	T	103909080	0.0662	++	5.632	8.48E-06
		rs10989829	9	A	C	103909525	0.0663	++	5.627	8.51E-06
		rs10989833	9	T	G	103909860	0.0663	--	-5.619	8.55E-06
		rs10989837	9	T	C	103910291	0.9336	++	5.607	8.65E-06
		rs10989791	9	A	G	103857420	0.058	++	5.696	9.26E-06
		rs7020246	9	T	G	103852964	0.9419	++	5.694	9.38E-06
		rs10760837	9	T	C	103879660	0.0579	--	-5.735	9.38E-06
		rs1449650	9	T	G	103883859	0.0578	--	-5.734	9.63E-06
		rs10820051	9	T	G	103884041	0.9422	++	5.734	9.67E-06
Region VII		rs7762018	6	A	C	169855169	0.1325	++	**3.647**	**7.50E-06**
		rs6923339	6	A	T	169869958	0.1326	++	3.642	7.71E-06
		rs6941766	6	A	G	169873578	0.8673	--	-3.629	8.25E-06
		rs6904601	6	T	G	169873830	0.8673	++	3.629	8.27E-06
		rs4716342	6	A	G	169879884	0.8673	--	-3.628	8.32E-06
Region VIII		rs1551678	10	C	G	127819002	0.3706	++	**2.645**	**9.30E-06**

Table 2.5.1 Top 48 SNPs in 8 different regions associated with glucose response in European Americans[94]

					GERA			PEAR			Meta-analysis		
SNP	chr	Nearby gene	minor alleles	MAF[a]	beta	s.e.	pvalue[b]	beta	s.e.	pvalue[b]	beta	s.e.	pvalue [bc]
rs11077614	17	SLC39A11	G	0.49	-3.24	1.27	1.1e-2	-3.25	0.83	8.34e-5	-3.25	0.68	2.83e-6
rs6870564	5	MYO10	A	0.13	4.55	1.09	2.81e-5	2.85	1.28	2.59e-2	3.84	0.83	3.57e-6
rs427576	16	-	C	0.43	-2.96	0.79	1.79e-4	-2.25	0.81	5.58e-3	-2.62	0.57	3.88e-6
rs1974942	4	PHF17	A	0.1	-6.12	1.97	1.91e-3	-4.87	1.42	5.76e-4	-5.29	1.15	4.09e-6
rs1669070	2	NMUR1/ HTR2B	T	0.4	-2.53	1.09	2.12e-2	-3.29	0.82	5.85e-5	-3.02	0.66	4.26e-6
rs1511453	4	PPARGC1 A	A	0.08	4.78	1.48	1.21e-3	5.14	1.59	1.19e-3	4.95	1.08	4.71e-6
rs10989824	9	GRIN3A	T	0.06	-6.76	1.74	9.93e-5	-4.69	1.96	1.68e-2	-5.85	1.3	6.82e-6
rs7762018	6	IDDM8	A	0.13	2.49	1.18	3.52e-2	4.69	1.12	2.96e-5	3.65	0.81	7.50e-6
rs890749	5	LOX	C	0.19	-2.90	1.02	4.39e-3	-3.59	1.04	5.14e-4	-3.25	0.73	7.93e-6
rs6859974	5	MGAT1	T	0.14	3.46	1.17	3.02e-3	3.89	1.17	8.52e-4	3.67	0.82	8.37e-6
rs1551678	10	ADAM12	C	0.37	2.67	0.83	1.35e-3	2.62	0.85	2.20e-3	2.65	0.59	9.30e-6
rs17644018	4	CSN1S2A	C	0.29	-2.41	0.85	4.45e-3	-3.03	0.89	6.34e-4	-2.70	0.61	1e-5

Table 2.5.2: Lead SNPs showing association with glucose response to hydrochlorothiazide in meta-analysis of European Americans.[94] [a]Minor allele frequency among combined sample of GERA and PEAR. [b]P values are based on a linear regression adjusted for sex, age, waist circumference, baseline insulin, baseline glucose and the two first principal components of genetic variation for each study. [c]P values are calculated using inverse-variance meta-analysis

Region		Name	chr	Allele1	Allele2	Position	Freq Allele1	Direction	β	P.value
Region I		rs17560407	5	A	G	88219407	0.7389	--	**-23.23**	**5.41E-07**
		rs17560451	5	T	C	88219759	0.7392	++	23.17	5.81E-07
		rs3850653	5	A	G	88222710	0.7332	--	-22.99	8.76E-07
		rs244760	5	C	G	88146965	0.2657	++	23.08	4.34E-06
Region II		rs2274788	1	T	C	94447314	0.7493	--	**-23.07**	**1.08E-06**
Region III	A	rs11216831	11	A	G	117622228	0.0675	++	**42.47**	**4.11E-06**
		rs12361791	11	T	G	117605386	0.9325	++	42.51	4.30E-06
	B	rs1559759	11	A	C	35079627	0.1451	++	**25.59**	**8.93E-06**
Region IV		rs2980003	8	T	C	66162517	0.2656	++	**22.07**	**4.80E-06**
		rs2956307	8	A	G	66166394	0.2496	--	-22.17	8.75E-06
Region V		rs12303914	12	A	G	10362317	0.3459	++	**20.39**	**6.83E-06**
Region VI		rs11648716	16	A	G	84464117	0.9213	--	**-39.68**	**7.05E-06**
		rs11861580	16	C	G	84456048	0.9255	--	-41.76	7.31E-06
Region VII		rs10943724	6	A	C	81359524	0.3051	--	**-19.77**	**7.15E-06**
		rs10943725	6	A	T	81359702	0.6949	++	19.77	7.19E-06
		rs10943726	6	T	G	81360629	0.3051	++	19.78	7.20E-06
		rs12207977	6	T	C	81284893	0.6971	--	-19.53	7.89E-06
		rs4706121	6	A	C	81302889	0.2947	--	-19.80	9.68E-06
Region VIII		rs17183114	4	T	G	182459721	0.1432	++	**26.49**	**7.58E-06**
Region IX		rs4722750	7	T	C	27970723	0.1	--	**-31.88**	**8.51E-06**
		rs17156099	7	C	G	27972967	0.1001	++	31.83	8.64E-06
		rs17156100	7	C	G	27974909	0.8999	--	-31.78	8.81E-06
		rs2057671	7	T	C	27965720	0.1003	--	-32.33	8.82E-06
		rs17156102	7	C	G	27975221	0.8999	--	-31.78	8.82E-06
Region X		rs1724611	15	A	G	50647845	0.7341	++	**30.09**	**1.05E-05**

Table 2.5.3: Top 25 SNPs in 10 different regions associated with triglyceride response in European Americans[94]

					GERA			PEAR			Meta-analysis		
SNP	chr	Nearby gene	minor alleles	MAF[a]	beta	s.e.	pvalue[b]	beta	s.e.	pvalue[b]	Beta	s.e.	pvalue [bc]
rs17560407	5	MEF2C	G	0.27	-18.3	7.35	1.26e-2	-26.4	5.97	9.45e-6	-23.23	4.64	5.41e-7
rs2274788	1	GCLM	C	0.25	-21.1	7.39	4.40e-3	-24.5	6.15	7.04e-5	-23.07	4.73	1.08e-6
rs11216831	11	PHLDB1	A	0.07	47.5	13.2	3.11e-4	37.6	12.9	3.56e-3	42.47	9.22	4.11e-6
rs2980003	8	CYP7B1	T	0.27	23.9	7.87	2.37e-3	20.9	6.11	6.03e-4	22.07	4.83	4.80e-6
rs12303914	12	KLRA1	A	0.35	19.3	7.82	1.36e-2	20.9	5.56	1.66e-4	20.39	4.53	6.38e-6
rs11648716	16	COX4I1	G	0.08	-53.0	21.3	1.29e-2	-36.9	9.70	1.42e-4	-39.68	8.83	7.05e-6
rs10943724	6	BCKDHB	A	0.31	-20.4	7.19	4.47e-3	-19.4	5.57	5.08e-4	-19.77	4.40	7.15e-6
rs17183114	4	-	T	0.14	33.9	8.99	1.56e-4	20.8	7.86	8.26e-3	26.49	5.92	7.58e-6
rs4722750	7	JAZF1	T	0.1	-7.59	10.2	4.57e-1	-55.4	10.05	3.47e-8	-31.88	7.16	8.51e-6
rs1559759	11	CD44	A	0.15	16.8	9.57	7.92e-2	30.6	7.22	2.24e-5	25.59	5.76	8.93e-6
rs1724611	15	ARPP19	G	0.27	36.7	12.9	4.40e-3	27.5	8.05	6.33e-4	30.09	6.83	1.05e-5

Table 2.5.4: Lead SNPs showing association with triglyceride response to hydrochlorothiazide in meta-analysis of European Americans.[94] [a]Minor allele frequency among combined sample of GERA and PEAR. [b]P values are based on a linear regression adjusted for sex, age, waist circumference, baseline insulin, baseline triglycerides and the two first principal components of genetic variation for each study.. [c]P values are calculated using inverse-variance meta-analysis**.**

Region	Name	chr	Allele1	Allele2	Position	Freq Allele1	Direction	β	P.value
Region I	rs225675	6	A	G	142553829	0.8121	--	**-5.286**	**1.09E-07**
	rs225666	6	C	G	142539169	0.8127	--	-5.276	1.18E-07
	rs225662	6	T	G	142532815	0.181	--	-5.137	2.88E-07
	rs225659	6	A	G	142529808	0.181	--	-5.134	2.95E-07
	rs225654	6	A	G	142528178	0.8192	--	-5.122	3.19E-07
	rs225653	6	T	G	142527112	0.1808	--	-5.119	3.23E-07
	rs225652	6	A	C	142526178	0.1808	--	-5.115	3.32E-07
	rs225665	6	A	C	142535483	0.1591	--	-5.176	8.22E-07
	rs225643	6	A	G	142523539	0.8254	--	-4.755	3.07E-06
	rs628311	6	A	C	142509959	0.1789	--	-4.613	5.13E-06
	rs225642	6	T	C	142523416	0.1766	--	-4.603	5.15E-06
	rs225641	6	T	C	142522696	0.8234	--	-4.593	5.43E-06
	rs225671	6	A	G	142542618	0.2914	--	-4.047	5.73E-06
	rs225674	6	A	T	142551860	0.2915	--	-4.047	5.74E-06
	rs225676	6	T	C	142555919	0.292	--	-4.046	5.79E-06
	rs225635	6	A	G	142518431	0.8237	--	-4.564	6.15E-06
	rs2842756	6	T	C	142472521	0.1745	--	-4.602	6.59E-06
	rs2842757	6	A	T	142474663	0.1745	--	-4.601	6.61E-06
	rs2842758	6	A	G	142477424	0.8255	--	-4.599	6.65E-06
	rs225630	6	C	G	142515724	0.8238	--	-4.543	6.78E-06
	rs2745470	6	T	C	142483233	0.8243	--	-4.559	6.86E-06
	rs639658	6	T	G	142509964	0.1761	--	-4.532	7.16E-06
	rs225606	6	A	G	142495600	0.8239	--	-4.529	7.23E-06
	rs225622	6	A	G	142509080	0.1761	--	-4.529	7.25E-06
	rs225673	6	T	C	142547983	0.8453	--	-4.934	9.80E-06
	rs225678	6	A	G	142559302	0.1553	--	-4.923	1.01E-05

RegionII	**A**	rs7077606	10	T	C	55077097	0.1193	--	**-5.439**	**4.78E-07**
		rs7904689	10	T	C	55118881	0.89	--	-5.691	5.61E-07
		rs17697015	10	A	G	55092707	0.8912	--	-5.402	1.39E-06
		rs2130372	10	A	G	55091939	0.1081	--	-5.405	1.43E-06
		rs17622628	10	A	T	55011608	0.1172	--	-5.667	1.48E-06
		rs7923617	10	A	C	55087496	0.1079	--	-5.365	1.64E-06
		rs7076633	10	T	C	55037365	0.8811	--	-5.489	1.77E-06
		rs7095328	10	T	C	55055968	0.1211	--	-5.371	2.29E-06
	B	rs11599315	10	T	C	1333017	0.3709	--	**-3.883**	**5.03E-06**
	C	rs2394354	10	T	C	68591112	0.1305	--	**-5.455**	**9.54E-06**
Region III		rs7801534	7	A	G	49300226	0.8348	++	**4.959**	**6.61E-07**
		rs6956707	7	A	G	49287553	0.8385	++	4.722	4.06E-06
Region IV	**A**	rs7964748	12	A	G	101489459	0.7181	++	**4.057**	**2.45E-06**
		rs7955640	12	T	C	101492429	0.2707	++	3.926	3.02E-06
		rs1350356	12	T	C	101502655	0.3161	++	3.463	4.62E-06
		rs10860887	12	A	G	101509514	0.6486	++	3.344	4.74E-06
		rs1457594	12	T	C	101511768	0.6591	++	3.314	7.97E-06
	B	rs10778699	12	A	G	78987215	0.2428	++	**4.277**	**4.06E-06**
		rs7961750	12	T	C	79011536	0.5911	++	3.589	6.41E-06
		rs7976086	12	C	G	79070824	0.64	++	3.549	8.58E-06
	C	rs7965364	12	T	C	25375460	0.3713	++	**3.799**	**6.00E-06**
Region V		rs12507634	4	A	G	175308177	0.8093	--	**-4.842**	**3.70E-06**
Region VI	**A**	rs11785622	8	T	C	99386915	0.0733	--	**-7.215**	**3.97E-06**
		rs17360909	8	A	C	99387233	0.9268	--	-7.198	4.06E-06
		rs17288913	8	T	C	99387921	0.0721	--	-7.462	4.79E-06
	B	rs2944755	8	A	G	141643376	0.0598	--	**-9.447**	**7.78E-06**
Region VII		rs13402330	2	A	T	102878724	0.1879	--	**-5.269**	**5.58E-06**
		rs10188760	2	C	G	102878339	0.188	--	-5.271	5.58E-06
Region	**A**	rs16951120	17	T	C	47648637	0.1544	++	**4.499**	**6.16E-06**

VIII		rs11867974	17	A	G	47649397	0.1544	++	4.499	6.19E-06
	B	rs12947365	17	T	C	2969720	0.7408	++	**3.881**	**9.81E-06**
Region IX		rs4808260	19	C	G	20638111	0.3024	--	**-3.713**	**6.95E-06**
Region X		rs6493487	15	A	G	49301021	0.9093	++	**5.959**	**8.01E-06**
Region XI		rs17668704	16	A	T	70815200	0.6052	--	**-3.383**	**8.50E-06**
Region XII		rs6118476	20	A	T	9012332	0.0707	--	**-7.148**	**9.52E-06**

Table 2.5.5 Top 61 SNPs in 12 different regions associated with glucose response in African-Americans[94]

					GERA			PEAR			Meta-analysis		
SNP	Chr	Nearby gene	minor alleles	MAF[a]	beta	s.e.	pvalue[b]	beta	s.e.	pvalue[b]	beta	s.e.	pvalue[bc]
rs225675	6	GPR126	G	0.19	-4.12	1.38	2.84e-3	-6.56	1.44	5.11e-6	-5.29	0.99	1.09e-7
rs7077606	10	PCDH15	T	0.12	-6.47	1.49	1.45e-5	-4.30	1.57	5.98e-3	-5.44	1.08	4.78e-7
rs7801534	7	CDC14C	G	0.17	5.14	1.22	2.58e-5	4.6	1.73	7.75e-3	4.96	0.99	6.61e-7
rs7964748	12	IGF1	G	0.28	4.29	1.13	1.45e-4	3.74	1.33	5.05e-3	4.06	0.86	2.45e-6
rs12507634	4	FBXO8	G	0.19	-3.43	1.50	2.26e-2	-6.18	1.46	2.28e-5	-4.84	1.05	3.70e-6
rs11785622	8	NIPAL2	T	0.07	-5.86	2.13	6.02e-3	-8.79	2.30	1.32e-4	-7.21	1.56	3.97e-6
rs10778699	12	PPP1R12A	A	0.24	3.04	1.28	1.76e-2	5.65	1.35	2.76e-5	4.28	0.93	4.06e-6
rs11599315	10	WDR37	T	0.37	-2.99	1.22	1.44e-2	-4.73	1.19	6.75e-5	-3.88	0.85	5.03e-6
rs13402330	2	MFSD9/ SCL9A2	A	0.19	-4.97	1.69	3.38e-3	-5.53	1.59	5.05e-4	-5.27	1.16	5.58e-6
rs7965364	12	BCAT1	T	0.37	5.62	1.15	1.04e-6	1.73	1.23	1.59e-1	3.79	0.84	6e-6
rs16951120	17	CA10	T	0.15	3.72	1.31	4.44e-3	5.58	1.54	2.81e-4	4.49	0.99	6.16e-6
rs4808260	19	ZNF626	C	0.30	-3.99	1.07	1.78e-4	-3.29	1.31	1.19e-2	-3.71	0.83	6.95e-6
rs2944755	8	EIF2C2	A	0.06	-7.39	3.73	4.76e-2	-10.4	2.56	4.84e-5	-9.45	2.11	7.78e-6
rs6493487	15	CYP19A1	G	0.09	6.90	1.59	1.36e-5	3.68	2.47	1.36e-1	5.96	1.33	8.01e-6
rs17668704	16	PMFBMP1	A	0.61	-3.99	0.98	4.54e-5	-2.46	1.20	4.09e-2	-3.38	0.76	8.5e-6

rs6118476	20	PLCB4	A	0.07	-4.52	2.13	3.43e-2	-10.7	2.47	1.54e-5	-7.15	1.61	9.52e-6
rs2394354	10	CTNNA3	T	0.13	-2.19	1.79	2.21e-1	-8.38	1.69	7.87e-7	-5.46	1.23	9.54e-6
rs12947365	17	OR1G1	C	0.26	2.85	1.14	1.28e-2	5.36	1.37	9.03e-5	3.88	0.88	9.81e-6

Table 2.5.6 Lead SNPs showing association with glucose response to hydrochlorothiazide in meta-analysis of African-Americans[94]
[a]Minor allele frequency among combined sample of GERA and PEAR. [b]P values are based on a linear regression adjusted for sex, age, waist circumference, baseline insulin, baseline glucose and the two first principal components of genetic variation for each study.. [c]P values are calculated using inverse-variance meta-analysis.

Region		Name	chr	Allele1	Allele2	Position	Freq Allele1	Direction	β	P.value
Region I	A	rs12279250	11	t	c	21521655	0.8538	++	**28.20**	**6.60E-09**
		rs4319515	11	t	c	21534943	0.8629	++	27.44	4.05E-08
		rs4923633	11	t	c	21496421	0.1038	++	28.94	4.07E-07
	B	rs17133858	11	t	g	74649205	0.1393	--	**-20.99**	**5.79E-06**
		rs10899383	11	a	t	76888784	0.8691	--	-24.09	6.27E-06
		rs17133815	11	a	g	74591404	0.1909	--	-18.58	9.36E-06
Region II	A	rs2686586	3	t	c	169380515	0.4734	++	**18.93**	**6.14E-08**
		rs2593426	3	a	t	169386527	0.5734	++	18.45	2.75E-07
		rs2249391	3	a	g	169381484	0.4261	++	17.81	3.00E-07
		rs2593422	3	t	g	169380249	0.424	++	17.85	3.02E-07
		rs2249516	3	a	g	169380468	0.424	++	17.85	3.05E-07
		rs2593423	3	t	g	169381964	0.4246	++	17.75	3.41E-07
		rs2253178	3	a	c	169373412	0.274	++	20.22	9.26E-07
	B	rs6440957	3	t	c	155977646	0.8359	++	**22.16**	**7.42E-06**
Region III		rs12455924	18	t	c	11054409	0.3572	--	**-17.88**	**2.91E-07**
		rs7228002	18	a	g	11058227	0.2579	--	-22.55	5.01E-07
		rs8086223	18	a	g	11055122	0.3888	++	16.71	8.64E-07
		rs8090641	18	t	c	11061006	0.6303	--	-17.17	1.49E-06
		rs7231609	18	a	g	11055993	0.412	++	15.52	3.89E-06
		rs6505611	18	a	g	11054752	0.4137	++	14.77	4.39E-06
		rs2870108	18	a	g	11053871	0.5862	++	14.76	4.44E-06
		rs2870109	18	a	t	11053562	0.591	++	14.94	4.51E-06
		rs8085688	18	t	c	11060641	0.5586	++	14.79	7.09E-06
		rs8090173	18	a	g	11061287	0.5562	++	14.61	7.92E-06
		rs8089847	18	t	g	11059798	0.5562	++	14.57	7.97E-06
		rs8090019	18	a	g	11061197	0.5562	++	14.59	7.97E-06
		rs8085263	18	t	c	11060338	0.5562	++	14.58	7.98E-06

		rs6505614	18	a	g	11059659	0.5562	++	14.56	8.00E-06
Region IV		rs12307997	12	a	g	7746538	0.784	++	**22.11**	**6.24E-07**
		rs17789655	12	a	g	7741253	0.2192	++	20.54	8.30E-07
		rs11055277	12	a	g	743319	0.1843	++	19.77	3.77E-06
		rs7132821	12	a	g	7741698	0.8158	++	19.77	3.78E-06
		rs11055283	12	a	g	7744779	0.1842	++	19.76	3.82E-06
		rs7299581	12	a	g	7741482	0.1862	++	19.29	6.97E-06
Region V	**A**	rs10152811	15	a	c	84655594	0.6948	--	**-18.81**	**8.33E-07**
	B	rs12904863	15	t	c	69211063	0.9207	--	**-30.13**	**6.43E-06**
		rs1470266	15	c	g	69211686	0.9202	--	-29.68	6.81E-06
		rs12914813	15	a	t	69226766	0.0815	--	-29.49	7.16E-06
		rs2292854	15	a	g	69222676	0.9188	--	-29.47	7.21E-06
		rs16955207	15	a	g	69224644	0.9185	--	-29.40	7.31E-06
		rs16955200	15	a	g	69224609	0.9185	--	-29.38	7.32E-06
Region VI	**A**	rs10508921	10	t	c	51680714	0.1266	--	**-25.70**	**8.76E-07**
		rs10825492	10	a	g	51678736	0.1542	--	-22.41	1.57E-06
	B	rs9420790	10	a	g	102246187	0.0755	--	**-34.63**	**1.68E-06**
		rs4545488	10	a	g	102602763	0.054	--	-42.89	6.63E-06
Region VII	**A**	rs12127679	1	t	c	242234424	0.1874	++	**22.31**	**1.45E-06**
		rs12119867	1	t	c	242231620	0.804	++	22.00	2.39E-06
		rs12122270	1	t	g	242237078	0.2016	++	19.92	3.19E-06
		rs10927099	1	t	c	242236379	0.7931	++	19.12	5.55E-06
		rs1156644	1	a	g	242234958	0.2232	++	18.13	5.64E-06
		rs12119931	1	t	g	242234288	0.2049	++	19.33	5.92E-06
		rs1319790	1	t	c	242234859	0.2036	++	19.27	6.11E-06
		rs12126831	1	a	c	242234012	0.2047	++	19.31	6.16E-06
		rs12126763	1	t	c	242233793	0.2032	++	19.24	6.63E-06
	B	rs11810574	1	a	g	238350359	0.8463	--	**-23.12**	**3.60E-06**
Region		rs10985375	9	a	c	123589463	0.1032	--	**-33.94**	**2.49E-06**

VIII										
Region IX		rs2613514	17	a	g	73622113	0.7788	--	**-20.12**	**3.20E-06**
Region X		rs2116940	19	a	g	10171392	0.8057	--	**-19.71**	**3.39E-06**
		rs8112895	19	a	g	10166162	0.8001	--	-19.33	5.39E-06
		rs10423341	19	a	c	10128266	0.1948	--	-19.15	6.54E-06
		rs2241531	19	c	g	10132034	0.7997	--	-18.76	9.69E-06
		rs9305012	19	t	c	10121869	0.7996	--	-18.61	1.16E-05
Region XI		rs7203315	16	t	g	10095561	0.3399	++	**15.83**	**3.75E-06**
Region XII		rs878131	20	a	g	45113909	0.2494	++	**17.21**	**3.77E-06**
		rs947019	20	t	g	45107654	0.2534	++	17.00	3.80E-06
		rs1417449	20	a	g	45106953	0.2535	++	16.96	6.18E-06
Region XIII	**A**	rs166881	6	t	c	118725674	0.8222	--	**-22.26**	**3.95E-06**
	B	rs6928289	6	a	g	137714995	0.5512	--	**-15.56**	**9.43E-06**
		rs4142802	6	t	g	137716038	0.4494	--	-15.56	9.74E-06
		rs1521173	6	a	t	137718151	0.5504	--	-15.59	9.91E-06
Region XIV		rs9788333	13	c	g	20774096	0.8943	--	**-28.65**	**4.09E-06**
Region XV	**A**	rs2460504	5	c	g	76259927	0.1835	--	**-24.57**	**4.53E-06**
	B	rs2099077	5	a	c	102791947	0.2964	--	**-16.53**	**8.25E-06**
Region XVI	**A**	rs7147996	14	a	t	89466327	0.1163	--	**-28.09**	**5.70E-06**
		rs12889533	14	t	c	89439502	0.0841	--	-30.50	8.52E-06
	B	rs9323104	14	t	c	21648621	0.1028	--	**-28.78**	**9.81E-06**
Region XVII		rs12498735	4	t	c	185158293	0.2517	++	**19.13**	**7.86E-06**

Table 2.5.7 Top 77 SNPs in 27 different regions associated with triglyceride response in African-Americans[94]

					GERA			PEAR			Meta-analysis		
SNP	chr	Nearby gene	minor alleles	MAF[a]	beta	s.e.	pvalue[b]	beta	s.e.	pvalue[b]	beta	s.e.	pvalue[bc]
rs12279250	11	NELL-1	C	0.15	14.72	8.22	7.33e-2	35.46	6.03	4.09e-9	28.20	4.86	6.60e-9
rs2686586	3	GOLIM4	T	0.47	15.09	4.92	2.15e-3	22.87	4.97	4.27e-6	18.93	3.59	6.14e-8
rs12455924	18	FAM38B	T	0.36	-18.9	5.31	3.64e-4	-17.1	4.62	2.18e-4	-17.88	3.49	2.91e-7
rs12307997	12	GDF3	G	0.22	19.5	6.91	4.79e-3	23.9	5.78	3.51e-5	22.11	4.44	6.24e-7
rs10152811	15	AGBL1	A	0.69	-17.8	5.37	8.56e-4	-19.7	5.43	2.76e-4	-18.81	3.82	8.33e-7
rs10508921	10	ASAH2	T	0.13	-14.1	7.68	6.61e-2	-35.7	7.13	5.61e-7	-25.7	5.22	8.76e-7
rs12127679	1	ZNF238	T	0.19	22.85	5.98	1.32e-4	21.49	7.32	3.33e-3	22.31	4.63	1.45e-6
rs9420790	10	ERLIN1	A	0.08	-19.4	11.87	1.03e-1	-43.6	9.12	1.72-6	-34.63	7.23	1.68e-6
rs10985375	9	DAB2IP	A	0.1	-27.6	10.98	1.19e-2	-38.7	9.56	5.06e5	-33.94	7.21	2.49e-6
rs2613514	17	TNRC6C	G	0.22	-14.2	6.57	3.08e-2	-24.6	5.73	1.73e-5	-20.12	4.32	3.20e-6
rs2116940	19	ANGPTL6/ UBL5	G	0.19	-29.2	6.23	2.81e-6	-11.5	5.79	4.67e-2	-19.71	4.24	3.39e-6
rs11810574	1	CHRM3	G	0.15	-24.9	9.59	9.24e-3	-22.4	5.84	1.23e-4	-23.12	4.99	3.60e-6
rs7203315	16	GRIN2A	T	0.34	17.46	4.96	4.29e-4	14.34	4.73	2.43e-3	15.83	3.42	3.75e-6
rs878131	20	EYA2	A	0.25	14.84	5.74	6.71e-3	19.25	5.08	1.49e-4	17.21	3.72	3.77e-6
rs166881	6	SLC35F1	C	0.18	-18.7	7.25	9.86e-3	-25.1	6.46	1.04e-4	-22.26	4.83	3.95e-6
rs9788333	13	ZDHHC20	G	0.11	-39.6	9.76	4.89e-5	-21.1	8.07	8.79e-3	-28.65	6.22	4.09e-6
rs2460504	5	IQGAP2	C	0.18	-34.2	7.86	1.32e-5	-16.2	7.33	2.73e-2	-24.57	5.36	4.53e-6
rs7147996	14	C14ORF14	A	0.12	-28.2	9.74	3.81e-3	-28	8.02	4.74e-4	-28.09	6.19	5.70e-6

		3											
rs17133858	11	ARRB1	T	0.14	-17.3	6.83	1.12e-2	-24.1	6.29	1.29e-4	-20.99	4.63	5.79e-6
rs12904863	15	CT62	C	0.08	-43.4	10.42	3.12e-5	-20.9	8.70	1.64e-2	-30.31	6.68	6.43e-6
rs12498735	4	STOX2	T	0.25	24	7.13	7.61e-4	16.39	5.35	2.21e-3	19.13	4.28	7.86e-6
rs2099077	5	NUDT12	A	0.29	-12.4	5.53	2.46e-2	-19.9	4.99	6.95e-5	-16.53	3.71	8.25e-6
rs6928289	6	OLIG3	G	0.45	-14	5.15	6.49e-3	-16.9	4.81	4.33e-4	-15.56	3.51	9.43e-6
rs9323104	14	-	T	0.1	-47.2	11.8	5.80e-5	-20.6	7.82	8.41e-3	-28.78	6.51	9.81e-6

Table 2.5.8: Lead SNPs showing association with triglyceride response to hydrochlorothiazide in meta-analysis of African-Americans[94] [a]Minor allele frequency among combined sample of GERA and PEAR. [b]P values are based on a linear regression adjusted for sex, age, waist circumference, baseline insulin, baseline triglycerides and the two first principal components of genetic variation for each study. [c]P values are calculated using inverse-variance meta-analysis.

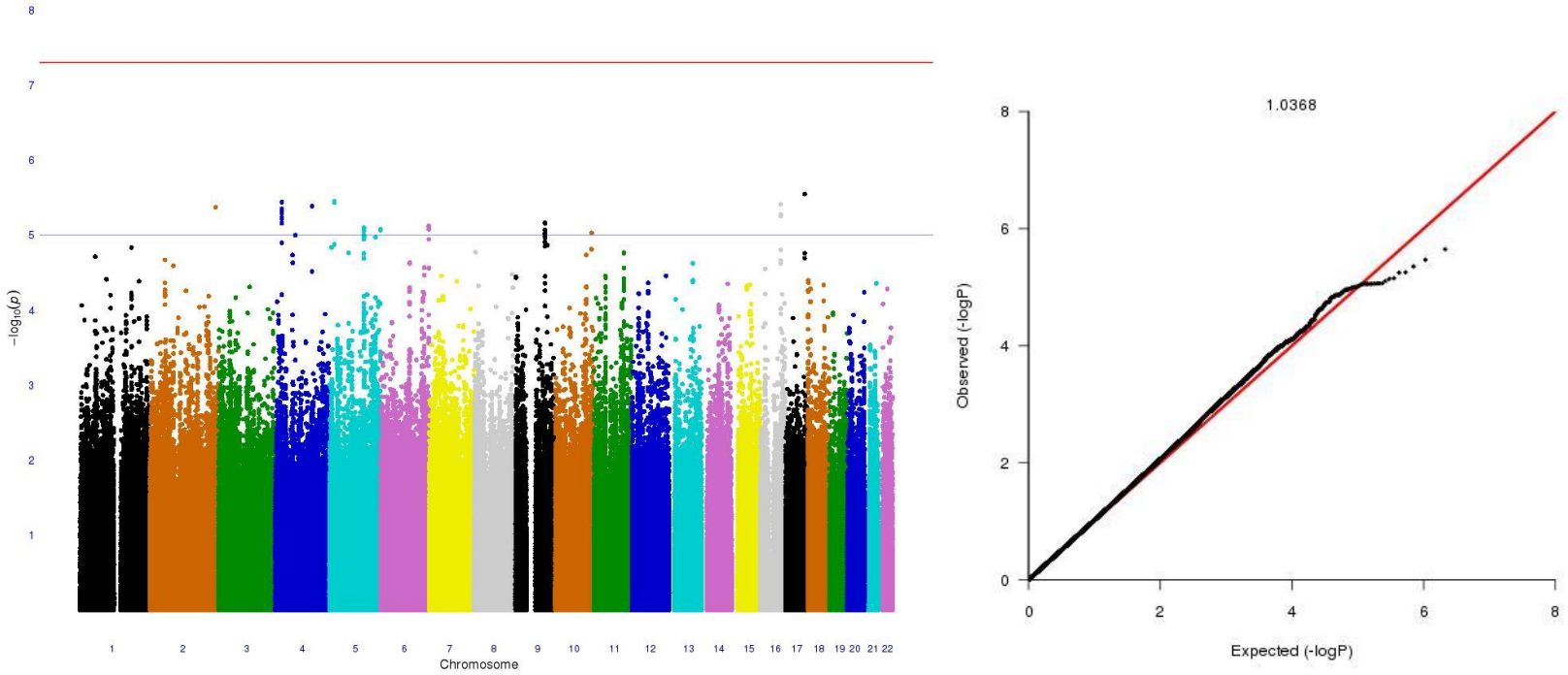


Figure 2.5.1 Manhattan and QQ plots of GWAS results for glucose responses to HCTZ in non-Hispanic Whites[94]

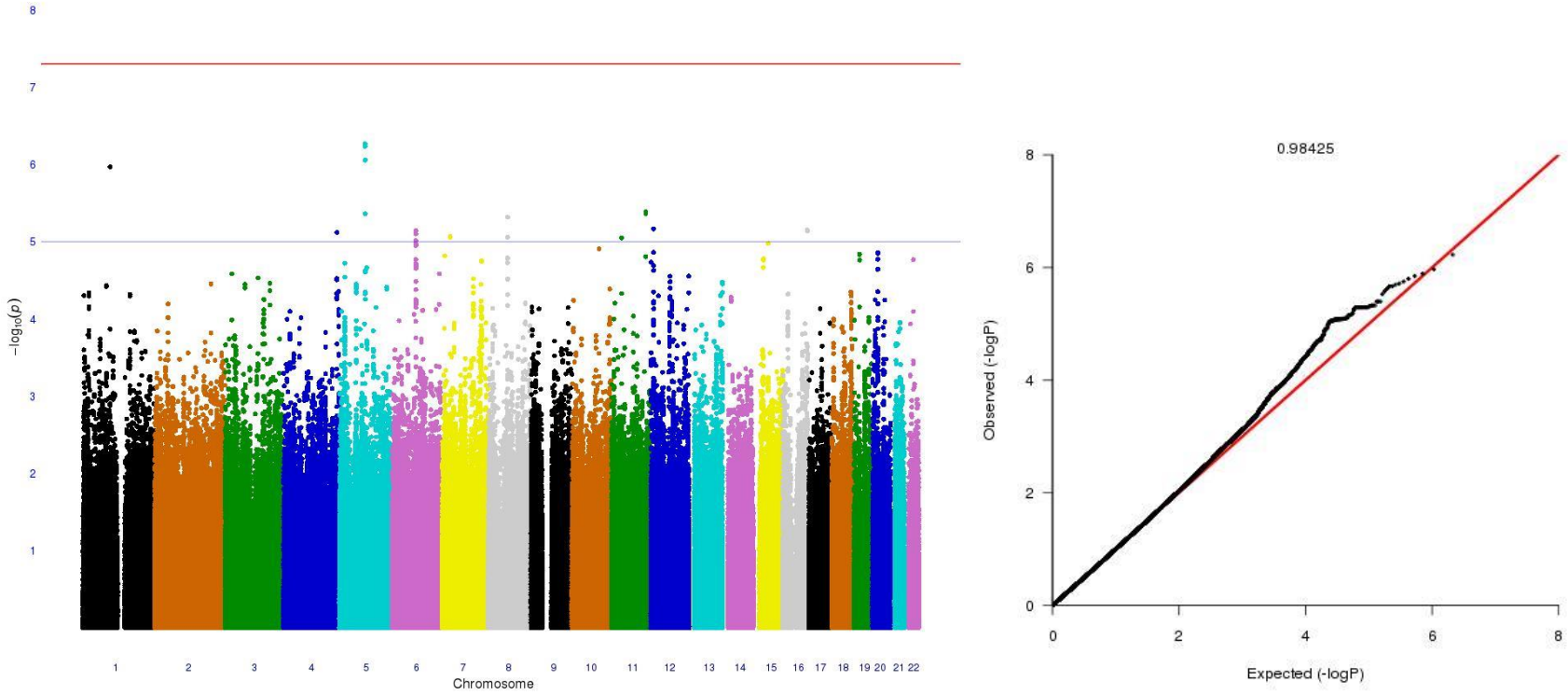


Figure 2.5.2 Manhattan and QQ plots of GWAS results for triglycerides responses to HCTZ in non-Hispanic Whites[94]

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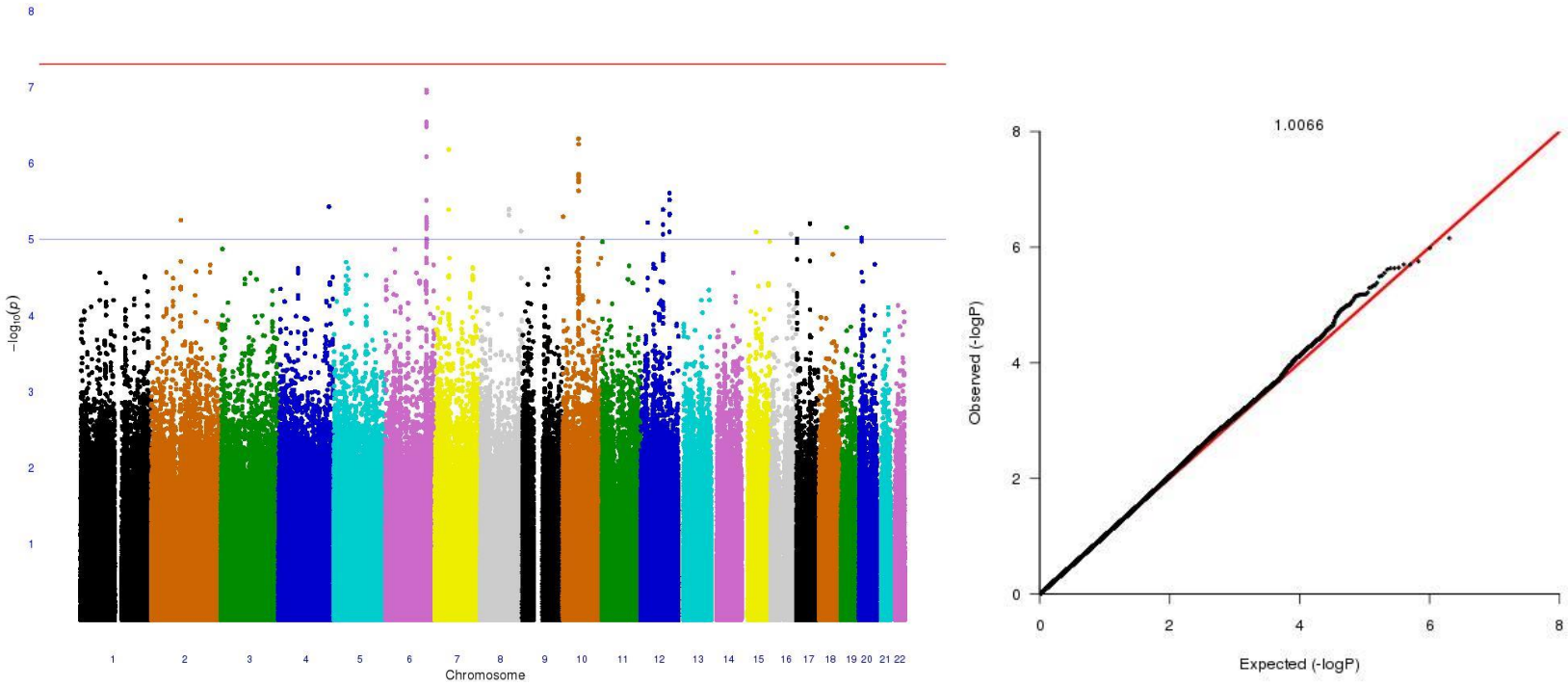

Figure 2.5.3 Manhattan and QQ plots of GWAS results for glucose responses to HCTZ in African-America[94]

CHAPTER 3: LONGITUDINAL GENOME-WIDE ASSOCIATION FOR INTERACTION OF SNPS WITH THIAZIDE DIURETIC USE ON FASTING GLUCOSE AND INSULIN LEVELS IN EUROPEAN AMERICANS IN THE CHARGE CONSORTIUM

3.1 INTRODUCTION

Diuretics are the most common prescribed antihypertensive drug in the USA[89]. Thiazide diuretics are the first line drug treatment for patients with essential hypertension[2] and 47.5 million filled prescriptions of this drug were dispensed in 2008[99]. Although the effectiveness and low cost of this drug are well known, so are its adverse metabolic effects. Pharmacogenomics studies (PGXs) of thiazide diuretics seek to identify genetic variants that may cause these adverse metabolic effects. Most PGXs are based on clinical trials or intervention studies in which the drug is taken, the response for each individual is assessed, and differences in response among genotypes are tested. Because of their intensity, these intervention studies often suffer from small sample size and low statistical power. However, it is possible to do PGX in an observational setting where the patterns of drug use and phenotypes levels are measured longitudinally. I used a genome-wide association approach with longitudinal data on fasting glucose and fasting insulin levels and diuretic use to identify genomic regions influencing adverse metabolic effect. These genome wide association studies were carried out in more than 40,000 individuals from the CHARGE consortium's population-based studies.

3.2 MATERIALS AND METHODS

3.2.1 Study populations

All participants were European American descendants that were enrolled in fourteen population based cohorts [the Atherosclerosis Risk in Communities (ARIC) study [103], the Age-Gene-Environment Susceptibility—Reykjavik Study(AGES)[104], Cardiovascular Health Study(CHS)[105], The Heart and Vascular Health Study(HVH)[106], Framingham Heart Study(FHS)[107], Multi-Ethnic Study of Atherosclerosis(MESA)[108], Rotterdam Study 1(RS1)[109-112], Rotterdam Study 2(RS2)[109-112], Prospective Study of Pravastatin in the Elderly at Risk / Pharmacogenomic study of Statins in the Elderly at risk for cardiovascular disease(PROSPER/PHASE)[113], the Health, Aging, and Body Composition Study(Health ABC)[114], Hypertension Genetic Epidemiology Network(HyperGEN)[115], Women's Health Initiative (WHI)[116], and Coronary Artery Risk Development in Young Adults (CARDIA) study[117]]. The analysis plan described in the statistical methods section was run for each cohort. Later on, the findings within each cohort were combined by meta-analysis.

Participants from each cohort provided written informed consent, and each study was approved by local ethics committees.

3.2.2 Study design exclusion and inclusion criteria

Participants with diabetes at baseline were excluded. The definition of diabetes was self-reported history, physician-reported diagnosis of diabetes, fasting glucose levels of more than 126mg/dL, non-fasting glucose more than 200mg/dL or use of anti-diabetic medications. We also excluded all participants treated with only loop diuretics, due to their different mechanism of action in the nephron, and all participants who did not give consent for use of their DNA. Participants missing both fasting glucose and fasting insulin or missing information on fasting state were excluded.

3.2.3 Definition of drug exposure

Drug exposure was assessed via the method of medication inventory in eleven cohorts and by pharmacy information in the other two (RS1 and RS2). We classified a participant as exposed to or users of the drug if he or she uses a thiazide or thiazide-like diuretic, at each visit, in a single or combination preparation with or without concomitant use of a loop diuretic, potassium-sparing diuretic, or potassium supplement.

3.2.4 Measurement of glucose and insulin

Fasting glucose (mmol/L) and fasting insulin (mmol/L) were quantified in each cohort by using similar procedures as described previously[118, 119]. Fasting insulin was natural log-transformed for analysis

3.2.5 Genotype arrays and imputation

Each cohort performed their own genome-wide SNP genotyping using different chip arrays from either Illumina (San Diego, CA, USA) or Affymetrix (Santa Clara, CA, USA). Common quality control steps were followed in all cohorts. SNP with minor allele frequency ≤1%, call rates <95% Hardy-Weinberg equilibrium p-values $<10^{-5}$ and individuals with more than 10% missing genotypes were excluded. To increase the coverage and facilitate interrogation of each SNP among the cohorts, the genotypes were imputed with

approximately 2.5 million HapMap SNPs using the Phase II release 22 build 36 and the HapMap CEU reference. Quality control for the imputed results was done using standard procedures (RSQ_HAT <0.3 and MAF ≤ 0.05) but different algorithms were used among cohorts to perform the imputation (Table 3.2.1), such as Bayesian Imputation-Based Association Mapping[120], Markov chain based haplotyper[121] or BEAGLE[122]. All studies inferred unobserved genotypes in a probabilistic manner.

Study[a]	Genotyping array	Genotype calling algorithm	Imputation software	GWAS statistical analysis software
AGES	Ilumina Hu370CNV	Bead Studio	MACH v.1.16	Boss
ARIC	Affymetrix 6.0	Birdseed	MACH v1.16	Boss
CHS	Illumina 370CNV	Genome Studio	BIMBAM 0.99	Boss
CARDIA	Illumina 6K/318K/370K	Bead Studio	MACH v 1.0.15	Boss
FHS	Affymetrix 500K + 50 MIP	BRLMM	MACH v 1.0.15	Boss
Health ABC	Illumina 1M	Bead Studio	MACH v1.16	Boss
HyperGEN	Illumina Human610-Quad BeadChip	GenCall	MACH	Boss
HVH	Illumina 370	Genome Studio	BIMBAM 0.99	Boss
MESA	Affymetrix 6.0	Birdseed	IMPUTE v2.1.0	Boss
PROSPER	Illumina 660K	Bead studio	MACH v1.15	Boss
RS1	Illumina 550k,	Bead Studio	MACH1 v 1.0.15,	ProbABEL/Boss
RS2	Illumina 550K Duo, 610KQuad	Genome Studio	MACH v 1.0.16	ProbABEL/Boss
WHI CORE white	Affymetrix 6.0	Birdseed	MACH v1.16	Boss
WHI BB white	Affymetrix 6.0	Birdseed	MACH v1.16	ProbABEL

Table 3.2.1: Genotyping characteristics of the 14 studies included in the pharmacogenomics analysis of fasting glucose and fasting insulin participants of European descent. [a]All studies used HapMap phase 2 release 22 build 36 and the HapMap CEU reference panel for imputation. . AGES, Age, Gene/Environment Susceptibility – Reykjavik Study. ARIC, Atherosclerosis Risk in Communities study. HVH, the Heart and Vascular Health Study. FHS, Framingham Heart Study. MESA, Multi-Ethnic Study of Atherosclerosis. RS, Rotterdam Study. PROSPER, Prospective Study of Pravastatin in the Elderly at Risk. Health ABC, Health, Aging, and Body and Composition Study. HyperGEN, Hypertension Genetic Epidemiology Network. WHI, Women's Health Initiative. CHS, Cardiovascular Health Study. CARDIA, Coronary Artery Risk Development in Young Adults.

3.2.6 Statistical analysis

Within each cohort and when the data were available, we performed either a longitudinal analysis or a cross-sectional analysis. We used the overall population design that includes drug users and non-users, to allow the separation of the SNP main effect and interaction effect estimates. Each drug-genotype interaction was estimated under additive genetic model, using the robust variance estimator and an independence working correlation (for longitudinal analysis). In the case of cohorts studies, the analysis was carried out using ProbABEL[123] and "Boss" for longitudinal analysis [124]. We used as covariates age, sex, study site, body mass index, and ancestry principal components. Two different phenotypes of interest were analyzed separately: fasting glucose and log fasting insulin levels.

For all of the studies we used the t-distribution as the reference distribution, because the number of genetic variants among participants on drug therapy was too small to use standard asymptotic results. The total degrees of freedom were calculated as the product of the number of drug users, the SNP imputation quality and the SNP minor allele frequencies. The p-values were calculated using the original beta over the standard error estimated under t-distribution.

The meta-analysis was done using METAL[125], applying the standard weighted Z-statistic method. The weights were based on the product of: the number of drug users and SNP imputation quality. The genome-wide threshold for significant drug-SNP interaction was $p<5X10-8$ and the suggestive threshold was defined as $p<1X10-5$

3.3 RESULTS

The total number of participants with European ancestry among the 14 cohorts for the fasting glucose study was 40226. HVH, WHI and FHS were cohorts that did not use longitudinal data for this analysis. In the case of fasting insulin, 12 cohorts out of the 14 (36923 total participants) have data for the analysis, and FHS, RS1, RS2 and WHI cohorts only have baseline phenotype information available. Some basic descriptive statistics are shown in Table 3.3.1. There were more women than men participants, and the sample was largely middle-aged to elderly. The average range of follow-up data for fasting glucose was

0.25 to 11.1 years and for fasting insulin it was 1.7 to 9.5 years. The total number of autosomal SNPs available for analysis after imputation was 2.5 million.

Figure 3.3.1 and Figure 3.3.2 show the Manhattan and Q-Q plots for fasting glucose and fasting insulin, respectively. They show a conservative distribution, with no early departures of the p-values from the null expectation. There were no genome-wide significant ($p<5*10^{-8}$) interactions detected for either phenotype. However, there were some suggestive signals scattered across the genome that may be of interest to other studies (Table 3.3.2 and Table 3.3.3).

Study/EA	Glucose total	Glucose Cases	insulin total	Insulin Cases	Glucose Follow-up time (mean -sd in years)	Insulin Follow-up time (mean-sd in years)	Age,y mean(SD)	%Female
ARIC	8619	1918	8349	1467	3.5 (0.9)	1.7 (0.4)	58.3 (6.5)	53%
AGES	1561	445	2620	574	5.19 (0.45)	0	74.6(4.75)/79.8(4.76)	62%
CHS	2752	733	2743	682	6.3 (4.7)	2.4 (1.2)	72.3 (5.3)	62%
HVH	450	168	-	-	0	-	65.7 (8.9)	54%
FHS	7382	329	6165	223	0	0	46.9(11.9)/47.0(11.9)	46.60%
MESA	2214	489	2214	415	4.9 (0.3)	9.5 (0.5)	62.4 (10.2)	52%
RS1	2275	372	5974	223	11.1(0.4)	0	71.1 (6.2)	63.10%
RS2	3315	144	2157	102	4.1 (0.5)	0	64.4 (7.6)/72.3 (8.6)	55.70%
PROSPER	4531	1263	-	-	0.25 (3 months)	-	75.3 (3.5)	52%
Health ABC	2091	358	1915	339	8.4(3.2)	7.6(4.4)	73.8(2.8)	50%
HyperGEN	1086	167	1086	166	0	0	48.3(13.7)	50%
WHI CORE white	855	168	855	180	3.4 (0.8)	3.31 (0.9)	66.1 (7.3)	100%
WHI BB white	1179	142	1151	140	0	0	66.7 (6.5)	100%
CARDIA	1703	96	1694	95	5.92(2.26)	5.55 (1.60)	25.7(3.6)	53%

Table 3.3.1: Baseline characteristics of all cohorts for the analyses of fasting glucose and fasting insulin. AGES, Age, Gene/Environment Susceptibility – Reykjavik Study. ARIC, Atherosclerosis Risk in Communities study. HVH, the Heart and Vascular Health Study. FHS, Framingham Heart Study. MESA, Multi-Ethnic Study of Atherosclerosis. RS, Rotterdam Study. PROSPER, Prospective Study of Pravastatin in the Elderly at Risk. Health ABC, Health, Aging, and Body and Composition Study. HyperGEN, Hypertension Genetic Epidemiology Network. WHI, Women's Health Initiative. CHS, Cardiovascular Health Study. CARDIA, Coronary Artery Risk Development in Young Adults

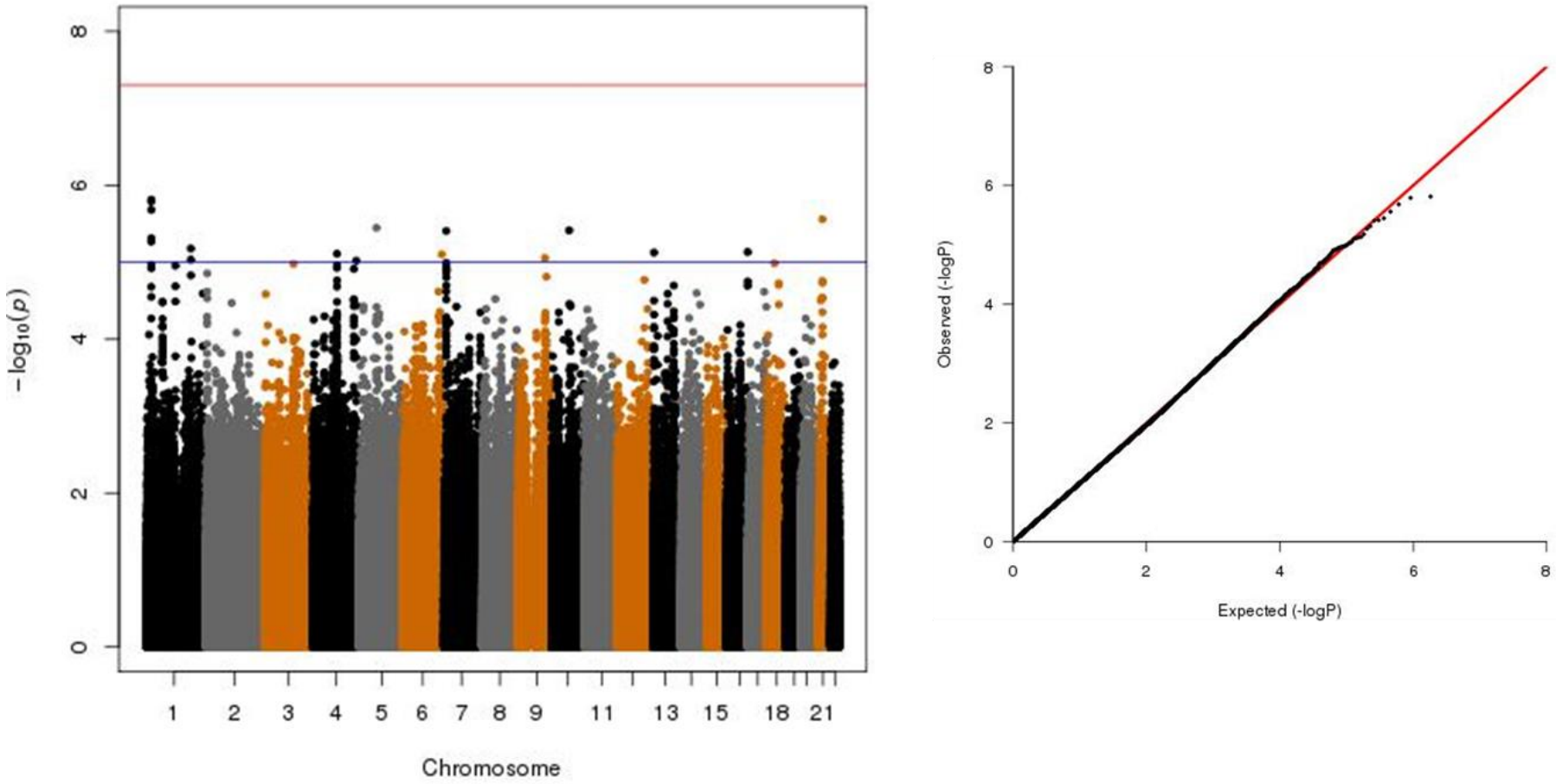

Figure 3.3.1 Manhattan and Q-Q plots of Thiazide SNP interaction estimates for fasting glucose after the meta-analysis from 14 cohorts of European ancestry

SNP	CHR	BP	Alleles	GENE	feature	Close left_gene	Close right_gene	Direction	P-value
rs6668463	1	19891367	T/G	TMCO4	Intron [NM_181719.3]	HTR6	RNF186	+?-+-+++++++++?	1.54E-06
rs11582254	1	19890664	T/C	TMCO4	Intron [NM_181719.3]	HTR6	RNF186	-?+-+-------??	1.62E-06
rs4912138	1	19866072	A/G	HTR6	Intron [NM_000871.1]	NBL1	TMCO4	?---+--------?	2.09E-06
rs2092018	21	28249480	A/C	NA	NA	RPL10P1	C21orf94	--+-------+---	2.76E-06
rs1217798	5	71411704	T/C	NA	NA	CARTPT	MAP1B	??--+----+----	3.58E-06
rs7072464	10	72456503	T/C	NA	NA	PCBD1	UNC5B	?---+-----+---	3.86E-06
rs2028401	7	9152388	A/G	NA	NA	LOC100129634	LOC100132601	??--+--+------	3.93E-06
rs6689496	1	19892631	T/C	TMCO4	Intron [NM_181719.3]	HTR6	RNF186	??+-+-------+?	4.88E-06
rs2050120	1	19889499	T/C	TMCO4	Intron [NM_181719.3]	HTR6	RNF186	??+-+-------+?	5.39E-06
rs4650679	1	183631346	T/G	NA	NA	IVNS1ABP	HMCN1	?+-++-++-+++++	6.64E-06
rs7191944	16	88521379	C/G	TUBB3	Intron [NM_006086.2]	MC1R	DEF8	??--?---------	7.38E-06
rs3117941	13	21420118	T/G	NA	NA	FGF9	FTHL7	+?+----+------	7.50E-06
rs12499530	4	99391302	A/G	NA	NA	C4orf37	RAP1GDS1	?-----+--++---	7.77E-06
rs4252166	6	161081894	C/G	PLG	Intron [NM_000301.1]	LPA	LOC100128831	?--+?+++++++++	7.90E-06
rs7867796	9	112160533	T/C	NA	NA	TXNDC8	SVEP1	-?++-+++-+++++	8.80E-06
rs17519029	1	183474050	A/T	C1orf26	Intron [NM_017673.6]	LOC100129295	IVNS1ABP	--?-----?---++	9.29E-06
rs12648583	4	179143587	A/G	LOC285501	Intron [NM_001085490.1]	LOC285500	LOC391719	??++-++++++++?	9.56E-06

Table 3.3.2 The SNPs with p-values that reached suggestive values ($p<1x10^{-5}$) for fasting glucose

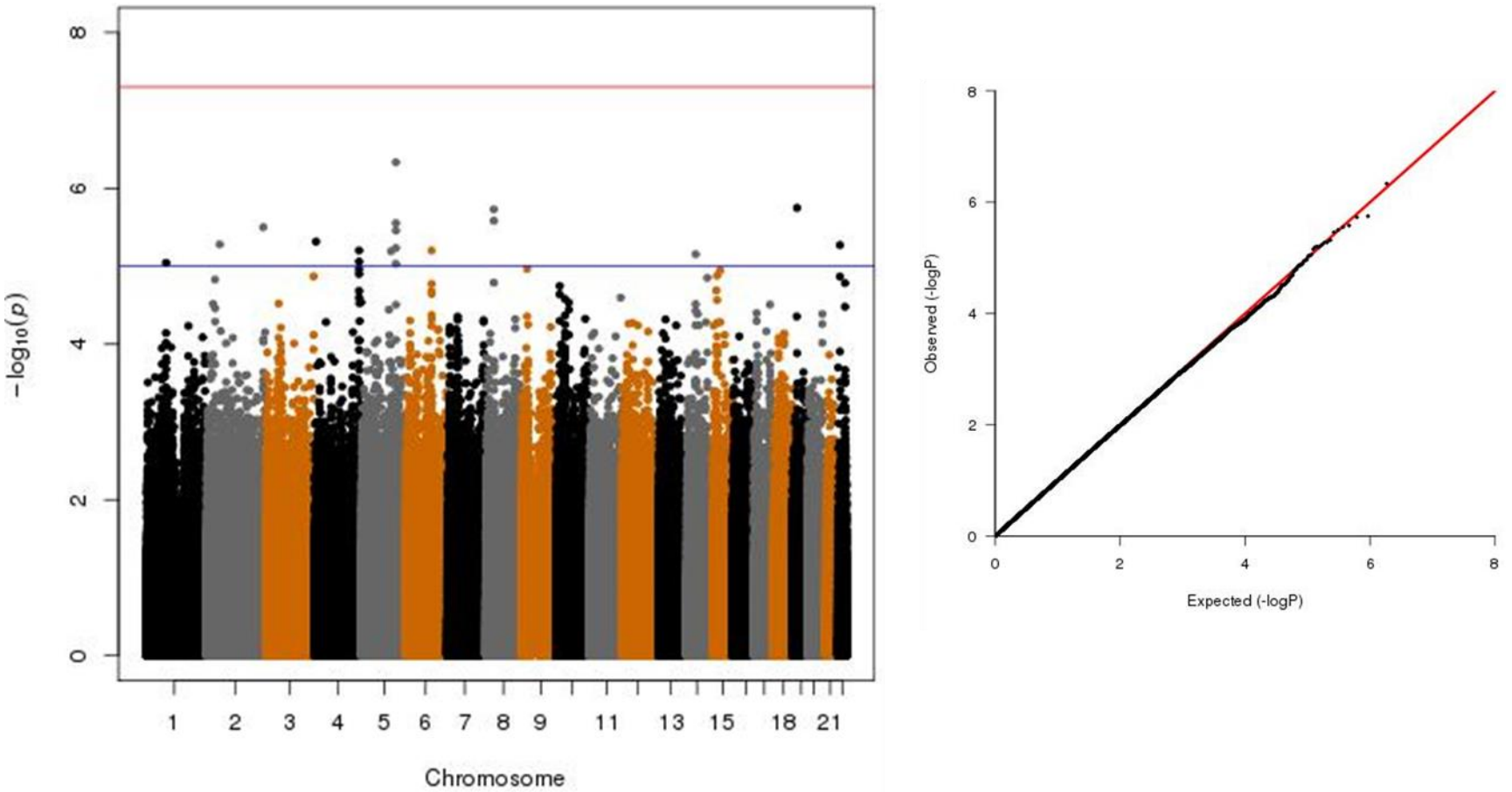

Figure 3.3.2 Manhattan and Q-Q plots of Thiazide SNP interaction estimates for fasting insulin after the meta-analysis from 12 cohorts of European ancestry.

SNP	CHR	BP	Alleles	GENE	feature	Close left_gene	Close right_gene	Direction	P-value
rs433623	5	141348082	C/T	RNF14	utr-3 [NM_183400.1]	PCDH12	GNPDA1	?------++--+	4.64E-07
rs3951363	19	21411491	C/T	NA	NA	ZNF493	LOC400680	?----+------	1.79E-06
rs2919378	8	32689211	C/T	NRG1	Intron [NM_013964.2]	LOC100127894	MST131	-?-+---+----	1.87E-06
rs2919379	8	32687507	C/T	NRG1	Intron [NM_013964.2]	LOC100127894	MST131	-?-+-------	2.62E-06
rs403075	5	141354978	C/T	NA	NA	RNF14	GNPDA1	-++++++--+??	2.80E-06
rs4973009	2	232169359	A/G	NA	NA	C2orf57	PTMA	?++-+++-++++	3.16E-06
rs7408	5	141360532	A/G	GNPDA1	utr-3 [NM_005471.3]	RNF14	MRPL11P2	?------++-++	3.48E-06
rs3856955	4	5825769	C/T	EVC	Intron [NM_153717.2]	EVC2	CRMP1	?-----+-----	4.82E-06
rs6758455	2	53307348	A/G	NA	NA	LOC129656	ASB3	++++++++++++	5.25E-06
rs5760783	22	23723897	C/T	NA	NA	TMEM211	CTA-221G9.4	-?------+-??	5.35E-06
rs164496	5	141329380	C/T	RNF14	Intron [NM_183400.1]	PCDH12	GNPDA1	?++++++-++++	5.83E-06
rs9386572	6	107161529	C/T	RTN4IP1	Intron [NM_032730.4]	AIM1	QRSL1	??--++------	6.29E-06
rs2546275	4	181804265	C/T	NA	NA	LOC391719	hCG_2025798	+?+++++++??	6.30E-06
rs959300	5	122070554	A/G	NA	NA	ARGFXP1	SNX2	?----+-+----	6.40E-06
rs173352	14	55826496	A/G	PELI2	Intron [NM_021255.2]	C14orf34	C14orf101	+?--------??	7.01E-06
rs2546270	4	181807433	C/T	NA	NA	LOC391719	hCG_2025798	??++++++++++	8.75E-06
rs11163056	1	80811644	A/G	NA	NA	COX6A1P	LOC646538	+++-+-++++++	9.07E-06
rs252141	5	141325246	A/G	RNF14	near-gene-5[NM_183399.1]	PCDH12	RNF14	?------+--+?	9.32E-06

Table 3.3.3 : The SNPs with p-values that reached suggestive values ($p<1x10^{-5}$) for fasting insulin.

3.4 DISCUSSION

Onset of adverse metabolic effects following thiazide or thiazide-like therapy is unpredictable. Therefore, if we can identify genomic markers that confer greater risk for these adverse effects, we can use them to better understand the biology underlying the phenomenon and perhaps predict those individuals who may be susceptible and advise them (and their physician) to use alternative antihypertensive therapies. However, one of the major limitations to identify the contributing genes (or genomic regions) is the small number of individuals in previous GWAS studies using a controlled intervention approach[94].

The CHARGE pharmacogenomics group with its 14 large multi-institutional cohorts represents the largest effort in detecting genomic loci influencing diuretic response by collecting a large number of participating individuals – 40226 in the case of fasting glucose and 36923 for fasting insulin. Our meta-analysis did not identify a common SNP (p-value < 5×10^{-8}) conferring high risk for hyperglycemia or hyperinsulinemia among European-American participants. Lack of positive results in our analysis suggests that it is unlikely that there is a single common SNP with a large influence on the adverse metabolic reaction to diuretic therapy. I speculate that there may be multiple variants spread throughout the genome each with modest effect sizes or a few rare variants with large effect that have a greater role in these phenotypes.

This study has limitations. For example, environmental factors such as salt intake were not controlled for in these observational studies. Confounding by contraindication resulting from comorbidities influencing thiazide diuretic use may also be a factor. However a previous simulation showed that these phenomena have very modest effects on estimates of interaction in pharmacogenomics studies[126]. Ascertainment of drug use was based on medication inventory or pharmacy data, and the latter is more accurate than the former to gauge usage. Finally, the fact that our drug definition is thiazide or thiazide-like diuretic, in a single or combination preparation with or without concomitant use of a loop diuretic, potassium-sparing diuretic, or potassium supplement may have led to heterogeneous effects. These realities may have reduced the sensitivity for detecting SNPs possessing important interactive effects.

CHAPTER 4: TRANS ETHNIC META ANALYSIS SUGGESTS GENETIC VARIATION IN THE HEME PATHWAY INFLUENCES POTASSIUM RESPONSE IN PATIENTS TREATED WITH HYDROCHLOROTHIAZIDE.

This chapter is based upon: Del-Aguila JL, Cooper-Dehoff RM, Chapman AB, Gums JG, Beitelshees AL, Bailey K, Turner ST, Johnson JA, Boerwinkle E Pharmacogenomics J. "in press" (permission from Nature Publishing Group)

4.1 INTRODUCTION

One adverse effect of thiazide diuretic treatment of hypertension is hypokalemia[49, 91]. Hypokalemia, in turn, may be responsible for impaired insulin secretion[56, 127], although this hypothesis is not universally supported[100, 128]. The process by which thiazide diuretics lead to potassium wasting and hypokalemia in some patients but not others is not well-understood.

This chapter is an effort to identify genes or pathways associated with hydrochlorothiazide (HCTZ)-induced hypokalemia. To accomplish this goal, I performed a genome-wide association study (GWAS) and a Multi-Ethnic Meta-Analysis (MANTRA)[129] in European-American and African-American hypertensive patients from two different pharmacogenetic studies.

The results presented here are the first GWAS to our knowledge of diuretic-induced potassium loss, and the analysis was conducted in four different study samples (European-Americans and African-Americans each from two different studies).

The data implicate the HEME pathway in affecting diuretic treatment-induced hypokalemia.

4.2 MATERIALS AND METHODS

4.2.1 Study population

Details of the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR, clinicaltrials.gov identifier NCT00246519) study and The Genetic Epidemiology of Responses to Antihypertensive (GERA, clinicaltrials.gov identifier NCT00005520) study have been previously published[130, 131]. PEAR and GERA study participants were African-Americans and European-Americans with mild to moderate essential hypertension without a history of heart disease or diabetes mellitus. In PEAR, after a wash out period of approximately 4 weeks, blood and urine were collected in a fasting state. After the baseline evaluations were completed, participants were randomized to HCTZ (thiazide diuretic 12.5mg orally once daily) or atenolol (β-blocker 50mg orally once daily) for three weeks, with dose doubling (to 25 mg HCTZ) for those with BP > 120/70 mmHg for an additional 6 weeks. More than 90% of PEAR participants received the higher 25 mg dose of HCTZ. For this analysis of potassium (K+) response, we used only those randomized to the HCTZ arm, referred to hereafter as PEAR HCTZ monotherapy.

In GERA, after a wash-out period of at least 4 weeks, blood samples were obtained for baseline biochemical measurement, and then qualifying participants were treated with HCTZ (25 mg orally once daily) for 4 weeks. At the end of the 4 week diuretic treatment period, blood pressure was measured and blood samples were again obtained for biochemical measurements. All blood collections were done in the morning after 8 hours of fasting.

All participants enrolled in PEAR and GERA provided written informed consent, and the institutional review boards of participating study centers approved the study protocols.

4.2.2 Phenotype and Genotype data

Serum potassium concentrations, in PEAR, were measured on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN, USA). In GERA, serum potassium concentrations were measured by flame photometer (Instrumentation Laboratories model 943, Lexington, MA). Plasma insulin, which was used as a covariate during statistical analyses, was measured in both studies with the Access Ultrasensitive Insulin immunoassay system.

In GERA, serum potassium concentration was determined after 2 weeks of HCTZ therapy. In PEAR, serum potassium concentrations were measured at the end of the wash-out period (baseline), after three weeks and at the end of the HCTZ monotherapy. In both studies, there were protocol-mandated potassium supplementation requirements based on serum potassium levels. Potassium supplementation was required for values < 3.6 mmol/L in GERA and < 3.2 mmol/L in PEAR. Potassium supplementation was also available for use by the study physician, at their discretion, at higher potassium levels. To avoid confounding from prescribed potassium supplementation and to promote patient safety, serum potassium response to HCTZ was defined as the difference between the levels measured after the first visit (two weeks in GERA case and three in PEAR) of treatment and the baseline visit, prior to initiation of potassium supplementation. Thirteen participants with potassium response values under or over 3 standard deviations from the mean response were removed from the analysis.

Participants were genotyped using the Illumina HumanOmni1-Quad (Illumina, San Diego, California, USA) in the case of PEAR and the GeneChip Human Mapping 500k Array, (Affymetrics, Santa Clara California, USA) in the case of GERA. Standard quality control steps were applied to the raw genotype data in both studies: single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) <1%, call rates <95%, Hardy-

Weinberg equilibrium p-values ≥10^{-5} and participants with more than 10% missing genotypes were removed from the analysis. The software MACH[95] (www.sph.umich.edu/csg/abecasis/MACH/download) was used to impute the approximate 2.5 million HapMap SNPs using the Phase II CEU as the reference panel for European-Americans and a cosmopolitan sample of CEU and YRI for African-Americans. Quality control filters for the imputed results were RSQ_HAT <0.3 and MAF ≤ 0.05. MACH generated a file with the highest posterior probabilities for each imputed SNP (i.e. dosage) which was used in the analysis. After quality control and imputation, there were more than 2 million SNPs used for the genotype-phenotype association studies in each race-study group.

4.2.3 Statistical analysis

Additive genetic association analyses of potassium response to HCTZ were performed by self-reported race within each study using ProbABEL[123], while adjusting for sex, age, waist circumference, the baseline values of potassium and insulin, and the two first principal component scores (PCS), to avoid the possibility of spurious association as the result of population substructure[132, 133]. The principal components were calculated using the EIGENSTRAT[132] software. The European-American and African-American GWAS analyses were combined via transethnic meta-analysis using MANTRA (Meta-Analysis of Transethnic Association studies)[129]. MANTRA clusters populations by the mean allele frequency difference. If populations are in the same cluster, MANTRA assumes that they have the same underlying allelic effects and a fixed effect model is performed. On the other hand, if populations are in different cluster, the assumption will be that they do not have the same underlying allelic effect and a random effect analysis is performed. MANTRA provides a combined association signal called a Bayes Factor (BF) instead of a traditional p-value to compare the null and alternative hypotheses. A BF of 10^5, roughly corresponding to a p-value of 7.9×10^{-7}, was considered significant evidence of association[134] after accounting for genome-wide multiple comparisons[135].

4.3 RESULTS

Table 4.3.1 A and Table 4.3.1B summarize the baseline characteristics of the European-American and African-American study participants, respectively. Both studies had a higher percentage of European-Americans male and African American female study participants. The average age and waist circumference were nearly identical between PEAR

and GERA. Baseline potassium was lower in African-Americans from GERA compared to the other groups. The mean response in European Americans was lower compared to African Americans. Figure 4.3.1 shows the distribution of potassium response in both populations after removing 13 outlier individuals.

No genome-wide significant association was found in each separate study and race group (supplementary information pp 77-90). After the multi-ethnic meta-analysis, the top seven SNPs from MANTRA are shown in Table 4.3.2, and four of them, located in three different loci, reached genome-wide significance. A Manhattan plot of the BFs is shown in Figure 4.3.2. These significant SNPs are: 1) rs10845697 (BF=5.560) on chromosome 12, near to the heme binding protein 1 gene (HEBP1), 2) rs1007869 and rs12596186 (LD: r^2>0.99, BF=5.347 and 5.114 respectively) on chromosome 16, near the junctophilin 3 gene (JPH3) and 3) rs11135740 (BF=5.258) on chromosome 8, near to the Mitoferrin-1 gene (SLC25A37 also known as MFRN1). The effects of rs10845697 were not consistent among study samples (Table 4.3.2). Whether this inconsistency is due to chance sampling variation, differences in linkage disequilibrium, or differences in allelic effects among the study samples is not known rs12596186 (LD: r^2>0.99, BF=5.347 and 5.114 respectively) on chromosome 16, near the junctophilin 3 gene (JPH3) and 3) rs11135740 (BF=5.258) on chromosome 8, near to the Mitoferrin-1 gene (SLC25A37 also known as MFRN1).

(A)

Variables	PEAR: European Americans	GERA: European Americans
Total	229	196
Gender(%men)	59.8	57.1
Age(y)	49.9±9.4	48.5±7.3
Waist Circumference (cm)	94.8±13.6	103.7±14.7
Insulin ((μIU/ml)	9.4±8.7	8.5±4.9
Plasma Glucose(mg/dL)	92.9±12.2	95.1±13.2
Serum Potassium(mEq/L)	4.39±0.441	4.06±0.246
Systolic blood pressure(mmHg)	151.8±13.5	142.6±12.6

(B)

Variables	PEAR: African Americans	GERA: African- Americans
Total	148	194
Gender(%men)	37.84	48.5
Age(y)	47.4±8.9	47.4±6.3
Waist Circumference (cm)	97.3±13.5	96.8±14.4
Insulin ((μIU/ml)	9.9±9.6	12.9±10.1
Plasma Glucose(mg/dL)	90.8±12.7	101.12±15.6
Serum Potassium (mEq/L)	4.13±0.48	3.76±0.307
Systolic blood pressure(mmHg)	151.8±13.14	150.8±14.9

Table 4.3.1 PEAR and GERA sample characteristics measured at baseline

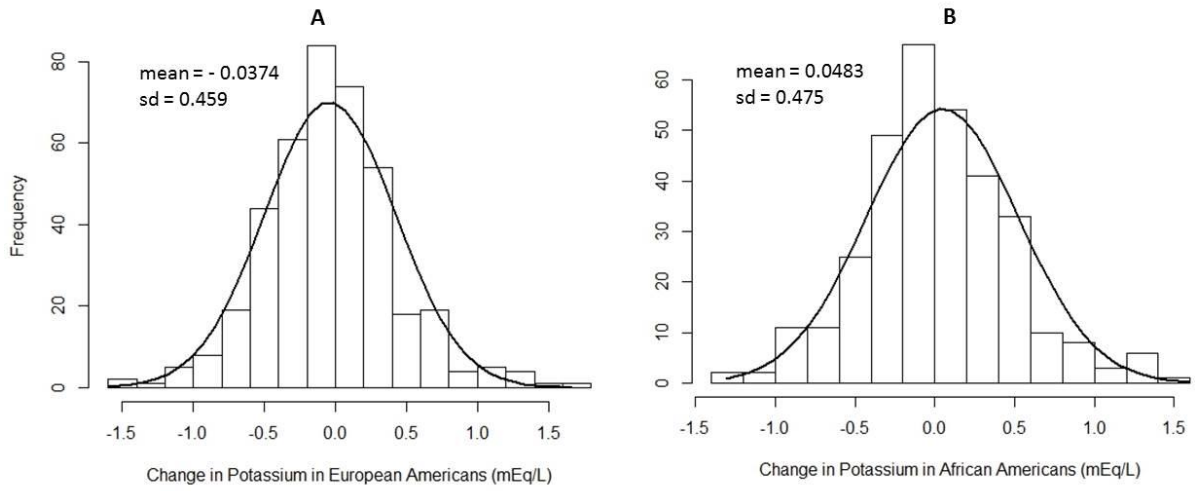

Figure 4.3.1: The distribution of potassium response due to Hydrochlorothiazide in both populations. The mean in the distribution was -0.03738(sd = 0.459) in European Americans (A), in the case of African Americans (B), it was 0.0483 (sd = 0.475).

SNP	CHR	Position	EA	Nearby Gene	Bayes Factor
rs10845697	12	13076153	G	HEBP1	5.560
rs1007869	16	86174420	T	JPH3	5.347
rs11135740	8	23423702	A	SLC25A37	5.258
rs12596186	16	86174068	A	JPH3	5.114
rs13250356	8	23415776	T	SLC25A37	4.984
rs721183	8	23441779	G	SLC25A37	4.982
rs12095896	1	81723330	C	--	4.980

Table 4.3.2: Top Seven SNPs from Multi-Ethnic Meta-Analysis. Log10 Bayes factor (BF) from the MANTRA analysis. A log10 BF of 5 and higher was considered as a conservative threshold for GWAS. EA=effect allele, AA=African American, EU=European American

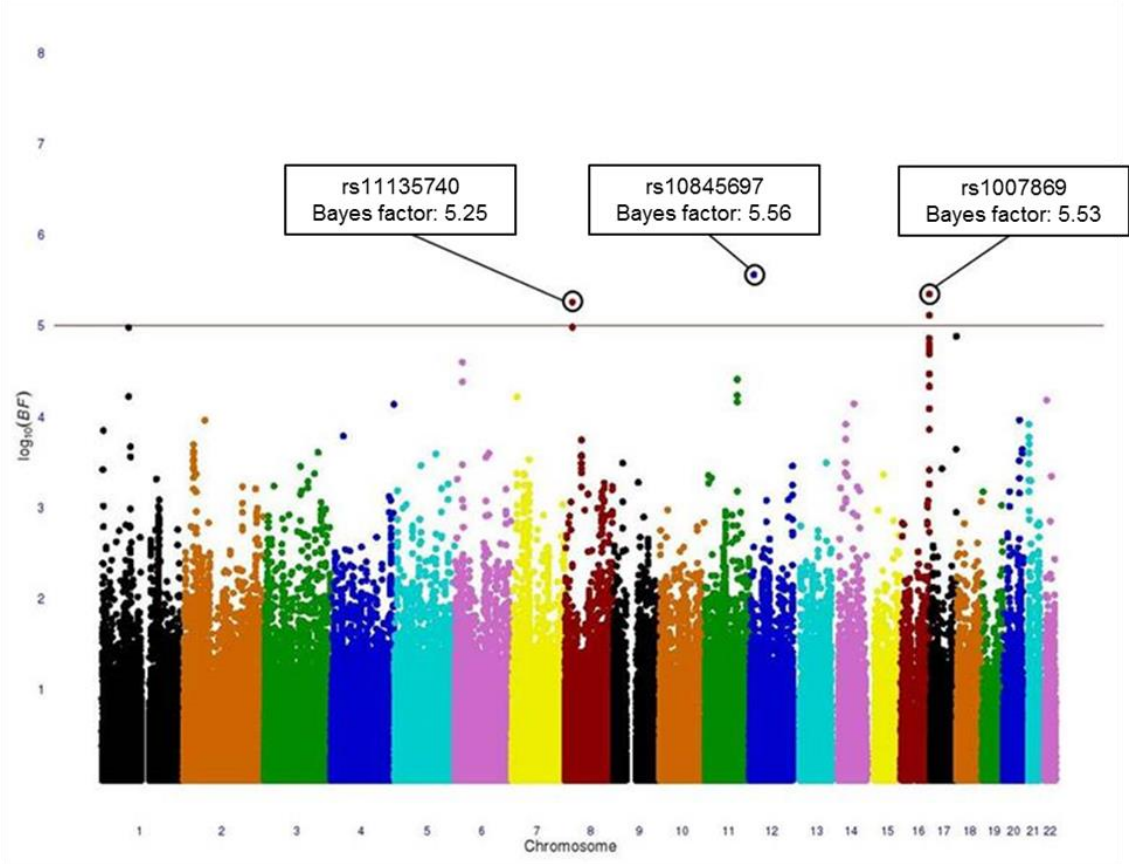

Figure 4.3.2: MANTRA Manhattan plot showing association of potassium response to HCTZ treatment in African Americans and European Americans in the PEAR and GERA studies. Three SNPs on chromosome 12, 8 and 16 showed genome-wide significance (BF > 5.00).

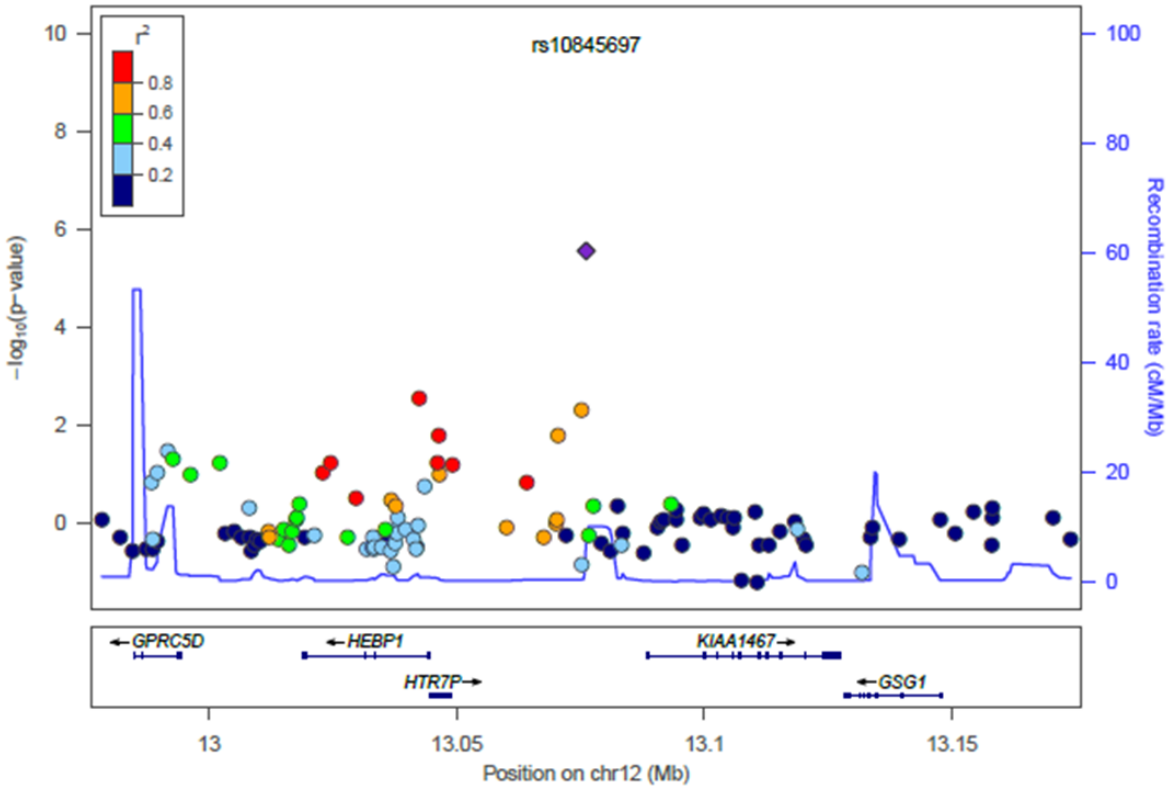

Figure 4.3.3: Regional Plot on chromosome 12 in which SNP rs10845697 is located. The gene HEBP1 is the gene related to the Heme pathway

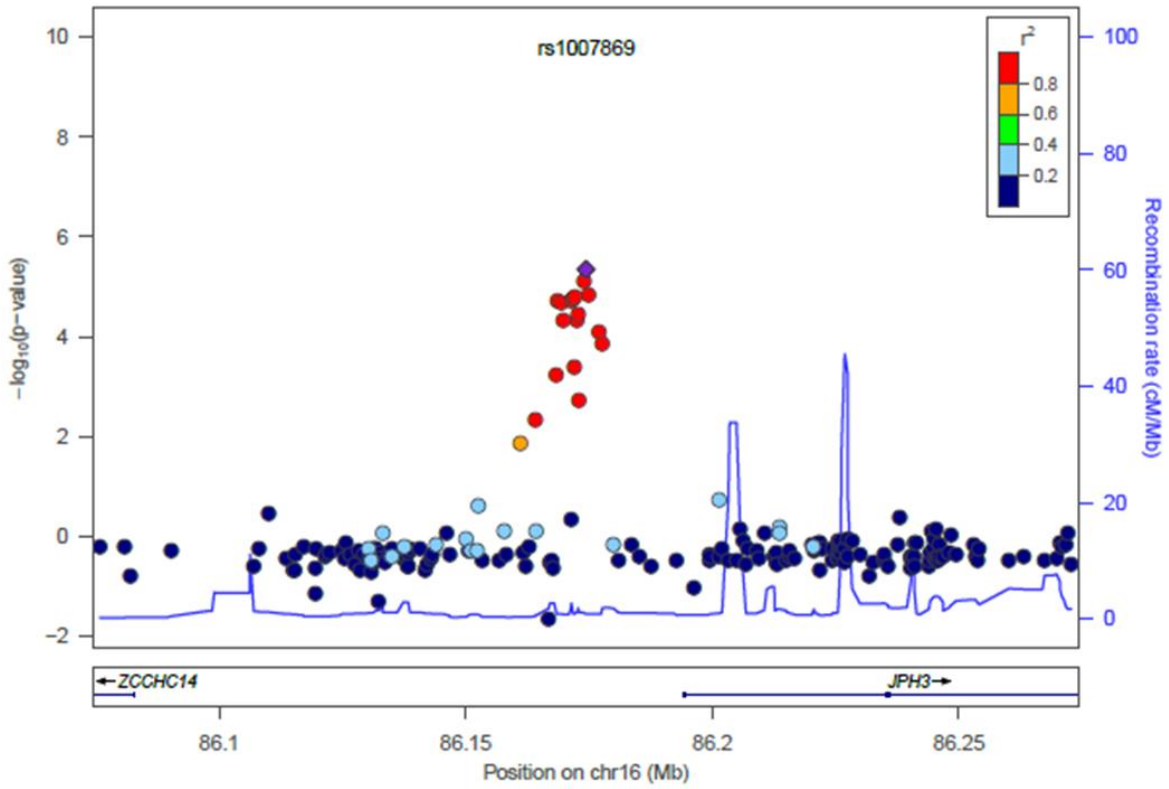

Figure 4.3.4: Regional Plot on Chromosome 16 shows the position of rs1007869. The gene JPH3 is related to Huntington Disease and chronic kidney disease.

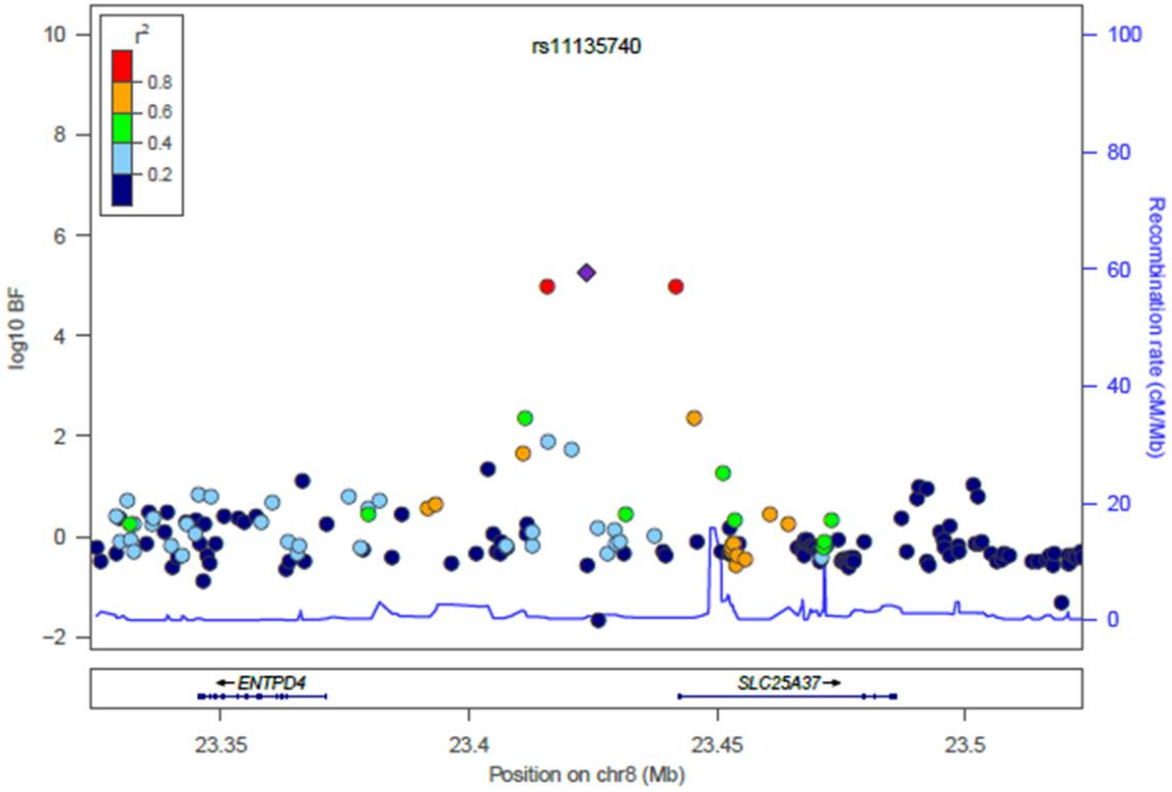

Figure 4.3.5: Regional Plot on Chromosome 18. The SNP rs11135740 is located close to SLC25A37 gene which is the gene related to the Heme pathway.

Because HEBP1 and SLC25A37 are related to HEME synthesis, we carried out a targeted analysis of other genes and SNPs related to HEME synthesis. Although rs12095896 on chromosome 1 did not reach GWAS significance (BF=4.980), it was found in region of the genome previously related to bilirubin levels (http://www.genome.gov/gwastudies/index.cfm?pageid=26525384#searchForm). Bilirubin is a bile pigment that is a degradation product of HEME. rs10845697 near HEBP1, rs11135740 near SLC25A37, and rs12095896 in the bilirubin locus combined accounted for 5% and 7% of the variance of potassium response in PEAR and GERA European Americans, respectively. In African Americans, these values were 16% for PEAR and 12% for GERA. The effect size estimates of the top signals from the HEME synthesis pathway are shown in Table 4.3.3

Effect size (se)		**rs10845697 (close to HEBP1)**	**rs11135740 (close to SLC25A37)**	**rs12095896 (close to Bilirubin QTL)**
African Americans	GERA	0.14218 (0.0454)	0.13685 (0.02667)	-0.09491 (0.05482)
	PEAR	-0.12882 (.0539)	0.13461 (0.02809)	0.21906 (0.05015)
European Americans	GERA	-0.05039 (0.0401)	0.13683 (0.02741)	-0.05655 (0.03434)
	PEAR	0.18161 (0.0459)	0.13410 (0.02835)	-0.06223 (0.03491)

Table 4.3.3 The effect size estimates from each separate GWAS for each of the top signals from trans-ethnic Meta-analysis related to HEME synthesis.

4.4 DISCUSSION

Hypokalemia is a well-known adverse effect in patients taking HCTZ. The purpose of this study was to identify genes or genomic regions associated with HCTZ-induced change in potassium. I conducted a GWAS and MANTRA from two studies of hypertensive European American and African American individuals. Four SNPs achieved a log 10 Bayes Factor greater than 5 which was defined *a priori* to be GWAS significant. Confirmation of these results will be required in an independent sample of hypertensive patients treated with HCTZ. Among the top SNPs, rs10845697 near HEBP1 and rs11135740, near Mitoferrin-1 (SLC25A37) have a role in heme biosynthesis[136-139]. Heme has been identified as a modulator of the large-conductance Ca^{2+}-activated K^+ channels (BK, or maxi K) channels[140]. BK channels are located in the cortical thick ascending limbs[141-143], the distal convoluted tubule(DCT)[144], connecting tubule (CNT)[145] and the medullary collecting tubule (MCD) [141-143] in the kidney. They are sensitive to change in both voltage and calcium concentration and they work as volume regulatory channels and secrete potassium[146]. In the CNT, Thiazide-induced increased ultrafiltrate delivery to the CNT, this will result in augmented tubular flow in CNT promoting BK-dependent K+ secretion at this site. However, they have not previously been considered important contributors to renal potassium secretion because of their low open probabilities (P_o) as well as their low concentration on the principal cell in the collecting ducts of the kidney [146]. The main route for potassium secretion is considered to be the inwardly-rectifying channel, subfamily J, member 1 (ROMK1) encoded by the gene KCNJ1 which has higher P_o and higher density on principal cells than BK channels[147]. Although KCNJ1 has shown significant associations with change in glucose in multiple studies[148, 149], SNPs in and around KCNJ1 were not associated with potassium response to HCTZ (data not shown) in this study.

I hypothesize that HCTZ affects serum K in part by influencing heme levels; the bulk of the effect would be inhibition of Na-Cl cotransporter. Heme has been shown to increase the *Po* of the BK channels at negative voltages and to decrease it at more positive voltages[150]. As a result, the secretion of potassium by BK channels would be positively associated with heme levels.

Herein, we report the first GWAS to our knowledge of HCTZ-induced hypokalemia and provide evidence for a novel mechanism of HCTZ-induced hypokalemia. Our study is unique in that it included a washout of all antihypertensive agents prior to initiation of HCTZ and collected detailed data regarding potassium supplementation and levels. However, there are weaknesses that must be acknowledged, for instance, although MANTRA can

provide an overall Bayes factor as the combined association signal, it fails in estimating a proper combined effect size from the joint analysis of all the studies. Another weakness is the absence of replication sample. During this study, we contacted multiple potential collaborators in the United States and Europe, but none had appropriate potassium response data to serve as a replication for this study. This experience underscores the need for an international pharmacogenetics of antihypertinsives consortium.

4.5 SUPPLEMENTARY INFORMATION

Name	CHR	Allele1	Allele2	Freq Allele1	B	Position	P.value[b]
rs3860285	16	A	T	0.1366	0.334355	86177102	1.21E-07
rs3860286	16	A	C	0.1317	0.338087	86177727	1.83E-07
rs1007869	16	C	T	0.8683	-0.33765	86174420	1.84E-07
rs12596186	16	A	G	0.1317	0.33681	86174068	1.85E-07
rs4843634	16	A	T	0.1317	0.335339	86172805	1.88E-07
rs5026466	16	C	G	0.1318	0.334663	86172236	1.89E-07
rs6540062	16	A	C	0.8682	-0.33442	86172138	1.89E-07
rs4843633	16	G	T	0.1317	0.334877	86172592	1.89E-07
rs17781628	16	A	G	0.1318	0.334078	86171795	1.90E-07
rs17781622	16	A	G	0.8699	-0.33537	86171466	2.07E-07
rs12444907	16	C	T	0.8632	-0.33075	86174969	2.52E-07
rs4843635	16	C	G	0.1369	0.32919	86173026	2.52E-07
rs8059726	16	G	T	0.1327	0.328863	86169468	2.53E-07
rs12444269	16	C	G	0.8673	-0.32879	86168628	2.55E-07
rs17836461	16	A	G	0.8673	-0.32878	86168428	2.55E-07
rs3965791	16	C	G	0.8677	-0.32937	86169845	2.57E-07
rs7124500	11	C	T	0.8289	-0.27354	132816875	1.78E-06
rs12446100	16	C	T	0.8689	-0.3178	86164164	2.10E-06
rs295699	5	A	T	0.3745	-0.21468	73732248	2.72E-06
rs3909525	16	A	G	0.1362	0.309976	86161146	2.91E-06
rs4833034	4	C	T	0.3455	-0.2197	37994749	3.38E-06

rs17215757	4	C	T	0.0559	0.455013	182896313	3.64E-06
rs7481213	11	C	T	0.8288	-0.26326	132844528	4.11E-06
rs7481500	11	G	T	0.8277	-0.26268	132839560	4.13E-06
rs7926647	11	C	T	0.173	0.259554	132827521	4.55E-06
rs11223474	11	A	T	0.8267	-0.259	132824185	4.66E-06
rs10474415	5	A	C	0.3724	-0.21108	73730174	4.89E-06
rs3912201	14	C	T	0.591	0.206028	55664173	5.00E-06
rs7349056	22	A	G	0.8351	-0.49493	22061801	5.88E-06
rs7871887	9	C	T	0.1336	-0.319	99611263	7.00E-06
rs3911953	3	A	G	0.3848	-0.20546	73880249	7.56E-06
rs3845885	3	C	T	0.3848	-0.20544	73881112	7.63E-06
rs2309536	4	C	T	0.906	-0.35428	182870193	8.17E-06
rs2961071	7	A	T	0.3574	0.204126	141407299	8.18E-06
rs11586466	1	C	T	0.6833	0.349123	94892799	8.61E-06
rs17779898	4	A	C	0.2737	0.22088	73987351	9.21E-06
rs6590698	11	C	G	0.7902	-0.24367	132819450	9.44E-06
rs7127798	11	A	G	0.2093	0.244889	132816986	9.59E-06
rs7112051	11	C	T	0.2127	0.243601	132852125	9.97E-06
rs6590712	11	A	C	0.7874	-0.24358	132854916	9.98E-06

Table 4.5.1: Top 40 SNPs associated with potassium response in European Americans in the GERA study ($p<10^{-5}$)[b]P-values are based on a linear regression adjusted for sex, age, waist circumference, baseline insulin, baseline potassium and the two first principal components.

Name	CHR	Allele1	Allele2	Freq. Allele1	B	Position	P.value[b]
rs8121649	20	C	T	0.9	-0.277999	49514051	2.17E-07
rs6091326	20	C	T	0.8999	-0.277314	49514749	2.27E-07
rs17728712	20	A	G	0.2259	0.222402	49497628	2.71E-07
rs6096436	20	A	T	0.2267	0.222387	49497963	2.72E-07
rs880324	20	A	G	0.2066	0.226564	49487597	3.87E-07
rs1457501	3	G	T	0.0787	0.409515	90391748	1.41E-06
rs12415289	10	G	T	0.945	-0.396245	65594256	1.82E-06
rs7962710	12	C	T	0.5521	-0.185898	38650369	1.85E-06
rs6722679	2	A	G	0.1274	0.301391	233641663	1.92E-06
rs9865917	3	A	G	0.1263	0.295705	112649698	1.97E-06
rs9856827	3	C	T	0.8734	-0.295608	112642842	1.99E-06
rs1909095	3	C	T	0.1349	0.294155	112636191	2.73E-06
rs7807828	7	C	T	0.63	-0.195253	42533677	3.03E-06
rs984809	7	C	G	0.938	-0.425247	16324952	3.18E-06
rs4720429	7	A	G	0.3686	0.194047	42524143	3.23E-06
rs6708049	2	C	T	0.8761	-0.292945	233651537	3.23E-06
rs10877877	12	C	G	0.4509	0.180851	38653720	3.32E-06
rs4428577	7	G	T	0.6267	-0.192542	42564495	3.83E-06
rs757522	7	C	G	0.052	0.419761	16330134	4.09E-06
rs10237886	7	C	T	0.0518	0.419453	16335031	4.15E-06
rs2158485	7	C	T	0.0518	0.419453	16332864	4.15E-06
rs7779204	7	A	G	0.0519	0.419453	16335490	4.15E-06

rs2389595	7	C	T	0.9481	-0.419468	16338850	4.15E-06
rs6949613	7	A	G	0.0519	0.419468	16341806	4.15E-06
rs6461240	7	A	G	0.9482	-0.420007	16341948	4.18E-06
rs6785666	3	A	C	0.5222	-0.187893	22551549	4.34E-06
rs1074612	3	A	C	0.5224	-0.18788	22554572	4.36E-06
rs9874439	3	A	T	0.4771	0.187885	22556225	4.44E-06
rs12638304	3	G	T	0.477	0.187885	22556385	4.45E-06
rs4520070	7	G	T	0.6268	-0.190643	42554149	4.58E-06
rs16858101	3	C	T	0.121	0.301383	112664498	5.11E-06
rs12667487	7	A	G	0.6239	-0.190391	42545153	5.57E-06
rs1818903	7	C	T	0.3775	0.189731	42565109	5.75E-06
rs11232492	11	C	T	0.8678	-0.304302	80531648	5.75E-06
rs9873792	3	A	C	0.867	-0.297479	112704293	5.96E-06
rs4634534	7	C	T	0.627	-0.188859	42579215	6.69E-06
rs1589794	7	A	C	0.627	-0.188775	42570889	6.79E-06
rs6506850	18	A	C	0.9321	-0.359879	74594768	6.80E-06
rs4355689	7	C	T	0.3775	0.187896	42554082	6.82E-06
rs1357772	7	C	T	0.3774	0.187767	42552488	6.90E-06
rs1524798	3	C	T	0.8174	-0.227436	83849028	6.97E-06
rs10032745	4	G	T	0.7925	-0.21749	110118152	6.97E-06
rs4856466	3	C	T	0.8174	-0.22736	83856031	6.99E-06
rs9828223	3	C	T	0.8781	-0.29863	112707615	7.04E-06
rs11663063	18	C	T	0.0541	0.412155	27827523	7.07E-06
rs1357797	3	A	G	0.1825	0.227114	83857768	7.13E-06
rs2941831	18	A	G	0.0744	0.329674	74623976	7.19E-06

rs3863122	4	G	T	0.2078	0.216103	110118295	7.36E-06
rs3863125	4	A	G	0.2077	0.216218	110118726	7.42E-06
rs1949050	11	C	T	0.5388	0.174517	108437543	7.58E-06
rs13433798	3	A	G	0.8892	-0.312413	112678830	7.68E-06
rs1524790	3	A	T	0.8157	-0.226553	83836660	8.05E-06
rs1873407	3	C	T	0.5052	-0.182601	195176275	9.40E-06
rs1729791	12	C	T	0.5351	-0.171664	57051720	9.43E-06
rs1349272	3	C	T	0.5024	0.1851	22502129	9.51E-06
rs1949052	11	A	G	0.5688	0.172612	108437872	9.72E-06

Table 4.5.2: Top 56 SNPs associated with potassium response in European Americans in the PEAR study ($p<10^{-5}$) [b]P-values are based on a linear regression adjusted for sex, age, waist circumference, baseline insulin, baseline potassium and the two first principal components.

Name	CHR	Allele1	Allele2	Freq. Allele1	B	Position	P.value[b]
rs7715999	5	A	G	0.8832	-0.532961	82111381	7.63E-08
rs1842249	5	G	A	0.8988	-0.430565	25773621	5.32E-07
rs2885404	17	C	T	0.9433	-0.786045	63931228	9.08E-07
rs583807	6	G	A	0.8991	0.343969	4100791	1.69E-06
rs6891558	5	C	G	0.889	0.447258	132682700	2.31E-06
rs4474027	8	A	G	0.8967	-0.309752	9514775	3.27E-06
rs9693430	8	G	T	0.9444	-0.49321	130034016	4.21E-06
rs11128214	3	C	T	0.8886	0.327577	71545572	4.30E-06
rs17242435	8	G	A	0.9442	-0.491735	130039930	4.36E-06
rs17820673	8	C	A	0.9442	-0.491208	130043811	4.44E-06
rs8063994	16	A	G	0.2708	-0.253213	82678219	4.75E-06
rs12700592	7	G	A	0.892	-0.354379	24987645	4.97E-06
rs12537228	7	C	T	0.8922	-0.35382	24986390	5.10E-06
rs9537937	13	A	T	0.7981	-0.232819	57546600	6.17E-06
rs11022391	11	G	A	0.9446	-0.514256	12506759	6.23E-06
rs1453830	11	A	G	0.4072	0.197476	132485784	6.44E-06
rs10750531	11	A	G	0.4072	0.197391	132486275	6.47E-06
rs1431780	11	A	G	0.4072	0.197274	132486656	6.50E-06
rs9537878	13	G	A	0.7986	-0.235714	57433793	6.59E-06
rs260555	3	C	A	0.591	-0.233258	590153	7.18E-06
rs4767276	12	T	A	0.8753	-0.497512	113798811	7.39E-06
rs7704137	5	A	C	0.3885	-0.241164	174582300	8.98E-06

rs11096569	2	A	G	0.7156	-0.233028	19075754	9.01E-06
rs7369021	2	A	G	0.7156	-0.23304	19076521	9.02E-06
rs13180965	5	G	A	0.866	-0.26733	7240278	9.32E-06
rs10060705	5	C	T	0.7368	-0.226592	63937060	9.74E-06

Table 4.5.3 Table S3: Top 26 SNPs associated with potassium response in African Americans in the GERA study ($p<10^{-5}$). [b]P-values are based on a linear regression adjusted for sex, age, waist circumference, baseline insulin, baseline potassium and the two first principal components.

Name	CHR	Allele1	Allele2	Freq. Allele1	Beta	Position	P.value[b]
rs6047645	20	A	G	0.8442	-0.340436	21748445	1.31E-07
rs2722265	7	G	A	0.3501	0.246094	37653661	4.52E-07
rs2598064	7	C	A	0.35	0.245905	37655181	4.59E-07
rs10216297	7	T	C	0.3452	0.248814	37653087	4.80E-07
rs16842009	2	C	T	0.9278	-0.51318	158419778	4.86E-07
rs6113381	20	C	T	0.8968	-0.409101	21732950	6.80E-07
rs12625990	20	A	G	0.8968	-0.40913	21726684	6.96E-07
rs10243276	7	G	T	0.355	0.238874	37648975	8.91E-07
rs2722236	7	T	G	0.3549	0.238803	37649086	8.94E-07
rs13220395	6	G	A	0.9259	-0.432178	26163347	9.08E-07
rs2722235	7	C	T	0.3547	0.238435	37649155	9.20E-07
rs10265204	7	G	A	0.3546	0.238241	37650935	9.34E-07
rs2722269	7	G	A	0.3544	0.237915	37652206	9.59E-07
rs2598054	7	T	C	0.3459	0.236483	37657880	1.00E-06
rs2722252	7	A	G	0.3451	0.236408	37658017	1.02E-06
rs2598053	7	G	A	0.3458	0.23628	37658209	1.02E-06
rs6971034	7	A	C	0.3463	0.236205	37666837	1.04E-06
rs2722333	7	G	A	0.3462	0.23616	37666459	1.04E-06
rs2598046	7	G	A	0.3457	0.235914	37662047	1.05E-06
rs2722360	7	A	T	0.3457	0.23591	37662160	1.05E-06
rs2598042	7	G	A	0.3558	0.237593	37663829	1.15E-06
rs2598043	7	G	A	0.3557	0.237583	37663821	1.15E-06
rs2598044	7	T	C	0.3557	0.237534	37663556	1.15E-06

rs2722372	7	A	G	0.3557	0.237534	37663507	1.15E-06
rs6888729	5	T	C	0.6548	-0.254523	35801938	1.68E-06
rs2722266	7	A	G	0.3341	0.240984	37652728	1.89E-06
rs2722260	7	C	T	0.3532	0.233249	37655782	1.92E-06
rs2722267	7	A	G	0.3339	0.241107	37652567	1.93E-06
rs12535173	7	G	T	0.3575	0.233175	37655846	1.94E-06
rs2722257	7	A	G	0.3531	0.233082	37656111	1.95E-06
rs2722256	7	A	G	0.353	0.232733	37656438	2.01E-06
rs2598061	7	A	C	0.353	0.232666	37656712	2.02E-06
rs13200797	6	G	A	0.9102	-0.43268	26230936	2.09E-06
rs10247167	7	C	T	0.9177	-0.433858	144716323	2.43E-06
rs10233894	7	A	G	0.9182	-0.436268	144734048	2.44E-06
rs9566765	13	T	C	0.8128	-0.387312	40902497	2.65E-06
rs17586553	6	G	A	0.9276	-0.426277	26036754	2.96E-06
rs13195279	6	T	A	0.9293	-0.42619	26027410	2.96E-06
rs2598052	7	G	T	0.3515	0.227805	37658294	3.01E-06
rs17587226	6	C	T	0.9312	-0.435172	26070283	3.43E-06
rs2328642	20	C	G	0.7638	-0.243085	21754161	3.65E-06
rs10519916	15	T	C	0.8907	-0.347048	32321272	3.65E-06
rs7205902	16	G	C	0.8368	-0.306934	25120016	3.71E-06
rs13207673	6	C	T	0.9345	-0.430981	26009112	3.82E-06
rs13208859	6	G	A	0.9345	-0.430874	26002588	3.84E-06
rs13220488	6	A	G	0.9346	-0.430874	26004886	3.84E-06
rs13241942	7	A	G	0.3384	0.23217	37651206	3.94E-06
rs11696327	20	C	T	0.7581	-0.237324	21757409	4.44E-06

rs457864	21	C	A	0.8957	-0.374412	27093797	4.48E-06
rs10484435	6	T	G	0.9279	-0.416692	26139790	4.63E-06
rs730549	16	G	A	0.2899	-0.238479	73788556	4.86E-06
rs2722254	7	C	G	0.367	0.227112	37657353	5.03E-06
rs12095896	1	A	C	0.605	-0.226253	81723330	5.40E-06
rs889511	16	C	T	0.5157	-0.215175	73789170	6.27E-06
rs643014	11	A	G	0.8853	-0.301209	117493433	6.39E-06
rs7870338	9	A	C	0.6603	0.254412	89432342	6.77E-06
rs10775183	15	C	A	0.8083	-0.275077	61077587	7.34E-06
rs1587837	8	T	G	0.5337	-0.204157	91897240	7.85E-06
rs1875901	8	T	C	0.5338	-0.204104	91895883	7.85E-06
rs12680488	8	G	A	0.5368	-0.203451	91891456	7.91E-06
rs1818193	8	A	G	0.5373	-0.202426	91886818	7.92E-06
rs1504791	8	G	A	0.5526	-0.209589	91904312	8.42E-06
rs1504792	8	A	C	0.5526	-0.209536	91904233	8.45E-06
rs1862736	16	G	C	0.4848	-0.212898	73786041	8.77E-06
rs4787696	16	G	A	0.8724	-0.318475	25108484	9.04E-06
rs1542931	8	C	G	0.5555	-0.20822	91892744	9.09E-06
rs1436849	12	C	T	0.3077	0.245046	64861385	9.09E-06
rs6498423	16	G	A	0.6164	-0.217535	13531381	9.22E-06
rs12681868	8	G	A	0.5556	-0.207585	91888527	9.43E-06

Table 4.5.4: Table S4: Top 69 SNPs associated with potassium response in African Americans in the PEAR study ($p<10^{-5}$). [b]P-values are based on a linear regression adjusted for sex, age, waist circumference, baseline insulin, baseline potassium and the two first principal components

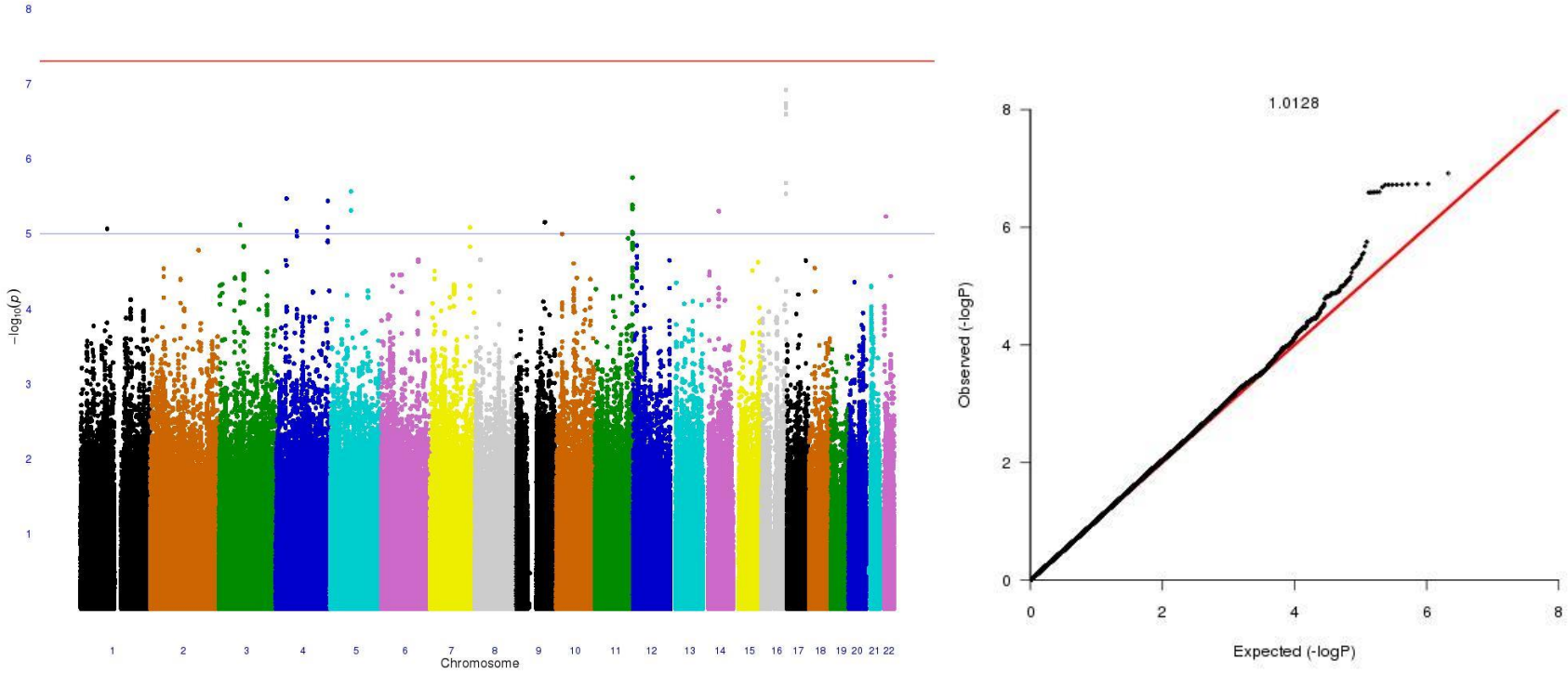

Figure 4.5.1: Manhattan and QQ plots of GWAS results for potassium responses to HCTZ in European Americans in the GERA Study.

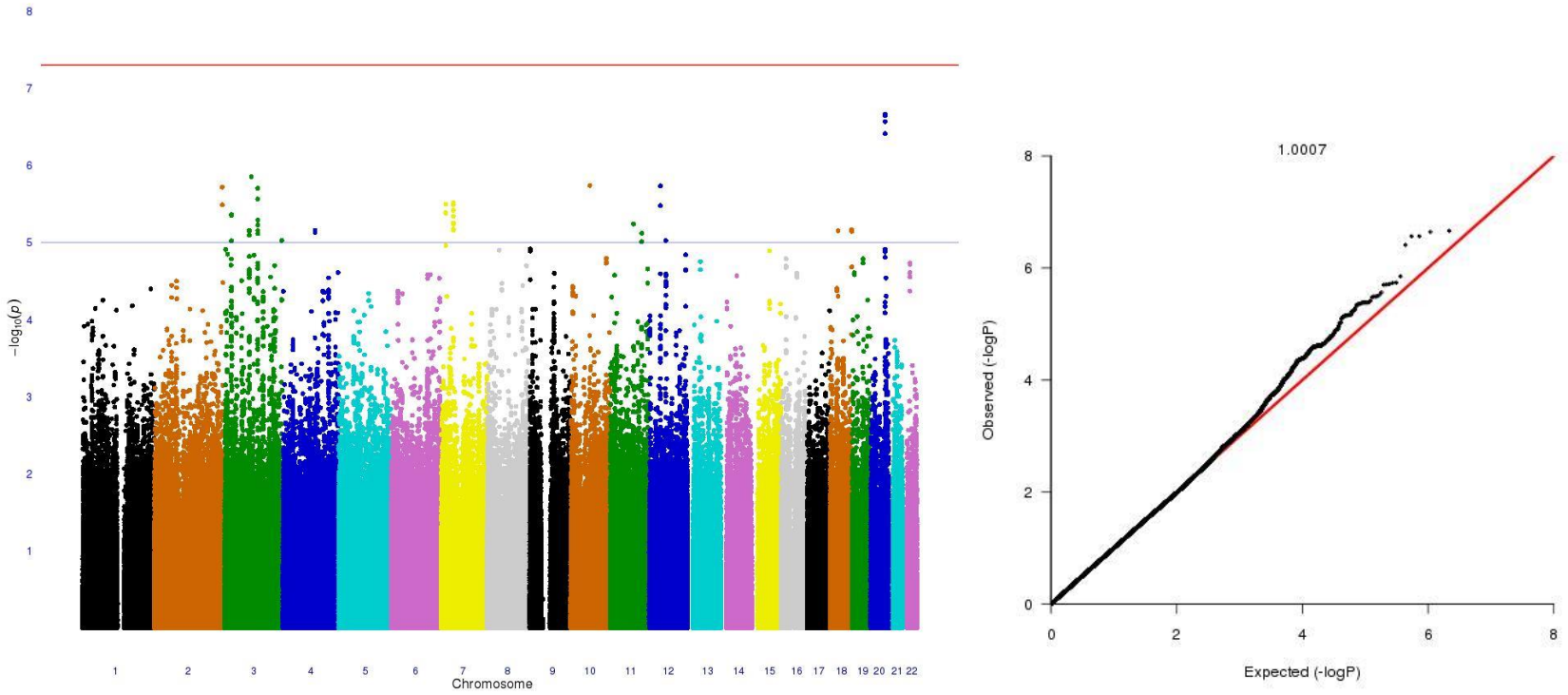

Figure 4.5.2: Manhattan and QQ plots of GWAS results for potassium responses to HCTZ in European Americans in the PEAR Study.

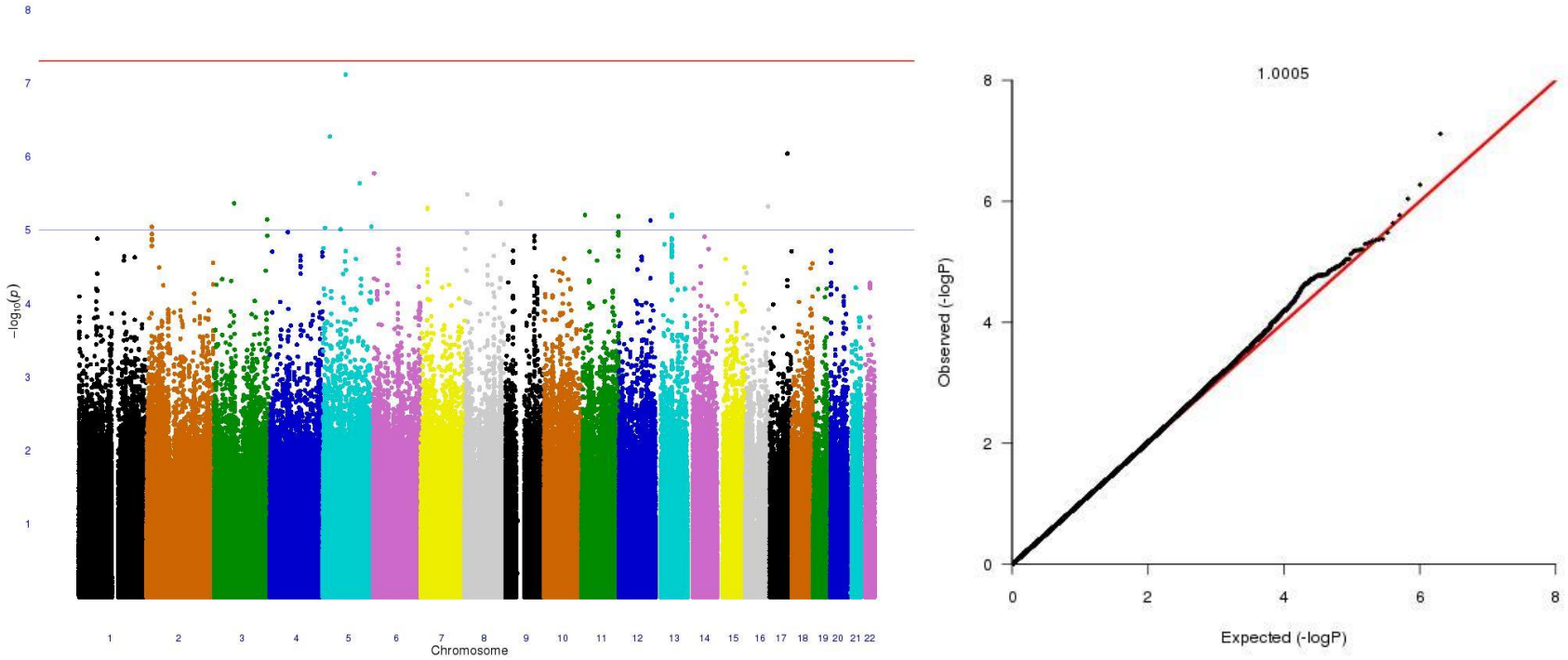

Figure 4.5.3Manhattan and QQ plots of GWAS results for potassium responses to HCTZ in African-Americans in the GERA Study.

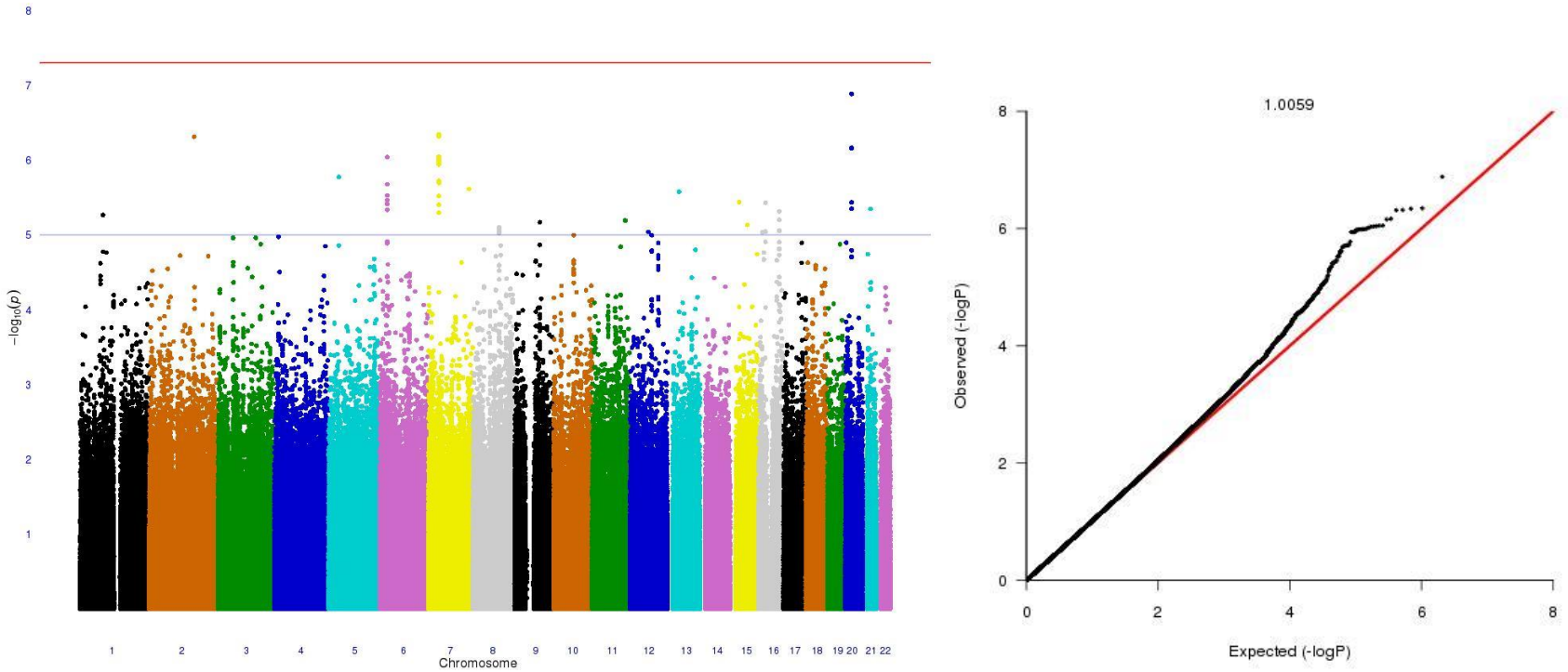

Figure 4.5.4: Manhattan and QQ plots of GWAS results for potassium responses to HCTZ in African-Americans in the PEAR Study.

CHAPTER 5: SYNTESIS AND FUTURE DIRECTIONS

5.1 SYNTHESIS

Pharmacogenomics is a promising component of the broader field of personalized medicine. The concept behind it is to use genomic information to prescribe the right drug for each person in order to increase efficacy and decrease side effects. This dissertation focused on identifying and quantifying genetic factors that contribute to hydrochlorothiazide adverse metabolic effects among European and African Americans. This final chapter provides a brief synthesis of information across the previous chapters and an indication of future directions of the field.

Chapter 2 showed the results of a GWAS performed to evaluate the association between each SNP and change in glucose and triglycerides due to HCTZ treatment. Only an intronic SNP at the NELL1 gene showed association between the change in triglycerides and HCTZ treatment in African Americans. This gene is related to adipocyte differentiation which led me to hypothesize that HCTZ could be modulating adipocyte biology through NELL1 leading to an accumulation of plasma triglycerides in susceptible patients. It is worth to mention that by the time that this dissertation was completed, a new GWAS paper was published by Rudkowska[151] in which NELL1 came up as a top hit in the association between triglyceride response to n-3 PUFA supplementation. No significant association was found in the case of change in glucose and HCTZ treatment.

The studies from the previous chapter were carried out in an interventional setting, but it is possible to test pharmacogenetic hypotheses in an observational setting. Chapter 3 used a longitudinal analysis approach in the CHARGE consortium to test hypotheses about the genetic predictors of the change in fasting glucose levels after taking a diuretic over time. This analysis consisted of 7,038 individuals from 14 cohort studies. No significant interaction was found between diuretic usage and any SNP with the change in glucose levels. This negative result suggested that the rare variant approach could have the answer instead of the common variant approach for my question.

It has been hypothesized that the mechanism by which HCTZ leads to hyperglycemia involves increased potassium excretion and hypokalemia leading to defects in insulin secretion (Duarte et. al. 2010 Expert Rev CardiovascTher; 8, 793-802), but the mechanisms underlying this phenomenon are not well-understood. Chapter 4 dealt with this hypothesis. To increase the power of the analysis, I ran a trans-ethnic meta-analysis which clusters populations by the mean allele frequency difference. If populations are in the same cluster, we will assume that they have the same underlying allelic effects and a fixed effect model is performed. On the other hand, if populations are in different clusters, the

assumption will be that they do not have the same underlying allelic effect and a random effect analysis is performed. In this analysis, I was able to find an interesting relationship between the Heme pathway and change in potassium. Heme has been identified as a modulator of the large-conductance Ca2+-activated K+ channels (BK, or maxi K channels) (Tang et. al. 2003 Nature; 425, 531-5). These channels are located in the medullary and, the cortical thick ascending limbs and the distal convoluted tubule in the kidney and they secrete potassium (Pluznick et. al. 2006; Am. J Physiol. Renal Physiol. 291, F517-F529). However, they have not previously been considered important contributors to renal potassium secretion because of their low open probabilities (Po). I hypothesize that HCTZ affects serum K in part by increasing Heme.

This dissertation research showed some interesting results, but the question about the genetic predictors of the change in glucose levels and the onset of diabetes levels following diuretic usage remains elusive. One explanation is that the primary genetic predictors are the result of rare variants with large effects. These rare variants would not have been detected by the GWAS methods used here. Sequencing is becoming the norm in modern human genetics[152], and it will likely move in that direction for pharmacogenetics. The field is moving from a common variant/common disease hypothesis or model to a rare variant/common disease hypothesis or model. However, this does not mean that AME is a simply inherited phenotype or Mendelian condition. Rather, this new hypothesis states that multiple rare variants influence the trait of interest. Over the population, these variants can reside in a single gene or multiple genes that have cumulative effects. Likewise, they may reside in multiple genes in the sample pathway. Pharmacogenetic researchers can use either whole-exome sequencing (WES) or whole-genome sequencing (WGS) to find association with drug response traits. Such large scale sequencing efforts have already begun in the CHARGE consortium.

5.2 THOUGHT AND FUTURE DIRECTIONS

Many polymorphisms associated with pharmacodynamics and pharmacokinetics have been identified for drugs such as antithrombotic agents, B-adrenergic receptor blockers, statins, and angiotensin-converting enzyme inhibitors and diuretics however most of these results do not replicate in other studies or are inconsistent. Therefore, the impact of this information in clinical outcomes as well as in clinical practice is still on hold

A possible reason for this inconsistent is the study design; some of them tend to be bias or more likely confounding problems. I agree with Kurland et al[153] that the ideal pharmacogenomic study should be prospective, use one drug at the time, include naïve hypertensive individuals and include a placebo arm. But, I know that followed these conditions are logistically difficult, costly and unethical (placebo-treatment).

Another problem is the definition of the phenotype across different studies. For instance, the use of clinical or ambulatory blood pressure measurements as outcome must be the same in all the different studies that are used for comparison.

Sample size, which leads to problems with insufficient statistical power, is another difficulty that we face in these pharmacogenomic studies and can produce spurious results.

As biologists, I tend to like a simple explanation for any disease or pathway, this oversimplification in thoughts tend to create hypotheses that exclude biological and environmental interaction, which gives us partial information of how diseases and its response to treatment really behave.

Important variables, sometimes over-looked during the comparison of pharmacogenomic antihypertensive studies, are not only drug dose but drugs within the same class (hydrochlorothiazide vs chlorthalidone or atenolol vs metoprolol) that may have different pharmacologic properties which lead to inconsistent results.

The formation of a new consortium known as International Consortium for Antihypertensive Pharmacogenomics Studies (ICAPS) promises to faced most of the problems describe in previous paragraphs in order to provide real genetic information that can be used for clinical practice. The consortium is formed by different cohorts in different continents. America is represented by Minesota-GERA and Florida-PEAR, Europe by Italy-PHSS, MILAN, Scotalnd-NORDIL and Finland-GENRES and Asia by China-FEVER_PGx. And although, most of the cohorts are based on European descends, other ethnicities are part of it. These cohorts are based on interventional studies, most of them used one drug, but if different drugs are used they were at different arms of the study or after some wash out period. I am hoping that these cohorts will increase our sample size and power to detect common and rare variants responsible for AMEs.

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