INVESTIGATING THE ROLE OF NUCLEAR AND MITOCHONDRIAL VARIATION IN CARDIOVASCULAR DISEASE

By

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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland

July, 2016

Abstract

Cardiovascular disease (CVD) refers to a complex heterogeneous cluster of diseases of the heart and blood vessels that often manifest clinically as outcomes of myocardial infarction and stroke. CVD is a major public health burden that affects 85 million people just in the United States. As a complex disease of high prevalence, CVD has been poised to benefit from large scale sequencing and genotyping studies. Therefore, understanding the underlying pathophysiology of CVD to identify biomarkers for diagnosis and drug targets for prevention and treatment are active areas of research. We attempt to approach this problem from a genetics perspective. First, we utilize large-scale genotyping in cohorts of patients with sudden cardiac arrest, a specific form of CVD, to carry out the largest genome-wide association study (GWAS) for SCA to date and identify genetic loci associated with SCA. Additionally, we used genetic risk score approach to determine the effect of traditional SCA risk factors on the genetically attributable risk for SCA, and hence identify QT interval and BMI as putative causal risk factors for SCA.

The second part of this work focuses on inter-individual variation in the number of copies of mitochondrial DNA (mtDNA), a measure we refer to as the mtDNA copy number (mtDNA CN). We assess the association of mtDNA CN with age and age related phenotypes like frailty and mortality, establishing mtDNA CN as significant predictor of all-cause mortality. Finally, we focus on determining the relationship between mtDNA CN and CVD and assessing the clinical utility of mtDNA CN as a predictor of different forms of cardiovascular disease.

Advisor: Dan E. Arking, PhD

Reader: Andrew S. McCallion, PhD

Preface

I have to start with, my mentor Dan Arking. Dan accepted me in the lab as a bright eyed, first year graduate student with no background in computational biology and all she knew was that, “genetics was cool”. In the many years since, Dan has taught me not only everything I know about genetics, but more importantly how to do good science and be a responsible scientist. Dan’s impact on my life will extend far beyond my scientific ventures, and I will cherish his mentorship and the time spent in his lab for the rest of my life.

Dan has also put together the best group of people who have made me look forward to coming in to work everyday for over 6 years. Pallav Bhatnagar and Simone Gupta, who welcomed me when I joined, providing much needed support and answering the million questions I had about working on a command line for the first time in my life. Anna Moes, who has an amazing lab manager, scientist and even better friend during my time here. Her incredible organizational skills and meticulous has affected almost every piece of data I have analyzed in my time here. My journey as a graduate would be very different without Shannon Ellis, who has been my lab sibling the entire time. From bouncing ideas, to helping me debug code and inspiring me to be a better person, Shannon has been an amazing colleague in lab, and one of my best friends outside of lab! Lastly, every member of the Arking lab—Naftali, Nathan, Ryan, Rebecca and Christina—who have been pivotal in my growth as a scientist during my time at Hopkins, and have supported me through the ups and downs of grad school.

Outside of lab, this work has been supported and influenced by a number of extraordinary collaborators. Andy McCallion and his lab—particularly Paul, Rebecca, Joey and Courtney—who have provided a second home for me in my time here. They have shaped and led the zebrafish work in ways I definitely could not have done on my own. Nona Sotoodehnia at the University of Washington, who has been a mentor and valued collaborator on a number of projects over the year. Her insight as a cardiologist and geneticist has taught me to consider and appreciate clinical utility of our work in ways I would never have been able to otherwise. Eliseo Guallar and Yiyi Zhang at the Welch Center for Aging, who have patiently introduced me to the world of epidemiology and biostatistics and have provided invaluable insight into our work with mitochondrial DNA copy number. A big thank you to the program and faculty for putting together the infrastructure for young scientists to grow and flourish. In particular, Dr Valle, Dr Kirby Smith and Sandy Muscelli, for supporting me and all the graduate students through stressful times of graduate school.

On a personal level, one of the highlights of graduate school will always be the friends I made here. Shannon, Nicole, Tim and Courtney have made Baltimore, and the US, feel like home for the last 6 years. It is hard to overstate their role they have played in keeping me sane through grad school. From all the football games we have watched together, many many pounds of chocolate we have consumed, to the parties, trips and weekends spent together, it is hard to imagine going through this them. I consider myself very lucky to be in a graduate program where I got to meet so many students I am glad to call friends. They have all helped shape my scientific, political and social views by the diversity of experiences they have exposed me to, and I can't wait to see the amazing things they accomplish in the future.

Finally, my family. My parents, Naresh and Shobha Ashar, who have pushed me to be the best version of myself my entire life, and knew the things I was capable of doing even when I didn't trust myself to be able to do them. To my brother Umang, who has grown into a thoughtful, compassionate person who I am so proud of. None of this would be possible without your love and support that keeps me going every day. I so lucky to be able to call you all family and depend on you to be there for me through all the adventures I get myself into!

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Chapter 1 Introduction

1.1 Genetics of cardiovascular disease

Cardiovascular disease (CVD) is a broad term that encompasses all diseases of the heart and blood vessels. Clinically, CVD is often defined by outcomes such as myocardial infarction and stroke. From a public health perspective, CVD is a major source of spending that affects over 85 million people in the United States and has is projected to have healthcare cost of 804 billion US dollars in 2020[1]. There are a number of very well established risk factors for CVD events, including elevated blood lipid levels, particularly total cholesterol and high density lipoproteins (HDL), elevated blood pressure, smoking, and sex, with men at higher risk for CVD events than women. Several of these risk factors modulate CVD risk by increasing risk for atherosclerosis, the primary pathological substrate for CVD outcomes.

From a genetics perspective, CVD has been the poster child for classic and modern genetics contributing directly to very successful therapies for disease. Seminal work from Goldstein and Brown in late 70's in elucidating the cholesterol synthesis pathway used cultured fibroblasts from patients with an autosomal recessive form of familial hypercholesterolemia[2–4]. More recently, as a complex disease of high prevalence, CVD genetic studies have benefitted from advances in technology, both microarray as well as next generation sequencing technologies. Genome wide association studies (GWAS) became possible as direct result of adaptation of microarray technology to genotyping that allowed for genotyping of thousands of SNPs across in the genome in a rapid, high throughput manner[5]. To date, GWAS for coronary artery disease, a subset of CVD defined clinically by myocardial infarction event, have identified over 50 loci associated with the disease[6]. These GWAS studies have been supplemented with targeted and whole-exome sequencing studies, like those that identified rare variants in *PCSK9*[7] directly leading to the development of newly approved *PCSK9* inhibitors[8].

In this work, we attempt to increase our understanding of CVD in two ways—to use nuclear variation to characterize the genetic architecture of a specific form of CVD called sudden cardiac arrest (SCA), and to identify novel risk factor for CVD that stems from variation in the mitochondrial DNA.

1.2 Genetics of sudden cardiac arrest (SCA)

While significant progress has been made in identifying loci associated with CVD from GWAS, identifying genes responsible for sudden cardiac arrest (SCA) has proved to be more challenging. SCA is broadly defined as an outcome of sudden unexpected loss of heart function due to ventricular arrhythmia that affects about 1% of the US population annually[1]. In contrast to coronary artery disease (CAD) or stroke where the underlying pathology is related to atherosclerosis, an SCA event is considered to be caused by underlying electrical instability that manifests clinically in the form of different arrhythmias. It is important to note however that majority of SCA occurs in people who have existing CAD. The challenge in SCA risk prediction arises from the fact that while the risk factors for both diseases are very similar, SCA has a much higher rate of mortality with average survival rate of about 10.6% after an SCA event[1].

The evidence for a genetic basis for SCA, first came from Paris Prospective Study in the late 1990's, where epidemiological data showed that family history of SCA, was independently associated with a two fold increase in SCA risk[9]. Additionally, patients with Mendelian forms of arrhythmias, like long QT Syndrome or Brugada Syndrome, have increased risk of SCA[10]. However, the vast majority of SCA occurs outside of this high risk population. In order to interrogate the role of common variants in SCA, we carried out the largest genome-wide association study (GWAS) for SCA, to date. This dataset allowed us to examine the role of genome-wide nuclear variation in SCA and common variation in candidate arrhythmia genes. Additionally, using cross-trait genetic risk score associations (GRSA) we were able to examine the contribution of SCA risk factors to the genetically attributable risk for SCA. Our findings demonstrate that common genetic variants reflecting multiple pathophysiologic pathways contribute to the genetic architecture of SCA. This approach allowed us to contrast and compare the genetic profiles for SCA and CAD, and present novel genetic evidence to show that SCA is not simply an extension of CAD, and that it might require different therapeutic interventions for those at risk.

1.3 Identifying mtDNA copy number as a novel risk factor for CVD

In the second part of this work, we explore the association between mitochondrial DNA copy number (mtDNA CN) and CVD. In addition to being the primary ATP-producing organelle in the eukaryotic cell, mitochondria also have their own mitochondrial DNA which is a 16.7kb maternally inherited circular DNA molecule. mtDNA encodes 37 genes that are essential for mitochondrial function. However, in contrast to the nuclear genome, where the copy number is fixed at two copies, the number of mitochondria per cell and the number of mtDNA molecules

per mitochondrion vary from 10 to 1000's to copies per cell. Several studies have shown that the amount of mitochondrial DNA has been shown to be an effective surrogate for mitochondrial function[11]. Given that mitochondrial dysfunction has well established role in atherosclerosis, we hypothesized that mtDNA CN, as a surrogate for mitochondrial function, could affect risk for several chronic diseases, including CVD.

To explore the association between mtDNA CN and general health, we use data from three well-established studies of cardiovascular health—the Atherosclerosis Risk in Communities (ARIC) study, Cardiovascular Health Study (CHS), and the Multiethnic Study of Atherosclerosis (MESA) study. As part of this work, we have develop a method to determine mtDNA CN from existing genotyping arrays that allows us to estimate mtDNA CN in large numbers of samples without additional DNA extraction.

Chapter 2 A Comprehensive Evaluation of the Genetic Architecture of Sudden Cardiac Arrest

2.1 Introduction

Sudden cardiac arrest (SCA) is a major cause of cardiac mortality, affecting approximately 300,000 people in the US every year[1]. Although SCA is the end result of a variety of molecular pathways, electrophysiologic characteristics, and pathologic conditions, clinical and autopsy studies have demonstrated a predominant, common pathophysiology in Western populations: the most common electrophysiologic mechanism for SCA is ventricular fibrillation (VF) and the most common pathologic substrate is coronary artery disease (CAD). Unfortunately, SCA survival remains low, and an important way to decrease SCA mortality is through risk stratification and prevention.

Family history of SCA and of myocardial infarction are both associated independently with a two–fold increase in SCA risk in the general population, suggesting that genetic variation, potentially in multiple pathophysiologic pathways, may influence SCA risk[12]. While patients with inherited Mendelian arrhythmias (e.g. those with mutations in ion channel genes leading to Long QT Syndrome) are at increased SCA risk[13–15], almost all SCA occurs outside of this high-risk population. Whether common variation in ion channel genes or other genomic regions influence SCA risk remains largely unknown, leaving physicians uncertain how best to evaluate patients with a known family history.

Genome-wide association studies (GWAS) have been a useful tool for gaining insights into the etiology of complex disease processes, such as SCA. In the post-GWAS era, Mendelian randomization methods, including multi-SNP cross-trait genetic risk score association (GRSA) methods, have emerged as powerful approaches to explore genetic relationships of risk factors and complex disease outcome[16–18]. Contrasting genetic risk score associations with known observational associations of risk factors is an effective way to understand the underlying pathways and processes that modulate SCA risk. A genetic association analysis that combines an agnostic GWAS with use of risk factor GRSAs may help shed light on the genetic architecture of SCA.

We therefore performed a SCA GWAS in 3,939 cases and 25,989 control participants of European descent, with replication genotyping in additional samples. We investigated whether

common variation in inherited arrhythmia genes were associated with SCA risk in the general population. We then evaluated the relationships between risk factors and SCA using multi-SNP GRSAs.

2.2 Methods

2.2.1 Study Population and Phenotype Definition.

We conducted a two-stage study, with 9 studies of European-descent individuals comprising the GWAS 'discovery' stage and 14 studies with individuals of European, African and Asian-descent comprising the 'replication' stage. Study descriptions, along with study-specific SCA definitions and genotyping methods, are detailed in the Supplementary methods. All studies were approved by appropriate institutional review boards.

2.2.2 GWAS

Genome-wide genotype data was imputed to HapMap2-CEU reference panel, following study-level quality control checks (**TableS1**). Each 'discovery' study performed regression analysis adjusted for age, sex, and study-specific covariates, and results were meta-analyzed using inverse variance meta-analysis implemented in METAL[19]. The top 25 SNPs were examined in a second 'replication' population. Findings from 'discovery' and 'replication' stages were then meta-analyzed (**TableS2, Figure 2-5A**). GWASs restricted to men; women; age under 65; and cases with VF/shockable rhythm, were performed (**TableS3, Figure 2-5B-E).**

2.2.3 Candidate genes

We examined variants in inherited arrhythmia genes using the 'logistic-minsnp-gene-perm' function in FASTv1.8[20]. This best single-SNP F-statistic within a gene serves as the test statistic to compute a permutation based p-value corrected for gene size by performing up to 1 million permutations per gene. Gene boundaries were defined by RefSeq gene coordinates on build GRCh37 with +/-10kb flank.

2.2.4 Genetic Risk Score Association (GRSA)

We calculated an estimate for GRSA for 17 SCA risk factors. **TableS4** details the 17 traits, and the source published GWAS study used to construct the GRSAs for these traits.

To construct the GRSAs for putative SCA risk factors, we first identify SNPs associated with the risk trait at five significance cutoffs (alpha=$5x10^{-8}$, $1x10^{-5}$, 0.001, 0.05 and 0.99) following stringent LD-pruning. The effects of these SNPs on the risk factors and SCA outcome are used to calculate an inverse-variance weighted multi-SNP GRSA as implemented in the R-package 'gtx'[21]. We used fixed-intercept linear regression with effect of SNPs on SCA (β_{SCA}) as the

dependent variable and effect of SNPs on the trait (β_{trait}) as the independent variable, weighted by the standard error of the β_{SCA} squared (SE_{SCA}^2) (for BMI, see **Figure 2-1A**). The resultant regression coefficient is the GRSA estimate or 'ahat,' for the trait on SCA (**Supplementary Information**). To test for heterogeneity in effect estimates between SNPs due to pleiotropy, we use Cochran's Q test[22]. Using an iterative approach, we excluded SNPs until the P-value for the risk score heterogeneity was >0.05.

We similarly computed risk factor GRSAs on the outcome of CAD. We use a modified two-sample Welch test to calculate p-value for difference in GRSA estimates between SCA and CAD.

2.2.5 Sex-specific analyses

We performed sex-stratified SCA GWAS analyses to construct trait GRSAs separately by sex. GRSAs were constructed from the same set of LD-pruned SNPs used for overall GRSA analyses, using sex-specific effect-estimates (and corresponding standard-error estimates) for SCA risk. P-values for difference in GRSAs between sexes were obtained from 1-degree of freedom Wald test for difference in regression coefficients of the sex-stratified analyses.

2.3 Results

2.3.1 GWAS

Meta-analysis was performed with results from 9 GWASs of 3,939 European-ancestry cases and 25,989 controls (**TableS1A, Figure 2-5A**) with additional genotyping of 26 SNPs in up to 4,918 cases and 21,879 controls of European, African, and Asian descent (**TableS1B**). No SNP associations passed genome-wide significance ($P<5x10^{-8}$) (**TableS2**) in the main analysis or in subgroup analyses limited to European-descent individuals, men, women, younger participants (<65 years), or cases with documented VF/shockable rhythm (**Tables S2** and **S3, Figure 2-5B-E**).

2.3.2 Candidate Gene and Candidate SNP Analyses

Despite sufficient power to detect relative risks of 1.15 (80% power, allele frequency 0.30, alpha=0.05)**,** we did not find common variants in inherited arrhythmia genes associated with SCA in the general population (**TableS5)**. Examining SNPs previously associated with SCA in small studies, 5/19 were nominally associated with SCA (P<0.05) in our study (**TableS6)**.

2.3.3 Genetic Risk Scores Associations (GRSAs)

To explore the underlying genetic architecture of SCA, we examined GRSAs with: (1) CAD and traditional CAD risk factors; (2) cardiac electrophysiologic factors; and (3) anthropometric traits.

In **TableS7,** we report the GRSA estimates (ahat) at five significance level cutoffs (alpha=$5x10^{-8}$, $1x10^{-5}$, 0.001, 0.05 and 0.99) from the risk factor GWAS variants.

CAD and CAD risk factors

Prevalent CAD is an important SCA risk factor with ~80% of male SCA survivors having underlying CAD[23]. As expected, CAD GRSA has a strong affect on SCA risk (**Figure 2-2**, **TableS7**), suggesting that genetic variants associated with CAD also influence SCA. For example, the GRSA with 39 SNPs associated with CAD at a genome-wide significant threshold (alpha=$5x10^{-8}$)[6] estimates that 50% increased CAD risk corresponds to 12.2% (95% CI=6.3-18.5%) increased SCA risk.

Examining traditional CAD risk factors, we show that while the diabetes GRSA was significantly associated with SCA at two of five alpha cutoffs tested, there was no significant association of GRSAs for fasting glucose or fasting insulin (**Figure 2-2, TableS7)**, suggesting the effect of diabetes variants on SCA risk may not be mediated by a direct effect of beta cell function (fasting glucose) or insulin resistance (fasting insulin). For lipid GRSAs, we found LDL, total cholesterol, and triglycerides variants that increase lipid levels were positively associated with SCA risk. Similarly, diastolic and systolic blood pressure GRSAs show variants that increase blood pressure also increase SCA risk (**Figure2, TableS7)**.

Cardiac electrophysiologic factors

To explore the influence of cardiac electrophysiologic risk factors on SCA, we examined the effect on SCA risk of variants associated with (1) atrial fibrillation (AF)[24], (2) QT interval (ventricular repolarization)[25]; (3) QRS interval (ventricular conduction)[26]; and (4) heart rate[27]. The GRSAs of AF and QT, both risk factors for SCA in the general population[28,29], showed significant association with SCA (**Figure 2-2, TableS7)**. By contrast, we did not identify a significant association of QRS or heart rate GRSAs with SCA (**Figure 2-2, TableS7, TableS8)**. In sensitivity analyses, we down-sampled the QT GWAS to reflect the smaller QRS GWAS sample size (**Supplemental Information)**, and found similar ahat estimates for the full and down-sampled QT dataset **(TableS8),** suggesting the lack of association for QRS GRSA is not simply due to decreased power and precision.

GRSAs of Anthropometric Measures

Height-increasing variants have a protective effect against CAD[30], and we correspondingly observed a negative association between SCA and height GRSA across all alpha cutoffs (**Figure 2-2, TableS7)**. Among the quantitative traits examined, the GRSA with 72 BMI variants (alpha=$5x10^{-8}$) had the largest effect on SCA risk, with a 1 standard deviation (4.83 BMI units)

increase in BMI corresponding to a 63.2% (95% CI=23.3-115.3%) increase in SCA risk. In contrast, no significant association was seen with GRSAs composed of variants associated with measures of central/abdominal adiposity, such as waist-to-hip ratio or waist circumference.

2.3.4 Contrasting SCA and CAD GRSAs

Comparing the effect of risk factor GRSAs on the outcomes of SCA (**Figure 2-2)** and CAD (**FigureS2),** we identified processes and risk factors that are common to, and differ between, SCA and CAD. While the GRSAs derived from traditional CAD risk factors were associated with both outcomes, the estimates of the diabetes and blood pressure GRSAs (**Figure 2-3A, TableS7**) were significantly larger for CAD than SCA. In contrast, GRSAs for electrophysiologic traits of QT interval and AF had significantly stronger association with SCA than CAD (**Figure 2-3B, TableS7**). BMI and height GRSAs were similarly associated with SCA and CAD. By contrast, while the waist-to-hip ratio GRSA was associated with CAD, it had no impact on SCA (**Figure 2-3A)**.

2.3.5 Sex differences

Sex differences in SCA incidence, underlying SCA pathophysiology, and prevalence of certain risk factors have been well documented[31], yet little is known about the differences by sex of the effect of risk factors on SCA. Using sex-stratified GRSAs, we found that diabetes, AF, and QT interval have larger effect on SCA among women than men (**Figure 2-4, TableS9**). The largest sex difference was seen with the diabetes GRSA, where the effect among women at alpha=$5x10^{-8}$ was ten times larger than among men (ahat=0.24 vs 0.026, respectively); and at alpha=0.99, this difference was highly statistically significant (interaction-P=$3.62x10^{-11}$, **TableS9**).

2.4 Discussion

SCA is a devastating and often fatal problem. A family history of SCA doubles SCA risk, but the genetics of SCA in the general population is poorly understood, leaving physicians uncertain how best to evaluate patients with a family history. Our large SCA GWAS sheds light on the underlying genetic architecture of SCA and is informative in both its negative findings as well as its positive ones. Despite adequate power to identify common variants associated with a modest increased risk, our study did not find variation in Mendelian arrhythmia genes associated with SCA risk in the general population. We do, however, establish that, in aggregate, genetic variants associated with SCA risk factors contribute to SCA. Using a GRSA approach, we characterize the effect of 17 putative SCA risk factors on SCA genetic risk. Specifically, we show that GRSA of CAD and CAD risk factors, electrophysiologic traits of AF and prolonged QT, and

anthropometric measures are associated with SCA. Finally, we present evidence supporting a sex-specific role for variants associated with diabetes, AF, and QT interval in influencing SCA risk in women.

Since underlying electrical instability is an important cause of SCA, prior studies have examined inherited arrhythmia genes or variants associated with electrophysiological traits to identify genetic variants that influence SCA risk [32–34]. While rare private mutations in ion-channel and other electrophysiology-related genes increase arrhythmia risk in families, our study suggests that common variants in these genes are not associated with SCA (OR>1.15) in the general population. This may be due to differing underlying genetics between inherited arrhythmias versus SCA in the general population. By contrast, we do find that GRSAs of phenotypes associated with electrical instability (AF and QT) are associated with SCA more so than with CAD. This confirms our understanding of the pathophysiology of SCA: SCA is not simply fatal CAD, but rather, electrical instability also plays a prominent role in mediating SCA risk.

Intriguingly, not all electrophysiologic phenotypes are genetically significantly associated with SCA. QRS interval and heart rate, two traits observationally associated with SCA[35,36], did not show evidence of a shared genetic basis with SCA. This lack of association may be due to inadequate power to identify modest correlations. Alternatively, it may be that the associations from observational studies are confounded by other factors, and not causative **(Figures 2-1B and 2-1C)**. For instance, underlying CAD can lead to both longer QRS interval and increased SCA risk, and thus while observational studies show an association between SCA and both traits (CAD and QRS interval), a genetic association would not be seen with SCA and QRS-associated SNPs. Similarly, the observational association of higher heart rate with SCA risk may be confounded by higher adrenergic state due to underlying heart disease and not itself causative. The GRSA approach to examining observational risk factors may help differentiate causative factors from those that may be confounded.

CAD is the most common underlying pathologic substrate for SCA. It is reassuring, therefore, that we find significant associations with GRSAs constructed from CAD and traditional CAD risk factors. That diabetes and blood pressure trait GRSAs had a larger effect on CAD than on SCA genetic risk is consistent with the interpretation that these risk factors influence CAD more strongly, and that some of the effect on SCA of GRSAs constructed from these traits may be mediated through their impact on CAD risk.

Anthropometric measures appear to share some common genetic basis with SCA. Shorter stature is associated with increased SCA risk in observational studies, consistent with our findings that

height GRSA is inversely associated with SCA. Observational data on BMI and SCA risk have been conflicting, perhaps due to confounding from smoking status and frailty. Previously[37], we have shown that BMI is associated with increased SCA risk in non-smokers, but not smokers. In this study, we find that genetic variants associated with BMI, but not central/abdominal obesity, were associated with SCA risk. This finding is especially interesting in the context of recent data that imply different biological process underlying BMI and central obesity[38,39].

Finally, we found that diabetes, AF, and QT interval GRSAs had stronger associations among women than men. While diabetes and AF are SCA risk factors among both sexes, previous observational studies have consistently suggested a stronger, albeit not statistically different, effect among women than men[40,41]. QT interval is longer in women than men after puberty[42], and women are more susceptible to fatal arrhythmias when using QT-prolonging medications (acquired QT prolongation)[43], consistent with our findings of a stronger association of QT GRSAs with SCA among women than men. These findings may reflect different underlying SCA pathophysiology between men and women. Moreover, the stronger genetic effects in women may suggest that men accumulate more environmental risk factors than women (e.g. underlying heart disease, tobacco use), therefore diminishing the relative impact of genetic factors.

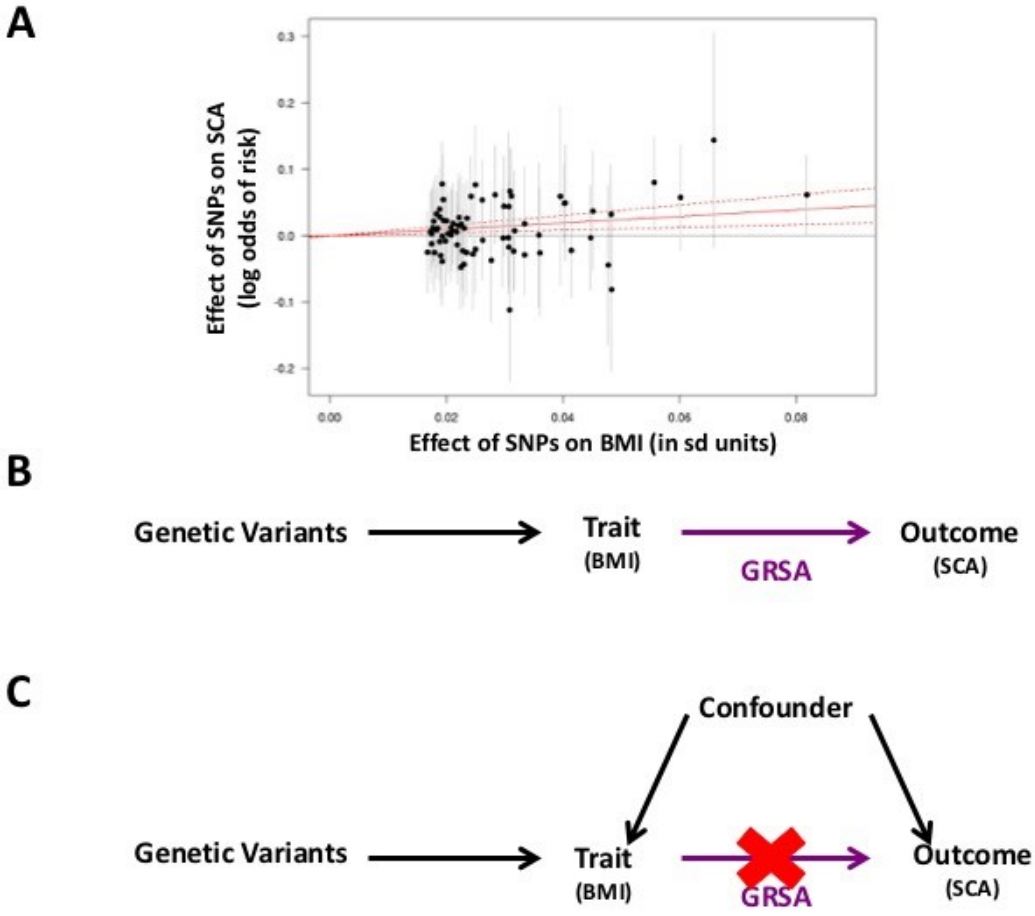
Several limitations deserve consideration. Despite being the largest exploration of SCA genomics, the sample size of ~4,000 cases limited our ability to find associations of modest effect or low frequency. Hence, while our data do not support screening individuals with a family history of SCA for common variation in inherited arrhythmia genes, much larger samples sizes are needed to address whether rare variation of modest effect in these genes influence SCA risk. Second, the validity of the GRSA method as a Mendelian randomization instrument is predicated on the effect of the variant on the outcome being mediated only through the risk factor of interest, and not via other confounders or directly on the outcome. Although we did not directly exclude SNPs associated with multiple risk factors (genetic pleiotropy), we did utilize a goodness-of-fit approach to exclude putative "pleiotropic" effects from all GRSATs. Furthermore, while genetic pleiotropy can confound these findings and interpretations, this is less likely when using multiple SNPs aggregated in a genetic risk score.

In conclusion, findings from the largest GWAS for SCA show that common genetic variants influence SCA risk. This genetic risk is largely not due to common variation in specific inherited arrhythmia genes, but rather, we show that common variation throughout the genome influencing electrical instability, CAD and its risk factors, and height and BMI all influence SCA risk. While SCA is a complex disease with multiple influencing factors, a comprehensive genetic approach

can untangle risk factor relationships, enhancing our understanding of SCA pathophysiology. Ultimately, genetic studies will lead to improved risk stratification and will enhance efforts to prevent SCA in high-risk populations and the general community.

2.5 Figures for Chapter2

Figure 2-1 BMI Genetic Risk Score association for SCA

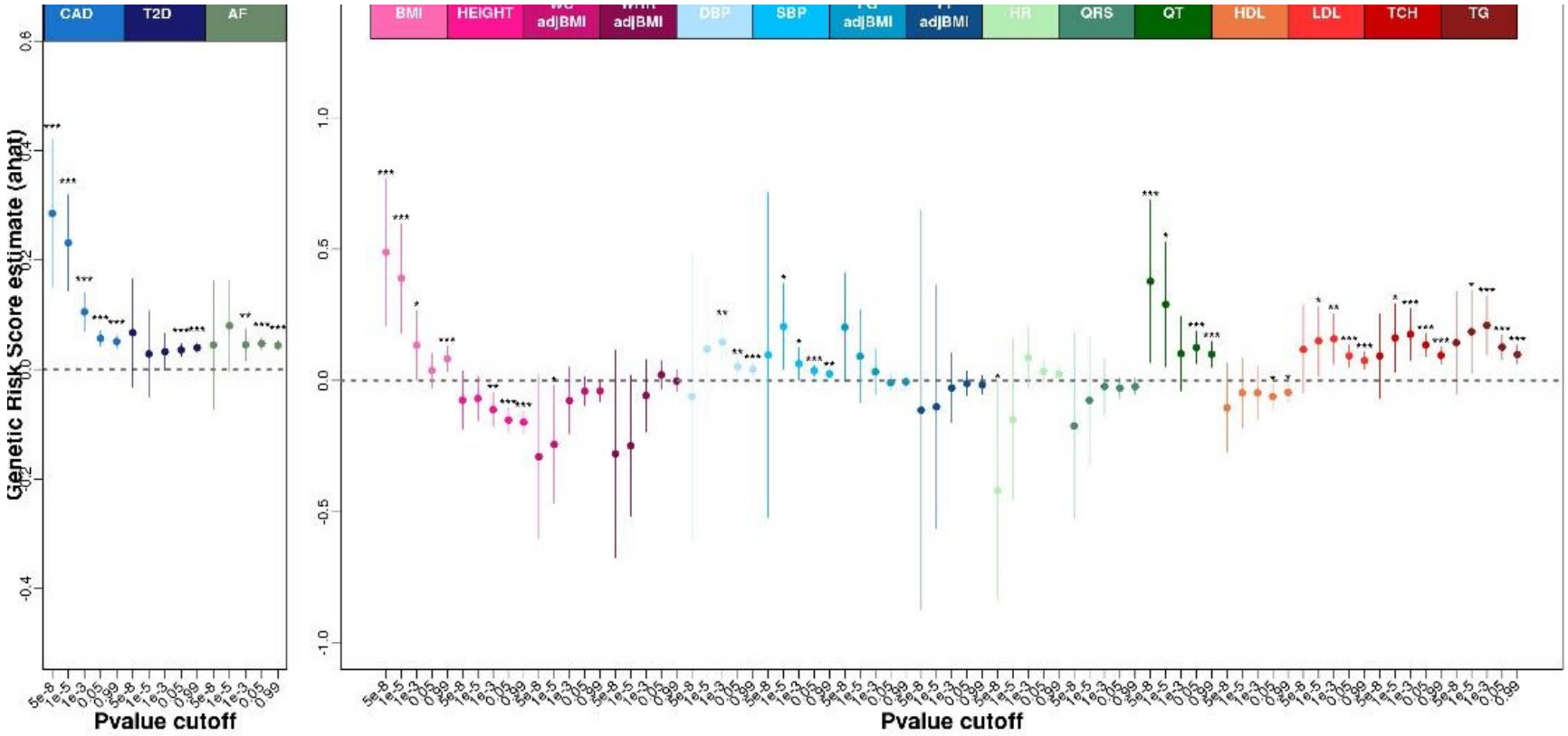


A. This figure presents the data used to calculate the BMI-SCA GRSA at an alpha cutoff of $5x10^{-8}$. The points represent the effect of each SNP on BMI (in units of standard deviation of BMI) on the x-axis, and the log odds effect on SCA risk (corresponding 95% confidence intervals in grey) on the y-axis. The estimate of the genetic risk score association (ahat) is the slope of the fixed intercept weighted regression line (solid red line in the above figure).

B. The directed acyl graph represents a scenario in which trait of interest has a causal effect on the outcome. If the GRSA comprising of trait-associated variants (e.g., BMI) has a significant effect on the outcome (e.g., SCA), it supports a causal role for trait in the outcome.

   This figure presents the case where there is an observational association between the trait and outcome, but the GRSA comprised of trait-associated variants is not significantly associated with the outcome, suggesting that observational association is likely being mediated by a confounding variable and the trait does not have a causal impact on the outcome.

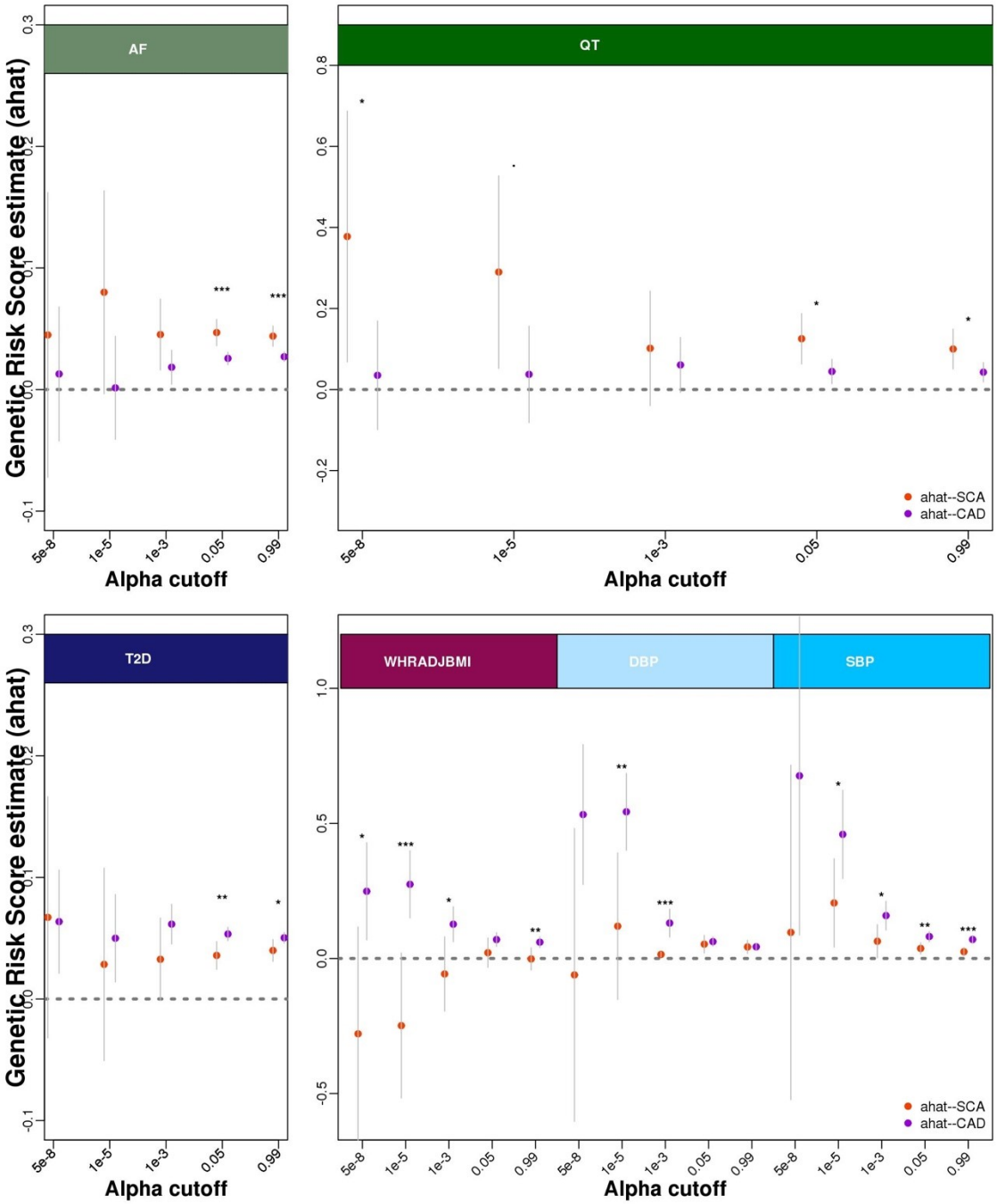
Figure 2-2 Genetic Risk Scores Association (GRSA) estimates for Sudden Cardiac Arrest



These data points represent the GRSA estimates (ahat) of 17 traits† on sudden cardiac arrest (SCA) and their corresponding 95% confidence interval values, from models that include SNPs at five different significance cutoffs (alpha=$5x10^{-8}$, $1x10^{-5}$, 0.001, 0.05, or 0.99). The ahat values in the left panel for the binary traits are in log odds units. Values in right panel are in SD units of the quantitative trait. The significance of the ahat estimates are represented as "*" for P<0.05, "**" for P<0.01, and "***" for P<0.001. For details on values of ahat estimates and pvalues, see **TableS1**.

†CAD denotes coronary artery disease, T2D type 2 diabetes, AF atrial fibrillation, BMI body mass index WCadjBMI waist circumference adjusted for BMI, WHRadBMI waist to hip ratio adjusted for BMI, DBP diastolic blood pressure, SBP systolic blood pressure, FGadjBMI fasting glucose adjusted for BMI, FIadjBMI fasting insulin adjusted for BMI, HR heart rate, QRS QRS interval, QT QT interval, HDL high density lipoproteins, LDL low-density lipoproteins, TCH total cholesterol, and TG triglyceride

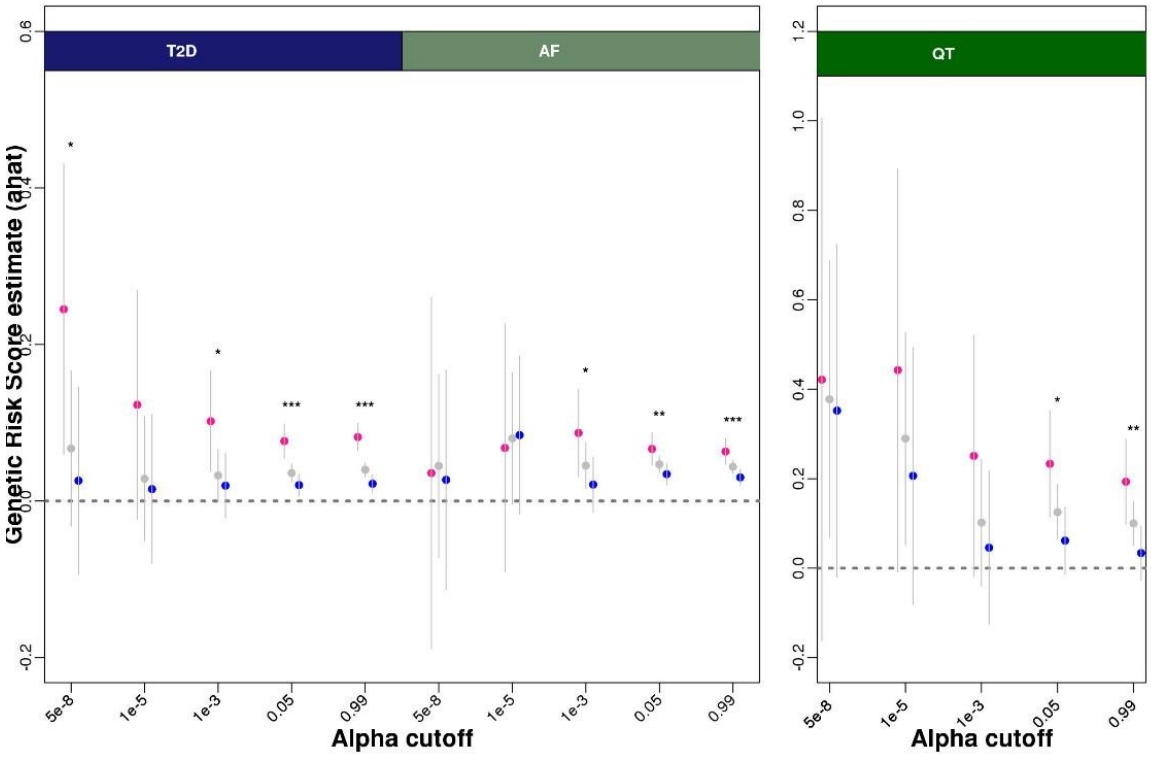
Figure 2-3 Comparison of risk scores for SCA and CAD for selected traits



These data represent GRSAs of 6 selected traits† on SCA and CAD. The top panel shows traits with larger effect on SCA than CAD risk. Traits in bottom panel have larger effects on CAD risk. Ahat estimates of effect of trait on SCA risk and CAD risk, are plotted in orange and purple respectively. Bars around the ahat estimates represent the 95% confidence interval for ahat

estimates. Ahat values for binary traits (left panels) are in log odds units, and for quantitative traits (right panels) are in standard deviation units
of the quantitative trait. The level of significance for a Welch test of difference in ahat values between SCA and CAD is represented "*" for P<0.05, "**" for P<0.01, and "***" for P<0.001. †AF denotes atrial fibrillation, QT QT interval, T2D type 2 diabetes, WHRadBMI waist to hip ratio adjusted for BMI, DBP diastolic blood pressure, SBP systolic blood pressure.

Figure 2-4 Sex stratified SCA genetic risk scores for selected risk factors

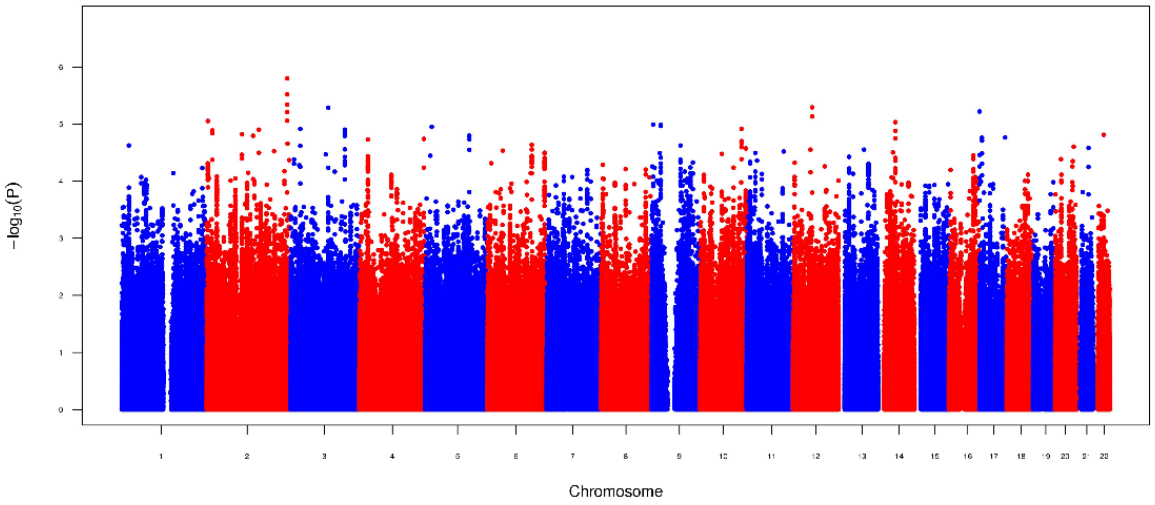


Points in grey represent genetic risk score estimates (ahat) for SCA risk from overall data for select traits†. Ahat estimates of effect of trait on SCA risk in women and men, are plotting in pink and blue respectively. Bars around the ahat estimates represent the 95% confidence interval for ahat estimates. All Ahat values for binary traits (T2D, and AF) are in log odds units, and for QT interval is in SD units. The level of significance for a 1 degree of freedom Wald test of difference in ahat values between the sexes is represented "*" for P<0.05, "**" for P<0.01, and "***" for P<0.001.

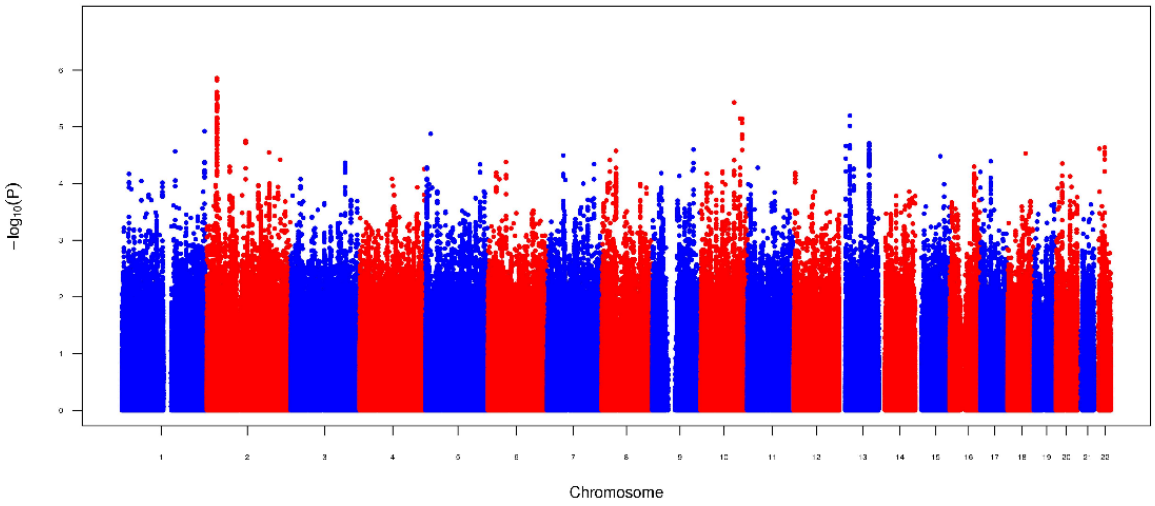
† T2D=type 2 diabetes, AF=atrial fibrillation, QT=QT interval.

Figure 2-5 Manhattan plots showing results from GWAS for sudden cardiac arrest

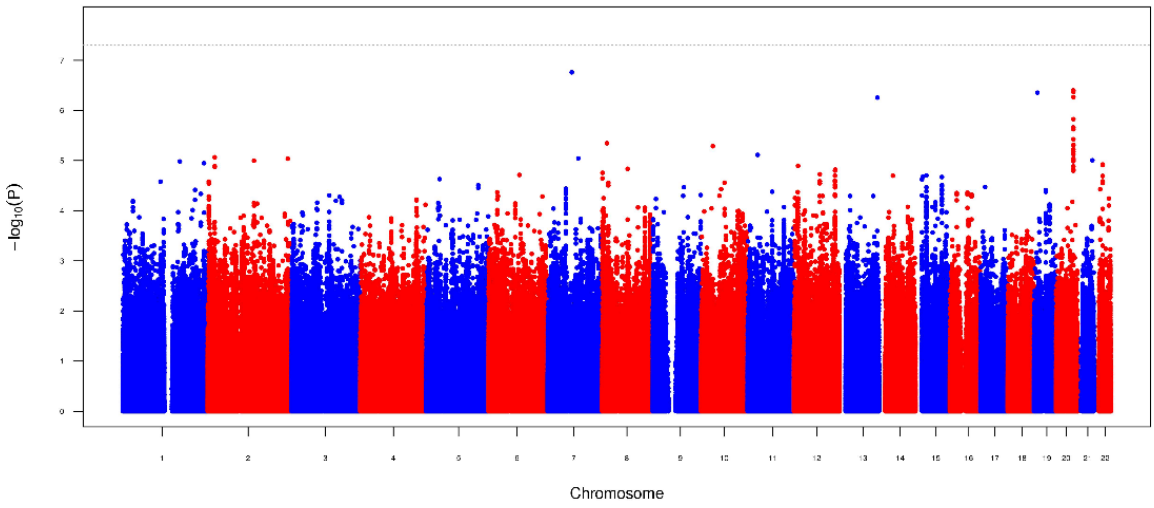
A. GWAS results from Stage1 Discovery



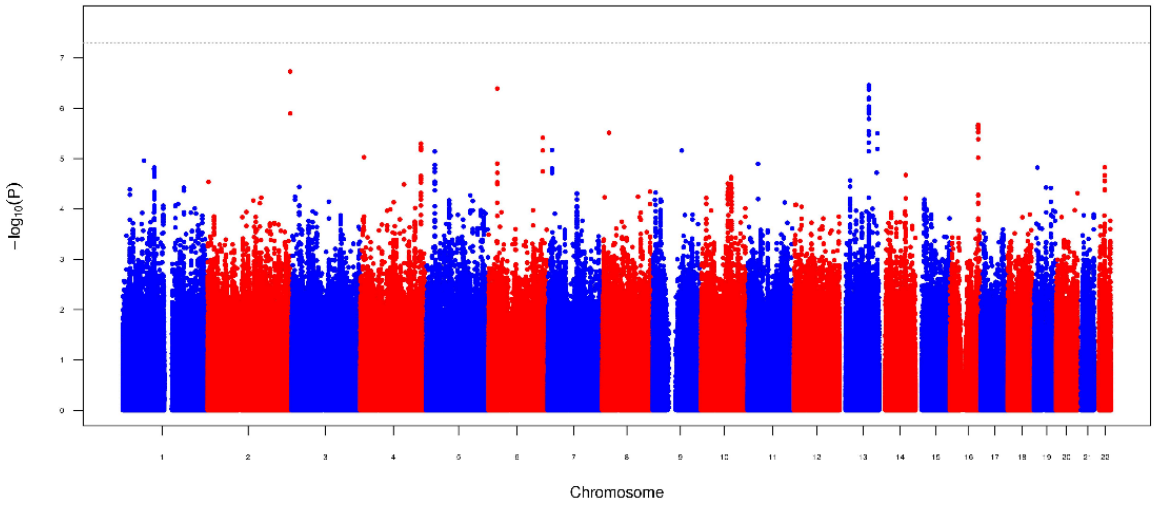
B. GWAS results from sex stratified analyses, restricted to males



C. GWAS results from sex stratified analyses, restricted to females



D. GWAS results from younger participants, restricted to age<=65



E. GWAS results from participants with VF/shockable rhythm.

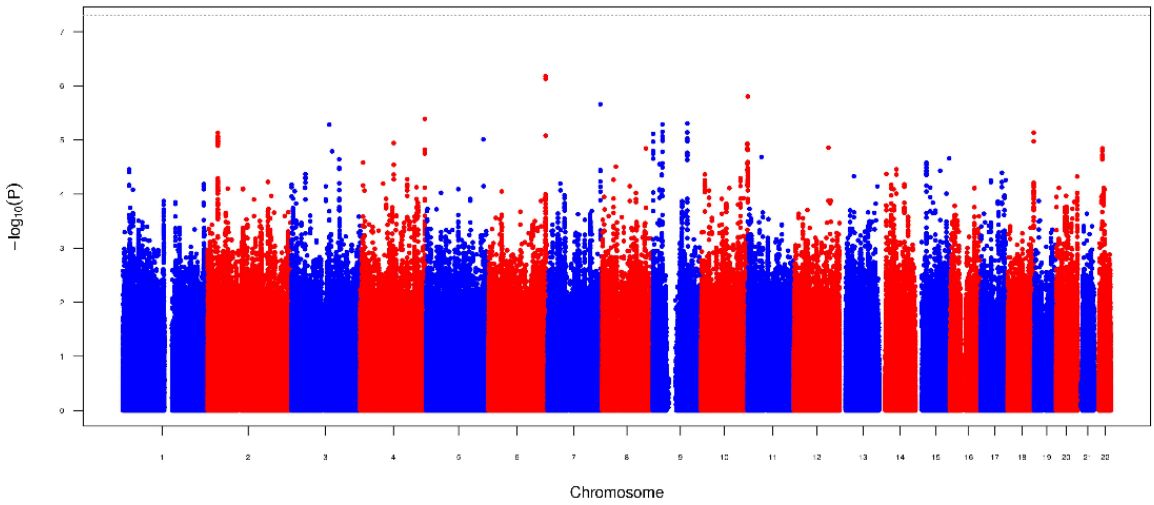
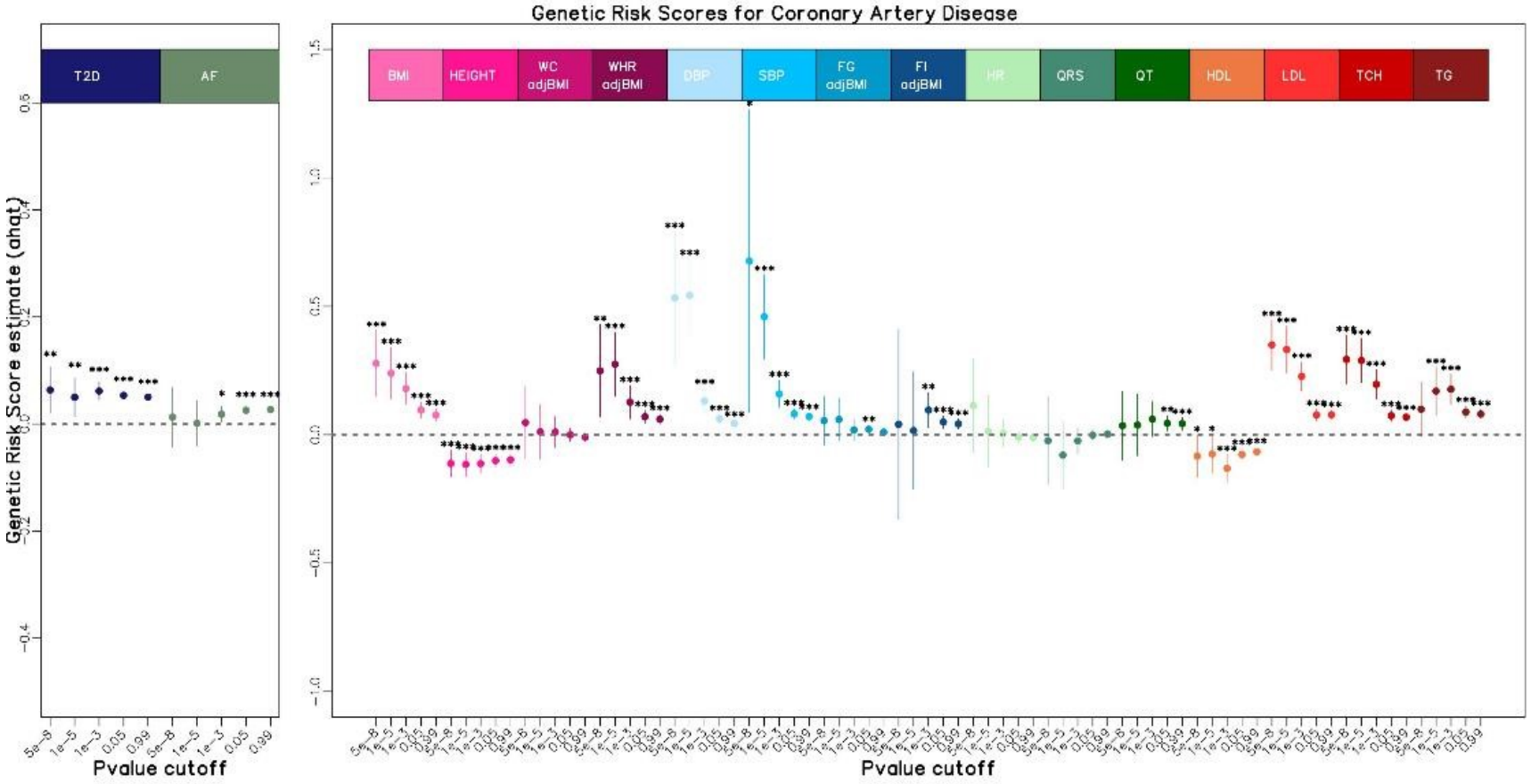


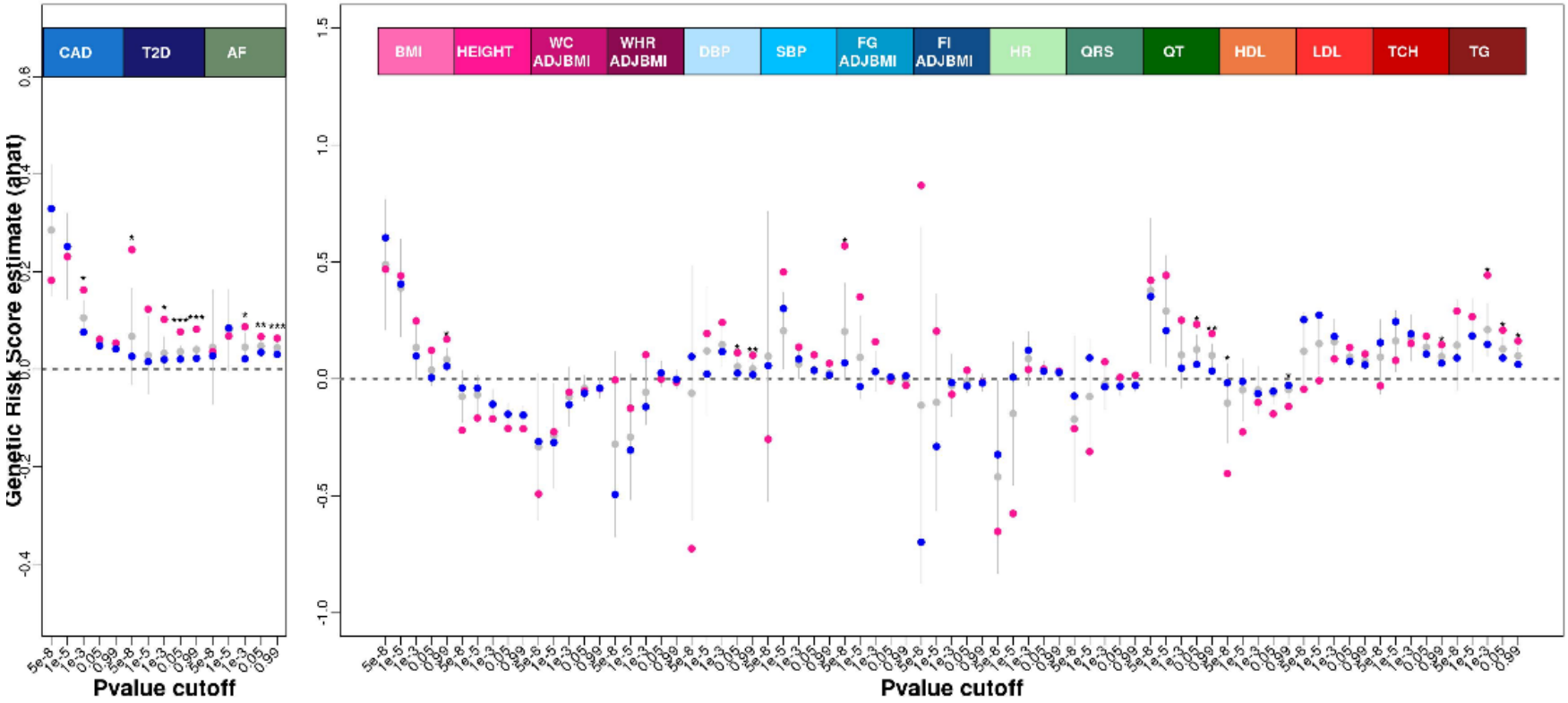
Figure 2-6 Genetic Risk Scores Association estimates for CAD



These data represent genetic risk scores association (GRSA) estimates of 17 traits† on coronary artery disease (CAD). Data points represent the estimates and their corresponding 95% confidence interval values from models that include SNPs at five different alpha cutoffs (alpha=$5x10^{-8}$, $1x10^{-5}$, 0.001, 0.05, or 0.99). The ahat values in the left panel for the binary traits are in log odds units. Values in right panel are in SD units of the quantitative trait. . The significance of the ahat estimates are represented as "*" for P<0.05, "**" for P<0.01, and "***" for P<0.001. For details on values of ahat estimates and pvalues, see **TableS1**.

† T2D denotes type 2 diabetes, AF atrial fibrillation, BMI body mass index WCadjBMI waist circumference adjusted for BMI, WHRadBMI jwaist to hip ratio adjusted for BMI, DBP diastolic blood pressure, SBP systolic blood pressure, FGadjBMI fasting glucose adjusted for BMI, FIadjBMI fasting insulin adjusted for BMI, HR heart rate, QRS QRS interval, QT QT interval, HDL high density lipoproteins, LDL low-density lipoproteins, TCH total cholesterol, and TG triglycerides

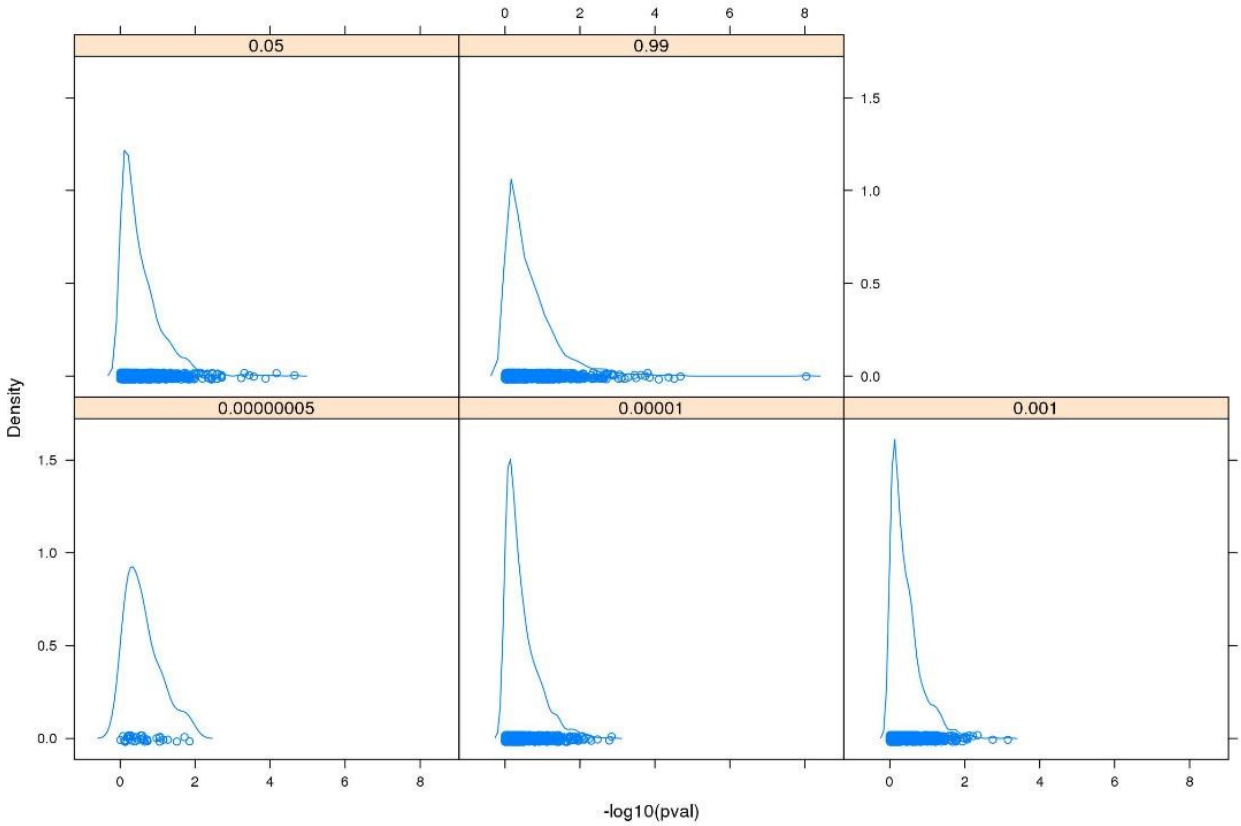
Figure 2-7 Sex stratified GRSA estimates for SCA



Points in grey represent GRSA estimates and corresponding 95% confidence intervals of traits† on SCA from overall data. Ahat estimates of effect of trait on SCA risk in women and men, are plotting in pink and blue respectively. Ahat values for binary traits (CAD, T2D, and AF) are in ln(odds) units, and for all other quantitative traits in SD units. The level of significance for a 1 degree of freedom test of difference in ahat values between the sexes is represented by "*" for P<0.05, "**" for P<0.01, and "***" for P<0.001.

† CAD denotes coronary artery disease, T2D type 2 diabetes, AF atrial fibrillation, BMI body mass index WCadjBMI waist circumference adjusted for BMI, WHRadBMI jwaist to hip ratio adjusted for BMI, DBP diastolic blood pressure, SBP systolic blood pressure, FGadjBMI fasting glucose adjusted for BMI, FIadjBMI fasting insulin adjusted for BMI, HR heart rate, QRS QRS interval, QT QT interval, HDL high density lipoproteins, LDL low-density lipoproteins, TCH total cholesterol, and TG triglycerides.

Figure 2-8 P-value distributions from 1000 null datasets



1000 dummy GWAS datasets were created using genotypes of **9,533** European participants from the ARIC cohort and 1000 randomly generated quantitative phenotypes (mean=0, sd=1). These datasets were subsequently used to compute a GRSA estimate for SCA at 5 alpha cutoffs. Each panel plots the -log10(p-value) of GRS's constructed from these datasets at the different alphas, and represents the null distribution of GRS pvalues. These null distributions were used to determine a permutated P-value in Table**S4.**

2.6 Tables for Chapter2

Table 2-1 Sample characteristics for discovery cohorts

Cohort	ARIC	CABS	CARTAGENE	CARTAGENE/ KORA F3	CHS	FHS	Fingesture	Harvard	Rotterdam study
Stage	Stage1--Discovery	Stage1--Discovery	Stage1--Discovery	Stage1--Discovery	Stage1--Discovery	Stage1--Discovery	Stage1--Discovery	Stage1--Discovery	Stage2=Extension
N, number of cases with genotype data	124	2165	166	169	138	32	340	420	385
N, number of controls with genotype data	8882	2430	241	338	3157	4358	570	424	5589
QC criteria, per sample	Sex-check, Removed duplicates, checks for cryptic relatedness and genetic outliers from PCA	Sex-check, Removed duplicates, checks for cryptic relatedness and genetic outliers from PCA	Sex-check, Removed duplicates, checks for genetic outliers from PCA	Sex-check, Removed duplicates, checks for genetic outliers from PCA	Array call rate <95%, sex check		Sex-check, Removed duplicates, checks for cryptic relatedness and genetic outliers from PCA, genotyping call rate > 90%	Checks for cryptic relatedness and genetic outliers from PCA, genotyping call rate > 95%	Sex-check, Removed duplicates, checks for cryptic relatedness and genetic outliers from PCA
Genotyping platform	Affy 6.0	Affymetrix Axiom	Illumina Human660K	Illumna HumanOmniExpress+HumanOmni25	Illumina CNV370	Affymetrix500K+ 50K Human Gene Focused Panel	Affy 6.0	Affy 6.0	Illumina / HumanHap610
Genotype calling algorithm	Birdseed	apt-probeset-genotype	Illumina beadstudio	Illumina Genomestudio	Illumina beadstudio	BRLMM	Birdseed	Birdseed	Beadstudio Genecall

Cohort	ARIC	CABS	CARTAGENE	CARTAGENE/ KORA F3	CHS	FHS	Fingesture	Harvard	Rotterdam study
Inclusion criteria--MAF	>1%	>1%	>=1%	>=0.1%	>=0%	> 1%	< 1%	> 1%	>1%
Inclusion criteria--Call Rate per SNP	>95%	>95%	>=95%	>=98%	>=97%	> 95%	> 95%	> 95%	>95%
Inclusion criteria--pvalue HWE	> 10 x 10-5	> 1E-5	>=5*10e-6	NA	HWE P < 10-5	> 1E-6	> 1E-6	> 1E-6	> 10e-6
Autosomal SNPs after QC	668,450	522,986	522,537	585,733	306,655		707,418	2,402,071	512349
Imputation Reference Panel	Hapmap.v2	Hapmap.v2	Hapmap.v2	not imputed	Hapmap.v2	Hapmap.v2	Hapmap.v2	Hapmap.v2	Hapmap.v2
Imputation Software	Mach1	Beagle	impute v1.0.0	not imputed	BimBam	Mach	Mach	Mach	Mach
Sex, number of women among cases	4739	496	30	29	70		52	127	203
Sex, number of women among controls	34	537	50	58	1935		135	134	3344
Age, mean age at baseline among cases	57.1	67.55	56.2	58	72.34		63.85	64.3	71.7
Age, age-range at baseline among cases	45-65	20-101	22-77	19-76	64-98		35-92	40.3-91.9	55.2-95.5
Age, mean age at time of SCD among cases	64.9	66.65	56.6	59.5	74.09		61.22	64.2	69.3
Age, age-range at time of SCD among cases	50.4-77.2	23-96	28-86	35-84	65-94		28-83	48.1-96.6	54.5-99.5

Cohort	ARIC	CABS	CARTAGEN E	CARTAGENE/ KORA F3	CHS	FHS	Fingesture	Harvard	Rotterdam study
Average time to SCD (for prospective studies)	7.82				9.17	3.78		7.49	9.2 years
Mean followup time (for prospective studies)	16.24				12.9	5.56	NA	11.02	13.2 years
Study design	Prospective	Case-control	Case-control	Case-control	Prospective	Prospective	Case-control	Case/control from prospective studies and clinical trials	Prospective, Cox PH
SCD definition/Ascertainment	Sudden, pulseless condition from a cardiac origin in a previously stable individual, review of death and medical records	Sudden, pulseless condition from a cardiac origin in a previously stable individual, review of death and medical records. Pt in VF or asystole (NO PEA)	Sudden, pulseless condition from a cardiac origin in a previously stable individual, review of death and medical records.	Sudden, pulseless condition from a cardiac origin in a previously stable individual, review of death and medical records.	Sudden pulseless condition presumed due to a cardiac arrhythmia, without evidence for a non-cardiac condition as a cause of the arrest, in an otherwise stable patient, after review of events	Coronary heart disease death within one hour of onset of symptoms adjudiated by panel of physicians.	Sudden, pulseless condition from a cardiac origin in a previously stable individual, review of death and medical records.	a cardiac death is considered a definite SCD if the death or cardiac arrest that precipitated death occurred within one hour of symptom onset as documented by medical records or next-of-kin reports or had an autopsy	Death <1 hour of cardiovascular symptoms or found dead and seen <24 hours earliers in stable medical condition. Based on review of medical records.

Cohort	ARIC	CABS	CARTAGEN E	CARTAGENE/ KORA F3	CHS	FHS	Fingesture	Harvard	Rotterdam study
					surrounding arrest / death and medical records.			consistent with SCD (i.e. acute coronary thrombosis or severe coronary artery disease without myocardial necrosis or other pathologic findings to explain death)	
Control definition	Population based	Population based	French registry of Acute ST elevation or non-ST-elevation Myocardial Infarction (FastMI)	Population based	Population based		MI survivors	Controls from population studies and clinical trials matched on on study cohort, sex, age (+/−1 year), ethnicity, smoking status (current, never, past), time and date of blood sampling,	Population based

Cohort	ARIC	CABS	CARTAGENE	CARTAGENE/ KORA F3	CHS	FHS	Fingesture	Harvard	Rotterdam study
								fasting status, and presence or absence of cardiovascular disease (MI, angina, CABG, or stroke) prior to death.	
Software used for GWAS statistical analysis	ProbABEL	R	snptest v2.1.1	PLINK v1.07	R		Mach2dat	Plink/Eigenstrat	ProbABEL
Model with covariates	Cox proportional hazards, with age, sex, and PCs as covariates	age, sex	age, sex	age, sex, PCs	age, sex, clinic	age,sex	age, sex, 10 PC	20 PCs, cohort	age, sex, PCs

Table 2-2 Sample Characteristics for replication cohorts

Cohort	**AGNES**	**ARREST**	**CHS**	**Fingesture**	**FINRISK**	**GEVAMI**	**Mayo**	**SMART**	**UMEA**	**OCME**	**CABS-African Americans**	**CABS-Asian Americans**
Stage	Stage2--Extension	Stage2--Extension	Stage2=Extension	Stage2=Extension	Stage2=Extension	Stage2=Extension	Stage2=Extension	Stage2=Extension	Stage2=Extension	Stage2=Extension	Stage2-Replication	Stage2-Replication
N, number of cases with genotype data	672	1409	78	559	209	533	124	368	470	119	152	225
N, number of controls with genotype data	761	1659	813	490	7976	265	139	8086	931	378	176	199
QC criteria, per sample	sex check, principle component analysis, removing of outliers,	Sex-check, Removed duplicates, checks for cryptic relatedness and genetic outliers	genotyping call rate > 50%	genotyping call rate > 90%	Genotyping call rate >98% / >95% / >95 %		genotyping call rate > 50%	Sex-check, Removed duplicates, checks for cryptic relatedness and genetic outliers from PCA	NA	genotyping call rate > 50%	Sex-check, Removed duplicates, checks for cryptic relatedness and genetic outliers from PCA	Sex-check, Removed duplicates, checks for cryptic relatedness and genetic outliers from PCA

Cohort	AGNES	ARREST	CHS	Fingesture	FINRISK	GEVAMI	Mayo	SMART	UMEA	OCME	CABS-African Americans	CABS-Asian Americans
		from PCA										
Genotyping platform	Illumina Human610- Quad & Illumina HumanOmni2.5		Sequenom	Sequenom	Illumina, subsets done by HumanCore Exome / 610K / Omni Express	Taqman	Sequenom		NA	Sequenom		
Genotype calling algorithm	BeadStudio & GenomeStudio		Sequenom	Sequenom	Illumina Bead Studio		Sequenom		NA	Sequenom	apt-probeset-genotype	apt-probeset-genotype
Inclusion criteria--MAF	>0.001	>1%	N/A	N/A	MAC<2 / >0.01 / >0.01	NA	N/A	>1%	NA	N/A	dose variance > .01	dose variance > .01
Inclusion criteria--Call Rate per SNP	>95%	>95%	>50%	>90%	> 95%	NA	>50%	>95%	NA	>50%	>95%	>95%
Inclusion criteria--pvalue HWE	> 1E-4	> 1E-5	N/A	N/A	> 10e-6	NA	N/A	> 1E-3	NA	N/A	> 1E-5	> 1E-5

Cohort	AGNES	ARREST	CHS	Fingesture	FINRISK	GEVAMI	Mayo	SMART	UMEA	OCME	CABS-African Americans	CABS-Asian Americans
Autosomal SNPs after QC	507,436 for Illumina Human610- Quad & 2,209,801 for Illumina HumanOmni2.5	23	26	25	Asked to replicate just 1.	1	26	17	NA	26	8,573,931	16,820,556
Imputation Reference Panel	Hapmap.v3	NA	N/A	N/A	1,000 Genomes haplotypes -- Phase I integrated	NA	N/A	-	NA	N/A	1000G PhaseIv3	1000G PhaseIv3
Imputation Software	Mach & minimac	NA	N/A	N/A	SHAPEIT2 (pre-phasing) and IMPUTE2	NA	N/A	-	NA	N/A	minimac	minimac
Sex, number of women among cases	135	301	23	100	45	34	41	69	121	25	43	70

Cohort	AGNES	ARREST	CHS	Fingesture	FINRISK	GEVAMI	Mayo	SMART	UMEA	OCME	CABS-African Americans	CABS-Asian Americans
Sex, number of women among controls	156	355	363	261	3983	131	29	2674	247	96	90	91
Age, mean age at baseline among cases	56.70	64.10	72.40	64.82	59.83	59.38	52.81	63.00	56.01	48.87	64.95	63.44
Age, age-range at baseline among cases	30-84	0 - 95	65-84	28-91	27-74	52.65-66.81	3-83	31-82	30-71	21-83	25-95	20-102
Age, mean age at baseline among controls	58.30	58.44	73.7	51.14	48.15	60.61	33.25	56	55.57	74	61.02	61.59
Age, age-range at baseline among controls	32-83	32 - 82	63-100	40-62	24-74	52.26-66.81	1-82	17-81	30-74	64-92	39-86	32-89
Average time to SCD (for prospective studies)	NA	NA	6.4	NA	6.78	NA	NA	NA	7.24	NA	NA	NA
Mean followup time (for prospective studies)	NA	NA	9.8		10.5	NA	NA	NA	NA	NA	NA	NA

Cohort	AGNES	ARREST	CHS	Fingesture	FINRISK	GEVAMI	Mayo	SMART	UMEA	OCME	CABS-African Americans	CABS-Asian Americans
Study design	Case-control	Case-control	Prospective cohort	Case-control	Prospective cohort	Case-control	Case-control	Case-control	Nested case-control	Case only (analysed with CHS controls)	Case-control	Case-control
SCD definition/Ascertainment	ECG-registered VF that occurred within 24 hours after the onset of symptoms and before reperfusion therapy in the setting of an acute and first ST-segment elevation MI.	out-of-hospital cardiac arrest with VT/VF documented by emergency medical services during resuscitation attempt	Sudden pulseless condition presumed due to a cardiac arrhythmia, without evidence for a non-cardiac condition as a cause of the arrest, in an otherwise stable patient, after review of events surroundin	Sudden, pulseless condition from a cardiac origin in a previously stable individual, review of death and medical records.	Review of death and medical records and other phenotypic data.	VF within first 12 hours of symptoms of STEMI before primary PCI		Sudden, pulseless condition from a cardiac origin in a previously stable individual, review of death and medical records. Pt in VF or asystole (NO PEA)	Unexpected death without obvious extracardiac cause that occurred within 24 hours of symptom onset in subjects with probable myocardial infarction		Sudden, pulseless condition from a cardiac origin in a previously stable individual, review of death and medical records. Pt in VF or asystole (NO PEA)	Sudden, pulseless condition from a cardiac origin in a previously stable individual, review of death and medical records. Pt in VF or asystole (NO PEA)

Cohort	AGNES	ARREST	CHS	Fingesture	FINRISK	GEVAMI	Mayo	SMART	UMEA	OCME	CABS-African Americans	CABS-Asian Americans
			g arrest / death and medical records.									
Control definition	patients with a first acute ST-segment elevation MI without VF		Population based	Population based	Population based	No VF during same time period		Population based	Population based	Sex matched controls from CHS	Population based	Population based
Software used for GWAS statistical analysis	ProbABEL	R	R	Plink	R	STATA	R	SPSS, Plink	NA	R	R	R
Model with covariates	age,sex, principle	age, sex	age, sex	age, sex	age, sex, 10 PCS	age, sex	age,sex	age, sex	age, sex	sex	age, sex, 2 PCs	age, sex, 2 PCs

Cohort	AGNES	ARREST	CHS	Fingesture	FINRISK	GEVAMI	Mayo	SMART	UMEA	OCME	CABS-African Americans	CABS-Asian Americans
	components											

Table 2-3 Results from GWAS analysis

							Discovery					Replication				
Chr	Position	gene	left gene	right gene	Risk Allele	Other Allele	Risk Allele Frequency	OR [95% CI]	P.value	Direction	HetPVal	Risk Allele Frequency	OR [95% CI]	P.value	Direction	HetPVal
2	233467128	NGEF	C2orf82	LOC729940	t	g	0.108	1.25 [1.37-1.14]	3.00E-06	++-++?++-	0.002605	0.117	1.12 [1.21-1.03]	0.01033	++++-+-+---	0.009947
9	83220552	NA	LOC100128222	TLE1	t	c	0.016	1.56 [1.97-1.24]	1.42E-04	+--++-++?	0.743	0.017	1.08 [2.55-0.46]	0.8561	???-+???????	0.2503
14	50620280	TRIM9	PYGL	TXNDC1	a	g	0.405	0.88 [0.94-0.83]	3.88E-05	--?--+---	0.3232	0.411	0.94 [1-0.89]	0.06847	-?++-??+?--	0.2218
2	119933926	SCTR	TMEM37	hCG_17324	t	c	0.089	0.8 [0.9-0.72]	7.12E-05	--?------	0.94	0.086	0.91 [1.01-0.81]	0.08369	-?+--??-?--	0.0565
12	4691160	NA	NDUFA9	GALNT8	t	c	0.957	0.74 [0.86-0.64]	4.74E-05	--?-+---+-	0.9375	0.961	0.88 [1.07-0.73]	0.1965	-?--+??-?+-	0.1324
20	51149410	TSHZ2	RPL36P1	LOC728805	a	g	0.983	0.58 [0.75-0.46]	1.76E-05	--------??	0.2976	0.979	0.89 [1.38-0.58]	0.608	???-???--?+	0.4891
5	82090500	NA	FLJ41309	LOC100127911	a	g	0.399	1.12 [1.18-1.05]	3.15E-04	+-+++++-+	0.00214	0.396	1.03 [1.22-0.87]	0.7224	?-?+-???????	0.8163
10	72999813	CDH23	SLC29A3	C10orf105	t	c	0.209	1.16 [1.26-1.07]	3.11E-04	+-?+++--+	0.01539	0.176	1.04 [1.26-0.85]	0.7258	+??+???????	0.2672
10	72979896	CDH23	SLC29A3	C10orf105	t	c	0.444	0.89 [0.95-0.83]	3.21E-04	-+-----+-	0.2134	0.360	1.02 [1.22-0.86]	0.8017	?+?-+???????	0.3565
2	172521464	HAT1	SLC25A12	MAP1D	a	g	0.546	1.11 [1.18-1.05]	3.83E-04	++--++--+	0.5164	0.546	1.02 [1.12-0.92]	0.7687	???-+??-?++	0.9913
3	157134105	GMPS	SLC33A1	LOC389168	t	c	0.943	0.75 [0.85-0.66]	1.25E-05	--?---+--	0.4719	0.944	0.98 [1.11-0.87]	0.7535	-?++-??++-+	0.6803
2	64449026	NA	LOC130773	LOC100128607	a	g	0.654	1.12 [1.2-1.05]	5.88E-04	+-+++++++	0.1907	0.645	0.98 [1.17-0.82]	0.8165	?+?--??????	0.5073
13	42194563	NA	TNFSF11	C13orf30	a	g	0.731	0.87 [0.93-0.82]	7.38E-05	-+?--+---	0.379	0.735	0.99 [1.06-0.92]	0.6864	-?+--??-+-+	0.09311
1	5659269	NA	AJAP1	NPHP4	a	g	0.349	1.13 [1.21-1.06]	2.84E-04	++?++++++	0.4831	0.326	0.97 [1.1-0.85]	0.6211	-+?-???????	0.509
6	68597786	NA	NUFIP1P	LOC100128757	a	g	0.055	1.28 [1.45-1.13]	1.54E-04	++?++++++	0.9988	0.057	1.03 [1.17-0.91]	0.6312	+?+-+??+-+-	0.5697
10	119580791	NA	EMX2	RAB11FIP2	t	g	0.093	1.25 [1.39-1.13]	1.20E-05	+-?++++?+	0.02519	0.089	0.97 [1.09-0.87]	0.618	?+-++??--+-	0.8576
5	30877798	NA	HPRTP2	LOC391774	t	g	0.810	0.87 [0.94-0.81]	4.01E-04	-+?------	0.6384	0.820	1.06 [1.24-0.91]	0.444	+-?+-??????	0.4649
5	30875088	NA	HPRTP2	LOC391774	a	g	0.802	0.87 [0.94-0.81]	2.28E-04	-+-------	0.7393	0.800	1.09 [1.26-0.94]	0.2521	+-?-+??????	0.8199
10	97061998	RBS1 SORE	PDLIM1	LOC643981	t	c	0.578	1.13 [1.2-1.06]	5.80E-05	+-?--++++	0.05339	0.533	0.99 [1.05-0.93]	0.7441	-+++-+??--+-	0.218
8	3844825	CSMD1	MYOM2	LOC780813	a	g	0.290	1.15 [1.22-1.07]	5.17E-05	+++-+++-+	0.5324	0.273	0.99 [1.06-0.92]	0.7198	+?--+??-+++	0.264
4	63299057	NA	LOC644534	LOC644548	a	c	0.026	1.37 [1.64-1.14]	8.86E-04	++?-+++?+	0.1561	0.026	1.03 [1.24-0.86]	0.7297	+?++-??+?-+	0.1426
16	75948773	ADAMTS18	VN2R10P	NUDT7	t	c	0.416	0.89 [0.95-0.84]	1.87E-04	--?+?----	0.005763	0.419	1.02 [1.08-0.95]	0.6122	-?+-+??+-++	0.5257
13	109857568	COL4A2	COL4A1	LOC100129836	a	g	0.137	1.17 [1.28-1.07]	9.12E-04	++?++++++	0.1236	0.118	1 [1.1-0.91]	0.9668	+?++-??-?-+	0.1101
3	157213758	NA	LOC389168	VN2R1P	a	c	0.044	1.37 [1.58-1.18]	2.76E-05	++?+++--+	0.3408	0.045	0.92 [1.08-0.79]	0.3102	-?--???--?-	0.7877
16	14114250	MKL2	ERCC4	CGI-148P	a	g	0.195	1.14 [1.23-1.06]	7.54E-04	++?--++?+	0.5168	0.190	0.98 [1.07-0.9]	0.6892	??--+??-++-	0.3031
14	29581604	NA	PRKD1	LOC100128358	a	c	0.946	0.7 [0.85-0.57]	3.52E-04	??--?+---	0.5738	0.960	1.06 [1.23-0.91]	0.4788	-?+++??+-+-	0.1215

							Combined (European only)					Combined (MultiEthnic)				
Chr	Position	gene	left gene	right gene	Risk Allele	Other Allele	Risk Allele Frequency	OR [95% CI]	P.value	Direction	HetPVal	Risk Allele Frequency	OR [95% CI]	P.value	Direction	HetPVal
2	233467128	NGEF	C2orf82	LOC729940	t	g	0.113	1.17 [1.25-1.1]	5.09E-07	++++-+-+---+	0.005641	0.1122	1.17 [1.1-1.24]	0.00000104	++++-+-+---+?-+	0.005564
9	83220552	NA	LOC100128222	TLE1	t	c	0.016	1.62 [2.02-1.3]	1.43E-05	???-+??????+	0.327	0.0805	1.35 [1.15-1.58]	0.0002456	???-+??????++-+	0.1085
14	50620280	TRIM9	PYGL	TXNDC1	a	g	0.408	0.91 [0.95-0.87]	2.49E-05	-?++-??+?---	0.151	0.4047	0.91 [0.87-0.95]	0.00002237	-?++-??+?---?--	0.2828
2	119933926	SCTR	TMEM37	hCG_17324	t	c	0.088	0.85 [0.92-0.79]	4.84E-05	-?+--??-?---	0.04243	0.0873	0.85 [0.79-0.92]	0.00004383	-?+--??-?---?-?	0.06825
12	4691160	NA	NDUFA9	GALNT8	t	c	0.958	0.79 [0.89-0.7]	5.71E-05	-?--+??-?+--	0.1063	0.9469	0.8 [0.72-0.89]	0.00007287	-?--+??-?+--?-+	0.152
20	51149410	TSHZ2	RPL36P1	LOC728805	a	g	0.982	0.68 [0.84-0.55]	3.32E-04	???-???--?+-	0.3414	0.9819	0.68 [0.55-0.84]	0.0003322	???-???--?+-???	0.3414
5	82090500	NA	FLJ41309	LOC100127911	a	g	0.399	1.11 [1.17-1.05]	4.43E-04	?-?+-??????+	0.7729	0.4247	1.1 [1.04-1.16]	0.000531	?-?+-??????+++-	0.7395
10	72999813	CDH23	SLC29A3	C10orf105	t	c	0.204	1.14 [1.23-1.06]	5.37E-04	+??+???????+	0.3082	0.229	1.12 [1.04-1.2]	0.002555	+??+???????+?--	0.196
10	72979896	CDH23	SLC29A3	C10orf105	t	c	0.434	0.9 [0.96-0.85]	1.01E-03	?+?-+??????-	0.2366	0.4175	0.91 [0.86-0.97]	0.002383	?+?-+??????-++-	0.2315
2	172521464	HAT1	SLC25A12	MAP1D	a	g	0.546	1.09 [1.14-1.03]	1.34E-03	???-+??-?+++	0.7456	0.5341	1.08 [1.03-1.13]	0.002647	???-+??-?+++?--	0.7219
3	157134105	GMPS	SLC33A1	LOC389168	t	c	0.944	0.87 [0.95-0.79]	1.43E-03	-?++-??++-+-	0.08631	0.9399	0.86 [0.79-0.94]	0.0007807	-?++-??++-+-?+-	0.1562
2	64449026	NA	LOC130773	LOC100128607	a	g	0.653	1.1 [1.17-1.04]	1.59E-03	?+?--?????+	0.357	0.6468	1.07 [1.01-1.14]	0.01605	?+?--??????+---	0.03214
13	42194563	NA	TNFSF11	C13orf30	a	g	0.733	0.93 [0.97-0.88]	1.79E-03	-?+--??-+-+-	0.01866	0.735	0.92 [0.88-0.97]	0.0007382	-?+--??-+-+-?--	0.01864
1	5659269	NA	AJAP1	NPHP4	a	g	0.345	1.1 [1.16-1.03]	2.49E-03	-+?-???????+	0.1302	0.375	1.1 [1.04-1.16]	0.000981	-+?-???????++++	0.3348
6	68597786	NA	NUFIP1P	LOC100128757	a	g	0.056	1.15 [1.25-1.05]	2.74E-03	+?+-+??+-+-+	0.1829	0.0658	1.14 [1.05-1.24]	0.002945	+?+-+??+-+-+?-+	0.3067
10	119580791	NA	EMX2	RAB11FIP2	t	g	0.091	1.12 [1.21-1.04]	3.47E-03	?+-++??--+-+	0.07805	0.0901	1.12 [1.04-1.2]	0.003554	?+-++??--+-+-++	0.05712
5	30877798	NA	HPRTP2	LOC391774	t	g	0.812	0.91 [0.97-0.85]	4.40E-03	+-?+-??????-	0.1105	0.766	0.9 [0.85-0.96]	0.001137	+-?+-?????----	0.2723
5	30875088	NA	HPRTP2	LOC391774	a	g	0.801	0.91 [0.97-0.85]	5.13E-03	+-?-+??????-	0.09091	0.7711	0.91 [0.85-0.96]	0.001761	+-?-+??????----	0.3081
10	97061998	RBS1 SORE	PDLIM1	LOC643981	t	c	0.556	1.06 [1.11-1.02]	7.33E-03	-+++-+??--+-+	0.01938	0.5569	1.06 [1.01-1.1]	0.009475	-+++-+??--+-++--	0.06258
8	3844825	CSMD1	MYOM2	LOC780813	a	g	0.281	1.06 [1.12-1.02]	8.74E-03	+?--+??-++++	0.01805	0.2837	1.06 [1.02-1.11]	0.00803	+?--+??-++++?-+	0.006828
4	63299057	NA	LOC644534	LOC644548	a	c	0.026	1.18 [1.35-1.04]	1.02E-02	+?++-??+?-++	0.04809	0.0298	1.18 [1.04-1.33]	0.008859	+?++-??+?-++?++	0.1113
16	75948773	ADAMTS18	VN2R10P	NUDT7	t	c	0.417	0.95 [0.99-0.91]	1.71E-02	-?+-+??+-++-	0.06549	0.4232	0.96 [0.92-1]	0.03863	-?+-+??+-++-?++	0.05359
13	109857568	COL4A2	COL4A1	LOC100129836	a	g	0.128	1.08 [1.16-1.01]	1.81E-02	+?++-??-?-++	0.02719	0.1401	1.09 [1.02-1.17]	0.00799	+?++-??-?-++?++	0.05448
3	157213758	NA	LOC389168	VN2R1P	a	c	0.045	1.13 [1.26-1.02]	2.11E-02	-?--???--?-+	0.01531	0.0501	1.12 [1.01-1.24]	0.02986	-?--???--?-+?-+	0.008446
16	14114250	MKL2	ERCC4	CGI-148P	a	g	0.193	1.07 [1.13-1.01]	2.53E-02	??--+??-++-+	0.05653	0.1961	1.07 [1.02-1.13]	0.01154	??--+??-++-+?++	0.08189
14	29581604	NA	PRKD1	LOC100128358	a	c	0.955	0.91 [1.02-0.81]	1.14E-01	-?+++??+-+--	0.004586	0.9531	0.9 [0.8-1.01]	0.0682	-?+++??+-+--?+-	0.005856

Table 2-4 Results from subgroup analysis

MALE

rsnum	chromosome	position	gene	left_gene	right_gene	Risk Allele	Other Allele	Risk Allele Frequency	OR [95% CI]	P-value	Direction	HetPVal
rs1019878	2	28822315	NA	PLB1	PPP1CB	t	c	0.575	1.19 [1.28-1.11]	1.39E-06	+++-+?++	0.47
rs319397(	10	97061998	SORBS1 SC	PDLIM1	LOC64398	t	c	0.576	1.18 [1.27-1.1]	3.72E-06	+++++?++	0.2274
rs1225263	10	114449263	VTI1A	ZDHHC6	LOC14318	a	g	0.934	0.69 [0.81-0.59]	7.07E-06	---?-?--	0.64
rs1119813	10	119580320	NA	EMX2	RAB11FIP2	t	g	0.906	0.76 [0.86-0.68]	7.12E-06	--+-----	0.0773
rs1410816	13	30992193	NA	B3GALTL	RXFP2	t	g	0.504	1.18 [1.27-1.1]	6.37E-06	++++-?++	0.3089

FEMALE

rsnum	chromosome	position	gene	left_gene	right_gene	Risk Allele	Other Allele	Risk Allele Frequency	OR [95% CI]	P-value	Direction	HetPVal
rs754570	2	19766139	NA	OSR1	CISD1B	a	g	0.230	1.34 [1.53-1.18]	8.47E-06	++++?++	0.6699
rs757263(	2	232243339	NA	C2orf57	PTMA	c	g	0.889	0.49 [0.68-0.36]	9.32E-06	+---?--	0.2042
rs2188683	7	89211935	NA	ZNF804B	DPY19L2P4	a	g	0.0164	13.18 [41.12-4.22]	8.95E-06	+??+?	0.4682
rs2810758	9	38257842	NA	SHB	ALDH1B1	a	g	0.991	0.06 [0.2-0.02]	5.53E-06	-?-??-?	0.7004
rs1099959	10	53243936	PRKG1	CSTF2T	LOC44055	a	c	0.990	0.02 [0.1-0]	3.40E-06	-???--?	0.3046
rs1280511	11	29085910	NA	OR2BH1P	LOC40167	a	t	0.1261	1.43 [1.68-1.22]	7.71E-06	++++?++	0.807
rs120569(	20	49760501	ATP9A	NFATC2	SALL4	a	c	0.1088	1.52 [1.79-1.29]	3.86E-07	++++++-+	0.01703

AGE65

rsnum	chromosome	position	gene	left_gene	right_gene	Risk Allele	Other Allele	Risk Allele Frequency	OR [95% CI]	P-value	Direction	HetPVal
rs1261518	2	238723021	KLHL30	ESPNL	C2orf19	a	g	0.0165	7.43 [15.8-3.5]	1.86E-07	+?++?+	0.5663
rs959233	4	10511257	NA	MIST	LOC64344	a	g	0.535	1.24 [1.37-1.13]	9.36E-06	+++++-+	0.3103
rs683865(	4	175636679	NA	KIAA1712	HPGD	a	c	0.2251	1.3 [1.46-1.16]	5.01E-06	++++?+	0.2242
rs1046181	5	24659860	CDH10	LOC10013	MSNL1	a	g	0.2737	1.27 [1.42-1.15]	7.22E-06	++++++	0.9314
rs303001	6	25164748	NA	LOC44216	CMAH	a	g	0.0556	1.71 [2.1-1.39]	4.06E-07	++++?+	0.1184
rs938381(	6	156895326	NA	NOX3	ARID1B	a	g	0.0184	2.42 [3.53-1.66]	3.84E-06	++++?+	0.5722
rs6974659	7	12788046	NA	ARL4A	LOC10013	a	g	0.7163	0.78 [0.87-0.7]	6.77E-06	------	0.388
rs2672247	8	19111189	NA	LOC10012	LOC44238	t	c	0.9858	0.11 [0.28-0.04]	3.06E-06	+?+-?-	0.006524
rs1199922	9	83756559	NA	FLJ44082	LOC10013	a	t	0.0133	3.56 [6.19-2.05]	6.89E-06	+?+-?+	0.135
rs1354032	13	84546973	NA	LOC38793	SLITRK6	a	g	0.3545	0.76 [0.84-0.68]	3.48E-07	----?-	0.3478
rs477317(	13	109858751	COL4A2	COL4A1	LOC10012	a	t	0.7636	1.32 [1.49-1.18]	3.14E-06	++++?+	0.1982
rs7404645	16	81578029	CDH13	MPHOSPH	HSBP1	a	g	0.2978	1.27 [1.42-1.14]	9.59E-06	++++++	0.6797

VF

rsnum	chromosome	position	gene	left_gene	right_gene	Risk Allele	Other Allele	Risk Allele Frequency	OR [95% CI]	P-value	Direction	HetPVal
rs4638745	2	28947307	NA	FLJ20628	WDR43	a	g	0.5553	1.22 [1.32-1.12]	7.40E-06	++-?	0.03045
rs204268(	3	108549433	NA	LOC34459	CCDC54	a	g	0.6621	0.81 [0.89-0.74]	5.21E-06	----	0.6567
rs1003039	4	186508148	SNX25	KIAA1430	LRP2BP	a	g	0.2106	1.27 [1.41-1.15]	4.09E-06	++++	0.9454
rs830684	5	165532119	NA	LOC72884	LOC44111	t	c	0.9302	0.69 [0.81-0.58]	9.77E-06	-++?	0.08123
rs9459122	6	164834802	NA	LOC72827	LOC72831	t	c	0.7624	1.3 [1.43-1.17]	6.65E-07	++++	0.5995
rs1373517	7	152688323	NA	ACTR3B	FLJ42291	a	c	0.5253	1.23 [1.34-1.13]	2.19E-06	++++	0.3854
rs1736902	9	735289	KANK1	LOC64235	DMRT1	t	c	0.1992	0.76 [0.86-0.67]	7.73E-06	----	0.895
rs185467(	9	27632744	NA	C9orf72	LINGO2	c	g	0.11	1.47 [1.73-1.24]	5.16E-06	+++?	0.1908
rs7855251	9	99908010	TRIM14 TE	NANS	CORO2A	t	c	0.7405	1.26 [1.39-1.14]	9.42E-06	++++	0.2428
rs2795489	9	99949752	CORO2A	TRIM14	TBC1D2	a	g	0.239	0.78 [0.87-0.7]	4.95E-06	----	0.1891
rs1276613	10	134519674	C10orf92	NKX6-2	LOC10012	a	g	0.5457	0.8 [0.88-0.73]	1.57E-06	--+?	0.03982
rs612927	18	75432295	LOC10012	NFATC1	FLJ25715	a	g	0.5125	1.64 [2.03-1.32]	7.32E-06	+?+?	0.9524

Table 2-5 Details of GWAS that were used for GRSA analyses

Trait	Consortium	Title	Reference in main manuscript	Sample Size
CAD	CARDIOGRAM+C4D	Large-scale association analysis identifies new risk loci for coronary artery disease (Deloukas et al., 2012)	16	194,427
T2D	DIAGRAM	Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes (Morris et al, 2012)	17	149,821
AF	CHARGE AF	Meta-analysis identifies six new susceptibility loci for atrial fibrillation (Ellinor et al., 2012)	23	59,133
BMI	GIANT	Genetic studies of body mass index yield new insights for obesity biology (Locke et al., 2015)	30	339,224
HEIGHT	GIANT	Defining the role of common variation in the genomic and biological architecture of adult human height (Wood et al., 2014)	29	253,288
WCADJBMI	GIANT	New genetic loci link adipose and insulin biology to body fat distribution (Shungin et al., 2015)	31	224,459
WHRADJBMI	GIANT	New genetic loci link adipose and insulin biology to body fat distribution (Shungin et al., 2015)	31	142,762
DBP	ICBP	Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk (Ehret et al., 2011)	20	69,395
SBP	ICBP	Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk (Ehret et al., 2011)	20	69,395
FGADJBMI	DIAGRAM	A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance (Manning et al., 2012)	18	58,074
FIADJBMI	DIAGRAM	A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance (Manning et al., 2012)	18	51,750
HR	CHARGE HR	Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders (den Hoed et al., 2013)	26	88,823
QRS	CHARGE QRS	Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction (Sotoodehnia et al., 2010)	25	40,407
QT	QT-IGC	Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization (Arking et al., 2014)	24	76,061

HDL	Global Lipids Genetics Consortium	Discovery and refinement of loci associated with lipid levels (Willer et al., 2013)	19	188,577
LDL	Global Lipids Genetics Consortium	Discovery and refinement of loci associated with lipid levels (Willer et al., 2013)	19	188,577
TCH	Global Lipids Genetics Consortium	Discovery and refinement of loci associated with lipid levels (Willer et al., 2013)	19	188,577
TG	Global Lipids Genetics Consortium	Discovery and refinement of loci associated with lipid levels (Willer et al., 2013)	19	188,577

Table 2-6 Results from lookup of genes associated with Mendelian forms of arrhythmias

Gene	Associated Disease*	Chr	Length	SNPs.in.gene	Tests	Best.SNP	SNP.maf	SNP.chi2	N.total.permutati	N.better	Pval
ABCC9	DCM	12	139,306	105	16.8289	rs11612749	0.0235	5.15704	100	39	0.39604
ACTC1	DCM, HCM	15	7,631	20	5.00555	rs6495883	0.3383	0.308642	100	99	0.990099
ACTN2	DCM, HCM	1	77,789	134	14.0981	rs16837283	0.1	3.60429	100	72	0.722772
CACNA1C	LQTS,BS,SQTS,CPVT	12	644,700	719	81.5763	rs10848666	0.1684	9.80455	100	29	0.29703
CACNA2D1	LQTS,BS,SQTS,CPVT	7	493,614	641	49.0956	rs7796271	0.4063	7.3521	100	62	0.623762
CACNB2	LQTS,BS,SQTS,CPVT	10	401,083	594	49.9443	rs11014084	0.2067	7.03784	100	63	0.633663
CASQ2	HCM,LQTS,BS,SQTS, CPVT	1	68,803	85	7.09843	rs699758	0.0509	6.17813	100	17	0.178218
CSRP3	DCM, HCM	11	20,012	40	9.58053	rs11025063	0.0101	7.08765	100	8	0.0891089
DES	DCM	2	8,363	67	5.53544	rs714132	0.1446	6.49428	100	8	0.0891089
DSC2	ARVC	18	36,447	57	6.45125	rs17738889	0.3535	1.8496	100	83	0.831683
DSG2	ARVC	18	50,788	51	2.93125	rs12606061	0.1511	1.71667	100	54	0.544554
DSP	ARVC	6	45,077	115	11.3373	rs6910468	0.4895	4.91042	100	36	0.366337
DTNA	LVN	18	398,555	513	48.4524	rs492392	0.23	5.64715	100	87	0.871287
EYA4	DCM	6	290,764	392	23.7326	rs9402497	0.2282	4.96821	100	66	0.663366
GPD1L	BrS	3	62,205	93	17.9548	rs6804760	0.096	7.92125	100	16	0.168317
JPH2	HCM	20	75,882	46	16.5222	rs927785	0.0577	4.4124	100	50	0.50495
JUP	ARVC	17	32,106	13	2.32466	rs9807088	0.0606	0.982684	100	58	0.584158
KCND3	LQTS,BS,SQTS,CPVT	1	213,324	328	29.7323	rs12060607	0.0191	4.59423	100	76	0.762376
KCNE1	LQTS	21	64,626	130	6.46547	rs2049798	0.0823	3.16049	100	59	0.594059
KCNE2	LQTS	21	7,118	15	7.49148	rs7275858	0.1778	3.02861	100	47	0.475248
KCNE3	BrS	11	12,715	23	5.2544	rs572656	0.0927	1.28587	100	86	0.861386
KCNH2	LQTS, SUD/SIDS	7	33,359	46	13.7827	rs10252799	0.1077	2.84124	100	85	0.851485
KCNJ2	CPVT	17	10,510	32	11.3036	rs8080540	0.2285	5.08983	100	33	0.336634
KCNQ1	LQTS, SUD/SIDS	11	404,120	345	51.6311	rs10766437	0.3438	12.4487	100	10	0.108911
LDB3	DCM, HCM, LVN	10	67,620	66	11.8822	rs3740345	0.2608	4.30305	100	47	0.475248
LMNA	DCM, DCM-CCD	1	25,418	65	10.2156	rs10489683	0.214	5.72857	100	20	0.207921
MYBPC2	DCM, HCM	19	33,424	27	5.60055	rs12982642	0.1121	0.898159	100	93	0.930693
MYBPC3	DCM, HCM	11	21,297	27	4.86832	rs1377416	0.3849	2.5888	100	45	0.455446
MYH6	DCM, HCM	14	26,288	64	8.5915	rs3742511	0.0376	5.83579	100	18	0.188119
MYH7	DCM, HCM, LVN, RCM	14	21,550	41	6.45408	rs7148384	0.178	2.73521	100	58	0.584158
MYL2	HCM	12	9,782	5	3.92527	rs10774652	0.0313	3.18464	100	31	0.316832
MYL3	HCM	3	5,617	9	4.77547	rs7651753	0.0223	4.41789	100	16	0.168317
MYOZ2	HCM	4	51,999	78	7.69757	rs4834729	0.244	2.62046	100	81	0.811881
PKP2	ARVC	12	106,101	176	15.8279	rs4479073	0.0139	7.48833	100	17	0.178218
PLN	DCM, HCM	6	12,146	25	6.36297	rs6903632	0.0224	2.23482	100	62	0.623762
PRKAG2	HCM	7	321,117	208	30.9595	rs6464203	0.0226	4.33844	100	84	0.841584
PSEN1	DCM	14	87,257	111	8.66337	rs12147044	0.1418	11.5698	417	3	0.00956938
PSEN2	DCM	1	25,532	20	8.76258	rs1341715	0.3722	2.58254	100	71	0.712871
RANGRF	LQTS,BS,SQTS,CPVT	17	1,441	11	4.10811	rs8069620	0.0618	1.6391	100	71	0.712871
SCN1B	BrS	19	9,762	16	4.9603	rs4804925	0.1935	2.35529	100	59	0.594059
SCN3B	BrS	11	25,421	58	9.55347	rs12289539	0.1582	4.02776	100	47	0.475248
SCN5A	BrS, PCCD, DCM, DCM-CCD, LQTS, SUD/SIDS	3	101,612	111	15.5989	rs6783517	0.0233	4.38592	100	69	0.693069
SGCD	DCM	5	441,033	380	22.8655	rs13171055	0.0597	4.3609	100	81	0.811881
TCAP	DCM, HCM	17	1,209	14	4.50318	rs12600758	0.2022	3.03105	100	41	0.415842
TGFB3	ARVC	14	23,651	28	8.58101	rs2362751	0.2	3.52896	100	49	0.49505
TMEM43	ARVC	3	18,741	46	13.2403	rs3731095	0.0274	4.1919	100	52	0.524752
TMPO	DCM	12	34,807	32	6.47055	rs3168500	0.1057	2.99254	100	58	0.584158
TNNC1	DCM, HCM	3	2,951	21	2.66845	rs13094915	0.3948	1.21781	100	64	0.643564
TNNI3	DCM, HCM, RCM	19	5,966	16	4.99771	rs2463248	0.0807	1.1911	100	85	0.851485
TNNT2	DCM, HCM	1	18,664	25	6.53986	rs34408707	0.0267	2.28891	100	62	0.623762
TPM1	DCM, HCM	15	29,277	33	5.25814	rs920687	0.414	1.58587	100	71	0.712871
TTN	DCM, HCM	2	281,435	270	24.1718	rs6732629	0.039	7.35244	100	37	0.376238
VCL	DCM, HCM	10	122,047	83	5.06803	rs12416497	0.0861	5.32544	100	13	0.138614

***Disease abbreviations**

ARVC: Arrhythmogenic right ventricular dysplasia

BS: Brugada Syndrome

CPVT: Catecholaminergic polymorphic ventricular tachycardia

DCM: Dilated cardiomyopathy
DCM-CCD: DCM with cardiac conduction disorder
HCM: Hypertrophic cardiomyopathy
LQTS: Long QT Syndrome
LVN: Left ventricular noncompaction-1
PCCD: Progressive cardiac conduction defect
RCM: Restrictive cardiomyopathy
SQTS: Short QT Syndrome
SUD/SIDS: Sudden Unexpected Death/Sudden Infant Death Syndrome

These data are results from a gene-based test that tests for enrichment of common variants associated with SCD implemented by the 'logisitic-minsnp-gene-perm' function in FASTv1.810. This best single-SNP F-statistic within a gene serves as the test statistic to compute a permutation based p-value corrected for gene size by performing up to 1 million permutations per gene. Gene boundaries were defined by RefSeq gene coordinates on build GRCh37 with +/-10kb flank. The gene list consisted of genes associated with a variety of inherited arrythmias (details below table). None of the genes show significant association with SCA following multiple testing correction (significance threshold=9x10-4,alpha 0.05 corrected for 54 genes).

Table 2-7 Results from GWAS for previously published candidate loci for SCA

rsnum	chr	gene	left_gene	right_gene	Risk Allele	Other Allele	Risk Allele Frequency	Beta	OR	StdErr (Beta)	P-value	Direction	HetPVal	Title	Journal	Year	First Author
rs16847548	1	NA	OLFML2B	NOS1AP	t	c	0.7815	-0.1	0.901	0.0359	0.0035	--?+-----	0.2822	Genetic variations in nitric oxide synthase 1 adaptor protein are associated with sudden cardiac death in US white community-based populations.	Circulation	2009	Kao
rs12567209	1	NA	OLFML2B	NOS1AP	a	g	0.0765	-0	0.987	0.0574	0.826	---+-+-+++	0.8353				
rs3864180	13	GPC5	MIRHG1	GPC6	a	g	0.6178	-0	0.986	0.0311	0.6484	-++-++---	0.3412	Genome-Wide Association Study Identifies GPC5 as a Novel Genetic Locus Protective against Sudden Cardiac Arrest	PLoS One	2010	Arking
rs2824292	21	NA	C21orf34	C21orf37	a	g	0.5492	0	1.023	0.0298	0.444	+--+-+--+	0.2076	Genome-wide association study identifies a susceptibility locus at 21q21 for ventricular fibrillation in acute myocardial infarction	Nature Genetics	2010	Bezzina
rs4665058	2	BAZ2B	WDSUB1	OC10012792	a	c	0.0159	0.4	1.467	0.13	0.0032	+-?+++?+	0.5714	Identification of a Sudden Cardiac Death Susceptibility Locus at 2q24.2 through Genome-Wide Association in European Ancestry Individuals	Nature Genetics	2011	Arking
rs10918859	1	NOS1AP	OLFML2B	C1orf111	a	g	0.1911	0	1.047	0.0386	0.2345	++?+++---	0.5433	Common Variants in CASQ2, GPD1L, and NOS1AP Are Significantly Associated With Risk of Sudden Death in Patients With Coronary Artery Disease	Circ Cardiovascular Genetics	2011	Westaway
rs3010396	1	CASQ2	VANGL1	NHLH2	a	g	0.4665	-0	0.991	0.0298	0.7644	+-?--++-+	0.48				
rs9862154	3	NA	ZNF860	GPD1L	c	g	0.8204	-0.1	0.918	0.0407	0.03591	--?-+--++	0.7333				
rs4621553	5	NA	YTHDC2	KCNN2	a	g	0.7798	-0.1	0.909	0.0357	0.00777	--+------	0.5461	GWAS for discovery and replication of genetic loci associated with sudden cardiac arrest in patients with coronary artery disease	BMC Cardiovascular Disorders	2011	Aouizerat
rs12189362	5	GRIA1	NMUR2	FAM114A2	t	c	0.1151	0	1.029	0.0476	0.5422	++?-+-++-	0.5971				
rs12429889	13	NA	KLF12	hCG_1820717	t	c	0.8625	-0	0.955	0.0441	0.2938	-+?+----+	0.1155				
rs11624056	14	NA	LOC283585	LOC730121	a	t	0.852	-0	0.975	0.0531	0.6315	-+?+--+?-	0.7059				
rs597503	18	LAMA1	FLJ38028	LAMA1	a	g	0.8765	-0	0.976	0.046	0.6032	-+?---+++	0.6879				
rs16942421	18	KCTD1	TAF4B	CIAPIN1P	t	g	0.1068	-0.1	0.926	0.0534	0.1504	-++++--++	0.2877				
rs6730157	2	RAB3GAP1	YSK4	ZRANB3	a	g	0.6288	0	1.027	0.0315	0.3988	-+----++-	0.009546	Novel Loci Associated with Increased Risk of Sudden Cardiac Death in the Context of Coronary Artery Disease	PLoS One	2013	Huertas-Vazquez
rs2077316	10	ZNF365	RTKN2	ATQL4	a	c	0.9161	-0	0.991	0.0815	0.9109	+-?---+??	0.9273				
rs10503929	8	G1 NRG1 NR	OC10012789	MST131	t	c	0.7982	-0	0.963	0.0376	0.3126	---+--+-+	0.05066	A Common Missense Variant in the Neuregulin1 Gene is associated with Both Schizophrenia and Sudden Cardiac Death	Heart Rhythm	2013	Huertas-Vazquez
rs7737692	5	LPCAT1	SLC6A3	LPCAT1	a	g	0.6477	0.1	1.099	0.0318	0.00291	+++++++--	0.1734	Common variation in fatty acid metabolic genes and risk of incident sudden cardiac arrest	Heart Rhythm	2014	Lemaitre
rs12567209	1	NA	OLFML2B	NOS1AP	a	g	0.0765	-0	0.987	0.0574	0.826	---+-+-+++	0.8353	A Common NOS1AP Genetic Polymorphism, rs12567209 G>A, Is Associated With Sudden Cardiac Death in Patients With Chronic Heart Failure in the Chinese Han Population	Journal of Cardiac Failure	2014	Liu

Table 2-8 GRSA estimates for traits on SCA and CAD

	SCD Genetic Risk Score							CAD Genetic Risk Score						
TRAIT	Alphacutoff	m	Ahat estimate (95% CI)	R2rs	pval	pval.perm	phet	Alphacutoff	m	Ahat estimate (95% CI)	R2rs	pval	phet	pval for difference in
CAD	5E-08	39	0.29 [0.42,0.15]	0.002862	3.369E-05	<0.001	0.597							
	0.00001	111	0.23 [0.32,0.14]	0.004408	2.629E-07	<0.001	0.169							
	0.001	1078	0.11 [0.14,0.07]	0.005626	5.949E-09	<0.001	0.165							
	0.05	13071	0.057 [0.071,0.043]	1.05E-02	1.67E-15	<0.001	0.868							
	0.99	42980	0.051 [0.062,0.04]	1.33E-02	3.17E-19	<0.001	0.849							
T2D	5E-08	39	0.067 [0.17,-0.032]	0.000293	0.1848716	0.238095238	0.083	0.00000005	34	0.064 [0.11,0.021]	0.000143876	0.003300977	0.072	0.949
	0.00001	89	0.028 [0.11,-0.051]	8.28E-05	0.4808203	0.468571429	0.060	0.00001	71	0.05 [0.086,0.014]	0.000123389	0.006508612	0.066	0.628
	0.001	798	0.033 [0.067,-0.0013]	0.000593	0.0591616	0.061428571	0.094	0.001	740	0.062 [0.078,0.045]	0.000913021	1.33014E-13	0.060	0.132
	0.05	12651	0.036 [0.047,0.024]	6.21E-03	9.66E-10	<0.001	0.999	0.05	11955	0.053 [0.059,0.048]	5.90E-03	3.62E-79	0.059	0.007
	0.99	44851	0.04 [0.049,0.031]	1.26E-02	3.17E-18	<0.001	0.880	0.99	41728	0.05 [0.055,0.046]	8.33E-03	3.54E-111	0.767	0.041
AF	5E-08	10	0.045 [0.16,-0.072]	9.39E-05	0.4528473	0.619047619	0.945	0.00000005	10	0.013 [0.068,-0.042]	3.45856E-06	0.648723014	0.530	0.628
	0.00001	35	0.08 [0.16,-0.0035]	0.000587	0.0604404	0.067142857	0.382	0.00001	30	0.0014 [0.044,-0.041]	6.51719E-08	0.950138783	0.461	0.100
	0.001	752	0.045 [0.074,0.016]	0.001531	0.0024288	0.002857143	0.422	0.001	705	0.018 [0.032,0.0043]	0.000109727	0.010290079	0.801	0.104
	0.05	11508	0.047 [0.058,0.036]	1.19E-02	2.22E-17	<0.001	0.057	0.05	10851	0.026 [0.031,0.02]	0.001578069	2.15041E-22	0.069	0.001
	0.99	48251	0.044 [0.052,0.036]	1.75E-02	6.72E-25	<0.001	0.077	0.99	43973	0.027 [0.031,0.023]	0.002878538	1.66882E-39	0.996	0.000
BMI	5E-08	72	0.49 [0.77,0.21]	0.001961	0.0005987	<0.001	0.663	0.00000005	69	0.28 [0.41,0.15]	0.000297809	2.36375E-05	0.079	0.178
	0.00001	220	0.39 [0.6,0.18]	0.002233	0.0002497	<0.001	0.454	0.00001	187	0.24 [0.34,0.14]	0.000367816	2.6247E-06	0.051	0.203
	0.001	980	0.13 [0.27,0.0015]	0.000654	0.0474907	0.047142857	0.140	0.001	884	0.18 [0.24,0.12]	0.000530248	1.68852E-08	0.057	0.546
	0.05	8651	0.038 [0.1,-0.028]	2.08E-04	2.64E-01	3.66E-01	0.797	0.05	7877	0.096 [0.13,0.064]	0.000593438	2.40E-09	0.051	0.119
	0.99	45994	0.083 [0.13,0.034]	1.83E-03	9.04E-04	1.29E-02	0.969	0.99	41273	0.077 [0.1,0.053]	0.000682164	1.56671E-10	0.082	0.827
HEIGHT	5E-08	464	-0.075 [0.036,-0.19]	0.000293	0.1849608	0.238095238	0.189	0.00000005	443	-0.11 [-0.06,-0.16]	3.02E-04	2.04E-05	0.064	0.556
	0.00001	1328	-0.068 [0.015,-0.15]	0.000429	0.1086681	0.115714286	0.149	0.00001	782	-0.12 [-0.07,-0.16]	4.08E-04	7.48E-07	0.056	0.330
	0.001	4114	-0.11 [-0.046,-0.18]	0.001886	0.0007629	0.001428571	0.223	0.001	2520	-0.11 [-0.077,-0.15]	6.30E-04	7.83E-10	0.052	0.963
	0.05	16500	-0.15 [-0.1,-0.2]	6.43E-03	4.93E-10	<0.001	0.691	0.05	10735	-0.1 [-0.075,-0.13]	9.28E-04	8.35E-14	0.053	0.075
	0.99	45696	-0.16 [-0.12,-0.2]	8.82E-03	3.08E-13	<0.001	0.976	0.99	30953	-0.097 [-0.074,-0.12]	1.07E-03	1.07E-15	0.339	0.014
WCADJBMI	5E-08	67	-0.29 [0.022,-0.6]	0.000552	0.0686701	0.095238095	0.150	0.00000005	64	0.047 [0.19,-0.093]	7.24302E-06	0.509748908	0.111	0.054
	0.00001	179	-0.24 [-0.02,-0.47]	0.000757	0.0330188	0.031428571	0.094	0.00001	153	0.011 [0.12,-0.095]	7.14925E-07	0.835922084	0.083	0.044
	0.001	919	-0.077 [0.05,-0.2]	0.000234	0.2362583	0.222857143	0.440	0.001	796	0.01 [0.07,-0.05]	1.8568E-06	0.738546565	0.057	0.225
	0.05	10216	-0.041 [0.014,-0.095]	3.51E-04	1.47E-01	2.33E-01	0.272	0.05	9346	-0.00023 [0.026,-0.026]	5.08273E-09	9.86E-01	0.051	0.193
	0.99	46012	-0.039 [0.0022,-0.081]	5.75E-04	6.33E-02	1.59E-01	0.578	0.99	89805	-0.0094 [0.0059,-0.025]	2.42768E-05	2.27E-01	0.626	0.184
WHRADJBMI	5E-08	39	-0.28 [0.12,-0.67]	0.000318	0.1668706	0.238095238	0.153	0.00000005	36	0.25 [0.43,0.069]	0.00012204	0.006808386	0.063	0.017
	0.00001	121	-0.25 [0.02,-0.52]	0.000549	0.069531	0.072857143	0.100	0.00001	107	0.27 [0.4,0.15]	0.000311326	1.54394E-05	0.076	0.001
	0.001	793	-0.057 [0.08,-0.2]	0.000111	0.4134138	0.421428571	0.075	0.001	716	0.13 [0.19,0.062]	0.000246484	0.000120117	0.062	0.018
	0.05	10547	0.021 [0.075,-0.033]	9.87E-05	4.42E-01	5.34E-01	0.804	0.05	9794	0.07 [0.096,0.044]	4.72E-04	1.03E-07	0.052	0.112
	0.99	46295	-0.0018 [0.039,-0.043]	1.20E-06	9.32E-01	9.47E-01	0.819	0.99	42437	0.06 [0.08,0.041]	6.01E-04	1.91E-09	0.053	0.008
DBP	5E-08	8	-0.061 [0.48,-0.6]	8.09E-06	0.8255951	0.952380952	0.242	0.00000005	6	0.53 [0.79,0.27]	0.00271168	5.42876E-05	0.638	0.053
	0.00001	43	0.12 [0.39,-0.15]	0.000124	0.3890347	0.385714286	0.379	0.00001	39	0.54 [0.69,0.4]	0.009281275	7.44254E-14	0.104	0.007
	0.001	551	0.015 [0.024,0.0055]	0.001613	0.0018548	0.002857143	0.053	0.001	515	0.13 [0.18,0.079]	0.00412542	6.34749E-07	0.055	0.000
	0.05	10144	0.053 [0.086,0.02]	1.65E-03	1.63E-03	8.57E-03	0.919	0.05	9561	0.062 [0.08,0.045]	0.008126767	2.61261E-12	0.054	0.615
	0.99	47612	0.043 [0.067,0.019]	2.00E-03	5.35E-04	1.29E-02	0.796	0.99	43581	0.043 [0.056,0.03]	6.85E-03	1.36E-10	0.285	0.970
SBP	5E-08	7	0.096 [0.72,-0.52]	1.55E-05	0.7603749	0.952380952	0.083	0.00000005	1	0.68 [1.3,0.087]	0.00084217	0.024552861	NA	0.184
	0.00001	49	0.21 [0.37,0.042]	0.001008	0.013916	0.011428571	0.474	0.00001	36	0.46 [0.62,0.3]	0.005040924	3.65945E-08	0.211	0.031
	0.001	595	0.064 [0.13,0.0022]	0.000686	0.04247	0.042857143	0.418	0.001	555	0.16 [0.21,0.11]	0.005650642	5.51119E-09	0.061	0.022
	0.05	9973	0.037 [0.058,0.016]	1.97E-03	5.76E-04	7.14E-03	0.206	0.05	9435	0.081 [0.1,0.062]	0.011839188	2.83083E-17	0.058	0.002
	0.99	47834	0.025 [0.04,0.0095]	1.68E-03	1.48E-03	1.71E-02	0.416	0.99	43673	0.07 [0.084,0.056]	1.56E-02	2.52E-22	0.954	0.000
FGADJBMI	5E-08	22	0.2 [0.41,-0.0041]	0.000615	0.0547043	0.095238095	0.064	0.00000005	22	0.055 [0.15,-0.04]	0.000212875	0.258385362	0.789	0.202
	0.00001	47	-0.016 [0.37,-0.4]	1.11E-06	0.9348874	0.932857143	0.051	0.00001	47	0.059 [0.14,-0.023]	0.000335676	0.155813376	0.308	0.707
	0.001	699	0.033 [0.12,-0.052]	9.73E-05	0.4448162	0.452857143	0.155	0.001	681	0.018 [0.059,-0.023]	0.000128086	0.380661184	0.979	0.756
	0.05	11424	-0.0082 [0.024,-0.04]	4.30E-05	6.12E-01	6.77E-01	0.217	0.05	10806	0.021 [0.037,0.0053]	0.001142376	0.008823675	0.082	0.105
	0.99	47510	-0.005 [0.019,-0.029]	2.79E-05	6.82E-01	7.63E-01	0.710	0.99	43500	0.0099 [0.022,-0.0025]	0.000405796	0.118633373	0.562	0.282
FIADJBMI	5E-08	9	-0.11 [0.65,-0.87]	1.41E-05	0.7712824	0.952380952	0.729	0.00000005	8	0.041 [0.41,-0.33]	7.70364E-06	0.829772759	0.060	0.722
	0.00001	35	-0.1 [0.36,-0.56]	3.01E-05	0.6708693	0.665714286	0.153	0.00001	32	0.016 [0.24,-0.21]	3.09454E-06	0.891614393	0.087	0.659
	0.001	719	-0.029 [0.17,-0.23]	1.31E-05	0.7795027	0.79	0.085	0.001	690	0.096 [0.16,0.028]	0.001278832	0.005590107	0.055	0.249
	0.05	11520	-0.011 [0.036,-0.059]	3.51E-05	6.46E-01	7.00E-01	0.191	0.05	10895	0.049 [0.074,0.025]	0.002573775	8.41558E-05	0.060	0.027
	0.99	47851	-0.016 [0.02,-0.053]	1.30E-04	3.77E-01	4.99E-01	0.930	0.99	43718	0.042 [0.061,0.023]	0.00307838	1.70014E-05	0.908	0.005
HR	5E-08	12	-0.42 [-0.0061,-0.83]	0.000659	0.0467206	0.047619048	0.540	0.00000005	12	0.11 [0.3,-0.069]	2.48355E-05	0.222193037	0.807	0.021
	0.00001	37	-0.15 [0.16,-0.45]	0.000151	0.341319	0.342857143	0.286	0.00001	35	0.013 [0.15,-0.13]	5.98025E-07	0.849760451	0.492	0.345
	0.001	627	0.0078 [0.019,-0.0038]	0.000289	0.1882053	0.174285714	0.056	0.001	609	0.0052 [0.059,-0.048]	6.12062E-07	0.848028604	0.024	0.927
	0.05	10842	0.035 [0.076,-0.006]	4.67E-04	9.41E-02	1.69E-01	0.556	0.05	10325	-0.0098 [0.0096,-0.029]	1.62825E-05	0.322951507	0.039	0.053
	0.99	46764	0.023 [0.054,-0.0081]	3.51E-04	1.47E-01	2.86E-01	0.969	0.99	43382	-0.011 [0.004,-0.026]	3.43237E-05	0.15126416	0.987	0.054
QRS	5E-08	18	-0.17 [0.18,-0.53]	0.000152	0.3392046	0.571428571	0.455	0.00000005	17	-0.023 [0.15,-0.19]	1.18561E-06	0.789688703	0.169	0.455
	0.00001	55	-0.076 [0.17,-0.32]	6.09E-05	0.5454098	0.531428571	0.218	0.00001	42	-0.079 [0.053,-0.21]	2.2936E-05	0.240753861	0.070	0.980
	0.001	714	-0.022 [0.086,-0.13]	2.76E-05	0.684112	0.681428571	0.220	0.001	665	-0.024 [0.027,-0.075]	1.39789E-05	0.359757105	0.059	0.979
	0.05	11349	-0.029 [0.011,-0.069]	3.35E-04	1.56E-01	2.47E-01	0.244	0.05	10618	-0.0015 [0.018,-0.021]	3.95026E-07	0.877646649	0.063	0.224
	0.99	47942	-0.023 [0.0082,-0.054]	3.46E-04	1.49E-01	2.93E-01	0.722	0.99	43391	0.0023 [0.017,-0.013]	1.48E-06	7.66E-01	0.994	0.153
QT	5E-08	33	0.38 [0.69,0.068]	0.000951	0.0168587	<0.001	0.092	0.00000005	33	0.035 [0.17,-0.099]	4.38235E-06	0.608106266	0.210	0.047
	0.00001	111	0.29 [0.53,0.052]	0.000952	0.0167947	0.012857143	0.118	0.00001	82	0.037 [0.16,-0.082]	6.33398E-06	0.537581935	0.218	0.063
	0.001	946	0.1 [0.24,-0.04]	0.000331	0.1586925	0.151428571	0.318	0.001	857	0.061 [0.13,-0.0072]	5.10834E-05	0.079991127	0.101	0.608
	0.05	12249	0.13 [0.19,0.063]	2.60E-03	7.67E-05	1.43E-03	0.201	0.05	11291	0.045 [0.074,0.015]	1.42E-04	3.53E-03	0.326	0.022
	0.99	47969	0.1 [0.15,0.051]	2.62E-03	7.33E-05	4.29E-03	0.819	0.99	43053	0.043 [0.067,0.019]	2.03E-04	4.83E-04	0.926	0.041
HDL	5E-08	90	-0.1 [0.066,-0.27]	0.000238	0.231871	0.285714286	0.225	0.00000005	75	-0.083 [-0.0023,-0.16]	6.77546E-05	0.043769953	0.076	0.836
	0.00001	224	-0.047 [0.085,-0.18]	8.17E-05	0.4837929	0.471428571	0.182	0.00001	139	-0.075 [-9e-04,-0.15]	6.55887E-05	0.04727854	0.054	0.722
	0.001	870	-0.047 [0.055,-0.15]	0.000139	0.3617122	0.372857143	0.092	0.001	686	-0.13 [-0.077,-0.19]	0.000380919	1.74284E-06	0.060	0.154
	0.05	10221	-0.061 [-0.013,-0.11]	1.05E-03	1.21E-02	3.00E-02	0.052	0.05	9283	-0.077 [-0.054,-0.1]	6.97E-04	1.01E-10	0.056	0.551
	0.99	43920	-0.046 [-0.0099,-0.081]	1.04E-03	1.23E-02	6.14E-02	0.051	0.99	40240	-0.067 [-0.049,-0.084]	9.44E-04	5.16E-14	0.162	0.302
LDL	5E-08	68	0.12 [0.28,-0.048]	0.000324	0.1629304	0.238095238	0.057	0.00000005	61	0.35 [0.45,0.25]	8.28E-04	1.78E-12	0.063	0.019
	0.00001	169	0.15 [0.28,0.018]	0.00083	0.0256451	0.025714286	0.082	0.00001	105	0.33 [0.42,0.24]	8.52E-04	8.62E-13	0.140	0.027
	0.001	808	0.16 [0.26,0.062]	0.001725	0.0012875	0.001428571	0.053	0.001	655	0.23 [0.28,0.17]	1.06E-03	1.59E-15	0.059	0.232
	0.05	10466	0.094 [0.14,0.051]	3.01E-03	2.08E-05	<0.001	0.054	0.05	9631	0.077 [0.098,0.055]	8.24E-04	2.01E-12	0.057	0.485
	0.99	43706	0.075 [0.11,0.042]	3.36E-03	6.97E-06	1.43E-03	0.495	0.99	40549	0.078 [0.094,0.062]	1.54E-03	7.86E-22	0.062	0.895
TCH	5E-08	83	0.093 [0.25,-0.067]	0.000218	0.2525285	0.380952381	0.096	0.00000005	72	0.29 [0.39,0.2]	6.04E-04	1.73E-09	0.064	0.036
	0.00001	205	0.16 [0.29,0.032]	0.000992	0.0146773	0.011428571	0.055	0.00001	134	0.29 [0.37,0.2]	7.36E-04	3.04E-11	0.053	0.110
	0.001	818	0.18 [0.27,0.077]	0.002045	0.0004567	<0.001	0.070	0.001	641	0.2 [0.25,0.14]	7.97E-04	4.65E-12	0.057	0.723
	0.05	10437	0.14 [0.18,0.092]	6.10E-03	1.36E-09	<0.001	0.051	0.05	9448	0.074 [0.096,0.052]	7.43E-04	2.41E-11	0.055	0.014
	0.99	44140	0.095 [0.13,0.062]	5.27E-03	1.79E-08	1.43E-03	0.152	0.99	84905	0.068 [0.081,0.056]	1.89E-03	1.47E-26	0.075	0.134
TG	5E-08	60	0.14 [0.34,-0.051]	0.000349	0.1476443	0.238095238	0.060	0.00000005	43	0.099 [0.21,-0.0067]	5.61E-05	6.65E-02	0.056	0.693
	0.00001	163	0.19 [0.34,0.028]	0.000891	0.0207456	0.02	0.134	0.00001	102	0.17 [0.26,0.076]	2.11E-04	3.76E-04	0.086	0.865
	0.001	810	0.21 [0.32,0.099]	0.002272	0.0002208	<0.001	0.809	0.001	666	0.18 [0.24,0.12]	5.80E-04	3.67E-09	0.052	0.607
	0.05	10578	0.13 [0.18,0.079]	4.46E-03	2.24E-07	<0.001	0.935	0.05	9875	0.088 [0.11,0.065]	9.14E-04	1.30E-13	0.054	0.154
	0.99	43479	0.099 [0.14,0.063]	4.72E-03	9.96E-08	1.43E-03	0.984	0.99	40744	0.08 [0.098,0.063]	1.35E-03	2.16E-19	0.290	0.367

Table 2-9 Comparison of QT and QRS interval GRSAs for SCA

	Alphacutoff	m	ahat	aSE	R2rs	pval	phet
QT downsampled*	5E-08	33	0.377593	0.158003	0.000951	0.016859	0.092462
	0.00001	111	0.289885	0.121231	0.000952	0.016795	0.117796
	0.001	946	0.101754	0.072193	0.000331	0.158693	0.318396
	0.05	12249	0.125393	0.03171	0.002603	7.67E-05	0.200646
	0.99	47969	0.100051	0.025231	0.002617	7.33E-05	0.81905
QT	5E-08	33	0.374628	0.171048	0.000799	0.02851	0.077844
	0.00001	111	0.293087	0.129035	0.000859	0.023124	0.091878
	0.001	946	0.092464	0.072767	0.000269	0.203838	0.306683
	0.05	12247	0.117465	0.030439	0.002479	0.000114	0.117013
	0.99	47961	0.07727	0.022339	0.001992	0.000542	0.903271
QRS	5E-08	18	-0.17299	0.181003	0.000152	0.339205	0.455081
	0.00001	55	-0.07575	0.125286	6.09E-05	0.54541	0.217993
	0.001	714	-0.0224	0.055053	2.76E-05	0.684112	0.219622
	0.05	11349	-0.02904	0.020468	0.000335	0.15598	0.244008
	0.99	47942	-0.02291	0.015889	0.000346	0.149329	0.72195

*metanalysis of QT interval with ~41,000 participants as opposed to ~77,000 participants for the whole study

To overcome differences in power due to differences in GWAS sample size for QT and QRS intervals, we conducted a down-sampled GWAS for QT interval with approximately the same sample size as the QRS GWAS. Across all alpha cutoffs, the ahat estimates for the full and down-sampled QT dataset were remarkably similar, suggesting that limited power is unlikely to account for the difference in association between QT and QRS interval GRS with SCA.

Chapter 3 Association of Mitochondrial DNA levels with Frailty and All-Cause Mortality

3.1 Introduction

Age-related declines in mitochondrial function have long been hypothesized to underlie multiple biological changes that increase vulnerability to multiple disease states, functional and cognitive decline, and ultimately, mortality[44–46]. The mechanisms contributing to age-related mitochondrial functional change encompass multiple domains, including declines in energy (ATP) production/energy reserves[47,48], increased free radical production[49], altered rates of apoptosis and mitophagy, and altered fusion/fission[50]. Alterations in these crucial intracellular processes lead to dysfunctional cells, altered tissues, and increased risk of disease [51–53]. The link between age-related changes in mitochondrial function and altered phenotypes and disease states is bolstered by the observation that mice with deficiency of the proofreading mechanism of the mitochondrial polymerase display a premature aging phenotype[54,55] and that mitochondrial dysfunction is a core component of several neurodegenerative disorders in humans[56–58].

The role of mitochondrial DNA (mtDNA) in aging and late life decline has also been studied, with evidence that mtDNA variants modulate risk of several age-associated diseases[53,56,59–62]. We have previously implicated a specific mitochondrial genetic variant in frailty[63], a clinical syndrome prevalent in older individuals characterized by broad decline in resilience and increased risk for disability and all-cause mortality[64]. The variant was located in the control region (D-loop), which plays a key role in mitochondrial replication, and suggests the possibility of affecting the levels of mitochondrial DNA. We therefore hypothesized that mtDNA copy number, which is a marker of mitochondrial replication and cellular energy reserves, with low levels of mtDNA copy number likely reflecting mitochondrial depletion, is likely to play an important role in the aging process. While the role of mitochondrial depletion in severe disorders, such as MDS (mtDNA depletion syndrome) is well established, its effect on aging and mortality in the general population is less understood. Several studies have examined the correlation between age and mtDNA copy number with often ambiguous and conflicting results[65–68,11]. To address this gap in the literature, we examined mtDNA copy number in two large multi-center prospective studies—the Cardiovascular Health Study (CHS) and the Atherosclerosis Risk in Communities (ARIC) study—in a total of 16,401 samples of European and African descent.

3.2 Methods

3.2.1 Ethics

The ARIC and CHS studies have been approved by the Institutional Review Boards (IRB) of all participating institutions, including the IRBs of the University of Minnesota, Johns Hopkins University, University of North Carolina, University of Mississippi Medical Center, Wake Forest University, University of Pittsburgh, and University of California Davis, and all participants provided written informed consent.

3.2.2 Participants

CHS is a prospective multi-center study comprising of 5,888 older individuals aged 65 years and above (15.69% African American, 42.37% female), drawn from 4 US communities[69,70] with initial enrollment in 1989-90, and follow-up recruitment of a minority cohort comprising 687 participants in 1992-93. Participants were followed by annual telephone interviews and clinic visits through 1998-99 and semi-annual telephone interviews subsequently. Mortality information was obtained via contact with next of kin, death certificates, autopsy and coroner's reports. DNA was extracted by salt precipitation following proteinase K digestion of the buffy coat from whole blood. Only participants self-identifying as white or black were included in this analysis. Participants were included only if they consented to use of their DNA for studies of cardiovascular disease outcomes. DNA used for qPCR assay (see below) came from the first visit the participant entered the study.

ARIC is a prospective study of 15,792 individuals, 45-65 years of age, from 4 different US communities[71]. The first visit was carried out in 1987-89, with four subsequent in-person visits and annual telephone interviews after initial visit. DNA was isolated from whole blood using the Gentra Puregene Blood Kit (Qiagen)[72]. Mortality was tracked via telephone follow-ups, hospitalization records, state records, and the National Death Index. Cause of death was determined using cause of death on the death certificate (ICD-9 code). Only samples with a self-reported race of white or black were included in this analysis. DNA used for array-based genotyping was isolated at different visits, with majority of the samples coming from visit2 (1990-92) (detailed breakdown in Table 3-4).

3.2.3 Frailty and SF-12 metrics

We operationalized frailty in CHS participants as detailed previously by Fried et al.[64]. Briefly, participants were scored on a 0-1 scale (1 being at risk and 0 being not at risk for frailty) for 5

characteristics—slowness, exhaustion, shrinking, weakness, and low activity, and classified as robust (0 characteristics), pre-frail (1 or 2 characteristics), or frail (>=3 characteristics). Frailty was not measured in ARIC. However the SF12v2 questionnaire, one of the most commonly used measures of general health, was administered at visit 5 (2011-13). The SF12 physical and mental component scores (PCS and MCS respectively) are determined from self-reported answers on physical issues, pain, energy levels, and mental wellness[73]. The scores are on a 0-100 scale with higher scores corresponding to higher physical or mental wellness.

3.2.4 MtDNA Copy Number qPCR Assay

mtDNA copy number in the CHS samples was determined using a multiplexed real time quantitative polymerase chain reaction (qPCR) utilizing ABI TaqMan chemistry (Applied Biosystems). Each well consisted of a VIC labeled, primer-limited assay specific to a mitochondrial target (*ND1*) (Assay ID Hs02596873_s1), and a FAM labeled assay specific to a region of the nuclear genome selected for being non-repetitive with no known alternative splicing events (*RPPH1*) (Assay ID Hs03297761_s1). Each sample was run in triplicate on a 384 well plate in a 10µL reaction containing 20ng of DNA. The cycle threshold (Ct) value was determined from the amplification curve for each target by the ABI Viia7 software. A ΔCt value was computed for each well as the difference between the Ct for the *RPPH1* target and the Ct for the *ND1* target, as a measure of mtDNA copy number relative to nuclear DNA copy number. For samples with standard deviation of ΔCt values of the three replicates > 0.5, an outlier replicate was detected and excluded from analysis. If sample ΔCt standard deviation remained >0.5 post replicate exclusion, the sample was excluded completely from further analyses. Replicates with values of Ct for *ND1* >28, Ct for *RPPH1* >5 standard deviations from the mean, or ΔCt value >3 standard deviations from the mean, were removed from each plate. Additionally, we observed a linear increase in ΔCt value by order in which the replicate was pipetted onto the plate. This effect was adjusted for using a linear regression, and ΔCt values corrected for pipetting order were used for all subsequent analyses.

3.2.5 MtDNA Copy Number from Microarray Intensities

13,444 ARIC samples were genotyped on the Affymetrix Genome-Wide Human SNP Array 6.0. Genotypes were called using Birdseed (version 2) as implemented in the Affymetrix Power Tools software[74]**.** In addition to determining genotype calls, the software was used to compute probe intensities for each of the two alleles at every SNP (A and B alleles).

To determine mtDNA copy number, data for 119 mitochondrial SNPs were collected across all samples. For mitochondrial SNPs, the software assumes haploidy and hence all genotype calls are homozygous. At a SNP with genotype call AA, probe intensity corresponding to the A allele is considered the true signal, and probe intensity for B allele is considered background. At each SNP, the overall signal intensity was calculated as the absolute difference of the probe intensities of the two alleles (|A-B|). The median probe intensity difference across all mitochondrial SNPs was taken as a measure of the relative mtDNA copy number for each sample.

Additionally, we generated principal components (PC) on probe intensities for both alleles of a randomly chosen subset of 1,000 autosomal SNPs. PCs generated from these data allow for correction of both technical artifacts (plate and batch effects) and population substructure. The mtDNA copy number was adjusted for the first 20 PCs, age, sex, and collection site using a linear model. Standardized residuals generated from this model were used for all subsequent analyses.

3.2.6 Statistical Analysis

All statistical analyses were performed using R version 3.0.1. For the qPCR based assay, across plate normalization was performed using quantile normalization as implemented in the R package 'qpcrNorm'[75]. Plate layouts used were non-random with respect to race, requiring all analyses post-normalization and post-removal of plate effects to be stratified by race. Mean ΔCt value was calculated per sample and adjusted for age, sex and collection site using a linear regression model. Standardized residuals were used as the measure of mtDNA copy number. Effect estimates are expressed in terms of standard deviation units (sd) of mtDNA copy number. In CHS, this corresponds to ~0.82 ΔCt units following across plate normalization (sd for whites=0.82 [mean=6.64]; sd for blacks=0.83 [mean=6.63]). In ARIC, the raw probe intensities obtained from the array-based method used to determine copy number, cannot be interpreted without adjusting for PCs accounting for plate and batch artifacts.

All analyses were conducted initially in CHS and validated in ARIC. The frailty characteristics were treated as binary variables and overall frailty was treated as an ordered variable (0, 1, 2). The association with mtDNA copy number was determined using a logistic regression model for the individual frailty characteristics, and a proportional odds model for overall frailty. Prevalence ratios for the individual frailty components were estimated using marginal standardization of the logistic models as implemented by the 'prLogisticBootMarg' function in R package 'prLogistic' v1.2[76].

To assess the association of mtDNA copy number with mortality, a Cox proportional-hazards model was used, adjusting for age, sex, and collection site, as the baseline model. A secondary multivariate mortality analysis was run including age, sex, collection site, body mass index (BMI), high-density lipoprotein (HDL), total cholesterol, prevalent hypertension (defined by elevated systolic or diastolic blood pressure, or hypertension medication intake), and smoking status as covariates, and excluding participants with prevalent coronary heart disease (CHD), diabetes, or history of myocardial infarction (MI).

For our analyses, baseline was defined as time at which the blood sample that was used to determine mtDNA copy number was collected. Age, follow-up time, and other variables were adjusted accordingly. Samples for which time of DNA extraction was unavailable were excluded. Quintiles were calculated using residuals from age, sex, collection site (for both cohorts), and PCs (for ARIC) adjusted mtDNA copy number. The hazard ratios from both cohorts were pooled using a random effects, inverse-variance weighted meta-analysis, as implemented by the 'metagen' function in R package 'meta' (version 3.1-2).

3.2.7 Sample Exclusions

In CHS, a total of 996 samples were excluded from the final analysis, primarily due to insufficient amount of DNA to run the assay (442 samples) and concerns about data quality (554 samples). In ARIC, array genotyping data was available on 13,444 of the 15,792 total participants. Further, sample exclusions based on sample quality and relatedness have been previously described[77]. Additionally, samples not self-identifying as either black or white, in either cohort were excluded (39 participants in CHS and 48 in ARIC). Differences between included and excluded participants are available in Table 3-5.

3.3 Results

3.3.1 Sample characteristics

The baseline characteristics of the 4,892 participants (4108 whites, 784 blacks) from the CHS cohort included in the current analysis after sample exclusions and stratified by age-,sex- and collection site- adjusted quintiles, are detailed in Table 3-1 (Also see Table 3-3 for unadjusted quintiles). We observed an inverse association between mtDNA copy number and age at time of DNA collection in both racial groups—a reduction of 0.14 (95% CI, 0.08-0.19, P<0.001) and 0.19 (95% CI, 0.06-0.31, P=0.002) sd over 10 years in whites and blacks, respectively. Additionally, we noted a higher mtDNA copy number in women relative to men, (OR=1.21 for women relative to men, 95% CI, 1.14-1.28, P<0.001) in whites, with a consistent, but not

statistically significant effect in blacks (OR=1.14 for women relative to men, 95% CI, 0.99-1.31, P=0.08).

We used 11,509 samples (9,025 whites, 2,484 blacks) from ARIC to validate our initial findings from CHS (Table 3-1 and 3-3). As in CHS, we observed an inverse association of mtDNA copy number with baseline age with a reduction of 0.11 sd (95% CI, 0.07-0.14, P<0.001) in whites and 0.11 sd (95% CI, 0.04-0.17, P=0.001) in blacks, over a 10 year period, and a significantly higher mtDNA copy number in women relative to men (whites OR=1.52 for women relative to men, 95% CI 1.46-1.59, P<0.001; blacks OR=1.42 for women relative to men, 95% CI 1.31-1.54, P<0.001).

3.3.2 Frailty

In a race-stratified analysis of samples from CHS, we observed a statistically significant association between lower mtDNA copy number and frailty, adjusted for age and sex, in whites (OR 0.91, 95% CI, 0.85-0.97, P=0.005). Furthermore, this association was not driven by any single component of the frailty phenotype, with three out of five frailty characteristics showing statistically significant association with lower mtDNA copy number in whites (Figure 3-1), and a similar trend of association for the remaining characteristics. While we observed this association in whites, we see no association of mtDNA copy number on any of the frailty characteristics in CHS blacks.

While frailty characteristics were not measured in ARIC participants, the latest visit (2011-2013) included the SF12v2 mental component score (MCS) and physical component score PCS. Of the ARIC participants included in our study 4,961 (4,046 whites and 915 blacks) participants were interviewed at visit 5 with a mean MCS of 46.35 in whites and 43.94 in blacks. In white participants from ARIC we observe a significant association between higher PCS, adjusted for age at visit 5, sex, and collection site, and mtDNA copy number, with an increase of 0.51 PCS units per sd unit increase in mtDNA copy number (95% CI, 0.17-0.84, P=0.003). The same model in blacks showed a similar association with an increase of 0.76 PCS units per sd unit increase in mtDNA copy number (95% CI, 0.02-1.50, P=0.04). Secondary analyses adjusting for additional covariates--prevalent diabetes, CHD or hypertension at time of DNA collection—showed the same trend of association between high PCS score and mtDNA copy number with effect estimates of 0.42 PCS units in whites (95% CI, 0.09-0.75, P=0.01), and 0.83 PCS units in blacks (95% CI, 0.11-1.56, P=0.02).

3.3.3 Mortality

A total of 2,961 deaths (60.4% samples) were observed in the CHS participants during 26,770 person-years of follow-up. In an age, sex, and collection site adjusted, race-stratified analysis, we observed a statistically significant association between lower mtDNA copy number and mortality, with overall hazard ratio of 1.39 (95% CI, 1.23-1.58, P<0.001) for the lowest quintile of copy number relative to the highest quintile in whites (Figure 3-2; Model 1 from Table 3-2). A more stringent multivariate model adjusted for age, sex, collection center, BMI, HDL, total cholesterol, prevalent hypertension, and smoking status, and excluding all samples with prevalent CHD, diabetes or previous history of MI, yielded a hazard ratio of 1.33 (95% CI, 1.13-1.56, P<0.001) (Model 2 from Table 3-2). When stratified by sex, we observed no significant difference in the inverse association between mtDNA copy number and mortality in men and women (P for interaction=0.80). As in frailty, we fail to observe a statistically significant association between mtDNA copy number and risk for mortality in CHS blacks. (Table 3-2).

We observed a similar inverse association of mtDNA copy number with mortality in ARIC (3,362 deaths, 188,377 person-years of follow-up), as seen in CHS, with a hazard ratio of 1.63 (95% CI, 1.44-1.84, P<0.001) for the lowest quintile of mtDNA copy number relative to the highest quintile, in whites in an age, sex, and center adjusted analysis (Table 3-2). We also observed a significantly higher risk of mortality in blacks with hazard ratio of 1.47 (95% CI, 1.19-1.81, P<0.001) for the lowest quintile of copy number relative to the highest quintile. In the subsequent multivariate analyses, low copy number remained strongly associated with increased risk for mortality in whites (hazard ratio=1.38, 95% CI, 1.19-1.61, P<0.001). We observe a similar, albeit not statistically significant, inverse association in blacks (hazard ratio=1.25, 95% CI 0.95-1.66, P=0.056).

An inverse-variance weighted meta-analysis of race-stratified results from both cohorts for the age, sex and collection site adjusted effect of the lowest quintile relative to the highest quintile on mortality, yielded an overall hazard ratio of 1.47 (95% CI, 1.33-1.62, P<0.001), with no significant heterogeneity between the subgroups (P=0.26) (Model1 from Figure 3-3). A subsequent meta-analysis of the results from a more stringent multivariate model, gave a meta-analyzed hazard ratio of 1.32 (95% CI, 1.19-1.46, P<0.001) (Model2 from Figure 3-3).

Additionally, we evaluated the associations of mtDNA copy number with cause-specific mortality, and observed a consistent association of low mtDNA copy number in death due to diseases of the circulatory system, respiratory system or neoplasms (Figure 3-4). Heterogeneity

between effect estimates from cause of death subgroups was determined to be non-significant (P=0.21) using a random-effects model.

3.4 Discussion

We demonstrate that low mtDNA copy number is strongly associated with age, sex, and frailty, and an independent predictor of mortality in 16,401 samples from two large multi-ethnic cohorts, even after adjustment for traditional mortality risk factors and exclusion of prevalent disease states associated with high risk of mortality. The secondary analyses excluding participants with prevalent disease states at baseline allows us to eliminate the concern that these conditions lead to altered mtDNA copy number and hence drive the association with mortality (i.e. reverse causation). Furthermore, the fact that we see consistent effect estimates from both cohorts using independent methods of ascertaining the mtDNA copy number demonstrates the robustness of our findings. While the qPCR-based metric is well established in the literature, we believe that the measure derived from >100 mitochondrial markers on the genotyping array is likely to be a more accurate measure of copy number. Also given that majority of modern large-scale genotyping arrays include mitochondrial markers, this measure can be easily generated from other large cohorts with genotyping data.

Our results demonstrating a strong inverse association between age and mtDNA copy number are in line with previous studies that have shown decreased mtDNA copy number with age in different tissue types[67,11]. Recently, Mengel-From and colleagues report a marginal association between high mtDNA copy number, and better health and survival in 1,067 Danish samples[78]. In a much larger sample size from two independent cohorts, we replicate their findings on the protective effect of high mtDNA copy number with respect to survival and increased energy reserves. Additionally, our data indicating a higher mtDNA copy number in women relative to men across all the subgroups might suggest that a mito-protective effect may account for the disparity in life expectancy between men and women.

Frailty has been previously shown to be predictive of both incident disability and mortality[64]. While there has been considerable debate about what drives the onset of frailty, our findings add to the evidence of a role for mitochondria in this process. Given that energy utilization forms a core feature of the phenotype, and low copy number is associated with overall frailty and several of its components, it is not surprising that mtDNA levels might form part of the biological component of the phenotype. While we were unable to assess the frailty phenotype in both cohorts, in ARIC we show a striking association between the physical component score of the SF-12 metric, and mtDNA copy number measured 15-20 years prior. Interestingly, several groups

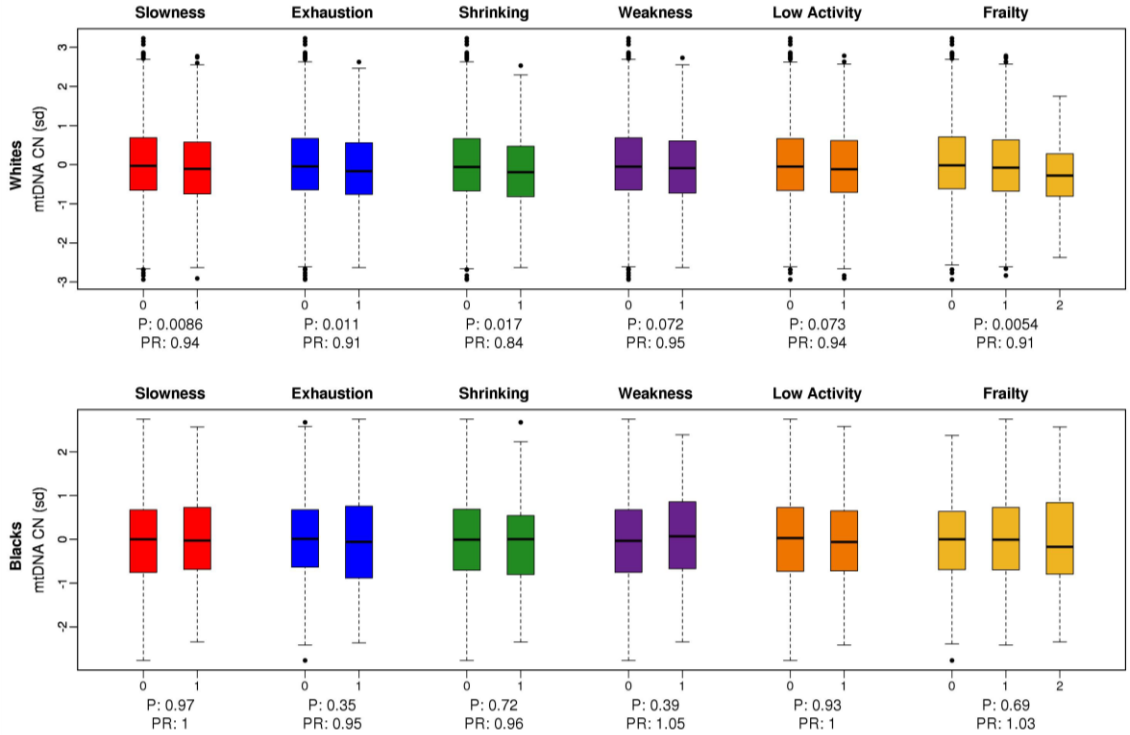
have published a link between mtDNA and cognitive function in the elderly[78–80], however we do not observe any association between mtDNA copy number and the cognitive component of the SF-12 (P for both race groups > 0.4) in ARIC participants.

Several limitations to the study should be noted. First, the mtDNA copy number used in this study is derived from a single time-point, and thus does not take into account the dynamic nature of mtDNA copy number during the life of an individual. Second, while mtDNA copy number has been associated with ATP production rate[11], it is an indirect measure, and further, does not account for acquired mutational burden-a mechanism that forms a critical part of the mitochondrial theory of aging. Third, while we are able to comment on differences between men and women with respect to mtDNA copy number, we cannot do so for race due to technical limitations of study design (see Methods Statistical analysis). This is an important issue, given the significant disparities in health outcomes in the U.S. between whites and blacks[81]. Finally, we were measuring mtDNA copy number in DNA derived from whole blood, which is not necessarily the relevant tissue with respect to many aging-related diseases.

In conclusion, while mitochondria have a central role in energy production, and thus the biological hypothesis for involvement in aging related decline (with energy utilization serving as a core feature of the phenotype) is readily apparent, this has been a neglected area of research with respect to general health outcomes. With recent changes in technology, including the ability to readily assess mtDNA copy number from existing genotyping array data, this is likely to become a rapidly emerging area of research. We highlight that a single, easily implemented, measure of mtDNA copy number, isolated from whole blood decades before the event of interest (death), is predictive of physical function later in life and all-cause mortality.

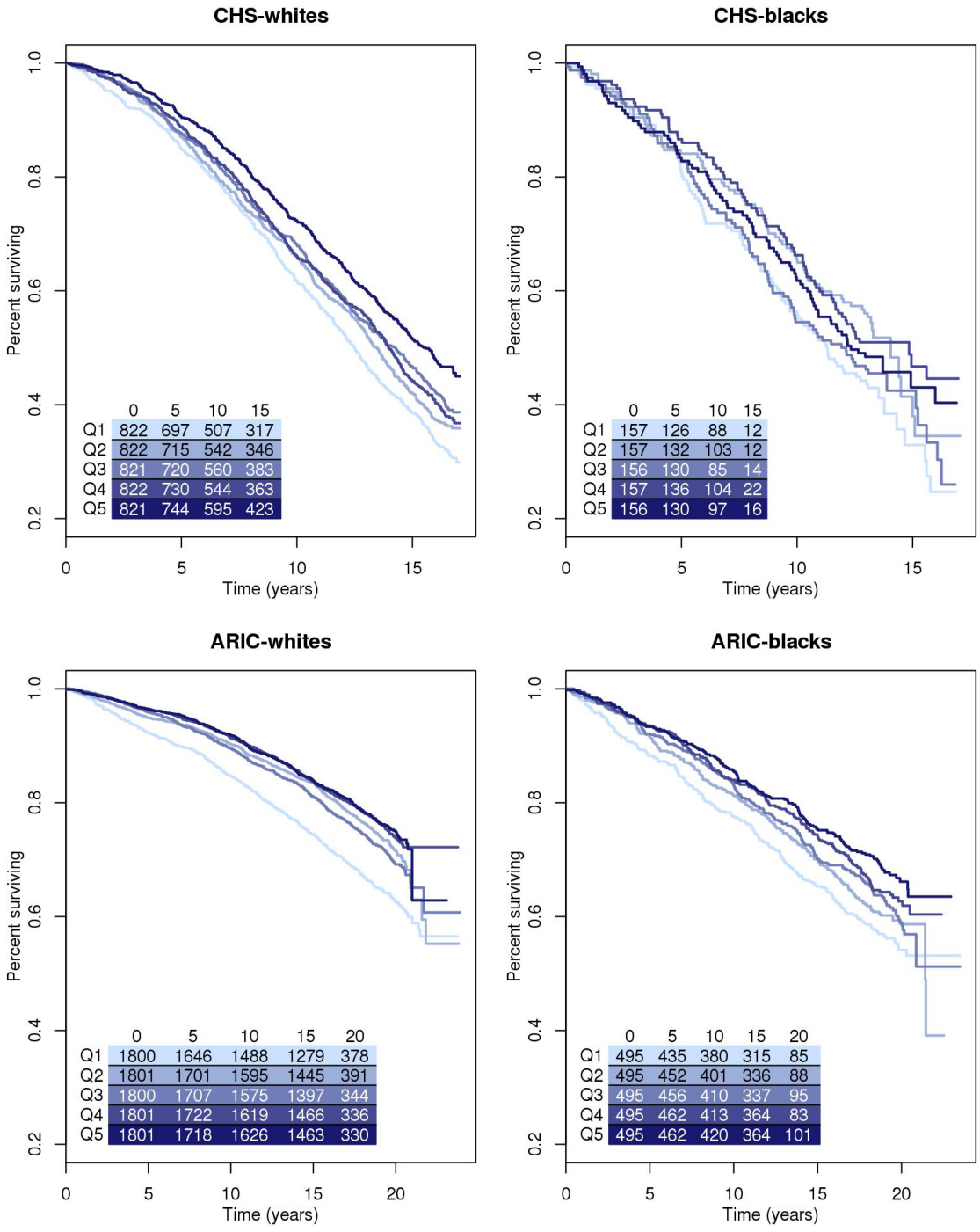
3.5 Figures for Chapter 3

Figure 3-1 Frailty components in CHS



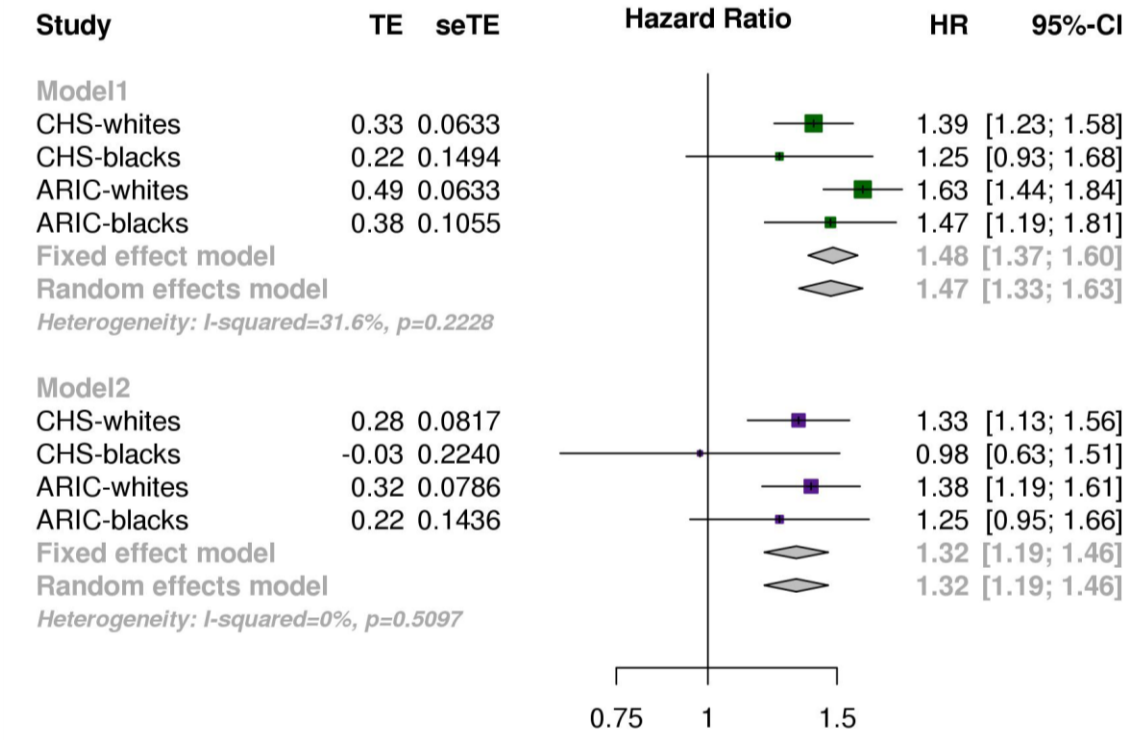
Association between age, sex and collection site adjusted mitochondrial copy number and frailty components in white samples (top panel) and black samples (bottom panel) from CHS. MtDNA copy number is expressed in terms of standard deviation units. Participants were scored as being at risk (1) or not at risk (0) for each characteristic of frailty. Overall frailty was scored in terms of number of characteristics that each participant was at risk for—robust 0 characteristics, pre-frail 1-2 characteristics and frail >2 characteristics. Effect size estimates are reported as prevalence ratios (details in Methods).

Figure 3-2 Kaplan-Meier survival curves by quintiles of mtDNA copy number



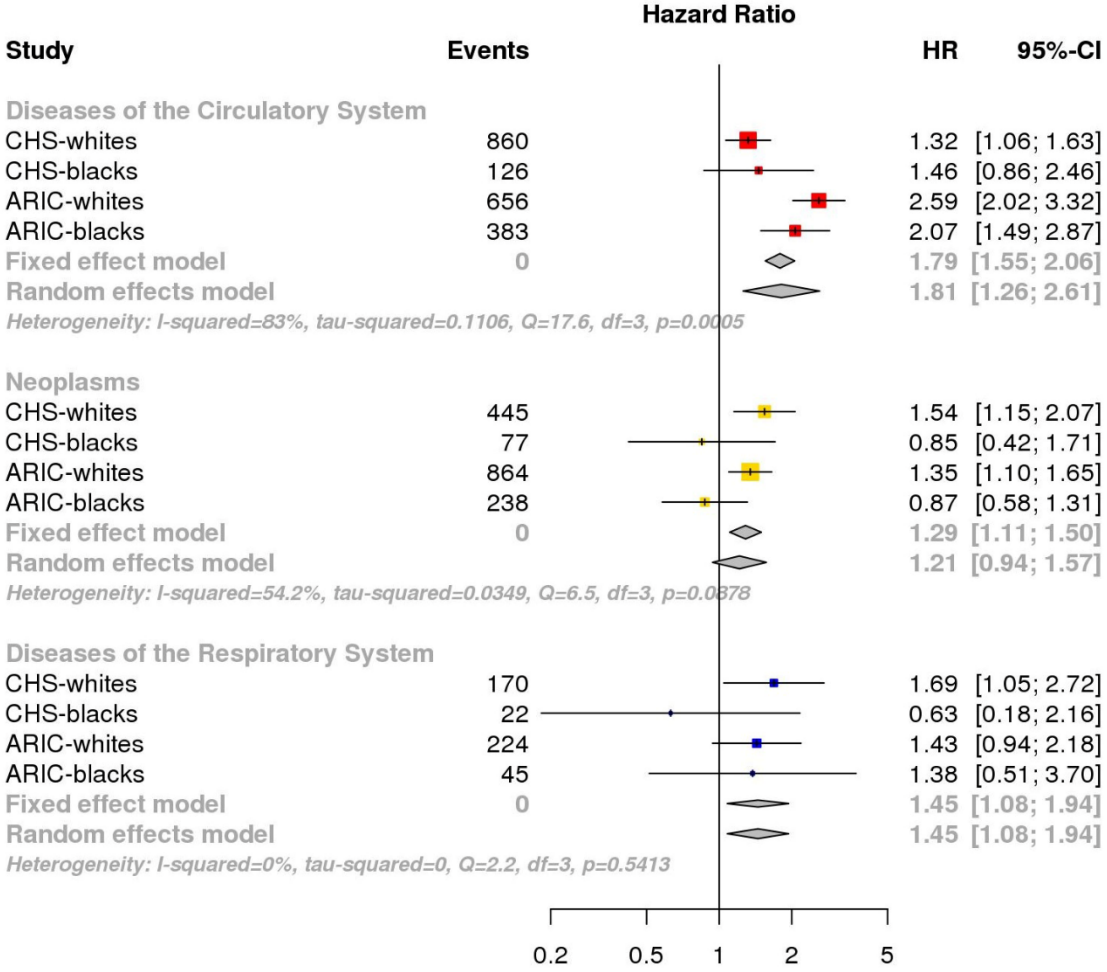
Kaplan-Meier estimates for all-cause mortality by quintile of mtDNA copy number were calculated for both race groups in CHS and ARIC. Table indicates the total number of people in the model at each time point.

Figure 3-3 Meta-analysis of effects mtDNA copy number on mortality



Effects of highest copy quintile of copy number relative to lowest quintile from race stratified analyses in each cohort were meta-analyzed using an inverse-variance weighted approach. Model 1 was the baseline model adjusted for age, sex and collection site. Model 2 was more stringent model that included age, sex, collection site, BMI, HDL, total cholesterol, hypertension, and smoking status as covariates, and excluded samples with prevalent CHD, diabetes or previous history of MI.

Figure 3-4 Effects of mtDNA copy number on cause-specific mortality



Hazards ratio reflect effect of lowest quintile of mtDNA relative to highest quintile on survival. Baseline models were adjusted for age, sex, and collection site. Heterogeneity between estimates of HR for subgroups of cause of death was evaluated using a random effects model. Diseases of the circulatory system were defined by ICD9 codes 390-459, neoplasms by 140-239 and diseases of the respiratory system by 460-519.

3.6 Tables for Chapter 3

Table 3-1 Sample characteristics stratified by age-, sex-, and collection site-adjusted quintiles

CHS-Whites

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
No. of samples	821	822	821	822	822
Age (in yrs)	72.4 ± 5.4	72.9 ± 5.6	72.3 ± 5.3	72.6 ± 5.6	72.3 ± 5.4
ber of males--no (%)	351 (42.7)	380 (46.2)	382 (46.5)	356 (43.3)	347 (42.2)
ow up time (in yrs)	11.6 ±4.66	11.7 ± 4.78	12.06 ± 4.99	12.41 ± 4.93	12.67 ± 5.04
of deaths--no (%)	551 (67.1)	539 (65.6)	492 (60.0)	491 (59.7)	458 (55.7)
age at death (in yrs)	82.85 ± 5.39	83.59 ± 5.42	83.04 ± 5.50	83.88 ± 5.48	83.69 ± 5.72

CHS-Blacks

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
No. of samples	156	157	156	157	157
Age (in yrs)	73.1 ± 6.1	73.0 ± 5.3	72.6 ± 5.6	72.6 ± 6.0	73.1 ± 5.8
ber of males--no (%)	68 (43.6)	59 (37.6)	48 (30.8)	59 (37.6)	68 (43.3)
ow up time (in yrs)	9.853 ± 4.46	10.59 ± 4.28	10.2 ± 4.43	10.81 ± 4.26	10.41 ± 4.39
of deaths--no (%)	96 (61.5)	79 (50.3)	88 (56.4)	81 (51.6)	85 (54.1)
age at death (in yrs)	81.97 ± 6.02	82.13 ± 5.84	81.81 ± 5.79	81.86 ± 6.21	82.08 ± 6.12

ARIC-Whites

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
No. of samples	1804	1804	1805	1804	1805
Age (in yrs)	58.4 ± 6.0	58.0 ± 5.9	58.1 ± 5.9	58.2 ± 6.0	58.1 ± 6.1
ber of males--no (%)	863 (47.8)	844 (46.8)	837 (46.4)	829 (46)	869 (48.1)
ow up time (in yrs)	15.82 ± 4.50	16.69 ± 4.46	16.59 ± 4.84	17 ± 4.83	16.95 ± 5.62
of deaths--no (%)	628 (34.8)	468 (25.9)	496 (27.5)	423 (23.4)	419 (23.2)
age at death (in yrs)	71.71 ± 6.54	72.44 ± 6.54	72.95 ± 6.47	72.32 ± 6.64	72.78 ± 7.05

ARIC-Blacks

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
No. of samples	496	496	497	496	497
Age (in yrs)	57.5 ± 5.9	57.2 ± 6.0	57.2 ± 5.8	57.7 ± 6.0	57.2 ± 6.1
ber of males--no (%)	182 (36.7)	181 (36.5)	191 (38.4)	190 (38.3)	177 (35.6)
ow up time (in yrs)	14.67 ± 5.16	15.46 ± 5.22	15.66 ± 5.43	15.84 ± 5.63	16.12 ± 6.15
of deaths--no (%)	213 (42.9)	193 (38.9)	188 (37.8)	174 (35.1)	158 (31.8)
age at death (in yrs)	68.87 ± 6.48	69.64 ± 6.82	70.37 ± 6.30	70.54 ± 6.66	70.35 ± 7.45

Data are presented as Mean±SD. Quintiles were calculated from age, sex, collection site adjusted mtDNA copy number (details in Methods). 'Pval for trend' is the pvalue for effect of trait on age, sex, collection site standardized mtDNA copy number as a continuous variable.

Table 3-2 Lower mtDNA copy number is associated with increased risk for all-cause mortality

		Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	Overall	Pval for trend
CHS								
Whites	**Model1**	821(551)	822(539)	821(492)	822(491)	822(458)	4109(2532)	
		1.39 (1.23-1.58)	1.29 (1.14-1.46)	1.17 (1.03-1.33)	1.09 (0.96-1.23)	1	0.89 (0.85-0.92)	<0.001
	Model2	509(302)	590(359)	586(314)	624(339)	610(301)	2902(1607)	
		1.33 (1.13-1.56)	1.38 (1.18-1.61)	1.17 (1-1.37)	1.09 (0.93-1.27)	1	0.89 (0.85-0.94)	<0.001
Blacks	**Model1**	156(96)	157(79)	156(88)	157(81)	157(85)	784(429)	
		1.25 (0.93-1.68)	0.92 (0.68-1.26)	1.21 (0.9-1.63)	0.94 (0.7-1.28)	1	0.96 (0.87-1.05)	0.35
	Model2	84(41)	100(48)	99(47)	102(49)	89(44)	469(227)	
		0.98 (0.63-1.51)	0.88 (0.58-1.33)	1.16 (0.76-1.79)	0.88 (0.58-1.34)	1	1.03 (0.9-1.19)	0.65
ARIC								
Whites	**Model1**	1800(629)	1801(467)	1800(496)	1801(423)	1801(416)	9004(2431)	
		1.63 (1.44-1.84)	1.18 (1.03-1.34)	1.28 (1.13-1.46)	1.02 (0.89-1.17)	1	0.84 (0.81-0.88)	<0.001
	Model2	1356(365)	1441(294)	1504(342)	1534(309)	1526(296)	7352(1603)	
		1.38 (1.19-1.61)	1.07 (0.91-1.25)	1.23 (1.05-1.44)	1.03 (0.87-1.2)	1	0.89 (0.85-0.94)	<0.001
Blacks	**Model1**	495(213)	495(195)	495(189)	495(173)	495(156)	2476(926)	
		1.47 (1.19-1.81)	1.36 (1.1-1.67)	1.28 (1.03-1.58)	1.08 (0.87-1.34)	1	0.86 (0.8-0.91)	<0.001
	Model2	313(101)	334(102)	348(102)	339(93)	360(95)	1678(485)	
		1.25 (0.95-1.66)	1.15 (0.87-1.52)	1.09 (0.82-1.44)	0.98 (0.74-1.31)	1	0.91 (0.83-1)	0.04

Numbers of events are presented as total number of subjects, followed by number of events in parentheses, for each quintile. Effect estimates are reported as hazards ratio for each quintile, followed by 95% confidence interval for the estimate of hazards ratio in parentheses.

Model 1 was the baseline model adjusted for age, sex and collection site.

Model 2 was more stringent model that included age, sex, collection site, BMI, HDL, total cholesterol, prevalent hypertension, and smoking status as covariates, and excluded samples with prevalent CHD, diabetes or previous history of MI.

Table 3-3 Sample characteristics stratified by collection site-adjusted quintiles

CHS-Whites

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	Pval
No. of samples	819	822	821	822	822	
Age (in yrs)	73.0 +/- 0.2	72.8 +/- 0.2	72.5 +/- 0.2	72.2 +/- 0.2	71.9 +/- 0.2	<0.001
Number of males--no (%)	407 (49.7)	409 (49.7)	368 (44.8)	328 (39.9)	302 (36.7)	<0.001
Follow up time (in yrs)	11.09 +/- 0.18	11.7 +/- 0.17	12.27 +/- 0.17	12.4 +/- 0.17	12.99 +/- 0.16	<0.001
No. of deaths--no (%)	580 (70.8)	539 (65.7)	494 (60.2)	487 (59.3)	430 (52.3)	<0.001
Mean age at death (in yrs)	83.15 +/- 0.27	83.4 +/- 0.26	83.7 +/- 0.28	83.26 +/- 0.27	83.49 +/- 0.3	0.42

CHS-Blacks

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	Pval
No. of samples	156	157	156	157	157	
Age (in yrs)	74.0 +/- 0.5	73.1 +/- 0.5	72.8 +/- 0.5	72.5 +/- 0.4	72.2 +/- 0.4	0.002
Number of males--no (%)	74 (47.44)	53 (33.76)	52 (33.33)	62 (39.49)	61 (38.85)	0.18
Follow up time (in yrs)	9.86 +/- 0.34	10.19 +/- 0.36	10.15 +/- 0.35	10.98 +/- 0.34	10.67 +/- 0.35	0.03
No. of deaths--no (%)	94 (0.6026)	87 (0.5541)	92 (0.5897)	73 (0.465)	83 (0.5287)	0.07
Mean age at death (in yrs)	82.77 +/- 0.73	82.18 +/- 0.71	81.72 +/- 0.74	81.86 +/- 0.77	81.2 +/- 0.67	0.09

ARIC-Whites

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	Pval
No. of samples	1804	1805	1805	1805	1805	NA
Age (in yrs)	58.85 +/- 0.14	58.23 +/- 0.14	58.13 +/- 0.14	57.84 +/- 0.14	57.56 +/- 0.14	<0.001
Number of males--no (%)	896 (49.7)	853 (47.3)	845 (46.8)	814 (45.1)	835 (46.3)	0.002
Follow up time (in yrs)	15.77+/-0.13	16.66+/-0.12	16.58+/-0.11	17.14+/-0.1	17.03+/-0.11	<0.001
No. of deaths--no (%)	638 (35.4)	497 (27.5)	501 (27.8)	399 (22.1)	400 (22.2)	<0.001
Mean age at death (in yrs)	72.05 +/- 0.31	72.7 +/- 0.34	72.97 +/- 0.32	71.91 +/- 0.38	72.26 +/- 0.38	0.68

ARIC-Blacks

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	Pval
No. of samples	496	497	496	497	497	NA
Age (in yrs)	57.99 +/- 0.28	57.28 +/- 0.27	57.3 +/- 0.27	57.18 +/- 0.27	56.64 +/- 0.26	0.002
Number of males--no (%)	188 (37.9)	178 (35.8)	193 (38.9)	185 (37.2)	177 (35.6)	0.68
Follow up time (in yrs)	14.5+/-0.28	15.44+/-0.25	15.77+/-0.24	15.87+/-0.23	16.32+/-0.23	<0.001
No. of deaths--no (%)	225 (45.4)	195 (39.2)	185 (37.3)	172 (34.6)	150 (30.2)	<0.001
Mean age at death (in yrs)	69.43 +/- 0.53	69.99 +/- 0.54	70.21 +/- 0.52	70.09 +/- 0.61	69.89 +/- 0.63	0.8

Table 3-4 Detailed breakdown of time of DNA collection by cohort

CHS

Whites	**BL**	**BL + 3**
	4,104	4
Blacks	**BL**	**BL + 3**
	194	578

ARIC

Whites	**visit1**	**visit2**	**visit3**	**visit4**
	367	7,201	1,395	32
Blacks	**visit1**	**visit2**	**visit3**	**visit4**
	117	1,922	398	35

CHS DNA was isolated at baseline (BL) for majority of the white participants, and at a subsequent visit 3 years following baseline for majority of the black participants.
ARIC visits were carried out 2-3 years apart—visit1 (1987-89), visit2 (1990-92), visit3 (1993-95), and visit4 (1996-98).

Table 3-5 Sample characteristics stratified by whether participants were included in the study.

CHS

	Included	**Excluded**
No. of samples	4,892	995
Age at visit1 (in yrs)	72.6 +/- 5.5	74.3 +/- 6.0
Number of males--no (%)	2,118 (43.3)	377 (37.9)
Follow up time from visit1 (in yrs)	11.8 +/- 4.9	10.8 +/- 4.9
No. of deaths--no (%)	2,960 (60.5)	726 (72.9)
Prevalent CHD at visit1--no (%)	917 (18.7)	237 (23.8)
Prevalent diabetes at visit1--no (%)	763 (15.6)	190 (19.1)
Prevalent hypertension at visit1--no (%)	2,858 (58.4)	599 (60.2)

ARIC

	Included	**Excluded**
No. of samples	11,509	4,283
Age at baseline (in yrs)	54.0 +/- 5.7	54.4 +/- 5.9
Number of males--no (%)	5,166 (44.9)	1,916 (44.7)
Follow up time from baseline (in yrs)	19.7 +/- 5.1	18.1 +/- 6.6
No. of deaths--no (%)	3,362 (29.2)	1,548 (36.1)
Prevalent CHD at baseline--no (%)	566 (5.0)	200 (4.8)
Prevalent diabetes at baseline--no (%)	1,267 (11.1)	603 (14.3)
Prevalent hypertension at baseline--no (%)	3,832 (33.5)	1,672 (39.3)

Data are presented as Mean +/- standard deviation.

Chapter 4 Mitochondrial DNA copy number is a predictor of cardiovascular disease

4.1 Introduction

Mitochondria play a critical role in energy homeostasis as the primary site of ATP production. Consequently, age-dependent mitochondrial dysfunction that disrupts energy homeostasis is a hallmark of aging process and forms a core component of several chronic conditions, including cardiovascular disease (CVD). This process of energy regulation is dependent on proteins that are translated from genes in the mitochondrial DNA (mtDNA), a 16.7kb circular DNA molecule. mtDNA copy number (mtDNA CN) is a measure of the levels of mtDNA per cell that has been shown to be correlated with mitochondrial enzyme activities and ATP production[82], establishing it as an indirect measure of mitochondrial function. From a practical perspective, mtDNA CN is measured using a low-cost, scalable assay and allows for rapid determination of mitochondrial function in large number of samples. Accordingly we and others, have shown a decline in mtDNA CN with age[78], and have shown mtDNA CN to be a significant predictor of all-cause mortality[83], and chronic kidney disease[84] in data from longitudinal studies.

In addition to the essential role of mitochondria in ATP production, there is increasing evidence to support a role for mitochondria in the initiation and progression of atherosclerotic processes. Atherosclerosis is the primary pathological lesion underlying cardiovascular disease and is initiated by an inflammatory response to damage in the endothelium. There are several lines of evidence supporting an pro-inflammatory role of damaged mtDNA in atherosclerosis. Circulating mtDNA molecules are known to activate an innate immune response following injury[85]. In ApoE knockout mouse models of hyperlipidemia, mtDNA damage has been demonstrated early in the atherosclerotic process before plaque formation with significant correlation between levels of mtDNA damage and extent of atherosclerosis[86]. In humans, white blood cell (WBC) mtDNA damage has been associated with high risk atherosclerotic plaques[86]. Therefore, measuring mtDNA CN in blood cells can capture important information about the atherosclerotic process and the development of CHD. Here, we used two methods to determine mtDNA CN in DNA derived from whole blood in 20,137 individuals from the Atherosclerosis Risk in Communities (ARIC) study, Cardiovascular Health Study (CHS), and the Multiethnic Study of Atherosclerosis (MESA) study. We used these data to explore the association of mtDNA CN with prevalent and incident hard CVD events, and its potential utility as a novel clinical biomarker of CVD.

4.2 Methods

4.2.1 Study Populations

The Atherosclerosis Risk in Communities (ARIC) study is a prospective cohort of 15,792 individuals from 4 US communities[71]. Participants were between 45-65 years of age when at the time of recruitment during the first visit in 1987-89, with three subsequent visits every four years. The final visit was conducted in 2011-13. In addition to study visits, information on hospitalization and health outcomes was collected by annual telephone interviews. For this analysis, baseline was considered time at DNA, and all other variables were adjusted accordingly.

The Cardiovascular Health Study (CHS) is a multicenter prospective study that focuses on studying cardiovascular health in older individuals, aged 65 years and above at baseline[69]. Following the first visit in 1989-90, a second round of recruitment was carried out in 1992-93 to increase minority enrolment in the study. Annual site visits were carried out till 1998-99 alternated with phone interviews every 6 months. Following the last site visit, biannual telephone interviews were used to monitor general health and hospitalization.

The Multiethnic Study of Atherosclerosis (MESA) study consists of 6,814 individuals in the 46-85 year age range who are free of prevalent cardiovascular disease at baseline (Exam1 in 2000-01)[87]. All participants are from one of four racial groups (self-identifying as White Caucasian, Black African American, Chinese-American or Hispanic) and were recruited from 6 centers across the US. There have been five in-clinic Exams, with the latest Exam in 2010-11. Additionally, participants are followed up by telephone interviews every 12months.

4.2.2 CVD risk factors

Traditional CVD risk factors were measured across all three cohorts at the baseline visit. Details for measurements of total cholesterol, high density lipoprotein (HDL), and blood pressure, and hypertension medication use for all three cohorts have been previously described. Diabetes was defined as fasting glucose level >=126mg/dl in accordance with the 2003 ADA Guidelines[88]. Smoking status was assessed by self-report across all three studies.

4.2.3 Measurement of mtDNA CN

mtDNA CN was measured using multiplexed Taqman-based qPCR assay in DNA isolated from whole blood from participants of the CHS study as previously described[83]. In participants from the ARIC and MESA cohorts, mtDNA CN was calculated from probe intensities of mitochondrial SNPs on the Affymetrix Genome-Wide Human SNP Array 6.0[84] using the GENVISIS software (www.genvisis.org). Briefly, this method uses median mitochondrial probe intensity of 25 high quality mitochondrial probes as a raw measure of mtDNA-CN. Data decomposition techniques (surrogate variable analysis in ARIC and

principal component analysis in MESA) were applied to probe intensities of 43,316 autosomal SNPs to adjust for technical artifacts.

For both methods, we used a linear regression model to adjust the effect of age, sex, site, and principal components/surrogate variables (for array-based metric only) on raw mtDNA-CN. Studentized residuals from this model were used as the mtDNA-CN metric for all analyses.

4.2.4 Outcome definition

Analyses for prevalent disease were limited to ARIC and CHS cohorts. In our analyses, we defined prevalent CHD as self- or physician-reported history of MI, or history cardiac procedures (coronary artery bypass grafting [CABG] or coronary artery angioplasty). Prevalent stroke was defined as self- or physician-reported stroke at the baseline visit.

For incident analyses across all three studies, we define coronary heart disease (CHD) as a MI or fatal CHD event. Incident stroke included definite/probable fatal and nonfatal outcomes following event adjudication. Incident CVD events included both incident CHD and stroke.

The event adjudication process in CHS[89], ARIC[90], and MESA[87] has been previously published, and broadly consisted of an expert committee review of hospital records, telephone interviews, and the National Death Registry.

4.2.5 Statistical Analyses

All statistical analyses were performed using R version 3.2.2. A multivariable logistic model was used to model the effect of mtDNA CN on prevalent outcomes (CHD, stroke, and CVD). To assess the effect of mtDNA CN on incident disease (incident and fatal CHD, stroke and CVD) we used Cox regression, excluded participants with prevalent disease at time of DNA collection. The baseline models for both prevalent and incident disease included age, sex, center, total cholesterol levels, HDL cholesterol levels, systolic blood pressure, hypertension medication use, current smoking and diabetes status as covariates. Secondary analyses for incident outcomes were conducted excluding all participants with prevalent CVD, AF and heart failure.

10 year CVD risk was calculated using the Pooled Cohort Equations (PCE) from the 2013 AHA/ACC Guideline on Assessment of Cardiovascular Risk[91]. To incorporate mtDNA CN in the risk score, we used Cox regression to compute race and sex stratified survival curves in participants from ARIC with the mtDNA CN and other covariates included in the PCE. Discriminative ability of the risk scores was compared using the Harrell's C statistic. P-value for difference in the C-statistics was obtained by bootstrapping as implemented by the function 'censboot' in the R package 'boot'[92].

4.3 Results

4.3.1 Sample characteristics

The baseline characteristics of the 20,137 participants from the three participating studies--Cardiovascular Health Study (CHS), the Atherosclerosis Risk in Communities (ARIC) and the Multi-Ethnic Study of Atherosclerosis (MESA), are detailed in Table S1 stratified by age, sex, and collection adjusted quintiles of mtDNA CN. The mean age of participants (55.2% female) across the studies was 62.4 years, with 1,707 participants in CHS and ARIC diagnosed with CVD at baseline. 7,636 participants were on hypertension medication, 2,830 participants had diabetes, and 3,750 participants identified as current smokers at baseline across the three studies. Over a mean follow-up time (±SD) of 13.7(±5.9) years, 3,572 participants had hard CVD events (MI, fatal CHD, and nonfatal and fatal stroke) (2,493 CAD and 1,737 stroke).

4.3.2 Prevalent disease

We examine the association of mtDNA CN with prevalent disease in race-stratified analyses adjusting for age, sex, collection site and traditional CVD risk factors—total cholesterol levels, HDL levels, systolic blood pressure, current smoking status, hypertension medication use, and prevalent diabetes. In data from self-identified white and black participants from ARIC and CHS, mtDNA CN is associated with prevalent CHD in 3 of 4 sub-groups (meta-analysis OR [95% CI]=0.85 [0.79-0.89], P<0.001) (Figure 4-1, Table 4-1). Focusing on prevalent stroke, lower mtDNA is significantly associated with the outcome in the CHS whites (OR [95% CI]=0.76 [0.64-0.90], P=0.002), with a consistent, albeit not statistically significant, direction of effect across the three other subgroups. Combining these phenotypes to examine the effect of mtDNA-CN on prevalent CVD, low mtDNA CN is associated with prevalent CVD (meta-analysis OR [95% CI]=0.87 [0.80-0.89], P<0.001). We also note that in analyses comparing the effect estimates of mtDNA CN on prevalent CVD in white participants from CHS and ARIC, CN has a significantly larger effect on prevalent CVD in CHS compared to ARIC (OR in CHS=0.73 vs ARIC=0.89, P for difference=0.03).

4.3.3 Incident disease

Baseline levels of mtDNA CN are associated with significantly increased risk of CHD and CVD events in participants from the ARIC cohort (Figure 4-2, 4-3). In contrast to the effect of mtDNA CN on prevalent disease in CHS, mtDNA CN is not a significant predictor of CHD in white participants (HR [95% CI]=0.95 [0.90-1.01], P=0.12), but has a significant effect in black participants (HR [95% CI]=0.85 [0.74-0.97], P=0.02) (Figure 4-3).

We then examine the effect of mtDNA CN on incident stroke in these data. While there is a nominal association between mtDNA CN and stroke in the ARIC whites (HR [95% CI]=0.84[0.77-0.92], P<0.001), this effect is not significant in any of the other subgroups tested.

From overall metaanalysis of 18,200 participants without prevalent analysis at baseline, we estimate that participants in the lowest quintile of mtDNA CN have 44% increased risk for CVD relative to participants in the highest quintile of copy number (Figure 4-5).

4.3.4 Age mediated effect of mtDNA CN on CVD

There are significant differences between the estimates of mtDNA CN obtained from ARIC and CHS whites, for both prevalent and incident disease. Given that the difference in the age distribution between the cohorts (mean [range] for ARIC =57.9 years [44.9-74.1] versus CHS=72.6 years [65.0-100.0]) is a major source of variation between the studies, we hypothesize that the effect of CN on disease is attenuated by age. The MESA cohort (mean age [range] =62.8 years [44-84]) includes participants that span the age distribution of both cohorts, and provides an ideal population to test our hypothesis. Since the MESA study design excludes participants with prevalent CVD at baseline, we limit our analysis to incident disease with two subgroups—young (participants younger than 65 years at baseline), and old (participant 65 years or older). While there is no statistical difference in effect estimates from both the subgroups, in both race groups across the three incident outcomes (CHD, stroke and CVD), there is a consistent trend of mtDNA CN having a larger effect in the young subgroup versus the older subgroup (Figure 4-4).

4.3.5 mtDNA CN improves risk discrimination and reclassification in ARIC

The recently released 2013 AHA/ACC Guidelines on the Assessment of Cardiovascular Risk provide coefficients for calculation of 10-year atherosclerotic CVD (ASCVD) risk in white and black participants. To evaluate the potential of mtDNA CN as a clinically informative predictor of CVD, we incorporate mtDNA CN in the 2013 AHA/ACC Pooled Cohort Equations (PCE) and generate a corresponding mtDNA CN+PCE CVD risk score. The effect of mtDNA CN on risk discrimination was evaluated by comparing the area under the receiver operating curves for the PCE, and mtDNA CN + PCE risk scores. In white participants from the ARIC cohort, adding mtDNA CN improved risk discrimination for CVD events, as measured by a change in Harrell's C statistic, by 0.014 units (95% CI from bootstrapping=0.0064-0.018).

Additionally, we assess the effect of the mtDNA CN on risk classification by the two metrics in ARIC participants who meet the risk evaluation criteria (participants free of type 2 diabetes, heart failure or angina at baseline, no previous TIA or CVD history, LDL levels >70mg/dl and <=190mg/dl). In the overall population, adding mtDNA CN to the PCE improves risk reclassification for CVD events, measured by continuous net reclassification index (NRI), by 21.2% (95% CI=10.92-31.52%, P<0.001) (Table 4-4). The guidelines specify 5% and 7.5% as two actionable risk cutoffs for therapy. Accordingly, a categorical NRI that quantifies reclassification across the 0-5%, 5-7.5% and >7.5% risk score categories in this population is 4.02% (95% CI=0.19-7.86%, P=0.04). When we stratify the outcomes as CHD or

stroke events, the reclassification effect for CHD (continuous NRI [95% CI]=26.36% [13.88-38.84%], P<0.001) is significant and larger than the effect on stroke (continuous NRI [95% CI]=11.91% [-4.46-28.19%], P=0.15).

4.4 Discussion

We explore the role of mtDNA CN in cardiovascular disease in 20,137 self-identified white and black participants from the ARIC, CHS and MESA studies. We show that mtDNA CN is inversely associated with prevalent CVD in 15,093 participants from the ARIC and CHS cohorts. We establish mtDNA CN as a predictor of incident CVD in ARIC cohort and in metaanalysis of data from 18,200 participants, without prevalent CVD, from the three studies. Finally, we demonstrate potential for mtDNA CN as a clinical useful predictor of CHD in improving risk prediction and risk reclassification according to the 2013 AHA/ACC Guidelines for ASCVD risk prediction.

While an association between prevalent CHD and mtDNA CN has been previously reported in a study with smaller size[93], to our knowledge this is the first time that mtDNA CN has been shown to be a predictor of incident CVD. Comparing the extremes of our study population, participants in the lowest quintile of copy number have a 44% increased risk of CVD compared to participants in the highest quintile of mtDNA CN. In analyses stratifying the events as CHD or stroke, we observe that the effect of mtDNA CN on incident CHD is larger than the effect on incident stroke (HR for Q1 relative to Q5 for CHD=1.51; HR for stroke=1.28). Biologically, there are two major categories of stroke events, ischemic and hemorrhagic stroke, that have different underlying pathologies that are required to precipitate the event leading to more heterogeneity in the stroke phenotype.

While this study is not designed to answer questions about the mechanism by which levels of mtDNA could affect CVD risk, our results are in line with evidence supporting a role of mitochondria in cardiovascular disease. Whether mtDNA CN directly modulates cardiovascular disease risk or is a biomarker for another CVD risk factor remains to be seen. However, given that all our analyses include traditional CVD risk factors—sex, blood lipids, blood pressure, smoking status, and hypertension medication use—as covariates, supports mtDNA as an independent risk factor for CVD.

Finally, we show that mtDNA CN improves risk discrimination and risk reclassification for CVD outcomes in the ARIC cohort. Risk reclassification are measured by categorical NRI is heavily dependent on the choice of risk categories. To provide an unbiased view of the data, we present the categorical, as well as continuous NRI. Indeed, mtDNA CN shows much higher rate of reclassification with the continuous NRI as compared to the categorical NRI. While the 2013 AHA/ACC Guidelines on the Assessment of Cardiovascular Risk define 5% and 7.5% as two actionable risk cutoffs, these data, taken together with issues of risk overestimation by the Pooled Cohort Equations, might suggest that the risk cutoffs warrant further evaluation.

This study has several strengths and limitations. We use data from three well-characterized prospective studies with a total sample size of 20,137 participants with a mean follow up time of 13.7 years to assess the role of mtDNA CN in cardiovascular disease. However, the marked difference in age distributions between the studies limits our power to detect an association between mtDNA CN and incident CHD in the CHS and MESA studies. The result in ARIC supporting mtDNA CN as a predictor of incident CHD requires replication in another cohort with sufficient sample size of middle-aged adults.

In conclusion, we examined the association between mtDNA CN and cardiovascular disease in 20, 137 participants from three well-characterized longitudinal studies. We show an association between mtDNA CN and prevalent CVD, and establish mtDNA CN as a predictor of incident CVD, that improves risk discrimination and reclassification in participants from the ARIC cohort.

4.5 Figures for Chapter4

Figure 4-1 Lower levels of mtDNA CN are associated with prevalent cardiovascular disease in ARIC and CHS

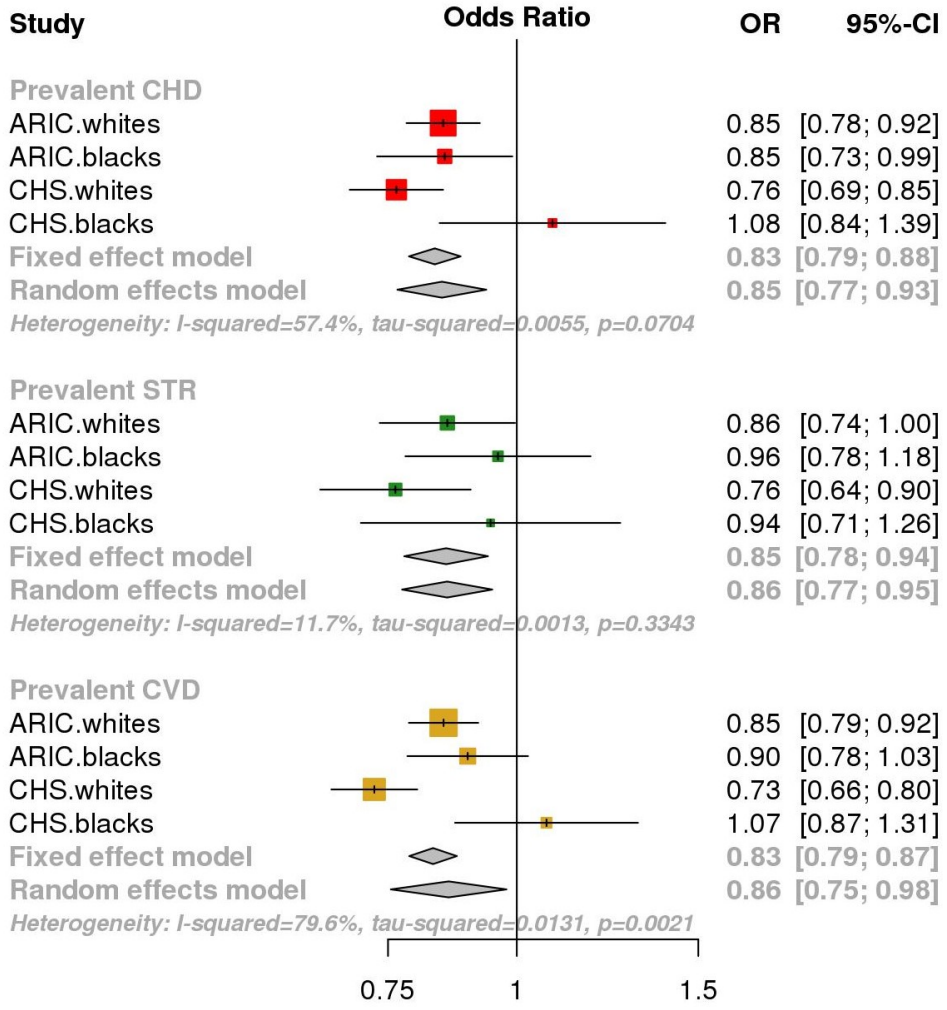


Figure 4-2 Lower mtDNA-CN is associated with higher risk of CVD in the ARIC cohort

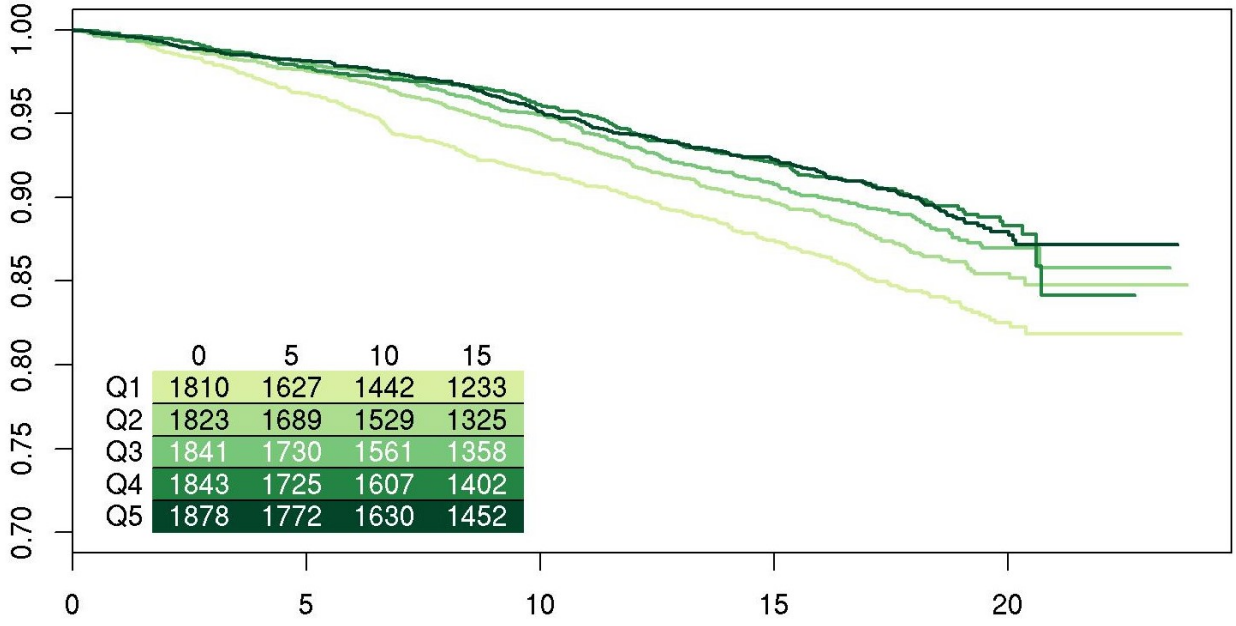


Figure 4-3 Effect of mtDNA CN on incident disease outcomes in ARIC, CHS and MESA cohorts

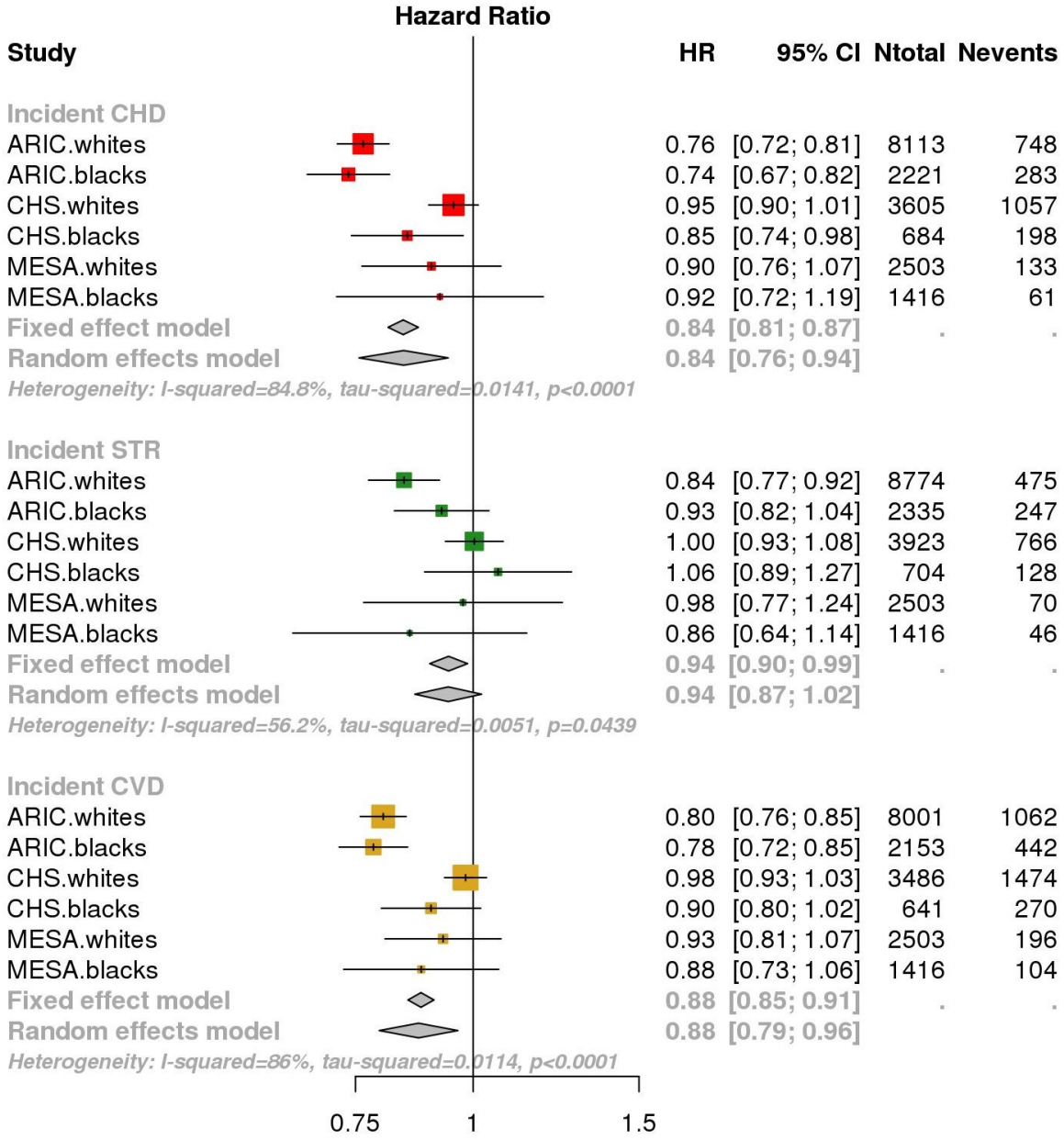


Figure 4-4 Effect of mtDNA CN on incident disease outcomes in MESA cohort stratified by age

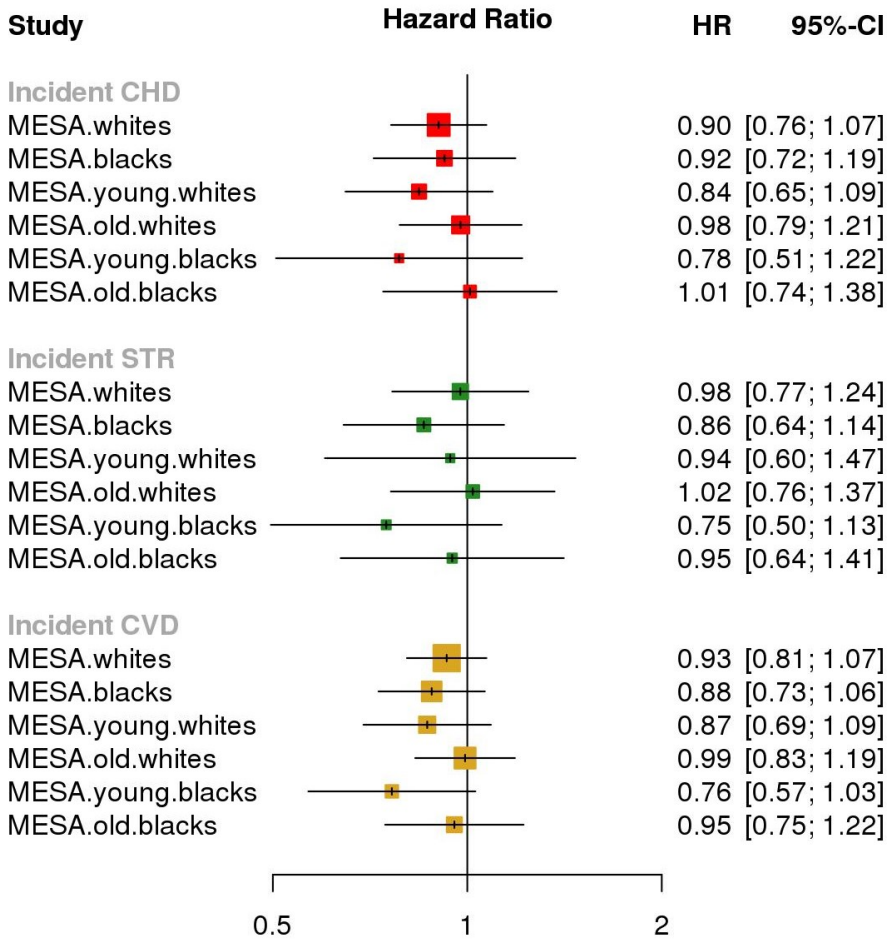
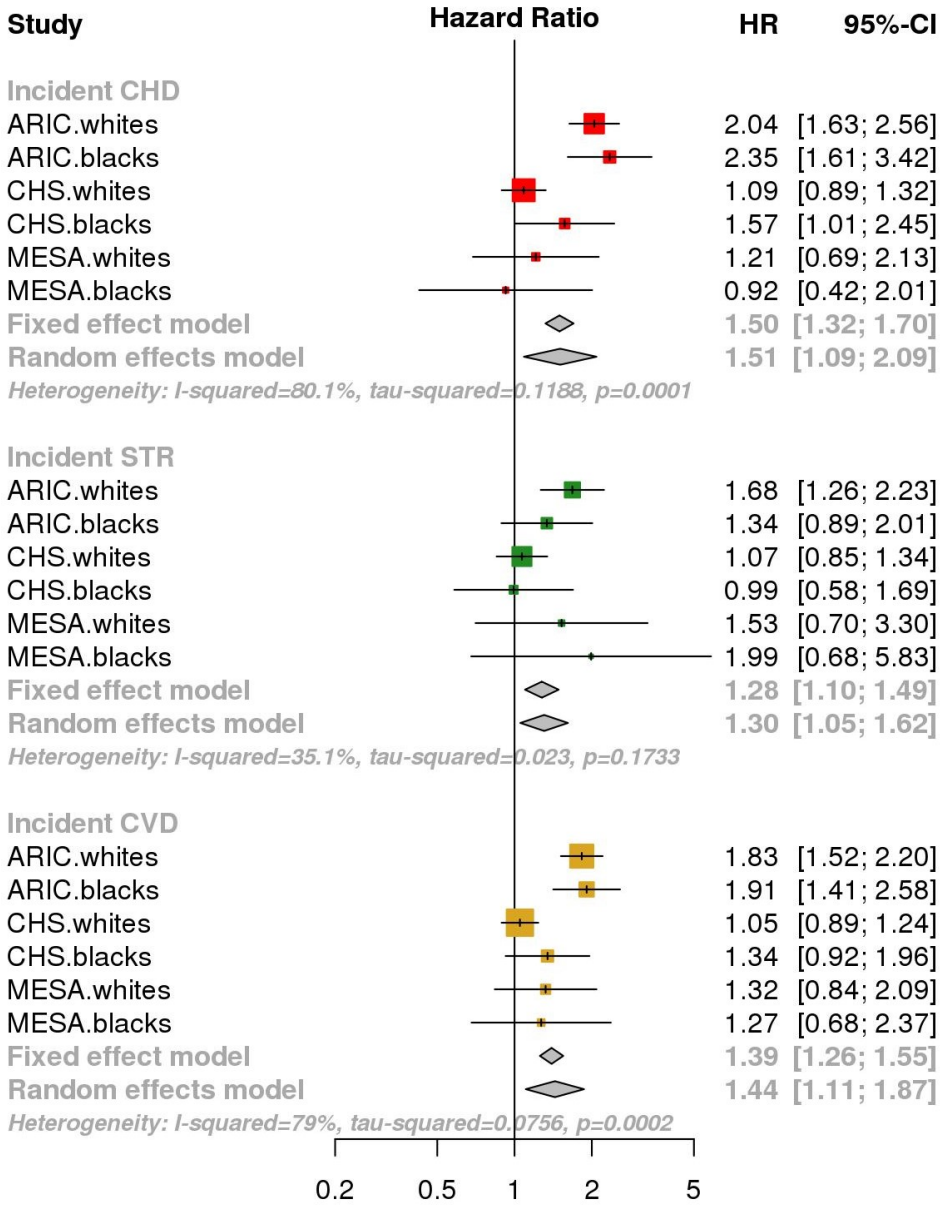


Figure 4-5 Effect of lowest quintile of mtDNA CN (Q1) relative to highest quintile (Q5) for incident disease outcomes



4.6 Tables for Chapter 4

Table 4-1 The effect of mtDNA CN on prevalent disease outcomes in the ARIC and CHS cohorts

	Q1	Q2	Q3	Q4	Q5 (Ref)	Continuous
Prevalent CHD						
ARIC Whites						
OR (95% CI)	1.67 (1.26-2.21)	1.36 (1.02-1.82)	1.53 (1.15-2.05)	1.17 (0.868-1.59)	1	0.848 (0.781-0.92)
N (Nevents)	1755 (185)	1754 (147)	1754 (143)	1754 (110)	1755 (93)	8772 (678)
ARIC Blacks						
OR (95% CI)	1.83 (1.09-3.14)	1.24 (0.715-2.19)	1.2 (0.688-2.12)	1.22 (0.695-2.16)	1	0.849 (0.731-0.991)
N (Nevents)	477 (46)	476 (33)	476 (31)	476 (30)	476 (24)	2381 (164)
CHS Whites						
OR (95% CI)	2.13 (1.55-2.96)	1.42 (1.01-1.99)	1.57 (1.13-2.21)	0.958 (0.661-1.39)	1	0.764 (0.688-0.848)
N (Nevents)	816 (137)	815 (98)	816 (104)	815 (64)	816 (70)	4078 (473)
CHS Blacks						
OR (95% CI)	0.918 (0.433-1.94)	0.471 (0.184-1.12)	0.858 (0.391-1.86)	0.757 (0.346-1.63)	1	1.08 (0.842-1.4)
N (Nevents)	151 (16)	150 (8)	151 (14)	150 (14)	151 (17)	753 (69)
Prevalent Stroke						
ARIC Whites						
OR (95% CI)	1.7 (1.02-2.91)	1.1 (0.623-1.95)	1.31 (0.754-2.31)	0.877 (0.471-1.62)	1	0.855 (0.736-0.998)
N (Nevents)	1755 (48)	1754 (29)	1754 (31)	1754 (20)	1755 (22)	8772 (150)
ARIC Blacks						
OR (95% CI)	1.09 (0.575-2.1)	0.618 (0.293-1.28)	0.932 (0.474-1.84)	0.59 (0.27-1.25)	1	0.948 (0.773-1.18)
N (Nevents)	477 (24)	476 (14)	476 (19)	476 (12)	476 (18)	2381 (87)
CHS Whites						
OR (95% CI)	2.12 (1.18-3.99)	2.48 (1.4-4.62)	2.19 (1.22-4.12)	1.8 (0.972-3.44)	1	0.763 (0.644-0.901)
N (Nevents)	816 (35)	815 (41)	816 (35)	815 (28)	816 (16)	4078 (155)
CHS Blacks						
OR (95% CI)	1.37 (0.573-3.39)	0.947 (0.344-2.53)	0.934 (0.339-2.51)	1.09 (0.424-2.81)	1	0.943 (0.704-1.26)
N (Nevents)	151 (13)	150 (8)	151 (8)	150 (10)	151 (10)	753 (49)
Prevalent CVD						
ARIC Whites						
OR (95% CI)	1.64 (1.27-2.13)	1.27 (0.973-1.67)	1.44 (1.1-1.89)	1.1 (0.83-1.46)	1	0.849 (0.786-0.918)
N (Nevents)	1755 (214)	1754 (164)	1754 (161)	1754 (124)	1755 (111)	8772 (774)
ARIC Blacks						
OR (95% CI)	1.43 (0.923-2.24)	1.03 (0.65-1.65)	1.03 (0.648-1.65)	0.968 (0.602-1.56)	1	0.899 (0.787-1.03)
N (Nevents)	477 (60)	476 (46)	476 (44)	476 (40)	476 (39)	2381 (229)
CHS Whites						
OR (95% CI)	2.35 (1.74-3.19)	1.72 (1.26-2.36)	1.75 (1.28-2.39)	1.06 (0.757-1.49)	1	0.727 (0.66-0.8)
N (Nevents)	816 (167)	815 (132)	816 (130)	815 (82)	816 (81)	4078 (592)
CHS Blacks						
OR (95% CI)	0.939 (0.508-1.73)	0.595 (0.295-1.17)	0.767 (0.397-1.47)	0.831 (0.44-1.56)	1	1.07 (0.871-1.31)
N (Nevents)	151 (26)	150 (16)	151 (20)	150 (23)	151 (27)	753 (112)

Table 4-2 The effect of mtDNA CN on incident disease outcomes in the ARIC and CHS cohorts

	Q1	Q2	Q3	Q4	Q5 (Ref)	Continuous
Incident CHD						
ARIC Whites						
OR (95% CI)	2.02 (1.62-2.54)	1.25 (0.975-1.59)	1.07 (0.824-1.38)	1.09 (0.848-1.41)	1	0.76 (0.713-0.81)
N (Nevents)	1619 (241)	1619 (145)	1618 (118)	1619 (123)	1619 (115)	8094 (742)
ARIC Blacks						
OR (95% CI)	2.34 (1.6-3.43)	1.54 (1.03-2.31)	1.16 (0.756-1.78)	1.05 (0.677-1.61)	1	0.736 (0.665-0.814)
N (Nevents)	444 (90)	443 (63)	443 (46)	443 (43)	444 (39)	2217 (281)
CHS Whites						
OR (95% CI)	1.11 (0.915-1.36)	1.23 (1.01-1.48)	1.08 (0.893-1.32)	1.14 (0.944-1.38)	1	0.953 (0.897-1.01)
N (Nevents)	721 (199)	721 (226)	721 (207)	721 (222)	721 (203)	3605 (1057)
CHS Blacks						
OR (95% CI)	1.56 (1.01-2.42)	1.54 (0.995-2.39)	1.47 (0.924-2.32)	0.842 (0.505-1.4)	1	0.851 (0.742-0.976)
N (Nevents)	137 (48)	137 (49)	136 (40)	137 (26)	137 (35)	684 (198)
Incident Stroke						
ARIC Whites						
OR (95% CI)	1.71 (1.29-2.28)	0.947 (0.688-1.3)	1.26 (0.934-1.71)	1.14 (0.833-1.55)	1	0.843 (0.772-0.919)
N (Nevents)	1725 (137)	1724 (77)	1724 (96)	1724 (85)	1725 (75)	8622 (470)
ARIC Blacks						
OR (95% CI)	1.41 (0.941-2.1)	1.22 (0.811-1.84)	1.07 (0.701-1.64)	1.03 (0.672-1.56)	1	0.928 (0.826-1.04)
N (Nevents)	459 (60)	459 (53)	458 (45)	459 (46)	459 (41)	2294 (245)
CHS Whites						
OR (95% CI)	1.08 (0.856-1.35)	1.04 (0.828-1.31)	1.13 (0.903-1.41)	1.14 (0.913-1.42)	1	1 (0.934-1.08)
N (Nevents)	785 (148)	784 (145)	785 (160)	784 (166)	785 (147)	3923 (766)
CHS Blacks						
OR (95% CI)	0.945 (0.554-1.61)	0.675 (0.389-1.17)	0.91 (0.532-1.55)	0.766 (0.444-1.32)	1	1.06 (0.888-1.27)
N (Nevents)	141 (26)	141 (23)	140 (26)	141 (24)	141 (29)	704 (128)
Incident CVD						
ARIC Whites						
OR (95% CI)	1.8 (1.49-2.17)	1.11 (0.901-1.36)	1.14 (0.926-1.4)	1.1 (0.893-1.35)	1	0.801 (0.757-0.848)
N (Nevents)	1600 (315)	1599 (197)	1600 (190)	1599 (185)	1600 (172)	7998 (1059)
ARIC Blacks						
OR (95% CI)	1.94 (1.44-2.62)	1.52 (1.12-2.07)	1.17 (0.844-1.62)	0.939 (0.668-1.32)	1	0.782 (0.719-0.85)
N (Nevents)	431 (125)	430 (103)	430 (79)	430 (67)	431 (67)	2152 (441)
CHS Whites						
OR (95% CI)	1.07 (0.912-1.27)	1.11 (0.947-1.31)	1.05 (0.896-1.24)	1.08 (0.917-1.27)	1	0.982 (0.932-1.03)
N (Nevents)	698 (285)	697 (298)	697 (296)	697 (303)	697 (292)	3486 (1474)
CHS Blacks						
OR (95% CI)	1.34 (0.926-1.95)	1.08 (0.739-1.57)	1.2 (0.819-1.77)	0.776 (0.512-1.18)	1	0.902 (0.798-1.02)
N (Nevents)	129 (64)	128 (59)	128 (55)	128 (40)	128 (52)	641 (270)

Table 4-3 Change in C statistic with mtDNA CN over base model

	Base Model	Base Model + mtDNA CN
All		
Cstatistic (95% CI)	0.756 (0.732-0.774)	0.768 (0.745-0.785)
DeltaC statistic (95% CI)		0.0123 (0.00639-0.0177)
Whites		
Cstatistic (95% CI)	0.748 (0.725-0.768)	0.762 (0.738-0.782)
DeltaC statistic (95% CI)		0.0142 (0.00508-0.0209)
Blacks		
Cstatistic (95% CI)	0.743 (0.716-0.771)	0.754 (0.724-0.778)
DeltaC statistic (95% CI)		0.0108 (-0.00419-0.0197)

Base model includes age, sex, collection center, total cholesterol, HDL, systolic blood pressure, current smoking status, hypertension medication use, and diabetes status as covariates.

Table 4-4 Net reclassification index (NRI) in ARIC participants comparing risk score with and without mtDNA CN

	CHD	STR	CVD
All			
NRI(Categorical) [95% CI], Model1	0.0529 [0.0168 - 0.0891] ; p-value: 0.00412	-0.0344 [-0.0873 - 0.0185] ; p-value: 0.20255	0.0212 [-0.01 - 0.0525] ; p-value: 0.18257
NRI(Categorical) [95% CI], Model2	0.073 [0.028 - 0.1179] ; p-value: 0.00146	-0.0188 [-0.0811 - 0.0436] ; p-value: 0.55541	0.0402 [0.0019 - 0.0786] ; p-value: 0.03967
NRI(Continuous) [95% CI]	0.2636 [0.1388 - 0.3884] ; p-value: 3e-05	0.1191 [-0.0436 - 0.2819] ; p-value: 0.15142	0.2122 [0.1092 - 0.3152] ; p-value: 5e-05
IDI [95% CI]	0.0158 [0.0099 - 0.0217] ; p-value: 0	0.0046 [-8e-04 - 0.01] ; p-value: 0.09267	0.0121 [0.0077 - 0.0165] ; p-value: 0
Whites			
NRI(Categorical) [95% CI], Model1	0.0568 [0.0141 - 0.0995] ; p-value: 0.00917	-0.0333 [-0.101 - 0.0345] ; p-value: 0.33545	0.0267 [-0.0107 - 0.0641] ; p-value: 0.16201
NRI(Categorical) [95% CI], Model2	0.064 [0.0107 - 0.1174] ; p-value: 0.01859	-0.0208 [-0.0993 - 0.0578] ; p-value: 0.60445	0.0358 [-0.01 - 0.0816] ; p-value: 0.12549
NRI(Continuous) [95% CI]	0.2776 [0.135 - 0.4201] ; p-value: 0.00014	0.1865 [-0.0102 - 0.3832] ; p-value: 0.06315	0.2397 [0.12 - 0.3593] ; p-value: 9e-05
IDI [95% CI]	0.0152 [0.0098 - 0.0205] ; p-value: 0	0.0056 [-3e-04 - 0.0115] ; p-value: 0.06389	0.0119 [0.0077 - 0.0162] ; p-value: 0
Blacks			
NRI(Categorical) [95% CI], Model1	0.0402 [-0.0267 - 0.1071] ; p-value: 0.23879	-0.0383 [-0.1207 - 0.0442] ; p-value: 0.36318	0.0053 [-0.0516 - 0.0623] ; p-value: 0.85392
NRI(Categorical) [95% CI], Model2	0.1062 [0.0248 - 0.1876] ; p-value: 0.01058	-0.0077 [-0.1095 - 0.0942] ; p-value: 0.88288	0.0588 [-0.0115 - 0.1292] ; p-value: 0.10121
NRI(Continuous) [95% CI]	0.2306 [-0.0275 - 0.4887] ; p-value: 0.07997	-0.006 [-0.2923 - 0.2804] ; p-value: 0.9675	0.1515 [-0.051 - 0.354] ; p-value: 0.14254
IDI [95% CI]	0.0176 [-3e-04 - 0.0355] ; p-value: 0.05459	0.002 [-0.0092 - 0.0132] ; p-value: 0.72997	0.0123 [5e-04 - 0.0241] ; p-value: 0.0406

Model1: NRI with 7.5% risk cutoff

Model2: NRI with 5% and 7.5% risk cutoff

Table 4-5 Sample characteristics for particpants from ARIC, CHS and MESA studies

ARIC--whites

	Q1	Q2	Q3	Q4	Q5	P trend
n	1788	1788	1788	1788	1788	
Age, mean(SD)	58.2 (0.1)	58.1 (0.1)	58.1 (0.1)	58 (0.1)	58.2 (0.1)	0.96
Sex,male, n(%)	825 (46.1)	861 (48.2)	832 (46.5)	834 (46.6)	841 (47)	0.93
Follow up time, mean(SD)	15.96 (0.13)	16.54 (0.12)	16.62 (0.11)	16.91 (0.11)	17.22 (0.1)	<0.001
CVD Risk factors						
Prevalent diabetes, n(%)	300 (16.8)	250 (14)	181 (10.1)	170 (9.51)	160 (8.95)	<0.001
Systolic blood pressure, mean(SD)	121.1 (0.44)	120.6 (0.43)	120.7 (0.43)	120 (0.41)	119.9 (0.42)	0.02
Hypertension medication, n(%)	603 (33.7)	549 (30.7)	507 (28.4)	473 (26.5)	449 (25.1)	<0.001
Current smoker, n(%)	573 (32)	432 (24.2)	350 (19.6)	338 (18.9)	277 (15.5)	<0.001
HDL, mean(SD)	47.07 (0.38)	48.63 (0.41)	49.74 (0.4)	49.88 (0.4)	50.75 (0.41)	<0.001
Total cholesterol, mean(SD)	208.7 (0.96)	209.3 (0.9)	208.6 (0.93)	209.8 (0.88)	211.1 (0.95)	0.18
CHD						
Prevalent CHD, n(%)	186 (10.4)	146 (8.17)	144 (8.05)	111 (6.21)	93 (5.2)	<0.001
Incident CHD, n(%)	240 (13.4)	149 (8.33)	119 (6.66)	128 (7.16)	122 (6.82)	<0.001
10-year incident CHD, n(%)	140 (7.83)	79 (4.42)	61 (3.41)	52 (2.91)	47 (2.63)	<0.001
Stroke						
Prevalent Stroke, n(%)	48 (2.68)	29 (1.62)	32 (1.79)	21 (1.17)	22 (1.23)	<0.001
Incident Stroke, n(%)	138 (7.72)	79 (4.42)	93 (5.2)	88 (4.92)	78 (4.36)	<0.001
10-year incident Stroke, n(%)	71 (3.97)	40 (2.24)	42 (2.35)	43 (2.4)	28 (1.57)	<0.001
CVD						
Prevalent CVD, n(%)	215 (12)	163 (9.12)	161 (9)	124 (6.94)	111 (6.21)	<0.001
Incident CVD, n(%)	312 (17.4)	202 (11.3)	188 (10.5)	194 (10.9)	184 (10.3)	<0.001
10-year incident CVD, n(%)	182 (10.2)	110 (6.15)	87 (4.87)	86 (4.81)	70 (3.91)	<0.001

ARIC--blacks

	Q1	Q2	Q3	Q4	Q5	Pval
n	486	486	485	486	486	
Age, mean(SD)	57.3 (0.3)	57.5 (0.3)	57.5 (0.3)	56.9 (0.3)	57.3 (0.3)	0.99
Sex,male, n(%)	174 (35.8)	191 (39.3)	175 (36.1)	187 (38.5)	167 (34.4)	0.97
Follow up time, mean(SD)	14.71 (0.27)	15.54 (0.25)	15.31 (0.25)	16.22 (0.24)	16.26 (0.24)	<0.001
CVD Risk factors						
Prevalent diabetes, n(%)	142 (29.2)	128 (26.3)	142 (29.3)	138 (28.4)	98 (20.2)	0.004
Systolic blood pressure, mear	131.1 (1.1)	129.9 (1)	127.5 (0.95)	128.7 (0.94)	127.6 (0.93)	0.002
Hypertension medication, n(%	256 (52.7)	256 (52.7)	243 (50.1)	240 (49.4)	236 (48.6)	0.14
Current smoker, n(%)	171 (35.2)	135 (27.8)	132 (27.2)	109 (22.4)	106 (21.8)	<0.001
HDL, mean(SD)	53.07 (0.77)	54.03 (0.8)	52.42 (0.77)	52.63 (0.77)	54.95 (0.83)	0.31
Total cholesterol, mean(SD)	209.7 (2)	208.7 (1.8)	210.1 (1.9)	212.3 (1.9)	214.4 (1.9)	0.08
CHD						
Prevalent CHD, n(%)	46 (9.47)	35 (7.2)	30 (6.19)	32 (6.58)	24 (4.94)	0.01
Incident CHD, n(%)	90 (18.5)	64 (13.2)	49 (10.1)	44 (9.05)	39 (8.02)	<0.001
10-year incident CHD, n(%)	55 (11.3)	34 (7)	30 (6.19)	24 (4.94)	21 (4.32)	<0.001
Stroke						
Prevalent Stroke, n(%)	24 (4.94)	15 (3.09)	19 (3.92)	12 (2.47)	20 (4.12)	0.39
Incident Stroke, n(%)	59 (12.1)	54 (11.1)	48 (9.9)	47 (9.67)	41 (8.44)	0.07
10-year incident Stroke, n(%)	40 (8.23)	33 (6.79)	28 (5.77)	26 (5.35)	21 (4.32)	0.03
CVD						
Prevalent CVD, n(%)	60 (12.3)	47 (9.67)	42 (8.66)	41 (8.44)	39 (8.02)	0.03
Incident CVD, n(%)	124 (25.5)	103 (21.2)	83 (17.1)	69 (14.2)	67 (13.8)	<0.001
10-year incident CVD, n(%)	83 (17.1)	57 (11.7)	50 (10.3)	41 (8.44)	36 (7.41)	<0.001

CHS--whites

	Q1	Q2	Q3	Q4	Q5	Pval
n	816	815	816	815	816	
Age, mean(SD)	72.4 (0.2)	72.9 (0.2)	72.3 (0.2)	72.6 (0.2)	72.3 (0.2)	
Sex,male, n(%)	350 (42.9)	379 (46.5)	380 (46.6)	352 (43.2)	345 (42.3)	
Follow up time, mean(SD)	12.86 (0.23)	13.04 (0.23)	13.71 (0.23)	13.98 (0.23)	14.47 (0.22)	<0.001
CVD Risk factors						
Prevalent diabetes, n(%)	152 (18.6)	101 (12.4)	97 (11.9)	115 (14.1)	110 (13.5)	0.02
Systolic blood pressure, mean(SD)	135 (0.78)	136 (0.74)	135.4 (0.75)	135 (0.72)	134.8 (0.75)	0.52
Hypertension medication, n(%)	384 (47.1)	372 (45.6)	370 (45.3)	323 (39.6)	340 (41.7)	0.002
Current smoker, n(%)	99 (12.1)	84 (10.3)	102 (12.5)	91 (11.2)	77 (9.44)	0.25
HDL, mean(SD)	52.59 (0.55)	53.65 (0.55)	53.76 (0.57)	53.92 (0.53)	54.14 (0.55)	0.28
Total cholesterol, mean(SD)	209.9 (1.4)	211.6 (1.3)	212.7 (1.4)	211.6 (1.3)	214.4 (1.3)	0.10
CHD						
Prevalent CHD, n(%)	137 (16.8)	98 (12)	104 (12.7)	64 (7.85)	70 (8.58)	<0.001
Incident CHD, n(%)	186 (22.8)	221 (27.1)	210 (25.7)	229 (28.1)	211 (25.9)	0.95
10-year incident CHD, n(%)	90 (11)	112 (13.7)	116 (14.2)	118 (14.5)	101 (12.4)	0.86
Stroke						
Prevalent Stroke, n(%)	35 (4.29)	41 (5.03)	35 (4.29)	28 (3.44)	16 (1.96)	<0.001
Incident Stroke, n(%)	147 (18)	141 (17.3)	161 (19.7)	167 (20.5)	150 (18.4)	0.40
10-year incident Stroke, n(%)	83 (10.2)	88 (10.8)	96 (11.8)	100 (12.3)	83 (10.2)	0.58
CVD						
Prevalent CVD, n(%)	167 (20.5)	132 (16.2)	130 (15.9)	82 (10.1)	81 (9.93)	<0.001
Incident CVD, n(%)	265 (32.5)	287 (35.2)	295 (36.2)	318 (39)	309 (37.9)	0.50
10-year incident CVD, n(%)	134 (16.4)	160 (19.6)	169 (20.7)	179 (22)	152 (18.6)	0.86

CHS--blacks

	Q1	Q2	Q3	Q4	Q5	Pval
n	151	150	151	150	151	
Age, mean(SD)	72.9 (0.4)	72.8 (0.5)	72.6 (0.4)	72.5 (0.4)	73 (0.5)	
Sex,male, n(%)	66 (43.7)	57 (38)	46 (30.5)	54 (36)	66 (43.7)	
Follow up time, mean(SD)	11.32 (0.49)	12.98 (0.51)	11.85 (0.5)	12.97 (0.51)	12.31 (0.51)	0.31
CVD Risk factors						
Prevalent diabetes, n(%)	44 (29.1)	34 (22.7)	31 (20.5)	37 (24.7)	39 (25.8)	0.80
Systolic blood pressure, mear	141.4 (1.8)	141.7 (1.9)	144.4 (1.9)	140.7 (1.9)	140.7 (1.8)	0.57
Hypertension medication, n(%	94 (62.3)	90 (60)	102 (67.5)	95 (63.3)	88 (58.3)	0.46
Current smoker, n(%)	24 (15.9)	25 (16.7)	26 (17.2)	23 (15.3)	23 (15.2)	0.89
HDL, mean(SD)	57.32 (1.2)	58.44 (1.3)	57.42 (1.2)	57.58 (1.2)	57.53 (1.3)	0.93
Total cholesterol, mean(SD)	211 (3.1)	211.3 (3.4)	208.1 (3.2)	206 (3)	209.4 (3.3)	0.37
CHD						
Prevalent CHD, n(%)	16 (10.6)	8 (5.33)	14 (9.27)	14 (9.33)	17 (11.3)	0.53
Incident CHD, n(%)	47 (31.1)	50 (33.3)	40 (26.5)	28 (18.7)	33 (21.9)	0.01
10-year incident CHD, n(%)	24 (15.9)	33 (22)	24 (15.9)	16 (10.7)	16 (10.6)	0.06
Stroke						
Prevalent Stroke, n(%)	13 (8.61)	8 (5.33)	8 (5.3)	10 (6.67)	10 (6.62)	0.80
Incident Stroke, n(%)	26 (17.2)	22 (14.7)	27 (17.9)	24 (16)	29 (19.2)	0.50
10-year incident Stroke, n(%)	16 (10.6)	13 (8.67)	17 (11.3)	18 (12)	19 (12.6)	0.62
CVD						
Prevalent CVD, n(%)	26 (17.2)	16 (10.7)	20 (13.2)	23 (15.3)	27 (17.9)	0.52
Incident CVD, n(%)	63 (41.7)	60 (40)	58 (38.4)	39 (26)	50 (33.1)	0.04
10-year incident CVD, n(%)	32 (21.2)	39 (26)	35 (23.2)	24 (16)	29 (19.2)	0.28

MESA--whites						
	Q1	Q2	Q3	Q4	Q5	Pval
n	503	502	503	502	503	
Age, mean(SD)	63.2 (0.5)	62.8 (0.5)	62.2 (0.4)	62.5 (0.4)	63.2 (0.5)	
Sex,male, n(%)	240 (47.7)	245 (48.8)	240 (47.7)	225 (44.8)	245 (48.7)	
Follow up time, mean(SD)	10.38 (0.11)	10.58 (0.11)	10.77 (0.1)	10.93 (0.089)	10.8 (0.096)	0.001
CVD Risk factors						
Prevalent diabetes, n(%)	26 (5.17)	25 (4.98)	24 (4.77)	26 (5.18)	32 (6.36)	0.19
Systolic blood pressure, mean(SD)	125.1 (0.88)	124.1 (0.9)	123.9 (0.92)	122.4 (0.92)	123 (0.96)	0.10
Hypertension medication, n(%)	174 (34.6)	177 (35.3)	178 (35.4)	161 (32.1)	149 (29.6)	0.07
Current smoker, n(%)	68 (13.5)	52 (10.4)	57 (11.3)	61 (12.2)	51 (10.1)	0.52
HDL, mean(SD)	52.19 (0.7)	50.59 (0.68)	51.49 (0.68)	53.64 (0.73)	54.48 (0.72)	0.003
Total cholesterol, mean(SD)	195.2 (1.6)	197 (1.6)	194.8 (1.6)	197.2 (1.5)	196.3 (1.5)	0.52
CHD						
Incident CHD, n(%)	27 (5.37)	27 (5.38)	34 (6.76)	22 (4.38)	23 (4.57)	0.32
10-year incident CHD, n(%)	22 (4.37)	22 (4.38)	30 (5.96)	16 (3.19)	18 (3.58)	0.19
Stroke						
Incident Stroke, n(%)	16 (3.18)	14 (2.79)	11 (2.19)	20 (3.98)	11 (2.19)	0.99
10-year incident Stroke, n(%)	15 (2.98)	12 (2.39)	10 (1.99)	17 (3.39)	10 (1.99)	0.91
CVD						
Incident CVD, n(%)	42 (8.35)	38 (7.57)	44 (8.75)	40 (7.97)	34 (6.76)	0.50
10-year incident CVD, n(%)	36 (7.16)	32 (6.37)	39 (7.75)	31 (6.18)	28 (5.57)	0.30

MESA--blacks						
	Q1	Q2	Q3	Q4	Q5	Pval
n	285	285	284	285	285	
Age, mean(SD)	62.7 (0.6)	63.5 (0.6)	62.3 (0.6)	63.2 (0.6)	62.3 (0.6)	
Sex,male, n(%)	124 (43.5)	141 (49.5)	125 (44)	125 (43.9)	130 (45.6)	
Follow up time, mean(SD)	3484 (68)	3577 (60)	3451 (73)	3701 (58)	3708 (61)	0.01
CVD Risk factors						
Prevalent diabetes, n(%)	53 (18.6)	44 (15.4)	43 (15.1)	48 (16.8)	40 (14)	0.49
Systolic blood pressure, mear	131.9 (1.3)	132.2 (1.3)	134.3 (1.3)	131.7 (1.3)	132.4 (1.4)	0.95
Hypertension medication, n(%	149 (52.3)	155 (54.4)	145 (51.1)	141 (49.5)	137 (48.1)	0.09
Current smoker, n(%)	62 (21.8)	52 (18.2)	45 (15.8)	58 (20.4)	47 (16.5)	0.07
HDL, mean(SD)	52.11 (0.86)	50.8 (0.87)	52.07 (0.89)	53.92 (1)	53.32 (0.91)	0.30
Total cholesterol, mean(SD)	188 (2.2)	190.6 (2)	188.9 (2.4)	190.8 (2.2)	188.5 (2.1)	0.94
CHD						
Incident CHD, n(%)	12 (4.21)	15 (5.26)	11 (3.87)	9 (3.16)	14 (4.91)	0.63
10-year incident CHD, n(%)	12 (4.21)	15 (5.26)	9 (3.17)	9 (3.16)	11 (3.86)	0.35
Stroke						
Incident Stroke, n(%)	10 (3.51)	13 (4.56)	9 (3.17)	9 (3.16)	5 (1.75)	0.29
10-year incident Stroke, n(%)	9 (3.16)	12 (4.21)	9 (3.17)	9 (3.16)	4 (1.4)	0.29
CVD						
Incident CVD, n(%)	22 (7.72)	28 (9.82)	19 (6.69)	17 (5.96)	18 (6.32)	0.21
10-year incident CVD, n(%)	21 (7.37)	27 (9.47)	17 (5.99)	17 (5.96)	14 (4.91)	0.11

Table 4-6 Sensitivity analysis examining the effect of mtDNA CN on incident disease outcomes in the ARIC and CHS cohorts, after excluding participants with heart failure, TIA or angina at baseline

	Q1	Q2	Q3	Q4	Q5	Continuous
Incident CHD						
ARIC Whites						
OR (95% CI)	1.99 (1.57-2.52)	1.18 (0.907-1.52)	1.01 (0.772-1.33)	1.11 (0.855-1.45)	1	0.771 (0.72-0.825)
N (Nevents)	1513 (216)	1513 (127)	1512 (103)	1513 (113)	1513 (105)	7564 (664)
ARIC Blacks						
OR (95% CI)	2.88 (1.88-4.41)	1.86 (1.18-2.93)	1.39 (0.863-2.25)	1.3 (0.805-2.11)	1	0.718 (0.646-0.798)
N (Nevents)	396 (81)	395 (55)	395 (40)	395 (39)	395 (29)	1976 (244)
CHS Whites						
OR (95% CI)	1.07 (0.842-1.35)	1.18 (0.944-1.48)	1 (0.796-1.27)	1.17 (0.939-1.47)	1	0.967 (0.899-1.04)
N (Nevents)	527 (138)	526 (162)	526 (139)	526 (164)	527 (145)	2632 (748)
CHS Blacks						
OR (95% CI)	1.81 (1.03-3.18)	1.69 (0.97-2.96)	1.29 (0.697-2.38)	0.721 (0.365-1.43)	1	0.765 (0.637-0.92)
N (Nevents)	92 (33)	92 (35)	91 (23)	92 (15)	92 (20)	459 (126)
Incident Stroke						
ARIC Whites						
OR (95% CI)	1.68 (1.22-2.31)	0.91 (0.635-1.3)	1.28 (0.917-1.8)	1.15 (0.812-1.62)	1	0.841 (0.762-0.927)
N (Nevents)	1513 (109)	1513 (59)	1512 (77)	1513 (68)	1513 (61)	7564 (374)
ARIC Blacks						
OR (95% CI)	1.38 (0.882-2.15)	1.31 (0.834-2.06)	1.06 (0.656-1.7)	1.03 (0.645-1.65)	1	0.932 (0.821-1.06)
N (Nevents)	396 (49)	395 (45)	395 (36)	395 (37)	395 (33)	1976 (200)
CHS Whites						
OR (95% CI)	0.993 (0.749-1.32)	0.912 (0.684-1.22)	1.08 (0.822-1.42)	1.04 (0.787-1.36)	1	1.04 (0.949-1.13)
N (Nevents)	527 (97)	526 (89)	526 (106)	526 (105)	527 (99)	2632 (496)
CHS Blacks						
OR (95% CI)	0.866 (0.414-1.81)	0.895 (0.445-1.8)	1.1 (0.531-2.29)	0.988 (0.479-2.04)	1	1.11 (0.875-1.41)
N (Nevents)	92 (14)	92 (17)	91 (15)	92 (16)	92 (15)	459 (77)
Incident CVD						
ARIC Whites						
OR (95% CI)	1.82 (1.5-2.22)	1.04 (0.841-1.3)	1.12 (0.905-1.39)	1.14 (0.917-1.41)	1	0.804 (0.758-0.852)
N (Nevents)	1513 (294)	1513 (173)	1512 (173)	1513 (174)	1513 (159)	7564 (973)
ARIC Blacks						
OR (95% CI)	2.26 (1.64-3.13)	1.74 (1.24-2.44)	1.28 (0.898-1.84)	1.08 (0.749-1.56)	1	0.762 (0.699-0.832)
N (Nevents)	396 (118)	395 (93)	395 (69)	395 (61)	395 (54)	1976 (395)
CHS Whites						
OR (95% CI)	1.04 (0.861-1.26)	1.06 (0.882-1.28)	0.997 (0.825-1.2)	1.05 (0.873-1.27)	1	0.989 (0.93-1.05)
N (Nevents)	527 (210)	526 (222)	526 (214)	526 (228)	527 (218)	2632 (1092)
CHS Blacks						
OR (95% CI)	1.37 (0.869-2.14)	1.22 (0.776-1.91)	1.12 (0.69-1.83)	0.77 (0.46-1.29)	1	0.883 (0.759-1.03)
N (Nevents)	92 (45)	92 (45)	91 (34)	92 (27)	92 (34)	459 (185)

Table 4-7 Change in C statistic with mtDNA CN over base model in sex stratified analyses

	Base Model	Base Model + mtDNA CN
All		
Men		
Cstatistic (95% CI)	0.717 (0.69-0.737)	0.734 (0.714-0.753)
DeltaC statistic (95% CI)		0.0179 (0.00118-0.0293)
Women		
Cstatistic (95% CI)	0.78 (0.755-0.808)	0.789 (0.764-0.815)
DeltaC statistic (95% CI)		0.00895 (-0.000555-0.0164)
Whites		
Men		
Cstatistic (95% CI)	0.706 (0.671-0.728)	0.728 (0.697-0.75)
DeltaC statistic (95% CI)		0.0221 (0.00505-0.039)
Women		
Cstatistic (95% CI)	0.766 (0.723-0.792)	0.779 (0.737-0.807)
DeltaC statistic (95% CI)		0.0125 (0.00114-0.0242)
Blacks		
Men		
Cstatistic (95% CI)	0.714 (0.658-0.764)	0.725 (0.664-0.774)
DeltaC statistic (95% CI)		0.0113 (-0.0228-0.0235)
Women		
Cstatistic (95% CI)	0.759 (0.706-0.794)	0.768 (0.714-0.803)
DeltaC statistic (95% CI)		0.0095 (-0.01-0.0188)

References

1. Mozaffarian, D. *et al.* Heart Disease and Stroke Statistics—2015 Update A Report From the American Heart Association. *Circulation* CIR.0000000000000152 (2014). doi:10.1161/CIR.0000000000000152
2. Brown, M. S. & Goldstein, J. L. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc. Natl. Acad. Sci.* **96,** 11041–11048 (1999).
3. Goldstein, J. L., Dana, S. E., Faust, J. R., Beaudet, A. L. & Brown, M. S. Role of lysosomal acid lipase in the metabolism of plasma low density lipoprotein. Observations in cultured fibroblasts from a patient with cholesteryl ester storage disease. *J. Biol. Chem.* **250,** 8487–8495 (1975).
4. Gil, G., Faust, J. R., Chin, D. J., Goldstein, J. L. & Brown, M. S. Membrane-bound domain of HMG CoA reductase is required for sterol-enhanced degradation of the enzyme. *Cell* **41,** 249–258 (1985).
5. Wang, D. G. *et al.* Large-Scale Identification, Mapping, and Genotyping of Single-Nucleotide Polymorphisms in the Human Genome. *Science* **280,** 1077–1082 (1998).
6. The CARDIoGRAMplusC4D Consortium *et al.* Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat. Genet.* (2012). doi:10.1038/ng.2480
7. Cohen, J. C., Boerwinkle, E., Mosley, T. H., Jr & Hobbs, H. H. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N. Engl. J. Med.* **354,** 1264–1272 (2006).
8. Natarajan, P. & Kathiresan, S. PCSK9 Inhibitors. *Cell* **165,** 1037 (2016).
9. Jouven, X., Desnos, M., Guerot, C. & Ducimetière, P. Predicting sudden death in the population: the Paris Prospective Study I. *Circulation* **99,** 1978–1983 (1999).

10. Spooner, P. M. *et al.* Sudden Cardiac Death, Genes, and Arrhythmogenesis Consideration of New Population and Mechanistic Approaches From a National Heart, Lung, and Blood Institute Workshop, Part I*. *Circulation* **103,** 2361–2364 (2001).
11. Friedlander, Y. *et al.* Sudden death and myocardial infarction in first degree relatives as predictors of primary cardiac arrest. *Atherosclerosis* **162,** 211–216 (2002).
12. Ackerman, M. J. Genotype-phenotype relationships in congenital long QT syndrome. *J. Electrocardiol.* **38,** 64–68 (2005).
13. Antzelevitch, C. *et al.* Loss-of-Function Mutations in the Cardiac Calcium Channel Underlie a New Clinical Entity Characterized by ST-Segment Elevation, Short QT Intervals, and Sudden Cardiac Death. *Circulation* **115,** 442–449 (2007).
14. Mohamed, U., Napolitano, C. & Priori, S. G. Molecular and electrophysiological bases of catecholaminergic polymorphic ventricular tachycardia. *J. Cardiovasc. Electrophysiol.* **18,** 791–797 (2007).
15. Jansen, H., Samani, N. J. & Schunkert, H. Mendelian randomization studies in coronary artery disease. *Eur. Heart J.* **35,** 1917–1924 (2014).
16. Smith, G. D. & Ebrahim, S. Mendelian randomization: prospects, potentials, and limitations. *Int. J. Epidemiol.* **33,** 30–42 (2004).
17. Voight, B. F. *et al.* Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet* **380,** 572–580 (2012).
18. Willer, C. J., Li, Y. & Abecasis, G. R. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* **26,** 2190–2191 (2010).
19. Chanda, P., Huang, H., Arking, D. E. & Bader, J. S. Fast Association Tests for Genes with FAST. *PLoS ONE* **8,** e68585 (2013).
20. Johnson, T. *gtx: Genetics ToolboX*. (2013).

21. Greco M, F. D., Minelli, C., Sheehan, N. A. & Thompson, J. R. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. *Stat. Med.* **34,** 2926–2940 (2015).
22. Albert, C. M., McGovern, B. A., Newell, J. B. & Ruskin, J. N. Sex Differences in Cardiac Arrest Survivors. *Circulation* **93,** 1170–1176 (1996).
23. Ellinor, P. T. *et al.* Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat. Genet.* **44,** 670–675 (2012).
24. Arking, D. E. *et al.* Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization. *Nat. Genet.* **46,** 826–836 (2014).
25. Sotoodehnia, N. *et al.* Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. *Nat. Genet.* **42,** 1068–1076 (2010).
26. den Hoed, M. *et al.* Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. *Nat. Genet.* **advance online publication,** (2013).
27. Chen, L. Y. *et al.* Atrial fibrillation and the risk of sudden cardiac death: the atherosclerosis risk in communities study and cardiovascular health study. *JAMA Intern. Med.* **173,** 29–35 (2013).
28. Straus, S. M. J. M. *et al.* Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. *J. Am. Coll. Cardiol.* **47,** 362–367 (2006).
29. Nelson, C. P. *et al.* Genetically Determined Height and Coronary Artery Disease. *N. Engl. J. Med.* **372,** 1608–1618 (2015).
30. Cupples, L. A., Gagnon, D. R. & Kannel, W. B. Long- and short-term risk of sudden coronary death. *Circulation* **85,** I11–18 (1992).
31. Arking, D. E. *et al.* Identification of a Sudden Cardiac Death Susceptibility Locus at 2q24.2 through Genome-Wide Association in European Ancestry Individuals. *PLoS Genet* **7,** e1002158 (2011).

32. Noseworthy, P. A. *et al.* Common Genetic Variants, QT Interval, and Sudden Cardiac Death in a Finnish Population-Based Study / Clinical Perspective. *Circ. Cardiovasc. Genet.* **4,** 305 –311 (2011).

33. Albert, C. M. *et al.* Common Variants in Cardiac Ion Channel Genes Are Associated With Sudden Cardiac Death. *Circ. Arrhythm. Electrophysiol.* **3,** 222–229 (2010).

34. Jouven, X., Zureik, M., Desnos, M., Guérot, C. & Ducimetière, P. Resting heart rate as a predictive risk factor for sudden death in middle-aged men. *Cardiovasc. Res.* **50,** 373–378 (2001).

35. Laukkanen, J. A. *et al.* T-wave inversion, QRS duration, and QRS/T angle as electrocardiographic predictors of the risk for sudden cardiac death. *Am. J. Cardiol.* **113,** 1178–1183 (2014).

36. Adabag, S. *et al.* Obesity related risk of sudden cardiac death in the atherosclerosis risk in communities study. *Heart Br. Card. Soc.* **101,** 215–221 (2015).

37. Locke, A. E. *et al.* Genetic studies of body mass index yield new insights for obesity biology. *Nature* **518,** 197–206 (2015).

38. Shungin, D. *et al.* New genetic loci link adipose and insulin biology to body fat distribution. *Nature* **518,** 187–196 (2015).

39. Chen LY, Sotoodehnia N, Bůžková P & et al. Atrial fibrillation and the risk of sudden cardiac death: The atherosclerosis risk in communities study and cardiovascular health study. *JAMA Intern. Med.* **173,** 29–35 (2013).

40. Jouven, X. *et al.* Diabetes, glucose level, and risk of sudden cardiac death. *Eur. Heart J.* **26,** 2142–2147 (2005).

41. Rautaharju, P. M. *et al.* Sex differences in the evolution of the electrocardiographic QT interval with age. *Can. J. Cardiol.* **8,** 690–695 (1992).

42. Makkar RR, Fromm BS, Steinman RT, Meissner MD & Lehmann MH. Female gender as a risk factor for torsades de pointes associated with cardiovascular drugs. *JAMA* **270,** 2590–2597 (1993).

43. ERNSTER, L., LOW, H., NORDENBRAND, K. & ERNSTER, B. A component promoting oxidative phosphorylation, released from mitochondria during aging. *Exp. Cell Res.* **9,** 348–349 (1955).

44. Honjo, I., Ozawa, K., Kitamura, O., Sakai, A. & Ohsawa, T. Rapid change of phospholipid in pancreas mitochondria during aging. *J. Biochem. (Tokyo)* **64,** 311–320 (1968).

45. Harman, D. The biologic clock: the mitochondria? *J. Am. Geriatr. Soc.* **20,** 145–147 (1972).

46. Conley, K. E., Jubrias, S. A. & Esselman, P. C. Oxidative capacity and ageing in human muscle. *J. Physiol.* **526 Pt 1,** 203–210 (2000).

47. Petersen, K. F. *et al.* Mitochondrial Dysfunction in the Elderly: Possible Role in Insulin Resistance. *Science* **300,** 1140–1142 (2003).

48. Cui, H., Kong, Y. & Zhang, H. Oxidative Stress, Mitochondrial Dysfunction, and Aging. *J. Signal Transduct.* **2012,** (2011).

49. Chan, D. C. Mitochondria: Dynamic Organelles in Disease, Aging, and Development. *Cell* **125,** 1241–1252 (2006).

50. Kabekkodu, S. P. *et al.* Mitochondrial DNA variation analysis in cervical cancer. *Mitochondrion* (2013). doi:10.1016/j.mito.2013.07.001

51. Liou, C.-W. *et al.* Mitochondrial DNA coding and control region variants as genetic risk factors for type 2 diabetes. *Diabetes* **61,** 2642–2651 (2012).

52. Kenney, M. C. *et al.* Mitochondrial DNA haplogroups confer differences in risk for age-related macular degeneration: a case control study. *BMC Med. Genet.* **14,** 4 (2013).

53. Kujoth, G. C. *et al.* Mitochondrial DNA Mutations, Oxidative Stress, and Apoptosis in Mammalian Aging. *Science* **309,** 481–484 (2005).

54. Trifunovic, A. *et al.* Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* **429,** 417–423 (2004).

55. Lezi, E. & Swerdlow, R. H. Mitochondria in neurodegeneration. *Adv. Exp. Med. Biol.* **942,** 269–286 (2012).

56. DuBoff, B., Feany, M. & Götz, J. Why size matters – balancing mitochondrial dynamics in Alzheimer's disease. *Trends Neurosci.* **36,** 325–335 (2013).

57. Gaweda-Walerych, K. & Zekanowski, C. Integrated pathways of parkin control over mitochondrial maintenance - relevance to Parkinson's disease pathogenesis. *Acta Neurobiol. Exp. (Warsz.)* **73,** 199–224 (2013).

58. van der Walt, J. M. *et al.* Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. *Am. J. Hum. Genet.* **72,** 804–811 (2003).

59. van der Walt, J. M. *et al.* Analysis of European mitochondrial haplogroups with Alzheimer disease risk. *Neurosci. Lett.* **365,** 28–32 (2004).

60. Hudson, G. *et al.* Two-stage association study and meta-analysis of mitochondrial DNA variants in Parkinson disease. *Neurology* **80,** 2042–2048 (2013).

61. Thyagarajan, B. *et al.* Mitochondrial DNA copy number is associated with breast cancer risk. *PloS One* **8,** e65968 (2013).

62. Moore, A. Z. *et al.* Polymorphisms in the Mitochondrial DNA Control Region and Frailty in Older Adults. *PLoS ONE* **5,** e11069 (2010).

63. Fried, L. P. *et al.* Frailty in older adults: evidence for a phenotype. *J. Gerontol. A. Biol. Sci. Med. Sci.* **56,** M146–156 (2001).

64. Clay Montier, L. L., Deng, J. J. & Bai, Y. Number matters: control of mammalian mitochondrial DNA copy number. *J. Genet. Genomics Yi Chuan Xue Bao* **36,** 125–131 (2009).

65. Liu, J. C. *et al.* Oxidative Stress-related Alteration of the Copy Number of Mitochondrial DNA in Human Leukocytes. *Free Radic. Res.* **37,** 1307–1317 (2003).

66. Welle, S. *et al.* Reduced amount of mitochondrial DNA in aged human muscle. *J. Appl. Physiol.* **94,** 1479 –1484 (2003).

67. Hebert, S. L., Lanza, I. R. & Nair, K. S. Mitochondrial DNA alterations and reduced mitochondrial function in aging. *Mech. Ageing Dev.* **131,** 451–462 (2010).

68. Short, K. R. *et al.* Decline in skeletal muscle mitochondrial function with aging in humans. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 5618 –5623 (2005).

69. Fried, L. P. *et al.* The cardiovascular health study: Design and rationale. *Ann. Epidemiol.* **1,** 263–276 (1991).

70. Psaty, B. M. *et al.* Methods of assessing prevalent cardiovascular disease in the Cardiovascular Health Study. *Ann. Epidemiol.* **5,** 270–277 (1995).

71. The ARIC Investigators. The Atherosclerosis Risk in Communities (ARIC) Study: Design and Objectives. *Am. J. Epidemiol.* **129,** 687–702 (1989).

72. ARIC Carotid MRI Study Investigators. Atheroschlerosis Risk in Communities Carotid MRI Study, Manual 7 DNA Determinations.

73. Fleishman, J. A., Selim, A. J. & Kazis, L. E. Deriving SF-12v2 physical and mental health summary scores: a comparison of different scoring algorithms. *Qual. Life Res. Int. J. Qual. Life Asp. Treat. Care Rehabil.* **19,** 231–241 (2010).

74. Korn, J. M. *et al.* Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs. *Nat. Genet.* **40,** 1253–1260 (2008).

75. Mar, J. C. *et al.* Data-driven normalization strategies for high-throughput quantitative RT-PCR. *BMC Bioinformatics* **10,** 110 (2009).

76. Amorim, R. O. and L. D. *prLogistic: Estimation of Prevalence Ratios using Logistic Models*. (2013).

77. Arking, D. E. *et al.* Genome-Wide Association Study Identifies GPC5 as a Novel Genetic Locus Protective against Sudden Cardiac Arrest. *PLoS ONE* **5,** e9879 (2010).

78. Mengel-From, J. *et al.* Mitochondrial DNA copy number in peripheral blood cells declines with age and is associated with general health among elderly. *Hum. Genet.* **133,** 1149–1159 (2014).

79. Lee, J.-W., Park, K. D., Im, J.-A., Kim, M. Y. & Lee, D.-C. Mitochondrial DNA copy number in peripheral blood is associated with cognitive function in apparently healthy elderly women. *Clin. Chim. Acta* **411,** 592–596 (2010).

80. Kim, M.-Y., Lee, J.-W., Kang, H.-C., Kim, E. & Lee, D.-C. Leukocyte mitochondrial DNA (mtDNA) content is associated with depression in old women. *Arch. Gerontol. Geriatr.* **53,** e218–221 (2011).

81. Kington, R. S. & Smith, J. P. Socioeconomic status and racial and ethnic differences in functional status associated with chronic diseases. *Am. J. Public Health* **87,** 805–810 (1997).

82. Jeng, J.-Y. *et al.* Maintenance of mitochondrial DNA copy number and expression are essential for preservation of mitochondrial function and cell growth. *J. Cell. Biochem.* **103,** 347–357 (2008).

83. Ashar, F. N. *et al.* Association of mitochondrial DNA levels with frailty and all-cause mortality. *J. Mol. Med.* **93,** 177–186 (2014).

84. Tin, A. *et al.* Association between Mitochondrial DNA Copy Number in Peripheral Blood and Incident CKD in the Atherosclerosis Risk in Communities Study. *J. Am. Soc. Nephrol.* ASN.2015060661 (2016). doi:10.1681/ASN.2015060661

85. Zhang, Q. *et al.* Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* **464,** 104–107 (2010).

86. Yu, E. *et al.* Mitochondrial DNA Damage Can Promote Atherosclerosis Independently of Reactive Oxygen Species Through Effects on Smooth Muscle Cells and Monocytes and Correlates With Higher-Risk Plaques in Humans. *Circulation* **128,** 702–712 (2013).

87. Bild, D. E. *et al.* Multi-Ethnic Study of Atherosclerosis: Objectives and Design. *Am. J. Epidemiol.* **156,** 871–881 (2002).

88. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up Report on the Diagnosis of Diabetes Mellitus. *Diabetes Care* **26,** 3160–3167 (2003).

89. Ives, D. G. *et al.* Surveillance and ascertainment of cardiovascular events: The Cardiovascular Health Study. *Ann. Epidemiol.* **5,** 278–285 (1995).

90. White, A. D. *et al.* Community surveillance of coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study: Methods and initial two years' experience. *J. Clin. Epidemiol.* **49,** 223–233 (1996).

91. Goff, D. C. *et al.* 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 01.cir.0000437741.48606.98 (2013). doi:10.1161/01.cir.0000437741.48606.98

92. Canty, A. & support), B. R. (author of parallel. *boot: Bootstrap Functions (Originally by Angelo Canty for S)*. (2015).

93. Chen, S. *et al.* Association between leukocyte mitochondrial DNA content and risk of coronary heart disease: a case-control study. *Atherosclerosis* **237,** 220–226 (2014).

Curriculum Vitae

FORAM N. ASHAR

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EDUCATION INFORMATION

Degree	Institution	Discipline	Year
Ph.D. Candidate	Johns Hopkins University	Human Genetics	2010-present
B.S.	University of Georgia	Genetics	2007-2010

SKILLS

Programming: Fluent in R, Perl, Bash; familiar with Python; proficient working in Unix environment on computing cluster.
Software: IMPUTE, METAL, FAST, birdseed suite, PLINK, samtools, PicardTools, GATK, Tuxedo suite, SNPEff.
Laboratory: NGS library prep, CRISPR/Cas9 mutagenesis in zebrafish, zebrafish breeding & maintenance, molecular cloning.

RESEARCH EXPERIENCE

Graduate Student Aug 2010-present
Johns Hopkins University School of Medicine • Baltimore, MD
Laboratory of Dan E. Arking, Ph.D.

- Implemented the largest GWAS to date for sudden cardiac death (SCD) as lead analyst for CHARGE-SCD consortium in collaboration with 30+ groups from 17 cohorts across the US and Europe.
- Utilized genetic risk score approach to integrate SCD GWAS with publicly available data for 17 SCD risk factors and identify causal SCD risk factors.
- Generated and analyzed targeted NGS data in 1,800 individuals to identify rare loss of function variants in *SLC35F1* associated with sudden cardiac death.
- Used morpholinos and CRISPR mutagenesis in zebrafish to identify roles of genes in cardiovascular development and cardiac electrophysiology.
- Developed a novel algorithm to calculate mitochondrial DNA copy number (mtDNA-CN) from genotyping arrays and utilized this method to establish mtDNA-CN as a predictor of all-cause mortality and cardiovascular disease.

Summer Undergraduate Research Fellow Jun 2009-Aug 2009
Mayo Clinic • Rochester, MN
Laboratory of Zhenkun Lou, Ph.D.

- Established the role of tumor supressor p53 in stress granule formation following cellular exposure to arsenite, a chemotherapeutic agent, in two cancer cell lines.

Undergraduate Researcher Sep 2007-May 2010
University of Georgia • Athens, GA
Laboratory of Daniel Promislow, Ph.D.

- Created novel assays to explore putative ecological interactions between *C. elegans* and *D. melanogaster* at different stages of development and in different environmental conditions.

RESEARCH ACTIVITIES

Original Research Publications:

- **Ashar, F.N.,** Albert, C.M., Newton-Cheh, C., Brody, J.A., Muller-Nurasyid, M., Moes, A., Mak, A., Huikuri, H., Junttila, M.J., Goyette, P., Pulit, S.L., Pazoki, R., Tanck, M.W., Blom, M.T., Zhao, X.Q., Havulinna, A.S., Jabbari, R., Glinge, C., Tragante, V., Escher, S.A., Chakravarti, A., Ehret, G., Coresh, J., Li, M., Prineas, R.J., Johnson, C.O., Franco, O.H., Kwok, P.Y., Lumley, T., Dumas, F., McKnight, B., Rotter, J.I., Lemaitre, R.N., O'Donnell, C.J., Hwang, S.J., Tardif, J.C., Kortelainen, M.J., VanDenBurgh, M., Uitterlinden, A.G., Hofman, A., Stricker, B.H.C., de Bakker, P.I. W., Franks, P.W., Jansson, J.H., Asselbergs, F.W., Halushka, M.K., Maleszewski, J.J., Tfelt-Hansen, J., Engstrom, T., Salomaa, V., Virmani, R., Kolodgie, F., Wilde,A.A.M., Tan, H.L., Bezzina, C.R., Eijgelsheim, M., Rioux, J.D., Rice, K., Jouven, X., Kaab, S., Psaty, B.M., Siscovick, D.S., Arking, D.E., Sotoodehnia, N., Comprehensive characterization of the genetic architecture of sudden cardiac death. *Manuscript in preparation.*
- **Ashar, F.N.**, Zhang, Y., Moes, A., Longchamps, R.J., Lane, J., Moore, A.Z., Grove, M.L., Chaves, P.H.M., Coresh, J., Newman, A.B., Matteini, A.M., Bandeen-Roche, K., Boerwinkle, E., Walston, J.D., Pankratz, N., Guallar, E., Arking, D.E., Mitocohndrial DNA copy number is a predictor of cardiovascular disease. *Manuscript in preparation.*
- Tin, A., Grams, M.E., **Ashar, F.N.,** Lane, J.A., Rosenberg, A.Z., Grove, M.L., Boerwinkle, E., Selvin, E., Coresh, J., Pankratz, N., Arking, D.E. (2016). Association between Mitochondrial DNA Copy Number in Peripheral Blood and Incident CKD in the Atherosclerosis Risk in Communities Study. J Am Soc Nephrol ASN.2015060661v1-ASN.2015060661.
- **Ashar, F.N.**, Moes, A., Moore, A.Z., Grove, M.L., Chaves, P.H.M., Coresh, J., Newman, A.B., Matteini, A.M., Bandeen-Roche, K., Boerwinkle, E., Walston, J.D., Arking, D.E. (2014). Association of Mitochondrial DNA levels with frailty and all-cause mortality. J Mol Med 93, 177-186.
- Gupta, S., Ellis, S.E**., Ashar, F.N**., Moes, A., Bader, J.S., West, A.B., and Arking, D.E. (2014). Transcriptome Analysis Reveals Deregulation of Innate Immune Response Genes and Neuronal Activity-Dependent Genes in Autism. Nat Commun 5, 5748.
- Ellis, S.E., Gupta, S., **Ashar, F.N.**, Bader, J.S., West, A.B., and Arking, D.E. (2013). RNA-Seq optimization with eQTL gold standards. BMC Genomics 14, 892.
- Provost, E., Wehner, K.A., Zhong, X., **Ashar, F.**, Nguyen, E., Green, R., Parsons, M.J., and Leach, S.D. (2012). Ribosomal biogenesis genes play an essential and p53-independent role in zebrafish pancreas development. Development 139, 3232–3241.

Invited Book Chapters:

- **Ashar, F.N.,** and Arking D.E (2014) Genomics of Complex Cardiovascular Disease. In Genomic Medicine. (Kumar D. and Weatherall D., eds). Oxford University Press. pp316-336.

Presentations:

- **Ashar, F.N.**, Albert, C., Cupples, A., Eigelsheim, M., Goyette, P., Huikuri, H., Jinttilla, M.J., Jouven, X., Kääb, S., Kortelainen, M-L., Kwok, P-Y., Müller-Nurasyid, M., Newton-Cheh, C., Psaty, B., Pulit, S., Siscovick, D., Stricker, B., Sotoodehnia, N., Arking, D.E., on behalf of the CHARGE-SCD Consortium. Comprehensive characterization of the genetic architecture of sudden cardiac death. Poster session presented at the 65th Annual Meeting of The American Society of Human Genetics, 2015 Oct 6-10, Baltimore, USA
- **Ashar, F.N,** Zhang, Y, Moes, A., Grove, M.L., Wilsdon, A.G., Chaves, P.H.M., Coresh, J., Newman, A.B., Bandeen-Roche, K., Boerwinkle, E., Walston, J.D., Guallar, E., Arking, D.E. Mitochondrial DNA copy number as a predictor of cardiovascular disease. Poster session presented at the Scientific Sessions of the American Heart Association, 2014 Nov 15-18, Chicago, USA

- **Ashar, F.N,** Moes, A., Moore, A.Z., Grove, M.L., Chaves, P.H.M., Coresh, J., Newman, A.B., Matteini, A.M., Bandeen-Roche, K., Boerwinkle, E., Walston, J.D., Arking, D.E. Association of Mitochondrial DNA levels with frailty and all-cause mortality. Poster session presented at 64th Annual Meeting of The American Society of Human Genetics, 2014 Oct 6-10, San Diego, USA
- **Ashar, F.N,** Moes, A., Moore, A.Z., Grove, M.L., Chaves, P.H.M., Coresh, J., Newman, A.B., Matteini, A.M., Bandeen-Roche, K., Boerwinkle, E., Walston, J.D., Arking, D.E. Association of Mitochondrial DNA levels with frailty and all-cause mortality. Poster session presented at the Genomics of Common Disease Meeting, 2014 Sept 17-20, Bethesda, USA
- **Ashar, F.N.**, Albert, C., Chugh, S.S., Cupples, A., Eigelsheim, M., Goyette, P., Huertas-Vazquez, A., Huikuri, H., Jinttilla, M.J., Jouven, X., Kääb, S., Kortelainen, M-L., Kwok, P-Y., Lehtimäki, T., Lyytikäinen, L., Müller-Nurasyid, M., Newton-Cheh, C., Psaty, B., Pulit, S., Siscovick, D., Stricker, B., Sotoodehnia, N., Arking, D.E., on behalf of the CHARGE-SCD Consortium. Sex-specific effects of CAD SNPs in sudden cardiac death. Poster session presented at the 63rd Annual Meeting of The American Society of Human Genetics, 2013 Oct 22-26, Boston, USA

Reviewer:
Ad hoc Peer Review for Journal: "***Heart***", Manuscript Topic: Genetic Risk Scores, Mendelian Randomization

PATENT

Arking, DE, Ashar, FN, Moes, A. Mitochondrial DNA Copy Number as a Predictor of Frailty, Cardiovascular Disease, Diabetes, and All-Cause Mortality. PCT/US15/28233. Filed April 2015. Patent Pending.

TEACHING EXPERIENCE

Teaching Assistant, Introduction to Computational Genetics Fall 2012, 2015
Johns Hopkins School of Medicine
- Collaborated with two instructors to develop in-class activities, take-home exercises and grading rubric for the first time class was offered, and received positive feedback from students in class evaluations.
- Developed test datasets and set up software for eight-week hands-on class on a high-performance computing cluster.

Tutor, Comprehensive Exam Preparation 2013-present
Johns Hopkins University School of Medicine
- Reviewed basics of linkage and association studies for over 30 second year graduate students as they prepared for their oral comprehensive exams, all of whom passed.

ORGANIZATIONAL ACTIVITIES

Organizing Member, Journeys of Women in Science August 2013-present
Johns Hopkins School of Medicine

Professional Societies
Member, The American Society of Human Genetics 2010-present

AWARDS

2007—2010 Charter Scholarship & Reagent's Waiver, University of Georgia, Athens
2007—2010 Dean's List, University of Georgia, Athens

2009	M.E. McCullough Scholarship
2009	Crane Leadership Scholarship
2008	Cordelia Anne Ellis Scholarship

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