

## Review of the Diabetes Heart Study (DHS) Family of Studies: A Comprehensively Examined Sample for Genetic and Epidemiological Studies of Type 2 Diabetes and its Complications

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### ■ Abstract

The Diabetes Heart Study (DHS) is a genetic and epidemiological study of 1443 European American and African American participants from 564 families with multiple cases of type 2 diabetes. Initially, participants were comprehensively examined for measures of subclinical cardiovascular disease (CVD) including computed tomography measurement of vascular calcified plaque, ultrasound imaging of carotid artery wall thickness, and electrocardiographic intervals. Subsequent studies have investigated the relationship between bone mineral density and vascular calcification, measures of adiposity, and biomarkers. Ongoing studies are carrying out an extensive evaluation of cerebrovascular disease using magnetic resonance imaging and cognitive assessment. A second, parallel study, the African American DHS, has expanded the sample of African Americans to investigate marked racial differences in subclinical CVD between European Americans and African Americans. Studies in development will evaluate the impact of social stress dur-

ing the lifecourse on CVD risk, and the prevalence of gastroparesis in this diabetes enriched sample. In addition, the ongoing high mortality rate in DHS participants provides novel insights into the increased risks for type 2 diabetes affected individuals. A comprehensive genetic analysis of the sample is underway using the genome-wide association study (GWAS) approach. Data from this GWAS survey will complement prior family-based linkage data in the analysis of genetic contributors to the wide range of traits in the sample. To our knowledge the DHS family of studies has created the most comprehensively examined sample of individuals with type 2 diabetes yet available, and represents a unique resource for the study people with type 2 diabetes. The aim of this review is to provide a collective overview of the major results from the DHS family of studies, and relate them to the larger body of biomedical investigations of diabetes and its complications.

**Keywords:** type 2 diabetes · adiposity · bone · cardiovascular disease · cerebrovascular disease · coronary calcium

### Introduction

he aim of this review is to summarize the major results of the Diabetes Heart Study (DHS) family of studies, and relate them, where appropriate, to the larger body of biomed-

ical investigation of diabetes and its complications. The development of the DHS family of studies reflects the high prevalence and medical relevance of diabetes. Over 25 million Americans are believed to be living with diabetes [1] with the great majority having type 2 diabetes (T2D). Prevalence is es-

pecially high in the southeastern region of the United States where, for example, more than 640,000 North Carolinians are affected, corresponding to 9.3% of the state population [2]. Diabetes contributes to almost 1 in 5 hospitalizations in this region [3]. The long term consequences of living with diabetes are dire, with the complications of diabetes reducing quality of life and life span. More than 50% of people with diabetes will succumb to vascular disease events, the majority of which are cardiovascular disease (CVD)-related. In people with diabetes, it is believed that genetic

susceptibility and environmental factors (hypertension, microalbuminuria, blood glucose control, etc.) ultimately culminates in diabetic macrovascular disease. Diabetes is widely recognized as an independent risk factor for the development of clinical atherosclerotic CVD [4-8]. For example, the relative risk of cardiovascular death was 2.1 for men and 4.9 for women, comparing diabetic subjects to non-diabetic subjects in the Framingham Study [9].

Patients with diabetes are at increased risk of mortality from coronary heart disease (CHD) [10]. In addition, studies have documented that a large proportion of patients with myocardial infarction (MI), without previous diagnosis of diabetes, have impaired glucose tolerance or frank diabetes [11]. The relationship between CVD risk and T2D has been recognized and extensively documented for over 50 years, yet the underlying mechanism of this association remains a subject of debate. For a problem of such magnitude, remarkably little is known about the origins of diabetic CVD. Risk for CVD and T2D is influenced by genetic and common clinical factors (e.g., insulin resistance and obesity). The Diabetes Heart Study was initiated with the goal of elucidating the genetic components of CVD in diabetes, and to investigate their correlation with environmental risk to help focus both treatment and intervention strategies.

CVD is one of the contributing factors to premature morbidity and mortality in people affected with diabetes. With the successful development of the initial DHS, we recognized capabilities in our interdisciplinary research team with expertise in modern human genetics, sophisticated imaging, epidemiology, biostatistics, and clinical sciences, which were applicable to multiple aspects of the study of individuals with diabetes. These additional studies have led to the development of a comprehensively examined ongoing cohort of T2D-affected individuals.

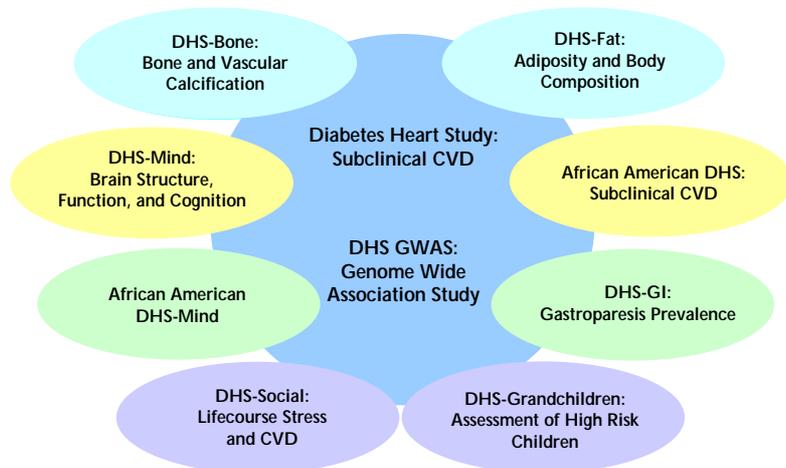
## Overview of the DHS family of studies

Figure 1 summarizes the completed, ongoing, and developing studies, which comprise the DHS family. Initially, three separate studies ran largely simultaneously: the parent DHS study, DHS-Bone, and DHS-Fat. Funding of the initial DHS, which focused on subclinical CVD, facilitated gaining support to evaluate bone mineral density (BMD) and vascular calcification (DHS-Bone), and quantity and distribution of adipose tissue (DHS-Fat). Data acquisition was centered upon thoracic

### Abbreviations:

AA - African American  
 AACP - abdominal aortic calcified plaque  
 ACR - albumin/creatinine ratio  
 BiVV - biventricular volume  
 BMD - bone mineral density  
 BMI - body mass index  
 CAC - coronary artery calcified plaque  
 CarCP - carotid artery calcified plaque  
 CARDIA - Coronary Artery Risk Development in Young Adults  
 CES-D - Center for Epidemiological Studies depression scale  
 CHD - coronary heart disease  
 cM - centimorgan (unit to measure genetic distance and linkage)  
 COWA - controlled oral word association task  
 CRP - C-reactive protein  
 CVD - cardiovascular disease  
 CT - computed tomography  
 DHS - Diabetes Heart Study  
 DSST - digit symbol substitution task  
 DXA - dual energy X-ray absorptiometry  
 ECG - electrocardiogram  
 FLAIR - fast fluid attenuated inversion recovery  
 GCRC - General Clinical Research Center  
 GI - gastrointestinal  
 GWAS - genome-wide association study  
 HbA1c - glycated hemoglobin  
 HDL - high-density lipoprotein  
 IMT - intima media thickness  
 LDL - low-density lipoprotein  
 LOD - logarithm (base 10) of odds  
 MALD - mapping by admixture linkage disequilibrium  
 MESA - Multi-Ethnic Study of Atherosclerosis  
 MI - myocardial infarction  
 MRI - magnetic resonance imaging  
 MS - metabolic syndrome  
 NCI - National Cancer Institute  
 NHLBI - National Heart, Lung, and Blood Institute's  
 NOS1AP - nitric oxide synthase 1 (neuronal) adaptor  
 QCT - quantitative computed tomography  
 QT interval - time between start of Q wave and end of T wave in the heart's electrical cycle  
 RAVLT - Rey auditory verbal learning task  
 SNP - single nucleotide polymorphism  
 SOLAR - sequential oligogenic linkage analysis routine  
 T2D - type 2 diabetes  
 vBMD - volumetric bone mineral density

and abdominal computed tomography (CT) scans to image vascular calcified plaque. The same scan data can also be evaluated for measures of BMD and adipose tissue.



**Figure 1.** The Diabetes Heart Study (DHS) family of studies. DHS comprises a series of studies to analyze vascular diabetic complications. Data collection in DHS-Bone and DHS-Fat are completed. DHS-GWAS, DHS-Mind, and African American DHS are currently ongoing. Through examining the same pool of participants, DHS represents the most comprehensively examined sample of T2D-affected individuals with heart disease and T2D-affected relatives. CVD: cardiovascular disease. GI: gastrointestinal.

One striking observation in initial analysis was that the amount of calcified plaque in vascular beds was quite different between African Americans and European Americans [12]. This observation supported and justified the development of the ongoing African American-DHS (AA-DHS), which has expanded the number of unrelated African Americans. In addition, we realized that the novel DHS sample was ideal for the study of cerebrovascular disease as determined by magnetic resonance imaging (MRI) and cognitive testing, which led to the ongoing DHS-Mind study. A long-term goal of these studies was to generate comprehensive genetic data on each participant. This is now underway with acquisition of genome-wide association study (GWAS) data on the original DHS sample. Finally, several studies are in the development stage, including an extension of the AA-DHS to encompass cerebrovascular disease and cognition (AA-DHS-Mind). Another study is designed to quantify and explore the impact of social stress on the development of CVD (DHS-Social), and to examine the risk of diabetes and its

complications for children and grandchildren of DHS participants.

The recruitment and examination phases of the initial Diabetes Heart Study, DHS-Bone, and DHS-Fat, have been completed. Ongoing studies are AA-DHS, DHS-Mind, and DHS-GWAS. Studies under development are the study of gastrointestinal pathologies (DHS-GI), AA-DHS-Mind, and DHS-Social.

## Recruiting in the DHS

The DHS is novel in its focus on CVD in a T2D-enriched population. In the initial study phase, recruiting and extensive phenotyping were carried out. A family-based genome-wide linkage scan was performed, and extensive data analysis was carried out. We recruited and phenotyped 1443 individuals from 564 European American, and African American, families with multiple T2D-affected members. Table 1 summarizes the results of recruiting, including recruitment by parameters ethnicity, number of families, number of subjects, number of T2D subjects, and number of sibling pairs. Ascertainment of families was based on at least two siblings concordant for T2D (defined as a clinical diagnosis of diabetes after the age of 34 years, in the absence of historical evidence of diabetic ketoacidosis). Unaffected siblings, similar in age to the siblings with T2D, were also invited to participate, as were any additional diabetes-affected siblings. African Americans made up 15.4% of the original participants. Recruiting was based upon family structure with no inclusions/exclusions based on prevalent CVD at the time of recruitment. The only individuals excluded were those with serious health conditions, e.g., advanced nephropathy. Thus DHS represents a cross section of the T2D population.

## Clinical evaluation in the DHS: measuring CVD in a diabetes-enriched sample

Participant examinations were conducted in the General Clinical Research Center (GCRC) of

**Table 1.** Patient recruitment in the Diabetes Heart Study

Back-ground	Families	Individuals	T2D	Non-T2D siblings	Total sibling pairs	T2D sibling pairs
All	564	1443	1208	234	1566	839
Caucasians	475	1220	1010	210	1367	701
African American	89	222	198	24	199	138

Wake Forest University School of Medicine. The examinations included interviews for medical history and health behaviors, anthropometric measures, resting blood pressure, a fasting blood draw and a spot urine collection. Laboratory assays included urine albumin and creatinine, total cholesterol, LDL, HDL, triglycerides, glycated hemoglobin (HbA1c), fasting glucose, and blood chemistries. A medical history was collected with emphasis on CVD history, procedures, etc. A summary of the major phenotypic variables is shown in Table 2.

#### *Subclinical cardiovascular disease: calcified atherosclerotic plaque*

Numerous reports document that vascular calcification, i.e. vascular calcified plaque, is an excellent surrogate marker of CVD. In particular, coronary artery calcified plaque (CAC) has long been considered a primary determinant of CVD [13-15], predicting both prevalent CVD and total mortality in asymptomatic individuals [13, 16, 17]. Primary phenotypes for DHS were CAC and vascular calcified plaque in other vascular beds: carotid artery calcified plaque (CarCP), and abdominal aortic calcified plaque (AACP). These were measured from thoracic and abdominal CTs in the corresponding arterial bed, using single and multidetector CT systems, and standardized protocols based on those contemporaneously implemented in the National Heart, Lung, and Blood Institute's (NHLBI) Coronary Artery Risk Development in Young Adults (CARDIA) and Multi-Ethnic Study of Atherosclerosis (MESA) studies [18, 19]. Technical aspects of the CT examination have been described in detail [20]. Images were obtained during suspended respiration and with electrocardiogram (ECG) gating. The image data was processed by experienced analysts producing measures of calcified plaque mass, volume, and the Agatston

score. Calcified plaque was measured using two thresholds for the presence of calcified plaque: a conventional 130 CT number threshold, and a more sensitive 90 CT number threshold.

#### *Other subclinical CVD measures: IMT, ECG, and heart size*

CVD is a multifaceted disease that can be measured subclinically in a variety of ways. In addition to calcified plaque, we have performed high-resolution B-mode carotid ultrasonography to measure intima media thickness (IMT) of the common carotid artery. In addition, a resting 12 lead ECG was performed to assess history of clinically significant CVD. ECGs were digitally recorded and coded using standardized procedures. ECG abnormalities were classified according to the Minnesota Code [21]. Heart failure is a major source of CVD events in individuals with diabetes [22, 23]. As a consequence, heart size, specifically biventricular volume (BiVV), was measured in an effort to assess left ventricular hypertrophy. BiVV was calculated using a modified Simpson's formula, which has been shown to be highly correlated ( $r = 0.804$ ) with left ventricular mass obtained from magnetic resonance imaging [24].

#### *Other measures: dietary intake and physical activity*

Measures of dietary intake and physical activity have also been acquired using the self-administered NCI food frequency questionnaire (FFQ) [25] and the seven-day physical activity recall (PAR) [26, 27].

### **Characteristics of the DHS family sample**

Table 3 summarizes major characteristics of DHS subjects included in the family-based genetic studies, comparing T2D-affected individuals and their non-diabetic siblings. These data are consistent with a conventional T2D population: mean age 59-61 years at examination, and overweight, or obese (mean BMI 29-32). In addition, more than 80% are hypertensive. Consistent with the high rates of CVD in diabetes, 96% of T2D-affecteds have detectable CAC (an important issue given the challenges presented by analysis of CAC in other studies with lower prevalence of CAC). CAC scores extend over an extraordinary range of zero to over 50,000. Non-diabetic siblings have significant CVD risk factor profiles also: 82% have de-

tectable CAC with a mean of 769 and median of 1691, 30% are on lipid-lowering medication and have evidence of significant CAC levels. Additional traits such as body composition (by dual energy X-ray absorptiometry, DXA), fat depots (visceral, subcutaneous, by CT), and multiple measures of bone density (by CT and DXA), supplemented the measures of subclinical CVD.

**Table 2.** Major phenotypic variables in the Diabetes Heart Study

Variable	Measurement
Calcified plaque (coronary, carotid, aorta)	CT
Carotid wall thickness	IMT
Electrocardiogram	
Glucose control	HbA1c, FG
Lipids	HDL, LDL, TG
Renal function	ACR, GFR
Biometry	Weight, height, etc.
Bone density	CT
Body composition	DXA
Adiposity (VAT, SAT)	CT
Blood pressure	SBP, DBP
Food frequency	Block
Heart size	LVH
Physical activity	Pfaffenbarger
Medication	
Biomarkers	

**Legend:** CT: computed tomography. IMT: intima media thickness. FG: fasting glucose. HDL/LDL: high-/low-density lipoprotein. ACR: albumin/creatinine ratio. GFR: glomerular filtration rate. DXA: dual energy X-ray absorptiometry. SBP: systolic blood pressure. DBP: diastolic blood pressure. LVH: left ventricular hypertrophy.

## Descriptive epidemiological studies

An important component of any population-based study is a detailed understanding of phenotypic relationships. This has been one focus of the DHS, especially evaluation of the relationship between vascular disease measures [28] and other phenotypes in the study. These have included assessments of contributors to CAC [29] and IMT [30]. Age, gender, and smoking were observed as significant contributors to phenotypic variation. These studies were followed with studies of vascular disease association with measures of renal function [31]. Whilst a clear association has been observed between albuminuria and CAC in European Americans [32, 33], no relationship was seen in African Americans [34]. In a related study, we

have shown that CAC is inversely associated with BMD [35].

Inflammation has been explored by evaluating association of C-reactive protein (CRP) with CAC, CarCP, and IMT [20]. Mean CRP levels in the DHS are more than twice the American Heart Association alert level consistent with average relative CVD risk. This reflects that DHS participants are living with the effects of inflammation on a daily basis. In the DHS study sample, there was no evidence for CRP association with measures of subclinical CVD, though the use of hormone replacement therapy increased CRP levels in women from DHS [36].

In addition to these studies, analyses evaluating the influence of physical activity and alcohol consumption have been performed. It shows that there are only modest contributions of these traits to vascular disease. Ongoing studies include explorations of the relationships between heart size and other measures in the study.

## What are the contributors to sub-clinical CVD?

The foundation of any genetic epidemiological study is an understanding of the contributors to the traits of interest. We have assessed the proportion of subclinical CVD variation explained by demographic and clinical characteristics in DHS. A range of demographic (i.e., age, gender), behavioral (i.e., physical activity, smoking), and clinical (i.e., T2D affection status, BMI, total cholesterol, LDL, HDL, urine albumin/creatinine ratio) characteristics were examined for their influence on the subclinical measures. The latter included CVD of CAC, CarCP, AACP, IMT, and a principal component of vascular calcium, a variable combining calcified plaque scores from the three vascular beds [37]. Models containing the covariates age, gender, and BMI explain a substantial amount of variation in subclinical CVD measures (Table 4), ranging between 0.26 and 0.37. Inclusion of the remaining characteristics raises the total proportion of variation explained by all characteristics to 0.31-0.43 (Table 4). The proportion of variation explained by an individual variable, after adjusting for all other covariates (the numbers reported for each individual trait in the table), suggests that most of these variables explain limited additional variation. That is, age, gender, and BMI together capture most of the variation contributed by other variables. In order of degree of effect, gender, age, duration of T2D, history of MI, and

smoking tended to explain the largest proportions of variation across subclinical CVD measures. Age and duration of T2D are highly correlated, i.e. collinear, and we have used age as the preferred covariate, since it explains the most variation in all of these subclinical CVD measures.

## Relationships between measures of vascular disease

We have completed a multivariate analysis, using generalized estimating equations, to compare risk factors for calcified atherosclerotic plaque in the three vascular beds (coronary, carotid, and abdominal aorta) [38]. The effect of each clinical and demographic risk factor was estimated after standardizing each measure of vascular calcium. Standardization permitted direct comparison of the magnitude of the regression parameters' coefficients. Risk factors included age, T2D duration, pack-years of smoking, LDL, HDL, triglycerides, urine albumin/creatinine ratio (ACR), HbA1c, CRP, BMI, gender, T2D status, hypertension, and previous MI. Significant ethnic-specific effects led to stratified analyses. In 1000 European Americans, age, duration of diabetes, pack-years, ACR, and MI were the strongest and most consistent correlates of vascular calcium

across the three beds (all  $p < 0.01$ , correlation coefficient ( $r$ ) ranging from 0.20-0.53). HDL and LDL (both inverse), and HbA1c, were also significantly correlated with vascular calcium in the three vascular beds, but with more modest correlation coef-

**Table 3.** Characteristics of DHS participants

Variable	T2D-affected		T2D-unaffected	
	Mean $\pm$ SD	Median (range)	Mean $\pm$ SD	Median (range)
<i>Phenotype</i>				
Gender (% female)	54.0		63.0	
Age (yr)	61.5 $\pm$ 9.3	61.4 (34 - 86)	59.3 $\pm$ 10.0	59.4 (34 - 83.4)
Diabetes duration (yr)	10.4 $\pm$ 7.3	8.0 (1 - 41)		
Insulin treatment (%)	29.7		0	
Oral antidiabetic med. (%)	75.4		0	
Lipid lowering med. (%)	43.9		28.0	
BMI (kg/m <sup>2</sup> )	32.7 $\pm$ 6.9	31.5 (17.1 - 59.8)	29.2 $\pm$ 5.3	28.3 (16.6 - 44)
Systolic BP (mmHg)	140.1 $\pm$ 19.2	138.5 (98 - 260)	135.5 $\pm$ 19.4	133.5 (69 - 197)
Diastolic BP (mmHg)	73.3 $\pm$ 10.6	72.5 (73 - 122)	74.5 $\pm$ 10.3	74.5 (37 - 104)
Hypertension (%)	88.3		68.3	
Hypertension med. (%)	76.0		42.3	
<i>Laboratory</i>				
Total cholesterol (mg/dl)	187.6 $\pm$ 43.0	183.0 (74 - 427)	195.1 $\pm$ 34.1	196.0 (104 - 325)
HDL (mg/dl)	43.5 $\pm$ 13.0	41.0 (8 - 115)	48.8 $\pm$ 14.3	47.0 (23 - 104)
LDL (mg/dl)	105.7 $\pm$ 33.0	103.0 (14 - 283)	114.3 $\pm$ 30.0	113.0 (43 - 221)
Triglycerides (mg/dl)	200.1 $\pm$ 139	168.0 (30 - 1310)	161.6 $\pm$ 84.6	145.0 (47 - 627)
HbA1c (%)	7.9 $\pm$ 2.0	7.5 (4.3 - 21.8)	5.6 $\pm$ 0.5	5.6 (3.5 - 6.9)
Fasting glucose (mg/dl)	152.2 $\pm$ 62.9	138.0 (16 - 568)	95.1 $\pm$ 14.6	94.0 (66 - 126)
C-reactive protein (mg/dl)	0.7 $\pm$ 1.0	0.3 (0 - 12.7)	0.5 $\pm$ 0.8	0.3 (0 - 7.1)
<i>Subclinical CVD</i>				
CA calcium	1875 $\pm$ 3391	479.8 (0 - 50415)	769 $\pm$ 1691	59.0 (0 - 11569)
CA calcium > 0 (%)	96.0		82.0	
Car calcium	357 $\pm$ 720	71.0 (0 - 6122)	159.5 $\pm$ 430	4.5 (0 - 3271)
CarCP > 0 (%)	80.0		63.3	
AA calcium	12118 $\pm$ 16458	(0 - 94156)	7456 $\pm$ 13450	(0 - 72745)
AACP > 0 (%)	97.0		92.2	
Carotid IMT (mm)	0.7 $\pm$ 0.1	0.7 (0.5 - 1.6)	0.7 $\pm$ 0.1	0.6 (0.5 - 1.3)
<i>CVD event (self reported)</i>				
Stroke (%)	9.3		4.5	
Heart attack (%)	19.6		7.6	
Myocardial infarction (%)	8.7		7.3	
Coronary angioplasty (%)	15.2		6.3	
Angina (%)	19.0		7.9	
Endarterectomy (%)	2.2		1.8	
CA bypass graft (%)	13.3		6.3	
Any CVD event (%)	44.6		22.2	

**Legend:** BMI: body mass index. BP: blood pressure. H/LDL: high-/low-density lipoprotein. CA: coronary artery. Car: carotid artery. CP: calcium plaque. AA: abdominal aortic. CVD: cardiovascular disease.

ficients ( $p < 0.05$ ; all  $r < 0.20$ ). Despite the large sample size, there were few instances where the risk factor relationships differed statistically across vascular beds.

**Table 4.** Proportion of variation in CVD outcome explained by the covariate after adjusting for other covariates

Covariate	Subclinical CVD measures explained by participants' characteristics				
	CAC	CarCP	AACP	IMT	VCCP
Age, gender, BMI (together)	0.33	0.27	0.33	0.26	0.37
All covariates together	0.43	0.31	0.43	0.32	0.42
Female	0.046	0.021	0.016	0.053	0.032
Age	0.08	0.113	0.11	0.094	0.138
BMI	0.003	0.003	0.003	0.001	0.001
<i>Diabetes traits</i>					
T2D status	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
T2D duration	0.038	0.033	0.011	0.0	0.045
Fasting glucose	< 0.001	0.001	0.002	0.001	< 0.001
HbA1c	0.001	< 0.001	0.001	< 0.001	0.002
<i>CVD traits</i>					
Hypertension	0.004	0.023	0.007	0.007	0.011
Hypertension med.	0.009	0.015	0.013	< 0.001	0.013
Total chol.	0.002	< 0.001	< 0.001	0.005	0.001
HDL	0.004	< 0.001	0.004	0.006	0.001
LDL	0.004	0.004	0.004	0.006	0.002
Statin use	0.017	0.015	0.026	< 0.001	0.029
History of MI	0.037	0.01	0.03	0.001	0.021
<i>Other</i>					
Physical activity	0.003	0.002	0.002	0.001	0.003
ACR	0.008	0.017	0.013	0.002	0.017
Smoking	0.018	0.008	0.045	0.028	0.022

**Legend:** CAC: coronary artery calcified plaque. CarCP: carotid artery calcified plaque. AACP: abdominal aortic calcified plaque. IMT: intima media thickness. VCCP: vascular calcium calcified plaque. BMI: body mass index. T2D: type 2 diabetes. CVD: cardiovascular disease. H/LDL: high-/low-density lipoprotein. MI: myocardial infarction. ACR: albumin/creatinine ratio.

## Racial differences: low levels of calcified plaque in African Americans

European American families make up 85% of DHS. The 15% fraction of African American DHS families enabled ethnic comparisons for presence and severity of subclinical CVD. Despite similar diabetes duration in the DHS, the prevalence of subclinical CVD in African Americans differed markedly from that in European Americans [12]. Relative to European American participants, African Americans had increased carotid artery IMT

and more conventional risk factors such as smoking, albuminuria, and poorer glycemic, lipid (LDL cholesterol), and hypertension control [12, 34, 39]. Despite these risk factors, African Americans had significantly lower amounts of CAC (mean of 866 vs. 1,915, respectively;  $p = 0.047$ ) and CarCP (179 vs. 355, respectively;  $p = 0.024$ ) relative to European Americans [12]. These results reveal that pathogenesis of large vessel atherosclerosis differs between ethnic groups.

Similar results have been reported in multiple studies of primarily non-diabetic subjects [40, 41], as well as in the subset of diabetic subjects in MESA [42]. In contrast, diabetic African Americans are clearly at significantly increased risk for developing diabetic nephropathy [43]. The marked ethnic disparities in risk for large vessel atherosclerosis (CVD) and small vessel disease (diabetic nephropathy) suggest different mechanisms underlying the development of vascular disease between ethnicities. These observations have had a major impact on the DHS. An important follow-up has been a search for factors that protect African Americans from large vessel calcified atherosclerotic plaque. This study of African Americans is being pursued as an independent ancillary study, in which an additional 566 unrelated African Americans with T2D have been recruited and phenotyped in a manner comparable to the initial DHS study. Mapping by admixture linkage disequilibrium (MALD) is being performed in the AA-DHS to detect genomic regions associated with CAC in African Americans. These regions are expected to contain excess European ancestry [44].

## Bone mineral density and diabetes

The relationship between T2D and BMD has been the source of some discussion in the literature. In an initial study, we measured trabecular volumetric bone mineral density (vBMD) of the thoracic and lumbar spine by quantitative computed tomography (QCT), as described previously [45], and areal BMD by DXA in 524 women and 425 men (age 36-87 years, BMI range 16-58, 82% with T2D) in the DHS [46]. Lumbar spine vBMD was positively associated with BMI ( $r = 0.24$ ,  $p < 0.0001$ ), and inversely associated with age ( $r = -0.50$ ,  $p < 0.0001$ ). The data were subjected to multivariate analysis adjusting for age, gender, ethnicity, BMI, physical activity, dietary intake, smoking, and alcohol use. No significant interaction between diabetes status and trabecular vBMD of the spine was observed. These data sug-

gest that BMD measured by DXA or QCT are not associated with T2D independent of BMI.

## Genetic epidemiology of cerebrovascular disease and cognition

T2D is associated with a decline in different domains of cognitive functioning. T2D individuals score lower on tests of memory and processing speed than unaffecteds [47-49]. Some studies have observed diminished executive functioning [50-52]. Also, those with T2D are at higher risk for developing dementia [53-56]. However, the physiological mechanisms responsible for this cognitive decline are not clear, with multiple paths such as glucose regulation, comorbidities (e.g. vascular disease), and genetic predisposition [57] being implicated. With its extensive phenotyping, the DHS is ideally situated to investigate the complex relationships between T2D and cognition.

The ongoing DHS-Mind study, which started in 2007, is an important extension of DHS. DHS-Mind is in the process of recruiting and re-examining 1000 subjects from the DHS sample, using MRI and cognitive testing. This study includes a range of MRI measures, such as high resolution T1-weighted images for computing brain volumes, and fast fluid attenuated inversion recovery (FLAIR) images for identification of white matter ischemic disease. In addition to the traditional measures, novel metrics are being acquired, including diffusion tensor imaging for calculation of fractional anisotropy of white matter tracts, cerebral blood flow maps using arterial spin labeling techniques, and functional MRI during performance of a cognitive task.

The primary cognitive outcome is the digit symbol substitution task (DSST), a measure that indexes processing speed and working memory. In addition, participants complete the Rey auditory verbal learning task (RAVLT), the controlled oral word association task (COWA), and the Stroop task to measure memory, word fluency, and executive functioning respectively. Depression is assessed using the Center for Epidemiological Studies depression scale (CES-D). Based on these accumulated data, future analyses will include the heritable component of cognition, MRI measures, and a comprehensive genetic analysis using genome scan and candidate gene data to map regions that contain genes contributing to cognition and cerebrovascular disease. This study is creating a unique data set for genetic and other studies of cerebrovascular disease and cognition, and will benefit from having GWAS data in the future.

Preliminary analyses of the relationship between vascular disease and cognitive functioning have shown intriguing first results. The DHS-Mind examination follows 3-10 years after initial recruitment, allowing the long-term effects of subclinical CVD to be related to current cognitive functions. The influence of diabetes and subclinical CVD in the DHS baseline examination has been evaluated for association with cognitive functioning, as measured by DSST. An interesting aspect of this analysis is that the family-based design is an advantage: individuals in families share both genetics and lifestyle. The relationship between DSST performance and subclinical CVD measures was assessed using mixed models that included age, sex, and education as covariates, along with the primary variables of interest, T2D status and measures of subclinical CVD. Family was included as a random effect to account for correlations induced by the family structure of the study. One model was calculated for each measure of subclinical CVD. Along with age ( $p < 0.001$ ), sex ( $p < 0.001$ ), and education ( $p < 0.001$ ), important predictors of cognitive performance were T2D status and measures of subclinical CVD (CAC ( $\beta = -0.68$ ,  $p = 0.008$ ), CarCP ( $\beta = -0.78$ ,  $p = 0.001$ ), and IMT ( $\beta = -21.25$ ,  $p = 0.01$ )). Measures of glucose control (fasting glucose and HbA1c) were not significant.

These results suggest that subclinical CVD burden and T2D status are potent independent predictors of cognitive decline, even within families where genes and environment are similar. That is, between family members, the diabetes state alone reduces cognitive performance. The lack of interaction between diabetes status and subclinical CVD suggests that diabetes acts independently to adversely impact cognition. Subclinical CVD in diabetics is a "second hit" to cognition.

## Genetic epidemiology in the DHS

### *Heritability of primary phenotypes*

A central DHS goal was to estimate heritability of measures of subclinical cardiovascular disease. Heritability studies estimate the genetic contributions to the traits under examination. Publication of these DHS heritability studies has been extensive: CAC [29], IMT [30], GFR and ACR [58], BMD [59], body composition [60], pulse pressure [61], and CRP [62]. Consistent with our expectations, CAC, adjusted for age, gender, ethnicity, and diabetes status, was heritable ( $h^2 = 0.50$ ;  $p = 0.009$ ). Similarly, age-, gender-, and ethnicity-adjusted

heritability for carotid IMT was 0.32 ( $p = 0.02$ ). Further adjustment for total cholesterol, hypertension status, and current smoking status resulted in  $h^2 = 0.41$  ( $p = 0.004$ ). The strongest predictors of carotid IMT, after adjusting for age and gender, were ethnicity (African American vs. European American), total cholesterol, and smoking status. These estimates of heritable components of the subclinical disease provide a solid foundation for the search for genes contributing to these traits.

#### *Genetic and phenotypic correlations and contributions to variance*

Many of the primary phenotypes in the DHS are significantly correlated. These correlations can be partitioned into genetic and environmental correlations. Strong genetic correlations were observed in vascular calcium between CAC, CarCP, and AACCP [37]. Although both the estimated environmental and genetic correlations among CAC, CarCP, and AACCP are highly significant, their genetic correlations are greater than their environmental correlations. Genetic correlations between vascular calcium (CAC, CarCP, and AACCP) and IMT, ACR, and BMD are modest. A principal component analysis based on the genetic correlations suggests that the vascular calcium measures form one strong principal component that is effectively the mean of CAC, CarCP, and AACCP, while IMT, ACR, and BMD largely form three separate components. T2D and metabolic syndrome (MS) affection status are correlated with the presence of vascular calcium.

#### *Candidate gene analysis*

The DHS provides a comprehensively phenotyped sample for evaluation of genetic contributions of specific genes with biological roles relevant to study phenotypes. Multiple studies have been performed. Over 700 single nucleotide polymorphisms (SNPs) in over 135 genes have been evaluated by genotyping multiple SNPs and testing for association with relevant phenotypes in DHS. Amongst others, genes have been targeted [73-76] in pathways for inflammation [63-69], lipid metabolism [63, 70], and calcification [71, 72].

A notable study is the evaluation of genetic variants in the nitric oxide synthase 1 (neuronal) adaptor (*NOS1AP*) gene with QT electrocardiographic interval [77]. An extensive record [78-80] demonstrates *NOS1AP* polymorphisms are profoundly associated with QT interval in individuals

of European ancestry. SNPs in the *NOS1AP* gene were genotyped in the DHS, and tested for association with QT interval duration. In European Americans, the SNPs were significantly associated with a longer QT interval, with  $p$ -values ranging from  $9.9 \times 10^{-5}$  to  $8.9 \times 10^{-7}$ , and genotypic means differing by 0.30-0.40 of the observed QT interval standard deviations. Importantly, the mean genotypic difference for QT interval in diabetes-affected members of DHS was double that observed for non-diabetic studies [78, 81]. This is consistent with the hypothesis that the T2D environment amplifies the genetic effect of a specific polymorphism.

#### *Genome linkage scans for CVD*

A goal of the initial phase of the DHS was to carry out family-based genome-wide linkage scans of quantitative measures of subclinical CVD. Extensive evaluations of both quantitative and qualitative traits have been carried out [36, 37, 82, 83].

Quantitative trait linkage analysis was performed using the variance component approach implemented in SOLAR [84] by adjusting for age, gender, BMI, ethnicity, and T2D status. The strongest evidence for linkage was observed with CarCP and markers in chromosome 16p13 in the genetic interval 0-15 cM [37]. In all European American subjects, the logarithm (base 10) of odds (LOD) score was 2.52, but increased to 4.39 when limited to T2D-affected European Americans. Thus, the T2D environment appears to amplify the genetic contribution to risk for accumulation of CarCP. In addition to the locus on chromosome 16, several other regions of the genome showed LOD scores over 2.0 for CAC, AACCP, and IMT.

In an effort to further evaluate the 16p linkage peak, an additional 59 SNPs were genotyped in the region to increase coverage. In the presence of true linkage, the results should be robust; whereas, for a false linkage, the evidence should be diminished. Evidence for linkage remained strong with a  $LOD = 4.86$  at 16 cM for CarCP. Also, fine mapping resulted in evidence for linkage to CAC ( $LOD = 2.27$  at 19 cM), thus providing additional evidence that this locus may influence vascular calcification more generally.

To explore the hypothesis of a common genetic influence on all subclinical CVD measures, we carried out a principal component analysis of vascular calcified plaque (CAC, CarCP, and AACCP). The resulting principal component was linked to 16p with  $LOD = 3.85$  in the European American T2D subjects in a follow-up fine mapping study [83].

## Future genetic studies in DHS

A GWAS is currently being performed in the DHS European American sample. This will provide a comprehensive genetic fingerprint of common variations in the DHS sample. The DHS is unique in its focus on T2D and CVD. Most other population-based studies of CVD have relatively modest numbers of T2D-affected individuals, with consequently limited statistical power. Our GWAS will enable us to participate in larger meta-analyses of quantitative subclinical CVD and other traits. Also, we will be able to contrast the genetic influences between diabetes and normal metabolic environments. One driving hypothesis, supported by the linkage studies, is that the T2D environment amplifies genetic risk for CVD.

In addition, we are re-evaluating our prior family-based linkage data acquired in the original DHS. In another (non-DHS study) [85], we have shown that a linkage peak from a family-based linkage analysis is due to a rare coding variant in a single gene. This may shed new light on the extensive body of linkage data for complex traits, and suggests that an exon-based search for mutations in the families contributing to the 16p linkage peak might be useful for identifying more influential rare variants. More broadly, the extensive phenotypic data is a source for studies of non-CVD traits such as adiposity, BMD, biomarkers, etc. Such studies should contribute to meta-analysis efforts, and provide a new perspective of diabetes in such studies.

## Mortality in the DHS

The first examination of a DHS participant took place in 1998. Thus, we have been following a cohort at high risk for complications of T2D for up to 12 years. Consistent with the health challenges of T2D, mortality is having an increasing impact. At the time of writing this article, 235 of the original 1442 participants are now deceased. This grim statistic has important research value [86]. Among those deceased, 55% of T2D-affected participants succumbed to vascular disease, 19% to cancer, and 26% to a variety of other causes. Participants were followed for an average of 7.4 years.

Subjects were classified into five groups based on CAC score: 0-9, 10-99, 100-299, 300-999, and  $\geq 1000$ . Logistic regression analysis was performed with adjustment for age, gender, race, smoking, and LDL. Consistent with the importance of subclinical CVD measures to mortality, the CAC score is a powerful independent predictor of all-cause

mortality. The odds ratio for all-cause mortality comparing the highest CAC sample ( $\geq 1000$ ) to the lowest (0-9) was 6.71 (CI 3.09-16.87,  $p = 0.0001$ ) [86]. Additional analysis showed that subjects with the highest CAC were over 3 times more likely to die during the follow-up period. Therefore, CAC  $\geq 1000$  defines a population at very high risk (2.7% annual mortality). Thus, in a sample characterized by high risk of death, CAC differentiates a group of subjects at particularly high risk. The mortality cohort will continue to grow, and add greater power to our efforts to identify factors that contribute towards high risk of fatal complications.

## The evolution of the DHS family of studies

The foundation of the DHS family of studies continues to foster novel investigations. One developing study, the African American Diabetes Mind study follows the pattern of DHS-Mind. Building upon the numerous racial differences observed in DHS-Mind, we are seeking to re-examine the African American DHS cohort with measures of cerebrovascular disease and cognition.

In the DHS-Social study, we are addressing the influence of the social underpinnings of subclinical CVD. It is routinely observed that genetic and clinically measurable contributors (e.g. age, gender, BMI, cholesterol, hypertension, etc.) to CVD do not completely explain observed variance in subclinical CVD. We hypothesize that assessments of exposure to stress and adversity across the life course using social sciences-based approaches may reveal a significant and important additional insights into CVD risk. There is broad acceptance that stress and adversity have deleterious influences on health, but there is surprisingly little research to support this supposition. DHS-Social will address this potential important influence of CVD health.

Another developing project is the study of gastroparesis in the DHS sample (DHS-GI). Gastroparesis, motility disorders, dyspepsia, and other gastrointestinal pathologies are common in diabetics [87-89]. Gastroparesis is a major negative influence on quality of life, and has few effective treatments. Its prevalence in T2D subjects and its associated risk factors, both genetic and clinical, are not well understood. The DHS family-based structure is ideal for assessing prevalence, familial aggregation, heritability, and clinical cor-

relates in a sample which broadly reflects the T2D population.

Mortality in the high-risk DHS sample is high. Early intervention, even in youth, may be a valuable approach for preventing chronic disease in later life. The DHS sample can contribute special value in this line of research: the children and grandchildren DHS families are the descendents of sibships enriched for diabetes, obesity, and related metabolic disorders. We hypothesize that these descendents will also be at risk, which is likely to be compounded by the social cohort effect of obesity. Such high-risk individuals may be an important group to target for early intervention.

## Conclusions

In this review we have sought to provide an overview of the DHS family of studies. The DHS comprises a series of studies on different kinds of diabetic complications, including heart and mind (DHS-Mind) diseases. With this extensively examined and followed up patient group, we attempt to give answers on potential causes and consequences of diabetic complications, including genome associations (DHS-GWAS), bone mineral density (DHS-Bone), and quantity and distribution of adipose tissue (DHS-Fat). The DHS series include completed studies, those which are ongoing, and those in planning stages. To our knowl-

edge the Diabetes Heart Study family of studies encompasses the most comprehensively examined group of individuals with T2D.

Our continued efforts to gather additional data, ranging from comprehensive genetic data to examinations using social science approaches should continue to extend the value of these studies to gain additional insights into the impact of diabetes on health. Undoubtedly, additional avenues of investigation will be pursued in the future. Novel and continued studies on the predictors of mortality, and on the impact of individual social history on health would be of special value. The latter aspect is included in a future DHS study design, called DHS-Social.

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