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Heritability and Genetic Association Analysis of Neuroimaging Measures in the Diabetes Heart Study

Laura M Raffield[a,b,c], **Amanda J Cox**[b,c,d], **Christina E Hugenschmidt**[e], **Barry I Freedman**[f], **Carl D Langefeld**[g], **Jeff D Williamson**[e], **Fang-Chi Hsu**[g], **Joseph A Maldjian**[h], and **Donald W Bowden**[b,c,d,*]

[a]Molecular Genetics and Genomics Program, Wake Forest School of Medicine, Winston-Salem, NC, USA

[b]Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Winston-Salem, NC, USA

[c]Center for Diabetes Research, Wake Forest School of Medicine, Winston-Salem, NC, USA

[d]Department of Biochemistry, Wake Forest School of Medicine, Winston-Salem, NC, USA

[e]Department of Gerontology and Geriatrics, Wake Forest School of Medicine, Winston-Salem, NC, USA

[f]Department of Internal Medicine - Nephrology, Wake Forest School of Medicine, Winston-Salem, NC, USA

[g]Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, NC, USA

[h]Department of Radiology, Wake Forest School of Medicine, Winston-Salem, NC, USA

Abstract

Patients with type 2 diabetes are at increased risk of age-related cognitive decline and dementia. Neuroimaging measures such as white matter lesion volume, brain volume, and fractional anisotropy may reflect the pathogenesis of these cognitive declines, and genetic factors may contribute to variability in these measures. This study examined multiple neuroimaging measures

[*]**Corresponding Author:** Dr. Donald W Bowden, Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Medical Center Boulevard, Winston Salem, NC, USA, 27157, Tel: +1 336-713-7507, Fax: +1 336-713-7544, dbowden@wakehealth.edu.

DISCLOSURE STATEMENT

The authors declare no conflicts of interest in relation to this work.

The data contained in the manuscript have not been published previously, have not been submitted elsewhere, and will not be submitted elsewhere while under review at *Neurobiology of Aging*.

Study protocols were approved by the Institutional Review Board at Wake Forest School of Medicine, and all study procedures were completed in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to participation.

All authors have reviewed the manuscript and approve of its contents and accuracy.

in 465 participants from 238 families with extensive genotype data in the type 2 diabetes enriched Diabetes Heart Study-Mind cohort. Heritability of these phenotypes and their association with candidate single nucleotide polymorphisms (SNPs) and SNP data from genome-and exome-wide arrays was explored. All neuroimaging measures analysed were significantly heritable ($\hat{h}^2$ =0.55–0.99 in unadjusted models). Seventeen candidate SNPs (from 16 genes/regions) associated with neuroimaging phenotypes in prior studies showed no significant evidence of association. A missense variant (rs150706952, A432V) in *PLEKHG4B* from the exome-wide array was significantly associated with white matter mean diffusivity ($p=3.66\times10^{-7}$) and gray matter mean diffusivity ($p=2.14\times10^{-7}$). This analysis suggests genetic factors contribute to variation in neuroimaging measures in a population enriched for metabolic disease and other associated comorbidities.

Keywords

Magnetic resonance imaging; type 2 diabetes; genetics; heritability

1. INTRODUCTION

Prior research has revealed that type 2 diabetes (T2D) accelerates age-related cognitive decline and increases risk of overt dementia (Reijmer, et al., 2010). A number of studies have investigated neuroimaging phenotypes using magnetic resonance imaging (MRI) in individuals with T2D as a way of assessing the pathogenesis of these cognitive declines. Prior studies have reported an increased risk of white matter lesions, which are associated with increased risk of cognitive decline and stroke, as well as reduced total brain volume (TBV) in patients with T2D when compared to non-diabetic controls, but these studies have in some cases produced conflicting results, and many are based on relatively limited sample sizes (Falvey, et al., 2013,Fornage, et al., 2011,Jongen and Biessels, 2008,Moran, et al., 2013,van Harten, et al., 2006). T2D has also been associated with reduced white matter fractional anisotropy (WMFA), a measure of the directionality of water molecule diffusion used to assess brain microstructure (Falvey, et al., 2013,Nucifora, et al., 2007). A number of factors may influence these neuroimaging phenotypes in individuals with T2D, including hypertension (Schmidt, et al., 2004), poor glycemic control (van Elderen, et al., 2010), and adiposity (Verstynen, 2013); however, few studies have examined the potential influences of genetic risk factors on these neuroimaging measures in individuals with T2D.

Prior estimates of the heritability of a variety of neuroimaging phenotypes, including TBV, gray and white matter volume (GMV, WMV) (Blokland, et al., 2012,Peper, et al., 2007), WMFA (Kochunov, et al., 2010), and total white matter lesion volume (WMLV) (Atwood, et al., 2004,Carmelli, et al., 1998,Turner, et al., 2004), have been high, increasing interest in genetic analysis of these measures. Heritable, quantitative neuroimaging measures are thought to be important endophenotypes for the analysis of genetic contributions to risk of cognitive decline and dementia, increasing power to detect genetic contributions to these complex clinical traits (Ge, et al., 2012,Gottesman and Gould, 2003). Multiple variants, including putatively functional coding variants in candidate genes, such as the *BDNF* V66M and the *COMT* V158M polymorphisms, have been reported previously to be associated with

neuroimaging measures in cohorts not enriched for T2D patients (Chiang, et al., 2011,Honea, et al., 2009), though these and other associations have proved difficult to replicate in additional studies (Barnes, et al., 2012,Lopez, et al., 2012). A limited number of genome-wide association studies (GWAS) of neuroimaging measures have also been performed, including analyses of WMFA (Lopez, et al., 2012), TBV (Furney, et al., 2011,Stein, et al., 2012), and white matter lesions (Fornage, et al., 2011). However, to our knowledge, no prior studies have focused on a cohort enriched for T2D, a high risk population for both cognitive decline and dementia.

The Diabetes Heart Study (DHS) is a family-based study of individuals with T2D designed to assess potential genetic and epidemiological risk factors for cardiovascular disease (CVD) in individuals with T2D. The DHS-Mind ancillary study to DHS performed cognitive testing and neuroimaging on 465 individuals from the original DHS cohort. This cohort provides a unique resource for examining genetic risk factors which may contribute to neuroimaging phenotypes of interest in a cohort enriched for T2D. In this study we evaluated the DHS-Mind neuroimaging dataset for heritability estimation and for associations with the comprehensive genetic data also available in the DHS. This included analysis of candidate SNPs from previously reported MRI-based neuroimaging studies and an exploratory, unbiased GWAS using data from both a traditional genome-wide array, designed to assay common variation across the genome, and an array enriched for exonic variants.

2. METHODS

2.1. Study Design and Sample

Participants in the DHS were recruited from outpatient internal medicine and endocrinology clinics and from the community from 1998 through 2005 in western North Carolina. Siblings concordant for T2D without advanced renal insufficiency were recruited, with additional non-diabetic siblings enrolled whenever possible. Ascertainment and recruitment have been described in detail previously (Bowden, et al., 2010,Bowden, et al., 2006,Lange, et al., 2002,Wagenknecht, et al., 2001). T2D was defined as diabetes developing after the age of 35 years treated with changes in diet and exercise and/or oral agents in the absence of initial treatment solely with insulin and without historical evidence of ketoacidosis. Diabetes diagnosis was confirmed by measurement of fasting glucose and glycated hemoglobin (HbA_{1C}) at the exam visit. Extensive measurements of CVD risk factors were obtained during baseline exams from 1998–2006.

The DHS-Mind study is an ancillary study to the DHS initiated in 2008 that performed cognitive testing and neuroimaging to investigate risk factors for cognitive decline in a cohort enriched for T2D. Participants returning from the original DHS investigation were re-examined on average 6.7 ± 1.5 years after their initial visit. Participant examinations were conducted in the General Clinical Research Center of the Wake Forest Baptist Medical Center. The current analyses are based on a subset of 465 participants (from 238 families) returning from the baseline DHS exam with neuroimaging phenotypes from the DHS-Mind study visit and available genome-wide SNP genotype data. Subjects were not excluded for Modified Mini-Mental State Examination (3MSE) scores or other indices of cognitive function indicative of mild cognitive impairment or dementia (Teng and Chui, 1987).

Study protocols were approved by the Institutional Review Board at Wake Forest School of Medicine, and all study procedures were completed in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to participation.

2.2. Neuroimaging

Magnetic resonance (MR) image acquisition—MR imaging was performed on a 1.5-T GE EXCITE HD scanner with twin-speed gradients using a neurovascular head coil (GE Healthcare, Milwaukee, WI). High-resolution T1 anatomic images were obtained using a 3D volumetric Inversion Recovery SPGR sequence (TR=7.36 ms; TE=2.02 ms; TI=600 ms; FA=20 degrees; 124 slices, FOV=24 cm, matrix size = 256×256, 1.5 mm slice thickness). Fluid-attenuated inversion recovery (FLAIR) images were acquired in the axial plane (TR=8002 ms; TE=101.29 ms; TI=2000 ms; FA=90 degrees; FOV=24 cm; matrix size = 256 × 256; 3-mm slice thickness). Whole brain diffusion tensor imaging (DTI) was performed using echo-planar imaging with 25 directions (TR=16000; TE=84.9; FA=90; b value = 0/1000, FOV = 280 cm, matrix size = 256×256, 3 mm slice thickness). Quantitative cerebral blood flow maps were generated using a Q2TIPS-FAIR sequence as previously described (Luh, et al., 1999). This sequence generates 60 tag and control image pairs. Imaging parameters are as follows: echo time 28ms, TI_1-800ms, TI_1s 1200ms, TI 2000ms, TR 3000ms, receiver bandwidth 62.5 kHz, flip angle 90 degrees, field of view 24 cm (frequency) × 18 cm (phase), an acquisition matrix 64×48 (11 slices, 8 mm thickness, 0mm slice gap), and frequency encoding direction anterior/posterior. A bipolar diffusion gradient with an equivalent b value of 5.25 mm^2/sec was added to suppress intra-arterial spins (Yang, et al., 1998).

Image segmentation—Structural T1 images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF), normalized to Montreal Neurologic Imaging (MNI) space, and modulated with the Jacobian determinants (non-linear components only) of the warping procedure to generate volumetric tissue maps using the Dartel high-dimensional warping and the SPM8 (Ashburner and Friston, 2000) new segment procedure as implemented in the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm.html). Intracranial volume (ICV) (GM + WM + CSF), TBV (GM + WM), GMV (GM), and WMV (WM) were determined from the VBM8 automated segmentation procedure which outputs values for native space total GM, WM, and CSF volumes. The normalized gray matter and white matter segmentation maps (without modulation) were binarized at a probability threshold of 0.5 to create segmentation masks for use in generating the tissue-specific measures of diffusion and cerebral blood flow.

Diffusion tensor processing—Diffusion tensor pre-processing was performed using FSL (Jenkinson, et al., 2012). Eddy current correction of the diffusion tensor images was performed using FSL dti_eddy by normalizing each image to the baseline (B0) image using the mutual information registration algorithm. The diffusion tensor was computed using the Camino software package (www.camino.org.uk). The resulting tensor images were converted to NIfTI symmetric positive orientation using the Diffusion Tensor Imaging ToolKit (DTI-TK) (http://www.nitrc.org/projects/dtitk). DTI scalar metrics, including FA and mean diffusivity (MD), were computed using DTI-TK. DTI scalars were normalized to

MNI space by coregistering the MD image to the T1 structural data using SPM8, and then combining this transformation matrix with the parameters computed in the VBM8 normalization procedure, allowing derivation of the global mean diffusivity and fractional anisotropy measures analyzed here (WMMD, GMMD, WMFA, GMFA).

Cerebral blood flow processing—Perfusion images were generated using a previously described fully automated data processing pipeline (Maldjian, et al., 2008). Quantitative data processing includes data cleaning (removal of individual images with noise spikes or severe motion artifact), realignment (separately for label and control images), spatial smoothing, and calculation of mean cerebral blood flow (CBF) maps; our methods and experience with this technique has been well documented (Deibler, et al., 2008a,Deibler, et al., 2008b,Deibler, et al., 2008c,Johnston, et al., 2013,Maldjian, et al., 2009,Maldjian, et al., 2008,McGehee, et al., 2012,Pollock, et al., 2008a,Pollock, et al., 2008b,Pollock, et al., 2009a,Pollock, et al., 2009b,Pollock, et al., 2008c,Pollock, et al., 2011,Pollock, et al., 2009c,Tan, et al., 2009,Watts, et al., 2013). The CBF maps were normalized to MNI space by coregistering to the T1 structural data using SPM8, and then combining this transformation matrix with the parameters computed in the VBM8 normalization procedure, allowing derivation of the gray matter cerebral blood flow (GMCBF) measure analyzed.

White Matter Lesion (WML) Segmentation—WML segmentation was performed using the lesion segmentation toolbox (LST) (Schmidt, et al., 2012) for SPM8 at a threshold (k) of 0.25. We previously validated the LST for use in the DHS-Mind in a sample of 100 subjects against expert manual segmentation, as well as identifying the optimum threshold in this population (Maldjian, et al., 2013). Normalization to MNI space was accomplished by coregistration with the structural T1 and applying the normalization parameters computed in the VBM8 segmentation procedure. The total WMLV measure used in these analyses was determined by summing the binary lesion maps and multiplying by the voxel volume.

2.3. Genotyping

Genotyping in the DHS has been described in detail previously (Cox, et al., 2014). Candidate SNPs previously associated with neuroimaging measures in other cohorts were investigated to determine their relationship with the neuroimaging measures available in the DHS. Genotype data for individual SNPs was obtained from several genetic datasets available in the DHS derived from (i) the Affymetrix Genome-wide Human SNP Array 5.0 (Affymetrix, CA, USA) (GWAS set; predominately common variants), (ii) the Illumina Infinium Human Exome Beadchip v1.0 (Illumina, CA, USA) (Exome set; predominately low-frequency and rare coding variants), and (iii) GWAS Imputed data (Imputed set) imputed from the 1,000 Genomes Project SNPs using IMPUTE2 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html) and the Phase I v2, cosmopolitan (integrated) reference panel, build 37 (Howie, et al., 2009). Genotype data for rs429358, in *APOE*, was not available from the array-based datasets and was directly genotyped using the MassARRAY SNP Genotyping System as described previously (Buetow, et al., 2001,Cox, et al., 2013).

For the GWAS set, exclusion criteria for SNP performance included a SNP call rate <95% (n=11,085), Hardy-Weinberg Equilibrium p-value $<1\times10^{-6}$ (n=344), and minor allele frequency <0.01 (n=57,382); 371,951 SNPs were retained for analysis. For the imputed set, SNPs that were used for imputation were required to have low missingness (<5%) and show no significant departure from Hardy-Weinberg expectations ($p>1\times10^{-4}$). Only imputed SNPs with a confidence score >0.90 and information score >0.50 were used, with a total of ~4.5 million SNPs passing imputation quality control. For the Exome set, exclusion criteria for SNP performance included SNP call rate <99% (n=972), monomorphic SNPs (n=157,754) and Hardy-Weinberg Equilibrium p-value $<1\times10^{-6}$ (n=26); 88,480 SNPs were retained for analysis.

2.4. Statistical Analyses

To determine the contribution of genetic factors to these neuroimaging phenotypes, heritability was estimated in family members using Sequential Oligogenic Linkage Analysis Routines (SOLAR) version 6.5.8 (Texas Biomedical Research Institute, TX, USA). SOLAR performs a variance components analysis of family data where the total phenotypic variation is partitioned into genetic and non-genetic sources of variation. For both the heritability analysis and genetic association analysis, neuroimaging measures were transformed to approximate the normality assumptions of the analysis if necessary. The natural logarithm of TBV and (total WMLV+1) and the square root of GMCBF were used. For the heritability analysis, if residual kurtosis remained, an inverse normal transformation was also used. This additional transformation was needed for GMCBF, WMMD, GMMD, and GMFA. The significance of the heritability ($\hat{h}^2$) estimates was obtained by likelihood ratio tests. Three models were developed that incorporated an increasing number of covariates to determine the extent that genetic factors contribute to variation in neuroimaging measures independent of other confounding variables. The first model was an unadjusted model; the second model was adjusted for age and sex; the third model was adjusted for age, sex, and T2D affected status, with additional adjustment for ICV for all WMLV, GMV, and WMV heritability analyses.

Targeted genetic association analyses were first performed for a set of 17 candidate SNPs, and genome-wide discovery analyses were next performed using the entire GWAS and Exome data sets. All single SNP association analyses were performed using variance components methods implemented in SOLAR version 6.4.1 (Texas Biomedical Research Institute, San Antonio, TX) to account for relatedness between subjects (Almasy and Blangero, 1998). Association was examined assuming an additive model of inheritance. For single variant analyses for both the GWAS set and the Exome set, association results based on a single observation of the rare allele and all associations with X and Y chromosome variants were excluded from the results. Gene-based tests of polymorphic exonic variants from the Exome set were also performed using the sequence kernel association test (SKAT) program with default weights using minor allele frequency. SKAT is a variance components based test that aggregates weighted test statistics for all variants in a gene which is applicable to family data for continuous traits, incorporating a kinship matrix into the models (Chen, et al., 2013,Lee, et al., 2013). Age, sex and T2D affected status were included as covariates in all analyses; ICV was also included as a covariate in analyses of

WMLV, GMV, and WMV. For the candidate SNPs statistical significance was accepted at $p<0.0029$ based on a Bonferroni correction for 17 SNPs tested. For the discovery analyses, genome-wide significance was accepted at the conventional level of $p<5\times10^{-8}$ for analysis of the GWAS set, and significance was accepted at $p<5.65\times10^{-7}$ for single variant analyses and at $p<4.84\times10^{-6}$ for the gene-based analyses for the Exome set based on a Bonferroni correction.

3. RESULTS

The goal of this study was to assess heritability and genetic associations for MRI-based neuroimaging measures in the T2D enriched DHS-Mind study. The clinical characteristics of the 465 individuals of European descent included in the study are summarized in Table 1. Risk factors including hypertension, high body mass and history of prior CVD events are prevalent, as might be expected in a T2D enriched cohort.

Heritability was first calculated for all neuroimaging phenotypes. All neuroimaging phenotypes analysed were significantly heritable ($\hat{h}^2$=0.55–0.99 in unadjusted models, $1.8\times10^{-15}<p<2.5\times10^{-5}$). Most heritability estimates were somewhat attenuated but remained significant upon adjustment for age, sex, and T2D affected status. However, some heritability estimates increased, notably that for TBV, upon further adjustment for these potential confounders.

A total of 17 candidate SNPs (from 16 genes/regions; Table 3) associated with neuroimaging phenotypes in prior studies were selected for targeted association analysis. Top associations from relevant GWAS studies were included in this analysis (Fornage, et al., 2011,Furney, et al., 2011,Lopez, et al., 2012), but the focus was on potentially functional coding polymorphisms previously associated with neuroimaging phenotypes (Braskie, et al., 2011,Chiang, et al., 2011,Godin, et al., 2009,Honea, et al., 2009,Jahanshad, et al., 2012,Kim, et al., 2013,Kohannim, et al., 2012,Kohara, et al., 2003,Penke, et al., 2010,Schmidt, et al., 2000,Schmidt, et al., 2001,Smith, et al., 2010,Sprooten, et al., 2011). The selected candidate SNPs were not associated with any of the neuroimaging phenotypes assessed in the DHS-Mind cohort, with no SNP reaching a Bonferroni corrected p-value threshold of p=0.0029. The strongest observed association was between rs903027, an intronic variant in CLVS1, and WMV (p=0.011). Nominal associations were also observed between rs1799945, a missense variant in HFE, and WMV (p=0.035) and rs1042714, a coding variant in the adrenoceptor beta 2 gene (ADRB2), and TBV (p=0.031).

Exploratory genome-wide analyses were performed using genotype data from the GWAS set, which contains mostly common, non-coding variants. No SNPs were associated with any of the neuroimaging phenotypes of interest at a traditional genome-wide significance threshold of 5×10^{-8}; however, there were multiple loci with nominal evidence of association (Figures S1A–S1I). The top 50 SNPs associated with each of the neuroimaging phenotypes assessed are included in Tables S1A–S1I. The most significant association was for rs10065017 with WMLV ($p=2.06\times10^{-7}$); this intergenic SNP is in weak linkage disequilibrium ($r^2>0.1$) with Homo sapiens early B-cell factor 1 (*EBF1*). This association of the *EBF1* locus with WMLV is also supported by an additional 12 SNPs

($1.69\times10^{-6}<p<6.20\times10^{-5}$). Several genes had multiple associated variants in or nearby a gene locus of potential relevance to the imaging phenotypes assessed, including 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*; five SNPs with GMCBF ($3.30\times10^{-5}<p<6.20\times10^{-5}$)), neuregulin 3 (*NRG3*; four SNPs associated with TBV ($2.10\times10^{-5}<p<7.00\times10^{-5}$)), cyclin-dependent kinase 8 (*CDK8*; five SNPs with WMFA ($5.90\times10^{-5}<p<1.16\times10^{-4}$), two SNPs associated with TBV ($p=1.26\times10^{-4}$ and $p=2.20\times10^{-4}$)), zinc finger and BTB domain containing 16 (*ZBTB16*; three SNPs with GMMD ($5.67\times10^{-6}<p<8.50\times10^{-5}$)), and thrombospondin, type I, domain containing 7A (*THSD7A*; three SNPs with GMFA ($4.30\times10^{-5}<p<7.10\times10^{-5}$), one SNP with GMCBF ($p=5.20\times10^{-5}$)).

Additional analyses were also completed using the Exome set, an array-derived set of approximately 88,000 polymorphic variants, most of them uncommon, potentially functional coding variants. Manhattan plots for each neuroimaging phenotype (Figures S2A–S2I) reveal that few variants were associated with the traits of interest at a level consistent with the Exome set array corrected p-value threshold (5.65×10^{-7}, $\alpha=0.05$). One missense variant (rs150706952, A432V) in pleckstrin homology domain containing, family G (with RhoGef domain) member 4B (*PLEKHG4B*) was significantly associated with WMMD ($p=3.66\times10^{-7}$) and GMMD ($p=2.14\times10^{-7}$). Other variants with potentially interesting functions were also identified as nominally associated with the neuroimaging phenotypes, for example two missense variants in *ZNF224* (rs2068061, M118V, and rs4239529, H162L) associated with WMLV ($p=8.71\times10^{-6}$). The top 50 SNPs from single variant analyses are displayed in Tables S2A–S2I.

Analysis of all genes with two or more polymorphic exonic variants (n=10,341 genes) in the Exome set was also performed using the SKAT program. The top 25 associated genes from SKAT analyses are displayed in Tables S3A–S3I. Some functionally interesting genes were nominally significant in these analyses, for example MAS-related GPR, member E (*MRGPRE*) with WMMD ($p=7.89\times10^{-6}$), CCR4-NOT transcription complex, subunit 6 (*CNOT6*) with GMCBF ($p=2.98\times10^{-5}$), and triggering receptor expressed on myeloid cells 2 (*TREM2)* with TBV (3.91×10^{-4}).

4. DISCUSSION

Given the acceleration of age-related cognitive decline and increased risk of overt dementia previously observed for individuals affected by T2D, determining which individuals with T2D are at higher risk for these comorbidities is of interest (Reijmer, et al., 2010). Neuroimaging phenotypes may aid in assessing the pathogenesis and progress of these cognitive declines and may be helpful as endophenotypes for identifying individuals at increased genetic risk (Ge, et al., 2012,Gottesman and Gould, 2003). To this end, the current study pursued genetic analysis of multiple neuroimaging phenotypes in the T2D-enriched DHS-Mind sample. All neuroimaging measures assessed were significantly heritable in the DHS-Mind cohort. However, candidate SNPs previously associated with neuroimaging phenotypes were not strongly replicated in the DHS-Mind, but our exploratory analyses of genome-wide genetic data revealed potentially interesting genetic risk loci that may

influence traits including TBV, GMV, WMV, WMLV, GMCBF, and FA and MD of the gray and white matter.

The family-based recruitment strategy for the DHS allowed us to assess the heritability of neuroimaging phenotypes in this cohort. A number of twin studies in the general population have previously calculated very high heritability for TBV (66–97%), with GMV and WMV also found to have estimated heritability greater than 70% (Blokland, et al., 2012,Peper, et al., 2007). Heritability of WMLV has been estimated to be between 55–71% in twin and family studies (Atwood, et al., 2004,Carmelli, et al., 1998,Turner, et al., 2004). Similarly, heritability estimates for white matter integrity as assessed by FA are generally high, with reported heritability of 52% for global WMFA in a large family study (Kochunov, et al., 2010), and high heritability of regional white matter FA also reported (Chiang, et al., 2009). The heritability of white matter MD and gray matter CBF, FA and MD has been less studied; one small twin study pointed to significant genetic influences on MD in specific brain regions during development (Chen, et al., 2009), and another study found significant heritability for regional differences in MD asymmetry (Jahanshad, et al., 2010), but to our knowledge no large scale studies of the heritability of global measures of MD, GMCBF and GMFA have been performed. However, these measures are correlated with more commonly examined neuroimaging measures, and we had hypothesized that high heritability estimates would be observed for these measures as well.

Consistent with this prior literature, heritability estimates were quite high for all neuroimaging measures assessed, with estimates remaining significant upon adjustment for the potential confounders of age, sex, and T2D affected status. Some heritability estimates, such as the estimate of 1 for TBV in models adjusted for age, sex, and T2D, are most likely inflated due to model instability at high heritability levels; however, this does not impact our essential conclusion that all neuroimaging measures assessed had a significant heritable component. Metabolic disease and associated comorbidities present in a T2D enriched cohort did not appear to confound the heritable component of these neuroimaging phenotypes in DHS-Mind.

The 17 candidate polymorphisms analysed here were drawn from several studies, including prior GWAS analyses of TBV, white matter hyperintensities and WMFA (Fornage, et al., 2011,Furney, et al., 2011,Lopez, et al., 2012), but our focus was on putatively functional coding polymorphisms (Braskie, et al., 2011,Chiang, et al., 2011,Godin, et al., 2009,Honea, et al., 2009,Jahanshad, et al., 2012,Kim, et al., 2013,Kohannim, et al., 2012,Kohara, et al., 2003,Penke, et al., 2010,Schmidt, et al., 2000,Schmidt, et al., 2001,Smith, et al., 2010,Sprooten, et al., 2011). In DHS-Mind, this set of candidate SNPs was not significantly associated with the neuroimaging phenotypes assessed. Three nominal associations were observed. The strongest was with rs903027, an intronic variant in CLVS1, with WMV ($p=0.011$); this variant was selected due to its nominal association ($p=6 \times 10^{-6}$) with whole brain volume in a GWAS study of MRI atrophy measures (Furney, et al., 2011). Nominal association was also observed between a missense variant in HFE (rs1799945, H63D), selected due to its prior associations with WM FA (Jahanshad, et al., 2012), and WMV ($p=0.035$), and between a missense variant in ADRB2 (rs1042714, E27Q), selected due to previous associations with regional changes in WM FA, as well as differences in cognitive

aging (Penke, et al., 2010), and TBV (p=0.031). One candidate locus of note is APOE; this locus has been associated with an elevated risk of Alzheimer's disease (AD) (Schipper, 2011), the rate of age-related cognitive decline (De Jager, et al., 2012), cognition in the general population (Izaks, et al., 2011), and multiple neuroimaging phenotypes. For example, in individuals without cognitive impairment, APOE genotype has been associated with reduced WMFA (Smith, et al., 2010), regional differences in CBF (Kim, et al., 2013), and WMLV (Godin, et al., 2009), though some of these associations are based on very small sample sizes. However, in our study, neither of the SNPs which indicate an individual's APOE haplotype, rs7412 and rs429358, were associated with any of the neuroimaging phenotypes assessed, nor was there association with the APOE haplotype. The small sample sizes of many of the previous genetic association studies, publication bias, variability in neuroimaging methods and phenotypes, variability in cohort ascertainment and confounding by environmental factors may all be contributing to our inability to replicate the associations of these selected candidate polymorphisms with neuroimaging measures in the DHS-Mind.

Exploratory analysis of genome-wide data from both the GWAS set, containing mostly common, non-coding variants, and the Exome set, containing mostly rare and low frequency coding variants, allowed us to further investigate potential genetic contributions to variation in neuroimaging phenotypes in T2D. While no variants from the GWAS set were associated with our neuroimaging measures at a traditional genome wide significance threshold ($p=5\times10^{-8}$), which is not unexpected given the sample size, nominal associations with several functionally interesting loci were identified. The most significantly associated SNP in these analyses implicated the *EBF1* locus as associated with WMLV, an association supported by an additional 12 SNPs in the top 50 association signals for WMLV. The transcription factor EBF1 regulates the commitment of cells to a B cell lineage and B cell function; EBF1 also regulates cell differentiation and neural migration during mouse brain development (Garel, et al., 2000,Garel, et al., 1999,Hagman, et al., 2012).

Several other loci supported by multiple SNPs in analyses of the GWAS set also have interesting functional roles with potential relevance to our neuroimaging phenotypes. NRG3, associated with TBV, has been implicated in neurodevelopment and in the function of the adult brain, with variants in the *NRG3* locus previously associated with developmental delay, cognitive impairment, autism, and schizophrenia(Kao, et al., 2010). CDK8, implicated in GWAS analyses of WMFA and TBV, is an oncogenic transcriptional regulator which is involved in cell cycle progression, WNT/beta-catenin signalling and other important pathways (Galbraith, et al., 2010); expression of CDK8 and its binding partner cyclin C is increased in the astrocytes of AD patients versus controls (Ueberham, et al., 2003). ZBTB16, a zinc finger transcription factor expressed in the brain which is protective against glutamate toxicity *in vitro* in human neuronal cells and is downregulated in experimentally induced stroke (Seidel, et al., 2011), was associated with GMMD. HMGCR, associated with GMCBF, is the rate-limiting enzyme for cholesterol synthesis; this locus has known impacts on total cholesterol and LDL cholesterol levels, which are risk factors for atherosclerosis and could thereby influence CBF (Claus, et al., 1998,Teslovich, et al., 2010). Lastly, THSD7A, a protein which may promote endothelial cell migration and tube formation during neuroangiogenesis (Kuo, et al., 2011), was associated with GMFA.

Analysis of the Exome set using both single variant and gene-based analyses also revealed interesting loci. A missense variant (rs150706952, A432V) in the *PLEKHG4B* locus was associated with WMMD and GMMD in the single variant analysis of the Exome set. Little is known about PLEKHG4B, but it is expressed in the adult and fetal brain (Kikuno, et al., 1999). A number of genes and variants which did not meet our strict significance thresholds also highlighted loci of potential interest in furthering understanding of brain structure and function. For example, TREM2, associated with TBV, has putative antiinflammatory functions in the brain, and a missense variant in this gene (rs75932628, R47H) was reported to be compellingly associated with risk of AD, with an OR of 2.90 (Jonsson, et al., 2013). MRGPRE, a locus associated with WMMD, is expressed in sensory neurons (Dong, et al., 2001) and was implicated in a recent GWAS study of brain lesions in multiple sclerosis (Gourraud, et al., 2013). Variants in CNOT6, a component of the CCR4-NOT complex which has been implicated in regulation of the differentiation of neural stem cells, were associated with GMCBF (Chen, et al., 2011). ZNF224, which contains two common missense variants associated with WMLV, is a brain-expressed transcriptional repressor with potential roles in regulating carbohydrate oxidation; another polymorphism in this gene (rs3746319, K640E) was previously associated with quantitative measures of global cognitive performance and AD pathology in the Religious Orders Study (Lupo, et al., 2011,Shulman, et al., 2010). These findings in the DHS-Mind support further genetic analysis of these loci in additional cohorts, in particularly other cohorts of patients affected by type 2 diabetes.

5. CONCLUSIONS

In conclusion, the current study observed high heritability of neuroimaging measures such as white matter lesion volume, brain volume, and fractional anisotropy in a T2D enriched cohort with significant metabolic and vascular disease. This study failed to replicate the association of previously identified candidate polymorphisms with neuroimaging measures such as TBV and WMLV. However, exploratory analyses using genome-wide sets of both common, mostly noncoding variants and less common coding variants revealed a number of biologically interesting loci that may be interesting targets for future analysis. While variants identified in these exploratory analyses were not replicated in additional T2D enriched cohorts, these results provide a useful starting point for future research and highlight loci to target in future analyses. This study in DHS-Mind provided a unique opportunity to examine genetic contributions to neuroimaging measures in a T2D enriched population at elevated risk for cognitive decline and dementia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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We examined the contribution of genetic factors to neuroimaging measures.

This analysis was performed in a cohort enriched for type 2 diabetes.

All neuroimaging measures assessed were significantly heritable.

Candidate SNPs did not associate with the neuroimaging measures assessed.

Variants associated with mean diffusivity were identified.

Table 1

Demographic characteristics of the 465 DHS-Mind participants with genotyping data.

	Mean ± SD or %	Median (range)
Demographics		
Age (years)	67.54 ± 8.92	67.56 (41.25 –89.21)
Sex (% female)	57.2%	
BMI (kg/m^2)	30.93 ± 5.87	30.11 (17.56 –57.68)
% smoking (current or past)	53.9%	
Hypertension (%)	88.4%	
Self-reported history of prior CVD	29.5%	
Type 2 Diabetes		
Type 2 diabetes affected (%)	74.4%	
Diabetes duration (years)	16.37 ± 6.60	14.23 (4.93 –44.32)
Glucose (mg/dl)	131.97 ± 47.94	119 (40 –349)
Hemoglobin A1C (%)	7.06 ± 1.33	6.8 (4.9 –14.8)
Medications		
Anti-diabetic medication	63.8 %	
Cholesterol-lowering medication	52.8 %	
Anti-hypertensive medication	72.5 %	
Education		
Less than high school	18.1%	
High school	53.3%	
Greater than high school	28.6%	
Cognition and Neuroimaging Measures		
Modified Mini Mental State Exam (3MSE)	90.54 ± 6.98	92 (62 –100)
Gray matter volume (GMV) (cc)		
White matter volume (WMV) (cc)		
Total brain volume (TBV) (cc)	1085.4 ± 112.6	1071.5 (775.0–1447.9)
Intracranial volume (ICV) (cc)	1340.7 ± 139.6	1326.9 (985–1758.8)
Fractional anisotropy gray matter (GMFA)	0.206 ± 0.016	0.206 (0.151 –0.255)
Fractional anisotropy white matter (WMFA)	0.356 ± 0.022	0.357 (0.290 –0.419)
Mean diffusivity gray matter (GMMD)	1.091 ± 0.094	1.090 (0.800 –1.376)
Mean diffusivity white matter (WMMD)	0.787 ± 0.051	0.789 (0.630 –0.951)
Total white matter lesion volume (WMLV) (cc)	4.29 ± 6.82	1.62 (0–59.57)
Gray matter cerebral blood flow (GMCBF) (mL/100g of tissue/min)	47.08 ± 19.35	44.15 (5.25 –134.93)

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Table 2

Heritability estimates for MRI imaging variables in related individuals from the Diabetes Heart Study Cohort.

Covariates	TBV	GMV	WMV	GMFA	WMFA	GMMD	WMMD	WMLV	GMCBF
None									
$\hat{h}^2$ (SE)	0.82 (0.11)	0.79 (0.12)	0.63 (0.12)	0.57 (0.13)	0.61 (0.11)	0.99 (0.11)	0.96 (0.11)	0.71 (0.12)	0.55 (0.14)
p-value	1.9×10^{-15}	2.8×10^{-10}	3.7×10^{-9}	2.0×10^{-7}	1.1×10^{-9}	1.8×10^{-14}	1.8×10^{-15}	9.3×10^{-10}	2.5×10^{-5}
Age, sex									
$\hat{h}^2$ (SE)	1.00	0.67 (0.12)	0.62 (0.12)	0.49 (0.13)	0.64 (0.11)	0.68 (0.12)	0.84 (0.11)	0.61 (0.13)	0.35 (0.14)
p-value	2.4×10^{-25}	2.7×10^{-9}	1.3×10^{-8}	9.6×10^{-6}	1.0×10^{-10}	9.1×10^{-9}	1.4×10^{-13}	2.0×10^{-7}	3.2×10^{-3}
Age, sex, T2D†									
$\hat{h}^2$ (SE)	1.00	0.66 (0.12)	0.65 (0.12)	0.50 (0.13)	0.64 (0.11)	0.73 (0.12)	0.85 (0.11)	0.61 (0.13)	0.31 (0.14)
p-value	6.8×10^{-25}	1.7×10^{-9}	3.7×10^{-9}	7.2×10^{-6}	1.5×10^{-10}	9.5×10^{-10}	6.7×10^{-14}	3.0×10^{-7}	0.01

TBV=total brain volume; GMV=gray matter volume; WMV= white matter volume; GMFA= fractional anisotropy gray matter; WMFA= fractional anisotropy white matter; GMMD= mean diffusivity gray matter; WMMD= mean diffusivity white matter; WMLV = total white matter lesion volume; GMCBF= gray matter cerebral blood flow

†WMLV, GMV, and WMV heritability additionally adjusted for intracranial volume (ICV). Note that the natural logarithm of TBV and (total WMLV+1) and the square root of GMCBF were used, with an inverse normal transformation also used for GMCBF, WMMD, GMMD, and GMFA.

Table 3

Genetic association (assuming an additive model of inheritance) between candidate SNPs and neuroimaging measures.

						DHS Analysis	Association p-values (covariates: age, sex, T2D affected status)										
Chr	**Pos**	**SNP**	**Gene**	**Location**	**Source**	**Alleles (maj/min)**	**MAF**	**TBV**	**WMV**	**GMV**	**GMFA**	**WMFA**	**GMMD**	**WMMD**	**WMLV†**	**GMCBF**	**Reference**
1	11856378	rs1801133	MTHFR	Exonic	Exome	G/A	0.333	0.356	0.661	0.840	0.976	0.236	0.328	0.155	0.346	0.823	(Kohara, et al., 2003)
1	48321221	rs946836	TRABD2B	Intronic	Imputed	C/T	0.285	0.371	0.746	0.150	0.472	0.588	0.575	0.646	0.421	0.628	(Lopez, et al., 2012)
1	156848918	rs6336	NTRK1	Exonic	Exome	G/A	0.076	0.422	0.318	0.862	0.315	0.113	0.380	0.326	0.923	0.717	(Kohannim, et al., 2012)
1	230845794	rs699	AGT	Exonic	Exome	A/G	0.396	0.594	0.886	0.758	0.239	0.288	0.429	0.414	0.965	0.946	(Schmidt, et al., 2001)
1	232144598	rs821616	DISC1	Exonic	Exome	A/T	0.273	0.701	0.948	0.435	0.158	0.785	0.524	0.626	0.195	0.408	(Sprooten, et al., 2011)
3	126993099	rs9871760	C3orf56	Downstream	Exome	C/A	0.241	0.868	0.823	0.835	0.233	0.567	0.509	0.837	0.224	0.440	(Furney, et al., 2011)
5	148206473	rs1042714	ADRB2	Exonic	Exome	G/C	0.413	**0.031**	0.559	0.180	0.937	0.878	0.239	0.336	0.848	0.095	(Penke, et al., 2010)
6	26091179	rs1799945	HFE	Exonic	Exome	C/G	0.169	0.838	**0.035**	0.630	0.988	0.575	0.728	0.422	0.298	0.773	(Jahanshad, et al., 2012)
7	94946084	rs854560	PON1	Exonic	Exome	A/T	0.370	0.085	0.335	0.559	0.959	0.997	0.563	0.550	0.361	0.257	(Schmidt, et al., 2000)
8	27464519	rs11136000	CLU	Intronic	Exome	G/A	0.412	0.119	0.825	0.902	0.132	0.415	0.911	0.962	0.294	0.617	(Braskie, et al., 2011)
8	62409428	rs903027	CLVS1	Intronic	Exome	A/C	0.169	0.837	**0.011**	0.164	0.641	0.404	0.555	0.093	0.734	0.536	(Furney, et al., 2011)
10	17142526	rs6602175	CUBN	Intronic	Exome	A/C	0.269	0.177	0.815	0.229	0.984	0.804	0.735	0.998	0.609	0.889	(Furney, et al., 2011)
11	27679916	rs6265	BDNF	Exonic	Exome	G/A	0.201	0.423	0.745	0.553	0.674	0.636	0.169	0.305	0.575	0.478	(Chiang, et al., 2011)
17	73872948	rs1055129	TRIM47	Intronic	Exome	A/G	0.281	0.386	0.202	0.816	0.415	0.726	0.835	0.318	0.264	0.178	(Fornage, et al., 2011)
19	45411941	rs429358	APOE	Exonic	Genotyped	T/C	0.047	0.842	0.661	0.891	0.831	0.140	0.991	0.355	0.892	0.824	(Godin, et al., 2009,Kim, et al., 2013,Smith, et al., 2010)
19	45412079	rs7412	APOE	Exonic	Exome	G/A	0.080	0.430	0.728	0.536	0.169	0.148	0.803	0.672	0.494	0.811	(Godin, et al., 2009,Kim, et al., 2013,Smith, et al., 2010)
			APOE haplotype					0.560	0.518	0.724	0.556	0.097	0.868	0.339	0.926	0.812	(Godin, et al., 2009,Kim, et al., 2013,Smith, et al., 2010)
22	19951271	rs4680	COMT	Exonic	Exome	G/A	0.473	0.853	0.721	0.957	0.488	0.485	0.990	0.551	0.507	0.190	(Honea, et al., 2009)

TBV=total brain volume; GMV=gray matter volume; WMV= white matter volume; GMFA= fractional anisotropy gray matter; WMFA= fractional anisotropy white matter; GMMD= mean diffusivity gray matter; WMMD= mean diffusivity white matter; WMLV = total white matter lesion volume; GMCBF= gray matter cerebral blood flow

†WMLV, GMV, WMV analyses additionally adjusted for intracranial volume (ICV).