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Heritability and genetic association analysis of neuroimaging measures in the Diabetes Heart Study

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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 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 were explored. All neuroimaging measures analyzed 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 ($p = 3.66 \times 10^{-7}$) and gray matter mean diffusivity ($p = 2.14 \times 10^{-7}$). This analysis suggests genetic factors contribute to variation in neuroimaging measures in a population enriched for metabolic disease and other associated comorbidities.

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 with nondiabetic 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 et al., 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 matter volume (GMV) and white

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matter volume (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), although 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 epidemiologic 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 data set 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 nondiabetic siblings enrolled whenever possible. Ascertainment and recruitment have been described in detail previously (Bowden et al., 2006, 2010; 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 to 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 reexamined 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 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 before participation.

2.2. Neuroimaging

2.2.1. Magnetic resonance image acquisition

MRI 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 (repetition time [TR] = 7.36 ms; echo time [TE] = 2.02 ms; time to inversion [TI] = 600 ms; flip angle [FA] = 20 degrees; 124 slices, field of view [FOV] = 24 cm, matrix size = 256 × 256, 1.5-mm-slice thickness). Fluid-attenuated inversion recovery 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 = 16,000; 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, 28 ms; TI_1, 800 ms; TI_1s, 1200 ms; TI, 2000 ms; TR, 3000 ms; 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, 0-mm slice gap); and frequency encoding direction anterior and/or posterior. A bipolar diffusion gradient with an equivalent b value of 5.25 mm^2/s was added to suppress intraarterial spins (Yang et al., 1998).

2.2.2. 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 (nonlinear 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.

2.2.3. Diffusion tensor processing

Diffusion tensor preprocessing 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 (http://www.nitrc.org/projects/dtitk). DTI scalar metrics, including FA and