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Obesity gene *NEGR1* associated with white matter integrity in healthy young adults

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ABSTRACT

Obesity is a crucial public health issue in developed countries, with implications for cardiovascular and brain health as we age. A number of commonly-carried genetic variants are associated with obesity. Here we aim to see whether variants in obesity-associated genes – *NEGR1*, *FTO*, *MTCH2*, *MC4R*, *LRRN6C*, *MAP2K5*, *FAIM2*, *SEC16B*, *ETV5*, *BDNF-AS*, *ATXN2L*, *ATP2A1*, *KCTD15*, and *TNN13K* – are associated with white matter microstructural properties, assessed by high angular resolution diffusion imaging (HARDI) in young healthy adults between 20 and 30 years of age from the Queensland Twin Imaging study (QTIM). We began with a multi-locus approach testing how a number of common genetic risk factors for obesity at the single nucleotide polymorphism (SNP) level may jointly influence white matter integrity throughout the brain and found a wide spread genetic effect. Risk allele rs2815752 in *NEGR1* was most associated with lower white matter integrity across a substantial portion of the brain. Across the area of significance in the bilateral posterior *corona radiata*, each additional copy of the risk allele was associated with a 2.2% lower average FA. This is the first study to find an association between an obesity risk gene and differences in white matter integrity. As our subjects were young and healthy, our results suggest that *NEGR1* has effects on brain structure independent of its effect on obesity.

Introduction

Obesity is a major public health issue facing developed countries. In the United States over a third of adults are classified as obese, and another third are considered to be overweight (Ogden et al., 2012). Obesity has well-established links to serious health issues such as diabetes, heart disease, and premature death (Must et al., 1999). High body mass index (BMI)[1] in midlife is linked to poorer cognitive functioning in old age (Fitzpatrick et al., 2009; Walther et al., 2009). Greater BMI is associated with lower brain volume (Walther et al., 2009; Ward et al., 2005; Taki et al., 2008), brain atrophy (Gustafson et al., 2004), and lower gray matter density (Pannacciulli et al., 2006), and neuronal and myelin abnormalities (Gazdzinski et al., 2010). Obesity is associated with abnormalities in white matter volume (Haltia et al., 2007; Raji et al., 2009), diffusivity (Alkan et al., 2008) and integrity across many brain regions (Stanek et al., 2009; Verstynen et al., 2012; Xu et al., 2013). These brain differences in obese people may be attributable to a less healthy diet and lifestyle, which negatively affect brain health (Molteni et al., 2002; Northstone et al., 2012; Ars, 2012). They may be partly due to genetic variants with joint effects on the brain and obesity risk. A gene may directly affect the brain, and its effects on appetite and physical activity could affect obesity. Alternatively, a gene could affect vascular health, reducing cerebral blood flow, and therefore delivery of oxygen and nutrients to the brain, with concomitant effects on brain function.

Diet and lifestyle are the most readily identifiable causes of obesity, yet it is highly heritable (Wardle et al., 2008), and genetic vulnerabilities interact with lifestyle factors. A number of genes have been repeatedly associated with obesity in cohorts worldwide (Frayling et al., 2007; Loos et al., 2008; Ng et al., 2012; Okada et al., 2012; Wen et al., 2012). We previously found that elderly carriers of the *FTO* risk allele had

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[1] Body mass index is a ratio of weight to height, intended as an approximate but readily computed assessment of fat mass. The equation to calculate BMI (in SI units) is BMI = mass (kg) / (height (m))^2.

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lower frontal and occipital lobe volumes (Ho et al., 2010), and a recent paper found that a locus near the obesity risk gene *MC4R* was associated with increased amygdalar, hippocampal, and medial orbitofrontal volume, as well as differences in eating behaviors (Horstmann et al., 2013). The obesity risk gene *Taq1A* has been associated with decreased striatal activation in response to receiving chocolate (Stice et al., 2008). Recent genome-wide association studies (GWAS) identified a number of loci associated with BMI (Speliotes et al., 2010; Thorleifsson et al., 2009; Willer et al., 2008).

Axonal integrity is vital for efficient brain function; well-myelinated tracts propagate signals quickly, but poor or impaired myelination can decrease the speed or reliability of neuronal transmission (Purves et al., 2001). FA is a widely accepted measure of white matter integrity, and evaluates the degree to which water diffuses along the primary direction of the axon rather than across it. Lower FA has been found in many diseases, such as Alzheimer's disease, multiple sclerosis, epilepsy, and many neuropsychiatric diseases (Ciccarelli et al., 2008). Genetic variants have also been discovered that may affect white matter integrity as measured by FA. Associations have been reported between FA and a number of genetic variants, including polymorphisms in *CLU*, *HFE*, *NTRK1*, and many other genes (Braskie et al., 2011; Jahanshad et al., 2012; Braskie et al., 2012). These are genes that are already closely tied to cognitive function or neuropsychiatric disorders.

Here we investigated whether 16 common variants in obesity-related genes (*NEGR1*, *FTO*, *MTCH2*, *MC4R*, *LRRN6C*, *MAP2K5*, *FAIM2*, *SEC16B*, *ETV5*, *BDNF-AS*, *ATXN2L*, *ATP2A1*, *KCTD15*, and *TNN13K*) relate to the brain's white matter integrity. We selected our SNPs based on three recent GWAS studies of obesity all with large sample sizes (Speliotes et al., 2010; Thorleifsson et al., 2009; Willer et al., 2008). Using a multi-locus approach to assess their combined effect, we tested whether obesity-related variants might predict differences in white matter integrity assessed using high angular resolution diffusion imaging (HARDI) (Kohannim et al., 2012). As a post-hoc test, we evaluated the most promising SNP (single nucleotide polymorphism) driving the effects in the multi-locus model. Analyses were completed in 499 healthy young adults (aged 20–30), to test if there was any evidence of a link between obesity-related genetic variants and white matter integrity. While the global incidence of obesity in developed countries is typically close to 30% (Ogden et al., 2012), our population was healthy with a lower obesity incidence, with around 6% obese and 20% overweight. Therefore, we did not map the effects of this biased population's BMI on the brain. Rather, we were interested in determining whether common genetic variants, which play a subtle role in obesity, and are also common in the general healthy population, continued to show effects on white matter integrity. We expected that variants associated with increased risk of obesity would be associated with lower white matter integrity.

Materials and methods

Participants

Participants were recruited as part of a 5-year project research project examining healthy Australian twins with structural MRI and diffusion-weighted imaging (de Zubicaray et al., 2008). Our analysis included 499 right-handed subjects (326 females/173 males, mean age = 23.8, SD = 2.5 years, range = 20–30 years). This sample included 163 monozygotic (MZ) twins, 274 dizygotic (DZ) twins, and 62 non-twin siblings, from 309 families. This information is summarized in Table 1, along with BMI information for each group. A histogram of BMI is shown in Fig. 1. All QTIM subjects were Caucasian, and ancestry outliers, defined as individuals more than 6 SD from the PC1/PC2 centroid after principal components analyses of the GWAS data (Medland et al., 2009), were excluded. Gene allele frequencies can differ between ethnicities, as can the risks associated with various alleles, so ethnically homogenous groups are generally preferred in genetic studies.

Table 1
Subject demographics. Number of subjects, female/male, mean BMI (body mass index) and standard deviation, across all genetic groups.

Genetic group	QTIM Subjects		
	N	F/M	BMI
AA	188	125/63	23.1 (3.80)
AG	233	154/79	23.4 (3.64)
GG	78	47/31	23.6 (3.97)

Additionally, the three published studies (Speliotes et al., 2010; Thorleifsson et al., 2009; Willer et al., 2008) – which we used to select our SNPs of interest – were analyses of sampled populations that were 99.7% Caucasian (one of the studies Thorleifsson et al., 2009 included a very small number of African American subjects as well).

Scan acquisition

Whole-brain anatomical and high angular resolution diffusion images (HARDI) were collected with a 4 T Bruker Medspec MRI scanner. T1-weighted anatomical images were acquired with an inversion recovery rapid gradient echo sequence. Acquisition parameters were: TI/TR/TE = 700/1500/3.35 ms; flip angle = 8°; slice thickness = 0.9 mm, with a 256 × 256 acquisition matrix. HARDI was also acquired using single-shot echo planar imaging with a twice-refocused spin echo sequence to reduce eddy-current induced distortions. Imaging parameters were: 23 cm FOV, TR/TE 6090/91.7 ms, with a 128 × 128 acquisition matrix. Each 3D volume consisted of 55 2-mm thick axial slices with no gap, and 1.79 × 1.79 mm^2 in-plane resolution. 105 images were acquired per subject: 11 with no diffusion sensitization (i.e., T2-weighted b_0 images) and 94 diffusion-weighted (DW) images (b = 1159 s/mm^2) with gradient directions evenly distributed on a hemisphere in the q-space. Scan time for the 105-gradient HARDI scan was 14.2 min.

Establishing zygosity and genotyping

Zygosity was objectively established by typing nine independent DNA microsatellite polymorphisms (polymorphism information content >0.7), using standard PCR methods and genotyping. Results were crosschecked with blood group (ABO, MNS, and Rh), and phenotypic data (hair, skin, and eye color), giving an overall probability of correct zygosity assignment >99.99%, and these were subsequently confirmed by GWAS. Genomic DNA samples were analyzed on the Human610-Quad BeadChip (Illumina) according to the manufacturer's protocols (Infinium HD Assay; Super Protocol Guide; Rev. A, May 2008). We imputed to Hapmap3. Information on the imputation protocols and quality control steps may be found at http://enigma.ini.usc.edu/wp-content/uploads/2010/09/ImputationProtocolsv1.2.pdf.

Diffusion tensor image (DTI) processing

Non-brain regions were automatically removed from each T1-weighted MRI scan using ROBEX (Iglesias et al., 2011) a robust brain extraction program trained on manually "skull-stripped" MRI data and FreeSurfer (Fischl et al., 2004), and from a T2-weighted image from the DWI set, using the FSL tool "BET" (Smith, 2002; FMRIB Software Library, http://fsl.fmrib.ox.ac.uk/fsl/). Intracranial volume estimates were obtained from the full brain mask, and included cerebral, cerebellar, and brain stem regions. All T1-weighted images were linearly aligned using FSL *flirt* (with 9 DOF) (Jenkinson et al., 2002) to a common space (Holmes et al., 1998) with 1 mm isotropic voxels and a 220 × 220 × 220 voxel matrix. Raw diffusion-weighted images were corrected for eddy current distortions using the FSL tool, "eddy_correct". For each subject, the eddy-corrected images with no diffusion sensitization were averaged (11 images), linearly aligned and resampled to a downsampled version