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Schizophrenia risk gene *ZNF804A* does not influence macroscopic brain structure: an MRI study in 892 volunteers

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The single nucleotide polymorphism (SNP) rs1344706 in the *ZNF804A* gene was the first SNP to reach unequivocal genome-wide significance for schizophrenia, but little is known about how it confers susceptibility. Here, we looked for neuroanatomical correlates of rs1344706, and of other SNPs across the gene, in a large structural MRI data set of healthy young adults, using brain volumetry and voxel-based morphometry (VBM). Neither rs1344706 nor the other SNPs, individually or in combination, affected any volumetric or VBM parameter. As such, the association of *ZNF804A* with psychosis seems unlikely to be mediated through an influence of rs1344706 or any other SNP on macroscopic brain structure.

Schizophrenia and bipolar disorder are highly heritable disorders. Data from genome-wide association studies (GWAS) provide compelling evidence for association to the *ZNF804A* (zinc finger protein 804A) gene, at chromosome 2q32.1. Association to schizophrenia was originally reported in 2008,[1] and a recent meta-analysis of >21 000 cases and 38 000 controls found an odds ratio (OR) of 1.10, $P=2.5\times10^{-11}$ for schizophrenia, and OR 1.11, $P=4\times10^{-13}$ for schizophrenia and bipolar disorder combined.[2] The genetic signal arises predominantly from a single, intragenic SNP, rs1344706[3] or possibly a haplotype that includes it.[2] The roles of *ZNF804A* and the functional correlates of rs1344706 are unclear, although the latter is predicted to create a transcription factor-binding site and to affect gene expression of *ZNF804A*.[4]

Several studies have used neuroimaging to examine whether rs1344706 impacts upon brain structure. Lencz *et al.*[5] showed in 39 healthy volunteers that carriers of the risk allele (A) of rs1344706 had significantly larger total white matter but reduced gray

Table 1 Brain volumes according to rs1344706 genotype

Genotype	*C/C*	*C/A*	*A/A*	P-*value*
N	*160*	*434*	*297*	
Frequency	*0.18*	*0.49*	*0.33*	
Accumbens	0.67 (0.12)	0.69 (0.12)	0.68 (0.11)	0.86
Amygdala	2.15 (0.39)	2.14 (0.36)	2.14 (0.37)	0.52
Brainstem	19.23 (2.32)	19.39 (2.32)	19.22 (2.33)	0.37
Caudate nucleus	5.45 (0.66)	5.52 (0.61)	5.52 (0.58)	0.45
Gray matter	829.9 (79.57)	838.0 (71.49)	838.0 (77.80)	0.27
Globus pallidus	1.88 (0.23)	1.89 (0.21)	1.89 (0.21)	0.84
Hippocampus	5.33 (0.68)	5.37 (0.64)	5.31 (0.63)	0.35
Putamen	6.21 (0.70)	6.24 (0.69)	6.23 (0.72)	0.82
Total brain volume	1304 (129.1)	1317 (113.4)	1314 (120.1)	0.70
Thalamus	11.43 (1.20)	11.55 (1.08)	11.44 (1.12)	0.32
White matter	474.1 (56.74)	479.5 (51.22)	476.2 (51.81)	0.56

Values are mm^3, mean (s.d.). Genotype data missing for one subject.

matter volumes in several regions comprising the 'default mode network'. Other studies reported that the risk allele affected volumes of the amygdala and hippocampus in 70 patients but not in 38 healthy controls,[6] and cortical thickness was reduced in the anterior cingulate, posterior cingulate and superior temporal gyrus in 62 healthy volunteers, with no changes in white matter tracts.[7] A Chinese study found decreased white matter density in the left prefrontal lobe of 69 healthy risk allele carriers.[8] However, all these studies were limited in terms of their sample sizes and no *ZNF804A* SNPs other than rs1344706 were examined.

Here, we studied the effects of *ZNF804A* on brain structure in a sample of 892 healthy young adults participating in the Brain Imaging Genetics (BIG) study (see Supplementary Table 1).[9] Of these, 422 volunteers were scanned at 1.5 Tesla (T) and 470 at 3T. We implemented two analysis methods—volumetry and VBM—to look at the effects of rs1344706, and the individual and combined effects of 266 other SNPs in *ZNF804A*. The genetic data were available from an Affymetrix Genome-Wide Human SNP Array 6.0 and a subsequent imputation step using HapMap2 as reference sample (CEU data, NCBI build 36, UCSC hg18, http://www.sph.umich.edu/csg/abecasis/MaCH/download/). The gene-wide analysis method used a statistical approach as described by Hoh *et al.*[10] where the analysis consisted of a SNP-by-SNP linear regression and the estimation of the effect of the complete gene on the volumes. The single SNP and gene-wide effects were assessed for gray matter, white matter and total brain volume, as well as eight circumscribed brain structures: nucleus accumbens, amygdala, brainstem, caudate nucleus, globus pallidus, hippocampus, putamen and thalamus. With VBM, we looked for genetic effects on regional differences across the whole brain in both the 1.5T and the 3T groups using a full-factorial ANCOVA with age, sex, scan protocol and total brain volume as covariates. To increase statistical sensitivity, we applied small volume corrections for regions of interest (dorsolateral prefrontal cortex, hippocampus, amygdala, anterior cingulate cortex and posterior cingulate cortex). See Supplementary Methods for details of imaging methods and genetic analysis.

The volumetry results for rs1344706 are presented in Table 1. They reveal no effects on either total brain volume, gray or white matter, or the regions of interest measured (all $P > 0.05$).

Similarly, VBM revealed no effects of rs1344706, either in the whole-brain analysis or in any of the regions of interest (Supplementary Table 2). Results for the gene-wide approach were also negative (Supplementary Tables 3–5). We also compared rs1344706 AA homozygotes versus CC homozygotes; again, there was no influence on any brain measure analysed (data not shown).

In summary, we have shown that genetic variation in *ZNF804A*, including the genome-wide significant psychosis risk allele rs1344706, does not affect total or regional brain volumes in healthy young adults. Compared with previous reports, we studied a far larger sample, used both volumetry and VBM and also conducted a gene-wide approach. The method has been shown previously to be effective for the detection of genetic effects on MRI volumes in the BIG database.[9] Together, our results strongly suggest that rs1344706 in particular, and sequence variation in *ZNF804A* in general, do not impact upon macroscopic brain structure. While we cannot rule out such effects occurring in childhood or in clinical populations, it is more likely that any pathophysiological correlates of genetic variation in *ZNF804A* occur via modulation of brain function or connectivity.

Conflict of interest

The authors declare no conflict of interest.

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Oxytocin receptor (OXTR) is not associated with optimism in the Nurses' Health Study

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The oxytocin system is implicated in complex social behavior such as socioemotional functioning, social recognition and bonding, and in modulating biological responses to stress.[1,2] The oxytocin receptor (*OXTR*) gene encodes the OXTR; a G-protein-coupled receptor that mediates the effects of the neurohormone oxytocin.[3,4] Prior studies have reported associations between an intronic single-nucleotide polymorphism (rs53576) of *OXTR* and various stress-related and psychological traits.[4,5] Most recently, Saphire-Bernstein *et al*[6] reported that rs53576 A allele carriage conferred lower optimism relative to G homozygotes, among 344 men and women aged 18–36 years of various ethnicities. We sought to replicate the association between *OXTR* rs53576 and optimism in 1229 women from the Nurses' Health Study (NHS).

The NHS was established in 1976 when 121 700 women registered as nurses aged 30–55 years from 11 US states completed a mailed questionnaire on medical history and lifestyle characteristics.[7] Every 2 years, follow-up questionnaires are sent. The 2004 and 2008 questionnaires included information on antidepressant use and the revised life orientation test,[8] the same dispositional optimism measure used in the Saphire-Bernstein *et al*.[6] study. We generated five different optimism scores to provide a thorough investigation of the previously reported association. Year-specific optimism scores were derived by taking the mean of all non-missing items in 2004 ($\alpha = 0.77$) and also in 2008 ($\alpha = 0.75$). Individuals with >2 items missing were excluded (up to 8% of analytic sample). Using both assessments, an overall mean optimism score was also derived, as were two subscale scores reflecting an 'optimistic' or 'pessimistic' outlook.[8] Genotypes for the current analysis were available from two case-control nested genome-wide association studies, initially designed to assess kidney stone disease and glaucoma (total $n = 1294$).[9,10] Samples were genotyped for *OXTR* rs53576 at the Broad Center for Genotyping and Analysis using the Illumina 610Q or 660Q array (Illumina, San Diego, CA, USA). Genotyping success rate for rs53576 was $>98\%$.[9,10] Principal components analyses using genome-wide data were conducted to assess self-reported race. Genetically inferred non-Caucasian samples were too few ($<3\%$ of analytic sample) for meaningful analysis and therefore excluded.

The 2004 and 2008 optimism scores were moderately correlated ($r = 0.65$) in the 1229 women with complete genetic and phenotype data (2004 mean age = 71.3 years, s.d. = 6.7). Overall optimism scores ranged from 1.33 to 4.25 (overall mean = 4.13, s.d. = 0.68). Frequencies (%) of the GG, GA and AA genotypes were 491 (40%), 559 (45%) and 179 (15%), respectively, and did not depart from Hardy–Weinberg equilibrium ($P = 0.33$). The A allele frequency was 38% in NHS versus 28% among white participants ($n = 87$) in the Saphire-Bernstein *et al*.[6] study.

Table 1 indicates no significant differences in means across genotypes for any optimism scores (all $P_{ADD} > 0.35$). Optimism scores also did not differ between G homozygotes and A carriers (all $P_{DOM} > 0.17$). Linear regression models adjusting for age, genotype platform, and case-control status yielded no significant association between rs53576 and any optimism score, regardless of genetic model (all