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ORIGINAL RESEARCH

Altered regional brain volumes in elderly carriers of a risk variant for drug abuse in the dopamine D2 receptor gene (*DRD2)*

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Abstract Dopamine D2 receptors mediate the rewarding effects of many drugs of abuse. In humans, several polymorphisms in *DRD2*, the gene encoding these receptors, increase our genetic risk for developing addictive disorders. Here, we examined one of the most frequently studied candidate variant for addiction in *DRD2* for association with brain structure. We tested whether this variant showed associations with regional brain volumes across two independent elderly cohorts, totaling 1,032 subjects. We first examined a large sample of 738 elderly participants with neuroimaging and genetic data from the Alzheimer's Disease Neuroimaging Initiative (ADNI1). We hypothesized that this addiction-related polymorphism would be associated with structural brain differences in regions previously implicated in familial vulnerability for drug dependence. Then, we assessed the generalizability of our findings by testing this polymorphism in a non-overlapping replication sample of 294 elderly subjects from a continuation of the first ADNI project (ADNI2) to minimize the risk of reporting false positive results. In both cohorts, the minor allele—previously linked with increased risk for addiction—was associated with larger volumes in various brain regions implicated in reward processing. These findings suggest that neuroanatomical phenotypes associated with familial vulnerability for drug dependence may be partially mediated by *DRD2* genotype.

Keywords Neuroimaging genetics · Dopamine D2 receptors · *DRD2* gene · Elderly brain structure · Structural MRI

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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Introduction

A large body of work has implicated dopamine in the etiology of drug addiction. Drugs of abuse produce their effects by interacting with many receptors in the brain, but, for many drugs, their effects on the activity of dopaminergic brain reward circuits are critical for their addictive properties (Wise 2004). The *DRD2* gene encodes the dopamine D2 receptor, and numerous preclinical animal studies suggest that these receptors mediate the rewarding effects of many addictive drugs (Le Foll et al. 2009). In human genetic studies, several single nucleotide polymorphisms (SNPs) in the *DRD2*

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gene have shown associations with drug abuse, and a few candidate variants have been the focus of most investigations.

A *DRD2* polymorphism commonly studied in relation to drug addiction lies at the rs1076560 locus. The minor A allele is more prevalent in addicts, including alcoholics (Lucht et al. 2010; Noble et al. 2000; Sasabe et al. 2007), opiate abusers (Doehring et al. 2009) and heavy smokers (Morton et al. 2006). This rs1076560 polymorphism is involved in emotional (Blasi et al. 2009) and cognitive (Nieoullon 2002) processes, and also relates to personality traits (Frank and Hutchison 2009; Koehler et al. 2011). Further, this particular variant is associated with differences in many neurological phenotypes–including EEG oscillations (Koehler et al. 2011), functional connectivity (Blasi et al. 2009; Sambataro et al. 2013), and functional brain activation during working memory (Bertolino et al. 2009, 2010; Zhang et al. 2007)–emotion processing (Blasi et al. 2009), and motor tasks (Fazio et al. 2011).

Several recent investigations suggest that the familial susceptibility for drug dependence may be associated with increased volumes in limbic and striatal structures, including the hippocampus, amygdala, and putamen (Ersche et al. 2012a, b, 2013). As many cellular processes in these brain regions are mediated by dopamine receptors, and given the large body of evidence linking the rs1076560 locus with elevated risk for substance abuse, we hypothesized that elderly carriers of the risk allele might show an altered pattern of regional brain volumes consistent with this brain phenotype. We first tested our hypothesis in a large sample of elderly subjects (n=738). Then, we assessed the generalizability of our findings by testing this polymorphism in a non-overlapping replication sample (n=294). The use of two separate samples was intended to reduce the risk of reporting false positive results.

Materials and methods

Subjects

Data used in preparing this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The initial goal of ADNI was to recruit 800 subjects but ADNI was followed by ADNI-GO and ADNI2; to date, these three projects have recruited over 1,500 adults, ages 55 to 90, consisting of cognitively normal older individuals, people with early or late MCI, and people with early AD. The follow-up duration for each group is specified in the protocols for ADNI1, ADNI2 and ADNI-GO. Subjects originally recruited for ADNI1 and ADNI-GO had the option to be followed in ADNI2, and new subjects were also recruited. For up-to-date information, see www.adni-info.org.

Here, we analyzed two independent samples of elderly subjects with neuroimaging and genome-wide genetic data from the ADNI1 and ADNI2 cohorts. In what follows, we refer to ADNI-GO and ADNI2 participants as ADNI2, as the only distinction was the grant funding mechanism that supported the data collection ("GO" denotes the "Grand Opportunity" award mechanism of the U.S. National Institutes of Health), and the data collection protocol was identical for them both. All ADNI studies are conducted according to the Good Clinical Practice guidelines, the Declaration of Helsinki, and U.S. 21 CFR Part 50 (Protection of Human Subjects), and Part 56 (Institutional Review Boards). Written informed consent was obtained from all participants before protocol-specific procedures were performed. To avoid the known effects of population stratification on genetic analysis (Lander and Schork 1994), we only included non-Hispanic Caucasian subjects identified by self-report and confirmed by multi-dimensional scaling (MDS) analysis (as in Stein et al. 2010) for both ADNI cohorts.

ADNI1

The ADNI1 cohort included three diagnostic groups: people with AD, MCI, and healthy elderly (cognitively normal) participants. We included participants from all diagnostic groups, as power is limited when performing any genetic association analysis. Typical effects of candidate genes on the phenotype are often around 1 % of the mean value per allele (Stein et al. 2012), so we have often been able to pick up effects only when ADNI's full sample is included. Even so, we have been able to replicate effects from ADNI in other non-overlapping samples, showing that effects found in ADNI can be robust and can generalize to different cohorts (Hibar et al. 2013; Roussotte et al. 2013; Stein et al. 2011). Effect sizes for individual genetic variants on brain structure in particular are expected to be small, so the genetic analysis would be underpowered if we further subdivided the sample. Our final analysis comprised 738 individuals (average age±s.d.=75.53±

6.78 years; 438 men/300 women) including 173 AD, 359 MCI, and 206 healthy participants (Table 1).

ADNI2

The ADNI2 cohort included an additional diagnostic group. To more precisely capture the cognitive status of elderly participants, people with MCI were further subdivided into participants with early-stage and late-stage mild cognitive impairment (EMCI and LMCI). At the time we conducted these analyses, just under 300 ADNI2 subjects had been genotyped and processed using tensor-based morphometry (see Minimal deformation target (MDT) and tensor based morphometry (TBM) section). Our final analysis comprised 294 individuals (average age±s.d.=73.16±7.33 years; 166 men/128 women) including 25 AD, 66 LMCI, 81 EMCI, and 122 healthy participants (Table 2).

Genotyping and SNP selection

In ADNI, genome-wide association study (GWAS) data was collected from 1,252 participants. All 818 subjects (including the non-Caucasians not used in this study) from the ADNI1 sample were genotyped using the Illumina Human 610-Quad BeadChip (San Diego, CA, USA), and DNA samples were genotyped from 434 ADNI-GO/ADNI2 participants using the Illumina OmniExpress genotyping array.

Data from both cohorts were separately imputed to a common reference set of genetic variants: the 1,000 Genomes CEU (Caucasian) reference set following freely available imputation protocols (ENIGMA2 2012, enigma.ini.usc.edu). The imputed data were filtered using standard quality criteria (imputation quality: Rsq<0.3) and minor allele frequency (MAF<0.05). The final, filtered genetic datasets were used for our analyses. We analyzed a common variant (C>A, minor allele frequency: A=0.215) previously implicated in drug abuse in the dopamine D2 receptor gene (rs1076560), for association with regional brain volumes in both ADNI cohorts.

Table 1 Demographic and genetic data for the **ADNI1** cohort

ADNI 1	Males	Females	Total
Total	438	300	738
Healthy Elderly	112	94	206 (28 %)
MCI	231	128	359 (49 %)
AD	95	78	173 (23 %)
rs1076560 0 A alleles	303 (69 %)	215 (71 %)	518 (70 %)
rs1076560 1 A alleles	127 (29 %)	80 (27 %)	207 (28 %)
rs1076560 2 A alleles	8 (2 %)	5 (2 %)	13 (2 %)
Mean Age (± sd)	75.90 (± 6.76)	74.98 (± 6.78)	75.53 (± 6.78)

Table 2 Demographic and genetic data for the **ADNI2** cohort

ADNI 2	Males	Females	Total
Total	166	128	294
Healthy Elderly	64	58	122 (41 %)
EMCI	47	34	81 (28 %)
LMCI	38	28	66 (22 %)
AD	17	8	25 (9 %)
rs1076560 0 A alleles	118 (71 %)	91 (71 %)	209 (71 %)
rs1076560 1 A alleles	44 (27 %)	33 (26 %)	77 (26 %)
rs1076560 2 A alleles	4 (2 %)	4 (3 %)	8 (3 %)
Mean Age (± sd)	74.49 (± 7.14)	71.45 (± 7.25)	73.16 (± 7.33)

Image acquisition

ADNI1 subjects were scanned with a standardized MRI protocol developed and evaluated for this cohort (Jack et al. 2008; Leow et al. 2006). Briefly, high-resolution structural brain MRI scans were acquired at 58 sites across North America, using 1.5 T MRI scanners. A sagittal 3D MP-RAGE sequence was used, optimized for consistency across sites (Jack et al. 2008) (TR/TE=2,400/1,000 ms; flip angle=8°; FOV=24 cm; final reconstructed voxel resolution=0.9375×0.9375×1.2 mm^3). Each ADNI2 subject received a 3 T accelerated T1-weighted MRI scan. By vendor, General Electric (GE) scanners use IR-SPGR sequences and Philips and Siemens use MP-RAGE sequences. Details of scan vendors and sequences for the ADNI2 sample may be found online (http://adni.loni.usc.edu/methods/documents/mri-protocols/).

Image correction and pre-processing

For both ADNI samples, image corrections were applied using a processing pipeline at the Mayo Clinic, consisting of: (1) a procedure termed GradWarp to correct geometric distortion due to gradient non-linearity (Jovicich et al. 2006), (2) a "B1-correction", to adjust for image intensity inhomogeneity due to B1 non-uniformity using calibration scans (Jack et al. 2008), (3) "N3" bias field correction, for reducing residual intensity inhomogeneity (Sled et al. 1998), and (4) geometrical scaling, according to a phantom scan acquired for each subject (Jack et al. 2008) to adjust for scanner- and session-specific calibration errors (also see http://adni.loni.usc.edu/methods/mri-analysis/mri-pre-processing/). To adjust for global differences in brain positioning and scale, all subjects' scans were linearly registered to the stereotaxic space defined by the International

Consortium for Brain Mapping (ICBM-53; Mazziotta et al. 2001), using a 9-parameter (9P) transformation (three translations, three rotations, three scales; Collins et al. 1994). For both ADNI cohorts, we used standard trilinear interpolation and resampled the resulting aligned scans to have 1 mm isotropic voxels. Subjects' brain images were not skull-stripped during pre-processing.

Minimal deformation target (MDT) and tensor based morphometry (TBM)

For ADNI1, we created a minimal deformation target (MDT), which serves as an unbiased average template image for automated image registration, and to reduce statistical bias. The MDT was created using the MRI scans of 40 randomly selected healthy elderly subjects, as detailed elsewhere (Hua et al. 2008a, b). The MDT image was calculated as a geometrically centered mean anatomical image, using a method called sKL-MI to align data to an average affine registered target image; this procedure leads to a fairly 'sharp' average brain image for a group and follows a procedure we developed and tested elsewhere (Hua et al. 2008a, b).

To quantify 3D patterns of volumetric tissue variations, all individual T1-weighted images (N=1,032) were non-linearly aligned to the MDT template created for ADNI1 with an inverse-consistent 3D elastic warping technique using a mutual information cost function (Leow et al. 2005). For each subject, a separate Jacobian matrix field was derived from the gradients of the deformation field that aligned that individual brain to the MDT template. The determinant of the local Jacobian matrix was derived from the forward deformation field to characterize local volume differences. Color-coded Jacobian determinants were used to illustrate regions of volume expansion, i.e., those with det J(r)>1, or contraction, i.e., det J(r)<1 (Chung et al. 2001; Freeborough and Fox 1998; Riddle et al. 2004; Thompson et al. 2000) relative to the template. All images were registered to the same template, so these Jacobian maps shared common anatomical coordinates, defined by the normal template. Individual Jacobian maps were retained for further statistical analyses.

Regression of structural brain differences with the candidate SNP

In both ADNI cohorts, we investigated how the rs1076560 variant was associated with regional brain volumes using multiple linear regression to associate the number of minor A alleles (0,1, or 2) with the Jacobian values (describing the amount of brain tissue deficit or excess relative to the standard template) at each voxel in the brain, after covarying for age, sex, and diagnosis (i.e., AD, MCI, and healthy elderly for ADNI1 and AD, LMCI, EMCI, and healthy elderly for ADNI2).

Multiple comparisons correction

Computing thousands of association tests across the brain can introduce a high Type I (false positive) error rate in neuroimaging studies, if not appropriately controlled. To control these errors, we used a searchlight method for false discovery rate (FDR) correction (Langers et al. 2007), which controls the false discovery rate in any reported statistical maps. We implemented this searchlight method to correct the maps of statistical associations between the image phenotype (morphometry) and genotype at the rs1076560 locus. For both cohorts, maps shown are thresholded at the appropriate corrected p-value, after performing searchlight FDR (q=0.05), to show only regions of significance that passed the multiple comparisons correction. For the ADNI2 sample, we further examined statistical associations between rs1076560 genotype and regional brain volumes using a less conservative threshold for multiple comparisons correction (q=0.10), since the genetic analysis may have been underpowered in this much smaller sample.

Results

In the ADNI1 sample, the *DRD2* polymorphism rs1076560 predicted differences in regional brain volumes, after covarying for sex, age, and diagnosis, and after multiple comparisons correction at q=0.05 (Fig. 1, *top panel*). Larger volumes in various brain regions were statistically related to carrying the minor A allele. These included the thalamus, cerebellum, precentral gyrus, lenticular nucleus (putamen and globus pallidus), medial temporal lobes (including the right hippocampus), and occipital association areas. Regional volume increases associated with each additional copy of the minor allele ranged from 2 to 4 %.

In the ADNI2 cohort, regional volume increases associated with the rs1076560 variant were marginally significant, after covarying for sex, age, and diagnosis, and after multiple comparisons correction at q=0.05 (Fig. 1, *middle panel*). Nonetheless, some of the same statistical associations between the minor A allele and larger brain volumes detected in the ADNI1 sample were partially replicated in the ADNI2 cohort in localized regions of the left precentral gyrus and medial temporal lobe, and in parts of the occipital association areas.

Since the ADNI2 sample was smaller and afforded less statistical power to detect small gene effects on the brain, we also examined statistical associations between the minor A allele at the rs1076560 locus and regional brain volumes after covarying for sex, age, and diagnosis, using a more liberal threshold for multiple comparisons correction at q=0.10 (Fig. 1, *bottom panel*). These less conservative maps revealed a more widespread, yet still partial replication of the SNP-brain associations detected in the ADNI1 cohort. In the

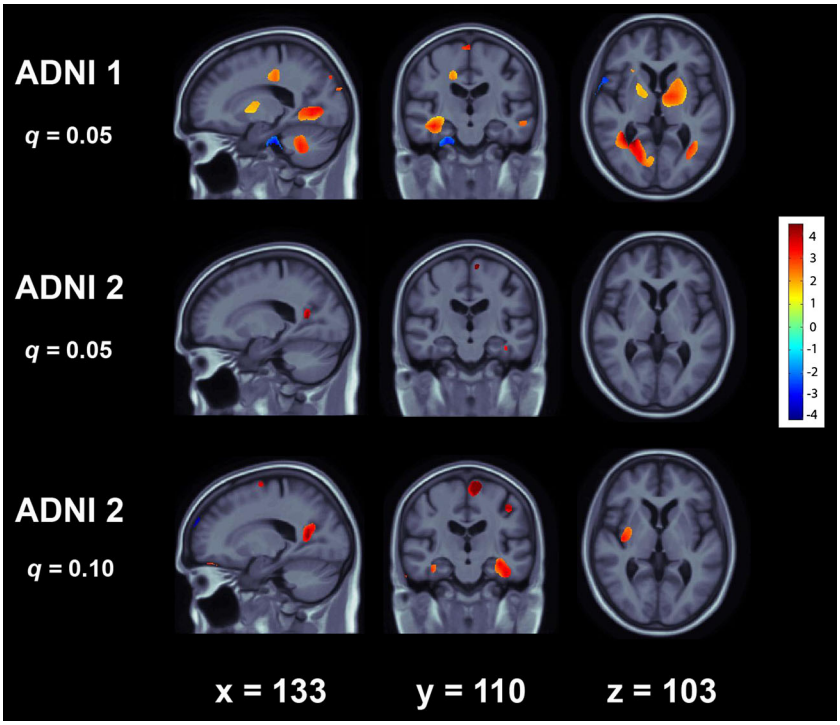

Fig. 1 Effects of the A allele at the rs1076560 locus in the *DRD2* gene on regional brain volumes in the **ADNI1** (*top panel*) and **ADNI2** (*middle* and *bottom panels*) cohorts. Positive beta values (*warm colors*) show regions where the minor A allele was associated with greater tissue volumes. Negative beta values *(cool colors*) show regions where the minor A allele was associated with lower tissue volumes. The color bar encodes the average percentage of volume difference associated with the minor allele, relative to the template. Tests for associations are adjusted for age, sex, and diagnosis; maps are corrected for multiple comparisons with the searchlight false discovery rate (FDR) method at $q=0.05$ (*top* and *middle panels*) and $q=0.10$ (*bottom panel*). Images are in radiological convention (*left side of the brain shown on the right*)

ADNI2 sample, the minor A allele predicted increased volumes in more extensive regions of the left precentral gyrus and medial temporal lobes (including the left hippocampus) and occipital association areas, and the association was also detected in the right putamen.

Discussion

This is the first large-scale study ($n=1,032$) to report an association, in two large non-overlapping cohorts, between brain structure and a commonly carried variant in the dopamine D2 receptor gene. We found that one of the most frequently studied drug addiction candidate variant in *DRD2* (rs1076560) predicted increased tissue volumes in various brain regions. As predicted, these differences in brain structure were generally consistent with the neuroanatomical phenotypes associated with familiar vulnerability for drug dependence (i.e., increased putamen and hippocampal volumes; Ersche et al. 2012a, b, 2013).

Addiction is often thought of as a disorder of reward sensitivity (Volkow et al. 2010). Some of the regions commonly associated with the reward system–such as the mesocortical dopaminergic pathway and the insula–did not appear to be affected by this *DRD2* variant, although in any statistical map, one cannot infer that there is no effect in a location, as the power can be insufficient to detect it if present. Even so, all of the brain regions that did show a significant association with the (minor) risk allele for drug abuse in both cohorts have been implicated in reward processing. The occipital cortex is engaged during reward anticipation (Krebs et al. 2012). The medial temporal lobes are involved in reward-based memory encoding, and activation patterns in the hippocampus discriminate between reward conditions and influence memory through the incorporation of information about motivational contexts into stored memory representations (Wolosin et al. 2013). The precentral gyrus sends extensive projections to the putamen (Kunzle 1975), the putamen projects back to cortical motor areas, and these neural circuits make up the "motor loop" of the basal ganglia (Alexander et al. 1986). In this basal ganglia loop, motor information is subcortically integrated with reward information through dopaminergic signals (Isomura et al. 2013).

The mechanisms by which rs1076560 affects the functioning of dopamine D2 receptors are well understood. Alternative splicing of *DRD2* results in two transcript variants encoding different isoforms: DRD2S (short), considered a presynaptic autoreceptor, and DRD2L (long), typically a postsynaptic

receptor. The minor allele at rs1076560 leads to decreased expression of DRD2S relative to DRD2L (Zhang et al. 2007), so minor allele carriers have reduced expression of presynaptic D2 receptors (Zhang et al. 2007). With fewer autoreceptors, synaptic levels of dopamine are increased, and since postsynaptic D2 receptor expression is dependent upon dopamine inputs, this is likely to result in reduced D2 receptor density in the striatum (or reduced bindings of radioligands; Bertolino et al. 2010).

A decrease in D2 receptor availability is commonly observed in drug abusers (Volkow et al. 1993, 2001, 2007). Further, several PET studies suggest that reduced dopamine D2 receptor availability in the striatum may be a predisposing neurobiological trait for substance dependence (Dalley et al. 2007; Morgan et al. 2002; Nader et al. 2006), rather than just reflecting neuroadaptations secondary to excessive dopaminergic stimulation from repeated drug abuse (Volkow et al. 2004). Mounting evidence suggests that individual differences in *DRD2* expression (which determines dopamine D2 receptor availability) relate to specific behavioral processes that confer a vulnerability to addiction. Specifically, low *DRD2* expression in the striatum predicts increased consumption of abused drugs (Dalley et al. 2007; Nader et al. 2006). These metabolic processes may represent a possible mechanism by which the rs1076560 variant contributes to the behavioral phenotype of addictive disorders.

Elevated levels of synaptic dopamine in carriers of the rs1076560 minor allele, perhaps secondary to a decrease in D2 autoreceptor function, may be responsible for the increased volumes we observed in striatal regions since dopamine can have trophic effects, especially during development (Nieoullon 2002). In addition, a net decrease in D2-mediated signaling resulting from reduced postsynaptic D2 receptor density in the striatum may also play a role in this process, since pharmacological blockade of D2 receptors has been consistently associated with increased striatal volumes in various animal (Benes et al. 1985; Chakos et al. 1998) and human (Corson et al. 1999; Keshavan et al. 1994; Scherk and Falkai 2006) studies.

The interpretation of the mechanisms underlying these findings and the theoretical framework presented here remain speculative. We are not able to provide mechanistic evidence that the rs1076560 variant affect brain volumes through the biological processes we describe. We cannot even conclude with certainty that the rs1076560 SNP is driving the observed associations, as variants in high linkage disequilibrium to this SNP may be responsible for the signals. Even so, the vast literature implicating this particular polymorphism in drug and alcohol abuse (Doehring et al. 2009; Lucht et al. 2010; Morton et al. 2006; Noble et al. 2000; Sasabe et al. 2007; Sasabe and Ishiura 2010), and the similarities between our findings and the neuroanatomical phenotypes associated with familial vulnerability for drug dependence (Ersche et al. 2012a, b, 2013) suggest that this particular variant may indeed be responsible for the brain volume differences reported here.

While the same allele was associated with greater tissue volumes in both samples, the regional replication was incomplete. The SNP-brain associations were sometimes detected in different hemispheres in the two cohorts, notably the right hippocampus in ADNI1 and the left hippocampus in ADNI2. However, this is quite unlikely to indicate a laterality effect. The power in imaging genetics is very low even with the large samples available today, and the most common situation is finding evidence for an effect in the brain using FDR, which does not require any one voxel to show a strong effect. As is usually the case when power is limited, many of the significant voxels after correction for multiple comparisons in the ADNI2 sample did not overlap with the voxels that survived statistical thresholding in the original ADNI1 cohort. In ADNI2, we performed an unbiased search across all voxels of the entire brain without incorporating prior information from our earlier tests in the ADNI1 dataset. The reason for this is that voxels that are significant after correction for multiple comparisons do not necessarily represent the only voxels where this variant may have an effect. Clearly, this is true of any statistical brain map—typically we are only able to detect some of the voxels where there is the strongest evidence a biological effect. We wanted to perform an analysis that allowed for the possibility to observe an effect anywhere in the brain, and SNP effects on brain structure are expected to be small. Thus, it may be that a weak effect is spread over large brain regions in both samples, but due to noise, sample size differences, and biological variability between the two cohorts, different locations in the brain (or similar locations in opposite hemispheres) provide the highest effect sizes to the statistical maps in each cohort. As a result, only a partial replication of the specific localization of the SNP-brain association is achieved on the thresholded maps. The replication of a specific localization of the SNP-brain association is extremely difficult to achieve in sample sizes available today. Finding an association effect at all is at the limit of statistical power in current samples such as ADNI. By using FDR, which does not make a strong hypothesis about regional localization, we are able to pick up a distributed effect. Even so, it would be very hard to implicate the same voxels without substantially more data, as FDR does not require a significant effect in any one voxel, just an aggregate effect on the brain.

An additional limitation is that, because no drug abuse measures were taken in these samples, we cannot directly establish a relationship between this particular variant and addiction, or between brain volumes and addiction in these particular cohorts, although some of these associations have been found in other cohorts. Further, our experimental design does not allow us determine if the variant of interest directly affects the brain or just modifies drug using behaviors that may in turn affect brain structure. Future studies relating drug

consumption measures to brain imaging and genetic data should clarify the direction of these relationships. Despite this limitation, our experimental design enabled us to demonstrate that carriers of an addiction risk allele in older cohorts not enriched in addiction traits show some of the same brain volume abnormalities as the non-dependent siblings of dependent individuals (Ersche et al. 2012a, b, 2013), in several brain regions implicated in reward processing (Isomura et al. 2013; Krebs et al. 2012; Wolosin et al. 2013).

To increase our confidence in these preliminary results, they should be replicated in a non-ADNI elderly sample. It is also important that these findings be replicated in different populations (e.g., young adults, non-Caucasians, etc.) to ascertain whether the SNP-brain associations reported here generalize to other age and ethnic groups, which the present study is unable to determine. ADNI participants may not be a representation of the community as a whole. For example, it is often noted that the ADNI cohort may not have so high an incidence of vascular disease, or co-morbidity as might be represented in the general elderly community. On the other hand, there are also advantages to studying homogenous and well-characterized cohorts like the ADNI samples. First, such cohorts may involve fewer confounding variables than truly random ascertainments intended to represent the broader community as a whole. Large variabilities in age, ethnicity, co-morbidity, and many other factors tend to complicate interpretation of association findings. Moreover, retention rates are relatively high in ADNI compared to other longitudinal investigations. It is not uncommon for certain studies to report attrition rates of 50 % (e.g., the anti-psychotic drug trial reported in Thompson et al. 2009), while the annual attrition rate in ADNI is only around 6 % (Aisen et al. 2010). Some researchers suggest a "5-and-20" rule of thumb, meaning that acceptable rates of attrition are between 5 and 20 % (Schulz and Grimes 2002). Clearly, ADNI rates are near the lower end of this range.

Notably, the individuals in this study are carriers of a "risk" gene, but as far as we know, they did not express the phenotype most commonly associated with the risk gene, namely drug abuse. However, studying gene-brain relationships in unaffected individuals is useful and informative, as it allows one to disentangle the gene effects from the effects of drug use on the brain. It was long thought that the neurological abnormalities in individuals with substance use disorders were the direct results of repeated drug use. Recently, several studies reported that the non-dependent siblings of dependent individuals also showed abnormalities in brain structure, and specific neuroanatomical phenotypes associated with *familial vulnerability* for drug dependence have been described (Ersche et al. 2012a, b, 2013). There is almost certainly a genetic contribution to this familial vulnerability and associated brain phenotypes. Here, we examined a polymorphism in the dopamine D2 receptor gene previously implicated in the propensity for drug abuse in many studies (Doehring et al. 2009; Lucht et al. 2010; Morton et al. 2006; Noble et al. 2000; Sasabe et al. 2007) for association with regional brain volumes in individuals who did not abuse drugs. This design allowed us to discover that non-abusers with a *genetic vulnerability* for drug dependence (i.e., carriers of the allele that is more prevalent in addicts) showed some of the same brain volume abnormalities as individuals who carry a *familial vulnerability* for drug dependence (i.e., the non-dependent siblings of dependent individuals). This suggests that neuroanatomical phenotypes associated with familial vulnerability for drug dependence (such as larger putamen and hippocampal volumes) may be partly mediated by *DRD2* genotype, at least in elderly Caucasians.

Understanding how genetic factors contribute to the susceptibility for substance abuse by affecting the brain independently of drug use is crucial. Such understanding provides an important piece of the puzzle in uncovering the etiology and mechanisms of addiction, and may help inform prevention and treatment. Several variants in the *DRD2* gene can predict the therapeutic response and the side-effect liability associated with various psychiatric medications (David et al. 2007; Hwang et al. 2005; Lawford et al. 1995, 2013; Mrazek 2010). By providing an objective intermediate phenotype between genes and complex multidimensional neuropsychiatric disorders such as addiction, brain structure could offer clues that may help predict the therapeutic outcome of certain medications in particular patients, especially where large epidemiological studies have not yet been conducted. Such neuroanatomical measures may eventually help clinicians make more informed decisions when prescribing psychotropic medications. This line of research may also help develop prevention strategies aimed specifically at carriers of particular genetic risk factors for drug abuse, before they express the behavioral phenotype, with important implications for public health.

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Conflict of interest Paul M. Thompson, Florence F. Roussotte, Neda Jahanshad, and Derrek P. Hibar declare that they have no conflicts of interest.

Informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of the investigation. Informed consent was obtained from all patients for being included in the study.

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