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ZNF804A variants confer risk for heroin addiction and affect decision making and gray matter volume in heroin abusers

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ABSTRACT

Drug addiction shares common neurobiological pathways and risk genes with other psychiatric diseases, including psychosis. One of the commonly identified risk genes associated with broad psychosis has been *ZNF804A*. We sought to test whether psychosis risk variants in *ZNF804A* increase the risk of heroin addiction by modulating neurocognitive performance and gray matter volume (GMV) in heroin addiction. Using case-control genetic analysis, we compared the distribution of *ZNF804A* variants (genotype and haplotype) in 1035 heroin abusers and 2887 healthy subjects. We also compared neurocognitive performance (impulsivity, global cognitive ability and decision-making ability) in 224 subjects and GMV in 154 subjects based on the *ZNF804A* variants. We found significant differences in the distribution of *ZNF804A* intronic variants (rs1344706 and rs7597593) allele and haplotype frequencies between the heroin and control groups. Decision-making impairment was worse in heroin abusers who carried the *ZNF804A* risk allele and haplotype. Subjects who carried more risk alleles and haplotypes of *ZNF804A* had greater GMV in the bilateral insular cortex, right temporal cortex and superior parietal cortex. The interaction between heroin addiction and *ZNF804A* variants affected GMV in the left sensorimotor cortex. Our findings revealed several *ZNF804A* variants that were significantly associated with the risk of heroin addiction, and these variants affected decision making and GMV in heroin abusers compared with controls. The precise neural mechanisms that underlie these associations are unknown, which requires future investigations of the effects of *ZNF804A* on both dopamine neurotransmission and the relative increases in the volume of various brain areas.

Keywords Decision making, genotype, gray matter volume, haplotype, heroin addiction, *ZNF804A* variants.

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INTRODUCTION

Opioid dependence is a relapsing psychiatric disorder characterized by withdrawal, tolerance and a compulsive drive to take drugs despite serious adverse consequences, including various health and social problems (Leshner 1997). Long-term addiction to opioids is associated with pathologic changes in brain functional circuits that regulate how a person interprets and behaviorally responds to motivationally relevant stimuli (Kalivas & O'Brien 2008). Identifying factors that drive relapse following abstinence remains one of the major challenges for clinicians and researchers.

Twin and family studies indicate that genetic risk factors may account for 40–60 percent of the etiology of addictive disorders (Uhl 2004). Epidemiologic studies

strongly suggest that genetic factors operate at all stages of addiction, including vulnerability to initiation, continued use and the propensity to become dependent (Kreek *et al.* 2005). These genetic vulnerabilities may reflect measurable neurobiology and personality traits related to reward and dependence (Robbins & Everitt 1999).

High comorbidity exists between addiction with psychiatry disorders, especially schizophrenia and bipolar disorder (Regier *et al.* 1990; Batel 2000). Drug addiction is related to other psychiatric diseases through genetically influenced common neurobiological pathways that modulate reward, behavioral control and stress responses (Goldman, Oroszi & Ducci 2005). Genetic association studies have shown overlap between addiction and psychiatric disorders (Hong *et al.* 2011; Lee *et al.* 2013).

The first risk gene that was identified as having a genome-wide significance for both schizophrenia and bipolar disorder was the zinc finger protein 804A gene (*ZNF804A*; O'Donovan *et al.* 2008; Purcell *et al.* 2009). *ZNF804A* is widely expressed in human brain neurons and has diverse interactions with genes related to dopaminergic transmission, such as the catechol-*O*-methyltransferase and dopamine receptor 2 genes, which are also critical for opioid reward and addiction (Demetrovics *et al.* 2010; Girgenti, LoTurco & Maher 2012; Martinez *et al.* 2012; Bernstein *et al.* 2014). Hence, the first aim of the present study was to examine the association between *ZNF804A* variants and heroin addiction in a sample of Han Chinese.

Endophenotypes may be more correlated than diagnostic phenotypes with the genetic liability to disease (Almasy & Blangero 2001). The detection of gene effects at the level of intermediate endophenotypes offers the opportunity to identify gene–environment interactions and understand the influence of environmental exposure (Goldman *et al.* 2005). Neuroimaging and cognitive traits have been used as intermediate endophenotypes to relate genetics to psychiatric disorders (Gottesman & Gould 2003). One recent neuroimaging study found that a *ZNF804A* variation affects gray matter volume (GMV) of the anterior insula (Nenadic *et al.* 2014) and plays a crucial role in conscious urges to take drugs and decision-making processes that involve uncertain risk and reward (Naqvi & Bechara 2009). Variants in *ZNF804A* that are associated with the risk for psychotic disorders also have significant effects on neurocognition (Balog, Kiss & Keri 2011; Van Den Bossche *et al.* 2012), which has been strongly linked with heroin addiction (Pau, Lee & Chan 2002). Therefore, the second aim of the present study was to test whether *ZNF804A* variants in heroin abusers are associated with neurocognitive performance (i.e. impulsivity, global cognitive ability and decision-making ability) and GMV.

METHODS AND MATERIALS

Subjects

We collected genetic samples from 1035 heroin abusers (726 men, 309 women; mean age, 35.67 ± 7.78 years) in drug addiction treatment centers who met the criteria for heroin dependence of the Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV), but did not have any other substance dependence history other than opioid drug use for not more than 1 month or other kinds of addictive drug use not more than three times, with the exception of nicotine (based on patient records and self-reported information). We also collected genetic samples from 2887 healthy controls (1313 men, 1574 women; mean age, 31.63 ± 9.70 years) who were recruited from the community through newspaper advertisements. Alcohol abusers were excluded based on the Michigan Alcoholism Screening Test. None of the heroin abusers received systemic pharmacologic substitution treatments at the time of this study. Only ethnic Han volunteers whose parents were natives of southern China were recruited. The participants had no past or current major medical conditions (e.g. cardiovascular, endocrinologic, oncologic or autoimmune diseases) or personal or family history of major psychiatric disorders diagnosed by the Structured Clinical Interview for DSM-IV Axis I disorders. The subjects did not use any prescription or over-the-counter medications within 2 weeks prior to enrollment in the study.

The neurocognitive subsample only came from drug addiction treatment centers and local communities in Zhongshan city, Guangdong province, China (121 male heroin abusers and 103 male controls). In addition to the requirement for genetic samples, the selection criteria for the neurocognitive subsample also included a level of education higher than primary school and an ability to understand Mandarin to ensure they could complete the test. The participants who underwent magnetic resonance imaging (MRI) were screened from the neurocognitive subsample. Subjects were excluded from MRI if they were left-handed or had contraindications for MRI, such as claustrophobia, dentures, head trauma and metal implants. A total of 76 male heroin abusers and 78 healthy male subjects were included in the statistic analysis of the MRI data.

The study was approved by the Peking University Research Ethics Board. All of the subjects were informed of the entire procedure and potential risks before signing a written informed consent form.

Genotyping

Genomic DNA samples were extracted from approximately 200 µl peripheral blood samples using the

QIAamp DNA Mini Kit (Qiagen, Inc., Valencia, CA, USA). Genotype was determined using the Sequenom MassArray system (Sequenom iPLEX assay, San Diego, CA, USA). Locus-specific polymerase chain reaction (PCR) and detection primers were designed using MassARRAY Assay Design 3.0 software (Sequenom, San Diego, CA, USA). The DNA samples were amplified by multiplex PCR reactions, and the PCR products were used for locus-specific single-base extension reactions. The resulting products were desalted and transferred to a 384-element SpectroCHIP array. Allele detection was performed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. The mass spectrograms were analyzed using MassARRAY TYPER software (Sequenom).

We focused on the following common polymorphic markers in *ZNF804A* that were previously associated with psychiatric disorders. The single-nucleotide polymorphism (SNP) rs1344706 in *ZNF804A* was the first genome-wide supported variant for the risk of schizophrenia and bipolar disorder in both Europeans and Asians (O'Donovan *et al.* 2008; Steinberg *et al.* 2011; Xiao *et al.* 2011). The rs7597593 SNP was associated with the strongest risk across multiple psychiatric disorders in a large-scale collaborative panel (Cross-Disorder Group of the Psychiatric Genomics Consortium 2013). We also selected some candidate tag SNPs in the coding exons that are close to the 3′ end of *ZNF804A*. Our total target SNPs included two synonymous SNPs in the intron (rs7597593 and rs1344706) and four missense SNPs in the coding region (rs12476147, rs4667001, rs1366842, and rs12477430). The total SNP span length sampled >80 percent of the *ZNF804A* gene sequence length.

MRI acquisition and image processing

Images were obtained using a GE Signa EXCITE 1.5T TwinSpeed MRI scanner at Zhongshan Traditional Chinese Medicine Hospital (Zhongshan, China). T1-weighted, sagittal three-dimensional images were acquired with spoiled gradient recalled echo sequence with coverage of the entire brain (1-mm slice thickness, 7.816 ms repetition time, 2.984 ms echo time, 450 ms inversion time, 13° flip angle, 256×256 acquisition matrix, 256×256 mm^2 field of view, number of averages = 2). T2-weighted images were also acquired to exclude subjects with a clinically abnormal brain structure.

Structural data were analyzed using voxel-based morphometry (Ashburner & Friston 2000) with FSL-VBM 4.1.4 (FMRIB Software Library – voxel based morphometry, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM; accessed January 13, 2015). Structural images were first brain-extracted using the Brain Extraction Tool (Smith 2002). Brain tissue segmentation was then performed using FAST (FMRIB's Automated Segmentation Tool; Zhang, Brady & Smith 2001). The resulting gray matter (GM) partial volume images were aligned to MNI-152 standard space using FLIRT (FMRIB's Linear Image Registration Tool; Jenkinson & Smith 2001), followed by non-linear registration using FNIRT (FMRIB's Nonlinear Registration Tool). The resulting images were averaged to create a study-specific template, to which the native GM images were then non-linearly re-registered. The registered partial volume images were modulated to correct for local expansion or contraction by dividing by the Jacobian of the warp field. The modulated GMV was smoothed with an isotropic Gaussian kernel with a sigma of 3 mm, corresponding to a full width at half maximum of 7 mm. The final voxel size for the smoothed images was $2 \times 2 \times 2$ mm^3. The cerebellum was excluded from the image analyses.

Neurocognitive performance assessments

We evaluated neurocognitive performance using four measures: (1) Montreal Cognitive Assessment (MoCA) to assess global cognitive ability; (2) Barratt Impulsiveness Scale (BIS-11) to determine the level of impulsivity; (3) Iowa Gambling Task (IGT) to evaluate decision-making ability; and (4) visual analog scale to assess self-reported average heroin craving during the past week. Additional details of the behavioral assessments are provided in the Supporting Information.

Statistic analysis

Deviation of the genotype counts from Hardy–Weinberg equilibrium was tested using a chi-square goodness-of-fit test. Pairwise linkage disequilibrium (LD) analysis was applied to detect inter-marker relationships. Case-control association analysis was performed using Haploview 4.1 (http://www.broad.mit.edu/mpg/haploview/; accessed January 13, 2015; Barrett *et al.* 2005). Individual haplotype information was acquired using Arlequin (Excoffier & Lischer 2010). Bonferroni corrections for multiple tests were performed to exclude type I errors. For the haplotype analyses, 10 000 permutation tests were performed to control for false positive results using Haploview. The results were considered significant at a two-tailed $P < 0.05$.

The effects of genetics and disease state on neurocognitive performance and GMV were analyzed using two-way analysis of variance (ANOVA), with cigarettes smoked and age as covariates. The data were analyzed using the Statistical Package for the Social Sciences (SPSS) 16.0 software (SPSS, Inc., Chicago, IL, USA). The characteristics of the subjects were analyzed using one-way ANOVA. We performed partial correlation analyses between GMV and behavioral performance, with age and cigarette use as regressors. *Post hoc* analyses were

Table 1 Allele distribution of *ZNF804A* single-nucleotide polymorphisms in heroin abusers and healthy controls.

SNP	*Position*	*Allele 1/2*	*HWE* P *value*	*MAF case*	*MAF control*	χ^2	p_{origin}	$p_{corrected}$	*OR*	*95 percent CI*
rs7597593	185533580	A/G	0.8214	0.4186	0.3866	6.51	0.06438	0.0207	1.142	1.03–1.27
rs1344706	185778428	A/C	0.7769	0.5116	0.4746	8.353	0.0231	0.0081	1.16	1.05–1.28
rs12476147	185800905	T/A	0.8389	0.1628	0.1568	0.4019	0.5261	NA	1.045	0.91–1.20
rs4667001	185801747	G/A	0.3696	0.156	0.1508	0.3169	0.5735	NA	1.041	0.91–1.20
rs1366842	185802243	C/A	0.4337	0.1565	0.1522	0.2123	0.645	NA	1.033	0.90–1.19
rs12477430	185802363	A/G	0.7383	0.1366	0.1231	2.518	0.1126	NA	1.127	0.97–1.31

CI = confidence interval; MAF = minor allele frequency; NA = not applicable; OR = odds ratio. The allele with MAF is underlined. *P* values were corrected using Bonferroni correction.

performed using SAS 9.0 software (SAS Institute, Cary, NC, USA). The statistic threshold was set at $P < 0.05$ using AlphaSim correction ($P < 0.01$; > 134 voxel cluster size). This correction was confined within the group GM mask (threshold at 0.2) and determined by 10 000 Monte Carlo simulations (Ledberg, Akerman & Roland 1998) using the AFNI AlphaSim program (http://afni.nih.gov/afni/docpdf/AlphaSim.pdf; accessed January 13, 2015).

Table 2 Haplotype distribution of *ZNF804A* rs7597593-rs1344706 in heroin abusers and controls.

Haplotype	*MAF case*	*MAF control*	χ^2	p_{origin}	$p_{corrected}$
G-C	0.457	0.494	8.503	0.0035	0.0109
A-A	0.387	0.356	6.245	0.0125	0.0385
G-A	0.125	0.119	0.445	0.5047	0.8836

MAF = minor allele frequency. *P* values were corrected after 10 000 permutation tests.

RESULTS

Subject characteristics

The imaging subsample was not different from the neurocognitive subsample in demographic, clinical or genetic characteristics. The distribution proportion of *ZNF804A* polymorphisms in the neurocognitive subsample was consistent with the genetic sample (Supporting Information Table S1). Although the neurocognitive subsample differed from the genetic sample in age, heroin dosage, duration and abstinence time (Supporting Information Table S2), these items were not correlated with any neurocognitive or imaging results in the correlation analysis (Supporting Information Tables S3 and S4). In the genetic sample, the gender distribution and age were substantially different between healthy controls and heroin abusers ($P < 0.001$), and cigarette use was significantly different in the neurocognitive and imaging subsamples ($P < 0.01$). Therefore, age and cigarette use were included as covariates in the statistic analysis. The demographic and heroin use characteristics are shown in Supporting Information Table S5.

Distribution of *ZNF804A* allele and haplotype frequencies

None of the individual proportions significantly deviated from Hardy–Weinberg equilibrium among controls and heroin abusers. The genotype call rate was >98 percent for each SNP. We found significant differences in allele frequencies between groups for rs7597593 [Bonferroni-corrected $P = 0.02$, 95 percent confidence interval (CI) = 1.03–1.27] and rs1344706 (Bonferroni-corrected $P = 0.008$, 95 percent CI = 1.05–1.28). The A allele frequencies of rs7597593 and rs1344706 were significantly higher in heroin abusers than in healthy controls. The allele distribution of the other four SNPs showed no evidence of associations with heroin addiction (Table 1).

Because only rs7597593 and rs1344706 were significantly associated with heroin addiction, and rs7597593-rs1344706 lied in a separate LD block with the other four SNPs (Supporting Information Fig. S1), we focused on the association of the rs7597593-rs1344706 haplotype with heroin addiction in the haplotype analysis. The rs7597593 and rs1344706 SNPs were in high LD ($D' = 0.84$, $r^2 = 0.50$; Supporting Information Fig. S1). The rs7597593-rs1344706 haplotype showed a significant association with heroin addiction ($P = 0.01$, corrected after 10 000 permutation tests). The frequency of the rs7597593-rs1344706 A-A haplotype was substantially higher in heroin abusers than in healthy controls, and the G-C haplotype was much higher in the healthy control group (Table 2).

Neurocognitive performance

Compared with healthy controls, heroin abusers had significantly worse performance on the MoCA and IGT and higher aggression scores on the BIS-11, especially on the motor behavior and non-planning dimensions (Supporting Information Table S6). Although the genotypes of rs7597593 and rs1344706 had no significant main

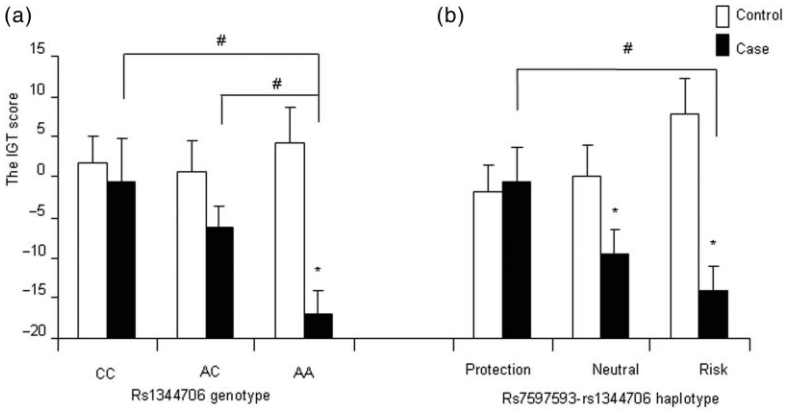

Figure 1 The *ZNF804A* variants interacted with heroin addiction to affect Iowa Gambling Task (IGT) performance. IGT scores were lower in heroin abusers than in controls and sequentially decreased with rs1344706 A allele dose among heroin abusers ($F_{2,216} = 3.35$, $P = 0.037$). IGT scores also sequentially worsened from the rs7597593-rs1344706 haplotype protective group to the risk group ($F_{2,216} = 4.59$, $P = 0.011$). $^{*}P < 0.05$, within genetic groups; $^{\#}P < 0.05$, within disease groups. The data are expressed as mean ± standard error

effects on any of the four behavioral tests, we found a robust effect of the interaction between heroin addiction and rs1344706 on IGT performance (AA: 26 controls and 39 heroin abusers; AC: 44 controls and 59 heroin abusers; CC: 33 controls and 23 heroin abusers). Impairments in decision-making ability in heroin abusers progressively worsened as the A allele of rs1344706 dose increased (Fig. 1a). A similar pattern of effects of rs7597593 was observed, but it did not achieve statistic significance ($P = 0.18$).

Based on the association results for the SNPs, we stratified all of the subjects who underwent the behavioral tests into three groups based on their individual rs7597593-rs1344706 haplotype (risk: A-A/A-A and A-A/G-A, 28 controls and 38 heroin abusers; neutral: A-A/G-C and G-A/G-A, 40 controls and 53 heroin abusers; protective: G-C/G-C and G-C/G-A, 35 controls and 30 heroin abusers). The haplotype of rs7597593-rs1344706 had no significant main effect on any of the four behavioral tests. However, a significant effect of the interaction between haplotype and heroin addiction on the IGT was found. IGT performance sequentially worsened in heroin abusers as the haplotype changed from the protective to risk type (Fig. 1b).

GMV

In addition to widespread GMV atrophy in heroin abusers (Supporting Information Fig. S2), we found a significant main effect of *ZNF804A* variants (rs1344706 genotype and rs7597593-rs1344706 haplotype) on GMV in the bilateral insular cortex, right temporal cortex and right superior parietal cortex (AlphaSim-corrected $P < 0.05$). GMV progressively increased as the dose of the *ZNF804A* risk variant increased. Heroin abusers exhibited no significant difference in the main effects of *ZNF804A* variants on GMV compared with controls (Fig. 2; Supporting Information Tables S7 and S8).

We also found a significant effect of the interaction between heroin addiction and *ZNF804A* variants (rs1344706 genotype and rs7597593-rs1344706 haplotype) on GMV in the left sensorimotor cortex (postcentral gyrus and precentral gyrus; AlphaSim-corrected $P < 0.05$). GMV in heroin abusers significantly increased as the risk allele and haplotype dose increased, and this GMV in heroin abusers was significantly different compared with controls in the respective homozygote genetic groups (Fig. 3; Supporting Information Tables S7 and S8).

DISCUSSION

The present study established an association between two intronic SNPs (rs7597593 and rs1344706) and a previously unexplored haplotype of *ZNF804A* with heroin addiction in a large sample of Han Chinese. We further found that abnormalities in decision making and GMV were associated with these same *ZNF804A* variants in heroin abusers.

Both rs7597593 and rs1344706 are located in the middle of introns of this gene, and no alternative splicing occurs when nucleotides are exchanged. The two risk SNPs have been associated with the regulation of *ZNF804A* mRNA expression (Riley *et al.* 2010; Zhang *et al.* 2011). Similar to findings in other psychiatric disorders (Dwyer *et al.* 2010), we failed to detect any non-synonymous rare variants within the *ZNF804A* locus that were strongly associated with heroin addiction. Thus, non-coding regulatory variants might carry inordinate weight in determining how *ZNF804A* leads to biologic susceptibility to psychotic disorders and heroin addiction. Considering the high LD in the detected common SNPs, interactions across multiple SNPs within *ZNF804A* may mediate overall biologic effects (Hess & Glatt 2014). Both the haplotype of rs7597593-rs1344706 A-A and two SNPs were significantly associated with heroin addiction, and other functional variations might confer risk for heroin addiction within the LD region between these two polymorphisms.

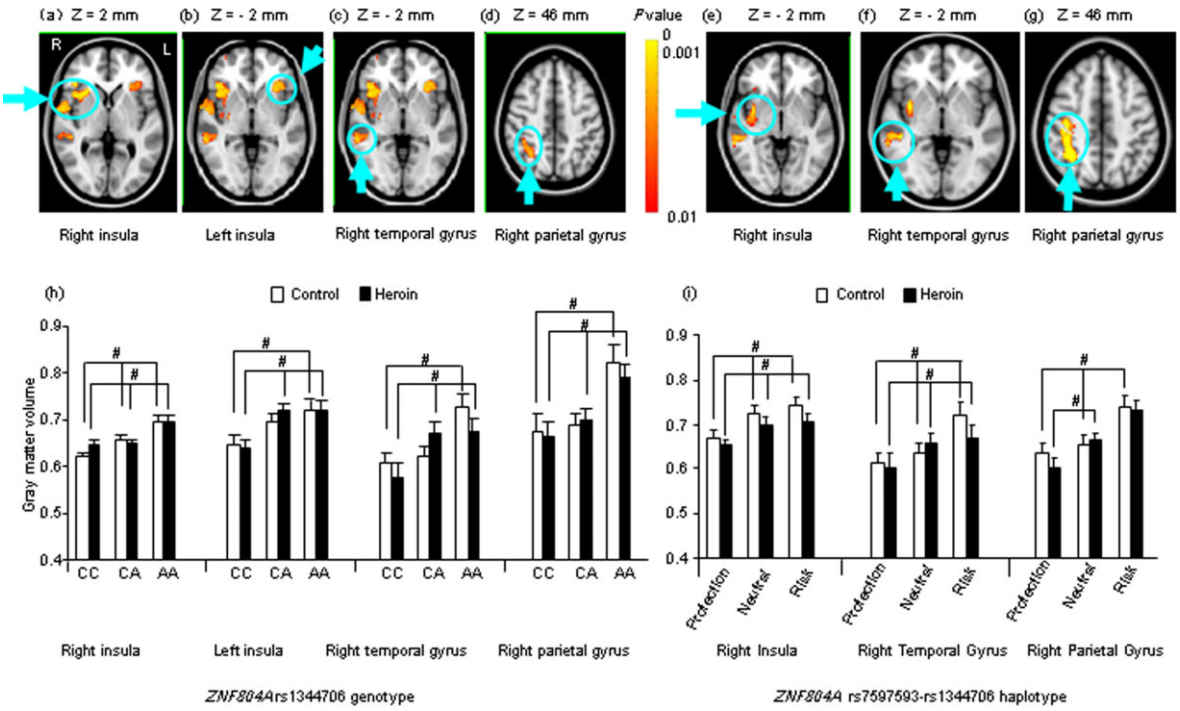

Figure 2 Main genetic effect of *ZNF804A* variants on gray matter volume (GMV). (h) GMV progressively increased as the rs1344706 A allele dose increased in the (a) right insular cortex extending to the frontal cortex and temporal pole, (b) left insular cortex extending to the orbitofrontal cortex, (c) right middle and superior temporal gyrus and (d) right superior parietal lobule (AlphaSim-corrected $P < 0.05$). (i) The *ZNF804A* rs7597593-rs1344706 haplotype sequentially increased GMV from the protective haplotype group to the risk haplotype group in the (e) right insular cortex, (f) right middle and superior temporal gyri, and (g) right superior parietal lobe (AlphaSim-corrected $P < 0.05$). Heroin abusers were not significantly different from controls within respective genetic subsamples. $^{\#}P < 0.05$, within disease groups. The data are expressed as mean ± standard error

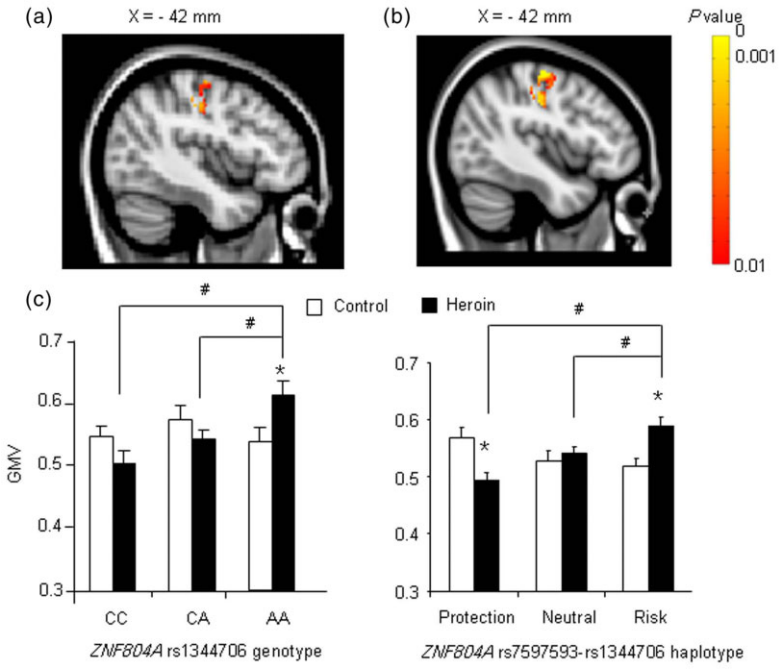

Figure 3 The *ZNF804A* variants interacted with heroin addiction to affect gray matter volume (GMV) in the left sensorimotor cortex. (a) Interaction between the rs1344706 genotype and heroin addiction. (b) Interaction between the rs7597593-rs1344706 haplotype and heroin addiction (AlphaSim-corrected $P < 0.05$). (c) GMV significantly increased from the risk variants to the protective variants. in heroin abusers, it was significantly different with controls but in opposite directions for the two homozygote genetic groups. $^{*}P < 0.05$, within genetic groups; $^{\#}P < 0.05$, within disease groups. The data are expressed as mean ± standard error

However, we did not obtain further evidence that the two SNPs that were associated with addiction are functional, so the possibility that they may simply be markers for causal variants cannot be excluded.

Consistent with previous behavioral studies (Verdejo-Garcia, Perales & Perez-Garcia 2007; Copersino *et al.* 2012), we confirmed striking deficiencies in neurocognitive tests among heroin abusers, including

the cognitive function, impulsivity and decision making. However, the implicated variants of *ZNF804A* had dose-dependent effects only on deficits in decision-making ability in heroin abusers. In previous reports, the association between *ZNF804A* and cognitive performance was not related to the degree of risk genetic loading (Van Den Bossche *et al.* 2012). The IGT simulates real-life decision making under conditions of uncertainty, reward and punishment (Bechara *et al.* 1994), *ZNF804A* appeared to correlate more with social deficits than with cognitive deficits in heroin addiction. Poor decision making reflects an inability to learn from previous mistakes, leading to negative consequences (Bechara 2005) that are characteristic of drug abusers and predict relapse and treatment dropout (Stevens *et al.* 2013). Therefore, decision making may be an intermediate phenotype that transfers genetic risk from *ZNF804A* to heroin addiction.

Neuroimaging studies have shown aberrant brain morphology after long-term repeated exposure to heroin (Liu *et al.* 2009; Yuan *et al.* 2010). Partially consistent with previous findings, we observed widespread decreases in GMV in heroin abusers. Imaging studies that selected patients based on the risk A allele of rs1344706 found relatively larger hippocampal volume among schizophrenia patients and increased dorsolateral prefrontal cortex connectivity in healthy subjects (Donohoe *et al.* 2011). These neuroimaging patterns partly overlapped with the neuroimaging findings in the present study. As expected, the *ZNF804A* variants mainly affected the insular cortex in both abusers and controls. Thus, the *ZNF804A* risk allele and haplotype may be related to pre-existing abnormal GMV in the insula, which is associated with susceptible traits in addiction, such as conscious interoception, emotional experience and decision making (Naqvi & Bechara 2010). We also found an interaction between *ZNF804A* variants and heroin addiction in the left sensorimotor cortex, which is highly relevant for the development and persistence of addiction (Yalachkov, Kaiser & Naumer 2010).

ZNF804A was reported to be expressed across the life span and have the highest expression prenatally (Tao *et al.* 2014). The mechanism for the genetic effect of *ZNF804A* rs1344706 on brain morphology may be related to its function as a transcription factor-binding site for brain-expressed transcription factors (Riley *et al.* 2010), whereas its effect on neurocognition may be related to dopamine neurotransmission (Volkow *et al.* 2004). *ZNF804A* can directly regulate the expression of dopaminergic genes (Girgenti *et al.* 2012), and the dopaminergic pathway may be another key mechanism that mediates the effects of *ZNF804A* variants on heroin addiction. Future neuroimaging studies using diffusion tensor imaging and resting-state connectivity, therefore, may provide important insights into these GMV abnormalities and genetic associations by focusing on dopaminergic pathways from midbrain areas, such as the nucleus accumbens and its connections to the sensory-motor cortex and insula.

Some limitations of the present study should be mentioned. First, it was a cross-sectional study, and our results could not differentiate the effects of genetic factors during different stages of addiction. Second, sex differences within the genetic sample may be a potential confounding factor, and female participants should be included in future studies to generalize the genetic effects on endophenotypes.

In conclusion, intronic polymorphisms of *ZNF804A* were associated with heroin addiction risk and abnormalities in GMV and decision-making ability among heroin abusers. Although the precise neural mechanisms that underlie these associations with genetic factors are unknown, the present results provide important leads for future investigations of these associations, including the effects of *ZNF804A* on both dopamine neurotransmission and relatively increase in the volume of various brain areas.

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Authors Contribution

JS chose the topic, defined the scope of the study and obtained funding for the study. YS designed the research, developed the protocol, performed the research and wrote the first draft of the article. LL provided guidance for the study design and data analysis, and assisted with the article writing. L-YZ and G-BW assisted with performing the research, analyzing the data and writing the article. J-LL, NC, H-MW and J-JF assisted with performing the trial and writing the article. W-HY, FW and Y-LT provided guidance for and assisted with the genetic analyses. YH, NS, Q-XL and JW provided guidance for and assisted with

analyzing the image data. Y-DT and S-X were responsible for the MRI. G-FD and G-HD assisted with performing the behavior tests and interpreting the findings. TRK and H-BH assisted with writing the article and provided critical revisions for important intellectual content. All of the authors critically reviewed the content and approved the final version for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1 Supplemental methods.

Figure S1 Linkage disequilibrium (LD) pattern between markers genotyped in *ZNF804A* in Han Chinese healthy populations. The LD structure between marker pairs is indicated by the shaded matrices. The figure was generated using HaploView 4.1. (a) D' value. (b) r^2 value

Figure S2 Main effects of heroin addiction on gray matter volume (GMV). Compared with healthy controls, heroin abusers showed smaller GMV in blue-light blue and larger regions in red-yellow (AlphaSim-corrected $P < 0.05$). The atrophied areas were mainly located in most of the bilateral frontal, temporal, occipital, and parietal cortices and sub-cortex (amygdala and putamen). Additionally, the left temporal occipital fusiform cortex had increased GMV in heroin abusers compared with controls

Table S1 Distribution of alleles of *ZNF804A* polymorphisms in genetic and neurocognitive samples

Table S2 Demographic and clinical characteristics of genetic sample and neurocognitive sample

Table S3 Correlation between neurocognitive tests and heroin clinical data

Table S4 Correlation between gray matter volume of significant clusters and heroin clinical data

Table S5 Subject characteristics

Table S6 Neurocognitive performance in the subjects

Table S7 Significant clusters of *ZNF804A* rs1344706 genotype effects on gray matter volume

Table S8 Significant clusters of *ZNF804A* rs7597593-rs1344706 haplotype effects on gray matter volume