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Sex-Specific Mediation Effect of the Right Fusiform Face Area Volume on the Association Between Variants in Repeat Length of *AVPR1A* RS3 and Altruistic Behavior in Healthy Adults

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Abstract: Microsatellite variants in the arginine vasopressin receptor 1A gene (*AVPR1A*) RS3 have been associated with normal social behaviors variation and autism spectrum disorders (ASDs) in a sex-specific manner. However, neural mechanisms underlying these associations remain largely unknown. We hypothesized that *AVPR1A* RS3 variants affect altruistic behavior by modulating the gray matter volume (GMV) of specific brain regions in a sex-specific manner. We investigated 278 young healthy adults using the Dictator Game to assess altruistic behavior. All subjects were genotyped and main effect of *AVPR1A* RS3 repeat polymorphisms and interaction of genotype-by-sex on the GMV were assessed in a voxel-wise manner. We observed that male subjects with relatively short repeats allocated less money to others and exhibited a significantly smaller GMV in the right fusiform face area (FFA) compared with male long homozygotes. In male subjects, the GMV of the right FFA exhibited a significant positive correlation with altruistic behavior. A mixed mediation and moderation analysis further revealed both a significant mediation effect of the GMV of the right FFA on the association between *AVPR1A* RS3 repeat polymorphisms and allocation sums and a significant moderation effect of sex (only in males) on the mediation effect. Post hoc analysis showed that the GMV of the right FFA was significantly smaller in male subjects carrying allele 426 than in non-426 carriers. These results suggest that the GMV of the right FFA may be a potential mediator whereby the genetic variants in *AVPR1A* RS3 affect altruistic behavior in healthy male subjects. *Hum Brain Mapp 37:2700–2709, 2016*.

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INTRODUCTION

Arginine-vasopressin (AVP) and oxytocin (OXT) are evolutionarily conserved, molecularly similar neuropeptides, differing at only two amino acids. Converging evidence from human studies indicates that AVP and OXT play a critical role in modulating complex social behaviors and are associated with several neuropsychiatric disorders characterized by impairment in social interactions, such as autism spectrum disorders (ASDs) [Meyer-Lindenberg et al., 2011].

The human AVP receptor 1A gene (*AVPR1A*) promoter-region contains three repeat polymorphisms, of which RS3 is a complex repeat of (CT)4-TT-(CT)8-(GT)n 3625 bp upstream of the transcription start site. The microsatellite variants in *AVPR1A* RS3 have been frequently linked to social behaviors, such as pair-bonding [Walum et al., 2008], altruistic behavior [Avinun et al., 2011; Knafo et al., 2008], and prepulse inhibition response [Levin et al., 2009]. Four independent studies in different population have revealed an association between RS3 microsatellite variants and ASDs [Kim et al., 2002; Wassink et al., 2004; Yang et al., 2010; Yirmiya et al., 2006]. However, the neural mechanisms underlying these associations remain uncertain.

Investigating genetic effects on brain imaging phenotypes may provide valuable information about potential pathways from gene to complex social behavior. So far, only one imaging genetics study has investigated the effects of microsatellite variants in *AVPR1A* RS3 on amygdala activation in healthy subjects. The authors observed that the longer alleles of RS3 were associated with greater amygdala activation in response to fearful/angry facial expressions and that subjects carrying allele 334 elicited the highest amygdala activation relative to subjects carrying other alleles [Meyer-Lindenberg et al., 2009]. Although this pioneering study has established the association between *AVPR1A* and brain function, it remains unknown whether genetic variations in *AVPR1A* RS3 affect the structural properties of the brain.

Here, we aimed to investigate the neural mechanisms underlying the association between *AVPR1A* RS3 variants and social behavior. Prosocial behavior is an integral component of daily life and helps us establish and maintain interpersonal relationships. Altruism, defined here as costly acts aimed at benefiting strangers, is a subsystem of prosocial behaviors that have been associated with *AVPR1A* RS3 variants in healthy subjects with European ancestry [Avinun et al., 2011; Knafo et al., 2008]. Our first hypothesis is that *AVPR1A* RS3 variants are also associated with individual differences in altruistic behavior in Chinese populations. If this hypothesis is correct, we further hypothesize that *AVPR1A* RS3 variants affect altruistic behavior by modulating the gray matter volume (GMV) of specific brain regions. In many species, AVP has shown a male-predominant effect on social behaviors, including aggression, pair-bond formation and stress-responsiveness [Carter et al., 2008; Donaldson and Young, 2008; Lim and Young, 2006; Young and Wang, 2004]. In human subjects, AVP selectively promotes agonistic responses in men but affiliative responses in women [Thompson et al., 2006]. Moreover, ASDs is four to five times more common in boys than in girls. As a result, we also hypothesize that the modulation effect of *AVPR1A* RS3 on brain GMV are more prominent in males than in females.

In this study, we systematically investigated relationships among *AVPR1A* RS3 repeat polymorphisms, brain GMV, and altruistic behavior. Then, we used a mixed mediation and moderation model with sex as a moderator and GMV as a mediator of gene-behavior association to test the hypothesis that the brain GMV may mediate the association between *AVPR1A* RS3 repeat polymorphisms and altruistic behavior in a sex-specific manner.

MATERIALS AND METHODS

Participants

A total of 324 young healthy subjects were recruited and underwent a series of behavioral assessments, genotyping, and MRI examinations. The participants were recruited through college and community advertisements and paid for their participation. The following inclusion criteria were used: (1) 18 to 30 years old to minimize aging effect on the brain; (2) Chinese Han populations and right-handedness to homogenize the sample; and (3) an intelligence quotient score greater than 90 to ensure that the participants could successfully complete our examinations. The following exclusion criteria were used: (1) the participant or their first-degree relatives had a history of a psychiatric disorder; (2) the participant had a history of neurological illness, psychiatric treatment, or drug or alcohol abuse; or (3) the participant had a contraindication to an MRI examination. This study was approved by the Medical Research Ethics Committee of Tianjin Medical University, and all participants provided written informed consent.

Behavioral Assessment

The dictator game (DG) is a popular paradigm used to test individual altruistic tendencies [Avinun et al., 2011].

The DG is a one-shot game with two players: a dictator and a recipient. The dictator is provided a fixed sum of money and has to decide how to split it between himself/herself and the recipient. The recipient has to accept the decision of the dictator and does not have an active role in the game. In our study, the DG was conducted by using the questionnaire "if you are given 100 Yuan, you have to decide how to distribute between you and a stranger." The sum that a participant allocated to a stranger was recorded which may somewhat reflect the altruism of the participant.

Because AVP has found to increase anxiety-related behaviors [Neumann and Landgraf, 2012], we also explored the effect of the *AVPR1A* RS3 on the anxiety-related personality trait, which were evaluated by harm avoidance (HA) subscale of a Chinese version of the Tridimensional Personality Questionnaire (TPQ) [Cloninger, 1987].

DNA Extraction and Microsatellite Genotyping

Peripheral venous blood samples were collected from these subjects, and DNA was extracted from white blood cells using standard procedures with the EZgeneTM Blood gDNA Miniprep Kit (Biomiga, Inc. San Diego, CA). Amplification of the RS3 microsatellite [corresponding to the $(CT)_4$-TT-$(CT)_8$-$(GT)_n$ marker, which is 3,625 bp upstream of the transcription start site] in the promoter region of *AVPR1A* was achieved using the following primers: (fluorescent) 5′-TTGAAGAGCTGCCTTTGAGC-3′ (forward) and 5′-AATGCTGACCCGCTTTTTC-3′ (reverse). The PCR reactions were performed with 1 μl DNA template, 0.4 μl each primer, 2 μl dNTP, 2 μl buffer solution, and 0.3 μl Taq DNA polymerase (1 unit/μl) and were increased to a final volume of 10 μl with water. The PCR reactions were run in a Gene Amp PCR system 9600 (PerkinElmer, Inc. MA) using the following cycling conditions: an initial 95°C incubation for 15 min followed by 35 cycles of 94°C (30 sec), 50°C (1 min 30 sec), and 72°C (1 min) and a final extension at 72°C (7 min). The fluorescently labeled DNA fragments were analyzed based on allele length with automated capillary electrophoresis using an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems) and GeneMapper software Version 4.0 (Applied Biosystems).

The allele frequencies for the *AVPR1A* RS3 microsatellite in the participants are shown in Table I. In Table I, we also provide the number of base pairs (bp) of the alleles between our study and other studies [Kim et al., 2002; Meyer-Lindenberg et al., 2009; Walum et al., 2008] because the observed length of the RS3 allele depends on the PCR primers employed. The distributions of allele frequencies were comparable to the previously reported *AVPR1A* RS3 genotypic distributions using ethnically similar samples [Yang et al., 2010] and did not deviate from Hardy-Weinberg equilibrium. Among the 12 alleles (410–436 bp), the 422 bp allele was the most frequent allele of the RS3 marker. The allele repeat distribution was characterized by relatively rare alleles at the extremes of the distribution (Table I). For example, the bins of 410 to 412 and 430 to 436 bp alleles were only included in 8/278 and 17/278 subjects, respectively. This characteristic distribution of microsatellite repeats has prompted many investigators to use a median cut method to group repeat lengths into short versus long in genetic analyses [Bisceglia et al., 2012; Knafo et al., 2008; Levin et al., 2009; Meyer-Lindenberg et al., 2009]. As a prior study stated [Knafo et al., 2008], the most optimal cutoff is thought to be the one that can classify subjects into two groups with approximately equal numbers of participants in each group. In line with this idea, a cutoff of >422 bp was selected to generate two genotypic subgroups with similar allele percentage [short (410–422 bp): 58.27%; long (424–436 bp): 41.73%]. The selection of the cutoff was after genotyping but before the imaging genetics analysis. Because each subject has two copies of this gene (one from their father and one from their mother), there were three possible genotypes for the *AVPR1A* RS3 microsatellite: short/short (SS), short/long (SL), or long/long (LL).

TABLE I. Allele frequencies for the *AVPR1a* RS3 microsatellite in subjects

Repeat numbers of the RS3	RS3 allele of our data (base pairs)	RS3 Allele of previous studies (base pairs)	Frequency	Percentage
17	410	318	7	1.26
18	412	320	1	0.18
20	416	324	19	3.42
21	418	326	39	7.01
22	420	328	125	22.48
23	422	330	133	23.92
24	424	332	119	21.40
25	426	334	68	12.23
26	428	336	28	5.04
27	430	338	5	0.90
28	432	340	11	1.98
30	436	344	1	0.18

Since the observed length of RS3 allele depends on the PCR primers employed, we also provided the correspondence of the number of base pairs (bp) of the alleles between our study and other studies [Kim et al., 2002; Meyer-Lindenberg et al., 2009; Walum et al., 2008]. Alleles are counted for each occurrence. There are twice as many alleles as participants because every individual has two alleles.

Image Acquisition

MR images were acquired using a Signa HDx 3.0 Tesla MR scanner (General Electric, Milwaukee, WI) with a standard quadrature head coil. Tight but comfortable foam padding was used to minimize head motion, and earplugs were used to reduce scanner noise. High-resolution

sagittal three-dimensional (3D) T1-weighted images were acquired by a brain volume (BRAVO) sequence with the following parameters: repetition time/echo time = 8.1/3.1 ms; inversion time = 450 ms; flip angle = 13°; field of view- = 256 × 256 mm; matrix = 256 × 256; slice thickness = 1 mm, no gap; and 176 slices. We checked image quality during two stages using the following criteria: (1) visible motion artifacts; (2) observable brain lesions; and (3) brain morphological abnormalities. All the 324 subjects underwent the first-stage image quality assessment by two junior researchers immediately after the MRI scan. Four subjects had visible motion artifacts in the 3D structural images and were asked to re-perform the MRI scan. One subject was excluded because of a visible lesion in the pons, and another subject because of a very large lateral ventricle. After further excluding 41 subjects because of genotyping failure, the remaining 281 subjects underwent the second-stage image quality assessment by a senior radiologist before image analyses. Two subjects were excluded because of suspected motion artifacts, and another subject because of the right temporal atrophy.

GMV Calculation

Adopting a state of the art registration technique, the diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL) [Ashburner, 2007], a voxel-based morphometry (VBM) analysis was performed using Statistical Parametric Mapping software (SPM8). For detailed preprocessing steps of the VBM analysis, please see our previous study [Wang et al., 2013]. After segmentation, registration, normalization, modulation, and smoothing, GMV maps were used for statistical analysis.

Statistical Assessment of the Imaging Data

We performed a whole-brain voxel-wise analysis to identify either a main effect of *AVPR1A* RS3 genotypes (SS, SL, and LL) or an interaction effect of genotype-by-sex on GMV using a two-way (genotype and sex) analysis of covariance (ANCOVA) after controlling for age and educational years. Monte Carlo simulations were used to correct for multiple comparisons ($P < 0.05$, 782 voxels). The threshold of cluster size was automatically estimated by the AlphaSim procedure included in the REST toolbox (http://restfmri.net/forum/index.php) with the following parameters: single voxel $P = 0.005$; an estimated smoothing kernel of FWHMx = 11.8 mm, FWHMy = 11.7 mm and FWHMz = 11.1 mm; cluster connection radius of $r = 5$ mm; and 1,000 simulations within a gray matter mask. For each significant cluster, we extracted the GMV of this cluster and investigated specific GMV differences between any two genotypic groups using a one-way analysis of variance (ANOVA) and post hoc analyses. We calculated Cohen's d [Parker and Hagan-Burke, 2007] to represent the effect size ("small: $d < 0.5$," "medium: $0.5 < d < 0.8$," and "large: $d > 0.8$") of each comparison. We also use post hoc analyses to compare the GMV of each significant cluster obtained from the whole-brain voxel-wise analysis between specific-allele carriers and noncarriers (such as 410 bp carriers vs. non-410 bp carriers) in males and females, respectively. Moreover, we calculated partial correlation coefficients between the GMV of the each significant cluster and allocation sums of DG with age, years of education, and HA scores as nuisance covariates in the male and female subjects, respectively. We then compared the difference between the two independent correlation coefficients using online software (http://www.quantpsy.org/corrtest/corrtest.htm).

A mixed mediation and moderation model was used to test whether the mediation effect of brain GMV on gene-behavior association is in a sex-dependent manner (http://www.afhayes.com/introduction-to-mediation-moderation-and-conditional-process-analysis.html). Here, the gene is *AVPR1A* RS3, the behavior is the allocation sums reflecting altruistic behavior, and the anatomy is the GMV of the significant cluster. This model was conducted in all qualified subjects ($n = 278$), the *AVPR1A* RS3 genotype was treated as independent variable (X) (SS, SL, and LL), the GMV as M variable, the allocation sums as outcome variable (Y), sex as proposed moderator (W), and age, educational years and HA scores as covariates of no interest. Bias-corrected bootstrapping 95% confidence intervals (CI) were calculated by 1,000 bootstrap samplings. A 95% CI not containing zero ($P < 0.05$) was considered a significant indirect effect.

Previous studies have associated *AVPR1A* with several subcortical nuclei, including the globus pallidus, nucleus accumbens, putamen, caudate nucleus and amygdala [Hammock and Young, 2005; Meyer-Lindenberg et al., 2009]. If the voxel-based whole-brain analysis did not observe any significant GMVs differences between genotypes in these regions, we directly compared the mean GMVs between genotypic groups in each of these regions using a one-way ANOVA in the Statistical Package for the Social Sciences version 18.0 (SPSS Inc., Chicago, IL) for Windows. Each of these subcortical nuclei was extracted using a threshold of 50% minimum probability from the Harvard-Oxford cortical structural atlas (http://www.fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases).

Statistical Analyses for Demographic, Genetic, and Behavioral Data

Statistical analyses for demographic, genetic and behavioral data were performed using the Statistical Package for the Social Sciences version 18.0 (SPSS Inc., Chicago, IL) for Windows. Generally, a χ^2 test was used to compare group differences in the categorical variables (sex). An ANOVA with genotype and sex as the two factors was used to examine group differences in the continuous variables (age, educational years and HA scores). Considering that

TABLE II. Demographic information of subjects for *AVPR1a* RS3 microsatellite*

	SS	SL	LL
No. subjects	90	45	143
Sex (males/females)	40/50	24/21	64/79
Age (yr)	22.5 ± 2.2	22.9 ± 1.9	22.0 ± 1.8
Years of education	15.9 ± 2.2	15.5 ± 2.1	15.4 ± 2.0
HA scores	14.4 ± 6.6	15.0 ± 6.4	14.1 ± 5.2

Data (age, years of education, and HA scores) are presented as the mean ± SD. No significant differences are found in any measures among individuals with SS, SL and LL repeats.
HA, harm avoidance.

the allocation sums of DG were not normally distributed, we adopted nonparametric tests to compare the amount differences between genotypic groups. Specifically, a Kruskal-Wallis test was used to compare differences in the allocation sums of DG among the SS, SL, and LL groups. A Mann-Whitney *U* test was used to compare differences in the allocation sums of DG between any two groups. All statistical analyses used a two-tailed α level of less than 0.05 for statistical significance.

RESULTS

Demographic, Genetic, and Behavioral Characteristics

After a careful evaluation of the exclusion criteria, a total of 278 subjects (128 males and 150 females, mean age = 22.3 ± 1.9 years, range: 18–27 years) were included. The demographic, genetic, and behavioral data are presented in Table II. There were no significant differences ($P > 0.05$) among the three genotypic groups with regard to sex, age, years of education or HA scores. Neither males ($F = 0.167$, $P = 0.846$) nor females ($F = 0.340$, $P = 0.712$) showed a significant difference in HA scores among genotypes.

In the male subjects, the mean rank of the allocation sums of DG was 55.9 for the SS group, 63.5 for the SL group, and 65.9 for the LL group. The allocation sums of DG exhibited significant genotypic differences in males ($\chi^2 = 6.342$, $P = 0.042$). A post hoc analysis demonstrated that male SS subjects allocated less money than male SL ($z = -2.088$, $P = 0.047$) and LL ($z = -2.277$, $P = 0.03$) subjects. In female subjects, the mean rank of the allocation sums of DG was 74.2 for the SS group, 74.7 for the SL group, and 74.3 for the LL group. No significant difference was observed among the three female genotypic groups ($\chi^2 = 0.010$, $P = 0.995$). Additionally, we did not observe any significant differences in the allocation sums of DG between any specific-allele carriers and noncarriers in either male or female subjects ($P > 0.05$) (Supporting Information Tables S1 and S2).

A Data-Driven Analysis of GMV Differences Between Genotypes

Although no significant main effect of genotype was identified, the right fusiform face area (FFA, Brodmann area 37, peak MNI coordinates: $x = 39$, $y = -52.5$, $z = -16.5$; cluster size = 841 voxels; peak $F = 8.90$) showed a significant interaction effect between genotype and sex ($P < 0.05$, AlphaSim corrected; Fig. 1a). Then, the GMV of the right FFA was extracted from each subject and compared among the three genotypic groups in males and females, respectively. We found a significant GMV difference in the right FFA among the three genotypic groups in males ($F = 10.825$, $P = 0.0000415$), but not in females ($F = 1.773$, $P = 0.174$). In males, a post hoc analysis demonstrated that both SS ($t = -4.808$, $P = 0.00000563$; Cohen's $d = 0.829$) and SL subjects ($t = -3.467$, $P = 0.001$; Cohen's $d = 0.657$) exhibited a smaller GMV in the right FFA than LL subjects (Fig. 1b). These differences were still significant following Bonferroni correction for multiple comparisons ($P < 0.05$).

In the post hoc analyses, we also compared the GMV of the right FFA between the specific-allele carriers and noncarriers in males and females, respectively. Only male subjects carrying allele 426 had a significantly smaller GMV ($t = -3.877$, $P = 0.000171$) in the right FFA compared with male non-carriers (Fig. 2 and Supporting Information Table S3), which can pass Bonferroni correction for multiple comparisons with a significance threshold of $P < 0.05$. We did not observe any significant differences ($P > 0.05$, uncorrected) between the specific-allele carriers and non-carriers in the GMV of the right FFA in female subjects (Supporting Information Fig. S1 and Table S4).

A Hypothesis-Driven Analysis of GMV Differences Between Genotypes

We also tested the effects of *AVPR1A* RS3 variants on the GMV of the globus pallidus, nucleus accumbens, putamen, caudate nucleus, and amygdala, which have been previously associated with these genetic variants. We extracted and compared the GMV of each region among the three genotypic groups in male and female subjects, respectively. One-way ANOVA did not show significant GMV differences ($P > 0.05$) among the genotypic groups in any of these regions (Supporting Information Fig. S2 and Table S5).

The Relationship Between the GMV of the Right FFA and the Allocation Sums of DG

Partial correlation analysis showed that the GMV of the right FFA was significantly correlated with the allocation sums of DG in the male subjects ($n = 128$, $r = 0.380$, $P = 0.00001$); however, the GMV of the right FFA did not

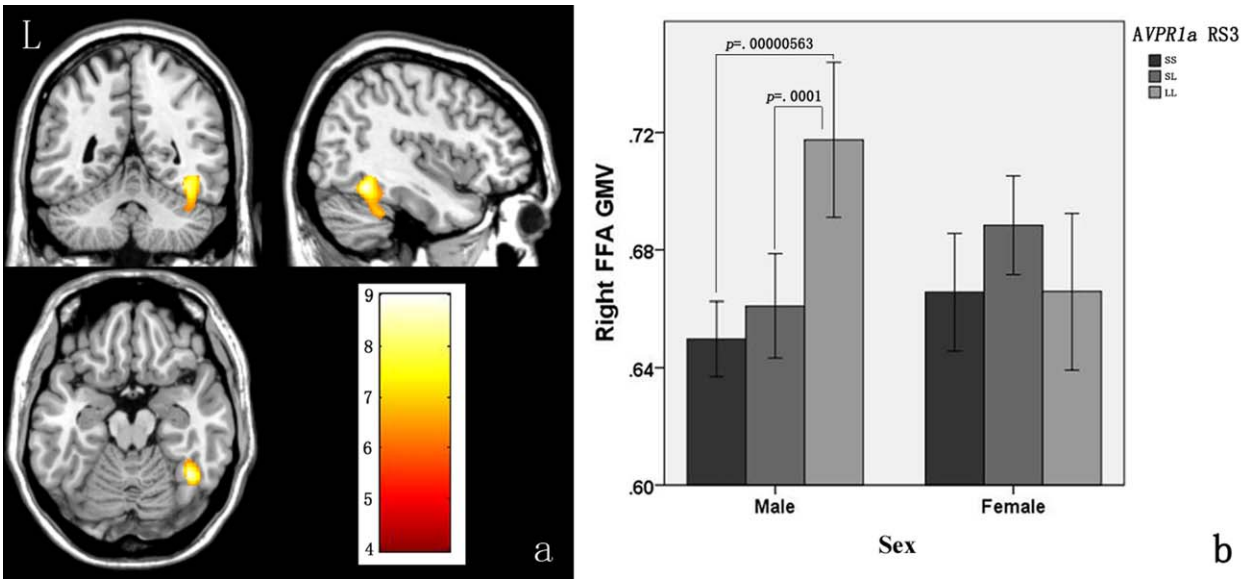

Figure 1.

RS3 allele repeat length variants of AVPR1A and regional gray matter volume (GMV) of the brain. Using a median cutoff threshold of ≥422 bp for long repeats, the whole-brain GMV analysis demonstrates a significant genotype × sex interaction in the right fusiform face area (FFA) ($P < 0.05$, AlphaSim corrected) (**a**). Post hoc tests show that both male subjects with short-shortcrepeats (SS) and short-long repeats (SL) exhibit a smaller GMV in the right FFA compared with male subjects homozygous for long repeats (LL) (**b**). No significant difference was observed for the right FFA GMV among the three genotypic groups in females (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

correlate with the allocation sums of DG in the female subjects ($n = 150$, $r = -0.032$, $P = 0.700$). There is a significant difference (z-score = 3.551, $P = 0.000383$) between these two correlation coefficients.

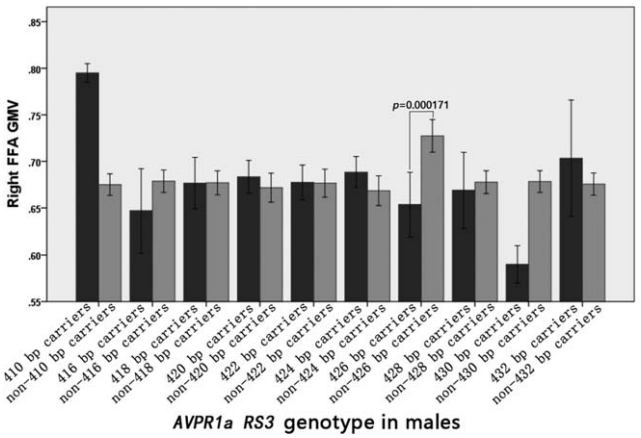

Figure 2.

The gray matter volume (GMV) differences in the right fusiform face area (FFA) between each specific-allele male carriers and male noncarriers. Only male subjects carrying allele 426 exhibit a significantly smaller GMV in the right FFA compared with those not carrying allele 426 ($P < 0.05$, Bonferroni correction).

A Mixed Mediation and Moderation Analysis

We used a mixed mediation and moderation model to examine whether the GMV of the right FFA was a mediator in the association between *AVPR1A* RS3 polymorphism and allocation sums of DG, and whether this mediation effect was moderated by sex. This model demonstrated a good fit and accounted for 22.1% of the variance in allocation sums of DG (Fig. 3). There was a direct effect of *AVPR1A* RS3 on allocation sums of DG ($b = 0.34$, $P = 0.001$). More importantly, we found a significant mediation effect of the GMV of the right FFA on the association between *AVPR1A* RS3 polymorphism and allocation sums of DG ($b = 0.25$, $P = 0.004$ for gene to GMV; $b = 0.32$, $P < 0.001$ for GMV to behavior). Sex significantly moderated the mediation effect ($P < 0.001$). Only in males, long *AVPR1A* RS3 repeats predicted increased GMV of the right FFA, which further predicted increased allocation sums of DG.

Correlations Between the GMV of the Right FFA and HA Scores

We also investigated possible correlations between the GMV of the right FFA and HA scores, neither males ($n = 128$, $r = 0.147$, $P = 0.086$) nor females ($n = 150$, $r = -0.114$, $P = 0.195$) showed a significant correlation.

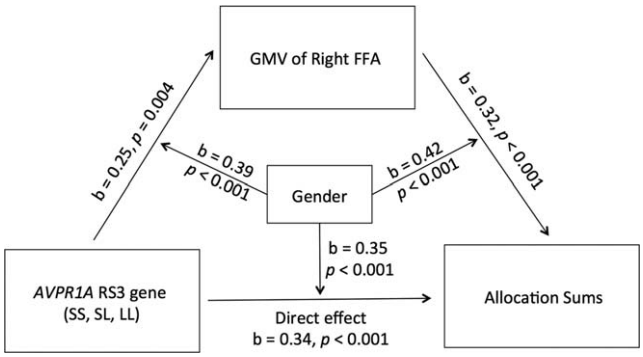

Figure 3.

Mediation effect of the gray matter volume (GMV) of the right fusiform face area (FFA) on the association between AVPR1A RS3 repeat polymorphisms and allocation sums and moderation effect of sex on this mediation effect.

DISCUSSION

This study revealed an association between *AVPR1A* RS3 and altruistic behavior only in male subjects, which is consistent with previously reported sex-dependent modulation of *AVPR1A* RS3 on social behaviors. In humans, *AVPR1A* RS3 variations have been associated with pair-bonding [Walum et al., 2008] and prepulse inhibition [Levin et al., 2009] in male subjects. In prairie voles, *AVPR1A* RS3 variants are associated with social engagement and bonding behavior only in males [Hammock and Young, 2005]. In chimpanzees carrying one copy of the long allele containing RS3, males had significantly higher dominance and lower conscientiousness scores than females [Hopkins et al., 2012]. ASDs, as a putative disorder characterized by impairment of social behavior, are 4-fold more common in males than in females [Chakrabarti and Fombonne, 2005].

In healthy subjects, individuals with short RS3 repeats have been consistently associated with a worse performance in social behaviors. We observed that individuals with short RS3 repeats tended to allocate less money to anonymous subjects in the DG, which is consistent with the report of a previous study [Knafo et al., 2008]. Individuals with short RS3 repeats are also associated with lower prepulse inhibition responses of the startle response to auditory stimuli [Levin et al., 2009]. In male prairie voles, animals with short repeats in *avpr1a* microsatellite are more difficult to form partner preferences than ones with long repeats [Hammock and Young, 2005].

The molecular genetic basis for the association between RS3 length variations and social behaviors has been investigated. Prairie voles with short repeats had significantly reduced gene expression compared with those with long repeats [Hammock and Young, 2002]. The length of the RS3 microsatellite could also affect gene expression in human hippocampal specimens. Specifically, shorter alleles reduced the transcription of the *AVPR1A* gene [Knafo et al., 2008]. Recently, the length of the RS3 microsatellite has been observed to affect relative promoter activity [Tansey et al., 2011; shorter repeat alleles of RS3 decreased relative promoter activity in human neuroblastoma cell lines [Tansey et al., 2011]. These results suggest that *AVPR1A* RS3 variants may affect social behaviors by modulating *AVPR1A* transcription.

Inspired by the only functional imaging genetics study of *AVPR1A* [Meyer-Lindenberg et al., 2009], we adopt brain volume variations as an intermediate phenotype between RS3 microsatellite variants and altruistic behavior since previous studies have shown that brain GMV is highly heritable [Thompson et al., 2001] and human's prosocial behavior is closely associated with genetic variations [Penner et al., 2005]. Although the exact biological mechanisms underlying genetic variants of *AVPR1A* RS3 on brain GMV remain unknown, it may be related the effect of *AVPR1A* RS3 variants on cortical development [Chen et al., 2000a,b; Wang et al., 1997]. Because exposure to AVP receptor agonist significantly increased neurite length, number of branches, branch length, number of branch bifurcation points and number of microspikes of neurons in the cerebral cortex [Chen et al., 2000a], *AVPR1A* RS3 variants may also affect neuronal development by modulating the AVPR1A.

We observed that male individuals with relatively short RS3 alleles had a smaller GMV in the right FFA compared with male long homozygotes; and the difference was more prominent between male SS and LL subjects. The human FFA serves to process facial information [Kanwisher et al., 1999] and exhibits a right-hemispheric dominance [Barton et al., 2002; Pitcher et al., 2007]. This brain region is also involved in processing socially relevant information [Singer et al., 2004; Winston et al., 2002]. In patients with ASDs, the FFA exhibits both reduced GMV [Kwon et al., 2004; Toal et al., 2010] and weakened activation during facial discrimination tasks [Sato et al., 2012; Weisberg et al., 2012]. The structural and functional impairments in the FFA may underlie the deficits in social communication in ASDs because the FFA is important for processing emotional facial expressions [Lauvin et al., 2012; Sato et al., 2012]. We also observed a significantly positive association between the GMV of the right FFA and individual altruistic behavior in male subjects, suggesting that the right FFA is one of the important brain regions for altruistic behavior. More importantly, using a mixed mediation and moderation model, we confirmed the mediation effect of the GMV of the right FFA on the association between *AVPR1A* RS3 and altruistic behavior and the moderation effect of sex on this pathway. These results indicate that RS3 polymorphism affects altruistic behavior via modulating the GMV of the right FFA in healthy males, which is a potential pathway from *AVPR1A* RS3 to social behavior.

Several studies have shown associations between specific alleles of *AVPR1A* RS3 and behavioral or imaging phenotypes. For example, males carrying allele 334

(corresponding to allele 426 in our study) had a lower score on pair-bonding scales than those not carrying this allele [Walum et al., 2008]. The only one imaging genetics study demonstrated that individuals carrying allele 334 elicited greater amygdala activation than noncarriers in a face-matching task [Meyer-Lindenberg et al., 2009]. In patients with ASDs, the RS3 allele 334 of *AVPR1A* has shown the most significant transmission disequilibrium for autism [Kim et al., 2002]. In the present study, we also compared differences in the allocation sums of DG and the GMV of the right FFA between specific-allele carriers and non-carriers. Although we did not observe significant differences in the allocation sums, we observed that males carrying allele 426 had a smaller GMV in the right FFA than non-carriers. This finding provides further evidence for the modulation of RS3 polymorphism on the GMV of the right FFA in healthy male subjects. From Figure 2, it seems that the male subjects carrying allele 410 had a rather larger GMV of the right FFA compared with male non-carriers ($P = 0.011$), but it cannot pass Bonferroni correction for multiple comparisons. Moreover, only 5 male subjects carry an allele 410, but 123 male subjects are non-carriers. The rather large difference in numbers of subjects between the two groups would make the statistical comparison unreliable.

Prairie voles have shown sex-specific differences in vasopressin receptor densities in the globus pallidus, nucleus accumbens, putamen, and caudate nucleus [Hammock and Young, 2005]. Functional imaging has revealed an association between *AVPR1A* RS3 variants and amygdala activation in humans [Meyer-Lindenberg et al., 2009]. These results might predict GMV differences in these regions between *AVPR1A* RS3 genotypes. However, in the present study, we did not observe significant GMV differences in any of these nuclei. The absence of structural differences in these regions might be because the differences are too small to be detected in this sample of young healthy adults or might be attributed to species-specific differences in the distribution of vasopressin receptors [Loup et al., 1991]. Social perception/interactions in rodents depend mostly on olfactory cues, whereas social behavior in humans is considerably complex and depends on multimodal sensory inputs, particularly visual input. This difference may also aid the understanding of differential neural mechanisms of *AVPR1A* on social behaviors in humans and animals.

We neither found significant between genotypic differences in HA nor significant correlations between the GMV of the right FFA and HA scores. The absence of HA effects on the right FFA seems a bit surprising in light of a recent finding of an association between a personality trait (neuroticism) and the amygdala-FFA functional connectivity [Kruschwitz et al., 2015a]. The discrepancy may be explained by the between-study differences in: (1) imaging metrics: the GMV used in our study is a measure of structural property, whereas, the amygdala-FFA connectivity used in the prior study reflects functional property and is affected by both the amygdala and FFA; (2) personality scales: our study used the HA subscale of the TPQ, but the prior study used neuroticism subscale of NEO; and (3) subregional specificity of the FFA: the coordinate ($x = 39$, $y = -52.5$, $z = -16.5$) of our FFA region is largely different from that ($x = 48$, $y = -75$, $z = -21$) of the prior study. A subregion-specific effect may account for the discrepancy [Kruschwitz et al., 2015b].

Several limitations should be noted when interpreting our results. First, this sample was exclusively composed of young adults with low variation in age (18–27 years), which may prevent us from generalizing these results to other age groups. Second, only investigating *AVPR1A* RS3 polymorphism is a limitation of this study because complex traits including brain structure and altruistic behavior are thought to be modulated by multiple genetic and environmental factors. Other genetic and environmental factors and their possible interactions should be further studied. Third, it has suggested that identification of a reliable genetic association with brain structure require considerable large number of subjects [Hibar et al., 2015]. Thus, our findings should be validated in a large sample with enough power. Moreover, the lack of significant difference in allocation sums between 426 bp carriers and non-426 bp carriers and the lack of reliable evidence for the GMV difference in the right FFA between 410 bp carriers and non-410 bp carriers may be also attributed to the relatively small sample size of the present study. Finally, as an outgrowth of TPQ, Temperament and Character Inventory (TCI) may provide more information about personality characteristics. But a Chinese version of TCI was not available when our experiment was performed.

In summary, using a mixed mediation and moderation model, we found that the GMV of the right FFA mediated the association between *AVPR1A* RS3 and altruistic behavior. Moreover, this mediation effect was significant only in male subjects. These results suggest that genetic variants in the RS3 repeat length of *AVPR1A* may affect altruistic behavior by modulating the GMV of the right FFA in a sex-dependent manner. However, the biological mechanisms underlying this gene-brain-behavior pathway are still unknown and need to be further investigated.

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