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The cortical surface area of the insula mediates the effect of *DBH* rs7040170 on novelty seeking

Running head：*DBH* gene, insula, and novelty seeking

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Abstract

Novelty seeking (NS) is a personality trait important for adaptive functioning, but an excessive level of NS has been linked to psychiatric disorders such as ADHD and substance abuse. Previous research has investigated separately the neural and genetic bases of the NS trait, but results were mixed and neural and genetic bases have yet to be examined within the same study. In this study, we examined the interrelationships among the dopamine beta-hydroxylase (*DBH*) gene, brain structure, and the NS trait in 359 healthy Han Chinese subjects. We focused on the *DBH* gene because it encodes a key enzyme for dopamine metabolism, NS is believed to be related to the dopaminergic system and has been reported associated with *DBH* variation. Results showed a significant positive association between the cortical surface area of the left insula and NS score. Furthermore, the *DBH* genetic polymorphism at the SNP rs7040170 was strongly associated with both the surface area of the left insula and NS score, with G carriers having a larger left insula surface area and a higher NS score than AA homozygotes. Subsequent path analysis suggested that the insula partially mediated the association between the *DBH* gene and the NS trait. Our data provided the first evidence for the involvement of the insula in the dopamine-NS relationship. Future studies of molecular mechanisms underlying the NS personality trait and related psychiatric disorders should consider the mediation effect of the neural structure.

Key words

Novelty seeking, surface area, cortical thickness, insula, *DBH*

Introduction

Novelty seeking (NS) is a personality trait characterized by a preference for exploratory and novel activities, avoidance of monotony and routine, and extravagance in approach to reward cues (Cloninger, 1987; Cloninger et al., 1993). This trait contributes to adaptive functioning. High NS individuals are impulsive, excitable, and quick-tempered, while low NS individuals are rigid, stoic, and slow-tempered (Cloninger, 1986; Cloninger et al., 1993). However, excessive NS has been linked to psychiatric disorders (Richter and Brandstrom, 2009), such as ADHD (Instanes et al., 2013; Jacob et al., 2014), pathological gambling (Kim and Grant, 2001) and substance abuse (Milivojevic et al., 2012), while reduced levels of NS are correlated with obsessive-compulsive disorders (Lyoo et al., 2001). A number of genetic and neuroimaging studies have attempted to examine the biological basis of NS, but results have been mixed and the combined genetic-neural mechanism of the NS remains to be explored.

Following Cloninger's model (Cloninger, 1986), much of the genetic work of the NS trait has focused on candidate genes affecting the dopamine (DA) system, and their genetic effects on NS (Davila et al., 2013; Montag et al., 2010; Munafo et al., 2008). In this study we focused on the *DBH* gene, located on chromosome 9q34. It encodes

dopamine β-hydroxylase (DβH) protein (Kaufman and Friedman, 1965) and is a major quantitative trait locus of plasma DβH activity (Zabetian et al., 2001). DβH protein catalyzes the conversion of DA to norepinephrine (NE) (Levin et al., 1960) in synaptic vesicles and thereby influences extracellular DA level. Hence, one might speculate that variation in the *DBH* gene could impact NS by virtue of its effect on dopamine levels. This hypothesis has been supported by recent studies linking the *DBH* gene to NS (Hess et al., 2009) and several psychiatric disorders related to excessive NS, such as ADHD (Carpentier et al., 2013) and addiction (Preuss et al., 2013). However, the mechanism for these gene-behavior associations, especially neural structure as mediating factors (or neural endophenotypes (Goldberg and Weinberger, 2004; Meyer-Lindenberg and Weinberger, 2006), remains unclear. Therefore, this study aimed to study the role of *DBH* gene on neural structure underlying NS. Our results should help to better elucidate the influence of the *DBH* gene on NS as well as related neuropsychiatric disorders.

Neuroimaging studies have examined the correlation between NS and cortical volume in several brain regions (Gardini et al., 2009; Iidaka et al., 2006; Schilling et al., 2013b; Van Schuerbeek et al., 2011), however, these findings are mixed. Some research found that NS was associated with cortical volume in the middle frontal gyrus (Iidaka et al., 2006) and orbitofrontal cortex (Schilling et al., 2013b), whereas some others found associations with regions such as the posterior cingulate regions (Gardini et al., 2009; Van Schuerbeek et al., 2011) and cerebellum (Van Schuerbeek et

al., 2011). These inconsistent results might be attributed to differences in sample size, methods, or statistical model specifications (Hu et al., 2011). Another possible issue is that most studies have examined cortical volume as a whole, while ignoring the two individual components that comprise it, cortical thickness and surface area. Cortical volume is the product of cortical thickness and surface area, which are genetically (Panizzon et al., 2009) and phenotypically (Winkler et al., 2010) independent. Moreover, these two measures are distinct aspects of the neural architecture and have different developmental trajectories (Lyall et al., 2014). Thus far, to our knowledge, the majority of structural neuroimaging studies of the NS trait have investigated the volume measure as a whole (Gardini et al., 2009; Iidaka et al., 2006), except for one study which estimated the cortical thickness measure but only focused on a facet of the NS trait (impulsiveness) (Schilling et al., 2013a). Given this gap in the literature, the respective contributions of surface area and cortical thickness to the NS trait should be investigated. This could not only help us to clarify the neural structure basis of the NS trait, but could also provide appropriate endophenotypes for imaging genetics studies (Winkler et al., 2010), to better understand the genetic-neural basis of the NS trait.

The aim of the present study was to examine the brain structure underpinnings of NS trait and to understand the gene-brain-behavior relationships among the *DBH* gene, brain structure, and the NS score. Therefore, we first evaluated the respective effects of cortical surface area and cortical thickness on the NS trait in a large healthy Han

Chinese sample. Then we tested the association between the single nucleotide polymorphisms (SNPs) in and nearby the *DBH* gene and NS-related brain structures. Finally, to clarify the role of the brain in the complex interrelations, we performed path analyses to test whether brain structure served as a mediator between the *DBH* gene and the NS trait. We hypothesized that variations in the *DBH* gene would be related to cortical structure and NS score, and furthermore, that cortical structure would mediate the association between the genetic variations and NS score.

Material and methods

Subjects. Three-hundred and fifty nine healthy Han Chinese subjects (173 females, mean age = 19.4±1.1 years) participated in the study. All subjects had no history of neurologic or psychiatric disorders, and were not taking any medications that could interfere with their ability to complete a questionnaire or provide structural MRI data. This study was approved by the Ethics Committee of School of Life Science and Technology at the University of Electronic Science and Technology of China. All participants gave informed written consent.

Genotyping. After blood sample collection, genomic DNA was extracted using the E.Z.N.A.™ Blood DNA Kit (Omega Bio-Tek, Georgia, US). All samples were genotyped using the standard Illumina genotyping protocol (Illumina, Inc). As described in Supplemental Materials **Table S1**, 51 SNPs located in or within 100kb

around the *DBH* gene on chromosome 9 were selected. These SNPs covered most of the linkage disequilibrium (LD) blocks in this region, as defined for the Chinese sample included in the HapMap Project (http://www.hapmap.org[phase 3]). Five of 51 SNPs failed to pass minor allele frequency (MAF) > 0.05 and were excluded for further analyses. The remained 46 SNPs met the criteria for Hardy–Weinberg equilibrium (HWE) $p > 0.01$ and genotype call rate > 0.98 and were included in subsequent analyses. When fewer than five participants were categorized as either heterozygotes or minor allele homozygotes for a SNP, the two genotype groups were combined in further analysis.

Novelty Seeking measure. Each subject was asked to complete the Chinese version of the Temperament and Character Inventory-Revised (TCI-R) (Chen et al., 2011; Cloninger, 1994). This inventory was translated from English to Chinese and back translated and verified through a bilingual group discussion, and the resulting Chinese versions had high internal consistency (Chen et al., 2011). The NS subscale of the TCI-R measures individual differences in the extent to which a person is quick tempered, impulsive, extravagant, and disorderly versus rigid, stoical, and orderly (Cloninger et al., 1993). The total score on the NS subscale was used in the current study.

Image acquisition and preprocessing. MRI scans were performed with an MR750 3.0 Tesla magnetic resonance scanner (GE Healthcare). High-resolution 3D

T1-weighted brain volume (BRAVO) MRI sequence was performed with the following parameters: TR = 8.16 ms, TE = 3.18 ms, flip angle = 7°, FOV = 256 mm × 256 mm, voxel size = 1 × 1 × 1 mm^3, and 188 slices. All the raw MRI data were inspected by two experienced radiologists who were blind to genotype information.

MRI data were analyzed with atlas-based FreeSurfer software (http://surfer.nmr.mgh.harvard.edu, version 5.3.0). The cortical surface was constructed through an automated procedure, involving segmentation of the white matter, classification of the gray/white matter boundary, inflation of the folded surface, and automatic correction of topological defects(Dale et al., 1999; Fischl and Dale, 2000). After the initial surface model had been constructed, measures of cortical surface area were calculated by computing the area of each triangle of a standardized tessellation. Then all of the individual reconstructed cortical surfaces were aligned to an average template with a surface-based registration algorithm. Quality control of scan images and segmentation was assured by visual inspection of the whole cortex of each subject and manual editing following the standard editing rules. Any inaccuracies in Talairach-transformation, skull stripping, and segmentation were also manually corrected, and re-inspected. A high correlation between these automatic measures and manual measures in vivo and ex vivo has been demonstrated (Desikan et al., 2006). Cortical thickness and surface area maps were then smoothed using a Gaussian kernel (20 mm FWHM).

Statistical analysis. After surface reconstruction, vertex-by-vertex analyses of

cortical thickness and surface area were performed separately, by using a general linear model to estimate the the association between the morphological measure at each vertex and the NS score. Gender and age were included as covariates to avoid potential confounding effects (Smith et al., 2007). Significance maps were then corrected for multiple comparisons with cluster-based Monte Carlo simulations with 5,000 permutations (using the *FreeSurfer* program mri_glmfit-sim, corrected for two spaces). Finally, because thickness analysis did not yield any significant results, the region significantly correlated with the NS score (corrected $p < 0.05$) in the cortical surface area analysis, the left insula, was extracted. We mapped this region onto the average reconstructed surface for visual display, and calculated the mean surface area for each subject for subsequent analysis.

Linear regression models were then used to detect the associations between each SNP and the mean surface area of the left insula. Additive genetic models (i.e., additive effects of allele dosage) were used, with sex and age as covariates. Statistical significance level was set at $p < 1.09\times10^{-3}$ (0.05/46 [SNPs], i.e., Bonferroni correction). Only one SNP (rs7040170) passed the significance level and was selected for further analysis. Allelic association tests were carried out by using plink v1.07 (Purcell et al., 2007). The genotype frequencies (AA = 304, AG = 53, GG = 2) were within the Hardy-Weinberg equilibrium ($\chi2 = 0.036$, $p = 0.850$). Given that there were only two GG homozygotes, we combined the two subjects with the AG group and comparisons were carried out between AA homozygotes and G carriers.

Mediation model. A mediation model was set up, with the surface area of the left insula as a mediator, to test the direct and indirect effects of *DBH* rs7040170 on NS score. We used the PROCESS macro developed by Hayes (2012) to test for a mediation model (model 4), with NS score as the outcome variable, the *DBH* rs7040170 polymorphism as the predictor, and the average surface area of the left insula as the mediator, with sex and age as covariates. The model estimates the total, direct (path from *DBH* genotype to NS score), and indirect (path from *DBH* genotype to NS score through the left insula) relations, to clarify whether the effect of *DBH* on NS was significantly mediated through the surface area of the left insula. Statistical significance for the mediators was established by bootstrapped 95% confidence intervals (CI) with 5000 iterations.

Results

The participants' NS scores (M = 101.2, SD = 11.3) were comparable to those of another Chinese sample in a previous study that used the same inventory (Lei et al., 2014).

Then the cortical surface maps for each individual was calculated, and regression analyses with NS scores were conducted. Significant positive correlations between the surface area in the left insular cortex (including a few parts of the superior temporal

gyrus) and NS score were observed (corrected cluster-wise p = 0.009, Monte-Carlo correction, see **Figure 1A**), whereas no significant correlation was found between cortical thickness and NS score.

Then we conducted a series of association tests using Plink, to test the association between the polymorphism at each *DBH* SNP and the mean surface area of the left insula. As presented in **Figure 1B** and Supplemental Materials **Table S2**, only one SNP (rs7040170) located about 62 kb downstream of the *DBH* gene showed a significant association with the left insula surface area after Bonferroni correction (t = 3.48, uncorrected $p = 5.7 \times 10^{-4}$), after controlling for the potential confounding effects of sex and age. Further regression analyses also showed a significant correlation between this SNP and the NS score (t = 2.63, $p = 9.0 \times 10^{-3}$, again with sex and age as covariates). For rs7040170, G carriers showed both larger left insula surface area and higher NS score than AA homozygotes (see **Table 1** and **Figure 1C**, **1D**).

Given the three concurrent relationships between the gene, neural structure and behavior, a path model was applied to examine whether the insula mediated the effect of *DBH* rs7040170 on NS score. Analysis in PROCESS revealed significant direct paths including a path from *DBH* rs7040170 genotype to NS score (B = 0.31, SE = 0.15, p = 0.04), a path from *DBH* rs7040170 genotype to insula surface area (B = 0.45, SE = 0.13, p = 0.0006) and a path from the insula surface area to NS score (B = 0.16,

SE = 0.06, p = 0.0058) (model depicted in **Figure 2**). There was also a significant indirect path from *DBH* rs7040170 genotype to NS score through insula surface area ($\alpha\beta$ = 0.07, SE = 0.03, p = 0.03, 95% CI = [0.02, 0.17]). This suggested that the effect of *DBH* rs7040170 genotype on the NS trait was partially accounted for by genotype-related variations in the insula's surface area.

Discussion

The present study found significant associations between the *DBH* rs7040170, surface area of the left insula, and NS scores. Furthermore, path analyses indicated that neural structure (i.e., surface area of the left insula) mediated the effect of the *DBH* rs7040170 polymorphisms on the NS trait. These results together provided possible genetic and neural mechanisms underlying the NS trait and psychiatric disorders related to abnormal NS scores, such as ADHD, pathological gambling and substance abuse (Instanes et al., 2013; Jacob et al., 2014; Kim and Grant, 2001; Milivojevic et al., 2012).

One of the major findings of this study was the correlation of NS score with cortical surface area but not with cortical thickness. This result confirmed our conjecture that these two measurements should be considered separately in studies of NS trait. According to the radial unit hypothesis (Rakic, 1988), these two phenotypes have distinct neurodevelopmental mechanisms, as cortical surface area is influenced by the

number of columns, whereas cortical thickness is influenced by the number of cells within a column. The significant contribution of surface area in our study might be supported by one or more of the following. First, individual differences in cortical gray matter volume were found to be more closely related to differences in cortical surface area than cortical thickness (Winkler et al., 2010). In addition, cortical surface area became dramatically larger during evolution while cortical thickness remained relatively conserved (Rakic, 2009). Last but not least, reduced surface area has been found to be linked to psychiatric disorders (Rimol et al., 2012), and has been proven more useful than cortical thickness in elucidating the associations between brain structure and cognitive endophenotypes of psychiatric disorders (Vuoksimaa et al., 2014).

The positive association between the left insular surface area and NS scores in our study is consistent with previous research implicating the insula in impulsiveness (Lee et al., 2008) and risk and reward processing (Hauser et al., 2014; Mohr et al., 2010). Our results are also in line with previously documented positive correlations between regional cerebral blood flow (rCBF) in the insula and NS (Sugiura et al., 2000), and insular surface area and impulsivity (Kaag et al., 2014). Besides, this anatomical association is consistent with a recent study on patients suffering substance abuse who were found to score high on NS (Bell et al., 2014) and showed increased surface area in the insular cortex (Kaag et al., 2014). Previous research has indicated that lesions to the insula to be associated with a reduction in addictive behaviors (Naqvi et al., 2007).

In that case, it is clear that the anatomy of the insula may constrain its function. Hence, we speculate that a small regional surface area of insular cortex could lead to decreased tendency of impulsivity and to risky behaviors through the insula's role in the processing of interoceptive cues as conscious feeling, such as urge (Naqvi and Bechara, 2009), thus associated with a lower NS score. Thus, we believe that the left insular surface area represents a promising biomarker for delineating the brain structural substrate for human NS trait and for understanding the neurobiological correlates for NS-related psychiatric conditions, such as ADHD (Jacob et al., 2014) and substance abuse (Bell et al., 2014).

Our study also found a lateralized association between the left insular structure and the NS trait. This finding is consistent with earlier studies that documented positive correlations between NS and rCBF in the left but not the right insula (Sugiura et al., 2000) and cigarette smoking induced cortical dopamine release in the left but not the right insula (Wing et al., 2014). This lateralization could be due to hypothesized differences in functionality of the left and right sides of the forebrain (Craig, 2005). Specifically, the left forebrain is predominantly associated with parasympathetic activity, which may underlie positive affect and approach behaviors, whereas the right forebrain is thought to be associated with sympathetic activity, which may underlie negative affect and avoidance behaviors. This left-lateralized result also concurs with previous EEG (Sutton and Davidson, 1997), fMRI (Berkman and Lieberman, 2010) and repetitive transcranial magnetic stimulation studies [rTMS, (van Honk and

Schutter, 2006)] and reviews (Harmon-Jones et al., 2010), all of which demonstrated the lateralization of approach tendencies to the left hemisphere. Moreover, such leftward lateralization has also been reported in psychiatric disorders related to excessive NS, such as ADHD (Bedard et al., 2014) and substance abuse (Krmpotich et al., 2013).

We also found that the rs7040170 G allele was associated with a larger insular surface area. There are two possible explanations for this association. One came from the influence of rs7040170 variation on DβH activity. Rs7040170 is localized in the *SARDH* gene, which is adjacent to, bounded with, and inhabits the same topological domain with the *DBH* gene (Dixon et al., 2012). In a recent genome-wide association study (GWAS), rs7040170 was identified as one of the SNPs significantly associated with plasma DβH activity ($p = 1.31\times10^{-14}$) (Mustapic et al., 2014). Specifically, the G allele is associated with higher plasma DβH activity and correlated with higher DβH activity in cerebrospinal fluid (CSF) (O'Connor et al., 1994). DβH is expressed early in embryonic development (Yew et al., 1995) and increases in late gestation, and may influence the cortical surface area through its roles in cell proliferation, migration and differentiation (Tiu et al., 2003). Another possibility is that rs7040170 G allele may be associated with larger insula surface area through its influence on lower DA level, and then a negative effect of dopamine on insula. The hypothesized effect is supported as the rs7040170 G allele related to higher DβH activity could lead to lower dopamine levels, since DβH catalyzes the conversion of dopamine to noradrenaline (Levin et al.,

1960). Moreover, experimental studies have found that the administration of DA receptor agonists decreased rCBF in the insula (Black et al., 2002) and that DAergic depletion increased insular activity (da Silva Alves et al., 2011). The rs7040170-insula relationship can also be inferred as the human insula receives relatively rich DA innervation (Gaspar et al., 1989), expresses DA receptors (Hurd et al., 2001), and is strongly affected by variation in DAergic neurotransmission. These findings together support the influence of the *DBH* rs7040170 polymorphism on insular surface area.

Additionally, a positive correlation was found between the G allele of *DBH* rs7040170 and NS score. A possible explanation for this finding is that the G allele may lead to higher DβH activity and reduced dopamine level, which could in turn lead to higher NS. Several studies support this hypothesis. First, as previously mentioned, the G-allele of rs7040170 has been found to be positively associated with DβH activity (Mustapic et al., 2014). Second, studies suggest that DβH function is positively associated with NS-related behaviors. For instance, higher plasma DβH activity has been found to be related to extraversion (Roy and Brockington, 1987), a trait that is positively correlated with NS (Kristensen et al., 2009), and DβH inhibitors have been found to suppress addictive behaviors (Schroeder et al., 2010). These aforementioned associations are likely due to the negative effects of DβH on dopamine levels (Levin et al., 1960). According to Cloninger's theory (1986), lower basal dopaminergic activity may be associated with more intense orienting responses to novel stimuli, thus resulting in higher NS. These studies together indicated that

DβH function may underlie NS-related behaviors through its role on DA level.

In the subsequent path analysis, the insula mediated the effect of *DBH* rs7040170 on NS scores. This result echoed the widely accepted hypothesis in gene-brain-behavior research (Goldberg and Weinberger, 2004; Green et al., 2008) and existing studies (Buckholtz et al., 2008; Fakra et al., 2009; Green et al., 2013) that genetic effects on behaviors are mediated by neural substrates. Many studies have found that the insular cortex regulates dopaminergic activity associated with the NS trait. For instance, a negative correlation between insular cortex DA receptor binding and the NS score has been demonstrated (Suhara et al., 2001). A later study also reported an inverse correlation between DA receptor availability in the insula and NS (Kaasinen et al., 2004). In short, the observed mediation effect was consistent with the reported rich DA innervation in the insula (Gaspar et al., 1989) and further supported Cloninger's model regarding associations between the NS trait and central dopaminergic pathways (Cloninger, 1986).

Further research is needed to support our findings. First, although associations between *DBH* rs7040170, the insula, and NS score were identified in the present research, these correlational results are phenomenological and do not imply causation. For instance, brain-behavior associations could reflect either a biological predetermination or experience-driven plasticity (Hyde et al., 2009). More systematic studies using animal models and other techniques are required to explain these

relationships. Second, our sample was comprised of only one ethnic group (i.e., Han Chinese) and only young adults. While this homogeneity is typically considered as an advantage for genetic studies (i.e., in helping with avoidance of ethnic stratification and other confounding effects), it also limits the generalizability of our results to other populations. It has been demonstrated that the relationships between gene, brain and behavior can be modulated by ethnicity (Long et al., 2013), age (Richter-Schmidinger et al., 2011), and gender (Kazantseva et al., 2014). Also, rs7040170 has different minor allele frequencies (MAF) in different ethnic populations based on the HapMap Data (www.hapmap.org, see **Table S1**). Therefore, to obtain confirmatory evidence, this association should be explored in future studies in samples with different age groups and ethnicity.

Conclusions

In conclusion, this study provided the first evidence of interrelationships between the *DBH* gene, insula morphology, and the NS trait, and the mediating effect of the brain between the gene and behavior in a sample of healthy Chinese individuals. Our findings suggest that cortical surface area may be a promising endophenotype in imaging genetic studies, and that future studies of gene-personality associations should consider the brain mediation effect.

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Conflict of interest

The authors declare no conflict of interest.

Figure Legends

Figure 1 Associations among the *DBH* gene, cortical surface area, and novelty seeking score. **A**, The region in red showed a significant linear increase in cortical surface area with increasing novelty seeking score, $p < 0.05$, Monte-Carlo corrected for two hemispheres. **B**, Associations between 46 SNPs in or within 100kb around the *DBH* gene region and surface area of the survived region with sex and age as covariates. All SNPs were plotted with their *p* values against their genomic position, with the most significant SNP in the region indicated as a diamond and other SNPs shaded according to their pair-wised correlation (r^2) with the signal SNP. The light blue line represented the estimated recombination rates. Gene annotations were shown as dark green lines. The regional plots were generated using the SNAP program (Johnson et al., 2008). **C** and **D**, Surface area (**C**) and novelty seeking score (**D**) in the survived region (means and standard errors) in rs7040170 AA homozygotes and AG heterozygotes. $^{**}p < 0.01$ (ANOVA). $^{***}p < 0.001$ (ANOVA).

Figure 2 rs7040170-insula-NS score Mediation Model after controlling for gender and age. Path coefficients in the graph were standardized regression weights. CI, confidence interval. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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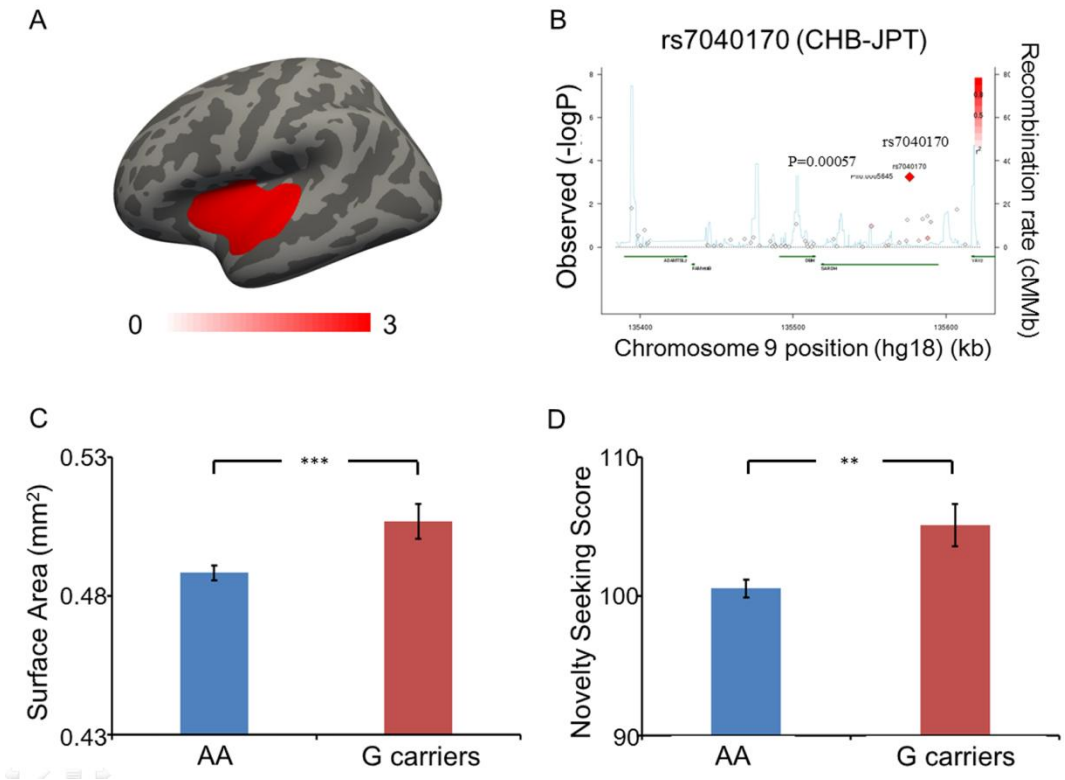

Figure 1

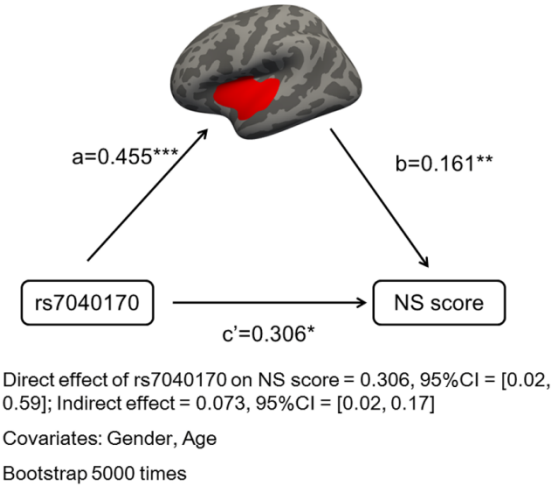

Figure 2

Table 1 Subject demographics by rs7040170 genotype

DBH	Mean(SD)		*p* value
Genotype	AA (N=304)	G carriers (N=55)	
Male/Female	160/144	26/29	0.468[a]
Age (yrs.)	19.37(1.07)	19.55(1.20)	0.270[b]
Average Surface Area of each vertex inInsula (mm^2)	0.49(0.05)	0.51(0.05)	5.662E-4[c]
Novelty seeking score	100.56(11.08)	105.11(11.84)	0.009[c]

[a] *p* value (Pearson χ^2 test).

[b] *p* value (ANOVA).

[c] *p* value (main effects of rs7040170 genotype, ANCOVA with gender and age as covariates).

Highlights

- Associations of cortical surface area and thickness with NS were tested separately.
- The surface area of the insula was positively correlated with NS score.
- *DBH* rs7040170 was associated with insula surface area.
- *DBH* rs7040170 AA homozygotes had smaller insula surface area than other genotypes.
- The insular surface area mediated the effect *DBH* rs7040170 variation on NS score.