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Original Article

# Thalamic gray matter volume mediates the association between *KIBRA* polymorphism and olfactory function among older adults: a population-based study

Lin Song[1,2], Xiaodong Han[2], Yuanjing Li[3], Xiaolei Han[2], Mingqing Zhao[1], Chunyan Li[1], Pin Wang[2], Jiafeng Wang[2], Yi Dong[2], Lin Cong[1,2], Xiaojuan Han[1,2], Tingting Hou[1,2], Keke Liu[1,2], Yongxiang Wang[1,2], Chengxuan Qiu[1,2,3,*], Yifeng Du[1,2,*]

[1]Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, 250021 Jinan, Shandong, PR China,
[2]Department of Neurology, Shandong Provincial Hospital, Shandong University, 250021 Jinan, Shandong, PR China,
[3]Department of Neurobiology, Care Sciences and Society, Aging Research Center and Center for Alzheimer Research, Karolinska Institutet-Stockholm University, 17177 Stockholm, Sweden
*Corresponding authors: Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324 Jingwuweiqi Road, Jinan, Shandong 250021, P.R. China. Email: duyifeng2013@163.com; chengxuan.qiu@ki.se

The kidney and brain expressed protein (*KIBRA*) rs17070145 polymorphism is associated with both structure and activation of the olfactory cortex. However, no studies have thus far examined whether *KIBRA* can be linked with olfactory function and whether brain structure plays any role in the association. We addressed these questions in a population-based cross-sectional study among rural-dwelling older adults. This study included 1087 participants derived from the Multidomain Interventions to Delay Dementia and Disability in Rural China, who underwent the brain MRI scans in August 2018 to October 2020; of these, 1016 took the 16-item Sniffin' Sticks identification test and 634 (62.40%) were defined with olfactory impairment (OI). Data were analyzed using the voxel-based morphometry analysis and general linear, logistic, and structural equation models. The *KIBRA* rs17070145 C-allele (CC or CT vs. TT genotype) was significantly associated with greater gray matter volume (GMV) mainly in the bilateral orbitofrontal cortex and left thalamus ($P < 0.05$) and with the multi-adjusted odds ratio of 0.73 (95% confidence interval 0.56–0.95) for OI. The left thalamic GMV could mediate 8.08% of the *KIBRA*-olfaction association ($P < 0.05$). These data suggest that the *KIBRA* rs17070145 C-allele is associated with a reduced likelihood of OI among older adults, partly mediated through left thalamic GMV.

*Key words*: MRI; old age; orbitofrontal cortex; thalamus; voxel-based morphometry.

## Introduction

The kidney and brain expressed protein (*KIBRA*), a postsynaptic protein encoded by the *WWC1* gene, could control synaptic plasticity and restrict brain tissue size (Genevet et al. 2010; Yu et al. 2010; Zhang et al. 2014). The common single-nucleotide polymorphism rs17070145 of *KIBRA* gene is located within the ninth intron of chromosome (Baumgartner et al. 2010). Previous studies have linked the *KIBRA* rs17070145 polymorphism with brain structure. For example, a cross-sectional study of 37 healthy older adults showed that *KIBRA* rs17070145 C-allele carriers had lower gray matter volume (GMV) of the prefrontal cortex and the para-hippocampal cortex than TT carriers, which further influenced cognitive function (Li et al. 2020). Another cross-sectional study of 288 healthy older people found that *KIBRA* rs17070145 C-allele carriers showed a lower GMV in the right medial prefrontal cortex and bilateral dorsal anterior cingulate cortices compared with carriers of the TT genotype (Wang et al. 2019). These 2 studies suggested that *KIBRA* rs17070145 polymorphism might influence the GMV of the prefrontal cortex, which consists of the orbitofrontal cortex, a secondary olfactory center (Edmund T. Rolls 2004; Karstensen et al. 2018; Rudebeck and Rich 2018). Neurons from the olfactory bulb project to several structures, including the amygdala, piriform cortex, and entorhinal cortex and further project to the orbital and orbitofrontal cortex (Courtiol and Wilson 2015). When the smell is perceived, odor memory and odor naming, modulated by several limbic regions, together contribute to the eventual integrated process of odor identification that involves the orbitofrontal cortex and other frontal lobe regions (Rolls 2019). However, whether *KIBRA* rs17070145 polymorphism is associated with olfactory function and whether brain structure plays any role in this association have not yet been investigated, especially in the large-scale population-based study.

In the present study, we aimed to explore the association of the *KIBRA* rs17070145 polymorphism with brain structure and olfactory function in older adults, and further to examine to what extent brain structure relevant to polymorphism of *KIBRA* rs17070145 could mediate the association between *KIBRA* rs17070145 genotype and olfactory function.

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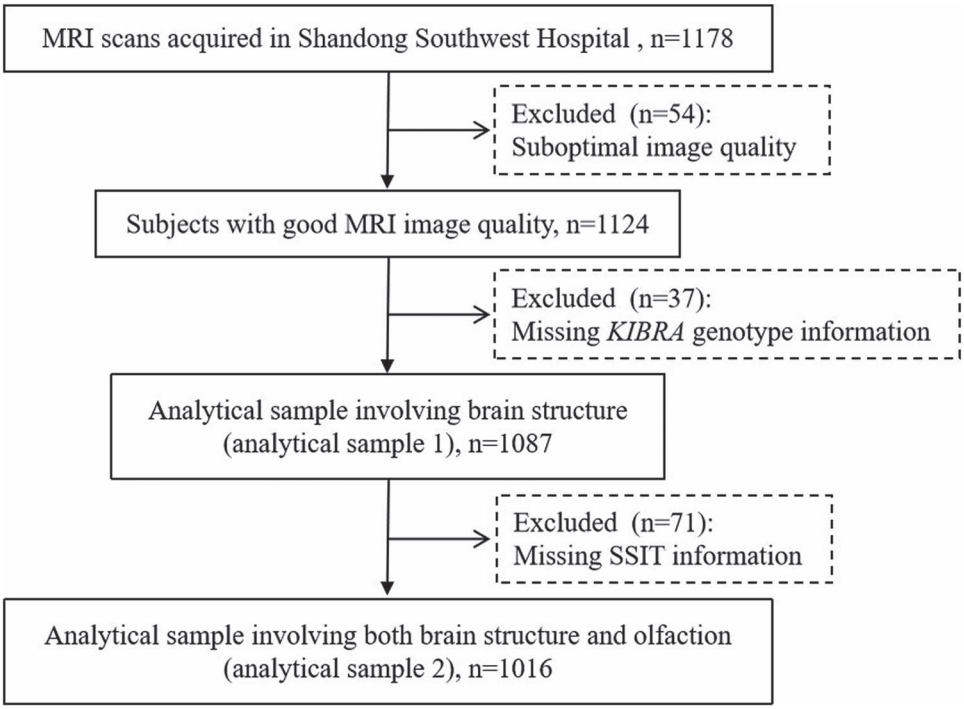


**Fig. 1.** Flowchart of the study participants. Note: *KIBRA*, kidney and brain expressed protein.

## Materials and methods

### Study design and participants

This population-based cross-sectional study used data derived from baseline assessments of the Multimodal INtervention to delay Dementia and disability in the rural China (MIND-China) study, as previously reported (Cong et al. 2022; Wang et al. 2022). In brief, the MIND-China study targeted people who were aged 60 years and older and living in the 52 rural communities of Yanlou Town, Yanggu County, western Shandong province, China. From March to September 2018, 5765 individuals were examined for MIND-China. A subsample underwent the structural brain magnetic resonance imaging (MRI) sub-study from August 2018 to November 2020. Participants in the MRI sub-study were recruited from 26 randomly selected villages. In total, 1178 individuals eventually agreed and accomplished the brain MRI scans in Southwestern Lu Hospital. Of these, 91 persons were excluded due to suboptimal image quality ($n = 54$) or missing information of *KIBRA* rs17070145 genotypes ($n = 37$), leaving 1087 participants for the analysis involving *KIBRA* gene and brain structure (analytical sample 1). Of these, we further excluded 59 participants who had missing information on olfactory function, leaving 1016 participants for investigating the association of *KIBRA* rs17070145 polymorphism with olfactory function (analytical sample 2). Figure 1 shows the flowchart of the study participants. Compared with participants who did not undertake brain MRI scan, participants in the MRI sub-study were younger and more educated, but the 2 groups did not differ significantly in the distribution of sex.

The protocols of MIND-China and MRI sub-study were reviewed and approved by the Ethics Committee at Shandong Provincial Hospital in Jinan, Shandong. Written informed consent was obtained from all the participants or from a proxy (usually a family member) if the participants were not able to give the consent. The MIND-China study was registered in the Chinese Clinical Trial Registry (registration no.: ChiCTR1800017758).

### MRI acquisition and preprocessing

All eligible participants were scanned on a Philips Ingenia 3.0 T MR System (Philips Healthcare, Best, The Netherlands) at the Southwestern Lu Hospital, as previously reported (Wang et al. 2022). During the MRI examinations, the participants were given comfortable foam padding to minimize head motion and earplugs to reduce the scanner noise. Sagittal T1-weighted structural images were acquired with the following scan parameters: repetition time (TR) = 8.3 ms; echo time (TE) = 3.8 ms; field of view (FOV) = 240 × 219 $mm^2$; acquisition matrix = 240 × 219; flip angle (FA) = 8°; and slice thickness = 1 mm.

The voxel-based morphometry analysis was performed in the Computational Anatomy Toolbox 12 (CAT 12; http://dbm.neuro.uni-jena.de/cat.html), which was installed in the package of Statistical Parametric Mapping (SPM) 12, on the platform of MATLAB R2013b (https://www.fil.ion.ucl.ac.uk/spm/) (Li et al. 2021). We used the default preprocessing pipelines, including: (i) All the 3D T1-weighted images were corrected for bias-field inhomogeneities. (ii) These brain images were segmented into gray matter, white matter, and cerebrospinal fluid.

(iii) A customized, study-specific template was generated with the Diffeomorphic Anatomical Registration using Exponentiated Lie algebra algorithm. (iv) Gray matter density images were warped to the customized template and affine registered to the Montreal Neurological Institute space. (v) The GMV map was obtained by multiplying the gray matter density map by the nonlinear determinants derived from the spatial normalization. (vi) The resultant GMV images were smoothed with a 6-mm full-width-at-half-maximum kernel. The smoothed GMV images were finally used in the following statistical analysis.

### *KIBRA* and *APOE* genotyping

The polymorphisms of *KIBRA* and *APOE* genes were detected using multiple-polymerase chain reaction amplification, as previously reported (Liang et al. 2022). In brief, the trained staff collected venous blood samples and extracted genomic deoxyribonucleic acid (DNA) from venous blood leukocytes using a TIANamp blood DNA kit (Tiangen, Beijing, China), and the DNA samples were quantified using Nanodrop 3300 spectrometer. Then, the sequencing libraries were generated using MultipSeqCustom Panel (iGeneTech, Beijing, China) following standard procedures, and qualified libraries were subjected to next-generation sequencing on a Novaseq system (Illumina). Genotyping was conducted by an operator who was blinded to all clinical data. The distribution of *KIBRA* rs17070145 and *APOE* genotypes conformed to the Hardy–Weinberg equilibrium ($P > 0.05$).

### Assessments of olfactory function

We used the 16-item Sniffin' Sticks identification test (SSIT, Burghardt Messtechnik GmbH, Tinsdaler Weg 175, 22880 Wedel, Germany) to assess olfactory identification function, as previously described in detail (Dong et al. 2021; Dong et al. 2022). Briefly, the original SSIT consisted of 16 felt-tip pens with common odors (i.e. orange, leather, cinnamon, peppermint, banana, lemon, liquorice, turpentine, garlic, coffee, apple, clove, pineapple, rose, anise, and fish). Three odors in the original test (i.e. cinnamon, turpentine, and coffee) were replaced with mushroom, soy sauce, and sesame oil because local residents were unfamiliar with these original odors. During the test, the participants were asked to smell the odor for 3–4 seconds and identify the correct odorant from 4 descriptors that were shown in a card with both the names and pictures of the 4 odors they just smelt. This could help people with no or low education to easily understand these concepts of odors. One point was given for each correct answer. The total SSIT score ranges from 0 to 16. We defined olfactory impairment (OI) as the SSIT score $\leq 10$. We also collected data on the history of sinonasal diseases, such as rhinitis, rhinosinusitis, nasal polyps, and nasal surgery.

### Data collection and assessments

Trained medical staff collected data via face-to-face interviews, clinical examinations, cognitive testing, and laboratory test following standard procedures, as previously reported (Wang et al. 2022). In brief, we collected data on sociodemographic features (e.g. age, sex, and education), behavioral factors (e.g. smoking, alcohol consumption, and physical activity), metabolic factors (e.g. hypertension, diabetes, and dyslipidemia), current use of medications, and clinical conditions (e.g. sinonasal disease). All medications were classified and coded according to the Anatomical Therapeutic Chemical (ATC) Classification System, as previously described (Cong et al. 2020). Hypertension was defined as having blood pressure $\geq$ 140/90 mm Hg or current use of antihypertensive drugs (ATC codes C02, C03, and C07–C09). Diabetes mellitus was defined as having a self-reported history of diabetes, or fasting blood glucose level $\geq$ 7.0 mmol/L, or use of glucose-lowering drugs, or insulin injection (ATC code A10). Dyslipidemia was defined according to the 2016 Chinese Guideline for the Management of Dyslipidemia in Adults: total cholesterol $\geq$6.2 mmol/L, triglycerides $\geq$2.3 mmol/L, LDL-C $\geq$ 4.1 mmol/L, HDL-C $\leq$ 1.0 mmol/L, or use of hypolipidemic agents (ATC code C10). The Mini-Mental State Examination (MMSE) was administered to assess global cognitive function.

### Statistical analysis

We presented mean (standard deviation, SD) for continuous variables and frequencies (%) for categorical variables. The characteristics of the study participants by the *KIBRA* rs17070145 polymorphism were compared using nonparametric Kruskal–Wallis H tests for continuous variables and chi-square tests for categorical variables. To identify GMV in the whole brain associated with the polymorphism of *KIBRA* rs17070145, we compared all regional GMV in the voxel level between *KIBRA* rs17070145 with or without C allele carriers, using the 2-sample *t* test in the SPM statistical software package, controlling for age, sex, and education. Multiple comparisons were corrected using an false discovery rate (FDR)-corrected *P*-value threshold of 0.05 and a cluster size of at least 100 voxels. We extracted the regional GMV that showed statistical difference between 2 groups and employed the general linear regression models to estimate association of the *KIBRA* rs17070145 polymorphism with regional GMV. Then, we used the binary logistic regression models to assess the association of the polymorphism of *KIBRA* rs17070145 with OI. We reported the main results from 2 models: model 1 was controlled for age, sex, education, and total intracranial volumes (ICV); model 2 was additionally controlled for physical exercise, smoking, alcohol intake, hypertension, diabetes, dyslipidemia, sinonasal disease, and when necessary, for *APOE* genotype. Finally, we performed the structural equation modeling to examine the mediation effect of GMV on the association between *KIBRA* 17070145

**Table 1.** Characteristics of the study participants by *KIBRA* rs17070145 genotypes.

| Characteristics | Total sample | *KIBRA* rs17070145 genotypes | | | |
|---|---|---|---|---|---|
| | (*n* = 1087) | TT (*n* = 633) | CT (*n* = 389) | CC (*n* = 65) | *P* value |
| **Age** (years) | 69.54 (4.35) | 69.61 (4.40) | 69.44 (4.31) | 69.51 (4.11) | 0.75 |
| **Female**, *n* (%) | 630 (57.96) | 370 (58.45) | 225 (57.84) | 35 (53.85) | 0.77 |
| **Education** (years) | 3.59 (3.61) | 3.63 (3.63) | 3.50 (3.59) | 3.71 (3.51) | 0.80 |
| **BMI** ($kg/m^2$) | 25.00 (3.48) | 24.91 (3.49) | 25.09 (3.48) | 25.20 (3.44) | 0.34 |
| **Physical exercise**, *n* (%) | | | | | 0.18 |
| Weekly | 737 (67.80) | 432 (68.25) | 255 (65.55) | 50 (76.92) | |
| Less than weekly | 350 (32.20) | 201 (31.75) | 134 (34.45) | 15 (23.08) | |
| **Ever smoking**, *n* (%) | 393 (36.15) | 22 (35.70) | 142 (36.50) | 25 (38.46) | 0.89 |
| **Ever alcohol intake**, *n* (%) | 361 (33.21) | 196 (30.96) | 141 (36.25) | 24 (36.92) | 0.22 |
| **Hypertension**, *n* (%) | 720 (66.24) | 411 (65.65) | 260 (67.71) | 49 (76.56) | 0.20 |
| **Diabetes**, *n* (%) | 161 (14.81) | 92 (14.53) | 58 (14.95) | 11 (16.92) | 0.87 |
| **Dyslipidemia**, *n* (%) | 263 (24.20) | 159 (25.12) | 86 (22.16) | 18 (27.69) | 0.45 |
| ***APOE* ε4 allele**, *n* (%) | 168 (15.46) | 104 (16.51) | 57 (14.73) | 7 (10.77) | 0.41 |
| **Sinonasal disease**, *n* (%) | 177 (16.28) | 100 (15.80) | 67 (17.22) | 10 (15.38) | 0.82 |
| **MMSE score** | 21.94 (5.70) | 22.09 (5.74) | 21.68 (5.61) | 21.97 (5.79) | 0.40 |

Data are mean (SD), unless otherwise specified. Note: *KIBRA*, kidney and brain expressed protein; *APOE*, apolipoprotein E gene.

genotypes and OI, with the bootstrapping of 5000 times, controlling for age, sex, education, ICV, cardiovascular risk factors, *APOE* genotype, and sinonasal disease. The standardized GMV was used in these models. RStudio version 4.1.2 (RStudio Inc. Boston, MA) was used for the mediation analysis. IBM SPSS Statistics 26.0 for Windows (IBM Corp., Armonk, NY) was used for all other analyses.

# Results

## Characteristics of the study participants

The mean age of the 1087 participants was 69.54 years (SD = 4.35; age range 60–88 years), 57.96% were women, and 34.50% were illiteracy (the average years of formal schooling = 3.59 years; SD = 3.61 years; range 0–18 years). Of all participants, 58.23%, 35.79%, and 5.98% carried the *KIBRA* rs17070145 TT, CT, and CC genotypes, respectively. There was no significant difference in demographic features, body mass index (BMI), physical exercise, cardiovascular risk factors, *APOE* ε4 allele, sinonasal disease, and MMSE score among *KIBRA* rs17070145 genotype groups (Table 1).

## Clusters of regional GMV associated with *KIBRA* genotypes (*n* = 1087)

In the voxel-wise whole gray matter analysis, we identified 5 clusters of regional GMV that were significantly associated with *KIBRA* genotypes (Fig. 2). Overall, compared with the *KIBRA* TT carriers, the C-allele carriers showed a higher GMV, mainly in the bilateral orbitofrontal cortex, left thalamus, right middle cingulate gyrus, and left supplementary motor area (Table 2). The biggest cluster was located mainly in the left orbitofrontal area, with 54.64% voxels in left orbital part of inferior frontal gyrus, 33.93% voxels in left orbital part of middle frontal gyrus, and 11.05% voxels in left orbital part of superior frontal gyrus. The second biggest cluster was located mainly in the right orbitofrontal area, with 66.98% voxels in right orbital part of inferior frontal gyrus and 28.98% voxels in right orbital part of middle frontal gyrus. Notably, all voxels in the third cluster were located in the left thalamus (Table 2).

## Associations of *KIBRA* rs17070145 with regional GMV (*n* = 1087)

We further examined the associations of the *KIBRA* rs17070145 polymorphism with GMVs of brain regions identified above. The general linear regression analysis suggested that after controlling for multiple potential confounders, *KIBRA* rs17070145 C-allele was significantly associated with increased GMVs of the left thalamus and bilateral orbitofrontal cortex, including left orbital part of inferior frontal gyrus, left orbital part of middle frontal gyrus, left orbital part of superior frontal gyrus, right orbital part of inferior frontal gyrus, and right orbital part of middle frontal gyrus (Table 3).

## Association of olfactory function with regional GMV (*n* = 1016)

Of the 1016 participants, 634 (62.40%) were defined with OI (SSIT score ≤ 10). We used binary logistic regression models to examine the association of regional GMV with olfactory function. After adjusting for demographics, vascular risk factors, and ICV, increased left thalamic GMV was significantly associated with a reduced likelihood of OI (multi-adjusted odds ratio [OR] = 0.84, 95% confidence interval [CI]: 0.74, 0.97) (Table 4). Furthermore, GMV of the left supplementary motor area was significantly associated with OI (multi-adjusted OR = 0.84, 95% CI: 0.71, 0.98). We did not find any significant association between the GMV of orbitofrontal cortex and OI.

## Association of *KIBRA* rs17070145 with olfactory function (*n* = 1016)

Logistic regression analysis showed that the *KIBRA* rs17070145 C-allele was significantly associated with

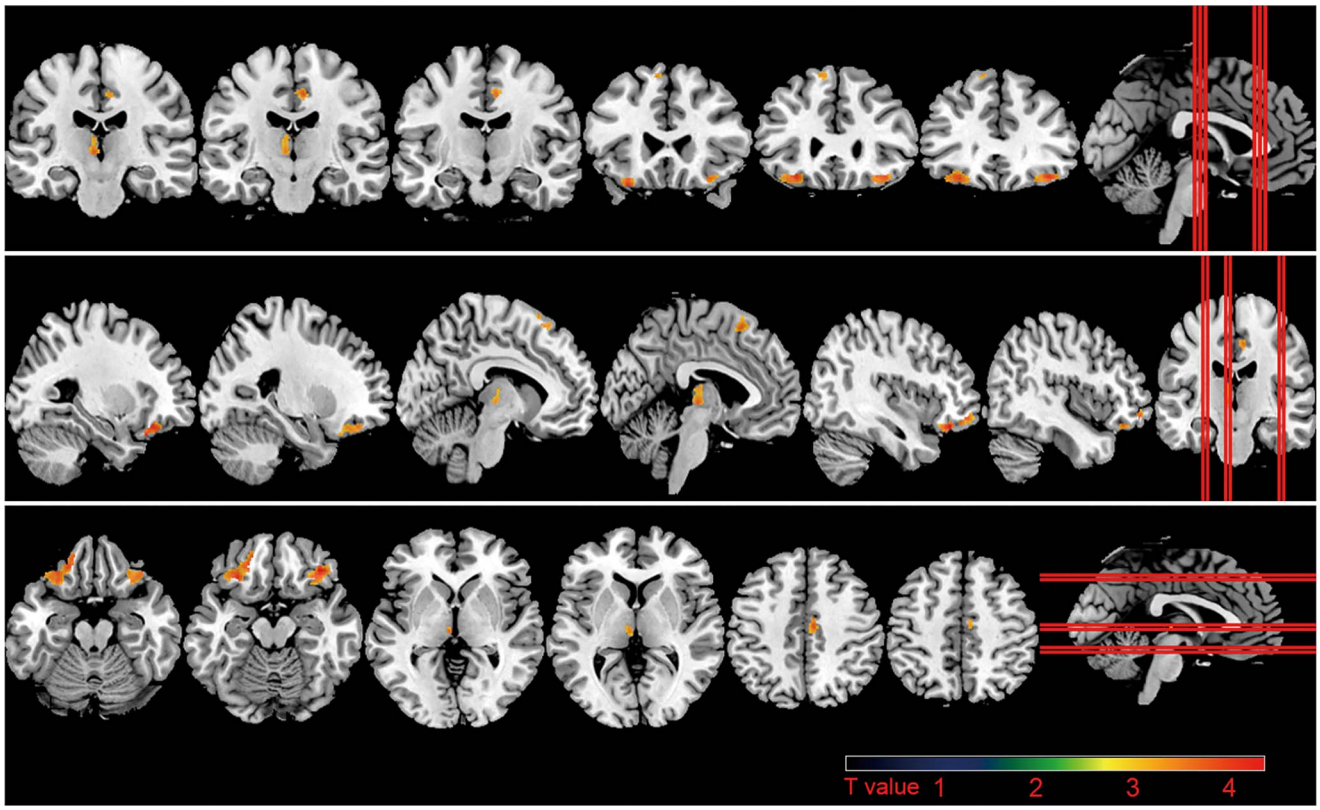


**Fig. 2.** Comparison of whole-brain voxel-wise GMV between *KIBRA* rs17070145 TT and C carriers after voxel-level FDR correction. The threshold was FDR-corrected $P < 0.05$.

**Table 2.** Characteristics of the clusters of brain regions with greater GMVs in *KIBRA* rs17070145 C-allele carriers than TT carriers.

| Characteristics | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 |
|---|---|---|---|---|---|
| **Co-ordinates of peak-voxel** | −28.5, 27, −16.5 | 42, 46.5, −10.5 | −4.5, −19.5, 1.5 | 7.5, −15, 45 | −4.5, 18, 57 |
| **Peak T value** | 4.14 | 4.34 | 3.71 | 3.80 | 3.66 |
| **Cluster size** | 507 | 421 | 142 | 152 | 166 |
| **Overlap of atlas region** | Frontal_Inf_Orb_L (54.64%)<br>Frontal_Mid_Orb_L (33.93%)<br>Frontal_Sup_Orb_L (11.05%)<br>Insula_L (0.39%) | Frontal_Inf_Orb_R (66.98%)<br>Frontal_Mid_Orb_R (28.98%)<br>Insula_R (4.04%) | Thalamus_L (100.00%) | Cingulum_Mid_R (96.71%)<br>Supp_Motor_Area_R (3.29%) | Supp_Motor_Area_L (68.67%)<br>Frontal_Sup_Medial_L (27.11%)<br>Frontal_Sup_L (4.22%) |

Note: *KIBRA*, kidney and brain expressed protein; Frontal_Inf_Orb_L, left orbital part of inferior frontal gyrus; Frontal_Mid_Orb_L, left orbital part of middle frontal gyrus; Frontal_Sup_Orb_L, left orbital part of superior frontal gyrus; Frontal_Inf_Orb_R, right orbital part of inferior frontal gyrus; Frontal_Mid_Orb_R, right orbital part of middle frontal gyrus; Insula_R, right insula; Thalamus_L, left Thalamus; Cingulum_Mid_R, right median cingulate and paracingulate gyri; Supp_Motor_Area_R, right supplementary motor area; Supp_Motor_Area_L, left supplementary motor area; Frontal_Sup_Medial_L, left superior frontal gyrus; Frontal_Sup_L, left superior frontal gyrus.

a reduced likelihood of OI, after controlling for demographics, vascular risk factors, and ICV (multi-adjusted OR = 0.73, 95% CI: 0.56, 0.95) (Fig. 3). Furthermore, we detected a statistically significant interaction between *KIBRA* rs17070145 and age group (age 60–69 vs. ≥70 years) on OI (*P* for interaction = 0.05). Stratifying analysis by age groups suggested that the association of *KIBRA* rs17070145 with OI was statistically evident only in older age group (≥70 years) (multi-adjusted OR = 0.54, 95% CI: 0.36, 0.79) (Fig. 3). In addition, *APOE* ε4 allele (vs. non-ε4 allele) was associated with the adjusted OR of 1.23 (95% CI: 0.86, 1.77) for OI in model 1 and 1.19 (95% CI: 0.82, 1.71) in model 2. We detected a marginally significant interaction between *KIBRA* rs17070145 and *APOE* ε4 allele on OI (*P* for interaction = 0.08 in model 1 and 0.05 in model 2). Stratifying analysis by *APOE* ε4 allele suggested that the association of *KIBRA* rs17070145 with OI was statistically evident among non-carriers of the *APOE* ε4 allele but not in *APOE* ε4 allele carriers (Fig. 3).

## Mediation of brain structure in the association of *KIBRA* genotypes with olfaction ($n = 1016$)

In the mediation analysis, we assessed the mediation effects of regional GMV on the association of *KIBRA* rs17070145 with OI. As shown in Fig. 4, 8.08% of the total association of *KIBRA* rs17070145 with OI was attributed to

**Table 3.** Associations of *KIBRA* rs17070145 with regional GMV ($n = 1087$).

| Brain regions | GMV | $\beta$-coefficient (95%CI), standardized GMV | |
|---|---|---|---|
| | Mean (SD) | Model 1[#] | Model 2[#] |
| **Frontal_Inf_Orb_L** | | | |
| TT | 0.29 (0.03) | 0.00 (reference) | 0.00 (reference) |
| CT + CC | 0.30 (0.03) | 0.12 (0.02, 0.23)* | 0.12 (0.02, 0.23)* |
| **Frontal_Mid_Orb_L** | | | |
| TT | 0.31 (0.04) | 0.00 (reference) | 0.00 (reference) |
| CT + CC | 0.31 (0.04) | 0.10 (0.00, 0.21)* | 0.11 (0.00, 0.21)* |
| **Frontal_Sup_Orb_L** | | | |
| TT | 0.31 (0.03) | 0.00 (reference) | 0.00 (reference) |
| CT + CC | 0.32 (0.04) | 0.13 (0.03, 0.23)* | 0.13 (0.03, 0.23)* |
| **Frontal_Inf_Orb_R** | | | |
| TT | 0.27 (0.03) | 0.00 (reference) | 0.00 (reference) |
| CT + CC | 0.27 (0.03) | 0.14 (0.03, 0.24)* | 0.14 (0.04, 0.25)** |
| **Frontal_Mid_Orb_R** | | | |
| TT | 0.30 (0.04) | 0.00 (reference) | 0.00 (reference) |
| CT + CC | 0.31 (0.04) | 0.15 (0.05, 0.26)** | 0.16 (0.06, 0.26)** |
| **Thalamus_L** | | | |
| TT | 0.41 (0.05) | 0.00 (reference) | 0.00 (reference) |
| CT + CC | 0.42 (0.05) | 0.15 (0.04, 0.27)* | 0.15 (0.04, 0.27)* |
| **Cingulum_Mid_R** | | | |
| TT | 0.37 (0.04) | 0.00 (reference) | 0.00 (reference) |
| CT + CC | 0.37 (0.04) | 0.11 (0.00, 0.21)* | 0.10 (−0.01, 0.20) |
| **Supp_Motor_Area_L** | | | |
| TT | 0.30 (0.03) | 0.00 (reference) | 0.00 (reference) |
| CT + CC | 0.30 (0.04) | 0.07 (−0.03, 0.17) | 0.08 (−0.02, 0.18) |
| **Frontal_Sup_Medial_L** | | | |
| TT | 0.29 (0.03) | 0.00 (reference) | 0.00 (reference) |
| T + CC | 0.30 (0.03) | 0.02 (−0.08, 0.12) | 0.03 (−0.07, 0.13) |

Note: *KIBRA*, kidney and brain expressed protein.[#]Model 1 was controlled for age, sex, education, and total intracranial volume; model 2 was additionally adjusted for ever smoking, alcohol intake, physical exercise, hypertension, diabetes, dyslipidemia, *APOE* genotype, and sinonasal disease. *$P < 0.05$, **$P < 0.01$.

**Table 4.** Associations of regional GMV with OI ($n = 1016$).

| Brain regions | GMV, mean (SD) | OR (95%CI), OI | | |
|---|---|---|---|---|
| | OI, no | OI, yes | Model 1[#] | Model 2[#] |
| **Frontal_Inf_Orb_L** | 0.30 (0.03) | 0.29 (0.03) | 0.94 (0.81, 1.09) | 0.93 (0.80, 1.09) |
| **Frontal_Mid_Orb_L** | 0.31 (0.03) | 0.31 (0.04) | 1.06 (0.91, 1.23) | 1.05 (0.90, 1.23) |
| **Frontal_Sup_Orb_L** | 0.32 (0.04) | 0.32 (0.04) | 1.03 (0.89, 1.20) | 1.04 (0.89, 1.21) |
| **Frontal_Inf_Orb_R** | 0.27 (0.03) | 0.27 (0.03) | 0.99 (0.85, 1.15) | 0.99 (0.86, 1.15) |
| **Frontal_Mid_Orb_R** | 0.31 (0.04) | 0.31 (0.04) | 1.03 (0.89, 1.21) | 1.04 (0.89, 1.21) |
| **Thalamus_L** | 0.42 (0.05) | 0.41 (0.05) | 0.85 (0.74, 0.98)* | 0.84 (0.74, 0.97)* |
| **Cingulum_Mid_R** | 0.37 (0.04) | 0.37 (0.04) | 0.90 (0.77, 1.04) | 0.89 (0.76, 1.03) |
| **Supp_Motor_Area_L** | 0.30 (0.03) | 0.30 (0.03) | 0.85 (0.73, 1.00) | 0.84 (0.71, 0.98)* |
| **Frontal_Sup_Medial_L** | 0.30 (0.03) | 0.29 (0.03) | 0.96 (0.82, 1.12) | 0.95 (0.81, 1.12) |

[#]Data were OR (95%CI) of OI associated with per 1-SD increase in the standardized GMV. Model 1 was controlled for age, sex, education, and total intracranial volume; model 2 was additionally adjusted for ever smoking, alcohol intake, physical exercise, hypertension, diabetes, dyslipidemia, *APOE* genotype, and sinonasal disease. *$P < 0.05$.

the left thalamic GMV after controlling for demographics, vascular risk factors, ICV, sinonasal disease, and *APOE* genotype.

## Sensitivity analysis

We repeated the analysis with regard to the associations of *KIBRA* rs17070145 polymorphism with brain structure and olfactory function after removing 111 participants with a history of sinonasal disease. This analysis yielded the main results that were essentially the same as those from the total sample (Fig. S1). For instance, wefound that among participants who had no history of sinonasal disease ($n = 905$), the *KIBRA* rs17070145 C-allele was still significantly associated with a reduced likelihood of OI (adjusted OR = 0.69 vs. 0.73 from the total sample). Furthermore, there was still a statistically significant interaction between *KIBRA* rs17070145 with age group (age 60–69 vs. ≥70 years) and a statistically marginal interaction with *APOE* $\varepsilon 4$ allele on OI (Fig. S1). In addition, the left thalamic GMV could mediate 6.65% of the total association of *KIBRA* rs17070145 with OI as compared with 8.08% from the analysis of the total sample (Fig. S2).

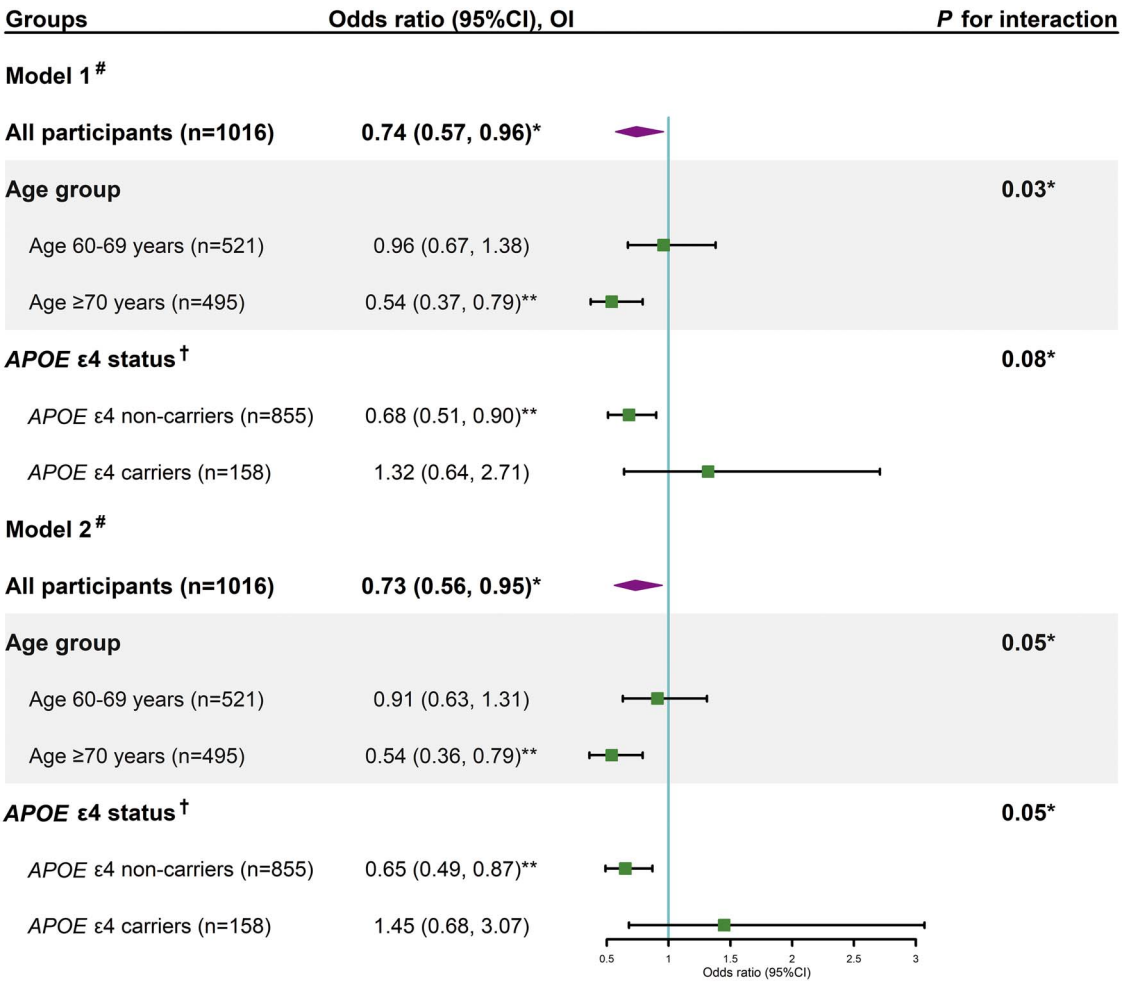


**Fig. 3.** Associations of *KIBRA* rs17070145 polymorphism with OI by age groups and *APOE ε4* allele status. Note: #The OR (95%CI) of OI was estimated by comparing *KIBRA* rs17070145 C-allele with TT genotype. Model 1 was controlled for age, sex, education; model 2 was additionally adjusted for ever smoking, alcohol intake, physical exercise, hypertension, diabetes, dyslipidemia, sinonasal disease, and if applicable, for *APOE ε4* allele. †Three persons were excluded due to missing information on *APOE* genotype. *$P < 0.05$, **$P < 0.01$.

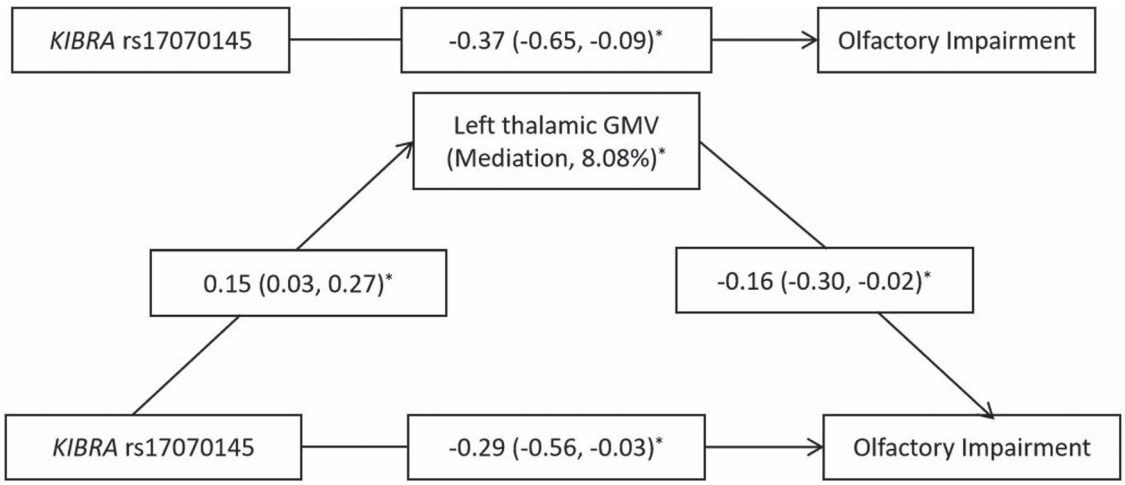


**Fig. 4.** Mediation effects of the left thalamic GMV on the associations of *KIBRA* rs17070145 with OI. Note: *KIBRA*, kidney and brain expressed protein. The mediation model was adjusted for age, sex, education, total intracranial volume, ever smoking, alcohol intake, physical exercise, hypertension, diabetes, dyslipidemia, *APOE* genotype, and sinonasal disease. *$P < 0.05$.

## Discussion

In this population-based study of rural-dwelling older adults in China, we had the unique opportunity to explore the associations of the *KIBRA* rs17070145 polymorphism with brain structure and olfactory function by integrating genetic data with olfactory test and

structural brain MRI data. The main findings were summarized as follows. (i) *KIBRA* rs17070145 C-allele carriers had a greater GMV than TT carriers, mainly in the bilateral orbitofrontal cortex and left thalamus. (ii) The greater left thalamic GMV was associated with better olfactory identification function. (iii) *KIBRA* rs17070145 C-allele was associated with a reduced likelihood of OI, especially in older adults aged ≥70 years or non-carriers of *APOE* ε4 allele. (iv) The association between *KIBRA* rs17070145 and OI was partly mediated by the left thalamic GMV.

In 2006, the genome-wide screening study first revealed the association of *KIBRA* rs17070145 CC homozygotes with poor episodic memory performance (Papassotiropoulos et al. 2006). However, this study did not find the allele-dependent differences in the volumes of the hippocampus, parahippocampal gyrus, or white and gray matter. In the past decade, several studies that examine the association of *KIBRA* genotypes with brain structure and cognitive function have yielded mixed results (Hayashi et al. 2010; Kauppi et al. 2011; Franks et al. 2014; Mazzeo et al. 2019;Wang et al. 2019 ; Li et al. 2020). Two cross-sectional studies of small samples from China indicated that *KIBRA* C-allele carriers, compared with TT carriers, had lower GMV of prefrontal cortex, parahippocampal cortex, and bilateral dorsal anterior cingulate cortices (Wang et al. 2019; Li et al. 2020). A longitudinal community-based study of nondemented older adults from Australia did not find any association between *KIBRA* genotypes and hippocampal volume (Boraxbekk et al. 2015). Several reasons may partly explain the discrepant results. Firstly, the frequency of *KIBRA* T-allele carriers differs substantially across ethnical populations, with the frequency being ∼25% in European populations, ∼50% in African Americans, and ∼75% in Asian populations (Papassotiropoulos et al. 2006), which may partly contribute to the different results among different racial and ethnic groups. Secondly, the relation of *KIBRA* with cognitive performance and brain structure may vary depending on characteristics of the study samples. For instance, previous studies showed an association of *KIBRA* CC genotype with episodic memory in patients with severe traumatic brain injury (Wagner et al. 2012), an association that was not observed in a study of healthy volunteers (Boraxbekk et al. 2015).

In the current study, the voxel-wise whole gray matter analysis revealed that *KIBRA* rs17070145 C-allele carriers showed a higher GMV, mainly in the bilateral orbitofrontal cortex and left thalamus. The orbitofrontal cortex received major projections from the piriform cortex and was correlated with olfactory functions (Seubert et al. 2013; Rudebeck and Rich 2018). However, we did not find a significant association between GMV in the orbitofrontal cortex and OI. In the cross-sectional study of healthy adults (mean age, 37 years) from Sweden, olfactory performance was assessed by measures of absolute odor threshold, cued olfactory identification, and olfactory quality discrimination (Seubert et al. 2013). The whole-brain analyses suggested that GMV in the orbitofrontal cortex was significantly associated with the composite score of olfactory Threshold, Discrimination, and Identification. However, consistent with our study, this Swedish study found no association between GMV in the orbitofrontal cortex and olfactory identification function measured with SSIT.

We further found that the association of *KIBRA* rs17070145 with OI was partly mediated by the GMV of the left thalamus. The thalamus, as a crucial crossroad structure involved in a variety of brain functions (e.g. sensory perception, sleep and arousal, and cognition), was initially considered as the major source of sensory information to the primary sensory cortex for all of the senses except olfaction (Rolls 2019). However, evidence has shown that the mediodorsal thalamic nucleus also has a somewhat unique sensory function because it receives direct input from primary olfactory cortex, projects to orbitofrontal associative cortex, and involves in translating odor inputs into recognizable and nameable (Olofsson et al. 2013; Courtiol and Wilson 2015; Anastasiades et al. 2021). Accordingly, a merged functional and structural olfactory network map was defined as follows: primary olfactory cortices included piriform cortex and amygdala; and secondary olfactory cortices included orbitofrontal cortex, caudate nucleus, anterior cingulate cortex, insula, putamen, pallidum, hippocampal–parahippocampal complex, and thalamus (Illig 2005; Scott et al. 2020). Consistently, our study showed that the brain region associated with *KIBRA* rs17070145 was located precisely in the medial nuclei of the left thalamus. Furthermore, these medial nuclei, primarily mediodorsal thalamic nucleus, partly mediated the association of *KIBRA* rs17070145 with OI.

Taken together, our large-scale population-based study provides evidence supporting a potential role of *KIBRA* in olfactory function, especially among older adults aged ≥70 years. We speculated that the following potential pathophysiological mechanisms might be involved in their association. Firstly, *KIBRA* might promote neuron survival and inhibit neuronal apoptosis in olfaction-related brain regions (Song, Tang, Dong, et al. 2019a). Secondly, *KIBRA* could influence long-term synaptic plasticity and postsynaptic receptor recycling, which was extremely important for the formation of olfaction (Makuch et al. 2011). Thirdly, *KIBRA* might affect olfaction through regulating the secretion of exosomes, which could deliver neurotransmitters as vehicles (Song, Tang, Han, et al. 2019b). In addition, the interaction between *KIBRA* and age could be explained by resource modulation hypothesis, which suggested that genetic effects were magnified in persons with constrained neural resources, such as older adults (Papenberg et al. 2015). We further detected a marginally statistical interaction between *KIBRA* rs17070145 and *APOE* ε4 allele on OI. Although biological mechanisms are unclear, both *APOE* and *KIBRA* genes have been found

to reduce long-term potentiation, a process related to synaptic plasticity and memory formation, by affecting ionotropic glutamate receptors (Zhang et al. 2017; Wang et al. 2019). The suggestive interaction between *KIBRA* and *APOE* genes on OI among older adults as well as the neurobiological mechanism deserves further investigation.

To the best of our knowledge, our population-based study is the first report showing that *KIBRA* rs17070145 was associated with OI among older adults and that their association was partly mediated by the brain structure. The major strengths of our study included the population-based design, with a relatively large sample, in which olfactory and genetic data were integrated with structural brain MRI data. Our study also has limitations. First, we were not able to establish the temporal relationships between structural brain measures and OI due to the cross-sectional nature of the study design, which should be kept in mind when interpreting the results. Second, we used only odor identification test (SSIT) to assess olfactory identification function, without odor threshold or odor discrimination tests, which could provide additional information for olfactory function. Finally, the study sample was relatively younger, healthier, and more educated than the average of the MIND-China total population. Thus, one should be cautious when generalizing our results to other populations.

## Conclusions

In conclusion, our population-based study demonstrated a strong association of the *KIBRA* rs17070145 genotype with olfactory function in rural older adults in China, such that carrying the *KIBRA* rs17070145 C-allele was associated with a reduced likelihood of OI. The GMV of the left thalamus could partly mediate the association of *KIBRA* rs17070145 with OI. The potential neuropathological mechanisms linking *KIBRA* rs17070145 with OI deserve further investigation.

## Supplementary material

Supplementary material is available at *Cerebral Cortex* online.

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*Conflict of interest statement*: None declared.

## Notes

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## Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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