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ORIGINAL ARTICLE

ALDH2 Glu504Lys Confers Susceptibility to Schizophrenia and Impacts Hippocampal-Prefrontal Functional Connectivity

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Abstract

Although previous evidence suggested that *ALDH2* is a candidate gene for schizophrenia, the association and underlying mechanisms have never been investigated. Therefore, we investigated *ALDH2* as a susceptibility gene for schizophrenia and explored the effect of its polymorphisms on brain functions. In the discovery stage, we detected a positive association between a dominant-negative mutant, Glu504Lys, and schizophrenia ($P = 8.01E{-}5$, OR = 1.34, 95% CI = 1.16–1.55). This association was confirmed in the validation stage ($P = 3.48E{-}6$, OR = 1.28, 95% CI = 1.15–1.42). The combined *P* reached a genome-wide significance ($P_{combined} = 1.32E{-}9$, OR = 1.30, 95% CI = 1.20–1.42). To investigate the neural mechanism linking Glu504Lys to schizophrenia, we calculated the functional connectivity (FC) and applied an imaging genetics strategy using resting-state fMRI data. The imaging analysis revealed a significant interaction of diagnostic group by genotype for FC between the left hippocampus and the prefrontal cortex. In the Glu homozygotes, hippocampal-prefrontal FC correlated inversely with memory performance in the healthy controls and with the PANSS negative score in the schizophrenia patients. Our results supported a role for *ALDH2* in the pathophysiology of schizophrenia. Moreover, variation at Glu504Lys disrupts hippocampal-prefrontal FC, which might be the neural mechanism linking it to the disease.

Key words: functional connectivity, Glu504Lys, hippocampus, PFC, schizophrenia

Introduction

Schizophrenia is a common, debilitating disorder characterized by psychosis, apathy and social withdrawal, and cognitive defects (Mueser and McGurk 2004). Compelling evidence from family, twin, and adoption studies has indicated that many genes significantly contribute to the etiology of schizophrenia. The estimated heritability is 80–85% (Sullivan et al. 2003). Despite the high heritability, the underlying genetic risk factors

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and the neural mechanisms behind those genetic factors are unclear.

Mitochondrial aldehyde dehydrogenase 2 (*ALDH2*) is a candidate gene for schizophrenia due to its positional and functional significance. The *ALDH2* gene is located on chromosome 12q24, a region frequently implicated in genetic association studies of schizophrenia and confirmed to harbor many schizophrenia susceptibility genes (Allen et al. 2008; O'Donovan et al. 2008). As a member of the aldehyde dehydrogenase family, ALDH2 is considered to be the principal enzyme in acetaldehyde metabolism and has been implicated in serotonin and dopamine oxidation (Keung and Vallee 1998). Serotonin and dopamine are 2 of the most important neurotransmitters influencing mental health. Convergent evidence suggests that both serotonin and dopamine play pivotal roles in the pathophysiology of schizophrenia and are effective therapeutic targets (Kapur and Remington 1996). Thus, ALDH2 dysfunction may lead to an imbalance in the neurotransmitter systems, impact normal neural functions, and eventually result in schizophrenia. One of our previous studies, which used a *cis*-expression quantitative trait loci (eQTL) analysis, suggested that *ALDH2* might be related to schizophrenia (Zhang et al. 2014). However, the association between *ALDH2* and schizophrenia has never been thoroughly investigated.

Schizophrenia is associated with disrupted brain functional connectivity (FC) along with related cognitive impairments (Cole et al. 2011; Fornito et al. 2011). A previous study reported that transgenic mice with Aldh2 activity defects displayed neurodegeneration in the hippocampus and correlated memory loss (Ohsawa et al. 2008). Other studies indicated that the hippocampus and prefrontal cortex (PFC) interact to support working memory, long-term memory, and other cognitive functions that are abnormal in schizophrenia patients (Axmacher et al. 2008; Supekar et al. 2013). Disrupted functional couplings between the hippocampus and the PFC have been observed in both schizophrenia patients and individuals at high genetic risk for the disease (Meyer-Lindenberg et al. 2005; Zhou et al. 2008; Benetti et al. 2009). Moreover, recent studies have showed that resting-state FC, which examines temporal correlations of intrinsic low-frequency fluctuations in the blood oxygenation level-dependent (BOLD) signals across brain regions, is useful for mapping brain networks and may even be able to predict individual cognitive performance (Biswal et al. 2010; Liu et al. 2014). Therefore, resting-state hippocampal-prefrontal functional coupling, which could be considered as a pivotal neuroimaging intermediate phenotype bridging the biological function of *ALDH2* with the neural mechanisms of schizophrenia, may help reveal potential mechanisms that link this gene to the risk of schizophrenia.

In light of those findings, we hypothesized that SNPs in *ALDH2* might confer susceptibility to schizophrenia and impact hippocampal-prefrontal FC. To investigate our hypotheses, we first conducted a 2-stage association study using 2 independent Han Chinese populations and then applied an imaging genetics strategy based on resting-state fMRI data collected from schizophrenia patients and healthy control subjects to explore the genetic influence of *ALDH2* polymorphisms on brain circuits.

Methods

Subjects

For the genetic association study, the initial sample consisted of 1316 unrelated schizophrenia patients (650 males and 666 females; mean age: 33.0 ± 6.1 years) and 1349 healthy control subjects (645 males and 704 females; mean age: 32.5 ± 6.7 years). An independent sample, consisting of 2514 unrelated schizophrenia patients (1128 males and 1386 females; mean age: 32.4 ± 8.6 years) and 2637 healthy control subjects (1187 males and 1450 females; mean age: 31.8 ± 9.3 years), was recruited for validation. For the imaging genetics study, we recruited a total of 100 schizophrenia patients and 100 healthy control subjects. All the subjects were Han Chinese from northern China. Consensus diagnoses were made by 2 experienced senior psychiatrists using the Diagnosis and Statistic Manual of Mental Disorders, 4th edition (DSM-IV) criteria for schizophrenia, based on the Structured Clinical interview for DSM-IV-TR Axis I Disorders (SCID-I, patient edition). Patients with any other neurologic disorder, a history of severe medical illness, substance dependence, pregnancy, or treatment with electroconvulsive therapy within the past 6 months and those with a diagnosis of any other Axis I disorder were excluded. Healthy controls were recruited from the community and screened using the SCID-I (non-patient edition). Individuals with any history of mental and/or neurological disorder were excluded. Written informed consent was obtained from all patients and their legal guardians (i.e., their parents) and all healthy controls. The study was approved by the Medical Research Ethics Committees of the local hospitals and institutes from which the patients and controls were recruited.

Genotyping

Peripheral blood samples were collected from all subjects. Genomic DNA was extracted from the blood using a Qiagen QIAamp DNA Mini Kit. Five SNPs spanning the selected genomic region, rs886205, rs441, rs4646777, Glu504Lys, and rs11066028, were selected from dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). Single SNPs were genotyped using TaqMan SNP genotyping assay on an ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, FosterCity, CA), as described previously (Zheng et al. 2014). For quality control purposes, all the genotypes were blind to the case or control status during the genotyping process. We repeated the genotyping assay for one percent of the samples and found that results were 100% concordant. The DNA extraction and genotyping were centrally processed at the Key Laboratory of Mental Health in Beijing, P.R. China.

Assessment of Symptomatology and Memory Performance

The symptom severity of the patients included in the imaging analysis was assessed by trained and experienced psychiatrists using the Positive and Negative Syndrome Scale (PANSS) within 1 week of MRI scanning. Based on previous finding of memory deficits in mice (Ohsawa et al. 2008), we assessed memory performance of participants with Wechsler Memory Scale-Revised (WMS-R) (Wechsler 1987).

Image Data Acquisition and Preprocessing

All of the subjects included in the imaging genetics study were scanned using the same Siemens 3.0 Tesla Trio magnetic resonance scanner with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, field of view (FOV) = 220 × 220 mm^2, matrix = 64 × 64, flip angle = 90°, voxel size = 3.4× 3.4 × 4.0 mm^3, 33 slices, and 240 volumes. Before the scanning, all the subjects were instructed to move as little as possible, keep their eyes closed, think of nothing in particular, and avoid falling asleep.

Data preprocessing was completed using Statistical Parametric Mapping 8 (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/software/

spm8/) and in-house software. The following preprocessing steps were performed: 1) discarding of the first 10 volumes; 2) slice timing correction; 3) realigning the volumes to the first volume to correct for intrascan movements; 4) spatially normalizing to a standard EPI template; 5) spatially smoothing using a 6 mm Gaussian kernel; 6) performing linear regression to remove the influence of head motion, whole brain signals, and linear trends; and 7) temporal band-pass filtration (0.01–0.08 Hz). Specifically, 19 schizophrenia patients and 2 healthy subjects were excluded because of genotyping failure, insufficient fMRI data, or a maximum displacement in any of the cardinal directions (x, y, z) of >2 mm, or a maximum spin (x, y, z) of >2°, leaving a total of 81 schizophrenia patients and 98 healthy subjects in the subsequent data analysis.

Analysis of Data

We employed independent sample *t*-tests and χ^2 tests for continuous and discrete variable comparisons. The case–control association analysis was performed using Haploview version 4.1 (http://www.broad.mit.edu/mpg/haploview/). A Bonferroni correction for multiple testing was carried out to exclude type I errors.

For the imaging analysis, we calculated individual voxel-wise hippocampal FC maps with the whole brain by taking the left hippocampus as the region-of-interest (ROI), based on previous findings of an aberrant left hippocampal FC in schizophrenia patients (Meyer-Lindenberg et al. 2005). The mask of the left hippocampus was constructed using the automated anatomical labeling atlas in Wake Forest University Pickatlas (Tzourio-Mazoyer et al. 2002). An FC map was computed for each individual by averaging the BOLD time series in the ROI and then computing the Pearson's correlation coefficient between the average time series and those from each voxel in the brain. The resulting correlations were transformed to approximate a Gaussian distribution using Fisher's z transformation. Thus, a whole brain FC map of the left hippocampus was created for each subject. A 2 × 2 ANOVA, with diagnostic category and genotype as between-subject factors and age and gender as covariates, was performed using SPM8. This full-factorial ANOVA allowed us to characterize the main effect of diagnostic group, the main effect of genotype, and the interaction between these factors. Given our interest in hippocampal-prefrontal FC, we used a prefrontal region, which included Brodmann areas 8, 9, 10, 11, 44, 45, 46, and 47 (Preuss 1995), with a small volume statistical correction (family-wise error, FWE correction with a threshold of $P < 0.05$). For each diagnostic group, a partial correlation analysis with 2 covariates (age and gender) was further performed by genotype groups to explore the associations between hippocampal-prefrontal FC and memory function as well as those between hippocampal-prefrontal FC and the PANSS score in patients with different genotypes.

Results

Glu504Lys Is Significantly Associated with Schizophrenia

The results of the 5 polymorphic SNPs are shown in Table 1. None of the genotype distributions of these SNPs in either the patients or the controls significantly deviated from Hardy–Weinberg equilibrium ($P > 0.05$, data not shown). Of these 5 SNPs, the allelic frequency of Glu504Lys ($P = 8.01E{-}5$, OR = 1.34, 95% CI = 1.16–1.55) was found to be significantly associated with schizophrenia. After a Bonferroni correction for multiple tests, the difference in allelic frequencies observed for Glu504Lys remained significant. In Stage 2, the finding of an association between Glu504Lys and schizophrenia was replicated in the validation sample ($P = 3.48E{-}6$, OR = 1.28, 95% CI = 1.15–1.42). In the combined study, the Mantel–Haenszel method was used, and the combine *P* value reached a genome-wide significance ($P_{combined} = 1.32E{-}9$, OR = 1.30, 95% CI = 1.20–1.42).

Effects of Glu504Lys on Hippocampal-Prefrontal FC

An independent sample with a total of 81 schizophrenia patients and 98 healthy subjects were included in the imaging analysis. No significant difference in age, gender, or years of education was observed between the diagnostic groups, as well as between the genotype subgroups in either diagnostic group (Table 2 and see Supplementary Tables 1 and 2). According to their PANSS ratings, the schizophrenic patients were moderately to severely impaired. Not surprisingly, the patients showed a significantly poorer performance than the healthy controls on the WMS-R (Table 2).

We investigated the influence of Glu504Lys polymorphisms on the FC of the hippocampus with the whole brain and found a significant interaction effect of diagnostic group by genotype for the FC between the left hippocampus and the frontopolar cortex (FPC), in that patients and controls showed diverging effects of genotype (Fig. 1, Brodmann area 10, Lateral FPC; peak voxel MNI coordinate: $x = -26$, $y = 48$, $z = 4$, *F*-score = 23.99, $P = 0.010$ after FWE correction; cluster size = 73). The main effect of neither diagnosis nor genotype could survive an FWE correction.

For the Glu homozygotes, the hippocampal-prefrontal FC was inversely correlated with the WMS score in the healthy control group ($R = -0.257$, $P = 0.039$) and with the PANSS negative score ($R = -0.404$, $P = 0.004$) in schizophrenia patients. However, these correlations disappeared in individuals who were Lys-allele carriers in either diagnostic group. These findings suggest that, depending on Glu504Lys genotype, the hippocampal-prefrontal FC may affect memory performance and schizophrenia negative symptoms in totally different ways.

Discussion

In the present study, we first conducted a 2-stage association study to investigate the role of *ALDH2* in schizophrenia

Table 1 Allele frequencies of 5 SNPs in the *ALDH2* gene in schizophrenia patients and controls in 2 independent Han Chinese populations

	SNP ID	Polymorphism[a]	MAF (Case)	MAF (Control)	OR (95% CI)	χ^2 (df = 1)	*P* value
Stage 1	rs886205	C/T	0.110	0.116	0.93 (0.79–1.11)	0.575	0.448
	rs441	T/C	0.277	0.295	0.92 (0.81–1.03)	2.123	0.145
	rs4646777	G/A	0.276	0.295	0.91 (0.81–1.03)	2.403	0.121
	Glu504Lys	Glu/Lys	0.185	0.145	1.34 (1.16–1.55)	15.555	8.01E–5
	rs11066028	C/A	0.077	0.085	0.90 (0.74–1.10)	1.177	0.278
Stage 2	Glu504Lys	Glu/Lys	0.180	0.146	1.28 (1.15–1.42)	21.583	3.48E–6

Note: [a]Polymorphism, the second allele is the minor allele in Asian populations, as indicated in the dbSNP database.

Table 2 Demographic and clinical characteristics of schizophrenia patients and healthy controls

Variables	Schizophrenia patients	Healthy controls	*P* value
Gender (Male/Female)	50/31	52/46	0.244
Age in years	27.33 (6.98)	25.77 (5.39)	0.093
Education in years	13.60 (2.98)	13.65 (3.37)	0.977
Genotype (Lys-carrier/GluGlu)	31/50	29/69	0.221
PANSS total score	77.21 (10.18)	n.a.	
PANSS positive score	23.62 (4.40)	n.a.	
PANSS negative score	18.68 (5.96)	n.a.	
PANSS general score	35.29 (5.43)	n.a.	
CPZ-eq at scan (mg/day)	438.13 (200.83)	n.a.	
WMS-R score[a]	94.40 (20.82)	108.42 (17.40)	<0.001

Note: Data are given as mean (standard deviation), unless otherwise indicated. PANSS, Positive and Negative Syndrome Scale; CPZ-eq, chlorpromazine equivalents; WMS-R, Wechsler Memory Scale-Revised; n.a., not applicable.
[a]Information from 24 patients was missing.

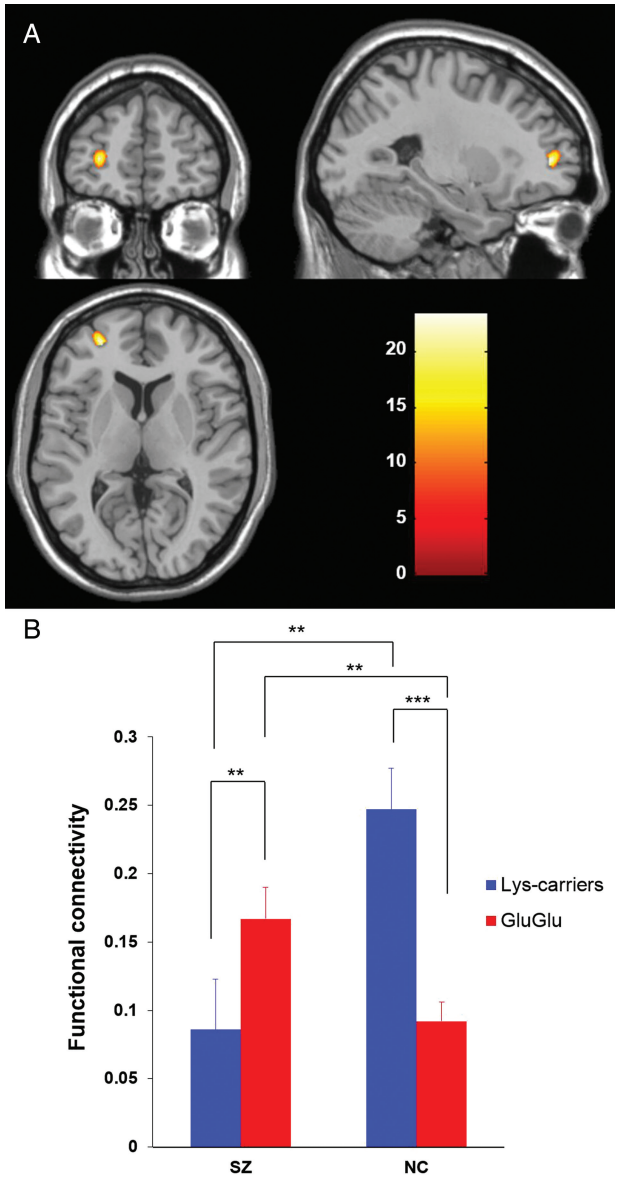

Figure 1. Results of group comparison analyses. (*A*) The figure shows the single frontal region with a significant genotype × diagnosis interaction effect for functional connectivity from the left hippocampus (Brodmann area 10, lateral frontopolar cortex; peak voxel MNI coordinate: x = −26, y = 48, z = 4, *F*-score = 23.99, *P* = 0.010 after FWE correction; cluster size = 73). The color bar indicates the *F*-score. (*B*) The bar charts show the functional connectivity (mean ± S.E.) between the left hippocampus and the survived frontal region in each group. The *Y*-axis indicates the *Z*-score. SZ, schizophrenia patients; NC, normal healthy controls. $^{**}P < 0.01$, $^{***}P < 0.001$.

susceptibility. Our results revealed that Glu504Lys was related to risk for schizophrenia. To further investigate the possible genetic influence of Glu504Lys polymorphisms on brain function, we applied an imaging genetics strategy based on resting-state fMRI data and found a significant interaction effect of diagnostic group by genotype for FC between the left hippocampus and the FPC. Moreover, higher FC may predict worse memory performance in healthy Glu homozygotes, but lower FC may predict a higher PANSS negative score in Glu homozygous patients. However, this predictive capability disappeared in the Lys-allele carriers. Taken together, the present findings confirmed our speculation that *ALDH2* confers susceptibility to schizophrenia and identified potential neural mechanisms linking Glu504Lys with the risk of developing schizophrenia via the intermediate phenotype of hippocampal-prefrontal FC.

To the best of our knowledge, this is the first study to report an association between *ALDH2* and schizophrenia. The observed significant SNP, Glu504Lys, is a missense mutation located in the 12th exon of the gene. This mutation results in a substitution of lysine for glutamate, acting in a dominant-negative manner, leaving a partially or completely inactive form of the enzyme (Bosron and Li 1986). Previous studies explored the associations between Glu504Lys and several mental disorders, including bipolar disorder and Alzheimer's disease. However, no significant association was detected (Hao et al. 2011; Wang et al. 2014). Other studies focusing on the molecular mechanisms found that overexpression of ALDH2 prevented acetaldehyde-induced oxidative stress, which is mediated, at least in part, through the ERK/MAP kinase stress signaling pathways (Li et al. 2004). Subsequent studies reported that mitochondrial ALDH2 deficiency itself might be viewed as a kind of oxidative stress (Ohta et al. 2004). Notably, during oxidative stress, the intracellular redox balance is disturbed, leading to an increased production and accumulation of reactive oxygen species, which may cause mitochondrial dysfunction (Marin-Garcia and Goldenthal 2008). In recent decades, mitochondrial dysfunction and oxidative stress have increasingly been implicated in the pathophysiology of schizophrenia (Sullivan and O'Donnell 2012). In addition, given the role of ALDH2 in serotonin and dopamine metabolism and given that the variation at Glu504Lys causes ALDH2 deficiency (Bosron and Li 1986), we may speculate that this variation leads to imbalance in the serotonin and dopamine systems plus an accumulation of neurotoxic metabolites, both of which would disturb normal brain functions and could eventually cause individuals to develop schizophrenia. Even though the pathway by which *ALDH2* could modulate the pathogenesis of schizophrenia is yet to be determined, given the results of the present association study, we concluded that *ALDH2* is a susceptibility gene for schizophrenia.

One of our principle findings was that the Lys-allele of Glu504Lys contributed to the risk of schizophrenia. However, little is known about how this mutation affects brain function to increase vulnerability to schizophrenia. Deciphering the neural mechanism underlying the association between Glu504Lys polymorphisms and schizophrenia may help us to establish a

pathway from the enzyme to the disease. Additionally, a previous study reported that transgenic mice expressing a mouse version of Glu504Lys exhibited neurodegeneration in the hippocampus accompanied by memory loss and cognitive impairment (Ohsawa et al. 2008). In the context of this finding and the important role of the hippocampus and the PFC in memory performance, we performed an imaging genetics analysis of resting-state fMRI data, based on the presumption that Glu504Lys affected the development and plasticity of the hippocampal-prefrontal circuitry. Intriguingly, we found a significant diagnosis-by-genotype interaction effect for FC between the hippocampus and the FPC, in which the patients and the controls showed diverging effects of genotype.

Emerging evidence has indicated that abnormal integration between the hippocampus and the PFC might underlie the cognitive impairments of schizophrenia (Fletcher 1998). In fact, as the anterior part of the PFC, the FPC has long been implicated in the pathologic features of schizophrenia. As illustrated by previous research, the FPC underwent a highly evolutionary expansion in humans and is believed to sit atop a prefrontal hierarchy (Boschin et al. 2015). Previous evidence supports a particularly important role of the FPC in performing a diverse range of high cognitive load functions, such as relational integration, multitasking, exploratory decision making, episodic memory retrieval, future thinking, and prospective memory (Boschin et al. 2015), most of which are impaired in schizophrenia patients. In addition, structural and functional impairments in the FPC, including decreased cortical thickness (Byun et al. 2012), smaller gray matter volume (Rosso et al. 2010), disrupted white matter integrity (Hao et al. 2009), and reduced FC (Zhou et al. 2015), have repeatedly been reported in schizophrenia patients and in individuals at high risk for schizophrenia. In addition, a prior study parcellated the human frontal pole (FP) into 2 subregions. Our identified region in the FPC is located in the lateral subregion FP1, which is preferentially activated by episodic and working memory tasks and thus implements the more "abstract cognitive" aspects of the FC (Bludau et al. 2014). Therefore, we can conclude that the observed FC between the hippocampus and the FPC may be a pivotal intermediate phenotype linking *ALDH2* to schizophrenia and to its related cognitive impairment. The lower hippocampal-FPC FC we found in Lys-allele carriers in the patient group may indicate a greater vulnerability to the well-documented effects of schizophrenia. The elevated FC observed in healthy risk Lys-allele carriers may represent a compensational mechanism for maintaining sufficient information processing and achieving normal behavior, given that left FPC hyperactivation and insufficiency have been found in patients with schizophrenia during working memory tasks (Glahn et al. 2005). Such a compensatory impact of genes on FC in the face of the effects of these genes on relevant brain region activity has been found in previous studies (Mohnke et al. 2014; Jaspar et al. 2016). Nevertheless, more data and different experimental designs are required to explore this speculation in the future. In addition, since serotonin and dopamine are essential neurotransmitters regulating neuronal excitability in the PFC, an impact of ALDH2 deficiency on hippocampal-prefrontal coupling is conceivable. Taken together, our findings may suggest a neural mechanism that underlies the association between Glu504Lys and the risk for schizophrenia.

Another interesting finding of the present study was that individual hippocampal-prefrontal FC reflected memory performance in healthy controls and symptom severity in schizophrenia patients in distinct ways, depending on the Glu504Lys genotype. Hippocampal-prefrontal FC was inversely correlated with memory performance in the healthy controls and with the PANSS negative score as well as the total PANSS score in the schizophrenia patients only in Glu homozygotes, who may have a lower genetic risk for schizophrenia. Such anticorrelations suggest that 1) in healthy Glu homozygotes, individuals with a higher amount of hippocampal-prefrontal FC have a worse memory performance, an interpretation that is compatible with our previous finding in healthy subjects (Liu et al. 2014); 2) in Glu homozygous schizophrenia patients, individuals with less hippocampal-prefrontal FC have more severe negative symptoms. These correlations corroborate prior studies linking the hippocampal-prefrontal pathway to the negative and cognitive symptoms of schizophrenia (Ghoshal and Conn 2015). The absence of the inverse relationship between FC and memory performance in healthy high-risk Lys-allele carriers may result from the compensatory effect mentioned above, while the disappearance of the one between FC and the PANSS score in Lys-carrier patients suggests a disrupted modulation of the hippocampal-prefrontal FC with an increase in the severity of psychiatric symptoms due to the effects of an interaction between the risk allele and the illness. We did not find any correlation between FC and memory performance in either genotype group in the patients, yet this is quite understandable given that memory performance impairment may stem from the core capacity limitations inherent to schizophrenia and the functional abnormalities detected represent a psychotic signature.

Our findings seem to shed light on the genetic contribution of Glu504Lys to the risk of developing schizophrenia and related brain functions. However, there are several limitations to the interpretation of our results. First, according to the HapMap database, SNP Glu504Lys in *ALDH2*, which showed a significant association with schizophrenia in our study, is likely to be present primarily in Asian populations and increase disease susceptibility. However, as far as we know, no existing evidence indicates that there is a higher prevalence of schizophrenia in Asian populations. This is quite understandable given that schizophrenia is a complex heritable disorder with numerous susceptibility genetic loci, each of which only exerts a minor effect on the pathogenesis of schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics 2014). In addition, the possibility cannot be excluded that there are other variations in this gene, which may confer a risk for schizophrenia in other ethnic populations. Thus, further studies, including high-density mapping and deep sequencing, are required to identify other susceptibility loci across populations. Second, the large sample size of the present study makes it susceptible to population stratification. While stratification cannot be completely ruled out as an explanation for our positive findings, it seems unlikely. Apart from reasons that have been discussed in detail elsewhere (Zheng et al. 2014), the present study used a 2-stage approach in which the positive association from the discovery stage was confirmed in an independent validation population, suggesting that our positive result was not a false-positive due to confounding factors such as population stratification. Third, based on previous experimental findings (Meyer-Lindenberg et al. 2005; Ohsawa et al. 2008), we specifically focused on FC from the hippocampus and did not explore FC from other brain regions. However, how to obtain the best seed region is still a question for the region-based functional connectivity analyses. Future studies focused on other theoretically important regions are needed. Last, but not the least, our neuroimaging evidence is based on resting-state fMRI, so future studies using different experimental paradigms are necessary to validate our findings.

In conclusion, our results are the first, to our knowledge, to reveal that Glu504Lys of *ALDH2* confers susceptibility to

schizophrenia, possibly via modulation of the hippocampal-prefrontal functional connectivity. In spite of the limitations and future areas for study mentioned above, our findings lend support to a role for *ALDH2* in the pathophysiology of schizophrenia.

Supplementary Material

Supplementary material can be found at http://www.cercor.oxfordjournals.org/online.

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Notes

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