ORIGINAL ARTICLE

GABAergic Abnormalities Associated with Sensorimotor Cortico-striatal Community Structural Deficits in ErbB4 Knockout Mice and First-Episode Treatment-Naïve Patients with Schizophrenia

Chengcheng Zhang^{1,2} · Peiyan Ni^{1,2} · Yikang Liu³ · Yang Tian^{1,2} · Jinxue Wei^{1,2} ·
Bo Xiang^{1,2} · Liansheng Zhao^{1,2} · Xiaojing Li^{1,2} · Xiaohong Ma^{1,2} ·
Wei Deng^{1,2} · Wanjun Guo^{1,2} · Rongjun Ni^{1,2} · Yamin Zhang^{1,2} · Qiang Wang^{1,2} ·
Hailiang Huang^{4,5} · Nanyin Zhang³ · Tao Li^{1,2}

Received: 24 December 2018 / Accepted: 1 May 2019 / Published online: 6 August 2019
© Shanghai Institutes for Biological Sciences, CAS 2019

Abstract The current study was designed to explore how disruption of specific molecular circuits in the cerebral cortex may cause sensorimotor cortico-striatal community structure deficits in both a mouse model and patients with schizophrenia. We used prepulse inhibition (PPI) and brain structural and diffusion MRI scans in 23 mice with conditional ErbB4 knockout in parvalbumin interneurons and 27 matched controls. Quantitative real-time PCR was used to assess the differential levels of GABA-related transcripts in brain regions. Concurrently, we measured structural and diffusion MRI and the cumulative contribution of risk alleles in the GABA pathway genes in first-

episode treatment-naïve schizophrenic patients ($n = 117$) and in age- and sex-matched healthy controls ($n = 86$). We present the first evidence of gray and white matter impairment of right sensorimotor cortico-striatal networks and reproduced the sensorimotor gating deficit in a mouse model of schizophrenia. Significant correlations between gray matter volumes (GMVs) in the somatosensory cortex and PPI as well as glutamate decarboxylase 1 mRNA expression were found in controls but not in knockout mice. Furthermore, these findings were confirmed in a human sample in which we found significantly decreased gray and white matter in sensorimotor cortico-striatal networks in schizophrenic patients. The psychiatric risk alleles of the GABA pathway also displayed a significant negative correlation with the GMVs of the somatosensory cortex in patients. Our study identified that ErbB4 ablation in parvalbumin interneurons induced GABAergic dysregulation, providing valuable mechanistic insights into the sensorimotor cortico-striatal community structure deficits associated with schizophrenia.

Chengcheng Zhang and Peiyan Ni have contributed equally to this work.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12264-019-00416-2>) contains supplementary material, which is available to authorized users.

✉ Nanyin Zhang
nuz2@psu.edu

✉ Tao Li
litaohx@scu.edu.cn

¹ Mental Health Center and Psychiatric Laboratory, State Key Laboratory of Biotherapy, West China Hospital of Sichuan University, Chengdu 610041, China

² West China Brain Research Center, West China Hospital of Sichuan University, Chengdu 610041, China

³ Department of Biomedical Engineering, Pennsylvania State University, University Park, PA 16802, USA

⁴ Analytic and Translational Genetics Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

⁵ Broad Institute of MIT and Harvard, Cambridge, MA 02141, USA

Keywords ErbB4 · Schizophrenia · MRI · Gene pathway · Mouse

Introduction

Schizophrenia is a heritable psychiatric disorder characterized by a heterogeneous collection of symptoms including altered perception and cognitive deficits [1–3]. Previous association studies, including by our group, have uncovered susceptibility genes for schizophrenia, such as the genes encoding neuregulin-1 (NRG1) and the only autonomous receptor tyrosine kinase erbB-4 (ErbB4) [4–8]. NRG1-ErbB4 signaling has been implicated in

neural development, and variation of the ErbB4 gene is associated with abnormal brain structure in schizophrenics [9]. Null mutations of the NRG1 and ErbB4 genes cause a spectrum of abnormal behaviors in mice, including hyperactivity, disrupted pre-pulse inhibition (PPI), and spatial learning and memory deficits, which are thought to be associated with schizophrenia [10, 11]. The PPI deficit, which has been widely shown in the ErbB4 knockout rodent model [12], is a characteristic feature of schizophrenia [13].

In addition to evidence at the behavioral level, transgenic rodent models have shown that GABAergic transmission, a major target of NRG1-ErbB4 signaling, is also critically involved in schizophrenia [14]. Almost all ErbB4-positive cells in the cortex, basal ganglia, and most of the amygdala in neonatal and adult mice are GABAergic with the highest enrichment in parvalbumin (PV) fast-spiking interneurons [15]. Specific ErbB4 deletion in PV neurons results in schizophrenia-relevant phenotypes in mice similar to those found in NRG1- or ErbB4-null mutant mice, including hyperactivity, impaired working memory, and a deficit in PPI. Using single-cell RNA sequencing of striatal cells, studies have shown that the main interneuron types are GABAergic, and most striatal interneurons signal *via* releasing GABA to inhibit target cells [16]. Thus, ErbB4-knockout (KO) in PV interneurons might provide a useful animal model in which to investigate how disturbances of the GABAergic system underlie the neurodevelopmental condition, schizophrenia, at the mechanistic level [17–19].

Despite the molecular- and behavioral-level evidence found in the transgenic rodent model of ErbB4 that is associated with key aspects of schizophrenia, we still do not understand the system-level alterations in this model and their relationship with behavioral and molecular changes. Specifically, there has been no research aiming to investigate the effects of knocking out the ErbB4 gene in PV neuron on brain structure in animals. Elucidating the relationship between ErbB4 and brain structural network change in a non-invasive and not hypothesis-driven MRI scanning manner is essential for understanding the pathophysiology of schizophrenia. In addition, it remains unknown whether gene expression abnormalities are present in the brain regions that exhibit morphological changes. Furthermore, how structural changes in the brain of KO animals are linked to clinical relevance remains elusive [20].

Previous studies have highlighted the importance of eliminating the confounder of drugs, particularly when investigating the molecular mechanisms of schizophrenia. Using both a mouse model with specific depletion of ErbB4 in PV-expressing interneurons and first-episode, treatment-naïve patients with schizophrenia, we set out to

analyze the brain sensorimotor-striatum network and gene expression abnormalities, which can completely eliminate the effects of antipsychotic treatment.

Materials and Methods

A flowchart of the experimental design is shown in Figure S1. Specific details of the methods and materials are provided in Supplementary Materials. All methods were in accordance with the relevant guidelines and regulations approved by the Institutional Review Board and local animal committee of West China Hospital of Sichuan University.

Animals

Conditional ErbB4-KO mice were generated by breeding PV-Cre mice with mice carrying loxP-flanked ErbB4 alleles [19]. Twenty-three KO mice (PV-Cre; ErbB4^{-/-}) and 27 age- and sex-matched controls (PV-Cre; ErbB4^{+/+}) were subjected to behavioral procedures and neuroimaging scans at ~ 3 months postnatal.

Behavioral Test and Mouse MRI

As many behavior tests have been conducted on the KO mice, we focused on their sensorimotor gating by measuring PPI. All MRI data were acquired on a 7.0 T MRI scanner (Bruker Biospec 70/30, Ettlingen, Germany). Brain structures were assessed using T2-weighted MRI and diffusion tensor imaging (DTI). T2-weighted anatomical images were analyzed using optimized voxel-based morphometry following Diffeomorphic Anatomical Registration Through Exponentiated Lie (DARTEL) algebra [21]. DTI images were analyzed using the FMRIB Diffusion Toolbox in FSL (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>) [22] as well as custom-written scripts in MatLab (MathWorks, Natick, MA).

Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (PCR)

As sensorimotor gating deficits may be relevant to schizophrenia, we next addressed the question of whether schizophrenia-related neuropathological and molecular changes exist. We analyzed the brain mRNA expression levels of the NRG1, PLCG1, GABRα3, GABRα2, and GAD1 genes, which have frequently been reported to be associated with the etiology of schizophrenia [23–27]. Total RNA was extracted from brain regions based on the results of image analysis, using TRIzol reagent (Life Technologies, Carlsbad, CA) [28]. The relative mRNA

levels in individual brain regions were measured in 19 KO mice and 21 controls. Details are provided in the Supplementary Materials. All assays were performed in triplicate.

Human Study

We also performed MRI scanning and examined cumulative contribution of risk alleles in the GABA pathway genes using polygenic risk scores (PRS) in 117 first-episode treatment-naïve patients with schizophrenia and 86 age- and sex-matched healthy controls (details in Supplementary Information).

Human MRI and Genetic Analysis

All participants were scanned on a 3.0 T MRI scanner (GE EXCITE, General Electric, Milwaukee, 8-channel head-coil). T1 images were processed using the DARTEL toolbox in Statistical Parametric Mapping (SPM8) (<https://www.fil.ion.ucl.ac.uk/spm/>) [29]. Diffusion anisotropy indices and diffusion shape measures were calculated from the tensor element output of the FMRIB Software Library in FSL (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>). Detailed procedures of scanning and pre-processing are presented in the Supplementary Materials.

DNA from whole-blood samples was obtained with the standard isolation method and genotyped using the HumanOmniZhongHua-8 BeadChip (Illumina, San Diego, CA). The resulting single-nucleotide polymorphism set was then used to calculate four multidimensional scaling (MDS) components to assess population stratification. Genome-wide summary statistics for schizophrenia were generated using fixed-effect meta-analysis across 13 cohorts, consisting of 13,305 individuals with schizophrenia and 16,244 healthy controls of Asian ancestry (unpublished work). We used these results to calculate the pathway-based PRS for GABAergic pathway genes selected from Reactome [30, 31], a well-annotated molecular pathway database, including genes involved in the synthesis, release, receptor-reuptake, and degradation of GABA. For each individual, a PRS was calculated using PRSice software [32] (http://prsice.info/PRSice_MANUAL_v1.25.pdf), which runs a linkage disequilibrium (LD)-based clumping for multiple stepwise P -value thresholds (P_T) to find the optimal P_T based on the largest variance explained (Nagelkerke R^2). We used the default settings for clumping (LD window: 250 kb, $r^2 < 0.1$) and generated six sets of scores using P_T cut-offs at 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5. For each P_T cut-off, a set of SNPs with P values below the cutoff were selected. All PRS quantifications were controlled for MDS.

Statistical Analysis

Imaging data were compared between control and KO mice using two-sample t -test after controlling for age and the whole brain. Imaging data across the whole brain were compared on a voxel-by-voxel basis. To further evaluate the correlation between brain morphometric changes and behavioral measures, we first defined each cluster of voxels that showed significant differences between the control and KO groups as a region of interest (ROI). We set the significance of differences at a threshold of $P < 0.05$ at a false discovery rate (FDR) corrected and cluster > 100 voxels. For each mouse, the gray matter volume (GMV) of each ROI was quantified by averaging the GMVs of all voxels within the ROI. Since PPI was measured at three noise amplitudes, principal component analysis (PCA) was applied to the three results, and the first principal component was used to quantify the PPI measure for each mouse. The participant-specific ROI GMV and PPI values were then separately correlated in control and KO mice, after controlling for age. Owing to skewed distributions of NRG1, PLCG1, GABR α 3, GABR α 2, and GAD1 values, their mRNA values were log-transformed [33]. Multivariate analysis of covariance was next performed on each of the normalized gene expression values and the effect size was calculated using Cohen's d . Age was set as covariate. The correlations between ROI GMV and mRNA expression level were separately calculated in control and KO mice after controlling for age.

For human imaging data, the GMVs of the left and right somatosensory cortex, as well as the GMVs and fractional anisotropy values (FAs) in the caudate were separately extracted and compared between healthy controls and patients with schizophrenia using univariate analysis with age, sex, education years, and the whole brain as covariates. For each P_T cutoff, the GABA-pathway PRS and the GMV of the somatosensory cortex were separately correlated in controls and schizophrenics after controlling for age, sex, education years, and the first four MDS components. All statistical analyses were performed using SPSS 22 statistical software (SPSS Inc., Chicago, IL) and MatLab (MathWorks, Natick, MA).

Results

In this study, we characterized alterations in behavior, brain structure, and gene expression of the GABAergic pathway in KO mice. We also examined the clinical relevance of our preclinical findings in first-episode patients with schizophrenia.

PPI Deficit

KO mice demonstrated impairment in somatosensory gating, reflected by significantly lower PPI amplitudes for

all three pre-pulse intensities relative to controls (Fig. 1B, $P = 0.02$, 0.004 , and 0.04 for 74 dB, 78 dB, and 86 dB, respectively). There was no significant difference in age

Fig. 1 Relationship between brain structures and PPI in ErbB4-KO mice. **A** Colored voxels represent clusters showing significant gray matter volume difference between controls and ErbB4-KO mice. **B** PPI tests in ErbB4-KO and control mice, quantified as the percentage decrease of the startle response amplitude at three pre-pulse intensities (74 dB, 78 dB, and 86 dB). Values are the mean $\pm$ SEM. **C** The first PCA component of PPI amplitude in ErbB4-KO

and control mice. **D–F** Significant correlations of the first PCA component of PPI and the GMVs of the right S1BF, S1ULp, and S2 in control mice. However, these correlational relationships did not occur in KO mice. Age was included as the covariate. PCA, principal component analysis; S1BF and S1ULp, primary somatosensory cortex, barrel field and upper lip region; S2, secondary somatosensory cortex. * $P < 0.05$, *** $P < 0.001$.

between control and KO mice. The PPI data in both groups are summarized in Table S1.

Changes in Brain Structure

KO mice exhibited altered brain structures in both GMV and white matter integrity. Voxel-wise analysis of T2-weighted structural images revealed a significantly smaller GMV in an extended area covering the barrel field (S1BF) and upper lip region (S1ULp) in the right primary somatosensory area, the right secondary somatosensory

cortex (S2), and the right lateral striatum (CPU) ($P < 0.05$, FDR-corrected, Fig. 2A–F; details in Table S2). In addition, significantly smaller FAs in the right parasubiculum (Pas) and CPU were found in KO mice relative to the controls (Fig. 2G–J; $P < 0.05$, FDR-corrected). Detailed information of these clusters is summarized in Table S3.

mRNA Expression Levels

The mRNA expression levels of both *NRG1* and *PLCG1* genes in the right somatosensory cortex were significantly

Fig. 2 Gray matter volume (GMV) and white matter integrity changes in ErbB4-KO mice. **A** Mouse brain atlas. **B** Colored voxels represent significant regional GMV decreases in ErbB4-KO mice ($P < 0.05$, FDR-corrected, after controlling for age and the whole brain), overlaid on structural MRI images. **C–F** GMVs of clusters. L, Left; R, Right; S1BF, barrel field of primary somatosensory cortex; S1ULp, upper lip region of primary somatosensory cortex; S2, secondary somatosensory cortex; CPU, lateral striatum. Values are

the mean $\pm$ SEM. **G** Mouse brain atlas. **H** Colored voxels represent significant regional decreases in fractional anisotropy values in ErbB4-KO mice ($P < 0.05$, FDR-corrected, after controlling for age and the whole brain), superimposed on anatomical MRI atlas. **I, J** Fractional anisotropy values of clusters. L, left; R, right; Pas, parasubiculum; CPU, lateral striatum. Values are the mean $\pm$ SEM. The colorbar shows the T -values of the statistical analysis. *** $P < 0.001$.

lower in KO mice relative to controls (for *NRG1*, $t = 2.38$, $P = 0.02$, medium Cohen's d effect size = 0.77; for *PLCG1*, $t = 2.46$, $P = 0.019$, medium Cohen's d effect size = 0.8, Fig. 3A, D). Similarly, in the right striatum the mRNA levels of both of these genes were significantly lower in KO mice than in controls (for *NRG1*, $t = 3.01$, $P = 0.005$, medium Cohen's d effect size = 0.97; for *PLCG1*, $t = 2.80$, $P = 0.001$, medium Cohen's d effect size = 0.91, Fig. 3F, I). However, as internal controls, we did not find differences in the mRNA levels of either gene

in the left somatosensory cortex or left striatum between KO mice and controls. Table S4 summarizes the ROI mRNA expression levels in both groups.

The mRNA levels of both *GAD1* and *GABR α 2* genes were significantly higher in the right striatum in KO mice than in controls (for *GAD1*, $t = -3.51$, $P = 0.008$, medium Cohen's d effect size = -1.14; for *GABR α 2*, $t = -2.40$, $P = 0.022$, medium Cohen's d effect size = -0.78, Fig. 3G, H). Again, no significant

Fig. 3 NRG1-ErbB4 signaling, GABA transmission, and their relationship to brain structure in ErbB4-KO mice. **A–J** mRNA levels in the right striatum and somatosensory cortex in ErbB4-KO mice and controls. **K** Colored voxels represent clusters showing significant gray matter volume differences between controls and ErbB4-KO mice. The *GAD1* mRNA levels in the right somatosensory cortex showed a

positive correlation with the GMV of the right (L) S1BF, (M) S1ULp, and (N) S2 in control, but not in KO mice. Age was included as the covariate. S1BF and S1ULp, primary somatosensory cortex, barrel field and upper lip region; S2, secondary somatosensory cortex. $*P < 0.05$; $**P < 0.01$.

difference in the mRNA levels of these genes was found in the left striatum (Table S4).

By using these genes as query genes (i.e., *NRG1*, *GABRa2*, *GABRa3*, *GAD1*, *ErbB4*, and *PV*), we further expanded the set of genes examined and assessed functional gene networks in the somatosensory cortex in *ErbB4*-KO mice. Functional gene networks were constructed by connecting query and related genes including co-expression, co-localization, predicted, and shared protein domains. The 6 query genes noted above were given the maximum node size. The size of nodes for related genes was inversely proportional to the rank of the gene in a list sorted by the gene scores, which were assessed by using GeneMANIA software (<http://genemania.org/>). By analyzing the gene networks using GeneMANIA, we found prominent networks, including the GABA A receptor activity network (Coverage [number of genes in the network with a given function/all genes in the genome with that function] = 7/14, FDR $q = 6 \times 10^{-14}$) and the GABAergic synaptic transmission network (Coverage = 4/31, FDR $q = 1.9 \times 10^{-4}$). Taken together, these results indicated that GABA receptor activity and GABAergic synaptic transmission in the somatosensory cortex were altered in KO mice.

Correlations Between Brain Structure, Behavior, and mRNA Levels

We found that both the behavioral data and mRNA levels of the *GAD1* gene were significantly correlated to brain morphometric measures in control animals. By contrast, these correlation relationships were absent in KO mice. Specifically, PPI was positively associated with the GMV of S1BF ($r = 0.58$, $P = 0.001$), S1ULp ($r = 0.58$, $P = 0.001$) and S2 ($r = 0.56$, $P = 0.002$) in controls but not in KO mice (for S1BF, $r = -0.22$, $P = 0.31$; for S1ULp, $r = -0.21$, $P = 0.33$; for S2, $r = -0.24$, $P = 0.26$; Fig. 1D–F). In addition, the mRNA level of the *GAD1* gene was positively associated with the GMV of S1BF ($r = 0.55$, $P = 0.01$), S1ULp ($r = 0.52$, $P = 0.02$) and S2 ($r = 0.52$, $P = 0.02$) in controls, but again, not in KO mice (for S1BF, $r = -0.07$, $P = 0.79$; for S1ULp, $r = -0.11$, $P = 0.68$; for S2, $r = -0.12$, $P = 0.68$; Fig. 3L–N). No other significant associations between behavioral data, mRNA levels, and changes in brain structure were found.

GMV and FA Differences Between Patients with Schizophrenia and Controls

To further examine the clinical relevance of our preclinical findings in mice, we compared GMV in the somatosensory cortex between first-episode patients with schizophrenia

and healthy controls. Our data demonstrated reduced GMV in the left primary somatosensory cortex ($t = 2.93$, $P = 0.001$), right somatosensory cortex ($t = 2.86$, $P = 0.002$), and right caudate body ($t = 3.1$, $P < 0.001$), after controlling for age, sex, and education years. We also compared FA in the striatum region in first-episode patients with schizophrenia and healthy controls. Our data showed reduced FA in the right caudate ($t = 4.45$, $P < 0.001$), after controlling for age, sex, and education years (Table S5 and Fig. 4A–E). There was no significant difference in age, sex, and education years between controls and patients. Details of intelligence quotient and the duration of untreated psychosis in both groups are summarized in Table S5. These data suggest that our brain imaging findings in KO mice have significant translational value in patients.

Relationship Between PRS and GMV

The PRS of GABAergic pathway genes for schizophrenia was negatively associated with the GMV of the left somatosensory cortex at all five GWAS (genome-wide association study) P_T cutoffs (P_T ranging from 0.05 to 0.5) in patients with schizophrenia, but not in controls. In addition, the PRS values of GABAergic pathway genes were negatively associated with the GMV of the right somatosensory cortex at three GWAS P_T cutoffs (0.05, 0.1 and 0.2) in patients with schizophrenia, but not in controls (Table S6 and Fig. 4F–N). These results suggested that, like *ErbB4*-KO mice, altered GABA function might contribute to morphological changes in sensorimotor areas in schizophrenia patients.

Discussion

Here, we showed for the first time that a mouse model of schizophrenia (*ErbB4*-KO in PV neurons) displays significantly reduced GMVs and white matter integrity in the right sensorimotor cortico-striatal networks and reduced mRNA expression in the *ErbB4*-*NRG1* signaling pathway, as well as increased mRNA expression in the GABAergic pathway in the right somatosensory cortex and striatum. In addition, we found that correlations between GMVs of the S1BF, S1ULp, S2, and PPI as well as *GAD1* mRNA expression that were present in control mice, were absent in KO mice. The data provide a possible link between the GABA pathway and schizophrenia-related pathobiology. In parallel, we also confirmed changes in the schizophrenia-associated GABAergic pathway in sensorimotor structures of patients. We found reduced GMVs and white matter integrity in regions in the same sensorimotor cortico-striatal networks in patients with schizophrenia and this structural alteration was further associated with the PRS of

Fig. 4 Clinical relevance. **A** Patients with schizophrenia showed significant reductions in the gray matter volume in the sensorimotor cortico-striatal community. **B–E** GMV and FA values of clusters. Values are the mean $\pm$ SEM. GMV, gray matter volume; FA, fractional anisotropy; L_SS, left somatosensory cortex; R_SS, right somatosensory cortex; R_CAU, right lateral caudate body; R_CPU, right caudate. **F–N** Polygenic risk scores of genes in the GABAergic pathway showing negative correlations with the GMV of the somatosensory cortex in patients with schizophrenia. Age, sex, and education years and the whole brain were included as covariates. P_T cutoffs, P_T ranging from 0.05 to 0.5.

Fig. 5 Schematic illustration of major findings in the present study. Using both a mouse model and first-episode, treatment-naïve patients with schizophrenia, we identified sensorimotor cortico-striatal community structure deficits associated with schizophrenia. **A** ErbB4 deletion in PV interneurons might be critically involved in schizophrenia as manifested by the genes in the GABAergic pathway, abnormal structure in the sensorimotor cortical-lateral striatal networks and sensory gating deficit. **B** Abnormal sensorimotor cortico-striatal network structure in patients with schizophrenia associated with the polygenic risk score of genes in the GABAergic pathway. GMV, gray matter volume; FA, fractional anisotropy; CPU, lateral striatum; PRS, polygenic risk scores.

genes in the GABAergic pathway. All these findings are summarized in Fig. 5. Taken together, these results suggest that ErbB4 in PV interneurons is critically involved in schizophrenia at the molecular (genes in the GABAergic pathway), systems (abnormal brain structure in the somatosensory-striatum regions), and behavioral levels (the PPI deficit).

Relationship between ErbB4 and Sensorimotor Gating Deficit in Schizophrenia

PPI provides an operational method to measure sensorimotor gating, a process by which an organism filters extraneous information from the internal and external milieu. A PPI deficit is a characteristic feature of schizophrenia both in patients and animal models [34–37]. However, less is known about the possible genetic underpinnings of this phenotypic PPI deficit in schizophrenia [38]. In the present study, we demonstrated a significant PPI deficit in ErbB4-KO mice. Our results confirmed that the ErbB4 gene plays an important role in the impaired sensorimotor gating in schizophrenia.

Relationships of ErbB4, Brain Structure, and PPI in Schizophrenia

The association between ErbB4 gene polymorphism and brain structure alterations in schizophrenia has been investigated in several studies [39, 40]. However, most of these studies investigated this relationship at the correlational level, while the causal effect of knocking out ErbB4 on brain structures remained unclear. Our study uncovered this effect on the sensorimotor cortical-lateral striatal networks. ErbB4-KO mice showed decreased GMVs of the right somatosensory cortex and CPU, as well as decreased FA in the right CPU relative to control mice, highlighting the impact of ErbB4 deletion on brain structures subserving sensorimotor-related functions.

These changes in brain structure in ErbB4-KO mice also echoed their behavioral deficit in sensorimotor gating. We found that in control mice, the GMV of the somatosensory cortex was positively correlated with PPI. This result is consistent with the human data demonstrating a linkage between the somatosensory cortex and PPI in healthy participants [41]. By contrast, this correlation disappeared in KO mice. These data collectively suggest that the somatosensory cortex is critical to somatosensory gating, and alteration in this brain structure can lead to a loss of a brain-behavior association in a right-hemisphere function. This brain-behavior relationship is consistent with anatomical studies in humans linking the right sensorimotor striatum to cognitive control, and suggests a loss of a brain-behavior association in schizophrenia [42]. Considering

that both the structural and behavioral changes are consequences of ErbB4-KO, ErbB4 might be the key genetic basis of somatosensory cortex changes and the somatomotor gating deficit in schizophrenia.

In contrast to the somatosensory cortex, we did not find any significant correlation between the deficit in the CPU and PPI, although the GMV of the CPU was also reduced in KO mice. These data are in line with a study that reported no significant correlation between the synaptic density in the striatum and PPI in either healthy participants or those at high risk of psychosis [35]. Taken together, these results indicate that the structural change in the striatum is not directly related to the PPI deficit in schizophrenia.

NRG1-ErbB4 Signaling, GABA Transmission, and Their Relationship to Brain Structure

On the basis of our imaging results, we identified abnormal mRNA levels of genes in the NRG1-ErbB4 and GABA pathways in the right somatosensory cortex and right striatum. It has been reported that NRG1 and PLCG1 are tightly connected to the ErbB4 pathway, and they are highly expressed in cortical interneurons and the striatum [43]. Our data confirmed that the NRG1 and PLCG1 mRNA levels were significantly decreased in the right somatosensory cortex and striatum in ErbB4-KO mice, suggesting changes in ErbB4-NRG1 signaling in this schizophrenia-like mouse model. In line with this result, previous studies have also reported decreased ErbB4 protein and NRG1-related cellular activity in the somatosensory cortex in schizophrenic patients [17, 44].

In addition, we found that the GAD1 (GAD67, an enzyme associated with GABA synthesis) [45] and GABR α 2 mRNA levels were increased in the right striatum in KO mice. This result is consistent with a previous report of stronger GABA expression in the dorsal caudate in ultra-high-risk patients with schizophrenia relative to healthy controls [46]. Moreover, previous researchers have shown that ErbB4 is enriched in GABAergic neurons. Although the exact mechanisms underlying the increases in GABR α 2 mRNA and GAD1 are unknown, it is possible that they are an up-regulated response to compensate for the deficit in GABA transmission [47–49]. Moreover, we found that the GMV of the right somatosensory cortex was positively correlated with the GAD1 mRNA expression in the same region in control mice, but this correlation was absent in ErbB4-KO mice. These data suggest that the structural alterations resulting from ErbB4 deletion can lead to impairment of transcriptional changes in NRG1-ErbB4 signaling and GABA transmission. These results demonstrate that anomalous NRG1-ErbB4 signaling and GABA transmission might be important molecular mechanisms

underlying the anatomical changes in the sensorimotor-striatum network in schizophrenia [50–52].

Clinical Relevance

Since ErbB4-KO mice displayed behavioral phenotypes that are characteristic of schizophrenia (e.g. PPI deficit) as shown in present study and others [17, 38], this animal model has been suggested to be a valuable model of schizophrenia. The sensorimotor gating deficits in ErbB4 mice provide an opportunity to understand the mild sensorimotor dysfunctions frequently reported in patients with schizophrenia. However, it is unclear whether our imaging and genetic findings in this animal model are clinically relevant. Thus, we concurrently examined in patients with schizophrenia the GMV and FA in the same sensorimotor cortical-lateral striatal networks identified in KO mice. Interestingly, we found decreased GMV in the somatosensory cortex and dorsal caudate in first-episode, treatment-naïve patients with schizophrenia relative to matched healthy controls. The results agree with our previous studies that showed decreased GMV of the somatosensory cortex in first-episode patients with schizophrenia [53]. It has also been shown that the GMV of the right primary somatosensory is still significantly smaller in schizophrenic patients than in healthy controls at 1-year follow-up [54]. Furthermore, we found that the FA in the right dorsal caudate was lower in patients than in controls, and this result is consistent with the report that the avolition in schizophrenia is linked to dorsal caudate hypoactivation [55–59]. Taken together, these results show that ErbB4-KO mice are a valuable model of schizophrenia and have high translational utility. Our results also indicate that abnormalities in the sensorimotor-striatum network might be a key systems-level marker of schizophrenia.

Since invasive procedures could not be considered in such a large and unique sample of first-episode and treatment-naïve patients with schizophrenia, brain-specific changes in gene expression could not be obtained. To further assess the clinical relevance of our molecular-level findings in mice, we used the polygenic risk-profiling method. Schizophrenia is a highly polygenic disorder, so PRS based on risk alleles derived from GWAS provides an ideal method to quantify the overall burden of the many risk allele carried by individuals. In addition, at the biological level, imaging genetics has demonstrated that psychiatric risk alleles are more closely tied to variance at the systems level as compared with clinical diagnoses [60]. Consistent with our findings in ErbB4-KO mice, we found that the additive contribution of risk alleles of genes in the GABAergic signaling pathway is clearly associated with decreased GMV of the somatosensory cortex in patients with schizophrenia. Our findings further support the role of

GABA dysfunction in schizophrenia and suggest that anomalous GABA transmission might be a significant factor contributing to the structural changes in the somatosensory cortex. This finding is striking because humans and mice use different modalities and levels (structural and diffusion MRI, expression levels and genetic variation) as a primary means of sensorimotor gating, suggesting that the physiological underpinnings of the sensorimotor network have remained evolutionarily conserved at the multi-system level and further supporting the use of such intermediate phenotypes as a way to enhance translational analyses.

The results of this study should be interpreted in the context of the following limitations. As we did not measure PPI or the ErbB4 expression level in the schizophrenic patients, further studies to confirm the relationship between the structural change in the somatosensory cortex and the PPI deficit as well as ErbB4 alterations in patients as in our animal study need to be conducted. The preliminary findings of the current study supported the possibility that deregulated ErbB4 signaling may be a joint mechanism between sensorimotor gating and GABAergic dysfunction, providing a working hypothesis for future research. It should be noted that the correlations between the PRS of GABAergic pathway genes and GMV of somatosensory cortex in schizophrenic patients reported in the current study are uncorrected with multiple comparisons. Further longitudinal studies in patients are required to verify the multi-system level changes in schizophrenia. In addition, the present study has several advantages. The whole-brain imaging method avoids the empirical preselection of brain regions. Animal models also avoid the confounding effects of medications. Indeed, researchers have measured GABA levels in the medial prefrontal cortex of medicated and unmedicated schizophrenics. They found significant elevations of the neurotransmitter only in un-medicated but not medicated patients [61], highlighting the importance of eliminating the confounder of drugs, particularly when investigating the molecular mechanisms of schizophrenia. As a result, in the human study, we used first-episode, treatment-naïve patients (or patients on drugs for < 3 days) and completely eliminated the effects of antipsychotic treatment on the sensorimotor-striatum network.

Conclusions

In this study, ErbB4 KO mice showed deficits in PPI, neuroanatomical changes (GMV and FA) in the sensorimotor cortical-lateral striatal networks, and abnormalities in the ErbB4-*NRG1* signaling and GABAergic pathways. The structural changes were replicated in first-episode treatment-naïve patients with schizophrenia. In addition,

the cumulative contribution of risk alleles of genes in the GABAergic pathway was negatively associated with the GMV of the somatosensory cortex in schizophrenic patients. We identified a critical function of ErbB4 in balancing brain circuits, and identified GABAergic abnormalities as a target of *NRG1*/ErbB4 signaling in sensorimotor cortico-striatal community structural deficits and key behaviors relevant to schizophrenia.

Acknowledgements This work was supported by the National Natural Science Foundation of China (81630030, 81130024, and 81528008); the National Natural Science Foundation of China/Research Grants Council of Hong Kong Joint Research Scheme (81461168029); the National Basic Research Development Program of China (2016YFC0904300); the Science and Technology Project of the Health Planning Committee of Sichuan (19PJ090); and the National Natural Science Foundation of China for Distinguished Young Scholars (81501159).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kavanagh DH, Tansey KE, O'Donovan MC, Owen MJ. Schizophrenia genetics: emerging themes for a complex disorder. *Mol Psychiatry* 2015, 20: 72–76.
2. Fioravanti M, Carlone O, Vitale B, Cinti ME, Clare L. A meta-analysis of cognitive deficits in adults with a diagnosis of schizophrenia. *Neuropsychol Rev* 2005, 15: 73–95.
3. Silverstein SM, Keane BP. Perceptual organization impairment in schizophrenia and associated brain mechanisms: review of research from 2005 to 2010. *Schizophr Bull* 2011, 37: 690–699.
4. Stefansson H, Petursson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, *et al.* Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet* 2002, 71: 877–892.
5. Silberberg G, Darvasi A, Pinkas-Kramarski R, Navon R. The involvement of ErbB4 with schizophrenia: association and expression studies. *Am J Med Genet B Neuropsychiatr Genet* 2006, 141: 142–148.
6. Lu CL, Wang YC, Chen JY, Lai IC, Liou YJ. Support for the involvement of the *ERBB4* gene in schizophrenia: a genetic association analysis. *Neurosci Lett* 2010, 481: 120–125.
7. Li T, Stefansson H, Gudfinnsson E, Cai G, Liu X, Murray RM, *et al.* Identification of a novel neuregulin 1 at-risk haplotype in Han schizophrenia Chinese patients, but no association with the Icelandic/Scottish risk haplotype. *Mol Psychiatry* 2004, 9: 698–704.
8. Yang JZ, Si TM, Ruan Y, Ling YS, Han YH, Wang XL, *et al.* Association study of neuregulin 1 gene with schizophrenia. *Mol Psychiatry* 2003, 8: 706–709.
9. Zuliani R, Moorhead TWJ, Bastin ME, Johnstone EC, Lawrie SM, Brambilla P, *et al.* Genetic variants in the ErbB4 gene are associated with white matter integrity. *Psychiatry Res Neuroimaging* 2011, 191: 133–137.
10. Barros CS, Calabrese B, Chamero P, Roberts AJ, Korzus E, Lloyd K, *et al.* Impaired maturation of dendritic spines without disorganization of cortical cell layers in mice lacking *NRG1*/ErbB signaling in the central nervous system. *Proc Natl Acad Sci U S A* 2009, 106: 4507–4512.

11. O'Tuathaigh CMP, Babovic D, O'Meara G, Clifford JJ, Croke DT, Waddington JL. Susceptibility genes for schizophrenia: characterisation of mutant mouse models at the level of phenotypic behaviour. *Neurosci Biobehav Rev* 2007, 31: 60–78.
12. Powell SB, Zhou X, Geyer MA. Prepulse inhibition and genetic mouse models of schizophrenia. *Behav Brain Res* 2009, 204: 282–294.
13. Cadenhead KS, Swerdlow NR, Shafer KM, Diaz M, Braff DL. Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. *Am J Psychiatry* 2000, 157: 1660–1668.
14. Woo RS, Li XM, Tao Y, Carpenter-Hyland E, Huang YZ, Weber J, *et al.* Neuregulin-1 enhances depolarization-induced GABA release. *Neuron* 2007, 54: 599–610.
15. Bean JC, Lin TW, Sathyamurthy A, Liu F, Yin DM, Xiong WC, *et al.* Genetic labeling reveals novel cellular targets of schizophrenia susceptibility gene: distribution of GABA and non-GABA ErbB4-positive cells in adult mouse brain. *J Neurosci* 2014, 34: 13549–13566.
16. Muñoz-Manchado AB, Gonzales CB, Zeisel A, Munguba H, Bekkouche B, Skene NG, *et al.* Diversity of interneurons in the dorsal striatum revealed by single-cell RNA sequencing and patchseq. *Cell Rep* 2018, 24: 2179–2190. e2177.
17. Del Pino I, García-Frigola C, Dehorter N, Brotons-Mas JR, Alvarez-Salvado E, de Lagrán MM, *et al.* ErbB4 deletion from fast-spiking interneurons causes schizophrenia-like phenotypes. *Neuron* 2013, 79: 1152–1168.
18. Chen YJ, Zhang M, Yin DM, Wen L, Ting A, Wang P, *et al.* ErbB4 in parvalbumin-positive interneurons is critical for neuregulin 1 regulation of long-term potentiation. *Proc Natl Acad Sci U S A* 2010, 107: 21818–21823.
19. Wen L, Lu YS, Zhu XH, Li XM, Woo RS, Chen YJ, *et al.* Neuregulin 1 regulates pyramidal neuron activity via ErbB4 in parvalbumin-positive interneurons. *Proc Natl Acad Sci U S A* 2010, 107: 1211–1216.
20. Donaldson ZR, Hen R. From psychiatric disorders to animal models: a bidirectional and dimensional approach. *Biol Psychiatry* 2015, 77: 15–21.
21. Biedermann S, Fuss J, Zheng L, Sartorius A, Falfan-Melgoza C, Demirakca T, *et al.* In vivo voxel based morphometry: detection of increased hippocampal volume and decreased glutamate levels in exercising mice. *Neuroimage* 2012, 61: 1206–1212.
22. Sahara N, Perez PD, Lin WL, Dickson DW, Ren Y, Zeng H, *et al.* Age-related decline in white matter integrity in a mouse model of tauopathy: an in vivo diffusion tensor magnetic resonance imaging study. *Neurobiol Aging* 2014, 35: 1364–1374.
23. Yu H, Bi W, Liu C, Zhao Y, Zhang D, Yue W. A hypothesis-driven pathway analysis reveals myelin-related pathways that contribute to the risk of schizophrenia and bipolar disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2014, 51: 140–145.
24. Gebicke-Haerter PJ. Differential expression of presynaptic genes in a rat model of postnatal hypoxia: relevance to schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 2010, 260 Suppl 2: S81–89.
25. Straub RE, Lipska BK, Egan MF, Goldberg TE, Callicott JH, Mayhew MB, *et al.* Allelic variation in GAD1 (GAD 67) is associated with schizophrenia and influences cortical function and gene expression. *Mol Psychiatry* 2007, 12: 854–869.
26. Marengo S, Geramita M, van der Veen JW, Barnett AS, Kolachana B, Shen J, *et al.* Genetic association of ErbB4 and human cortical GABA levels *in vivo*. *J Neurosci* 2011, 31: 11628–11632.
27. Volk DW, Pierri JN, Fritschy JM, Auh S, Sampson AR, Lewis DA. Reciprocal alterations in pre- and postsynaptic inhibitory markers at chandelier cell inputs to pyramidal neurons in schizophrenia. *Cereb Cortex* 2002, 12: 1063–1070.
28. Simms D, Cizdziel PE, Chomczynski P. TRIzol: A new reagent for optimal single-step isolation of RNA. *Focus* 1993, 15: 532–535.
29. Ashburner J, Barnes G, Chen C, Daunizeau J, Flandin G, Friston K, *et al.* SPM8 manual. Functional Imaging Laboratory, Institute of Neurology, UCL 2012.
30. Power RA, Steinberg S, Bjornsdottir G, Rietveld CA, Abdellaoui A, Nivard MM, *et al.* Polygenic risk scores for schizophrenia and bipolar disorder predict creativity. *Nat Neurosci* 2015, 18: 953–955.
31. Jia P, Wang L, Meltzer HY, Zhao Z. Common variants conferring risk of schizophrenia: a pathway analysis of GWAS data. *Schizophr Res* 2010, 122: 38–42.
32. Euesden J, Lewis CM, O'reilly PF. PRSice: polygenic risk score software. *Bioinformatics* 2014, 31: 1466–1468.
33. Pillai AK, Howell KR, Ahmed AO, Weinberg D, Allen KM, Bruggemann JM, *et al.* Association of serum VEGF levels with prefrontal cortex volume in schizophrenia. *Mol Psychiatry* 2016, 21: 686–692.
34. Cheng CH, Chan PY, Niddam DM, Tsai SY, Hsu SC, Liu CY. Sensory gating, inhibition control and gamma oscillations in the human somatosensory cortex. *Sci Rep* 2016, 6: 20437.
35. De Koning MB, Bloemen OJN, Van Duin EDA, Booij J, Abel KM, De Haan L, *et al.* Pre-pulse inhibition and striatal dopamine in subjects at an ultra-high risk for psychosis. *J Psychopharmacol* 2014, 28: 553–560.
36. Brody SA, Dulawa SC, Conquet F, Geyer MA. Assessment of a prepulse inhibition deficit in a mutant mouse lacking mGlu5 receptors. *Mol Psychiatry* 2004, 9: 35–41.
37. Mena A, Ruiz-Salas JC, Puentes A, Dorado I, Ruiz-Veguilla M, De la Casa LG. Reduced prepulse inhibition as a biomarker of schizophrenia. *Front Behav Neurosci*. 2016, 10: 202.
38. Lazar NL, Neufeld RW, Cain DP. Contribution of nonprimate animal models in understanding the etiology of schizophrenia. *J Psychiatry Neurosci* 2011, 36: E5–29.
39. Chong VZ, Thompson M, Beltaifa S, Webster MJ, Law AJ, Weickert CS. Elevated neuregulin-1 and ErbB4 protein in the prefrontal cortex of schizophrenic patients. *Schizophr Res* 2008, 100: 270–280.
40. Konrad A, Vucurevic G, Musso F, Stoeter P, Dahmen N, Winterer G. ErbB4 genotype predicts left frontotemporal structural connectivity in human brain. *Neuropsychopharmacology* 2009, 34: 641–650.
41. Nakagawa K, Inui K, Yuge L, Kakigi R. Inhibition of somatosensory-evoked cortical responses by a weak leading stimulus. *NeuroImage* 2014, 101: 416–424.
42. Levitt JJ, Nestor PG, Levin L, Pelavin P, Lin P, Kubicki M, *et al.* Reduced structural connectivity in frontostriatal white matter tracts in the associative loop in schizophrenia. *Am J Psychiatry* 2017, 174: 1102–1111.
43. Villarcervino V, Kappeler C, Nobregapereira S, Henkemeyer M, Rago L, Nieto MA, *et al.* Molecular mechanisms controlling the migration of striatal interneurons. *J Neurosci* 2015, 35: 8718–8729.
44. Barz CS, Bessaih T, Abel T, Feldmeyer D, Contreras D. Altered resonance properties of somatosensory responses in mice deficient for the schizophrenia risk gene Neuregulin 1. *Brain Struct Funct* 2016, 221: 4383–4398.
45. Addington AM, Gornick M, Duckworth J, Sporn A, Gogtay N, Bobb AJ, *et al.* GAD1 (2q31.1), which encodes glutamic acid decarboxylase (GAD67), is associated with childhood-onset schizophrenia and cortical gray matter volume loss. *Mol Psychiatry* 2005, 10: 581–588.
46. de la Fuente-Sandoval C, Reyes-Madrigal F, Mao X, León-Ortiz P, Rodríguez-Mayoral O, Solís-Vivanco R, *et al.* Cortico-striatal GABAergic and glutamatergic dysregulations in subjects at ultra-

- high risk for psychosis investigated with proton magnetic resonance spectroscopy. *Int J Neuropsychopharmacol* 2015, 19: pyv105.
47. Frankle WG, Cho RY, Prasad KM, Mason NS, Paris J, Himes ML, *et al.* *In vivo* measurement of GABA transmission in healthy subjects and schizophrenia patients. *Am J Psychiatry* 2015, 172: 1148–1159.
 48. Deng C, Huang XF. Increased density of GABA A receptors in the superior temporal gyrus in schizophrenia. *Exp Brain Res* 2006, 168: 587–590.
 49. Geng HY, Zhang J, Yang JM, Li Y, Wang N, Ye M, *et al.* ErbB4 deletion from medium spiny neurons of the nucleus accumbens core induces schizophrenia-like behaviors via elevated GABAA receptor $\alpha 1$ subunit expression. *J Neurosci* 2017, 37: 7450–7464.
 50. Hintiryan H, Foster NN, Bowman I, Bay M, Song MY, Gou L, *et al.* The mouse cortico-striatal projectome. *Nat Neurosci* 2016, 19: 1100–1114.
 51. Yin DM, Sun XD, Bean JC, Lin TW, Sathyamurthy A, Xiong WC, *et al.* Regulation of spine formation by ErbB4 in PV-positive interneurons. *J Neurosci* 2013, 33: 19295–19303.
 52. Shamir A, Kwon OB, Karavanova I, Vullhorst D, Leiva-Salcedo E, Janssen MJ, *et al.* The importance of the NRG-1/ErbB4 pathway for synaptic plasticity and behaviors associated with psychiatric disorders. *J Neurosci* 2012, 32: 2988–2997.
 53. Xiao Y, Lui S, Deng W, Yao L, Zhang W, Li S, *et al.* Altered cortical thickness related to clinical severity but not the untreated disease duration in schizophrenia. *Schizophr Bull* 2013, 41: 201–210.
 54. Ferro A, Roiz-Santianez R, Ortiz-Garcia de la Foz V, Tordesillas-Gutierrez D, Ayesa-Arriola R, de La Fuente-Gonzalez N, *et al.* A cross-sectional and longitudinal structural magnetic resonance imaging study of the post-central gyrus in first-episode schizophrenia patients. *Psychiatry Res* 2015, 231: 42–49.
 55. Scanlon C, Anderson-Schmidt H, Kilmartin L, McInerney S, Kenney J, McFarland J, *et al.* Cortical thinning and caudate abnormalities in first episode psychosis and their association with clinical outcome. *Schizophr Res* 2014, 159: 36–42.
 56. Ebdrup B. Hippocampal and caudate volume reductions in antipsychotic-naïve first-episode schizophrenia. *J Psychiatry Neurosci* 2010, 35: 95–104.
 57. Mucci A, Dima D, Soricelli A, Volpe U, Bucci P, Frangou S, *et al.* Is avolition in schizophrenia associated with a deficit of dorsal caudate activity? A functional magnetic resonance imaging study during reward anticipation and feedback. *Psychol Med* 2015, 45: 1765–1778.
 58. Li H, Tang J, Chen L, Liao Y, Zhou B, He Y, *et al.* Reduced middle cingulate gyrus volume in late-onset schizophrenia in a Chinese Han population: a voxel-based structural MRI study. *Neurosci Bull* 2015, 31: 626–627.
 59. Zhou D, Pang F, Liu S, Shen Y, Liu L, Fang Z, *et al.* Altered motor-striatal plasticity and cortical functioning in patients with schizophrenia. *Neurosci Bull* 2016, 33: 307–311.
 60. Torkamani A, Wineinger NE, Topol EJ. The personal and clinical utility of polygenic risk scores. *Nat Rev Genet* 2018, 19: 581–590.
 61. Kegeles LS, Mao X, Stanford AD, Girgis RR, Ojeil N, Xu X, *et al.* Elevated prefrontal cortex γ -aminobutyric acid and glutamate-glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 2012, 69: 449–459.