Journal of Neuroscience and Cognitive Studies

Research Article
Published: 27 Oct, 2020

Replicated Risk *CACNA1C* Variants for Major Psychiatric Disorders May Serve as Potential Therapeutic Targets for the Shared Depressive Endophenotype

Xiaoyun Guo[1,2,3*], Yingmei Fu[1,4], Yong Zhang[5], Tong Wang[3], Lu Lu[6], Xingqun Luo[7], Kesheng Wang[8], Juncao Huang[9], Ting Xie[9], Chengchou Zheng[10], Kebing Yang[9], Jinghui Tong[9], Lingjun Zuo[2], Longli Kang[11], Yunlong Tan[9], Kaida Jiang[1*], Chiang-shan R. Li[2] and Xingguang Luo[2,9]

[1]*Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, China*

[2]*Department of Psychiatry, Yale University School of Medicine, USA*

[3]*Department of Cellular and Molecular Physiology, Yale University School of Medicine, USA*

[4]*Shanghai Key Laboratory of Psychotic Disorders, Shanghai Jiao Tong University School of Medicine, China*

[5]*Tianjin Mental Health Center, China*

[6]*Departments of Genetics, Genomics, Informatics, Anatomy and Neurobiology, University of Tennessee Health Science Center, USA*

[7]*Department of Clinical Medicine, College of Integrated Traditional Chinese and Western Medicine, Fujian University of Traditional Chinese Medicine, China*

[8]*Department of Biostatistics and Epidemiology, College of Public Health, East Tennessee State University, USA*

[9]*Biological Psychiatry Research Center, Beijing Huilongguan Hospital, China*

[10]*Minqing Psychiatric Hospital, Minqing, China*

[11]*Key Laboratory for Molecular Genetic Mechanisms and Intervention Research on High Altitude Diseases of Tibet Autonomous Region, Xizang Minzu University School of Medicine, Xiangyang, China*

Abstract

Genome-Wide Association Studies (GWASs) have reported numerous associations between risk variants and Major Psychiatric Disorders (MPDs) including Schizophrenia (SCZ), Bipolar Disorder (BPD), Major Depressive Disorder (MDD) and others. We reviewed all of the published GWASs, and extracted the genome-wide significant ($p<10^{-6}$) and replicated associations between risk SNPs and MPDs. We found the associations of 6 variants located in 6 genes, including L Type Voltage-Gated Calcium Channel (LTCCs) subunit *alpha1 C* gene (*CACNA1C*), that were genome-wide significant ($2.0 \times 10^{-8} \leq p \leq 1.0 \times 10^{-6}$) and replicated at single-point level across at least two GWASs. Among them, the associations between MPDs and rs1006737 within *CACNA1C* are most robust. Thus, as a next step, the expression of the replicated risk genes in human hippocampus was analyzed. We found *CACNA1C* had significant mRNA expression in human hippocampus in two independent cohorts. Finally, we tried to elucidate the roles of venlafaxine and ω-3 PUFAs in the mRNA expression regulation of the replicated risk genes in hippocampus. We used cDNA chip-based microarray profiling to explore the transcriptome-wide mRNA expression regulation by ω-3 PUFAs (0.72/kg/d) and venlafaxine (0.25/kg/d) treatment in Chronic Mild Stress (CMS) rats. ω-3 PUFAs and venlafaxine treatment elicited significant *CACNA1C* up-regulation. We concluded that *CACNA1C* might confer the genetic vulnerability to the shared depressive symptoms across MPDs and *CACNA1C* might be the therapeutic target for depressive endophenotype as well.

Keywords: *CACNA1C*; Major Psychiatric Disorders (MPD); Schizophrenia (SCZ); Bipolar Disorder (BPD); Major Depressive Disorder (MDD); Genome-Wide Association Study (GWAS)

Introduction

Severe psychiatric disorders could cause severe disability all over the world. It affects several millions of people worldwide. Further exploration of new treatment method and therapeutic target is needed. Since the heritability of psychiatric disorders is high, exploring genetic mechanism is a power route. Psychiatrists who are major in genetics found increasing evidence that severe psychiatric disorders shared genetic etiology [1]. Genome-Wide Association Studies (GWAS) have showed accumulated evidence for an association of *CACNA1C* and Bipolar Disorder (BD), Major Depressive Disorder (MDD), Schizophrenia (SCZ) and others. Some studies showed "replicable" results at individual marker or gene level. Exploring the potential relationships between those shared replicable markers might have great importance for the development of both clinical and basic psychiatry.

CACNA1C gene product, namely Cav1.2, belongs to the L-Type Voltage-Gated Calcium Channel (VDCC) alpha subunit. Cav1.2 (*CACNA1C*) accounts for ~85% of the L-type channels [1]. Cav1.2

OPEN ACCESS

Correspondence:

Xiaoyun Guo, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China, E-mail: Xiaoyun.Guo@yale.edu

Xingguang Luo, Biological Psychiatry Research Center, Beijing Huilongguan Hospital, Beijing, China, Tel: +86-21-64387250; E-mail: Xingguang.Luo@yale.edu

Received Date: *27 Sep 2020*

Accepted Date: *22 Oct 2020*

Published Date: *27 Oct 2020*

Citation:

Guo X, Fu Y, Wang T, Zhang Y, Lu L, Wang K, et al. Replicated Risk CACNA1C Variants for Major Psychiatric Disorders May Serve as Potential Therapeutic Targets for the Shared Depressive Endophenotype. J Neurosci Cogn Stud. 2020; 4(1): 1017.

activates as the result of membrane depolarization. It could conduct Calcium (Ca^{2+}) into the cell. Activation of Cav1.2 could initiate physiological responses such as secretion, and gene transcription, etc. Calcium (Ca^{2+}) is important for the normal function of immunity. Ca^{2+} could control the complex and crucial immune functions in lymphocytes [3]. Altered Ca^{2+} concentration in lymphocytes leads to various pathological conditions such as immunodeficiency, inflammatory and autoimmune syndromes [3]. Ca^{2+} is also required for microglial M1-like pro-inflammatory activation [4,5].

DSM-5 and ICD-10 have guided psychiatry since the early 1980s. They have discrete categories that are based largely on the symptoms. DSM-5 and ICD-10 have discrete categories such as schizophrenia, major-depressive disorder and bipolar disorder. So far biologists still have been unable to find any biological such as genetic or neuroscientific evidence to divide complex mental disorders into separate categories [6]. Some psychiatrists suggested to divide the disease into several endophenotypes, among which depressive symptom are commonly observed in BD, MDD, and SCZ [7]. Interestingly, pharmacological activation of VDCCs induces a depressive-like phenotype [8], while knockdown of or down regulation of *CACNA1C* (Cav1.2) in prefrontal cortex could induce the antidepressant-like effect in mice [9].

Omega-3 Polyunsaturated Fatty Acids (omega-3 PUFAs) have potential effect on the resolution of inflammation [10]. Omega-3 PUFAs include Eicosapentaenoic Acid (EPA; 20:5 omega-3) and Docosahexaenoic Acid (DHA; 22:6 omega-3) [11]. EPA and DHA serves as a substrate for the formation of the specialized pro-resolving mediator Resolvin E1 (RvE1) and Resolvin D1 (RvD1), which stimulates the resolution of inflammation. RvE1 and RvD1 produce antidepressant effects induced by the lipopolysaccharide induced depression model [12] and chronic mild stress model [13] rodents. Clinical studies have reported low levels of omega-3 PUFAs in patients with depression [14,15]. In Randomized Controlled Trials (RCTs), supplementation with omega-3 PUFAs has been reported to have beneficial effects on severe psychiatric disorders [16], esp. depressive symptoms [17].

In this context, we reviewed all of the published VDCCs (*CACNA1C*, *CACNA2D1*, *CACNB2*, CACNB3, and *CACNG5-G4*), GWASs, and extracted the genome-wide significant ($p<10^{-6}$) and replicated associations between risk SNPs and severe psychiatric disease including SCZ, BD, and MDD. These associations per se are not sufficient to suggest a cause-effect relationship. However, if the biological functions of the risk SNPs can be demonstrated, or if their regulatory targets are abundant in specific human brain regions which are related with depressive symptoms (e.g., hippocampus), we could have more clues for the mechanisms underlying depressive symptoms. Thus, in the next step, the expression of the replicated risk genes in human hippocampus was analyzed. Finally, we tried to elucidate the roles of antidepressant venlafaxine and omega-3 PUFAs in the mRNA expression regulation of the replicated risk genes in hippocampus.

Materials and Methods

Identification of replicated associations between risk genes and severe psychiatric disease

We searched for the literatures using the keywords "(GWAS OR Genome-wide association study) AND (schizophrenia OR depression OR bipolar disorder OR mood disorder) AND the following VDCC (*CACNA1C*, *CACNA2D1*, *CACNB2*, CACNB3 and *CACNG5-G4*)". From these literatures, the most reliable associations between VDCC and schizophrenia, depression or bipolar disorder were extracted. We notice that although most of the distinct VDCCs have been associated with schizophrenia, depression or bipolar disorder, the associations at individual marker level replicated across studies are not very common. Such replicated associations were reported in six genes located at five genomic regions by a total of seven studies (Table 1).

Detection of mRNA expression of risk genes in human hippocampus

mRNA expression of risk genes was examined in the human hippocampus by two independent cohorts free of neurodegenerative disorders. The first cohort included human hippocampus tissues of 134 Europeans from UK Brain Expression Consortium (UKBEC) [18]. mRNA expression of the risk genes in this first cohort was examined with Affymetrix Human ST 1.0 exon arrays, which were validated by qPCR. When a normalized intensity was >36, that is, a log2 (normalized intensity) was >5.17, it was taken as "expressed". The second cohort included 13 human hippocampus tissues extracted from 173 Americans (The Genotype-Tissue Expression (GTEx) project [19]). mRNA expression of the risk genes in this second cohort was examined using RNA-Seq (validated by qPCR). The expression levels with TPM values >1 were taken as "expressed".

Hippocampal expression of risk genes regulated by omega-3 PUFAs and venlafaxine in rats

Animals: 18 male Sprague-Dawley rats, weighing 100 g to 120 g, were purchased from the Laboratory Animal center (Shanghai, China). All rats were given at least 3 days to acclimate to the environment before the experimentation. All rats were housed single in cages (40 cm × 25 cm × 20 cm) with bedding. Food and tap water were available freely during the experiments unless otherwise noted. The housing environment maintained on a 12 h light/dark cycle (lights on at 7:00) under controlled temperature of (22°C ± 1°C) until otherwise noted. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and P.R. China legislation on the use and care of laboratory animals.

Materials: Fish oil and Venlafaxine were purchased from Sigma (St Louis, MO, USA) and Wyeth Pharmaceuticals, Inc (Collegeville, PA, USA), respectively. 10,891 cDNA clones were used in the cDNA chip (provided by Shanghai Biochip Ltd, Shanghai, China). RNA isolation and purification reagents were purchased from Qiagen, Inc. (Gaithersburg, MD, USA). Cy3, Cy5 fluorescent dye was purchased from Amersham Biosciences (Waltham, MA, USA). G2655AA chip scanners and G2938b analysis software were purchased from the U.S. Agilent Technologies (Santa Clara, CA, USA).

Chronic mild stress (CMS): The CMS procedure was performed as described previously with slight modification. Rats were subjected to two periods of food and water deprivation, 45°C cage tilt, soiled cage (300 ml water in sawdust bedding), paired housing, and low intensity stroboscopic illumination (300 flashes/min), and one period of white noise (90 db) and overnight illumination. The above procedure was applied randomly and continuously every week. Each period of stress was 12 h to 14 h duration.

Sucrose intake tests: After 14 h period of food and water deprivation, water and sucrose (1%) bottles were supplied. All rats consumed the sucrose for 1 h. Then the bottles were weighed, and

Table 1: Replicated association between VDCC genes and major psychiatric disorders by GWASs.

SNP	p-value	Reported gene(s)	Reported trait	Reported studies
rs1006737	7×10^{-8}	*CACNA1C*	BP	[21]
	3×10^{-8}	*CACNA1C*	BP and MDD	[22]
	7×10^{-8}	*CACNA1C*	BP	[23]
rs4765913	2×10^{-8}	*CACNA1C*	BP	[24]
rs17156280	2×10^{-7}	*CACNA2D1*	MDD	[25]
rs2799573	4×10^{-8}	*CACNB2*	ASD, ADHD, BP, MDD, SCZ	[26]
rs2070615	1×10^{-6}	*CACNB3*	BD	[24]
rs17645023	6×10^{-7}	*CACNG5-CACNG4*	BD, SCZ	[27]

BD: Bipolar Disorder; MDD: Major Depressive Disorder; SCZ: Schizophrenia; ASD: Autism Spectrum Disorder; ADHD: Attention Deficit-Hyperactivity Disorder

sucrose intake was calculated. Four-baseline sucrose intake tests, with a 7 day break between, were conducted and averaged. Sucrose intake tests were performed once a week throughout the whole experiment [20].

Procedure: At week 0, all rats were divided into 2 groups randomly: CMS group (N=18) and CON (n=6). Rats in CMS group went through the 4-week chronic mild stress procedure. Rats in CON group were treated normally without any additional treatment. At the end of the week 4, rats in CMS group were subdivided into 3 groups: CMS+omega-3 PUFAs group, CMS+VEN and CMS+NS group. Rats in CMS+ omega-3 PUFAS group were administered with fish oil of menhaden (omega-3 PUFAs) at a dosage of 3.16 $mlkg^{-1}$ p.o., q.d. The menhaden oil contained 25% w/w omega-3 PUFAs, and the concentration was 0.91 g/ml. As such, rats in CMS+ omega-3 PUFAS group received a dose of 0.72 g/kg/omega-3 PUFAs [21]. Rats in CMS+VEN group received a dose of 2.5 mg/kg/d venlafaxine. Rats in CMS+NS group were subjected 0.9% saline to 2.5 ml/d, which is equal volume with that in CMS+omega-3 PUFAs and CMS+VEN group. All drugs and saline were administered for a total of 4 weeks from week 5 to week 8 (Figure 1).

Microarray hybridization experiment: Total RNA was extracted and purified from the bilateral hippocampus tissues from CMS+omega-3 PUFAS and CMS+VEN group (3 rats in each group randomly) and saline group (3 rats randomly). The resulting RNA was used to measure the absorbance values (A260 and A280) at 260 nm and 280 nm with an ultraviolet spectrophotometer. RNA purity was determined by the A260/A280 ratio (standard range 1.8 to 2.1). The results showed that all RNA quality was high (RIN>7). A total of 3 control group RNAs were mixed as a negative control. The total volume of the reaction system was 20 μL. RNA was transfected with 1 μL of MMLV reverse transcriptase into cRNA. 4 μg cRNA was added to Cy3 and Cy5 fluorescent dyes. The omega-3 PUFA and venlafaxine group cRNA was labeled with Cy3, and the saline mixed control group was labeled with Cy5. A pair of samples of one omega-3 PUFA or venlafaxine group and mixed control group were used for each chip. RNA was labeled with the same amount of probe hybridization: 42°C 16 hour's hybrid, 55°C wash tablets. The chip results were scanned with Agilent microarray scanner. Finally, we used the GeneSpring to carry out the homogenization processing analysis. The final ratio of Cy3/Cy5 ≥ 1.5 or ≤ 0.6 indicates that the gene has a differential expression. According to the gene number provided by the microarray, we carried out Gene Informatics Analysis *via* U.S. National Medical Biology Information Center (NCBI).

Statistical analysis: Behavioral data were analyzed using Multivariate Analysis of Variance (MANOVA) and then Fisher Least Significant Difference (LSD) post hoc tests and Nyholt correction [20]. We used one-way ANOVA to test the antidepressant effect of omega-3 PUFAs and venlafaxine.

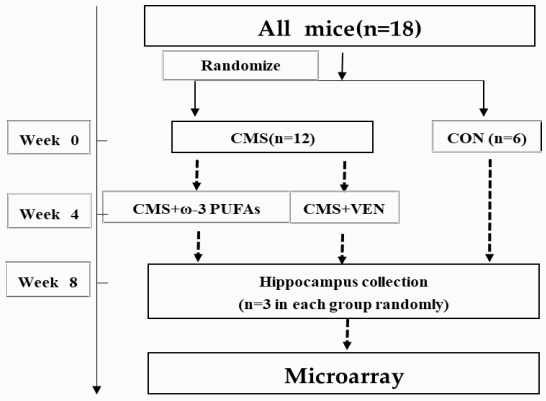

Figure 1: Procedure of the chronic mild stress experiment.
CMS: Chronic Mild Stress; VEN: Venlafaxine
NS: 0.9% Saline; ω-3 PUFAs: ω-3 polyunsaturated fatty acids; CON, normal control
At week 0, All rats were divided into 2 groups randomly: CMS group (N=18) and CON (n=6). Rats in CMS group were subjected to chronic mild stress procedure for 4 weeks. Rats in CON group were treated normally without any additional treatment. At the end of the week 4, rats in CMS group were subdivided into 3 groups: CMS+ omega-3 PUFAs group, CMS+VEN group and CMS+NS group. All drugs and saline were administered for a total of 4 weeks from week 5 to week 8. At the end of week 8, we collect hippocampus in 3 rats in each group randomly and stored for further hippocampus microarray detection.

Results

Genome-wide significant associations between SNPs of 6 VDCC genes and major psychiatric diseases

We only found the associations of 6 variants located in six genomic regions (*CACNA1C* [21-24], *CACNA2D1* [25], *CACNB2* [26], CACNB3 [24], and *CACNG5-G4* [27]) that were genome-wide significant and replicated at single-point level across at least two GWASs ($2.0 \times 10^{-8} \leq p \leq 1.0 \times 10^{-6}$). The protein encoded by the above genes belongs to the voltage-gated calcium channel alpha (*CACNA1C*, *CACNA1D*, *CACNA1E*, *CACNA2D1* and *CACNA2D2*), beta (*CACNB2* and CACNB3) and gamma (*CACNG4* and *CACNG5*) subunit family. Among them, the associations between MPDs and variants rs1006737 within *CACNA1C* are most robust, because they were replicated across three GWASs ($3 \times 10^{-8} \leq p \leq 7 \times 10^{-8}$) [21-23]. Part of these associations has been validated by functional studies (Table 1).

Table 2: The mRNA expression of VDCC genes in hippocampus in normal human brain.

	UK European (n=134)	American (n=173)
CACNA1C	6.4	0.9
CACNA2D1	7.69	5.4
CACNB2	6.76	2.2
CACNB3	6.8	10.4
CACNG4	6.89	10.2
CACNG5	5.25	0.1

The numbers in UK European cohort are log2-transformed normalized intensity values from Affymetrix Human ST 1.0 exon arrays; the numbers in American cohort are TPM values from RNA-Seq.

Table 3a: Sucrose intake (mg/h) in ω-3 PUFAs and venlafaxine treated rats (n=6).

Week	CMS+ω-3 PUFAs	CMS+VEN	CMS+NS	CON+NS
Week 0	8.26 ± 3.37	8.48 ± 3.46	7.32 ± 2.99	8.81 ± 3.6
Week 1	7.48 ± 3.06	8.3 ± 3.39	7.73 ± 3.16	5.66 ± 2.31
Week 2	6.7 ± 2.74	8.12 ± 3.31	8.15 ± 3.33	10.69 ± 4.36
Week 3	7.96 ± 3.25[b]	5.78 ± 2.36[b]	5.83 ± 2.38[b]	12.08 ± 4.93
Week 4	4.38 ± 1.79[b]	5 ± 2.04b	4.88 ± 1.99[b]	10.35 ± 4.22
Week 5	3.58 ± 1.46[b]	8.55 ± 3.49	7 ± 2.86	8.96 ± 3.66
Week 6	6.85 ± 2.79[b]	9.42 ± 3.84	5.33 ± 2.18[b]	13.42 ± 5.48
Week 7	13.68 ± 5.58[a]	17.85 ± 7.29[a]	4.85 ± 1.98[b]	13.1 ± 5.35
Week 8	12.75 ± 5.21[a]	18.9 ± 7.72[a]	4.65 ± 1.9[b]	11.42 ± 4.66

Data are presented as mean sucrose intake ± SEM (n=6 ml/h)
CMS: Chronic Mild Stress; VEN: Venlafaxine (2.5 mg/kg/d diluted in 2.5 ml saline); NS: Equal volume with that in rats 2.5 ml/d; ω-3 PUFAs: ω-3 PUFAs (0.72 g/kg/d)
a: P<0.05, compared with CMS+NS group; b: P<0.05, compared with CON+NS group. 1% sucrose intake decreased after CMS from week 3 in CMS+ω-3 PUFAs, CMS+VEN and CMS+NS group. 1% sucrose intake was significantly improved by a single dose of n-3 PUFAs (0.72 g/kg/d), and venlafaxine (2.5 mg/kg) at week 7 and week 8 after Independent Samples t- test.

mRNA expression of risk genes in human hippocampus

We determined mRNA expression levels for the risk genes reported in GWAS. Six protein-coding genes, including *CACNA1C*, *CACNA2D1*, *CACNB2*, CACNB3, *CACNG4* and *CACNG5* had significant mRNA expression in human hippocampus in two independent cohorts (Table 2). All log2 (normalized intensity) values for nine genes in the UK Europeans >5.17 (5.25 to 7.76); TPM values for six genes in the Americans >1 (1.0 to 10.4).

Sucrose intake ratio in omega-3 PUFAs and venlafaxine treated rats

Unpredictable Chronic Mild Stress (CMS) produced anhedonia in rodents, a core symptom of depression that shows loss of interest in normally pleasurable and rewarding activities. The sucrose intake significantly decreased after CMS from week 3.1% sucrose intake was significantly improved by a single dose of n-3 PUFAs (0.72 g/kg/d), and venlafaxine (2.5 mg/ kg) after treatment of 3 weeks (Week 7) and 4 weeks (Week 8) (Tables 3a-3c).

Omega-3 PUFAs and venlafaxine treatment regulated gene expression

After the last sucrose test, all rats were sacrificed. Hippocampus was collected and total RNA was extracted and purified as we previously described [28]. To identify transcriptome-wide mRNA expression changes in hippocampus after omega-3 PUFAs and venlafaxine treatment, we used cDNA chip-based microarray profiling to explore the regulatory effects of omega-3 PUFAs and venlafaxine in CMS rats, by comparing to saline treatment. We focused on the six risk genes reported in GWAS. Omega-3 PUFAs treatment elicited significant upregulating genes including *CACNA1C*. Venlafaxine treatment elicited significant upregulating genes including *CACNA1C*, as well.

Table 3b: Hippocampal expression of VDCC genes regulated by omega-3 PUFAs in rats.

Gene bank ID	Unigene	Gene	Cytoband	Mean ± SEM
NM_012517	Rn.9827	*CACNA1C*	4q42	2.4 ± 0.17*
NM_012919	Rn.11276	*CACNA2D1*	4q11	1.01 ± 0.01
NM_053851	Rn.10739	*CACNB2*	17q12.3	0.54 ± 0.05
NM_012828	Rn.2808	*CACNB3*	7q36	1.35 ± 0.56

We set the 1.6 and 0.6 as the threshold for up or down regulation

Table 3c: Hippocampal expression of VDCC gene regulated by Venlafaxine in rats.

Gene bank ID	Unigene	Gene	Cytoband	Mean ± SEM
NM_012517	Rn.9827	*CACNA1C*	4q42	2.4 ± 0.17*
NM_012919	Rn.11276	*CACNA2D1*	4q11	0.98 ± 0.04
NM_053851	Rn.10739	*CACNB2*	17q12.3	1.07 ± 0.87
NM_012828	Rn.2808	*CACNB3*	7q36	0.88 ± 0.06
NM_080692	Rn.57184	*CACNG4*	10q32.1	1.34 ± 0.48

We set the 1.6 and 0.6 as the threshold for up or down regulation

Discussion

In the current study, we identified a significant gene, i.e. *CACNA1C*, for the shared etiology of severe psychiatric disease. We also found *CACNA1C* might be the therapeutic target for the depressive endophenotype in CMS rats. To the best of our knowledge, the present study is the first research in detecting the pharmaceutical target of *CACNA1C* gene expression for omega-3 PUFAs.

Severe psychiatric diseases are genetically complex diseases. Increasing evidence suggested a shared genetic etiology among them [1]. We first searched the GWASs and meta-analyses of GWASs literatures. We notice the most replicable six SNPs located in six VDCC genes (*CACNA1C*, *CACNA2D1*, *CACNB2*, CACNB3, *CACNG4* and *CACNG5*) contributed to the risk of severe psychiatric diseases. The protein encoded by the above genes belongs to the voltage-gated calcium channel alpha (*CACNA1C*, *CACNA2D1*), beta (*CACNB2* and CACNB3) and gamma (*CACNG4* and *CACNG5*) subunit family. We focused on these 6 replicable genes in the following study. Among which GWASs and meta-analyses of GWASs have shown the most robust associations of Single-Nucleotide Polymorphisms (SNPs) of *CACNA1C* with severe psychiatric diseases [21-24,29-31].

Secondly, we detect the mRNA expression of the 6 genes (*CACNA1C*, *CACNA2D1*, *CACNB2*, CACNB3, *CACNG4* and *CACNG5*) in hippocampus. We found all of the 6 genes are abundant in hippocampus in 134 Europeans [18] and 173 Americans individually [19] cohorts. VDCC is important in providing calcium influx to cortical pyramidal neuron in hippocampus [32]. VDCC channels are rich in the soma and proximal dendrites of hippocampal pyramidal neurons and in axons, glial processes and axon terminals in all hippocampal subfields as well [33]. Multiple VDCC also activates transcription regulators that regulate adult hippocampal neurogenesis [34]. Embryonic deletion of VDCC in glutamatergic neurons in forebrain could induce the emotional behavior endophenotypes [35].

Furthermore, based on the evidence that VDCC contribute to shared etiology among severe psychiatric disorders, including SCZ, BD and MDD, and the contribution of VDCC on the inflammatory etiology on the depressive like phenotype. In our following-up step, we tried to illustrate whether VDCC contributed to the therapeutic target of antidepressants. CMS paradigm produced anhedonia, a core feature of depression. 1% sucrose solution intake is a standard criterial to evaluate the anhedonia induced by CMS. We found 1% sucrose intake was significantly improved by venlafaxine (2.5 mg/kg) after treatment of 3 weeks and 4 weeks. Next, we used cDNA chip-based microarray profiling to explore the transcriptome-wide regulatory effects of venlafaxine in CMS rats, by comparing to saline treatment. We found *CACNA1C* was significantly upregulated by 2.4 times by venlafaxine. In accordance with our findings, Du's research also found the Cav1.2 mRNA expression was doubled after fluoxetine hydrochloride treatment (10 mg/kg) for two weeks [36]. All of the above findings suggested that Cav1.2 might be the pharmaceutic target of the antidepressants.

Lastly, since VDCC contribute to the normal function of immunity and etiology of depressive endophenotype, we hypothesis that VDCC might be the pharmaceutic target of omega-3 PUFAs, which has potential effect on the resolution of inflammation [10]. Our data showed 1% sucrose intake was significantly improved by omega-3 PUFAs after treatment of 3 weeks and 4 weeks. This result confirmed the previous hypothesis that omega-3 PUFAs have potential antidepressant effects [14,15]. In consistent with our findings, previous research also showed omega-3 PUFAs have potential effect on the resolution of inflammation [10] and potential antidepressant effect in both animal model of depression [13] and human patients [14,15]. cDNA chip-based microarray data showed that *CACNA1C* were most significantly regulated by omega-3 PUFAs among all 6 protein-coding genes we previously found. Among which *CACNA1C* was most highly upregulated by 2.4 times by omega-3 PUFAs. VDCC contribute to the normal glial function, which could produce active cytokine cells in the brain [37]. Drugs targeted at VDCC might have promising management for neuroinflammatory disease [38].

The modest sample size is one limitation of our study. We recruit 3 rats for the cDNA chip-based microarray analysis. However, our data consistently showed omega-3 PUFAs and venlafaxine treatment elicited significant upregulating genes *CACNA1C*. We concluded that there was a significant increase of *CACNA1C* mRNA expression in omega-3 PUFAs and venlafaxine treated CMS rats in hippocampus, suggesting *CACNA1C* might be a therapeutic target for antidepressants of anti-inflammatory biomarker in depressive endophenotype.

Acknowledgement

This work was supported in part by National Institute on Alcohol Abuse and Alcoholism (NIAAA) grants R21 AA021380, R21 AA023237 and R21AA020319, Chinese National Science Foundation (81201057, 81371491), Shanghai Clinical Center for Psychiatric Disease, and Shanghai Municipal Commission Award (20124109), Shanghai Health Talent Professional Project (XYQ2013114), the Shanghai Key Laboratory of Psychotic Disorders (No.13dz2260500).

Author Contributions

Conceived and designed the experiments: X.G. X.L. K.J. Performed the experiments: X.L. X.G. Y.F. Y.Z. Analyzed the data: X.G. X.L. L.L. K.W. Wrote the manuscript: X.G. X.L. K.J.

References

1. Crespi B, Stead P, Elliot M. Evolution in health and medicine Sackler colloquium: Comparative genomics of autism and schizophrenia. Proc Natl Acad Sci U S A. 2010;107(Suppl 1):1736-41.
2. Krug A, Nieratschker V, Markov V, Krach S, Jansen A, Zerres K, et al. Effect of *CACNA1C* rs1006737 on neural correlates of verbal fluency in healthy individuals. NeuroImage. 2010;49(2):1831-6.
3. Trebak M, Kinet JP. Calcium signalling in T cells. Nat Rev Immunol. 2019;19(3):154-69.
4. Di Lucente J, Nguyen HM, Wulff H, Jin LW, Maezawa I. The voltage-gated potassium channel Kv1.3 is required for microglial pro-inflammatory activation *in vivo*. Glia. 2018;66(9):1881-95.
5. Espinosa-Parrilla JF, Martinez-Moreno M, Gasull X, Mahy N, Rodriguez MJ. The L-type voltage-gated calcium channel modulates microglial pro-inflammatory activity. Mol Cell Neurosci. 2015;64:104-15.
6. Adam D. Mental health: On the spectrum. Nature. 2013;496(7446):416-8.
7. Kabir ZD, Martinez-Rivera A, Rajadhyaksha AM. From gene to behavior: L-type calcium channel mechanisms underlying neuropsychiatric symptoms. Neurotherapeutics. 2017;14(3):588-613.
8. Mogilnicka E, Czyrak A, Maj J. BAY K 8644 enhances immobility in the mouse behavioral despair test, an effect blocked by nifedipine. Eur J Pharmacol. 1988;151(2):307-11.
9. Kabir ZD, Lee AS, Burgdorf CE, Fischer DK, Rajadhyaksha AM, Mok E, et al. *CACNA1C* in the prefrontal cortex regulates depression-related behaviors *via* REDD1. Neuropsychopharmacology. 2017;42(10):2032-42.
10. Back M, Hansson GK. Omega-3 fatty acids, cardiovascular risk, and the resolution of inflammation. FASEB J. 2019;33(2):1536-39.
11. Tocher DR, Betancor MB, Sprague M, Olsen RE, Napier JA. Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: Bridging the gap between supply and demand. Nutrients. 2019;11(1):89.
12. Deyama S, Ishikawa Y, Yoshikawa K, Shimoda K, Ide S, Satoh M, et al. Resolvin D1 and D2 reverse lipopolysaccharide-induced depression-like behaviors through the mTORC1 signaling pathway. Int J Neuropsychopharmacol. 2017;20(7):575-84.
13. Ishikawa Y, Deyama S, Shimoda K, Yoshikawa K, Ide S, Satoh M, et al. Rapid and sustained antidepressant effects of resolvin D1 and D2 in a chronic unpredictable stress model. Behav Brain Res. 2017;332:233-36.
14. Edwards R, Peet M, Shay J, Horrobin D. Depletion of docosahexaenoic acid in red blood cell membranes of depressive patients. Biochem Soc Trans. 1998;26(2):S142.
15. Peet M, Murphy B, Shay J, Horrobin D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. Biol Psychiatry. 1998;43(5):315-9.
16. Pawelczyk T, Grancow-Grabka M, Kotlicka-Antczak M, Trafalska E, Pawelczyk A. A randomized controlled study of the efficacy of six-month supplementation with concentrated fish oil rich in omega-3 polyunsaturated fatty acids in first episode schizophrenia. J Psychiatr Res,. 2016;73:34-44.
17. Nemets B, Stahl Z, Belmaker RH. Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. Am J psychiatry. 2002;159(3):477-9.
18. Trabzuni D, Ryten M, Walker R, Smith C, Imran S, Ramasamy A, et al. Quality control parameters on a large dataset of regionally dissected human control brains for whole genome expression studies. J Neurochem. 2011;119(2):275-82.
19. Consortium GTEx. The Genotype-Tissue Expression (GTEx) project. Nat

Genet. 2013;45(6):580-5.

20. Parise EM, Alcantara LF, Warren BL, Wright KN, Hadad R, Sial OK, et al. Repeated ketamine exposure induces an enduring resilient phenotype in adolescent and adult rats. Biol Psychiatry. 2013;74(10):750-9.

21. Ferreira MA, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L, et al. Collaborative genome-wide association analysis supports a role for ANK3 and *CACNA1C* in bipolar disorder. Nat Genet. 2008;40(9):1056-8.

22. Liu Y, Blackwood DH, Caesar S, de Geus EJ, Farmer A, Ferreira MA, et al. Meta-analysis of genome-wide association data of bipolar disorder and major depressive disorder. Mol Psychiatry. 2011;16(1):2-4.

23. Ruderfer DM, Fanous AH, Ripke S, McQuillin A, Amdur RL; Schizophrenia Working Group of the Psychiatric Genomics Consortium, et al. Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia. Mol Psychiatry. 2014;19(9):1017-24.

24. Psychiatric GCBDWG Consortium Bipolar Disorder Working Group. Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nat Genet. 2011;43(10):977-83.

25. Ikeda M, Shimasaki A, Takahashi A, Kondo K, Saito T, Kawase K, et al. Genome-wide environment interaction between depressive state and stressful life events. J Clin Psychiatry. 2016;77(1):e29-30.

26. Cross-Disorder Group of the Psychiatric Genomics Consortium. Identification of risk loci with shared effects on five major psychiatric disorders: A genome-wide analysis. Lancet. 2013;381:1371-9.

27. Curtis D, Vine AE, McQuillin A, Bass NJ, Pereira A, Kandaswamy R, et al. Case-case genome-wide association analysis shows markers differentially associated with schizophrenia and bipolar disorder and implicates calcium channel genes. Psychiatr Genet. 2011;21(1):1-4.

28. Guo X, Fu Y, Xu Y, Weng S, Liu D, Cui D, et al. Chronic mild restraint stress rats decreased CMKLR1 expression in distinct brain region. Neurosci Lett. 2012;524(1):25-9.

29. Sklar P, Smoller JW, Fan J, Ferreira MA, Perlis RH, Chambert K, et al. Whole-genome association study of bipolar disorder. Mol Psychiatry. 2008;13(6):558-69.

30. Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium. Genome-wide association study identifies five new schizophrenia loci. Nat Genet. 2011;43(10):969-76.

31. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511(7510):421-7.

32. Sahu G, Asmara H, Zhang FX, Zamponi GW, Turner RW. Activity-dependent facilitation of CaV1.3 calcium channels promotes KCa3.1 activation in hippocampal neurons. J Neurosci. 2017;37(46):11255-70.

33. Tippens AL, Pare JF, Langwieser N, Moosmang S, Milner TA, Smith Y, et al. Ultrastructural evidence for pre- and postsynaptic localization of Cav1.2 L-type Ca2+ channels in the rat hippocampus. J Comp Neurol. 2008;506(4):569-83.

34. Ortega-Martinez S. A new perspective on the role of the CREB family of transcription factors in memory consolidation *via* adult hippocampal neurogenesis. Front Mol Neurosci. 2015;8:46.

35. Dedic N, Pohlmann ML, Richter JS, Mehta D, Czamara D, Metzger MW, et al. Cross-disorder risk gene *CACNA1C* differentially modulates susceptibility to psychiatric disorders during development and adulthood. Mol Psychiatry. 2018;23(3):533-43.

36. Du T, Liang C, Li B, Hertz L, Peng L. Chronic fluoxetine administration increases expression of the L-channel gene Cav1.2 in astrocytes from the brain of treated mice and in culture and augments K(+)-induced increase in [Ca(2+)]i. Cell Calcium, 2014;55(3):166-74.

37. Kato TA, Watabe M, Kanba S. Neuron-glia interaction as a possible glue to translate the mind-brain gap: A novel multi-dimensional approach toward psychology and psychiatry. Front Psychiatry, 2013;4:139.

38. Silva RBM, Greggio S, Venturin GT, da Costa JC, Gomez MV, Campos MM. Beneficial effects of the calcium channel blocker CTK 01512-2 in a mouse model of multiple sclerosis. Mol Neurobiol. 2018;55(12):9307-27.