Schizophrenia Related Variants in CACNA1C also Confer Risk of Autism

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Abstract

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders with a strong genetic component. Many lines of evidence indicated that ASD shares common genetic variants with other psychiatric disorders (for example, schizophrenia). Previous studies detected that calcium channels are involved in the etiology of many psychiatric disorders including schizophrenia and autism. Significant association between CACNA1C (calcium channel, voltage-dependent, L type, alpha 1C subunit) and schizophrenia was detected. Furthermore, rare mutation in CACNA1C is suggested to cause Timothy syndrome, a multi-system disorder including autism-associated phenotype. However, there is no evidence for association between CACNA1C and autism in Chinese Han population. To investigate the association between single nucleotide polymorphisms (SNP) in CACNA1C and autism, we first performed a family-based association study between eighteen SNPs in CACNA1C and autism in 239 trios. All SNPs were genotyped by using Sequenom genotyping platform. Two SNPs (rs1006737 and rs4765905) have a trend of association with autism. To further confirm the association between these two SNPs with autism, we expanded the sample size to 553 trios by adding 314 trios. Association analyses for SNPs and haplotype were performed by using family-based association test (FBAT) and Haploview software. Permutation tests were used for multiple testing corrections of the haplotype analyses (n=10,000). The significance level for all statistical tests was two-tailed (p<0.05). The results demonstrated that G allele of rs1006737 and G allele of rs4765905 showed a preferential transmission to affected offspring in 553 trios (p=0.035). Haplotype analyses showed that two haplotypes constructed from rs1006737 and rs4765905 were significantly associated with autism (p=0.030, 0.023, respectively; Global p=0.046). These results were still significant after permutation correction (n=10,000, p=0.027). Our research suggests that CACNA1C might play a role in the genetic etiology of autism in Chinese Han population.
Introduction

Autism is a neurodevelopmental disorder characterized by deficits in social interaction and communication, and the presence of repetitive or stereotypic behaviors [1]. These symptoms become apparent in the first three years of life. Twin studies have provided evidence for a strong genetic component for autism. The concordance rate for monozygotic twins is much higher than that for dizygotic twins (70%-82% vs. 0%-10%). The estimated heritability of autism is more than 90% [2]. The contribution of common variants is not only substantial but also highly polygenic. By analyzing common variations throughout the genome, a recent study showed that common variations, individually of small effect, exert substantial additive genetic effects on autism spectrum disorder (ASD) liability [3]. It provides evidence for the common disease-common variant hypothesis.

Calcium channels mediate the influx of calcium ions into the cell upon membrane polarization. CACNA1C (calcium channel, voltage-dependent, L type, alpha 1C subunit) encodes an alpha-1 subunit of a voltage-dependent calcium channel, which plays an important role in the development and function of the central nervous system. CACNA1C influences neuronal firing by modulating calcium channel functions. Moreover, it modulates γ-aminobutyric acid-transmitting interneuron function. Therefore, CACNA1C could affect brain regional activation and inter-regional connectivity [4]. Mice with a selective inactivation of Cacna1c gene in the hippocampus and neocortex show a defect in N-methyl-D-aspartate (NMDA) receptor-independent long-term potentiation in the CA1 region of the hippocampus that paralleled by a severe memory deficit. It indicated that CACNA1C may play a role in NMDA receptor-independent synaptic plasticity in hippocampus [5].

A previous meta-analysis of genome-wide association study (GWAS) identified that single nucleotide polymorphism (SNP) rs1006737 in CACNA1C was significantly associated with bipolar disorder ($p = 7.0 \times 10^{-8}$) [6]. While within the Wellcome Trust Case Control Consortium (WTCCC) bipolar disorder dataset, the significant level was $p = 7.0 \times 10^{-4}$ [7]. Another GWAS showed that rs1006737 was associated with bipolar disorder (OR = 1.21) [8]. Furthermore, CACNA1C was also associated with schizophrenia. One study found that the risk allele of rs1006737 conferred increasing risk for schizophrenia ($p = 0.034$) [9]. A previous GWAS demonstrated that rs4765905 in CACNA1C reached genome-wide significance in 16,374 cases with schizophrenia, schizoaffective disorder or bipolar disorder and 14,044 controls ($p = 7.0 \times 10^{-9}$) [10]. Recently, another independent GWAS identified that rs4765905 in CACNA1C was associated with schizophrenia ($p = 1.23 \times 10^{-8}$) [11]. Moreover, other independent studies replicated the association of the specific SNP rs1006737 in CACNA1C with schizophrenia in white subjects of self-identified European descent, Danish subjects, and Spanish population, respectively [12–14]. All these studies provide genetic evidence that CACNA1C may play a role in the etiology of psychiatric disorders.

For clinical features, autism and schizophrenia share the same neurocognitive defects such as impaired executive function and deficits in social functioning [15,16]. Some of the SNPs conferring risk for schizophrenia also appear to confer risk for autism. There is genetic evidence of shared loci and pathways in the genetic etiology of autism and schizophrenia [11,17–21]. A recent study published by the Psychiatric Genomics Consortium (PGC) has identified that several SNPs (including SNPs in CACNA1C) were significantly associated with five major psychiatric disorders including ASD, attention deficit-hyperactivity disorder, bipolar disorder, major depressive disorder, and schizophrenia in 33,332 cases and 27,888 controls of European ancestry [22]. These findings provide further support for some degree of overlap in the susceptibility to mental illness across schizophrenia and autism.
Genetic support of a role for calcium channel genes in ASD is the association of two SNPs (rs757415 and rs12603112) in CACNA1G encoding a T-type Ca$^{2+}$ channel subunit [23]. Rare mutation in CACNA1C is suggested to cause Timothy syndrome, a disorder whose features include ASD-related phenotypes and intellectual disability [24]. A previous GWAS suggest that SNPs surrounding CACNA1C show suggestive evidence of association with ASD [25]. These studies indicate that calcium channels might be involved in the etiology of autism. However, no replication association studies have yet been reported.

To investigate whether the genetic variants in CACNA1C are associated with autism, we performed a family based association study between CACNA1C and autism in Chinese Han population. Here we report the association of two SNPs (rs1006737 and rs4765905) and haplotypes in CACNA1C with autism. These results suggest that CACNA1C may be a susceptibility gene of autism.

Materials and Methods

Ethics statement

This research was approved by the Ethics Committee of Institute of Mental Health, The Sixth Hospital, Peking University. All subjects provided written informed consents, and informed written consents for children were obtained from their biological parents (the children’s legal guardians).

Subjects

Our study included 553 children affected with autism and their biological parents of Chinese Han descent. These trios were recruited at the Institute of Mental Health, Peking University, China. In the first discovery sample, we recruited 239 autism trios. Among the children affected with autism, 226 were male and 13 were female. The age of these children at the clinical assessment time ranged from 2 to 17 years old and the mean age was 7.5 years old. Then, we expanded our sample to 553 trios (1659 individuals) by recruiting additional 314 trios (median age of autistic children was 6.0 years old). Among all 553 autistic children, 513 were male and 40 were female. The assessments of autism were established by two senior psychiatrists using DSM-IV criteria. Autism Behavior Checklist (ABC) [26] and Childhood Autism Rating Scale (CARS) [27] were used for additional clinical assessment. All children had scored more than 53 for ABC and 35 for CARS scales. Exclusion criteria included children with phenylketonuria, fragile X syndrome, tuberous sclerosis, chromosomal abnormality by karyotyping analysis, and non-Han Chinese ancestry. To decrease the heterogeneity, children affected with Asperger disorder and Rett syndrome were excluded in our study.

Blood was obtained from autistic children and their biological parents after informed consents were obtained.

SNP selection and genotyping

Eighteen SNPs with minor allele frequency (MAF) >0.05 in CACNA1C were selected. These SNPs were distributed from 2011392bp to 2668602bp on chromosome 12 (cover 90.4% of the CACNA1C region) with a mean inter-SNP distance of 38.7 Kb (GRCh38, National Center for Biotechnology Information [NCBI]). Among these 18 SNPs, rs1006737 and rs476590 were selected for positive association with schizophrenia and bipolar in previous studies. Furthermore, Genotype data in Chinese Han in Beijing (CHB) from the HapMap phase II and III was downloaded from Hapmap genotype dataset (http://hapmap.ncbi.nlm.nih.gov/). Then pairwise tagging in the Tagger module in Haploview version 4.2 program (http://www.broad.mit.tername.com).
edu/mpg/haploview/) was considered to select these SNPs that could capture the known common genetic variation.

Genomic DNA was extracted from blood using Qiagen QIAamp DNA Kits. All SNPs were genotyped using Sequenom genotyping platform, which uses the MALDI-TOF primer extension assay. Primers were designed according to the sequence of the forward strand from dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/). We used iPLEX genotyping assay, which has increased plexing efficiency and flexibility for the MassARRAY system through single base primer extension with mass-modified terminators [28–30].

To confirm the genotype results by Sequenom genotyping platform, all these eighteen SNPs were re-genotyped in 10% of the whole samples.

Statistical analysis
To decrease population stratification, we performed a family based association study. All those SNPs with MAF greater than 5% were used as genetic markers in this study. The Hardy-Weinberg Equilibrium (HWE) for genotype frequency distributions was tested by using the chi-square goodness-of-fit test. Mendelian inconsistencies were checked using family-based association test (FBAT) software v1.7.2 (http://www.biostat.harvard.edu/~fbat/default.html) [31]. Genotypes of families with Mendelian errors have been reset to zero.

Association analyses for SNPs and haplotype were performed by using FBAT software. Single marker association tests were performed under an additive model. The FBAT program uses generalized score statistics to perform a variety of transmission disequilibrium tests (TDT), including haplotype analyses. Moreover, the global haplotype tests of association were performed under “multiallelic” mode in haplotype based association test (HBAT). Meanwhile, the individual haplotype tests were conducted under “biallelic” mode in HBAT. Permutation tests were used for multiple testing corrections of the haplotype analyses (n = 10,000). The significance level for all statistical tests was two-tailed (p<0.05). Haploview software provides estimation of pairwise linkage disequilibrium (LD) between the specified markers by calculating \( r^2 \) value. The single SNP association analyses and haplotype association were also performed by Haploview.

The power for this association study was calculated by using Quanto software version 1.2.4 (http://biostats.usc.edu/software) [32]. The population risk is 0.006 and relative risk was set to 1.5 for power calculation.

Results
The concordance rate of genotype in the re-genotyped samples by Sequenom was more than 99%. All of these eighteen SNPs in CACNA1C were successfully genotyped in 239 nuclear families and polymorphic with minor allele frequency (MAF) more than 5%. None of the genotype distributions of these SNPs in parents and affected children deviated from Hardy-Weinberg equilibrium (S1 Table). The power to detect these risk alleles was ranged from 69% to 86.6% except for rs1006737 and rs4765905 in 239 trios.

Univariate (single marker) test demonstrated that no SNPs were associated with autism in 239 trios. The LD structure constructed from 18 SNPs is shown in S1 Fig. Two SNPs rs1006737 and rs4765905 have a trend of association with autism (\( p = 0.071, 0.096 \), respectively) (Table 1). The association results calculated by Haploview were similar to those calculated by FBAT (S2 Table). To further confirm the association between rs1006737 and rs4765905 and autism, we expanded the sample size to 553 trios by adding 314 trios. The power to detect risk alleles for rs1006737 and rs4765905 was increased to 58% in 553 trios. None of the genotype
distributions of these two SNPs in parents and affected children deviated from Hardy-Weinberg equilibrium in 553 trios (S3 Table).

Single marker association test demonstrated that G allele of rs1006737 showed a preferential transmission from parents to children affected with autism (G > A, Z = 2.105, \( p = 0.035 \)). Moreover, rs4765905 was nominal significantly associated with autism (G > C, Z = 2.105, \( p = 0.035 \)).

Allele frequencies and the results of FBAT for single SNPs analysis are shown in Table 2.

### Table 1. Results of family-based association test between 18 SNPs in CACNA1C and autism in 239 trios.

| Marker   | position | Allele | Afreq | Families | S       | E (S) | Var (S) | Z       | p       |
|----------|----------|--------|-------|----------|---------|-------|---------|---------|---------|
| rs11062065 | 2011392  | C      | 0.811 | 126      | 175.0   | 175.5 | 35.25   | -0.084  | 0.933   |
|          |          | T      | 0.189 | 126      | 77.0    | 76.5  | 35.25   | 0.084   | 0.984   |
| rs917365  | 2043005  | A      | 0.720 | 143      | 181.0   | 184.0 | 45.00   | -0.447  | 0.655   |
|          |          | G      | 0.280 | 143      | 105.0   | 102.0 | 45.00   | 0.447   | 0.447   |
| rs4765663 | 2069594  | C      | 0.163 | 111      | 66.0    | 63.5  | 30.75   | 0.451   | 0.652   |
|          |          | G      | 0.837 | 111      | 156.0   | 158.5 | 30.75   | -0.451  | 0.652   |
| rs1558322 | 2120889  | A      | 0.253 | 138      | 95.0    | 98.5  | 42.75   | -0.535  | 0.592   |
|          |          | G      | 0.747 | 138      | 181.0   | 177.5 | 42.75   | 0.535   | 0.535   |
| rs7298845 | 2175167  | A      | 0.711 | 156      | 203.0   | 194.0 | 48.50   | 1.292   | 0.196   |
|          |          | G      | 0.289 | 156      | 109.0   | 118.0 | 48.50   | -1.292  | 0.222   |
| rs2239031 | 2227003  | G      | 0.754 | 141      | 190.0   | 182.5 | 43.75   | 1.134   | 0.257   |
|          |          | T      | 0.246 | 141      | 92.0    | 99.5  | 43.75   | -1.134  | 0.257   |
| rs1006737 | 2236129  | A      | 0.066 | 56       | 23.0    | 30.0  | 15.00   | -1.807  | 0.071   |
|          |          | G      | 0.934 | 56       | 89.0    | 82.0  | 15.00   | 1.807   | 0.071   |
| rs4765905 | 2240418  | C      | 0.066 | 57       | 24.0    | 30.5  | 15.25   | -1.664  | 0.096   |
|          |          | G      | 0.934 | 57       | 90.0    | 83.5  | 15.25   | 1.664   | 0.096   |
| rs2238060 | 2316328  | A      | 0.658 | 158      | 202.0   | 194.0 | 48.50   | 1.149   | 0.251   |
|          |          | C      | 0.342 | 158      | 114.0   | 122.0 | 48.50   | -1.149  | 0.251   |
| rs2238070 | 2346949  | G      | 0.540 | 182      | 189.0   | 188.5 | 63.75   | 0.063   | 0.950   |
|          |          | T      | 0.460 | 182      | 175.0   | 175.5 | 63.75   | -0.063  | 0.950   |
| rs2238083 | 2377835  | C      | 0.231 | 123      | 82.0    | 79.0  | 37.50   | 0.490   | 0.624   |
|          |          | T      | 0.769 | 123      | 164.0   | 167.0 | 37.50   | -0.490  | 0.624   |
| rs2239062 | 2393406  | G      | 0.295 | 156      | 118.0   | 118.5 | 50.25   | -0.071  | 0.944   |
|          |          | T      | 0.705 | 156      | 194.0   | 193.5 | 50.25   | 0.071   | 0.944   |
| rs2239074 | 2429383  | C      | 0.796 | 132      | 172.0   | 175.0 | 39.50   | -0.477  | 0.633   |
|          |          | T      | 0.204 | 132      | 92.0    | 89.0  | 39.50   | 0.477   | 0.633   |
| rs4765686 | 2450917  | A      | 0.687 | 166      | 198.0   | 202.0 | 52.00   | -0.555  | 0.579   |
|          |          | G      | 0.313 | 166      | 134.0   | 130.0 | 52.00   | 0.555   | 0.579   |
| rs2239109 | 2519645  | G      | 0.267 | 152      | 104.0   | 104.5 | 47.25   | -0.073  | 0.942   |
|          |          | T      | 0.733 | 152      | 200.0   | 199.5 | 47.25   | 0.073   | 0.942   |
| rs2238090 | 2574166  | A      | 0.295 | 158      | 124.0   | 118.5 | 49.25   | 0.784   | 0.433   |
|          |          | G      | 0.705 | 158      | 192.0   | 197.5 | 49.25   | -0.784  | 0.433   |
| rs216008  | 2611971  | C      | 0.618 | 166      | 193.0   | 192.0 | 54.00   | 0.136   | 0.892   |
|          |          | T      | 0.382 | 166      | 139.0   | 140.0 | 54.00   | -0.136  | 0.892   |
| rs6489375 | 2668602  | A      | 0.339 | 169      | 138.0   | 131.0 | 52.50   | 0.966   | 0.334   |
|          |          | G      | 0.661 | 169      | 200.0   | 207.0 | 52.50   | -0.966  | 0.334   |

Afreq, allele frequency; Families, number of informative families; S, test statistics for the observed number of transmitted alleles; E(S), expected value of S under the null hypothesis (i.e., no linkage and no association).
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Single marker association test demonstrated that G allele of rs1006737 showed a preferential transmission from parents to children affected with autism (G > A, \( Z = 2.105, \ p = 0.035 \)). Moreover, rs4765905 was nominal significantly associated with autism (G > C, \( Z = 2.105, \ p = 0.035 \)).
results of each allele transmitted from heterozygous parents to affected children calculated by Haploview are shown in S4 Table.

We calculated the pairwise LD for all possible pairs of the SNPs. Strong LD block was detected between rs1006737 and rs4765905 (\( r^2 = 1 \)). Haplotype analyses showed that haplotype G-G (rs1006737-rs4765905) demonstrated an excess transmission (\( p = 0.030 \), Global \( p = 0.046 \)). While haplotype constructed from A allele of rs1006737 and C allele of rs4765905 was a protective haplotype (\( p = 0.023 \), Global \( p = 0.046 \)). To decrease false positive results, we performed permutation test for multiple testing correction. After using permutation test of 10,000 rounds, the results were still significant (\( p = 0.025 \)). The results of specific and global haplotype association are shown in Table 3. Moreover, haplotype association results calculated by Haploview are listed in S5 Table. The genotype data in our study are shown in S6 and S7 Tables.

### Discussion

Previous studies demonstrated that CACNA1C was associated with schizophrenia. To test whether CACNA1C is involved in the etiology of autism, we performed a family based association study. Our results identified a nominal significant association between two SNPs (rs1006737 and rs4765905) in CACNA1C and autism in 553 nuclear families of Chinese Han ancestry. Moreover, haplotype analyses indicated statistically significant association between CACNA1C and autism.

However, our study found that G allele of rs1006737 was associated with autism (\( p = 0.035 \)), while the risk allele in schizophrenia was A allele. The inconsistence results might be due to a few reasons. First, one reason was the genetic heterogeneity of ethnicity. The allele frequency of rs1006737 is different between CHB (Han Chinese in Beijing, China) and CEU (Utah residents with Northern and Western European ancestry from the CEPH collection) populations. Our results show that the MAF of rs1006737 is 0.063 in CHB population, while that is about 0.33–0.36 in CEU population [6,9]. The MAF of rs4765905 in CHB is also 0.063. These two SNPs

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Table 2. Results of association analyses between two SNPs in CACNA1C and autism in 553 trios.

| Marker     | Allele | Afreq | Families | S   | E (S) | Var (S) | Z   | \( p \) |
|------------|--------|-------|----------|-----|-------|---------|-----|-------|
| rs1006737  | A      | 0.063 | 124      | 53.0| 65.0  | 32.5    | -2.105| 0.035 |
|            | G      | 0.937 | 124      | 195.0| 183.0 | 32.5    | 2.105|        |
| rs4765905  | C      | 0.063 | 124      | 53.0| 65.0  | 32.5    | -2.105| 0.035 |
|            | G      | 0.937 | 124      | 195.0| 183.0 | 32.5    | 2.105|        |

Afreq, allele frequency; Families, number of informative families; S, test statistics for the observed number of transmitted alleles; E(S), expected value of S under the null hypothesis (i.e., no linkage and no association).

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Table 3. Results of association analyses for haplotype constructed from rs1006737 and rs4765905 in CACNA1C in 553 trios.

| Marker                  | Haplotypes | freq | Fam | S     | E (S) | Var (S) | Z   | \( p \) | Global \( p \) | Permutation \( a p \) |
|-------------------------|------------|------|-----|-------|-------|---------|-----|--------|--------------|----------------------|
| rs1006737-rs4765905     | G-G        | 0.937| 119 | 190.00| 178.00| 30.50   | 2.173| 0.030  | 0.046        | 0.027                |
|                         | A-C        | 0.062| 118 | 47.00 | 59.50 | 30.25   | -2.273|        |              |                      |

\( a \) Whole marker permutation test using chisq sum \( p \) value, the number of permutation is 10,000.; freq, Estimation of haplotype frequencies; Fam, number of informative families; S, test statistics for the observed number of transmitted alleles; E(S), expected value of S under the null hypothesis (i.e., no linkage and no association).

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rs4765905 and rs1006737 are in a strong LD block. These results are consistent with those of HapMap project. Second, it might be likely that the genetic signal is tagging a less common (and possibly rare) genetic variant which might contribute directly to autism risk, such as the rare mutation G406R in Timothy Syndrome. Third, the mechanism of genetic etiology of autism and schizophrenia is different despite the susceptibility genes overlap between these two diseases.

Two haplotypes constructed from rs1006737 and rs4765905 which are in a strong LD block were associated with autism. There are quite few other SNPs between these two SNPs. It will be interesting to investigate the association between other SNPs in this region and autism, and to explore whether there are differences of association results between the Chinese population and other population. In future, we will explore the association between autism and SNPs in this region by selecting SNPs in a high intensity. Moreover, it is important to perform mutation screening in CACNA1C to detect potentially deleterious rare variants.

Evidence for shared risk was observed for specific genes between schizophrenia and ASD. The susceptibility genes (such as DISC1 [33,34], RELN [35,36], GABA [37–39], SHANK3 [40,41], NRXN1 [20,42], NTNG1 [43,44], etc.), which were associated with schizophrenia, also confer risk to ASD. The cross-disorder analyses reveal a significant genetic overlap between schizophrenia and ASD [22,45–49]. Furthermore, epidemiological and neuroimaging studies provided further support for biological overlap between schizophrenia and ASD [50,51]. The most recent evidence for shared etiology comes from studies of rare copy number variants [52]. However, the risk variants were not completely overlap between autism and schizophrenia. These results indicated the existence of shared genetic susceptibility to schizophrenia and autism, suggesting the possibility that the genes may exert their effects through a biological pathway common to both disorders.

Recent studies suggested that calcium channel dysfunction may contribute to the pathogenesis of autism. Three rare missense mutations of CACNB2 which encodes a subunit of a voltage-dependent calcium channel protein were detected in ASD-affected families. Two of these mutations displayed significantly decelerated time-dependent inactivation as well as increased sensitivity of voltage-dependent inactivation [53]. Another study provided evidence that rs10848653 in CACNA1C was associated with ASD [54].

The calcium ion is one of the most versatile and universal of biological signaling molecules [55]. In brain, the subunit encoded by CACNA1C is the major constituent of brain L-type voltage gated calcium channels, and is a crucial regulator of dendritic calcium influx in response to synaptic activity [56]. It is most frequently implicated in coupling of cell membrane depolarization to transient increase of the membrane permeability for calcium, leading to activation and potentially changes in intracellular signaling pathway activity, gene transcription, and synaptic plasticity. Therefore, CACNA1C plays important roles in the proper function of numerous neurological circuits including hippocampus, amygdala, and mesolimbic reward system, which are strongly implicated in psychiatric disease pathophysiology [57].

Moreover, neuroimaging researches provided evidence that CACNA1C might affect brain regional activation and inter-regional connectivity. Previous study demonstrated that the effect of rs1006737 in CACNA1C on the brain converges on the neural circuitry involved in affect processing [4,58]. Strong evidence indicates that rs1006737 exerts pleiotropic effects on particular brain functions and affects different brain regions (such as amygdala, hippocampus, and ventrolateral prefrontal cortex). Moreover, alteration in CACNA1C expression may be a molecular mechanism of genetic risk [12]. There is converging evidence that patients with autism may have affected brain regional activation and inter-regional connectivity [59–64]. A recent study demonstrated that beta connectivity was reduced during emotional face processing in adolescents with autism [65]. These findings suggest that functional disconnection in brain
networks mediating emotional processes may contribute to deficits in social cognition in ASD. CACNA1C might potentially be related to alternations in intracellular calcium homeostasis and then confer risk of autism.

Other replication studies are needed. In addition, further studies are necessary to understand the underlying mechanisms the gene CACNA1C exerts on autism as well as other psychiatric disorders.

Conclusions

Our study indicates that CACNA1C is associated with autism in Han Chinese population. CACNA1C might play a role in the pathogenesis of autism.

Supporting Information

S1 Fig. Linkage disequilibrium block constructed from 18 SNPs in CACNA1C. Markers with linkage disequilibrium (0 < \( r^2 \) < 1) are shown in black through grey (color intensity decreases with decreasing \( r^2 \) value). The square is shown in black when \( r^2 = 1 \), while the square is white when \( r^2 = 0 \).

S1 Table. Information of 18 SNPs in CACNA1C and genotype frequencies in 239 autism trios. a Hardy-Weinberg equilibrium \( p \) value for genotype distributions in children affected with autism; b Hardy-Weinberg equilibrium \( p \) value for genotype distributions in parents.

S2 Table. Association results of 18 SNPs in CACNA1C and autism in 239 trios calculated by Haploview. SNPs, single nucleotide polymorphisms; Overtransmitted, the allele overtransmitted to affected offspring; T, transmitted; U, untransmitted; T:U is the ratio of transmissions to non transmissions of the overtransmitted allele.

S3 Table. Genotype frequencies of rs1006737 and rs4765905 in 553 autism trios. a Hardy-Weinberg equilibrium \( p \) value for genotype distributions in children affected with autism; b Hardy-Weinberg equilibrium \( p \) value for genotype distributions in parents.

S4 Table. Association analyses of two SNPs (rs1006737 and rs4765905) in 553 trios calculated by Haploview. SNPs, single nucleotide polymorphisms; Overtransmitted is the allele overtransmitted to affected offspring; T, transmitted; U, untransmitted; T:U is the ratio of transmissions to non transmissions of the overtransmitted allele.

S5 Table. Haplotype analyses of two haplotypes constructed from rs1006737 and rs4765905 in 553 trios calculated by Haploview. a the number of permutation is 10,000; SNPs, single nucleotide polymorphisms; Freq, frequency; T, transmitted; U, untransmitted; T: U is the ratio of transmissions to non transmissions of the overtransmitted allele.

S6 Table. Genotyping data of the selected 18 SNPs in CACNA1C in 239 trios of Han Chinese descent.
S7 Table. Genotyping data of rs1006737 and rs4765905 in additional 314 trios of Han Chinese descent.

(XLS)

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Author Contributions

Conceived and designed the experiments: DZ LFW. Performed the experiments: J. Li YY LFW YYY TLL LNZ. Analyzed the data: YY J. Li HY WHY. Contributed reagents/materials/analysis tools: LL. Wrote the paper: J. Li LFW YY LNZ. Collected the samples: MXJ J. Liu.

References

1. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord. 2000; 30: 205–223. PMID:11055457
2. Folstein SE, Rosen-Sh opley B. Genetics of autism: complex aetiology for a heterogeneous disorder. Nat Rev Genet. 2001; 2: 943–955. PMID: 11733747
3. Klei L, Sanders SJ, Murtha MT, Hus V, Lowe JK, Willese AJ, et al. Common genetic variants, acting additively, are a major source of risk for autism. Mol Autism. 2012; 3: 9. doi:10.1186/2040-2392-3-9 PMID:23067556
4. Dima D, Jogia J, Collier D, Vassos E, Burdick KE, Frangou S. Independent modulation of engagement and connectivity of the facial network during affect processing by CACNA1C and ANK3 risk genes for bipolar disorder. JAMA Psychiatry. 2013; 70: 1303–1311. doi: 10.1001/jamapsychiatry.2013.2099 PMID:24108394
5. Moosmang S, Haider N, Klugbauer N, Adelsberger H, Langwieser N, Muller J, et al. Role of hippocampal Cav1.2 Ca2+ channels in NMDA receptor-independent synaptic plasticity and spatial memory. J Neurosci. 2005; 25: 9883–9892. PMID:16251435
6. 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: 1056–1058. doi: 10.1038/ng.209 PMID:18711365
7. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447: 661–678. PMID: 17554300
8. 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: 558–569. doi: 10.1038/mp.4002151 PMID:18317468
9. Green EK, Grozeva D, Jones I, Jones L, Kirov G, Caesar S, et al. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. Mol Psychiatry. 2012; 15: 1016–1022.
10. Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium. Genome-wide association study identifies five new schizophrenia loci. Nat Genet. 2011; 43: 969–976. doi: 10.1038/ng.940 PMID:21926974
11. Hamshere ML, Walters JT, Smith R, Richards AL, Green E, Grozeva D, et al. Genome-wide significant associations in schizophrenia to ITIH3/4, CACNA1C and SDCCAG8, and extensive replication of associations reported by the Schizophrenia PGC. Mol Psychiatry. 2013; 18: 708–712. doi: 10.1038/mp.2012.67 PMID:22614287
12. Bigos KL, Mattay VS, Callicott JH, Straub RE, Vakkalanka R, Kolachana B, et al. Genetic variation in CACNA1C affects brain circuitries related to mental illness. Arch Gen Psychiatry. 2010; 67: 939–945. doi: 10.1001/archgenpsychiatry.2010.96 PMID:20819988
13. Ivorra JL, Rivero O, Costas J, Iniesta R, Arrojo M, Ramos-Rios R, et al. Replication of previous genome-wide association studies of psychiatric diseases in a large schizophrenia case-control sample from Spain. Schizophr Res. 2014; 159: 107–113. doi: 10.1016/j.schres.2014.07.004 PMID:25124521
14. Nyegaard M, Demontis D, Foldager L, Hedemand A, Flint TJ, Sorensen KM, et al. CACNA1C (rs1006737) is associated with schizophrenia. Mol Psychiatry. 2010; 15: 119–121. doi: 10.1038/mp.2009.69 PMID: 20098439

15. Baron-Cohen S, Belmonte MK. Autism: a window onto the development of the social and the analytic brain. Annu Rev Neurosci. 2005; 28: 109–126. PMID: 16033325

16. Craddock N, Owen MJ. Data and clinical utility should be the drivers of changes to psychiatric classification. Br J Psychiatry. 2010; 197: 158; author reply 158–159. doi: 10.1192/bjp.197.2.158 PMID: 20679271

17. Burbach JP, van der Zwaag B. Contact in the genetics of autism and schizophrenia. Trends Neurosci. 2009; 32: 69–72. doi: 10.1016/j.tins.2008.11.002 PMID: 19135727

18. Iossifov I, Zheng T, Baron M, Gilliam TC, Rzhetsky A. Genetic-linkage mapping of complex hereditary disorders to a whole-genome molecular-interaction network. Genome Res. 2008; 18: 1150–1162. doi: 10.1101/gr.075622.107 PMID: 18417725

19. Kakinuma H, Ozaki M, Sato H, Takahashi H. Variation in GABA-A subunit gene copy number in an autistic patient with mosaic 4 p duplication (p12p16). Am J Med Genet B Neuropsychiatr Genet. 2008; 147B: 973–975. doi: 10.1002/ajmg.b.30663 PMID: 19889066

20. Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, et al. Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet. 2008; 82: 477–488. doi: 10.1016/j.ajhg.2007.12.009 PMID: 18252227

21. 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: 1371–1379. doi: 10.1016/S0140-6736(12)62129-1 PMID: 23453885

22. Krug DA, Arick J, Almond P. Behavior checklist for identifying severely handicapped individuals with high levels of autistic behavior. J Child Psychol Psychiatry. 1980; 21: 221–229. PMID: 7430288

23. Schopler E, Reichler RJ, DeVellis RF, Dale K. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). J Autism Dev Disord. 1990; 10: 91–103. PMID: 6927682

24. Gauderman WJ, Morrison JM. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies, http://hydra.usc.edu/gxe, 2006.

25. Rabinowitz D, Laird N. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. Hum Hered. 2000; 50: 211–223. PMID: 10782012

26. Kilpinen H, Ylisaukko-Oja T, Hennah W, Palotie A, Vanhala R, et al. Association of DISC1 with autism and Asperger syndrome. Mol Psychiatry. 2008; 13: 187–196. PMID: 17579608

27. Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, et al. Disruption of two novel genes by a translocation co-segregating with schizophrenia. Mol Hum Genet. 2000; 9: 1415–1423. PMID: 10814723

28. Impagnatiello F, Guidotti AR, Pesold C, Dwivedi Y, Caruncho H, Pisu MG, et al. A decrease of reelin expression as a putative vulnerability factor in schizophrenia. Proc Natl Acad Sci U S A. 1998; 95: 15718–15723. PMID: 9861036
36. Persico AM, D’Agruma L, Maiorano N Totaro A, Militerni R, Bravaccio C, et al. Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. Mol Psychiatry. 2001; 6: 150–159. PMID: 11317216

37. Coon H, Sobell J, Heston L, Sommer S, Hoff M, Holik J, et al. Search for mutations in the beta 1 GABAA receptor subunit gene in patients with schizophrenia. Am J Med Genet. 1994; 54: 12–20. PMID: 10861500

38. Martin ER, Menold MM, Wolpert CM, Bass MP, Donnelly SL, Ravan SA, et al. Analysis of linkage disequilibrium in gamma-aminobutyric acid receptor subunit genes in autistic disorder. Am J Med Genet. 2000; 96: 43–48. PMID: 10686550

39. Schroer RJ, Phelan MC, Michaelis RC, Crawford EC, Skinner SA, Cuccaro M, et al. Autism and maternally derived aberrations of chromosome 15q. Am J Med Genet. 1998; 76: 327–336. PMID: 9545097

40. Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, et al. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet. 2007; 39: 25–27. PMID: 17173049

41. Gauthier J, Champagne N, Lafreniere RG, Xiong L, Spiegelman D, Brustein E, et al. De novo mutations in the gene encoding the synaptic scaffolding protein SHANK3 in patients ascertained for schizophrenia. Proc Natl Acad Sci U S A. 2010; 107: 7863–7868. doi:10.1073/pnas.0906232107 PMID: 20385823

42. Kim HG, Kishikawa S, Higgins AW, Seong IS, Donovan DJ, Shen Y, et al. Disruption of neurexin 1 associated with autism spectrum disorder. Am J Hum Genet. 2008; 82: 199–207. doi:10.1016/j.ajhg.2007.09.011 PMID: 18179900

43. Aoki-Suzuki M, Yamada K, Meerabux J, Iwayama-Shigeno Y, Ohba H, Iwamoto K, et al. A family-based association study and gene expression analyses of netrin-G1 and-G2 genes in schizophrenia. Biol Psychiatry. 2005; 57: 382–393. PMID: 15705354

44. O’Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature. 2012; 485: 246–250. doi:10.1038/nature10989 PMID: 22495309

45. Carroll LS, Owen MJ. Genetic overlap between autism, schizophrenia and bipolar disorder. Genome Med. 2009; 1: 102. doi:10.1186/gm102 PMID: 19886976

46. Guilmatre A, Dubourg C, Mosca AL, Legallic S, Goldenberg A, Drouin-Garraud V, et al. Recurrent rearrangements in synaptic and neurodevelopmental genes and shared biologic pathways in schizophrenia, autism, and mental retardation. Arch Gen Psychiatry. 2009; 66: 947–956. doi:10.1001/archgenpsychiatry.2009.80 PMID: 19736351

47. Kenny EM, Cormican P, Furlong S, Heron E, Kenny G, Fahey C, et al. Excess of rare novel loss-of-function variants in synaptic genes in schizophrenia and autism spectrum disorders. Mol Psychiatry. 2014; 19: 872–879. doi: 10.1038/mp.2013.127 PMID: 24126926

48. Lee SH, Ripke S, Neale BM, Faraone SV, Perlis RH, et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet. 2013; 45: 984–994. doi: 10.1038/ng.2711 PMID: 23933621

49. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet. 2011; 88: 76–82. doi: 10.1016/j.ajhg.2010.11.011 PMID: 21167468

50. King BH, Lord C. Is schizophrenia on the autism spectrum? Brain Res. 2011; 1380: 34–41. doi: 10.1016/j.brainres.2010.11.031 PMID: 21078305

51. Sullivan PF, Magnusson C, Reichenberg A, Boman M, Dalmann C, Davidson M, et al. Family history of schizophrenia and bipolar disorder as risk factors for autism. Arch Gen Psychiatry. 2012; 69: 1099–1103. PMID: 22752149

52. Saus E, Brunet A, Armengol L, Alonso P, Crespo JM, Fernandez-Aranda F, et al. Comprehensive copy number variant (CNV) analysis of neuronal pathways genes in psychiatric disorders identifies rare variants within patients. J Psychiatr Res. 2010; 44: 971–978. doi: 10.1016/j.jpsychires.2010.03.007 PMID: 20398908

53. Breitenkamp AF, Matthes J, Nass RD, Sinzig J, Lehmkuhl G, Numberg P, et al. Rare mutations of CACNB2 found in autism spectrum disease-affected families alter calcium channel function. PLoS One. 2014; 9: e95579. doi: 10.1371/journal.pone.0095579 PMID: 24752249

54. Lu AT, Dai X, Martinez-Agosto JA, Cantor RM. Support for calcium channel gene defects in autism spectrum disorders. Mol Autism. 2012; 3: 18. doi: 10.1186/2040-2392-3-18 PMID: 23241247

55. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol. 2000; 1: 1–21.
56. Vacher H, Mohapatra DP, Trimmer JS. Localization and targeting of voltage-dependent ion channels in mammalian central neurons. Physiol Rev. 2008; 88: 1407–1447. doi: 10.1152/physrev.00002.2008 PMID: 18923186

57. Bhat S, Dao DT, Terrillion CE, Arad M, Smith RJ, Soldatov NM, et al. CACNA1C (Cav1.2) in the pathophysiology of psychiatric disease. Prog Neurobiol. 2012; 99: 1–14. doi: 10.1016/j.pneurobi.2012.06.001 PMID: 22705413

58. 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: 1831–1836. doi: 10.1016/j.neuroimage.2009.09.028 PMID: 19781653

59. Boersma M, Kemner C, de Reus MA, Collin G, Snijders TM, Hofman D, et al. Disrupted functional brain networks in autistic toddlers. Brain Connect. 2013; 3: 41–49. doi: 10.1089/brain.2012.0127 PMID: 23259692

60. Noonan SK, Haist F, Muller RA. Aberrant functional connectivity in autism: evidence from low-frequency BOLD signal fluctuations. Brain Res. 2009; 1262: 48–63. doi: 10.1016/j.brainres.2008.12.076 PMID: 19401185

61. Radulescu E, Minati L, Ganeshan B, Harrison NA, Gray MA, Beacher FD, et al. Abnormalities in fronto-striatal connectivity within language networks relate to differences in grey-matter heterogeneity in Asperger syndrome. Neuroimage Clin. 2013; 2: 716–726. doi: 10.1016/j.nicl.2013.05.010 PMID: 24179823

62. Shukla DK, Keehn B, Lincoln AJ, Muller RA. White matter compromise of callosal and subcortical fiber tracts in children with autism spectrum disorder: a diffusion tensor imaging study. J Am Acad Child Adolesc Psychiatry. 2010; 49: 1269–1278, 1278 e1261–1262. doi: 10.1016/j.jaac.2010.08.018 PMID: 21093776

63. Supekar K, Musen M, Menon V. Development of large-scale functional brain networks in children. PLoS Biol. 2009; 7: e1000157. doi: 10.1371/journal.pbio.1000157 PMID: 19621066

64. Verly M, Verhoeven J, Zink I, Mantini D, Peeters R, Deprez S, et al. Altered functional connectivity of the language network in ASD: Role of classical language areas and cerebellum. Neuroimage Clin. 2014; 4: 374–382. doi: 10.1016/j.nicl.2014.01.008 PMID: 24567909

65. Leung RC, Ye AX, Wong SM, Taylor MJ, Doesburg SM. Reduced beta connectivity during emotional face processing in adolescents with autism. Mol Autism. 2014; 5: 51. doi: 10.1186/2040-2392-5-51 PMID: 25371811