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ULK4 Genetic Variants Have Pleiotropic Effect on Risk of Autism, Associated with Brain mRNA Expression and Antipsychotic Treatment Response

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ABSTRACT

Background: *ULK4* genetic variants have been implicated for adult-onset psychiatric disorders, and common variants are associated with hematologic and cardiologic disorders at genome-wide significance. This study aimed to examine the pleiotropic effect of *ULK4* on the risk of autism, *cis*-association with mRNA and impact on antipsychotic treatment response in humans.

Methods: The clinical genetic data comprised one cohort of autism case-parent triad sample in the Han Chinese and three cohorts of family-based samples in the European ancestry, from Autism Genetic Research Exchange, the Autism Genome Project and the Simons Foundation for Autism Research Initiative; mRNA expression in postmortem human prefrontal cortex across the lifespan and different brain regions of postmortem human brain and other tissues from two independent datasets were used for examining the *cis*-association with *ULK4* variants. Antipsychotic treatment response data were from the Clinical Antipsychotic Trials in Intervention Effectiveness in patients with chronic schizophrenia. Transmission disequilibrium test was used to examine the genetic association with autism. General linear regression analysis was

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performed for *cis*-association with mRNA expression. The Cox proportion hazard model was used to analyze the primary outcome, the time to discontinued use of antipsychotics.

Results: Multiple functional SNPs including rs2272007 in strong linkage disequilibrium at *ULK4* were associated with autism in the Han Chinese sample (minimum $p < 0.00071$) which survived the Bonferroni correction for multiple testing. SNP rs2272007 and other SNPs were significantly associated with *ULK4* expression in postmortem human prefrontal cortex in subjects across the lifespan and multiple brain areas in two independent datasets. In addition, two SNPs rs7651623 (Hazard Ratio, HR = 16.33; $p = 5.00 \times 10^{-4}$) and rs2030431 (HR = 17.25; $p = 3.00 \times 10^{-4}$) in strong LD were associated with the risk of discontinuing use of antipsychotic medications in the patients with schizophrenia. SNP rs2272007, perfect LD with rs7651623, was associated with treatment response in olanzapine only (HR = 4.22; $p = 0.0034$).

Conclusion: We provide evidence at multiple layers for *ULK4* common genetic variants associated with the risk of autism. This may have clinical implication for translational research and precision psychiatry.

KEYWORDS: *ULK4*; autism; brain mRNA expression; antipsychotic treatment response; olanzapine

INTRODUCTION

Autism is a pervasive developmental disorder characterized by the presence of atypically developing or impaired development in social interaction and communication, and markedly restricted, repetitive activities and interests [1]. While autism has been incorporated into the diagnosis of Autism Spectrum Disorder (ASD) in DSM-V [2], patients with autism generally have a moderate to severe level of illness often with a clinically significant intellectual impairment that requires substantial care or support. The prevalence of ASD diagnosis has increased rapidly in recent decades [3], reaching 16.8 per 1000 children (one in 59) according to the latest report from the Autism and Developmental Disabilities Monitoring (ADDM) Network in the United States [4], although this increase might be in part attributed to the change in the diagnostic criteria. Even though twin studies of autism suggest a strong genetic component [5,6], the genetic etiology of autism is yet evident. Genetic research about autism has uncovered many moderately penetrant rare variants that may explain up to 35% of cases with this diagnosis. While recent genome-wide association studies suggest that the genetic risk of autism mostly resides with common variants, which may act additively [7,8], so far only a few common genetic variants have been associated with autism through several sizeable genome-wide association studies [9–13]. A recent large-scale genome-wide combined analysis of multiple major psychiatric

disorders indicate that common genetic variants shared between ADHD and ASD are mainly located at genomic regions with CNVs that have been associated with ASD [14].

Previous studies have implicated genetic variants in *ULK4* as risk factors for multiple psychiatric disorders [15–17]. Unc-51-like kinase 4 (ULK4), located at 3p22.1 (Chr3: 41, 246, 599–41, 962, 440, hg38) is comprised of 37 exons and plays an important role in neuronal development [15]. Deletions spanning exons 21–34 of *ULK4* have been found in 4 out of 3,391 patients with schizophrenia from the International Schizophrenia Consortium but none of 3181 control subjects [17]. The CNVs, deletions removing exons 33 and 34 of a large splice variant of *ULK4*, have been found in patients with schizophrenia (2/708), bipolar disorder (2/1136) and autism (1/507) compared with 37 of 98,022 controls in the Icelandic population [15]. Another study detected an additional seven cases of CNVs involving *ULK4* in 5891 patients with developmental disorders (DD) that often have co-occurrence with ASD [16]. While common genetic variants at *ULK4* have not been associated with schizophrenia in the past genome-wide association studies from either the Psychiatric Genomic Consortium (PGC) or a sizeable study of Han Chinese populations [18], the data from the PGC showed potentially suggestive association signals (p = 0.0001) at rs17210774 in intron 33 of *ULK4* with bipolar disorder and rs1722850 (p = 0.001) flanking *ULK4* with major depressive disorder [15].

Apart from these reports, previous studies have associated *ULK4* common variants with hematologic and cardiologic disorders such as multiple myeloma, acute aortic dissections, and hypertension at genome-wide significance. A meta-analysis of 19 studies (n = 29,378 subjects) identified rs1717027 at *ULK4* associated with diastolic blood pressure [19]. The missense variants rs1052501 and rs2272007 in complete linkage disequilibrium (LD) in *ULK4* (r^2 = 0.98 and D' = 1) have been associated with multiple myeloma at genome-wide significance [20]. In addition, genome-wide association studies have identified *ULK4* variants associated with blood pressure trait and hypertension in both European ancestry and Han Chinese populations [21,22]. Therefore, *ULK4* might have a pleiotropic effect on complex human disorders including autism.

We conducted the present study to examine associations of common variants in *ULK4* with autism using a family cohort of samples in Han Chinese, followed by an analysis of three family cohorts of European ancestry samples. SNP rs2272007, a non-synonymous variant was associated with autism in the Han Chinese. SNP rs2272007 and several others in LD were strongly associated with *ULK4* expression in postmortem human prefrontal cortex, multiple brain areas, and other human tissues including artery and whole blood in an independent dataset. Finally, the minor risk allele of rs2272007 for autism was associated with poorer treatment response in patients with schizophrenia treated with olanzapine in an antipsychotic trial.

METHODS

Clinical Subjects

Chinese autism sample: The subjects of autism case-parent triad sample were initially recruited for a genome-wide association study (GWAS) and all were Han Chinese [9]. A senior child psychiatrist made the diagnosis of Autistic Disorder according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR). An independent child psychiatrist confirmed the diagnosis with a semi-structured interview, which included a parent interview in which a psychiatrist reviewed children's symptoms with parents and a child interview [23]. Subjects with Asperger's syndrome and pervasive developmental disorder not otherwise specified (PPD-NOS) were excluded. Therefore, the cases were typically autism, belonging to autism spectrum disorder according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V). Neurological examination and check of features and signs were performed to exclude specific genetic diseases such as Down's syndrome, Angel's syndrome, and Fragile X syndrome. The karyotype analysis was performed for each subject to eliminate chromosomal abnormalities, and CNVs were screened.

European ancestry sample: Three cohorts of European ancestry samples were also analyzed, and they were from previous GWAS of autism [9]. The samples were the Autism Genetic Research Exchange (AGRE) sample, the Autism Genome Project (AGP) and the Simons Foundation for Autism Research Initiative (SAFRI) Base samples. All three cohorts are family-based samples including multiplex pedigree. The recruitments have been described in the previous studies [10,24,25].

Subjects for antipsychotic treatment: Data from the Clinical Antipsychotic Trials in Intervention Effectiveness (CATIE) [26] was used to examine the association of genetic variant at *ULK4* with antipsychotic treatment response in patients with schizophrenia, a neurodevelopmental disorder that may share common genetic etiology with autism. CNVs that disrupted *ULK4* have been found in schizophrenia. Considering the sample size, we only included patients with schizophrenia of European ancestry and who completed the Phase 1/1A of the CATIE study, in which patients were randomly assigned to one of the five medications (olanzapine, perphenazine, quetiapine, ziprasidone, and risperidone), for the analysis. The primary outcome of the CATIE study was the time to discontinuing use of antipsychotic medication.

SNP Genotype Data

All data of SNPs were from previous genome-wide studies and genotyped with microarray chips. The Chinese autism triads were genotyped using the HumanHap CNV370K. SNPs located in the genomic region of *ULK4* (upstream and downstream 100 kb) were selected for this study. The region was on purpose defined wider in order to include more

SNPs for analysis. The detailed genotype data of three European ancestry cohorts in AGRE, AGP, and SFARI have been described in previous studies [10,24,25]. In brief, the AGRE samples were genotyped with the Illumina HumanHap 550 K chip; and the AGP and SFARI samples were genotyped with the HumanHap 1M chip [9]. Genome-wide quality control was applied as follows: individuals with missing SNP call rate > 2% were excluded. SNPs were zeroed out if Mendelian errors > 5% for a SNP and individuals were removed if Mendelian errors > 3% for an individual from the analysis. Sample duplications and cryptic relatedness were checked by the Identity by State (IBS) analysis of genotypes data in 22 chromosomes. One of each related pair (IBD-sharing coefficients > 0.10) was excluded. No sex error was found based on the heterozygote of the X chromosome. Individual genotyping and quality control of the CATIE sample were conducted for a genome-wide association study of schizophrenia [27], and the samples were genotyped with Affymetrix 500 K chip and 164 K chip.

Gene Expression Data

One dataset of the mRNA expression was based on postmortem human prefrontal cortex of fetal and postnatal subjects. The sample was collected at the Clinical Brain Disorders Branch of the National Institute of Mental Health of NIH and data were developed by the Lieber Institute for Brain Development (http://braincloud.jhmi.edu) [28]. Sample subjects included fetal, neonatal, child and adolescent, and adults.

The other dataset was the Genotype-Tissue Expression (GTEx), which consists of whole-genome sequence and RNA-Seq data from about 650 deceased adult donors, with multiple tissue samples collected per donor (https://commonfund.nih.gov/gtex). The GTEx dataset included mRNA expression from many different regions of the brain with variable sample size in specific tissues, which included anterior cingulate cortex (BA24), Caudate nucleus (basal ganglia), Cerebellar hemisphere, Cerebellum, Cortex, Frontal cortex (BA9), Hippocampus, Hypothalamus, Nucleus accumbens (basal ganglia), Putamen (basal ganglia) and Pituitary [29].

Statistical Analysis

Transmission disequilibrium test (TDT) was performed to identify common genetic variants of *ULK4* associated with autism in the Chinese and European ancestry samples using PLINK 1.9. The *cis*-association analysis was performed to test for the association of SNP genotypes with the expression of a gene where the SNPs are located. In the Braincloud gene expression dataset, general linear regression analysis was performed to test the association of SNP genotypes with the mean level of gene expression while adjusting for age, sex, race, and post-mortem interval (in h) and RNA integrity number (RIN). In the expression data of Genotype-Tissue Expression (GTEx), associations of SNPs at *ULK4* with its mRNA expression were performed through the online analysis, in which linear regression was used while adjusting for PEER factors, sex, genotyping

platform, and three genotype-based principal components in the gene expression dataset of GTEx (https://gtexportal.org/).

The association analysis of *ULK4* variants with antipsychotic treatment response was based on the SNPs genotyped in the original genotyping arrays [27] in a 160-kb genomic region around the top signals. Cox proportional hazard model was used to estimate the hazard ratio of discontinued use of antipsychotics [30].

RESULTS

Common Genetic Variants Associated with Autism in Han Chinese Triads

TDT analysis was performed for 15 SNPs in 275 autism case-parent triads of Han Chinese ancestry. The mean age of assessment was 60.69 (SD = 22.29) months and 85% of children were boys. Three cohorts of European ancestry samples were also analyzed as replication samples, including 285 case-parent triads of AGRE, 325 case-parent triads of AGP, and 689 case-parent triads of SAFRI. SNPs in the analysis were located at the genomic region (upstream and downstream 100kb) of *ULK4* and genotyped with the Illumina HumanHap 370K Chip. Multiple association signals (OR > 1.65; $p < 0.005$) were observed at SNPs rs1052501, rs1716975, rs2272007, rs12635286, rs17066958 for allelic transmission disequilibrium from parents to patients with autism (Table 1). Even though some of these SNPs were in strong LD, they passed through the Bonferroni correction for multiple testing. The SNPs rs2272007, rs1716975, and rs1052501 are located in exon 2, 7 and 17 of *ULK4*, respectively, whereas rs12635286 and rs17066958 are located at an intergenic region between *ULK4*||*TRAK1*. The three SNPs in the exons are non-synonymous and in complete LD with each other (pairwise $r^2 = 1$ and $D' = 1$) according to the CHBJPT sample of 1000 Genomes, of which SNPs rs1052501 and rs2272007 are conserved in vertebrates. However, we did not find a consistently nominal association in three family-based samples of the European ancestry ($p > 0.05$), likely due to that our patient subjects were limited to a diagnosis of simple form of autism rather than autism spectrum disorders that were included in other three samples, some of which were from multiplex families [9].

Multiple *ULK4* Variants Show Strong *cis*-Association with mRNA Expression in Postmortem Human Brains and Other Human Tissues

All SNPs showing signals for association with autism had significant *cis*-association with mRNA expression in the tissue sample of postmortem human prefrontal cortex ($n = 261$). The most significant *cis*-association was observed at rs1052501 ($p = 1.77 \times 10^{-8}$), rs1716975 and rs2272007 ($p = 4.94 \times 10^{-7}$) (Figure 1a), which are also in strong LD (pairwise $r^2 > 0.9$ and $D' = 1$) in the CEU sample. We selected rs2272007 as a proxy for all three SNPs for examining *cis*-association in the sample by the stage of life. Interestingly, SNP rs2272007 was associated with mRNA expression consistently in the sample of fetal ($p = 0.039$; $n = 37$), child and adolescent

(p = 0.005; n = 30), and adult (p = 0.001; n = 162) comprising of adult African American (p = 0.001; n = 80) and adults of European ancestry (p = 0.018; n = 82) (Figure 1b).

SNP rs2272007 had consistent *cis*-associations in multiple brain areas and other human tissues in an independent GTEx dataset. We found the most significant *cis*-association with *ULK4* expression for rs2272007 in the sample of tibial nerve (NES = −0.69; $p = 1.4 \times 10^{-37}$), pituitary (NES = −1.2; $p = 9.6 \times 10^{-31}$); and rs2272007 had *cis*-association in broad brain regions including putamen (NES = −1.3; $p = 9.6 \times 10^{-20}$), frontal cortex-Brodmann area (BA9) (NES = −1.1; $p = 1.6 \times 10^{-19}$), anterior cingulate (BA24) (NES = −1.1; $p = 1.96 \times 10^{-16}$), cerebellum (NES = −0.88; $p = 3.4 \times 10^{-14}$), caudate of basal ganglia (NES = −0.75; $p = 3.5 \times 10^{-14}$), nucleus accumbens of basal ganglia (NES = −0.82; $p = 9.6 \times 10^{-13}$), cerebellar hemisphere (NES = −0.93; $p = 3.7 \times 10^{-13}$), hypothalamus (NES = −0.59; $p = 3.6 \times 10^{-11}$), hippocampus (NES = −0.60; $p = 3.2 \times 10^{-10}$), amygdala (NES = −0.61; $p = 8.3 \times 10^{-8}$) and substantia nigra (NES = −0.91; $p = 1.4 \times 10^{-7}$) (Figure 1c). In addition, we noted that rs2272007 had a strong *cis*-association in other human tissues such as transformed fibroblast cells, artery and whole blood. In all these, the risk-associated alleles were associated with relatively higher expression.

ULK4 Variants Associated with Antipsychotic Treatment Response in Patients with Schizophrenia

Antipsychotics have been approved for treating behavior problem of autism [31]. Based on the treatment data available in the European ancestry sample, we performed a fast-track analysis to examine the impact of *ULK4* variants on antipsychotic drug response in patients with schizophrenia. Of 16 SNPs in the 100kb around the top association signal at rs1716975, we found that only rs2272007, which showed both associated with risk of autism and *cis*-regulatory effect in postmortem human brain, was genotyped in the CATIE clinical trial data (n = 419). SNP rs2272007 was associated with time to discontinuing the use of antipsychotics in patients treated with olanzapine (p = 0.0034) (Table 2). Two other SNPs rs1717020 and rs9856633, in complete LD with rs2272007 (r^2 = 0.996 and D' = 1), were also associated (p = 0.0034) with time to the discontinuation in patients treated with olanzapine (n = 95).

In the overall sample, SNPs rs2030431 (p = 0.0003) and rs7651623 (p = 0.0005) were associated with the antipsychotic response. Specifically, the rs2030431 heterozygous TC (minor allele C, HR = 17.25; $p = 3.0 \times 10^{-4}$) and rs7651623 heterozygous GA (minor allele A, HR = 16.33; $p = 5 \times 10^{-4}$) were shown a high risk of discontinuing the use of antipsychotics before completing the Phase 1/1A trial (Table 3). Two SNPs rs2030431 and rs7651623 were non-polymorphic (MAF = 0) in the CHBJPT and CEU samples of the 1000 Genomes, but they are also in strong LD (pairwise r^2 = 0.799 and D' = 1) in the patient sample. SNP rs7651623 is at a putative transcriptional factor binding site (TFBS) and perfect LD with rs2272007

(pairwise r^2 = 0.027 and D' = 1). In addition, we also found that some SNPs were associated with treatment response in individual drugs (Table 2). For example, compared with the homozygote CC of the major allele that conferred a risk of autism, the heterozygous (CT) of rs2272007 had more than the 4-fold likelihood for discontinuing use of olanzapine medication (HR = 4.22; p = 0.0034). However, we did not find a significant effect of rs2272007 homozygote TT on the time to discontinuation, likely due to the small sample size (n = 4) for a non-continuous outcome.

DISCUSSIONS

Our study provides several lines of evidence that *ULK4* may be a susceptibility gene for autism and this has implications for precision medicine. Three nonsynonymous SNPs in complete LD were associated with autism in a Han Chinese sample, and they were shown *cis*-association with *ULK4* expression in postmortem human prefrontal cortex from samples in the different stage of life and different brain regions in an independent dataset. SNP rs2272007, one of the three non-synonymous in LD, also had an impact on antipsychotic treatment response in the primary outcome, time to discontinuing use of medication, in chronic patients with schizophrenia treated with olanzapine.

The association of three nonsynonymous SNPs with autism is likely real, but they are located at three exons, different from those disrupted by the CNVs reported in the previous studies of schizophrenia. First, SNP rs2272007 and the other two were associated with autism and were shown *cis*-association with the gene expression in postmortem human brain. Although the *cis*-association analysis performed in the sample of European ancestry populations, the MAFs of rs2272007 are similar in the East Asian (0.149), European (0.191) and American (0.19) populations. Therefore, the gene expression results provide molecular evidence for the genetic association with autism that is found in the sample of Han Chinese. In addition, SNP rs2272007 is a non-synonymous variant causing a lysine-to-arginine substitution. Multiple rare variants, including rs7651623 in a TFBS, were associated with the time to discontinuing use of antipsychotics in the overall sample and were in perfect LD with rs2272007, which was only associated with time to the discontinuation in patients treated with olanzapine. This suggests that the three functional SNPs associated with the risk of autism may play a role in the antipsychotic response. We noted that individuals with the heterozygous of risk allele have a poorer response to olanzapine than those homozygotes of non-risk allele C. However, the mechanism of this relationship between antipsychotic treatment response and the autism-associated variant at *ULK4* is not clear. This warrants a further study of the molecular mechanism.

Table 1. Associations of ULK4 genetic variants with autism in Han Chinese triad sample.

SNP	BP	Gene	A1	A2	T	U	OR	*P*-value	nsSNP	Conserved	Allele Frequency [a]		
											Alter allele	CEU	CHB
rs4973978	41642549	*ULK4*	C	A	45	27	1.667	0.03389			T	0.6919	0.9078
rs9824775	41645175	*ULK4*	G	A	36	24	1.500	0.12130			T	0.7121	0.9272
rs6783612	41649598	*ULK4*	A	G	76	62	1.226	0.23340			G	0.7273	0.8155
rs9852303	41734529	*ULK4*	A	G	96	59	1.627	0.00296			C	0.7626	0.8010
rs6599175	41761013	*ULK4*	G	A	96	59	1.627	0.00296			T	0.7626	0.8010
rs4973893	41811474	*ULK4*	A	G	71	103	0.689	0.01527			G	0.8485	0.7670
rs900569	41834977	*ULK4*	A	G	163	133	1.226	0.08121			C	0.5051	0.5437
rs1716670	41885575	*ULK4*	A	G	75	104	0.721	0.03019			C	0.8030	0.7718
rs1052501	41900402	*ULK4*	G	A	96	58	1.655	0.00220	Y	0.97	T	0.7626	0.7961
rs1716975	41935010	*ULK4*	A	G	98	56	1.750	0.00071	Y		C	0.7626	0.7913
rs2272007	41971140	*ULK4*	A	G	98	58	1.690	0.00136	Y	1	C	0.7626	0.7913
rs12635286	42026840	*ULK4*||*TRAK1*	G	A	96	57	1.684	0.00162			T	0.7879	0.8107
rs17066958	42048961	*ULK4*||*TRAK1*	A	G	110	62	1.774	0.00025			G	0.8838	0.8010
rs904269	42051626	*ULK4*||*TRAK1*	A	C	151	117	1.291	0.03781			C	0.5253	0.5194
rs9847688	42054115	*ULK4*||*TRAK1*	C	A	113	69	1.638	0.00111			T	0.7273	0.7670

Note: nsSNP, non-synonymous SNPs; Conserved, SNPs are conserved in vertebrates. [a] Allele frequency from samples of 1000 Genomes; A1, minor allele; A2, major allele.

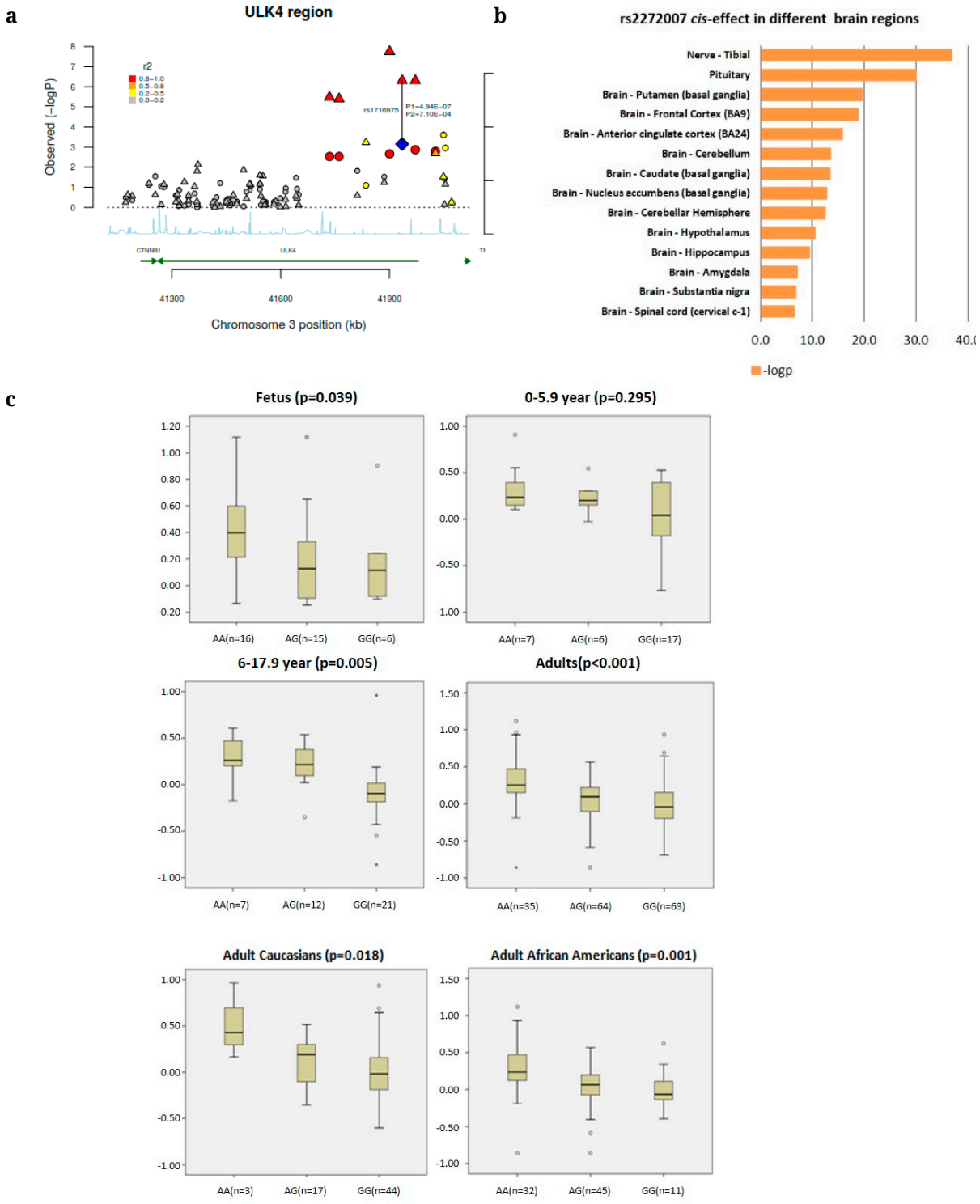

Figure 1. Genetic association of SNPs at *ULK4* with the risk of autism and *cis*-association in postmortem human brain. **(a)** overall genetic association of all SNPs with autism and *ULK4* expression (circle, indicates −log *p*-value of SNPs for association with autism in Han Chinese sample; Triangle, indicates −log *p*-value of SNPs for *cis*-association in postmortem human prefrontal cortex; Blue diamond, indicates the top signals for association with autism, and red colored, indicates LD (r^2) of top SNPs with other signals; **(b)** *cis*-association of rs2272007 in prefrontal cortex across different stage of life and in adult sample by race; **(c)** *cis*-association of rs2272007 in multiple tissues of the central nevus system and different brain regions.

Table 2. Autism-associated SNPs at *ULK4* associated with antipsychotic treatment response in patients with schizophrenia in the primary outcome, the time to discontinued use.

							The *p*-value for SNPs with time to discontinuation				
Chr	**Loc**	**BP**	**SNP**	**A1**	**A2**	**MAF**	***P***	***P*_olanz (*n* = 95)**	***P*_perph (*n* = 79)**	***P*_quet (*n* = 97)**	***P*_zipra (*n* = 50)**	***P*_risp (*n* = 98)**
3	downstream	41882783	rs1716667	C	G	0.0072	0.0818					0.2652
3	downstream	41895339	rs2030431	C	T	0.0060	0.0003					0.0710
3	upstream	41896323	rs9830599	C	T	0.0191	0.5000	0.2726	0.7454		0.2807	0.0710
3	intron	41900305	rs1716685	T	C	0.1471	0.4460	0.2524	0.0782	0.1404	0.2426	0.5695
3	intron	41901152	rs17283677	G	A	0.1283	0.6160	0.4784	0.0850	0.1404	0.0462	0.2452
3	intron	41915452	rs1717006	C	A	0.1492	0.4460	0.2524	0.0782	0.1404	0.2426	0.5695
3	intron	41928336	rs1716681	C	T	0.0024	0.0150					
3	intron	41950851	rs17218264	C	T	0.1301	0.2830	0.0196	0.1574	0.1404	0.0462	0.3383
3	intron	41956494	rs1717020	C	A	0.1527	0.1870	0.0034	0.1680	0.1404	0.2426	0.5695
3	intron	41958619	rs1716691	G	C	0.0072	0.0417				0.8450	0.0710
3	intron	41961791	rs17284313	C	A	0.1277	0.3000	0.0235	0.1574	0.1404	0.0462	0.3383
3	CDS	41971140	rs2272007	T	C	0.1514	0.2170	0.0034	0.1680	0.1623	0.2426	0.3467
3	5UTR	41971279	rs7651623	A	G	0.0048	0.0005					0.0710
3	intron	41971308	rs17284472	A	G	0.1331	0.2580	0.0196	0.1705	0.1138	0.0462	0.3383
3	upstream	41982343	rs10510732	A	G	0.1329	0.2920	0.0196	0.1574	0.1404	0.0462	0.1971
3	upstream	41988854	rs9856633	A	G	0.1542	0.2340	0.0034	0.2378	0.1404	0.5876	0.5695

Note: MAF, minor allele (A1) frequency based on 419 patients with schizophrenia; all SNPs passed the Hardy-Weinberg equilibrium test ($p > 0.01$); *P*, *p*-value for SNPs associated with the time to discontinuation in an antipsychotic trial; *P*_olanz, *P*_perph, *P*_quet, *P*_zipra, *P*_risp are *p*-value for response to olanzapine, perphenazine, quetiapine, ziprasidone, and risperidone, respectively.

Table 3. Cox proportional hazard model estimated the hazard ratio of discontinued use of antipsychotics in patients with schizophrenia.

SNP	Genotype	*N*	Beta	SE	*P*	HR	95% CL		*P* *
							Low	Upper	
rs2030431	TT (ref)	412	0			1			0.0003
	TC	5	2.85	0.78	0.0003	17.25	3.75	79.74	
rs7651623	GG (ref)	415	0			1			0.0005
	GA	4	2.79	0.8	0.0005	16.33	3.39	78.10	
rs2272007 **	CC (ref)	71	0			1			0.0034
	CT	20	1.44	0.49	0.0034	4.22	1.62	11.03	
	TT	4	−1.07	0.72	0.1385	0.34	0.08	1.41	

Note: * *P* value for the overall genotypic association; ** only for patients treated with olanzapine, an atypical antipsychotic; ref: reference genotype group.

It is interesting to mention that previous studies have shown multiple *ULK4* functional variants including rs2272007 associated with multiple myeloma and blood pressure at genome-wide significance. A meta-analysis of 19 studies (n = 29,378) identified rs1717027 associated with diastolic blood pressure [19], which is in strong LD (r^2 > 0.90 and D' = 1) with two non-synonymous SNPs, rs1716975 and rs2272007 that we found here associated with autism. The missense variants rs1052501 and rs2272007 (r^2 = 0.98 and D' = 1) in *ULK4* have been associated with multiple myeloma at genome-wide significance [20]. In addition, genome-wide association studies also identify that genetic variants at *ULK4* are associated with blood pressure trait and hypertension in both European ancestry and Han Chinese populations [21,22]. The SNP rs2272007 had stronger *cis*-association with mRNA expression of *ULK4* in the tissue sample of the artery and whole blood, providing molecular evidence for these genetic associations of *ULK4* with both diastolic blood pressure and multiple myeloma. These four SNPs at *ULK4* are in strong or complete pairwise LD in the CEU (r^2 > 0.909 and D' = 1), YRI (r^2 > 0.8 and D' = 1), and CHBJPT (r^2 = 1 and D' = 1) sample of the 1000 Genomes, which correspond to European ancestry, African and Asian population, respectively.

We do not know the exact biological mechanisms that *ULK4* may implicate in the development of neurodevelopment disorders like autism. *ULK4* and related genes are involved in cell cycle progression, which influences cell apoptosis and tumorigenesis [20], especially in the nervous system [32]. Autism risk-associated allele was associated with a higher level of gene expressions in postmortem prefrontal cortex and other brain areas or human tissues including artery and whole blood. Because multiple SNPs at *ULK4* were associated with multiple myeloma, this suggests that the associated SNPs may result in gain-of-function. In addition, *ULK4* deficiency mice are shown to have a significant decrease in thickness and superficial layers of the cortex, and this phenomenon was a uniform distribution in six sections of frontal cortex [16]. As neurons in six layers are generated from subventricular zone (SVZ) progenitors, *ULK4* deficiency mice show reduction state of neural progenitors in the S phase, which is pivotal for DNA synthesis, and then influence normal progress of

cell cycle. The principal role of G2 in the cell cycle is RNA and microtubules synthesis and it provides raw materials for spindle fiber assembly. Both *in vivo* and *in vitro the ULK4* gene is highly expressed in G2/M phase of neural proliferating cells [16].

AUTHOR CONTRIBUTIONS

FZ designed this study. The clinical genetic data was from previous genome-wide association study of autism designed by KX, JZ and FZ, in which the Han Chinese cohort was designed KX and JZ and carried out by HG, ZH and JO; FZ obtained three cohorts of European ancestry samples (with Dr. David St Clair), gene expression of human brain samples and antipsychotics treatment response dataset. FZ, OJ, HG performed data analysis; JO, FZ and KL drafted the manuscript and all authors contribute to the manuscript significantly.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th ed. Washington (DC, US): American Psychiatric Association Publishing; 2000.
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington (VA, US): American Psychiatric Association Publishing; 2013.
3. Lai MC, Lombardo MV, Baron-Cohen S. Autism. Lancet. 2014;383(9920):896-910.
4. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years—Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. MMWR Surveill Summ. 2018;67(6):1-23.
5. Hallmayer J, Glasson EJ, Bower C, Petterson B, Croen L, Grether J, et al. On the twin risk in autism. Am J Hum Genet. 2002;71(4):941-6.
6. Devlin B, Scherer SW. Genetic architecture in autism spectrum disorder. Curr Opin Genet Dev. 2012;22(3):229-37.
7. Klei L, Sanders SJ, Murtha MT, Hus V, Lowe JK, Willsey AJ, et al. Common genetic variants, acting additively, are a major source of risk for autism. Mol

Autism. 2012;3(1):9.
8. Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, Lee AB, et al. Most genetic risk for autism resides with common variation. Nat Genet. 2014;46(8):881-5.
9. Xia K, Guo H, Hu Z, Xun G, Zuo L, Peng Y, et al. Common genetic variants on 1p13.2 associate with risk of autism. Mol Psychiatry. 2014;19(11):1212-9.
10. Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, et al. Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature. 2009;459(7246):528-33.
11. Torrico B, Chiocchetti AG, Bacchelli E, Trabetti E, Hervas A, Franke B, et al. Lack of replication of previous autism spectrum disorder GWAS hits in European populations. Autism Res. 2017;10(2):202-11.
12. Grove J, Ripke S, Als TD, Mattheisen M, Walters RK, Won H, et al. Identification of common genetic risk variants for autism spectrum disorder. Nat Genet. 2019;51(3):431-44.
13. Ou J, Liu R, Shen Y, Xia K, Zhao J. An Overview of Genetic and Environmental Risk of Autism Spectrum Disorder. Glob Clin Transl Res. 2019;1(1):37-44.
14. Xia L, Xia K, Weinberger DR, Zhang F. Common genetic variants shared among five major psychiatric disorders: a large-scale genome-wide combined analysis. Glob Clin Transl Res. 2019;1(1):21-30.
15. Lang B, Pu J, Hunter I, Liu M, Martin-Granados C, Reilly TJ, et al. Recurrent deletions of ULK4 in schizophrenia: a gene crucial for neuritogenesis and neuronal motility. J Cell Sci. 2014;127(Pt 3):630-40.
16. Liu M, Guan Z, Shen Q, Flinter F, Dominguez L, Ahn JW, et al. Ulk4 Regulates Neural Stem Cell Pool Stem Cells. 2016;34(9):2318-31.
17. International Schizophrenia Consortium. Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature. 2008;455(7210):237-41.
18. Shi Y, Li Z, Xu Q, Wang T, Li T, Shen J, et al. Common variants on 8p12 and 1q24.2 confer risk of schizophrenia. Nat Genet. 2011;43(12):1224-7.
19. Franceschini N, Fox E, Zhang Z, Edwards TL, Nalls MA, Sung YJ, et al. Genome-wide association analysis of blood-pressure traits in African-ancestry individuals reveals common associated genes in African and non-African populations. Am J Hum Genet. 2013;93(3):545-54.
20. Broderick P, Chubb D, Johnson DC, Weinhold N, Forsti A, Lloyd A, et al. Common variation at 3p22.1 and 7p15.3 influences multiple myeloma risk. Nat Genet. 2011;44(1):58-61.
21. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet. 2009;41(6):677-87.
22. Lu X, Wang L, Lin X, Huang J, Charles Gu C, He M, et al. Genome-wide association study in Chinese identifies novel loci for blood pressure and hypertension. Hum Mol Genet. 2015;24(3):865-74.
23. Chen C, Shen YD, Xun GL, Cai WX, Shi LJ, Xiao L, et al. Aggressive behaviors and treatable risk factors of preschool children with autism spectrum disorder. Autism Res. 2017;10(6):1155-62.
24. Anney R, Klei L, Pinto D, Regan R, Conroy J, Magalhaes TR, et al. A genome-

wide scan for common alleles affecting risk for autism. Hum Mol Genet. 2010;19(20):4072-82.

25. Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, et al. Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron. 2011;70(5):863-85.
26. Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med. 2005;353(12):1209-23.
27. Sullivan PF, Lin D, Tzeng JY, van den Oord E, Perkins D, Stroup TS, et al. Genomewide association for schizophrenia in the CATIE study: results of stage 1. Mol Psychiatry. 2008;13(6):570-84.
28. Colantuoni C, Lipska BK, Ye TZ, Hyde TM, Tao R, Leek JT, et al. Temporal dynamics and genetic control of transcription in the human prefrontal cortex. Nature. 2011;478(7370):519-23.
29. Aguet F, Brown AA, Castel SE, Davis JR, He Y, Jo B, et al. Genetic effects on gene expression across human tissues. Nature. 2017;550(7675):204-13.
30. Apud JA, Zhang F, Decot H, Bigos KL, Weinberger DR. Genetic variation in KCNH2 associated with expression in the brain of a unique hERG isoform modulates treatment response in patients with schizophrenia. Am J Psychiatry. 2012;169(7):725-34.
31. Posey DJ, Stigler KA, Erickson CA, McDougle CJ. Antipsychotics in the treatment of autism. J Clin Invest. 2008;118(1):6-14.
32. Dominguez L, Schlosser G, Shen S. Expression of a novel serine/threonine kinase gene, *Ulk4*, in neural progenitors during Xenopus laevis forebrain development. Neuroscience. 2015;290:61-79.

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