A Study of Genetic Variants in Genes of Glutamate Signaling and Risk of Childhood Autism Spectrum Disorder

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Abstract

Background: Dysfunction of glutamate signaling has been implicated in the etiology of autism spectrum disorder (ASD). This case-control study was to examine the association between childhood ASD and single nucleotide polymorphisms (SNPs) in genes of the glutamate signaling pathway in a Chinese Han population.

Methods: A total of 12 SNPs in the SLC1A1, SLC25A12, GRM7 and GRM8 genes were examined. The Children Autism Rating Scale (CARS) was applied to evaluate the severity of the disease. The relationship between SNPs and the risk of ASD or the severity of the disease was determined by logistic regression.

Results: The T allele of rs2292813 in the SLC25A12 gene was significantly associated with an increased risk of ASD (odds ratio (OR) =1.7, 95% confidence interval (CI): 1.1-2.6, $P=0.0107$). Other examined SNPs were not associated with the risk of ASD. None of the SNPs examined were associated with the severity of ASD.

Conclusions: Our findings support the involvement of SNPs in the SLC25A12 gene, but not SNPs in the SLC1A1, GRM7 and GRM8 genes, in the development of childhood ASD in the Chinese Han population.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorders with core symptoms of defects in reciprocal social interactions and language communication, repetitive and restricted behaviors or interests [1]. The onset of the disease is before the age of three. Most ASD patients need life-time support or care due to the lack of a cure for the disease. This disease brings huge economic and emotional burdens to families and society. Its increasing prevalence worldwide further emphasizes the need to develop early diagnosis and more effective treatments [1].

Genetics plays an important role in the etiology of ASD [2, 3]. A large body of genetic and genomic studies have identified a wide spectrum of genetic variants that contribute to the pathogenesis of ASD, such as single nucleotide polymorphisms (SNPs), chromosomal abnormalities, copy number of variations (CNVs) and epigenetic alterations [4, 5]. SNPs are the most common type of genetic variant and may impact individual susceptibility to diseases and sensitivity to treatments. Hence, these genetic variants have the potential to become biomarkers in predicting disease risk and response to treatments.

Glutamate is the main excitatory neurotransmitter in the central nervous system. Glutamate carriers and receptors play important roles in signaling and defects of these components are associated with ASD and other neurological diseases [6, 7]. The carrier, SLC1A1, functions to transport glutamate into cells to maintain low extracellular glutamate concentration. Homozygous SLC1A1 knockout in mice led to altered locomotor activity and age-dependent behavioral abnormalities [8]. Excessive biallelic loss of-function and missense mutations of SLC1A1 were observed in patients with ASD [9]. The carrier SLC25A12, involved in the transport of glutamate and aspartic acid into mitochondria as well as calcium

homeostasis, and is implicated in pathogenesis of ASD [10–12]. GRM7 and GRM8 are the members of the metabotropic glutamate receptors [13]. GRM7-deficient mice demonstrated an increased susceptibility to seizure and impairment in memory acquisition and spatial working memory [14]. Haploinsufficiency of GRM7 contributes to ASD and hyperactivity [15]. Microdeletion of the GRM8 gene was associated with attention deficit hyperactivity disorder [16, 17].

The correlation between SNPs in SLC1A1, SLC25A12, GRM7 and GRM8 genes has been reported in previous studies with inconsistent results [18, 19] [20–23]. It is noted that most studies investigated these genes individually. It is still unknown whether these SNPs have an additive effect in predicting the risk of ASD. Our current case-control study was to examine multiple SNPs in these genes and determine their correlation with ASD and the disease severity in a Chinese Han Population.

Patients And Methods

All autistic children and age- and gender-matched healthy children from the Chinese Han population were recruited from September 2012 to November 2017. A total of 249 cases and 353 controls were enrolled. There were no significant difference in mean age and ratio of gender between the two groups [24]. Based on the scores of Childhood Autism Rating Scale (CARS), 133 children were classified as mildly/moderately (score < 36) affected and 116 as severely (score $\geq$ 36) affected. This study was approved by the Medical Ethics Committee of Zhejiang Xiaoshan Hospital. Informed consent was obtained from parents or guardians of all children.

Using a TaqMan probe-based real-time PCR approach, the genotypes of select SNPs were examined in DNA extracted from fasting blood samples. TaqMan probes were designed and synthesized by Applied Biosystems (Beijing, China). Real-time PCR was conducted following the manufacturer's protocol as described previously [25]. A total of 12 SNPs were analyzed: SLC1A1 rs301979, rs301430, rs3780412 and rs301443; SLC25A12 rs2056202 and rs2292813; GRM7 SNPrs779867 and rs6782011; GRM8 rs1800656, rs712723, rs2237731 and rs17862331. The genetic information of these SNPs is listed in Table 1.

Table 1
Genetic information of SNPs examined.

SNP	Gene	Chromosome	Genotypes	Region	mRNA
rs301979	SLC1A1	9	G/C	Intron 10	NM_004170
rs301430	SLC1A1	9	T/C	Exon 10	NM_004170
rs3780412	SLC1A1	9	T/C	Intron 7	NM_004170
rs301443	SLC1A1/SPATA6L	9	C/G	Inter-gene	NA
rs2056202	SLC25A12	2	T/C	Intron 3	NM_003705
rs2292813	SLC25A12	2	T/C	Intron 16	NM_003705
rs779867	GRM7	3	T/C	Intron 5	NM_000844
rs6782011	GRM7	3	C/T	Intron 6	NM_000844
rs1800656	GRM8	7	G/A	Downstream	NA
rs17862331	GRM8	7	T/G	Upstream	NA
rs712723	GRM8	7	A/G	3'UTR	NM_000845
rs2237731	GRM8	7	G/A	Intron 8	NM_000845
NA: not applicable; 3'UTR: 3' untranslated region					

The χ^2 analysis was used to determine whether the genotypic distributions of SNPs satisfied the Hardy-Weinberg equilibrium. Logistic regression analysis was used to determine whether genotypes or alleles of SNPs were correlated with the risk of childhood ASD or disease severity. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. All data were analyzed using SAS software V9.3 (SAS Institute Inc., Cary, NC). A P value < 0.05 was considered to be statistically significant.

Results

In both autistic children and healthy controls, genotypic distributions of examined SNPs were all in line with the Hardy-Weinberg genetic equilibrium (Table 2).

Table 2. Hardy-Weinberg equilibrium tests for SNPs in case and control groups.

SNP	Cases	Control
rs301979	0.8692	0.8077
rs301430	0.6831	0.8087
rs3780412	0.0579	0.2705
rs301443	0.9659	0.6063
rs2056202	0.1568	0.6054
rs2292813	0.3177	0.6009
rs779867	0.7459	0.5896
rs6782011	0.4962	0.0573
rs1800656	0.3277	0.9123
rs712723	0.8973	0.3018
rs2237731	0.3433	0.9367
rs17862331	0.3461	0.3566

Logistic regression analyses showed that the T allele of rs2292813 on SLC25A2 was significantly associated with an increased risk of ASD (OR = 1.7, 95% CI = 1.1–2.6, P = 0.0107), and genotypes of two SNPs rs2056202 and rs2292813 had a trend of association with the risk of ASD (Table 3). Neither the genotypes nor allele distributions of other SNPs were significantly associated with risk of childhood ASD (Table 3).

Table 3
Correlation between genotypes and allele frequencies of SNPs and childhood ASD.

SNPs	Genotype/ Allele	Cases n (%)	Controls n (%)	OR (95% CI)	Pvalue
rs301979	C/C	135 (54.2)	181 (57.8)	1	
	C/G	96 (38.6)	113 (36.1)	1.1 (0.8–1.6)	0.4681
	G/G	18 (7.2)	19 (6.1)	1.3 (0.6–2.5)	0.4920
	C	366 (73.5)	475 (75.9)	1	
	G	132 (26.5)	151 (24.1)	1.1 (0.9–1.5)	0.3603
rs301430	C/C	98 (39.3)	119 (38.1)	1	
	C/T	114 (45.8)	149 (47.8)	0.9 (0.6–1.3)	0.6904
	T/T	37 (14.9)	44 (14.1)	1.0 (0.6–1.7)	0.9361
	C	310 (62.3)	387 (62.0)	1	
	T	188 (37.7)	237 (38.0)	1.0 (0.8–1.3)	0.9372
rs3780412	T/T	129 (52.7)	172 (57.0)	1	
	T/C	89 (36.3)	107 (35.4)	1.1 (0.8–1.6)	0.5755
	C/C	27 (11.0)	23 (7.6)	1.5 (0.9–2.9)	0.1441
	T	347 (70.8)	451 (74.7)	1	
	C	143 (29.2)	153 (25.3)	1.2 (0.9–1.6)	0.1540
rs301443	C/C	67 (26.9)	79 (25.2)	1	
	C/G	124 (49.8)	161 (51.4)	0.9 (0.6–1.4)	0.6371
	G/G	58 (23.3)	73 (23.3)	0.9 (0.6–1.5)	0.7867
	C	258 (51.8)	319 (51.0)	1	
	G	240 (48.2)	307 (49.0)	1.0 (0.8–1.2)	0.7773
rs2056202	C/C	194 (78.2)	256 (81.8)	1	
	C/T	48 (19.4)	55 (17.6)	1.2 (0.7–1.8)	0.5196
	T/T	6 (2.4)	2 (0.6)	4.0 (0.8–19.8)	0.0942
	C	436 (87.9)	567 (90.6)	1	
	T	60 (12.1)	59 (9.4)	1.3 (0.9–1.9)	0.1498
rs2292813	C/C	196 (78.7)	269 (85.9)		

SNPs	Genotype/ Allele	Cases n (%)	Controls n (%)	OR (95% CI)	Pvalue
	T/C	48 (19.3)	43 (13.7)	1.5 (1.0-2.4)	0.0637
	T/T	5 (2.0)	1 (0.3)	6.9 (0.8–59.2)	0.0798
	C	440 (88.4)	581 (92.8)	1	
	T	58 (11.6)	45 (7.2)	1.7 (1.1–2.6)	0.0107
	C/C	79 (32.1)	107 (35.0)	1	
rs779867	C/T	123 (50.0)	144 (47.1)	1.2 (0.8–1.7)	0.4491
	T/T	44 (17.9)	55 (17.9)	1.1 (0.7–1.8)	0.7490
	C	281 (57.1)	358 (58.5)	1	
	T	211 (42.9)	254 (41.5)	1.1 (0.8–1.3)	0.6644
rs6782011	C/C	190 (76.3)	223 (71.9)	1	
	C/T	51 (20.5)	78 (25.2)	0.7 (0.5–1.1)	0.1949
	T/T	8 (3.2)	9 (2.9)	1.0 (0.4–2.8)	0.9319
	G	431 (86.6)	524 (84.5)	1	
	A	67 (13.4)	96 (15.5)	0.8 (0.6–1.2)	0.3053
rs1800656	G/G	100 (40.6)	111 (35.5)	1	
	G/A	108 (43.9)	150 (47.9)	0.8 (0.6–1.2)	0.2304
	A/A	38 (15.5)	52 (16.6)	0.8 (0.5–1.3)	0.4101
	G	308 (62.6)	372 (59.4)	1	
	A	184 (37.4)	254 (40.6)	0.9 (0.7–1.1)	0.2802
rs712723	A/A	102 (41.0)	104 (35.0)	1	
	A/G	114 (45.8)	150 (50.5)	0.8 (0.5–1.2)	0.1720
	G/G	33 (13.2)	43 (14.5)	0.8 (0.5–1.3)	0.3639
	A	318 (63.9)	358 (60.3)	1	
	G	180 (36.1)	236 (39.7)	0.9 (0.7–1.1)	0.2131
rs2237731	G/G	95 (38.6)	105 (33.8)	1	
	G/A	110 (44.7)	152 (48.9)	0.8 (0.6–1.2)	0.2374
	A/A	41 (16.7)	54 (17.4)	0.8 (0.5–1.4)	0.4847

SNPs	Genotype/ Allele	Cases n (%)	Controls n (%)	OR (95% CI)	<i>P</i> value
rs17862331	G	300 (61.0)	362 (58.2)	1	0.3416
	A	192 (39.0)	260 (41.8)	0.9 (0.7–1.1)	
	T/T	220 (88.7)	282 (90.1)	1	0.5953
	T/G	28 (11.3)	31 (9.9)	1.2 (0.7-2.0)	
	T	468 (94.4)	595 (95.1)	1	
	G	28 (5.6)	31 (4.9)	1.1 (0.6–1.9)	0.6057

The relationship between these SNPs and childhood ASD was further analyzed using dominant and recessive models. Our study showed that the C/C genotype was associated with significantly reduced risk compared to other genotypes (OR = 0.6, 95% CI = 0.4–0.9, *P* = 0.0252). No other SNPs were significantly associated with the risk of childhood ASD in dominant and recessive models (Table 4).

Table 4
Correlation between genotypes and allele frequencies of SNPs and childhood ASD
under the dominant and recessive models of inheritance.

SNP	Genotype/ Allele	Cases n (%)	Controls n (%)	OR (95% CI)	Pvalue
rs301979	C/C + C/G	231 (92.8)	294 (93.9)	1	
	G/G	18 (7.2)	19 (6.1)	1.2 (0.8–1.6)	0.3915
	G/G + G/C	114 (45.8)	132 (42.2)	1	
	C/C	135 (54.2)	181 (57.8)	0.8 (0.4–1.6)	0.5826
rs301430	C/C + C/T	212 (85.1)	268 (85.9)		
	T/T	37 (14.9)	44 (14.1)	1.1 (0.7–1.7)	0.7995
	T/T + C/T	151 (60.6)	193 (61.8)	1	
	C/C	98 (39.4)	119 (38.1)	1.1 (0.7–1.5)	0.7687
rs3780412	T/T + T/C	218 (89.0)	279 (92.4)	1	
	C/C	27 (11.0)	23 (7.6)	1.5 (0.8–2.7)	0.1717
	C/C + C/T	116 (47.3)	130 (43.0)		
	T/T	129 (52.7)	172 (57.0)	0.8 (0.6–1.2)	0.3148
rs301443	C/C + G/C	191 (76.7)	240 (76.7)	1	
	G/G	58 (23.3)	73 (23.3)	1.0 (0.7–1.5)	0.9934
	G/G + CG	182 (73.1)	234 (74.8)	1	
	C/C	67 (26.9)	79 (25.2)	1.1 (0.7–1.6)	0.6536
rs2056202	C/C + C/T	242 (97.6)	311 (99.4)	1	
	T/T	6 (2.4)	2 (0.6)	3.9 (0.8–19.3)	0.1002
	T/T + C/T	54 (21.8)	57 (18.2)	1	
	C/C	194 (78.2)	256 (81.8)	0.8 (0.5–1.2)	0.2933
rs2292813	C/C + C/T	244 (98.0)	312 (99.7)	1	
	T/T	5 (2.0)	1 (0.3)	6.4 (0.7–55.1)	0.0913
	T/T + C/T	53 (21.3)	44 (14.1)	1	
	C/C	196 (78.7)	269 (85.9)	0.6 (0.4–0.9)	0.0252
rs779867	C/C + C/T	202 (82.1)	251 (82.1)	1	

SNP	Genotype/ Allele	Cases n (%)	Controls n (%)	OR (95% CI)	Pvalue
rs6782011	T/T	44 (17.9)	55 (17.9)	1.0 (0.6–1.5)	0.9787
	T/T + C/T	167 (67.9)	199 (65.0)	1	
	C/C	79 (32.1)	107 (35.0)	0.9 (0.6–1.3)	0.4809
	C/C + C/T	241 (96.8)	301 (97.1)	1	
rs1800656	T/T	T/T	8 (3.2)	1.1 (0.4–2.9)	0.8315
	T/T + C/T	59 (23.7)	87 (28.1)	1	
	C/C	190 (76.3)	223 (71.9)	1.3 (0.9–1.8)	0.2429
	G/G + G/A	208 (84.5)	261 (82.4)	1	
rs712723	A/A	38 (15.5)	52 (16.6)	0.9 (0.6–1.4)	0.7107
	A/A + G/A	146 (59.4)	202 (64.5)	1	
	G/G	100 (40.6)	111 (35.5)	1.2 (0.9–1.8)	0.2094
	A/A + A/G	216 (55.8)	254 (89.9)	1	
rs2237731	G/G	33 (13.2)	43 (14.5)	0.9 (0.6–1.5)	0.6816
	G/G + A/G	147 (59.0)	193 (65.0)	1	
	A/A	102 (41.0)	104 (35.0)	1.3 (0.9–1.8)	0.1536
	G/G + G/A	205 (83.3)	257 (82.6)	1	
	A/A	41 (16.7)	54 (17.4)	1.0 (0.6–1.5)	0.8285
	A/A + G/A	151 (61.4)	205 (66.2)	1	
	G/G	95 (38.6)	105 (33.8)	1.2 (0.9–1.7)	0.2358

Children affected with ASD were classified into mild/moderate and severe groups based on CARS scores. Logistic regression analysis showed that there was no significant association between presence of these SNPs and the severity of childhood ASD (Table 5).

Table 5
Correlation between genotypes or allele frequencies of the examined SNPs and the severity of ASD.

SNP	Genotype/ Allele	Mild-Moderate n (%)	Severe n (%)	OR (95% CI)	P value
rs301979	C/C	73 (54.9)	62 (53.5)	1	
	C/G	49 (36.8)	47 (40.5)	1.1 (0.7–1.9)	0.6492
	G/G	11 (8.3)	7 (6.0)	0.7 (0.3–2.1)	0.5740
	C	195 (73.3)	171 (73.7)	1	
	G	71 (26.7)	61 (26.3)	1.0 (0.7–1.5)	0.9659
rs301430	C/C	50 (37.6)	48 (41.4)	1	
	C/T	62 (46.6)	52 (44.8)	0.9 (0.5–1.5)	0.6246
	T/T	21 (15.8)	16 (13.8)	0.8 (0.4–1.7)	0.5519
	C	162 (60.9)	148 (63.8)	1	
	T	104 (39.1)	84 (36.2)	0.9 (0.6–1.3)	0.5559
rs3780412	T/T	70 (53.0)	59 (52.2)	1	
	T/C	50 (37.9)	39 (34.5)	0.9 (0.5–1.6)	0.7798
	C/C	12 (9.1)	15 (13.3)	1.5 (0.6–3.4)	0.3546
	T	190 (72.0)	157 (69.5)	1	
	C	74 (28.0)	69 (30.5)	1.1 (0.7–1.7)	0.5025
rs301443	C/C	39 (29.3)	28 (24.1)	1	
	C/G	69 (51.9)	55 (47.4)	1.1 (0.6–2.0)	0.7311
	G/G	25 (18.8)	33 (28.5)	1.8 (0.9–3.7)	0.0933
	C	147 (55.3)	111 (47.8)	1	
	G	119 (44.7)	121 (52.2)	1.4 (1.0–2.0)	0.0805
rs2056202	C/C	101 (75.9)	93 (80.9)	1	
	C/T	27 (20.3)	21 (18.2)	0.8 (0.4–1.6)	0.6030
	T/T	5 (3.8)	1 (0.9)	0.2 (0.03–1.9)	0.1670
	C	229 (86.1)	207 (90.0)	1	
	T	37 (13.9)	23 (10.0)	0.7 (0.4–1.2)	0.1848

SNP	Genotype/ Allele	Mild-Moderate n (%)	Severe n (%)	OR (95% CI)	P value
rs2292813	C/C	106 (79.7)	90 (77.6)		
	T/C	24 (18.0)	24 (20.7)	1.2 (0.6–2.2)	0.6117
	T/T	3 (2.3)	2 (1.7)	0.8 (0.1–4.8)	0.7935
	C	236 (88.7)	204 (87.9)	1	
	T	30 (11.3)	28 (12.1)	1.1 (0.6–1.9)	0.7565
rs779867	C/C	48 (36.1)	31 (27.4)	1	
	C/T	59 (44.4)	64 (56.6)	1.7 (0.9-3.0)	0.0764
	T/T	26 (19.5)	18 (15.9)	1.1 (0.5–2.3)	0.8562
	C	155 (58.3)	126 (55.8)	1	
	T	111 (41.7)	100 (44.2)	1.1 (0.8–1.2)	0.6032
rs6782011	C/C	106 (79.7)	84 (72.4)	1	
	C/T	25 (18.8)	26 (22.4)	1.3 (0.7–2.4)	0.3895
	T/T	2 (1.5)	6 (5.7)	3.8 (0.7–19.2)	0.1085
	C	237 (89.1)	194 (83.6)	1	
	T	29 (10.9)	38 (16.4)	1.6 (0.9–2.6)	0.0918
rs1800656	G/G	56 (42.1)	44 (38.9)	1	
	G/A	61 (45.9)	47 (41.6)	1.0 (0.6–1.7)	0.9422
	A/A	16 (12.0)	22 (19.5)	1.8 (0.8–3.7)	0.1465
	G	173 (65.0)	135 (59.7)	1	
	A	93 (35.0)	91 (40.3)	1.2 (0.9–1.8)	0.2260
rs712723	A/A	59 (44.4)	43 (37.1)	1	
	A/G	59 (44.4)	55 (47.4)	1.3 (0.7–2.2)	0.3699
	G/G	15 (11.2)	18 (15.5)	1.6 (0.7–3.6)	0.2160
	A	177 (66.5)	141 (60.8)	1	
	G	89 (33.5)	91 (39.2)	1.3 (0.9–1.8)	0.1900
rs2237731	G/G	55 (41.4)	40 (35.4)	1	
	G/A	60 (45.1)	50 (44.3)	1.1 (0.7 – 2.0)	0.2374

SNP	Genotype/ Allele	Mild-Moderate n (%)	Severe n (%)	OR (95% CI)	<i>P</i> value
rs17862331	A/A	18 (13.5)	23 (20.3)	1.8 (0.8–3.7)	0.6300
	G	170 (63.9)	130 (57.6)	1	
	A	96 (36.1)	96 (42.5)	1.3 (0.9–1.9)	0.1533
	T/T	118 (88.7)	102 (88.7)	1	
	T/G	15 (11.3)	13 (11.3)	1.0 (0.5–2.2)	0.9948
	T	251 (94.4)	217 (94.4)	1	
	G	15 (5.6)	13 (5.6)	1.0 (0.5–2.2)	0.9950

Discussion

Aberrant glutamate signaling leads to excitatory/inhibitory imbalance which is associated with the development of ASD [7, 26, 27]. This study examined SNPs in genes related to the glutamate signaling pathway and analyzed their relationships with the risk of ASD and its severity in a Chinese Han population. Our results revealed that only SNP rs2292813 in the SLC25A2 gene, but not the other SNPs, was associated with ASD. None of the SNPs examined was associated with disease severity.

A Finnish case-control study genotyped rs2228622, rs12682807, rs2072657, rs301430, rs1471786 and rs301979 of the SLC1A1 gene in 175 patients with ASD and 216 controls. Similar to our study, no association was found between these SNPs and ASD [28]. Another family-based study examined rs301430, rs301979 and rs301434 in 86 strictly defined trios. Their result revealed that the G allele of rs301979 and haplotype T-G of rs301430 -rs301979 were undertransmitted to individuals with ASD, although this was not evident after correction for multiple comparisons [29]. These results suggest that these SNPs in the SLC1A1 gene are not biomarkers for ASD.

SLC25A12 has been considered a candidate gene for the development of ASD [11, 12]. Several studies conducted one decade ago reported an association between ASD and SNPs rs2292813, rs2056202 [30–32] and rs908670 [33] in the SLC25A12 gene, though a few others found no association between ASD and these SNPs [34, 35], including one study conducted within a Han population in Taiwan [18]. Two Recent meta-analyses, including one from our group, summarized studies published before 2014 and both concluded a strong association between SNPs rs2056202 and rs2292813 and ASD [20, 36]. With a smaller sample size, we have previously demonstrated a positive association with rs2056202 and rs2292813 and the risk of ASD [22]. Findings in this study present further supportive evidence that SNP rs2292813 is associated with the risk of ASD.

Although findings from this study didn't reveal a significant association between SNPs of the GMR7 gene and childhood ASD, at least two separate groups did demonstrate a significant correlation. Yang et al [19]

utilized Affymetrix Genome-Wide Human SNP microarrays to analyze 297 SNPs in the GRM7 gene in 22 patients with ASD, 14 non-ASD patients, and 18 healthy controls from a Chinese Han population. Their study found that genotypes of rs779867 and rs6782011, and their haplotype (T-C) were statistically significantly correlated with ASD. In another population based case-control study, both rs6782011 and rs779867 were examined in 518 Iranian ASD patients and 472 control individuals and results showed that genotypes of rs779867, and haplotypes of rs779867-rs6782011 were significantly associated with ASD [21]. A recent study extracted genomic data for 487 ASD patients and 455 healthy individuals. Among the assessed SNPs, rs6782011 of GRM7 was recognized as the one of most significant risk factors related to ASD [23]. The discrepant finding in our study may be due to differences in phenotypic or genetic characteristics.

Serajee et al [37] first reported a nominally significant association of the autistic disorder with SNPs 2237731, rs712723 and rs1800656 of the GRM8 gene. In contrast, a case-control study analyzed rs1800656, rs712723, rs2237731, rs17862331 and rs17862331 of the GRM8 gene and 7 SNPs in the RFLN gene in 213 children with ASD and 160 controls in a Chinese Han population. Neither the single SNP nor the haplotype analysis showed significant association between ASD and the SNPs of the GRM8 gene [38]. Likewise, no significant association between SNPs in the GRM8 gene and ASD was observed in our study on a Chinese Han population.

Using the CARS, we assessed overall severity of disease in children with ASD. None of the SNPs examined were associated with the severity of the disease. In contrast, Gadow et al [39] reported that SNP rs301430 in the SLC1A1 gene was associated with severity of repetitive behaviors and anxiety in children with ASD. Other studies found that rs2056202 and rs2292813 in the SLC25A12 gene were associated with restricted repetitive behavior [40], and rs2056202 was associated with severity of routines and rituals [41]. These results suggest that some SNPs may be associated with severity of individual symptoms in patients with ASD.

There are several limitations of this case-control study. The sample size is still relatively moderate. All patients were from the local hospital and mental health facility and were receiving treatments. These untreated children with ASD were not included. Most of these untreated autistic children commonly have less severe symptoms. Furthermore, only a limited number of SNPs were selected. The severity of disease was only evaluated by the CARS. The individual symptoms of CARS were not analyzed.

Conclusions

Our findings support the involvement of SNPs in the SLC25A12 gene, but not SNPs in the SLC1A1, GRM7 and DRM8 genes, in the development of childhood ASD in the Chinese Han population.

Declarations

Ethical approval and consent to participate

This study was approved by the Medical Ethics Committee of Zhejiang Xiaoshan Hospital. Informed consent was obtained from parents or guardians of all children.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author (JL) upon reasonable request.

Competing interest

The authors have declared no competing interests with respect to the research, authorship, and/or publication of this article.

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Author's contributions

LJ and HY designed the study and selected the SNPs. HY and ZZ diagnosed and recruited patients and controls. LJ, AY and PH collect samples, extracted DNA and genotyped SNPs. LJ, HY and AY analyzed data and prepared the manuscript. PH and ZZ provided critical feedback and contributed to revisions. All authors read and approved the final manuscript.

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