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

Jun Liu (✉ 18967167212@163.com)  
Zhejiang Xiaoshan Hospital  https://orcid.org/0000-0003-4132-4450

Hong Yu  
hang zhou shi xiao shan qu di yi ren min yi yuan: Hangzhou Xiaoshan No 1 People's Hospital

Pingfang Hu  
Zhejiang Xiaoshan Hospital

Aiping Yang  
Zhejiang Xiaoshan Hospital

Zengyu Zhang  
hang zhou shi xiao shan qu di yi ren min yi yuan: Hangzhou Xiaoshan No 1 People's Hospital

Primary research

Keywords: Autism, SNP, glutamate, SLC1A1, SLC25A12, GRM7, GRM8, severity.

DOI: https://doi.org/10.21203/rs.3.rs-85019/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
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 (OD) =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 DRM8 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 ≥ 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 SNPs rs779867 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 $\chi^2$ 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) | P value |
|----------|-----------------|-------------|----------------|-------------|---------|
| 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)     | 1           |         |
| SNPs       | Genotype/Allele | Cases n (%) | Controls n (%) | OR (95% CI) | P value |
|------------|-----------------|-------------|----------------|-------------|---------|
|            | 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  |
| rs779867   | C/C             | 79 (32.1)   | 107 (35.0)     | 1           |         |
|            | 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) | P value |
|--------|-----------------|-------------|----------------|-------------|---------|
|        | G               | 300 (61.0)  | 362 (58.2)     | 1           |         |
|        | A               | 192 (39.0)  | 260 (41.8)     | 0.9 (0.7–1.1) | 0.3416  |
| rs17862331 | T/T          | 220 (88.7)  | 282 (90.1)     | 1           |         |
|        | T/G             | 28 (11.3)   | 31 (9.9)       | 1.2 (0.7–2.0) | 0.5953  |
|        | 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) | P value |
|---------|-----------------|-------------|----------------|-------------|---------|
| 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)       | P value |
|------------|-----------------------|-------------|----------------|-------------------|---------|
|            | 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  |
| rs6782011  | C/C + C/T             | 241 (96.8)  | 301 (97.1)     | 1                 |         |
|            | 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  |
| rs1800656  | G/G + G/A             | 208 (84.5)  | 261 (82.4)     | 1                 |         |
|            | 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  |
| rs712723   | A/A + A/G             | 216 (55.8)  | 254 (89.9)     | 1                 |         |
|            | 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  |
| rs2237731  | 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)    | 1.2 (0.6–2.2) | 0.6117  |
|          | T/C             | 24 (18.0)           | 24 (20.7)    | 0.8 (0.1–4.8) | 0.7935  |
|          | T/T             | 3 (2.3)             | 2 (1.7)      |              |         |
|          | 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) | P value |
|-------------|-----------------|---------------------|--------------|-------------|---------|
|             | 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  |
| rs17862331  | 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.

Funding

This work was supported by grants from Xiaoshan Science & Technology Commission of Hangzhou (2018220) and Health Commission of Zhejiang Province (2018ZD038).

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.

Acknowledgement

Not applicable.

References

1. Lavelle TA, Weinstein MC, Newhouse JP, Munir K, Kuhlthau KA, et al. (2014) Economic burden of childhood autism spectrum disorders. Pediatrics 133: e520-529.
2. Tick B, Bolton P, Happe F, Rutter M, Rijsdijk F (2016) Heritability of autism spectrum disorders: a meta-analysis of twin studies. J Child Psychol Psychiatry 57: 585-595.
3. Rylaarsdam L, Guemez-Gamboa A (2019) Genetic Causes and Modifiers of Autism Spectrum Disorder. Front Cell Neurosci 13: 385.
4. De Rubeis S, Buxbaum JD (2015) Genetics and genomics of autism spectrum disorder: embracing complexity. Hum Mol Genet 24: R24-31.
5. Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, et al. (2014) Most genetic risk for autism resides with common variation. Nat Genet 46: 881-885.

6. Choudhury PR, Lahiri S, Rajamma U (2012) Glutamate mediated signaling in the pathophysiology of autism spectrum disorders. Pharmacol Biochem Behav 100: 841-849.

7. Kawada K, Kuramoto N, Mimori S (2020) Possibility that the Onset of Autism Spectrum Disorder is Induced by Failure of the Glutamine-Glutamate Cycle. Curr Mol Pharmacol.

8. Peghini P, Janzen J, Stoffel W (1997) Glutamate transporter EAAC-1-deficient mice develop dicarboxylic aminoaciduria and behavioral abnormalities but no neurodegeneration. EMBO J 16: 3822-3832.

9. Doan RN, Lim ET, De Rubeis S, Betancur C, Cutler DJ, et al. (2019) Recessive gene disruptions in autism spectrum disorder. Nat Genet 51: 1092-1098.

10. Lepagnol-Bestel AM, Maussion G, Boda B, Cardona A, Iwayama Y, et al. (2008) SLC25A12 expression is associated with neurite outgrowth and is upregulated in the prefrontal cortex of autistic subjects. Mol Psychiatry 13: 385-397.

11. Napolioni V, Persico AM, Porcelli V, Palmieri L (2011) The mitochondrial aspartate/glutamate carrier AGC1 and calcium homeostasis: physiological links and abnormalities in autism. Mol Neurobiol 44: 83-92.

12. Anitha A, Nakamura K, Thanseem I, Yamada K, Iwayama Y, et al. (2012) Brain region-specific altered expression and association of mitochondria-related genes in autism. Mol Autism 3: 12.

13. Ribeiro FM, Vieira LB, Pires RG, Olmo RP, Ferguson SS (2017) Metabotropic glutamate receptors and neurodegenerative diseases. Pharmacol Res 115: 179-191.

14. Callaerts-Vegh Z, Beckers T, Ball SM, Baeyens F, Callaerts PF, et al. (2006) Concomitant deficits in working memory and fear extinction are functionally dissociated from reduced anxiety in metabotropic glutamate receptor 7-deficient mice. J Neurosci 26: 6573-6582.

15. Liu Y, Zhang Y, Zhao D, Dong R, Yang X, et al. (2015) Rare de novo deletion of metabotropic glutamate receptor 7 (GRM7) gene in a patient with autism spectrum disorder. Am J Med Genet B Neuropsychiatr Genet 168B: 258-264.

16. Asadollahi R, Oneda B, Joset P, Azzarello-Burri S, Bartholdi D, et al. (2014) The clinical significance of small copy number variants in neurodevelopmental disorders. J Med Genet 51: 677-688.

17. Sangu N, Shimojima K, Takahashi Y, Ohashi T, Tohyama J, et al. (2017) A 7q31.33q32.1 microdeletion including LRRC4 and GRM8 is associated with severe intellectual disability and characteristics of autism. Hum Genome Var 4: 17001.

18. Chien WH, Wu YY, Gau SS, Huang YS, Soong WT, et al. (2010) Association study of the SLC25A12 gene and autism in Han Chinese in Taiwan. Prog Neuropsychopharmacol Biol Psychiatry 34: 189-192.

19. Yang Y, Pan C (2013) Role of metabotropic glutamate receptor 7 in autism spectrum disorders: a pilot study. Life Sci 92: 149-153.
20. Liu J, Yang A, Zhang Q, Yang G, Yang W, et al. (2015) Association between genetic variants in SLC25A12 and risk of autism spectrum disorders: An integrated meta-analysis. Am J Med Genet B Neuropsychiatr Genet 168B: 236-246.

21. Noroozi R, Taheri M, Movafagh A, Mirfakhraie R, Solgi G, et al. (2016) Glutamate receptor, metabotropic 7 (GRM7) gene variations and susceptibility to autism: A case-control study. Autism Res 9: 1161-1168.

22. Liu J, Mo W, Zhang Z, Yu H, Yang A, et al. (2017) Single Nucleotide Polymorphisms in SLC19A1 and SLC25A9 Are Associated with Childhood Autism Spectrum Disorder in the Chinese Han Population. J Mol Neurosci 62: 262-267.

23. Ghafouri-Fard S, Taheri M, Omrani MD, Daaee A, Mohammad-Rahimi H, et al. (2019) Application of Single-Nucleotide Polymorphisms in the Diagnosis of Autism Spectrum Disorders: A Preliminary Study with Artificial Neural Networks. J Mol Neurosci 68: 515-521.

24. Yu H, Zhang Z, Liu J, Hu P, Liu Z (2020) Association study between genetic variants in vitamin D metabolism related genes and childhood autism spectrum disorder. Metab Brain Dis 35: 971-978.

25. Yu H, Liu J, Yang A, Yang G, Yang W, et al. (2016) Lack of Association Between Polymorphisms in Dopa Decarboxylase and Dopamine Receptor-1 Genes With Childhood Autism in Chinese Han Population. J Child Neurol 31: 560-564.

26. Hadley D, Wu ZL, Kao C, Kini A, Mohamed-Hadley A, et al. (2014) The impact of the metabotropic glutamate receptor and other gene family interaction networks on autism. Nat Commun 5: 4074.

27. Gao R, Penzes P (2015) Common mechanisms of excitatory and inhibitory imbalance in schizophrenia and autism spectrum disorders. Curr Mol Med 15: 146-167.

28. Kantojarvi K, Onkamo P, Vanhala R, Alen R, Hedman M, et al. (2010) Analysis of 9p24 and 11p12-13 regions in autism spectrum disorders: rs1340513 in the JMJD2C gene is associated with ASDs in Finnish sample. Psychiatr Genet 20: 102-108.

29. Brune CW, Kim SJ, Hanna GL, Courchesne E, Lord C, et al. (2008) Family-Based Association Testing of OCD-associated SNPs of SLC1A1 in an autism sample. Autism Res 1: 108-113.

30. Ramoz N, Reichert JG, Smith CJ, Silverman JM, Bespalova IN, et al. (2004) Linkage and association of the mitochondrial aspartate/glutamate carrier SLC25A12 gene with autism. Am J Psychiatry 161: 662-669.

31. Segurado R, Conroy J, Meally E, Fitzgerald M, Gill M, et al. (2005) Confirmation of association between autism and the mitochondrial aspartate/glutamate carrier SLC25A12 gene on chromosome 2q31. Am J Psychiatry 162: 2182-2184.

32. Turunen JA, Rehnstrom K, Kilpinen H, Kuokkanen M, Kempas E, et al. (2008) Mitochondrial aspartate/glutamate carrier SLC25A12 gene is associated with autism. Autism Res 1: 189-192.

33. Anney R, Klei L, Pinto D, Regan R, Conroy J, et al. (2010) A genome-wide scan for common alleles affecting risk for autism. Hum Mol Genet 19: 4072-4082.

34. Blasi F, Bacchelli E, Carone S, Toma C, Monaco AP, et al. (2006) SLC25A12 and CMYA3 gene variants are not associated with autism in the IMGSAC multiplex family sample. Eur J Hum Genet 14: 123-
35. Rabionet R, McCauley JL, Jaworski JM, Ashley-Koch AE, Martin ER, et al. (2006) Lack of association between autism and SLC25A12. Am J Psychiatry 163: 929-931.

36. Aoki Y, Cortese S (2016) Mitochondrial Aspartate/Glutamate Carrier SLC25A12 and Autism Spectrum Disorder: a Meta-Analysis. Mol Neurobiol 53: 1579-1588.

37. Serajee FJ, Zhong H, Nabi R, Huq AH (2003) The metabotropic glutamate receptor 8 gene at 7q31: partial duplication and possible association with autism. J Med Genet 40: e42.

38. Li H, Li Y, Shao J, Li R, Qin Y, et al. (2008) The association analysis of RELN and GRM8 genes with autistic spectrum disorder in Chinese Han population. Am J Med Genet B Neuropsychiatr Genet 147B: 194-200.

39. Gadow KD, Roohi J, DeVincent CJ, Kirsch S, Hatchwell E (2010) Glutamate transporter gene (SLC1A1) single nucleotide polymorphism (rs301430) and repetitive behaviors and anxiety in children with autism spectrum disorder. J Autism Dev Disord 40: 1139-1145.

40. Kim SJ, Silva RM, Flores CG, Jacob S, Guter S, et al. (2011) A quantitative association study of SLC25A12 and restricted repetitive behavior traits in autism spectrum disorders. Mol Autism 2: 8.

41. Silverman JM, Buxbaum JD, Ramoz N, Schmeidler J, Reichenberg A, et al. (2008) Autism-related routines and rituals associated with a mitochondrial aspartate/glutamate carrier SLC25A12 polymorphism. Am J Med Genet B Neuropsychiatr Genet 147: 408-410.