J Hum Genet (2007) 52:985–989
DOI 10.1007/s10038-007-0207-5

ORIGINAL ARTICLE

No evidence for significant association between GABA receptor genes in chromosome 15q11–q13 and autism in a Japanese population

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Received: 22 August 2007 / Accepted: 26 September 2007 / Published online: 24 October 2007

Abstract The γ-aminobutyric acid (GABA) receptor genes *GABRB3*, *GABRA5*, and *GABRG3* located on chromosome 15q11–q13 have been major candidates for susceptibility genes for autism, a neurodevelopmental disorder with a complex genetic etiology. In this study, we first investigated the association between the GABA receptor genes and autism in a Japanese population by analyzing 11 single nucleotide polymorphisms (SNPs). Intron 3 of *GABRB3* was densely mapped because the previous studies observed the association of the microsatellite 155CA-2 located in the region. We observed no significant difference in allelic frequencies or genotypic distributions of the 11 SNPs between patients and controls. A permutation test showed no significant global differences in estimated haplotype frequencies between patients and controls. Analysis after confining the subjects to males showed similar results. Thus, this study provides no positive evidence of an association between the GABA receptor genes and autism in a Japanese population. However, in a SNP (rs3212337) located near the microsatellite 155CA-2, a significant deviation from the Hardy–Weinberg equilibrium was observed in patients ($p = 0.029$, corrected for multiple testing). This finding may suggest further studies around the markers for more definitive conclusions.

Keywords Autism · Chromosome 15 · GABA · *GABRB3* · SNP

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Introduction

Autism is a developmental disorder characterized by three areas of abnormality: impairment in social interaction, impairment in communication, and restricted and stereotyped pattern of interest or behavior. Impairment in all three areas is observed before age 3 years, and disrupted brain growth of unknown mechanism is implicated in the etiology. Twin and family studies have indicated a robust role of genetic factors in autism development, whereas few susceptibility genes have been elucidated (Freitag 2007). Chromosome 15q11–q13 has been a focus of genetic studies of autism susceptibility due to the presence of cytogenic abnormalities of this region in autistic patients. The γ-aminobutyric acid (GABA) receptor genes *GABRB3*, *GABRA5*, and *GABRG3* located on chromosome 15q11–q13 have received considerable attention because a

decreased GABA receptor density was observed in the hippocampus of autism, and a suppressed GABAergic inhibition has been implicated in autism etiology (Blatt et al. 2001; Hussman 2001).

To our knowledge, 12 studies to date have investigated the genetic association between GABA receptor genes and autism. Two of the studies (Cook et al. 1998; Buxbaum et al. 2002) observed the association of a microsatellite located in intron 3 of *GABRB3* (155CA-2), whereas the other four studies did not replicate the association (Salmon et al. 1999; Maestrini et al. 1999; Martin et al. 2000; Curran et al. 2005). Except for 155CA-2, only nominal significant associations were observed with respect to markers located in or around these three GABA receptor genes (Martin et al. 2000; Menold et al. 2001; McCauley et al. 2004; Ashley-Koch et al. 2006). In another three studies, no significant association was observed between the genes and autism (Nurmi et al. 2001; Ma et al. 2005; Kim et al. 2006). Thus, the results were inconclusive, and further investigation may be needed to elucidate the problem. In the study reported here, we investigated the association between GABA receptor genes in chromosome 15q11–q13 and autism in Japanese case-control subjects. To our knowledge, this is the first study to investigate the association in a Japanese population.

Subjects and methods

In this study, Japanese patients and control subjects around Tokyo, Japan, were recruited: 166 unrelated patients (147 males and 19 females; age 19.9 ± 9.8 years, mean ± SD) with autistic disorder diagnosed by the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria and 412 unrelated healthy volunteers (136 males and 276 females; age 36.0 ± 11.5 years). Patient diagnosis was confirmed by two experienced child psychiatrists independently through semistructured behavior observation and parent interview. At the interview, the Child Behavior Questionnaire Revised (Izutsu et al. 2001) was used to assist the evaluation of autism-specific behaviors and symptoms. After the initial observation and interview, patients were followed up for 6 months to confirm the diagnosis. In order to exclude other genetic syndromes, we performed standard karyotyping and fragile X testing for the trinucleotide repeat expansion in the *FMR-1* gene (Chong et al. 1994). The objective of our study was clearly explained, and written informed consent was obtained from all parents. The consent was also obtained from the patients when they were able to follow the explanation. The study was approved by the Ethical Committee of the Faculty of Medicine, the University of Tokyo.

Genomic DNA was extracted from leukocytes by using the standard phenol-chloroform method. We genotyped 11 single nucleotide polymorphisms (SNPs), as detailed in Table 1. Intron 3 of *GABRB3* was densely mapped because the previous studies observed the association of the microsatellite 155CA-2 located in the region (Cook et al. 1998; Buxbaum et al. 2002). The 155CA-2 is located between SNP4 and SNP5 in our study at chromosome position 5170956. All SNPs were analyzed using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, CA, USA). The chi-square test was used to compare SNP frequencies between patients and controls. Lewontin's D' was used to analyze pairwise linkage disequilibrium (LD) (Lewontin 1964). Haplotype block analysis was conducted with the Gabriel and the Four Gamete methods (Gabriel et al. 2002; Wang et al. 2002). SNP haplotypes and their frequencies were estimated by

Table 1 Allelic frequencies of 11 single nucleotide polymorphisms (SNPs) in the *GABR* genes

SNPs	db SNP ID	Location	Alleles (major/minor)	Minor allele frequency			Chromosome position (bp)
				Autism[a]	Control[a]	*p* value	
SNP1	rs11637141	*GABRB3* (3′-UTR)	C/T	0.042 (166)	0.055 (397)	0.36	4954325
SNP2	rs890317	*GABRB3* (intron 3)	A/C	0.49 (164)	0.47 (409)	0.60	5084462
SNP3	rs2059574	*GABRB3* (intron 3)	T/A	0.38 (166)	0.37 (407)	0.95	5159328
SNP4	rs11161335	*GABRB3* (intron 3)	A/T	0.35 (160)	0.34 (358)	0.74	5166381
SNP5	rs3212337	*GABRB3* (intron 3)	C/T	0.39 (166)	0.38 (399)	0.98	5173373
SNP6	rs8179184	*GABRB3* (5′ upstream)	C/T	0.38 (165)	0.38 (407)	0.89	5181451
SNP7	rs140682	*GABRA5* (exon 8)	C/T	0.31 (166)	0.35 (406)	0.19	5343538
SNP8	rs140685	*GABRA5* (exon 10)	C/T	0.32 (166)	0.37 (409)	0.14	5349640
SNP9	rs4887536	*GABRG3* (intron 3)	A/C	0.42 (166)	0.48 (411)	0.073	5508122
SNP10	rs28564251	*GABRG3* (intron 5)	G/A	0.40 (165)	0.35 (348)	0.16	5738611
SNP11	rs4778109	*GABRG3* (intron 5)	G/A	0.38 (164)	0.36 (409)	0.62	5884510

[a] Number of genotyped individuals for each SNP is given in parenthesis

the maximum likelihood method with an expectation-maximization algorithm (Excoffier and Slatkin 1995). Permutation p values were calculated in comparison with haplotype frequencies between patients and controls (Fallin et al. 2001). The SNPAlyze 5.1 standard software (DYNACOM, Japan) was used to conduct LD, haplotype block, and haplotype analyses.

Results

Table 1 shows allelic frequencies of the 11 SNPs in patients and controls. The distributions of all 11 SNPs follow the Hardy–Weinberg equilibrium in controls. In patients, however, distributions of SNPs 5, 6, and 9 significantly deviated from the Hardy–Weinberg equilibrium ($p = 0.0026$, 0.018, and 0.035, respectively), whereas distributions of the other eight polymorphisms were within the values expected from the Hardy–Weinberg equilibrium. No significant difference was observed in allelic frequencies of the 11 SNPs between the patients and controls. In genotypic distributions, there were significant differences between patients and controls in SNP5 (major homo/hetero/minor homo = 0.32/0.59/0.09 vs. 0.38/0.47/0.15, respectively, $\chi^2 = 6.65$, $df = 2$, $p = 0.036$ in codominant model) and SNP9 (0.30/0.57/0.13 vs. 0.26/0.52/0.22, respectively, $\chi^2 = 5.37$, $df = 1$, $p = 0.021$ in dominant model for major allele). No significant difference was observed in genotypic distributions of the other nine SNPs between patients and controls. Analysis after confining the subjects to males showed no significant difference between patients and controls in allelic frequencies or genotypic distributions of the 11 SNPs (data not shown).

LD strength denoted as D' between pairs of the SNPs is shown in Table 2. Two haplotype blocks, SNPs 3–4 and 7–8, were suggested by the Gabriel and Four Gamete methods of haplotype-block analysis (Gabriel et al. 2002; Wang et al. 2002). In those analyses, no significant difference was observed in frequencies of any estimated haplotype or in distributions of all estimated haplotypes between patients and controls. Similar results were obtained in LD, haplotype block, and haplotype analyses confining the subjects to males (data not shown).

Discussion

In this study, we first investigated the association between the three GABA receptor genes, *GABRB3*, *GABRA5*, and *GABRG3*, and autism in a Japanese population. Nominal significant differences were observed between patients and controls in genotypic distributions of SNPs 5 and 9, although the statistical levels became insignificant after Bonferroni correction. A permutation test showed no significant global difference in estimated haplotype frequencies between patients and controls. Analysis after confining the subjects to males showed similar results. Thus, this study provides no positive evidence of the association between GABA receptor genes and autism in a Japanese population.

Distributions of SNPs 5, 6, and 9 significantly deviated from Hardy–Weinberg equilibrium in patients but not in controls. Statistical levels of deviations in SNPs 6 and 9 became insignificant after Bonferroni correction; however, that in SNP5 was significant (corrected $p = 0.029$). This could suggest an association of SNP5 with autism, although it might be a chance observation. Also, a possibility of population stratification in the sample may not be completely ruled out. SNP5 is located in intron 3 of *GABRB3*, 2.4 kb telomeric from the microsatellite 155CA-2, which was suggested to be associated with autism in previous studies (Cook et al. 1998; Buxbaum et al. 2002). It

Table 2 Linkage disequilibrium (LD) strength between single nucleotide polymorphisms (SNP) pairs in patients and controls

	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11
SNP1		0.49	0.51	0.22	1.00	0.51	0.38	0.47	0.04	0.43	0.09
SNP2	0.42		0.05	0.08	0.13	0.05	0.14	0.15	0.08	0.04	0.02
SNP3	0.25	0.03		0.89	0.88	0.92	0.09	0.05	0.14	0.15	0.10
SNP4	0.07	0.22	0.94		0.93	0.96	0.05	0.07	0.12	0.29	0.17
SNP5	0.21	0.01	0.88	0.97		0.96	0.13	0.14	0.12	0.07	0.06
SNP6	0.23	0.03	0.94	0.98	0.93		0.07	0.08	0.14	0.13	0.07
SNP7	0.15	0.10	0.11	0.02	0.09	0.10		0.97	0.30	0.19	0.04
SNP8	0.11	0.11	0.11	0.04	0.07	0.07	0.99		0.24	0.16	0.09
SNP9	0.15	0.07	0.02	0.09	0.04	0.06	0.12	0.10		0.06	0.09
SNP10	0.29	0.06	0.11	0.05	0.09	0.08	0.07	0.07	0.05		0.12
SNP11	0.14	0.03	0.13	0.01	0.08	0.09	0.03	0.06	0.06	0.01	

LD strength is denoted as D'. D' values for patients are shown in the upper diagonal, and those for controls are shown in the lower diagonal

may be interesting to further investigate the region around SNP5 or the microsatellite, although no evidence for a significant association was obtained in our study.

Statistical power of this study was 0.77 ($\alpha = 0.05$) when assuming that the prevalence of autism is 0.21% in a Japanese population (Honda et al. 1996), genotypic relative risk is 1.8 (dominant model), and marker allele frequency is 0.1. Thus, our results might have adequate statistical power to detect the effect of the gene, with odds ratios of approximately 1.8 or more, although smaller effects might not have been detected. Caution may be advised for controls because they were not age-matched to patients. However, this likely may not significantly affect the results, considering no major effect of environmental factors in autism (Folstein and Rosen-Sheidley 2001). Imbalance in sex ratio between patients and controls may be overcome by analysis confining subjects to males, considering autism's higher prevalence in males than in females.

In conclusion, no significant association was observed between GABA receptor genes on chromosome 15q11–q13 and autism in the Japanese subjects. However, the significant deviation from Hardy–Weinberg equilibrium in SNP5 might suggest further search for a susceptibility variant around microsatellite 155CA-2.

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