Possible association of *CUX1* gene polymorphisms with antidepressant response in major depressive disorder

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Abstract

Association between response to antidepressant treatment and genetic polymorphisms was examined in two independent Japanese samples of patients with major depressive disorder (MDD). Genome-wide approach using the Illumina Human CNV370-quad Bead Chip was utilized in the analysis of the 92 MDD patients in the first sample. Eleven non-intergenic SNPs with uncorrected allelic *P* value < 0.0001 were selected for the subsequent association analyses in the second sample of 136 MDD patients. Difference in allele distribution between responders and nonresponders were found in the second stage sample for rs365836 and rs201522 of the *CUX1* gene (*P* = 0.005 and 0.004, respectively). The allelic *P* values for rs365836 and rs201522 in both samples combined were 0.0000023 and 0.0000040, respectively. Our results provide the first evidence that polymorphisms of the *CUX1* gene may be associated with response to antidepressant treatment in Japanese patients with MDD.

Keywords: antidepressant; genome-wide association study; major depressive disorder; *CUX1*

Introduction

Response to antidepressant treatment varies markedly between individuals. The genetic predictors of treatment response have been intensively searched for in recent years. However, previous pharmacogenetic studies on antidepressant response have not yielded consistent results.

Three previous studies have implemented genome-wide approaches to detect single nucleotide polymorphisms (SNPs) associated with antidepressant response. Garriock et al [1] identified SNPs associated with response to citalopram near the Ubiquitin protein ligase E3C (*UBE3C*) gene ($P = 4.65\times10^{-7}$) and the Bone morphogenic protein 7 (*BMP7*) gene ($P = 3.45\times10^{-6}$). Ising et al [2] reported a SNP in the Cadherin-17 gene (*CDH17*) to be associated with early partial response ($P = 7.6\times10^{-7}$). The Genome-based Therapeutic Drugs for Depression study (GENDEP) [3], which was specifically designed for pharmacogenetic investigation, reported a SNP in the Uronyl 2-sulphotransferase gene (*UST*) associated with nortriptyline response ($P = 3.56\times10^{-8}$) and a SNP in the interleukin-11 gene (*IL11*) associated with escitalopram response ($P = 2.83\times10^{-6}$).

The concordance to antidepressant response in members of the same family [4] suggests a genetic component. However, the published genome-wide association studies (GWAS) failed to identify SNPs consistently associated with antidepressant response. In the present study, a genome-wide approach was implemented for selecting the candidate SNPs associated with antidepressant response. Although the small GWAS sample was not appropriate for the purpose of detecting the genome-wide significance, the results of the GWAS were employed to narrow down the candidate polymorphisms for the subsequent analysis using an independent sample.

Materials and methods

Subjects

The first-stage GWAS sample consisted of 92 patients with major depressive disorder (MDD) that completed 8 weeks of antidepressant treatment. Subjects were recruited from the outpatient clinics in Tokyo, Japan. The second sample for the candidate SNP analysis consisted of 136 patients with MDD that completed 8 weeks of antidepressant treatment and were recruited from the outpatient clinics in Kyushu, Japan.

All subjects in the first and the second sample were biologically unrelated Japanese individuals. Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition criteria [5], on the basis of unstructured interviews and information from medical records. Participants were excluded if they had prior medical histories of central nervous system diseases or severe head injury or if they met the criteria for substance abuse or dependence or mental retardation. The study protocol was approved by the institutional ethics committees. After describing the study, written informed consent was obtained from every subject.

The severity of depression was assessed by trained psychiatrists by use of the Japanese version of the GRID Hamilton Rating Scale for Depression (HAM-D), 17-item version [6], which has been demonstrated to show excellent inter-rater reliability [7]. Patients with HAM-D score ≥ 15 were enrolled in the study. All patients were treated with a single antidepressant medication. The patients in the first sample were prescribed one of the following antidepressants: paroxetine, fluvoxamine, nortriptyline,or milnacipran. The patients in the second sample were prescribed either

paroxetine or sertraline. No concomitant psychotropic medications were allowed aside from benzodiazepines and hypnotics. Responders were defined as those whose HAM-D score on their 8-week clinical visit showed ≦ 50% reduction compared to baseline.

Genotyping of the genome-wide scan

Genomic DNA was prepared from the venous blood according to standard procedures. A total of 92 samples were genotyped using the Illumina Human 370-quad bead chip (Illumina, Inc., San Diego, CA, USA). A total of 356 075 autosomal SNPs were assessed for quality. SNPs were excluded if the call rate < 95 %, minor allele frequency < 0.01, or the deviation from Hardy-Weinberg equilibrium (HWE) was at an error level of $P < 0.001$. The remaining 291 512 SNPs were available for analysis. The total genotyping rate was 99.8%.

Candidate SNP selection

$P = 0.0001$ was used for the cut-off value of the first stage GWAS. Assuming a minor allele frequency (MAF) of 0.3 in nonresponders and a relative risk of 1.5 of the

minor allele being the risk allele of being a responder, the power to detect association was 11.4%. Assuming the same MAF and a relative risk of 2.0, the power increased to 62.8%. Intergenic SNPs were defined as SNPs that are located at least 0.5kb 3' to or 2kb 5' to a gene included in the HapMap data set release 28 (http://hapmap.ncbi.nlm.nih.gov/). All 11 non-intergenic SNPs with *P* values below the cut-off $P = 0.0001$ were included in the subsequent second-stage analysis.

Genotyping of the second stage sample

Genomic DNA of 136 subjects was prepared from venous blood according to standard procedures. The SNPs were genotyped using the TaqMan 5'-exonuclease allelic discrimination assay. Thermal cycling conditions for polymerase chain reaction were 1 cycle at 95°C for 10 minutes followed by 50 cycles of 92°C for 15 seconds and 60°C for 1 minute. The allele-specific fluorescence was measured with ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster city, CA, USA). Genotype data were read blind to the case-control status. Ambiguous genotype data were not included in the analysis. In 3 subjects, none of the SNPs were successfully genotyped

and thus were excluded from the subsequent analyses. The call rate for each SNP ranged from 97.0% to 100%. The genotyping failure rate for all SNPs combined was 1.6%.

Statistical analysis

Treatment outcome was evaluated binary and the association with genotypes and alleles were assessed by χ^2 test for independence. Deviations of genotype distributions from the HWE were assessed with the χ^2 test for goodness of fit. These statistical analyses were performed using PLINK v1.07 [8]. Differences between groups were compared using t-tests for continuous variables and chi-square or Fisher's exact test for categorical variables using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo, Japan). All statistical tests were two-tailed, and $P < 0.05$ indicated statistical significance unless otherwise specified.

Results

Demographics

In the first stage GWAS, 61 and 31 subjects were responders and

nonresponders, respectively. In the second sample for the candidate SNP analysis, 82 and 54 subjects were responders and nonresponders, respectively. The demographic characteristics are shown in Table 1. No significant difference was observed between responders and nonresponders in age, gender, depression subtype, the type of antidepressant used, and the baseline HAM-D score.

Genome-wide association analysis

A list of SNPs with P values < 0.0001 is shown in Table 2. Of the 37 SNPs with P values < 0.0001, 11 were introns and the remaining 26 were intergenic. The largest pharmacogenetic association was found for rs10516049 ($P = 4.3 \times 10^{-7}$). However, none of the SNPs reached the Bonferroni-corrected genome-wide significance of $P < 1.7 \times 10^{-7}$ (= 0.05 × 291,798).

Candidate SNP analysis

Table 3 presents the results of the association analysis of the 11 SNPs genotyped in the second-stage analysis. The genotype distributions did not significantly

deviate from the HWE in any of the SNPs examined. Significant difference in genotype and allele distribution was found between responders and nonresponders for rs365836 and rs201522 of the *CUX1* gene. The A allele of rs365836 and the G allele of rs201522 was associated with better response to antidepressant, consistent with the results of the first-stage GWAS. The allelic odds ratios in both first- and second-stage samples combined were 4.79 (95% CI = 2.38 to 9.64, $P = 0.0000023$) and 3.95 (95% CI = 2.13 to 7.31, $P = 0.0000040$) for rs365836 and rs201522, respectively. SNPs rs365836 and rs201522 were located in intron 9 and 8 of the *CUX1* gene, respectively, and were in linkage disequilibrium with each other (D' = 1.0, LOD = 19.84, $R^2 = 0.783$; calculated using Haploview 4.2).

Discussion

The present study examined the association between response to antidepressant treatment and genetic polymorphisms in two independent Japanese samples of patients with MDD. Although none of the SNPs reached the Bonferroni corrected genome-wide significance of $P < 1.7 \times 10^{-7}$ in the GWAS, rs385836 and rs201522 of the *CUX1* gene

with $P < 0.0001$ in the GWAS were also found to be significantly associated with response to antidepressant in the second sample.

The three previous studies [1-3] using genome-wide approaches to detect genetic variations associated with antidepressant response have not succeeded in identifying consistently significant SNPs. Although they have produced statistically significant associations between genetic polymorphisms and antidepressant response, the small effect size necessitates even larger sample size and high-quality studies. Determining which previous GWAS findings merit pursuit is not an easy task. Furthermore, none of the previous pharmacogenetic studies of antidepressant response in a Japanese population has utilized a genome-wide approach. Therefore, we performed a GWAS in our own sample and used its results to select the candidate SNPs for the subsequent analysis.

To our knowledge, the present study is the first to attempt a genome-wide approach to examine the association of genetic variations and antidepressant response in an Asian population. The homogenous sample specifically recruited for a pharmacogenetic investigation is one of the strength of this study. The unduly small

sample size of the genome-wide association analysis was inappropriate for detecting genome-wide significant findings. Assuming a minor allele frequency (MAF) of 0.3 in nonresponders and a relative risk of 1.5 to 2.0, however, the selection of SNPs with $P < 0.0001$ gave 11.4% to 62.8% sensitivity for detecting associated SNPs while excluding 99.99% of the unassociated SNPs. Therefore, utilization of our GWAS data was helpful for narrowing down the candidate SNPs.

The list of SNPs with $P < 0.0001$ in the GWAS included SNPs from both of the two Cux (also known as Cut and CDP) genes known in humans. The second stage analysis revealed that the polylmorphisms of *CUX1* were significantly associated with antidepressant response. Cux proteins are a family of transcription factors involved in the regulation of cellular proliferation and differentiation (reviewed in [9-11]. Cux plays a critical role in regulating neuronal function and cognition by controlling dendritic structures [11-13]. In humans, two Cux genes, *CUX1* [14] and *CUX2* [15], have been identified. Studies in mice showed that *Cux1* was expressed in many somatic tissues and also in the brain [16], whereas expression of *Cux2* was restricted to neural tissue [17]. Both *Cux1* and *Cux2* are known to be expressed in postmitotic pyramidal neurons of

upper cortical layers and in precursor cells of the proliferative ventricular and subventricular zones in the mouse cerebral cortex [18-21]. *Cux2* particularly regulates the proliferation of intermediate neuronal precursors in the subventricular zone of the developing brain [22]. *Cux2* deletion in mice resulted in altered dendritogenesis, synaptogenesis and spine formation in pyramidal glutamatergic/ GABAergic neurons [12]. It is worthy to note that a previous study has provided evidence for association between bipolar disorder and genetic variations of *CUX2* gene [23]. If Cux plays a role in the pathogenesis of bipolar disorder, it is reasonable that it may also affect antidepressant response in depressive patients, since unrecognized bipolar disorder is considered to be a contributor to apparent treatment resistant depression [24]. An alternative explanation can be that a common genetic factor may be associated with treatment resistant unipolar depression and bipolar disorder.

In contrast to *Cux2*, which is expressed only in neurons [17], *Cux1* is expressed in many somatic tissues [16]. However, *Cux1* also has a specific role in the development of cortical pyramidal neurons. A recent study in rats showed that *Cux1*, but not *Cux2*, can regulate dendritic morphology of cortical pyramidal neurons by reducing the

dendritic complexity [25].

The results of the present study must be interpreted cautiously in light of the following limitations. First, the small sample size of the GWAS may have missed a large proportion of truly associated SNPs. Although $P < 0.0001$ would include a certain proportion of associated SNPs while excluding a high percentage of unrelated SNPs, the power would be further decreased if the relative risk of the associated SNP is smaller than assumed. Next, only the non-intergenic SNPs with $P < 0.0001$ in the GWAS results were re-examined in the second sample. Another limitation is the heterogeneity of antidepressant treatment types used. However, these antidepressants have common mechanisms of action, i.e., the enhancement of monoaminergic neurotransmission. Therefore, the influence of drug-specific genetic effects may be negligible. However, our findings are limited by the absence of a placebo-comparison group, which did not allow us to determine whether the improvement was a natural course or due to the effect of antidepressants. Furthermore, as mentioned in the previous paragraph, the inclusion of patients with unrecognized bipolar depression may have also affected the results.

Our results provide the first evidence that SNPs of the *CUX1* gene may be

associated with response to antidepressant treatment in Japanese patients with MDD. The present study effectively utilized a genome-wide approach for the selection of the SNPs for the candidate SNP analysis. Further studies including a gene-wide tagging study of the *CUX1* gene are required to confirm our findings.

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Conflict of Interest

The authors report no conflicts of interest.

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Table 1: Demographic and Clinical Characteristics

Characteristics	GWAS sample							Second-stage SNP analysis sample						
	All (N=92)		Responders (N=61)		Nonresponders (N=31)		Responders vs Nonresponders	All (N=136)		Responders (N=82)		Nonresponders (N=54)		Responders vs Nonresponders
	N		N	%	N	%		N		N	%	N	%	
Female gender	48		32	52.5	16	51.6	χ^2=0.01, *P* =0.94	68		38	46.3	30	55.6	χ^2=1.1, *P* =0.29
Depression diagnosis														
MDD, single episode	54		37	68.5	17	31.5	χ^2=0.29, *P* =0.59	77		45	58.4	32	41.6	χ^2=0.25, *P* =0.61
MDD, recurrent	38		24	63.2	14	36.8		59		37	62.7	22	37.3	
Antidepressant used														
Paroxetine	28		16	26.2	12	38.7		64		35	54.7	29	45.3	
Fluvoxamine	29		21	34.4	8	25.8		0		0	0	0	0	
Sertraline	0		0	0	0	0	χ^2=2.1, *P* =0.55	72		47	65.3	25	34.7	χ^2=1.6, *P* =0.21
Nortriptyline	16		10	16.4	6	19.4		0		0	0	0	0	
Milnacipran	19		14	23.0	5	16.1		0		0	0	0	0	
	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD	
Age (years)	43.1	12.7	44.2	12.6	40.9	12.6	t=1.18, *P* =0.24	51.3	15.4	51.5	14.6	51.1	16.8	t=0.15, *P* =0.88
Baseline HAM-D (17 item) score	22.2	5.0	22.5	5.4	21.6	4.3	t=0.79, *P* =0.43	21.1	4.5	21.3	4.7	20.9	4.2	t=0.51, *P* =0.61
HAM-D score %decrease over 8 weeks	61.3	27.7	75.3	15.8	26.4	24.5	t=9.90, *P* <0.0001	52.7	27.1	71.0	13.3	25.0	17.3	t=17.5, *P* <0.0001

MDD: major depressive disorder; SD; standard deviation; HAM-D: Hamilton depression rating scale

Table 2: Association results of SNPs with an allelic P value < 0.0001

SNP	Chr	Position	MAF of Nonresponder	MAF of Responders	P value	OR	SE	L95	U95	Type	Gene
rs11165207	1	94908659	0.4667	0.1721	2.54E-05	4.208	0.3528	2.108	8.403	intergenic	
rs3753513	1	208066413	0.2419	0.02459	2.72E-06	12.66	0.6555	3.503	45.75	intergenic	
rs10489344	1	208090410	0.2419	0.02459	2.72E-06	12.66	0.6555	3.503	45.75	intron	*DIEXF*
rs724286	1	208103322	0.2419	0.04098	3.48E-05	7.468	0.5445	2.569	21.71	intergenic	
rs2347611	2	47179477	0.4483	0.1525	2.05E-05	4.514	0.3678	2.195	9.281	intergenic	
rs10514737	3	82231015	0.129	0	4.98E-05	NA	NA	NA	NA	intergenic	
rs10514738	3	82275974	0.129	0	4.98E-05	NA	NA	NA	NA	intergenic	
rs2053627	3	106787174	0.2742	0.5902	5.04E-05	0.2623	0.339	0.135	0.5099	intergenic	
rs1509620	4	95782995	0.5806	0.2705	4.00E-05	3.734	0.3283	1.962	7.106	intron	*PDLIM5*
rs1485458	5	96856499	0.2581	0.05738	1.00E-04	5.714	0.4856	2.206	14.8	intergenic	
rs2035550	5	96861245	0.2581	0.05738	1.00E-04	5.714	0.4856	2.206	14.8	intergenic	
rs1021224	5	96862809	0.2581	0.05738	1.00E-04	5.714	0.4856	2.206	14.8	intergenic	
rs1038491	5	96874779	0.2581	0.05738	1.00E-04	5.714	0.4856	2.206	14.8	intergenic	
rs11746295	5	168082337	0.3	0.07377	5.43E-05	5.381	0.4465	2.243	12.91	intron	*SLIT3*
rs10516049	5	168097247	0.3065	0.04098	4.33E-07	10.34	0.5333	3.635	29.41	intron	*SLIT3*
rs13202332	6	107573323	0.4355	0.1557	3.44E-05	4.182	0.3577	2.074	8.431	intergenic	
rs1837345	7	63149422	0.5806	0.2705	4.00E-05	3.734	0.3283	1.962	7.106	intergenic	
rs978661	7	63183209	0.5833	0.2705	4.11E-05	3.776	0.3318	1.97	7.235	intergenic	
rs201522	7	101558266	0.2903	0.07377	8.71E-05	5.136	0.4452	2.146	12.29	intron	*CUX1*
rs365836	7	101596571	0.2581	0.04918	3.66E-05	6.725	0.5094	2.478	18.25	intron	*CUX1*
rs9690295	7	139023506	0.2903	0.06557	3.52E-05	5.83	0.4605	2.364	14.38	intergenic	
rs3780126	8	64112466	0.371	0.1148	4.16E-05	4.549	0.387	2.131	9.714	intron	*GGH*
rs2353903	8	64179435	0.371	0.1148	4.16E-05	4.549	0.387	2.131	9.714	intergenic	
rs4471020	8	69073722	0.04839	0.3443	1.02E-05	0.09685	0.6218	0.02863	0.3276	intron	*PREX2*
rs9408013	9	24869019	0.2419	0.04098	3.48E-05	7.468	0.5445	2.569	21.71	intergenic	
rs998494	9	79476782	0.4516	0.1803	9.23E-05	3.743	0.3472	1.895	7.393	intergenic	
rs7030006	9	83600032	0.04839	0.3279	2.29E-05	0.1042	0.6225	0.03077	0.3531	intergenic	
rs11222749	11	99474024	0.1774	0.01639	5.60E-05	12.94	0.7867	2.769	60.48	intron	*CNTN5*
rs7300860	12	110238980	0.2903	0.07377	8.71E-05	5.136	0.4452	2.146	12.29	intron	*CUX2*
rs9518586	13	101308167	0.6613	0.3197	9.94E-06	4.155	0.3312	2.171	7.952	intron	*FGF14*
rs992734	16	72010854	0.3871	0.1311	6.93E-05	4.184	0.3741	2.01	8.71	intergenic	
rs1055164	16	72012373	0.3871	0.123	3.41E-05	4.505	0.3795	2.141	9.478	intergenic	
rs1110338	16	85807728	0.6613	0.3525	7.03E-05	3.587	0.3285	1.884	6.829	intergenic	
rs9896237	17	51005511	0.129	0	4.98E-05	NA	NA	NA	NA	intergenic	
rs7223150	17	51013132	0.3065	0.08197	7.81E-05	4.949	0.4299	2.131	11.49	intergenic	
rs1471408	18	11531256	0.3871	0.1311	6.93E-05	4.184	0.3741	2.01	8.71	intergenic	
rs554440	18	36814255	0.2258	0.04098	9.85E-05	6.825	0.5485	2.329	20	intergenic	

SNP: single nucleotide polymorphism;

Chr: chromosome; MAF: minor allele frequency; OR: odds ratio; SE: standard errors;

L95: lower limit of 95% confidence interval of OR; U95: upper limit of 95 % confidence interval of OR

Table 3: Results of the replication sample analysis

Gene	SNP	Allele 1/2		N	Genotype			Allele		P-value		Allele OR (95% CI)	HWE P-value
					1/1	1/2	2/2	1	2	Genotype	Allele		
DIEXF	rs10489344	G/A	Responder	76	64	11	1	139	13	0.70	0.77	0.87 (0.35-2.19)	0.51
			Nonresponder	53	45	8	0	98	8				0.55
PDLIM5	rs1509620	G/A	Responder	78	32	34	12	98	58	0.71	0.40	0.80 (0.47-1.34)	0.55
			Nonresponder	53	25	22	6	72	34				0.73
SLIT3	rs11746295	A/G	Responder	76	55	21	0	131	21	0.37	0.31	1.42 (0.73-2.77)	0.16
			Nonresponder	54	35	18	1	88	20				0.44
SLIT3	rs10516049	A/G	Responder	76	57	19	0	133	19	0.45	0.46	1.31 (0.65-2.65)	0.21
			Nonresponder	54	38	15	1	91	17				0.73
CUX1	rs201522	G/A	Responder	77	69	8	0	146	8	0.016	0.0042	3.41 (1.41-8.22)	0.63
			Nonresponder	54	38	15	1	91	17				0.73
CUX1	rs365836	A/G	Responder	79	73	6	0	152	6	0.023	0.0054	3.77 (1.40-10.16)	0.73
			Nonresponder	54	41	12	1	94	14				0.91
GGH	rs3780126	C/T	Responder	77	36	36	5	108	46	0.35	0.84	0.95 (0.55-1.62)	0.31
			Nonresponder	54	29	19	6	77	31				0.30
PREX2	rs4471020	A/G	Responder	79	55	20	4	130	28	0.45	0.28	1.40 (0.76-2.56)	0.24
			Nonresponder	54	32	19	3	83	25				0.94
CNTN5	rs11222749	G/A	Responder	75	66	9	0	141	9	0.39	0.41	0.60 (0.18-2.01)	0.58
			Nonresponder	54	50	4	0	104	4				0.78
CUX2	rs7300860	C/T	Responder	78	53	23	2	129	27	0.49	0.59	0.83 (0.42-1.63)	0.79
			Nonresponder	54	38	16	0	92	16				0.20
FGF14	rs9518586	G/A	Responder	78	28	34	16	90	66	0.80	0.90	0.97 (0.59-1.60)	0.34
			Nonresponder	53	21	20	12	62	44				0.10

SNP: single nucleotide polymorphism; HWE: Hardy-Weinberg Disequilibrium

OR: odds ratio; CI: confidence interval