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Table I. Distribution of the HTR4 genotypes in 198 schizophrenic and 211 control subjects.

	n	Genotype			Frequency of rare allele	Significance*
		26+14T>C				
		T/T	T/C	C/C	26+14C	
Schizophrenia	198	161 (81%)	36 (18%)	1 (1%)	0.096	NS†
Control	211	179 (85%)	31 (15%)	1 (0%)	0.078	
		353+6G>A				
		G/G	G/A	A/A	353+6A	
Schizophrenia	198	110 (56%)	70 (36%)	18 (9%)	0.268	NS†
Control	207	115 (56%)	75 (36%)	17 (8%)	0.263	
		508-36T>C				
		T/T	T/C	C/C	508-36C	
Schizophrenia	198	109 (55%)	69 (35%)	20 (10%)	0.275	NS†
Control	225	130 (58%)	84 (37%)	11 (5%)	0.236	
		d-25T>C				
		T/T	T/C	C/C	d-25C	
Schizophrenia	198	58 (29%)	95 (50%)	45 (23%)	0.467	NS†
Control	299	106 (35%)	135 (45%)	58 (19%)	0.42	

*Statistical analysis was performed using the computer program CLUMP. (†NS=not significant.)

Table II. Association between Schizophrenia and the possession of each HTR4 haplotype (353+6G>A and 508-36T>C).

	Schizophrenia	Control	*P*	Corrected *P**	OR (95% CI)
A-C					
With	86	79	NS†	NS	1.23 (0.83-1.84)
Without	112	127			
A-T					
With	2	15	0.001727	0.014	0.13 (0.03-0.58)
Without	196	191			
G-C					
With	4	2	NS	NS	2.1 (0.38-11.61)
Without	194	204			
G-T					
With	177	189	NS	NS	0.76 (0.39-1.48)
Without	21	17			

Table III. Primers Used in dHPLC, PCR-RFLP, and Primer Extension Analysis.

Exon	Primer	5'-3' sequence	Nucleotide position(5'-3')	PCR product (bp)	PCR condition	dHPLC Temperature (°C)
Exon1 (nt1-26)	5-HT4-1f	ACT TCC CCC ATT TTA GGA CCC	-63→-43	151	Mg2+ 1.5mM, pH8.5, 63°C	56, 58
	5-HT4-1b	TAG AGT CTT CAT AGC AGA AAT GTT CTC A	+62→+35			
Exon2 (nt27-152)	5-HT4-2f	CTG ATG GTG AAG TTA CCT TTC TGA AG	-66→-41	255	Mg2+ 1.5mM, pH8.5 60°C	59, 65 (+2)
	5-HT4-2b	AAA AGG TTC CCT CCT GCT GCT	+24→+4			
Exon3 (nt153-353)	5-HT4-3.1f	CCC TTT TTC CCT TCA TCC CTC	-56→-36	312	Mg2+ 1.5mM, pH8.5 60°C	58, 62 (+2.5)
	5-HT4-3.1b	CAT CAA GTC ATG TCT CCA GCA TG	+55→+33			
	5-HT4-3.2f	TCT TAC ACT TTT TCA CTC ACA GGA AA	-22→357	277	Mg2+ 3.5mM, pH8.5 60°C	54 (-2), 61, 62
	5-HT4-3.2b	CAT CAA GTC ATG TCT CCA GCA TG	+55→+33			
Exon4 (nt354-507)						
	5-HT4-4f	TTG CCC ATG CCT ATG CTC TG	-39→-20	251	Mg2+ 1.5mM, pH8.5 63°C	59, 62
	5-HT4-4b	CCT TGG GGT ACG TTT CAA CTA	+58→+38			

Exon h	5-HT4-hf 5-HT4-hb	ACA GGG AGC TGC CCT TTC CT CCG GCA TTT CTT TCA GAA TCC	-68→-49 +45→+25	155	Mg2+ 1.5mM, pH8.5 60°C	61 (+2), 62 (+3)
Exon5 (nt508-1167)	5-HT4-5.1f 5-HT4-5.1b	TCC CCA TTT TTC CCA CTT CTT CAT GCG ATG AGT GCT ATG CT	-82→-62 +132→+113	328	Mg2+ 1.5mM, pH8.5 60°C	57, 62 (+2), 64 (+4)
	5-HT4-5.2f 5-HT4-5.2b	ATA GAA AAG AGG AAG TTC AA CAT GCG ATG AGT GCT ATG CT	508→527 +132→+113	246	Mg2+ 3.5mM, pH8.5 57°C	59, 63, 66 (+2)
	5-HT4-5.3f 5-HT4-5.3b	GCC CAT CAG ATC CAG ATG TT TAT AGC CGA GCC AGA GGA AA	667→686 888→907	240	Mg2+ 1.5mM, pH8.5 63°C	62, 64, 66 (+2)
	5-HT4-5.4f 5-HT4-5.4b	CAT AGA CTA CAC TGT CCC TG CAA ATC AAT GAA CTC CCT TA	852→871 +30→+11	255	Mg2+ 3.5mM, pH8.5 60°C	59, 62
Exon b	5-HT4-b.1f 5-HT4-b.1b	GGT GGG CTC TTT CAG GAG ATG TCT TCT GGG TCA TTG TCC CAG	-75→-54 +25→+5	190	Mg2+ 1.5mM, pH8.5 62°C	60, 65 (+3)
	5-HT4-b.2f	GGA TGC AGT GGA GTG TGG TG	1→20	159	Mg2+ 1.5mM, pH8.5 63°C	65, 66
	5-HT4-b.2b	AAG CAG CAG CTT AGG ACC TGG CCC	+68→+45			
Exon c	5-HT4-cf	CTG TGG TTT AAT AGC ATC TCA GGA TTA	-98→-72	290	Mg2+ 1.5mM, pH8.5 58°C	54, 57 (+3.5), 58 (+3)

	5-HT4-cb	ACG AAT TCT GAA TAG CAT TTC TCT TTC	+32→+6			
Exon d	5-HT4-df	GTT CTT CTC CTG TGA CAT TTT GAT A	-76→-50	151	Mg2+ 1.5mM, pH8.5 60°C	58, 59 (+1.5), 61 (+2.5)
	5-HT4-db	CAA AAA CCT GTG TTG GGC ACT	+59→+39			
Exon gef	5-HT4-geff	TTC CCA AAT TCT GCT TGG CT	-58→-39	180	Mg2+ 1.5mM, pH8.5 60°C	62 (+3), 63 (+4)
	5-HT4-gefb	AAT AGG CAG ACA CAG ACA GAC TCA CA	+61→+36			
Exon a	5-HT4-a.1f	TGA CTT CGG TGC AGT TGG AG	-62→-43	174	Mg2+ 1.5mM, pH8.5 60°C	62 (+2), 63 (+3)
	5-HT4-a.1b	CTA AGT TGT GAG CCA TGT CCT CA	+24→+2			
	5-HT4-a.2f	TAC ACC GTT CTG CAC AGG GG	2→21	156	Mg2+ 1.5mM, pH8.5 60°C	61, 62
	5-HT4-a.2b	ATG CCA GGG TGA CCT GTT CA	+69→+50			
PCR-RFLP	5-HT4_353 +6(g-a)	CCG CTA TGC ACA TTG TTC GGT	-27→-7	250	Mg2+ 1.5mM, pH8.5 60°C, with 5-HT4-3.2f	
	5-HT4_508-36(t-c)	TTT TCA CTT TTT CTT TCC TTT TTA GT	-62→-37	308	Mg2+ 1.5mM, pH8.5 60°C, with 5-HT4-5.1b	

Primer Extension	5-HT4_PE_353+6G>A CAT TTC TCT GGA TAG GTA AG
	5-HT4_PE_508-36T>C CTT TTT CTT TCC TTT TTA CC

Table IV. SNPs Typing by PCR-RFLP and Primer Extension.

SNPs	PCR-RFLP primers	PCR product (bp)	Nucleotide position	Substitution	Enzyme for RFLP	Alleles	Fragment size(bp)	PCR primer for Primer Extension	Extension Primer	Nucleotide composition of primer extension reaction	v a
26+14T>C	5-HT4,1f	151	26+14	(tct-tcc)	BsrGI	26+14t	104+48				
	5-HT4,1b					26+14c	152				
353+6G>A	5-HT4-3.2f	250	353+6	(agg-aga)	AvaII	353+6g	229+21	5-HT4-3.2f	5-HT4_PE_353+6G>A	ddG,ddA	G/
	5-HT4_353 +6(g-a)					353+6a	250	5-HT4-3.2b			
508-36T>C	5-HT4_508-36(t-c)	309	508-36	(cct-ccc)	AluI	508-36t	185+98+25	5-HT4-5.1f	5-HT4_PE_508-36T>C	ddC,ddT	T/
	5-HT4-5.1b					508-36c	210+98	5-HT4-5.1b			
d-25C<T	5-HT4-df	151	-25	(tac-tat)	HpyCH4IV	d-25c	100+51				
	5-HT4-db					d-25t	151				

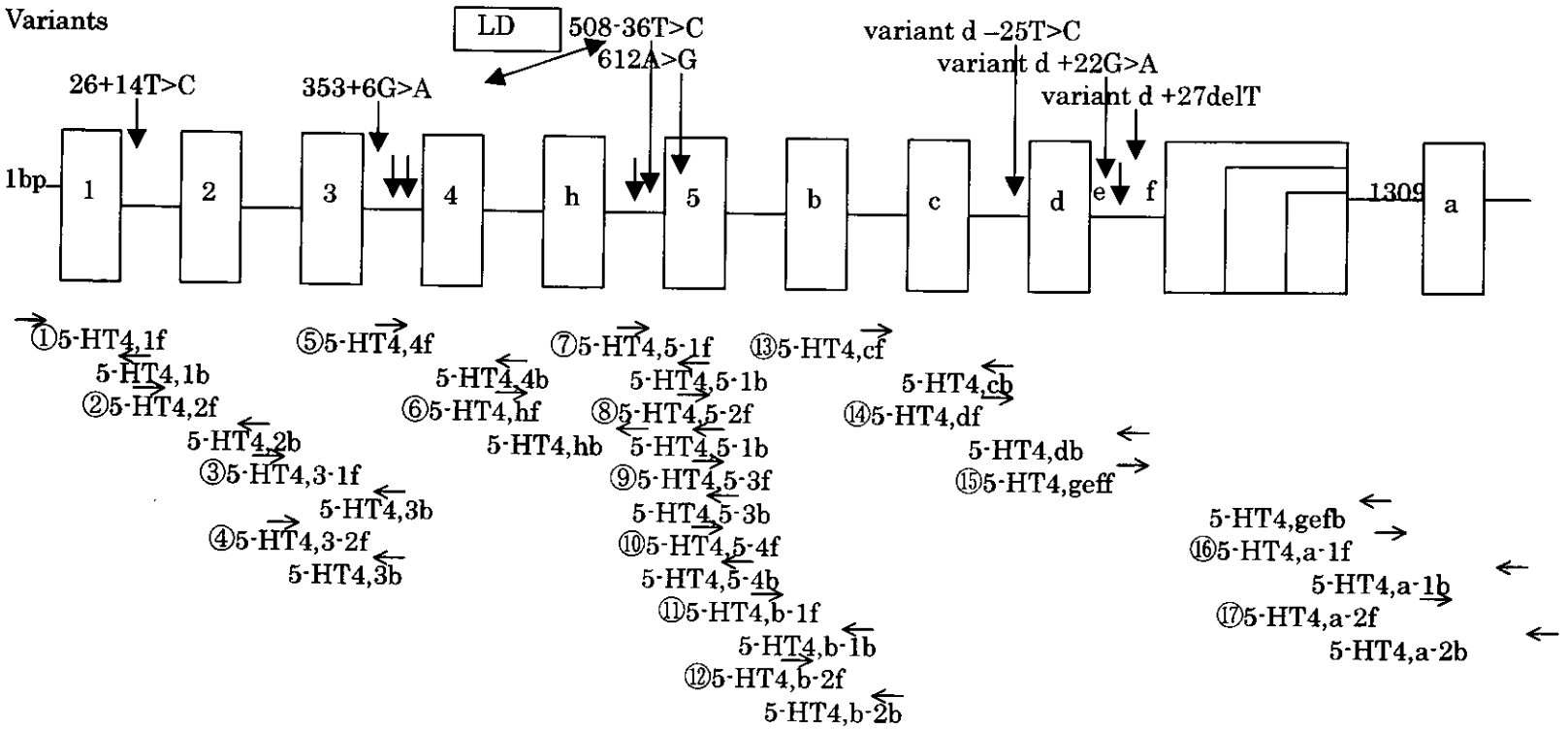

Figure. Direct sequencing screen of the human 5-HT$_4$ receptor locus. The base pair (bp) designations are relative to the start codon. PCR and Sequencing primers are indicated by horizontal arrows and sequence variation by vertical arrows. Alphabetical coding regions are splice variants. We detected one synonymous DNA substitution and six SNPs in intron, which were designated '26+14T>C', '353+6G>A', '508-36T>C', '612A>G', 'variant d-25tT>C, 'variant d+22G>A', 'variant d+27delT.

Effect of DRD2, 5-HT2A, and COMT Genes on Risperidone Treatment Response

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ABSTRACT

Risperidone is a widely used atypical antipsychotic with certain advantages over typical antipsychotics. Although variations in the efficacy of treatment with risperidone have been observed, no specific predictable marker has been identified as of yet. 64 patients with schizophrenia were given risperidone for eight weeks, and clinical symptoms were evaluated using the Positive and Negative Syndrome Scale (PANSS). Six candidate polymorphisms (in HTR2A -1438G>A, 102T>C, H452Y; in DRD2 -141delC, *Taq* I A; and in COMT V158M) were genotyped. The diplotype configuration for each individual was estimated by the maximum-likelihood method. Multiple linear regressions were used to analyze the effects of these haplotypes/genotype and other prognostic factors on PANSS scale performance. After adjustment for the effects of treatment duration and patient-related variables, a DRD2 haplotype was observed to correlate with better clinical performance. Compared with patients who had Ins-A1/Ins-A1 diplotype, PANSS total scores of patients with Ins-A2/Del-A2 diplotype showed more improvement. HTR2A diplotype and COMT genotype, as well as other potential prognostic factors, did not significantly influence clinical performance. The results suggest that DRD2 may influence individual response to risperidone.

INTRODUCTION

Risperidone, one of the most widely used atypical antipsychotics, has two major advantages over typical antipsychotics: 1) high efficacy in the treatment of negative symptoms of schizophrenia and 2) few side effects.[1] Dopamine D2 receptor blockade is thought to mediate antipsychotic action, while the blocking of serotonin 2A receptor has been implicated in its added efficacy as well as its reduced extrapyramidal side effects profile.[2] Recent studies reported that some polymorphisms in the dopamine D2 receptor gene (DRD2) and the serotonin 2A receptor gene (HTR2A) are associated with schizophrenia or antipsychotic drug responses. For example, –141delC[3] and *Taq* I A[4] polymorphism in DRD2 caused a functional change of the product, and both are reported to have an association with antipsychotic response.[5,6] In HTR2A, the 102T>C polymorphism showed an association with the presence of schizophrenia,[7] and -1438G>A polymorphism has been shown to be a clozapine response.[8] In addition, H452Y in HTR2A, which caused functional change of the expressed gene product [9], is reported to be involved in clozapine response.[10] Very recently, Lane and colleagues reported a significant association between HTR2A 102T>C polymorphism and risperidone efficacy for negative symptoms in Chinese Han patients.[11] However, the interaction of DRD2 and HTR2A to risperidone efficacy has not been discussed as pharmacodynamic-pharmacogenetics studies.

Moreover, a common functional polymorphism in the human catechol-O-methyltransferase gene (COMT), a methylation enzyme that metabolizes released dopamine, which accounts for a 4-fold variation in enzyme activity and dopamine catabolism, was shown to be associated with cognitive characteristics that are presented in schizophrenia.[12] Shifman and colleagues recently reported a highly significant association between schizophrenia and a COMT haplotype.[13] The biological functions of COMT, in addition to the genetic association findings, make it a popular

candidate because of the long hypothesized role of dopamine in schizophrenia.[14]

To explicate the variability of risperidone response from one individual to another using the pharmacodynamic-pharmacogenetic approach, we investigated the influence to polymorphisms in the DRD2, HTR2A, and COMT genes on risperidone effectiveness.

RESULTS

Of 64 schizophrenic patients, 27 were newly administered risperidone as a first-time prescription, and 37 consisted of a group that was switched from typical antipsychotics. There were no differences between the two groups for gender, age, and mean dose of risperidone at 8 weeks. Durations of illness and hospitalization as well as initial mean total score of the Positive and Negative Syndrome Scale (PANSS) varied because most of the newly administered patients were experiencing their first episode of illness and had been previously untreated (Table 1).

"Table 1 about here"

The proportions of genotype at each site were in agreement with the Hardy-Weinberg equilibrium among each group. There were no differences in genotypes and frequencies of each polymorphism between New Group and Switched Group (data not shown). Therefore, we combined the two groups together following analysis. The HTR2A H452Y polymorphism was excluded in further analysis because all subjects were H/H homozygote genotypes in our samples. The allele frequencies of the six polymorphisms in the 64 patients were similar to those calculated from reported data of Japanese populations, suggesting that our data was not biased with respect to allele frequency.[5-7]

Because -141delC and *Taq* I A (DRD2), -1438G>A and 102T>C (HTR2A) polymorphisms exist in the same gene loci, one of these two polymorphisms would be actually related to risperidone treatment response, and another might be in linkage disequilibrium. Using in silico methods, the haplotype configuration for each locus could be estimated; therefore, the diplotype of DRD2 and HTR2A were used for further analyses. The genotypic findings from all

patients were analyzed using the computer programs Haplotyper,[15] EMDecoder,[15] and PHASE[16] to estimate haplotype frequencies concerning the two-polymorphism DRD2 and HTR2A loci. The three programs estimated identical individual haplotype estimation. Estimated haplotype frequencies were not deviated from the maximum likelihood estimation. Table 2 indicates the number of individuals with each diplotype configuration for the DRD2 (a) and HTR2A (b) loci.

"Table 2 about here"

The distribution of the PANSS total and subscale scores were skew to the right (data not shown) and unsuitable for regression analyses. The value of each score was thus transformed to its natural logarithm to obtain normal distributions (data not shown).

Table 3 illustrates that after adjustment for the effects of treatment duration and patient-related variables, a DRD2 haplotype was related to better clinical performance. Compared with patients who had Ins-A1/Ins-A1 diplotype, the PANSS total scores of patients with Ins-A2/Del-A2 diplotype showed the better improvement (Table 3). Patients with other diplotypes did not differ significantly in any subscale scores or the total score. In addition, the HTR2A diplotype and the COMT genotype, as well as other potential prognostic factors, did not significantly influence clinical performance (data not shown).

"Table 3 about here"

DISCUSSION

Our finding suggests that DRD2 Ins-A2/Del-A2 diplotype compared with Ins-A1/Ins-A1 diplotype may predict superior risperidone response in schizophrenic patients. The −141delC and the *Taq* I A polymorphisms were both said to effect the density of the dopamine D2 receptor. In regard to the −141delC polymorphism, a positron emission tomography study showed that genotypes with the Del allele were associated with increased density of the receptor at human striatum.[17] In the *Taq* I A polymorphism, the genotype with homozygous A1 allele was reported to be associated with decreased density of the receptor.[18-21] According to these previous findings, patients with Ins-A2/Del-A2

diplotype hypothetically had the highest dopamine D2 receptor density; on the other hand, patients with Ins-A1/Ins-A1 diplotype had the lowest in our samples (Table 2). One explanation may be that patients who have higher dopamine D2 receptor density showed greater improvement in risperidone treatment because their dopamine receptor density rate was less occupied by risperidone than those with A1 allele and Ins allele.

Our results did not support the HTR2A positive findings by Lane and colleagues. There are several explanations: first, we combined the two groups because the proportion of genotype frequencies did not differ. However, risperidone acts as a potent serotonin 2A receptor antagonist while it exhibits a low affinity for dopamine D2 receptors. Thus, the desensitization effects may be higher in the previous typical antipsychotic users. Additionally, a small number of subjects may have caused the beta error to occur. Indeed the power to detect the PANSS total score for HTR2A diplotype was 0.2 and the least significant number was 186, which is the minimum number of samples needed to thoroughly evaluate our negative findings. Lastly, we used haplotype configuration instead of the each polymorphism genotype. That may mask the single gene effects on the analysis.

SUBJECTS AND METHODS

Subjects

The subjects were 52 patients with schizophrenia and 12 patients with schizophreniform disorder who lived in the central part of Japan. Consensus diagnosis according to DSM-IV by at least two experienced psychiatrists was made with double-blind evaluation for each patient on the basis of unstructured interviews and information from medical records. None had severe medical complications or other Axis-I disorders according to DSM-IV. All patients were unrelated to each other and ethnically Japanese. After description of the study, written informed consent was obtained from each subject. This study was approved by the Ethics Committee of the Fujita Health University.

Clinical protocols

Subjects received only risperidone as antipsychotic treatment for 8 weeks. No other drugs were given except benzodiazepine and barbiturate for insomnia, anticholinergic drugs for extrapyramidal symptoms, and less than 50 mg/day levomepromazine for agitation or excitement. The subjects belonged to either the "new group", comprised of subjects who took an antipsychotic for the first time or had not received any antipsychotics for at least one month before this study, or the "switched group", which consisted of subjects asked to change antipsychotic drugs from typicals to risperidone. The subjects who belonged to the "new group" took 2-6 mg/day of risperidone. Those who belonged to the "switched group" were administered their former drugs for at least four weeks. After this period, antipsychotics were gradually replaced with the equivalent potency of risperidone (maximum dose was 12 mg/day) in 2 weeks based on the antipsychotics conversion table by the Treatment Resistant Schizophrenia Research Group of the Japanese Ministry of Health and Welfare.

Clinical symptoms initially and after 8 weeks of treatment were evaluated using PANSS.[22] Psychiatrists and clinical psychologists, who were well trained, performed the PANSS assessments.

Laboratory methods

Six polymorphisms (HTR2A -1438G>A,[23] 102T>C,[24] H452Y[24]; in DRD2 -141delC,[3] *Taq* I A[4]; and in COMT V158M[25]) were genotyped using polymerase chain reaction - restricted fragment length polymorphism (PCR-RFLP). Seven ml of EDTA-anticoagulated venous blood sample was drawn from each subject, and DNA was isolated from leukocytes using the PUREGENE™ DNA isolation kit (Gentra Systems, Minneapolis, MN USA). All genotyping methods were followed by the previous descriptions.[3,4,23-25]

Statistical analysis

Statistical analysis to evaluate differences between groups was carried out by either Fisher's exact test for discrete data or by Mann-Whitney's test for continuous data.

Deviation from the genotype counts predicted by Hardy-Weinberg equilibrium expectations was tested using an exact test, as described by Weir[26] and implemented in software written by Lewis and Zaykin (2001; Genetic Data Analysis (GDA), version 1.0 (d16c)).

Estimation of the haplotype frequencies was performed by the expectation –maximization (EM) algorithm, a pseudo-Gibbs sampler (PGS) algorithm, and a partition ligation (PL) algorithm. Haplotyper,[15] which implements the PL algorithm, was kindly provided by T. Niu. PHASE,[16] which implements the PGS algorithm as described by Stephens et al. was downloaded from their website (Mathematics Genetics Group). EM-Decoder,[15] which implements the EM algorithm as described by Niu et al. was downloaded from their website. The genetic status of an individual concerning linked loci can be expressed by the combination of two haplotypes (diplotype configuration).

Total and subscale scores of PANSS were used as a measure of response to risperidone. Potential prognostic factors included DRD2 diplotype, HTR2A diplotype and COMT genotype as well as the administrated group (New or Switched), gender, age at illness onset, duration of illness, number of previous hospitalizations, baseline PANSS total score, and endpoint risperidone dose. Because there were repeated assessments, multiple linear regressions were used to adjust the within-subject dependence. Statistical significance was defined as $p<0.05$ and was not corrected for multiple analysis.

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