Table 2
First-set case control analysis of exon region

Markers	SNP ID	*P*-values[a]		
		1-window	2-windows	3-windows
C#1	rs10503915	.116		
C#2	rs7016691	.231	.0603	
C#3	rs11782671	.472	.371	.349
C#4	rs10103930	.168	.474	.296
C#5	rs10503917	.699	.322	.508
C#6	rs10107065	.765	.628	.0935
C#7	rs6468118	.138	.138	**.0244**
C#8	rs7000590	.0939	.154	.174
MS1	rs7820838	.110	.107	.158
MS2	rs7834206	.149	.142	.181
C#9	rs4236709	.0786	.0879	.145
C#10	rs13260545	.0994	.187	.352
C#11	rs4316112	.948	.248	.403
C#12	rs2439305	.196	.144	.0984
C#13	rs7826814	.715	.130	.132
C#14	rs2466064	.690	.851	.129
MS3	rs3924999	.162	.313	.436
C#15	rs10954864	.803	.113	.0699
C#16	rs2439281	.965	.969	.602
C#17	rs9642729	.0680	.0725	.301
C#18	rs12547858	.0801	.0988	.137
C#19	rs10098373	.801	.457	.523
C#20	rs10095694	.380	.835	.654
MS4	rs3735774	.762	.727	.872
C#21	rs2466058	.372	.727	.718
C#22	rs2466052	.379	.526	.587
C#23	rs2466046	.187	.286	.509
C#24	rs10503923	.546	.372	.431
C#25	rs2466084	.310	.473	.203
C#26	rs2976515	.253	.551	.197
C#27	rs4445183	.702	.654	.563
C#28	rs2919377	.151	.484	.500
C#29	rs2919375	.819	.341	.455
MS5	rs3735776	.740	.222	.182
C#30	rs7007436	.711	.758	.129
C#31	rs3757934	.758	.815	.866
MS7	rs4733376	.379	.421	.562
C#32	rs4360253	.357	.336	.357
C#33	rs7005288	.864	.893	.789
C#34	rs6992642	.569	.812	.738
MS6 (C#24–#30)[b]	rs17731664	.772		
C#5–#11–#14		1.00		
C#5–#14		.180		
C#16–#27		.751		
C#23–#26–#28		.245		

[a] Bold number represents significant *P*-value.
[b] MS6 could be represented by the haplotypes constructed by C#24–30.

2.5. Statistical methods for conventional association analysis

In the case–control samples, the marker–trait association was evaluated with the χ^2 test in allele- and genotype-wise analyses. Haplotype frequencies were estimated in a 2- to 3-marker sliding window fashion by EM algorithm and Log likelihood ratio tests were performed for Global *P*-values with COCAPHASE program version 3.06 (Dudbridge, 2003). In the family samples, the transmission disequilibrium test (TDT) and 3-marker haplotype analyses were performed with the TDTPHASE program version 3.06 (Dudbridge, 2003). In these haplotype-wise analyses, rare haplotypes (less than 0.05) of cases and controls were excluded from the association analysis in order to provide greater sensitivity and accuracy.

The significance level was set at $P<0.05$.

2.6. Imputation of ungenotyped SNPs

Our conventional haplotype-wise analysis was done in a sliding window fashion, since our selection for tagging SNPs was not based on the haplotype block concept. Although this type of haplotype-wise analysis does not adapt to the degree of LD, so that it is unclear which markers should be considered jointly, it results in a higher level of statistical power since it can reflect unknown SNPs that were not included in the analysis. Considering this, we included a recently developed method, imputation, to test for any SNPs that reflect the significant haplotypes (Marchini et al., 2007). The IMPUTE program imputes the genotypic distribution of un-observed SNPs using observed SNP information (60 SNPs used in the screening scan) and the HapMap database (fine-scale recombination map, haplotype for JPT/CHP).

The targeted region for imputation was limited to within known recombination hot spots, because our data targeted only the HAP_{ICE} and exon regions.

After imputation, we applied a Bayesian test with an additive model to assess the association using SNPTEST software (Marchini et al., 2007). Default values were used in all settings needed in IMPUTE and SNPTEST (e.g. effective population size for JPT/CHP, buffer, call threshold for calling genotyped SNPs and number of samples of genotypes that should be used for Bayesian tests).

Table 3
Individual haplotype analyses from significant Global *P*-values in first-set samples

	haplotypes	Case Freq (%)	Con Freq (%)	*P*-value	Global *P*-value
C#5–6–7	1–1–1	9.36	11.8	.0104	.0244
	1–1–2	15.6	13.6	.0896	
	1–2–2	65.8	65.5	.886	
	2–1–1	7.21	6.27	.300	

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2.7. Power calculation

Power calculation was performed with a web-based statistical program, Genetic Power Calculator (Purcell et al., 2003). Power was estimated under a multiplicative model of inheritance, assuming the disease prevalence to be 1% and the population susceptibility allele frequencies to be the values observed in control samples.

3. Results

3.1. Mutation scan and first-set association analysis

We detected seven SNPs through dHPLC analysis of the exon region (MS1–7: Table 2). One of them, MS3 (rs3924999), is a non-synonymous SNP (Gly38Arg) and had shown a significant association in the Chinese population (Yang et al., 2003). The other SNPs were located in an untranslated region (UTR) or branch site, and may therefore have a functional effect (Table 2).

Next, 49 SNPs and 7 haplotypes were selected as Tagging SNPs from the HapMap database. These SNPs are located in the HAP_{ICE}- and coding regions based on the HapMap database (Tables 1 and 2).

Consequently, by involving 11 SNPs (the 7 SNPs we detected and 4 SNPs reported in other papers (Stefansson et al., 2002; Walss-Bass et al., 2006)), a total of 60 SNPs were genotyped in the first-set screening samples (however, since we were unable to design a genotyping method for one SNP that we detected (MS6) by TaqMan Assay by Design (Applied Biosystems), we determined the genotype distribution of some samples (192 cases and 192 controls) using a direct sequencing method. With these samples we confirmed that MS6 could be represented by the haplotypes constructed by C#24–30 in LD evaluation.).

The SNP for which significance was shown in the report of Walss-Bass et al. (2006) was not polymorphic in our samples.

Allele- and genotype-wise analyses did not show association either the HAP_{ICE} region or the exon region. In this haplotype-wise analysis, 3-marker haplotypes of C#5–6–7 were associated with schizophrenia (Global P-value=0.0244, uncorrected: Tables 1, 2 and 3, Supplementary Tables 3 and 4). The genotyping of C#5, C#6, C#7 in a subset of the screening samples was re-confirmed by direct sequencing, and the results were perfectly identical to those shown by TaqMan assay. Hence, we speculate that it was unlikely that genotyping error had occurred.

3.2. Imputation of ungenotyped SNP for first-set samples

Data for ungenotyped SNPs could not provide sufficient evidence for association in either region (Fig. 1). In particular, the weights of evidence for the regions near the significant haplotypes in first-set samples were less than one. Since weights of evidence of at least four are required for evidence for association

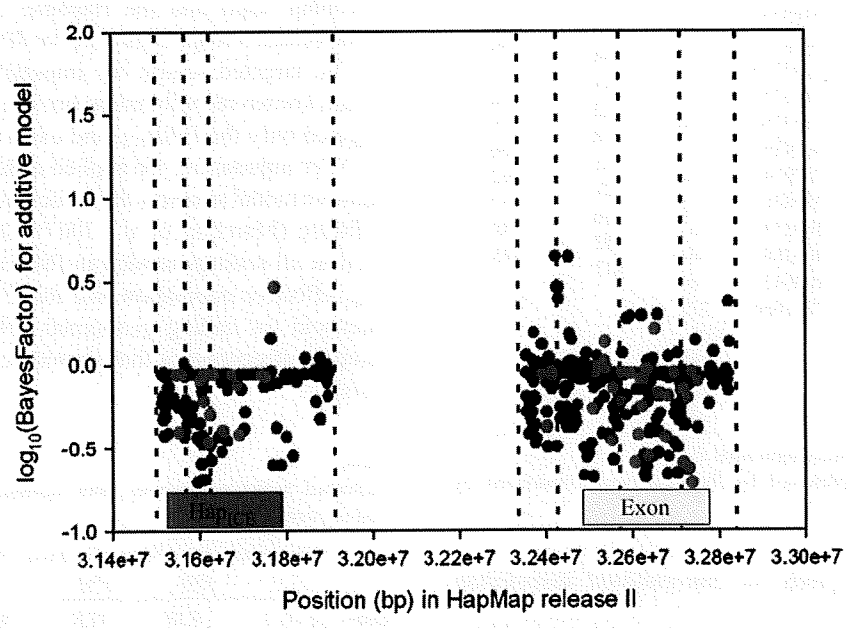

Fig. 1. Results of imputing SNP in the *NRG1* gene. The weights of evidence were calculated using imputed genotypes (red circles) and observed genotypes (black circles). Data from SNPs that constructed the significant haplotype in the first-set samples are shown in blue circles. Dotted lines indicate the estimated hot spots from the HapMap database. The SNP position from the HapMap release II database is plotted on the X axis.

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Table 4
Confirmation analysis of significant haplotypes from first-set analysis

Samples	SNPID	1-window	2-windows	3-windows
Case–control	C#5	.408		
	C#6	.362	.101	.120
	C#7	.371	.601	
Family samples	C#5	.107		
	C#6	.964	.323	.505
	C#7	.499	.846	
Combined samples	C#5	.976		
	C#6	.389	.591	.478
	C#7	.801	.303	

(if 1000 SNPs of 10,000,000 common human SNPs might be associated with a disease, we may assign a prior odds of association of 1/10,000. Therefore, a Bayes factor more than 10,000 (or $\log_{10}$ [Bayes factor] more than 4) is required (Balding, 2006)). Thus, these results indicate a low probability for association in our sample.

3.3. Confirmation analysis of the positive haplotypes using different case–control samples and family samples

To confirm the significance of exon region C#5–6–7 in the first-set samples, we conducted a confirmation analysis using independent case–control samples and family samples. In these analyses, we could not replicate this association. To increase the power, we combined samples (first-set and confirmation samples) but again we could not detect an association in this explorative analysis (Table 4).

4. Discussion

In the present study, using three large and independent samples, our data did not provide sufficient evidence for associations between tagging SNPs in the HAP_{ICE} and exon regions of *NRG1* and schizophrenia in the Japanese population.

We could not replicate previous reports for the HAP_{ICE} region (Stefansson et al., 2002; Stefansson et al., 2003); however, the results of this study are in concordance with our previous replication study in the Japanese population (schizophrenia=607, controls=515) (Iwata et al., 2004). Another study (Fukui et al., 2006), however, examined independent Japanese samples (belonging to one-third of confirmation case–control samples) and reported a positive association. Specifically, that study reported a significant association of haplotypes constructed by three core SNPs from Stefansson et al. (SNP8NRG221533 (HAP_{ICE}#3), SNP8NRG241930 (HAP_{ICE}#7) and SNP8NRG243177 (HAP_{ICE}#8)), and one more intronic SNP (rs1081062), as well as a trend for association of rs1081062. Since our tagging SNPs could not involve this SNP (rs1081062), we found by consulting the latest HapMap database (release#21a) that rs1081062 is tagged by rs13274954 (HAP_{ICE}#11); moreover, neither HAP_{ICE}#10 nor its haplotypes (HAP_{ICE}#3–7–8–11) were associated with schizophrenia (Global *P*-value= 0.540). Therefore, the aforementioned positive report could have been the result of type I error due to inadequate sample size (schizophrenia=349, controls=424) (Fukui et al., 2006). Or, as the authors speculated (Fukui et al., 2006), the different clinical backgrounds (e.g. genetic loading) in each sample could have led to inconsistent results. In this regard, a recent study reported that *DAOA/G30*, which is also a strong candidate gene for schizophrenia, influences susceptibility to the symptomatology of psychiatric disorders including schizophrenia and bipolar disorder, but not to diagnosis itself (Williams et al., 2006).

In the coding region, our results indicated the importance of controlling inflation of the type I error rate due to multiple testing, when a significant association is obtained in an analysis that involves several markers. In this study we found significant associations only from haplotype-wise analysis, not from allele- or genotype-wise analysis. It is generally accepted that a haplotype-wise analysis gives high power. At the same time, haplotype-wise analysis, especially multi-marker analysis or sliding-window analysis, tends to increase the chance of false positive results, since numerous hypotheses are examined. Bonferroni correction is typically used for solving multiple testing problems; however, since markers are not independent due to the existence of LD, Bonferroni correction is thought to be too conservative.

Therefore, we adopted two methods to validate the observed association; firstly, we imputed ungenotyped SNPs that might reflect a significant haplotype based on observations including our genotypic distribution of tagging SNPs and LD structure from the HapMap database. However, our simulation suggests that results for ungenotyped SNPs do not provide sufficient evidence for association. In other words, there was no SNP which could reflect a significant haplotype in the current data in HapMap release II. Secondly, we examined independent sets of samples for which a significant association was obtained in the initial screening analysis. We considered this to be the best strategy at present; however, the former significance of the exon region haplotype could not be replicated though independent case–control and family trios samples.

It is unlikely that negative results are due to type II error since a large sample size was used in this study; moreover, power analyses showed that the power was more than 80% when genotype relative risk (GRR) was set at 1.2–1.65 and 1.6–3.1 for confirmation case–

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control samples and family samples, respectively (MAF=2.4% and 47%), under a multiplicative model of inheritance in first-set screening samples.

Regarding interpretation of the results from this study, several limitations should be mentioned: Firstly, we did not screen the entire region of *NRG1*. On that point, Corvin et al. showed an independent 'at-risk' haplotype close to an EST cluster of unknown function (*Hs.*97362) within intron 1 of *NRG1* (Corvin et al., 2004). Secondly, our samples were not assessed with the use of the standard structured interview, and therefore have the possibility of false negatives due to misdiagnosis or sampling bias. Detailed association analyses with dense markers in the entire region of *NRG1* in well-phenotyped samples, including symptomatology, are essential in future study.

In conclusion, these results indicate that the positionally and functionally attractive regions of *NRG1* are unlikely to contribute to susceptibility to schizophrenia in the Japanese population. Moreover the nature of our results support that two-stage analysis with large sample size is appropriate to examine the susceptibility genes for common diseases; independent samples for examination of significance found in screening results should be an integral part of experimental design in genetic association analysis. Imputation methods should also be used when only haplotype association shows significance, in order to check for possible causal SNPs that can reflect the haplotype.

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Contributors

MI and NT designed the study, wrote the protocol and drafted the manuscript. MI, NT, SS, BA, YW, AN, YY, TK, YK, TK, and KK performed laboratory assays and the data-analysis. RH, HU, TI, TS, and MT advised on data-analysis. NO and NI participated in the design of the study, interpretation of the data, and drafting of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2008.01.010.

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Letter to the Editors

No association between the NDE1 gene and schizophrenia in the Japanese population

Dear Editors,

Series of studies have implicated that Disrupted in Schizophrenia 1 (DISC1) and its pathways are in the pathophysiology of schizophrenia (SZ) (Callicott et al., 2005; Hennah et al., 2003; Yamada et al., 2004). Recently, Hennah et al reported that the schizophrenic samples for the presence of SZ risk allelic haplotype (HEP3) of the DISC1 gene displayed an evidence for linkage of 16p13 (LOD=3.17) that contains the NDE1 gene. In addition, they also showed a significant association between specific haplotypes of the NDE1 gene (rs4781678-rs2242549-rs881803-rs2075512) and affected females with SZ spectrum disorders (Hennah et al., 2007). The NDE1 gene encodes a protein which interacts with the DISC1 protein (Millar et al., 2003; Brandon et al., 2004) and mouse models with NDE1 homozygous mutations displayed disordered cortex (Feng and Walsh, 2004). To confirm the association of the NDE1 gene with SZ, we performed the case-control association study in the Japanese population.

We used genomic DNA samples from 726 SZ patients: 406 male (mean age: 48.6±13.8 years), 320 female (mean age: 49.2±14.5 years) from the Tokushima University Hospital, affiliated psychiatric hospitals of the University of Tokushima, the Ehime University Hospital and the Osaka University Hospital in Japan. The diagnosis of SZ was made by at least two experienced psychiatrists according to DSM-IV criteria. 744 controls: 419 male (mean age: 45.8±11.3 years), 325 female (mean age: 45.2±10.5) were selected from volunteers without the psychiatric problems. All subjects were unrelated Japanese origin and signed written informed consent to participate in the genetic association studies approved by the institutional ethics committees. Genotyping was performed using commercially available TaqMan probes for the NDE1 gene with the Applied Biosystems 7500 Fast Real Time PCR System. We selected seven single nucleotide polymorphic (SNP) markers for genotyping from the public databases (dbSNP Home page) as reference for International Hap Map Project and Hennah's report (Hennah et al., 2007). The SNPs we selected includes six of seven of Hennah's because they are suitable for association study in the Japanese population. Haplotype block structure was determined using the HAPLOVIEW program (Barrett et al., 2005) defined according to the criteria of Gabriel et al. (Gabriel et al., 2002). Allelic and genotypic frequencies of patients and control subjects were compared using Fisher's exact test. The SNPAlyze 3.2Pro software (DYNACOM, Japan) was used to estimate haplotype frequencies, LD, permutation p values (10,000 replications) and deviation from Hardy–Weinberg (HW) distribution of alleles. Pair-wise LD indices (D' and r^2) were calculated for the control subjects. Power calculations for our sample size performed using the G*Power program (Erdfelder et al., 1996). The criterion for significance was set at $p<0.05$ for all tests.

Genotypic and allelic frequencies of the NDE1 gene are shown in Table 1. In power calculations using the G*Power program, our sample size had >0.97 power for detecting a significant association (alpha<0.05) when an effect size index of 0.2 was used. Genotypic distributions of these seven SNPs did not deviate significantly from HW equilibrium in either group ($p>0.05$). There were no significant differences in genotypic and allelic frequencies between cases and controls in all seven SNPs. LD between each pair of all the SNPs is relatively high ($D'\geqq 0.76$, $r^2\geqq 0.39$). There were two LD blocks in NDE1 with rs2242549 and rs881803 residing in block 1 and rs2075512 and rs2384933 residing in block 2. The two marker haplotypes of block 1 and block 2 were not associated with SZ (permutation $p=0.93$, 0.36, respectively). When the data were subdivided on the basis of gender, no significant association was observed in seven SNPs either in male or female samples. The two marker haplotypes of block 1 and block 2 were not associated with SZ either in male and female (permutation p of block 1=0.73 and 0.26, permutation p of block 2=0.49 and 0.21, respectively). In addition, a tag-haplotype (rs4781678-rs2242549-rs881803-rs2075512) that Hennah et al reported a significant association with SZ

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t1.1 Table 1
t1.2 Genotypes and allele frequencies for the seven polymorphism

t1.3 SNP	Total samples									Female			Male		
t1.4	Diagnosis	Allele		p-value genotype				p-value frequency		Allele		p-value	Allele		p-value
t1.5		C	A		C/C	C/A	A/A			C	A		C	A	
t1.6 rs4781678	SZ	923	519	0.56	299	325	97	0.75	0.36	408	226	0.91	515	293	0.54
t1.7	CT	963	517		321	321	98		0.349	420	228		543	289	
t1.8		C	T		C/C	C/T	T/T			C	T		C	T	
t1.9 rs6498567	SZ	814	632	0.5	226	362	135	0.58	0.437	347	289	0.26	467	343	0.96
t1.10	CT	851	627		249	353	137		0.424	372	272		479	355	
t1.11		T	G		T/T	T/G	G/G			T	G		T	G	
t1.12 rs2242549	SZ	762	690	0.61	196	370	160	0.35	0.475	324	316	0.22	438	374	0.69
t1.13	CT	793	691		221	351	170		0.466	352	298		441	393	
t1.14		C	T		C/C	C/T	T/T			C	T		C	T	
t1.15 rs881803	SZ	689	761	0.66	159	371	195	0.47	0.475	320	318	0.13	369	443	0.49
t1.16	CT	694	794		168	358	218		0.466	298	352		396	442	
t1.17		C	T		C/C	C/T	T/T			C	T		C	T	
t1.18 rs2075512	SZ	714	736	0.51	174	366	185	0.51	0.492	316	324	0.87	398	412	0.49
t1.19	CT	751	737		197	357	190		0.505	324	326		427	411	
t1.20		C	T		C/C	C/T	T/T			C	T		C	T	
t1.21 rs2384933	SZ	936	516	0.54	298	340	88	0.71	0.355	414	226	0.68	522	290	0.64
t1.22	CT	976	512		321	334	89		0.344	428	222		548	290	
t1.23		G	A		G/G	G/A	A/A			G	A		G	A	
t1.24 rs11130	SZ	699	741	0.55	162	375	183	0.07	0.485	313	323	1	386	418	0.43
t1.25	CT	738	748		197	344	202		0.497	320	330		418	418	

spectrum disorders in female was not associated with SZ either in male and female (permutation $p=0.90$, 0.054, respectively) of the Japanese population.

Although an association between specific haplotypes of NDE1 and a broad spectrum of SZ specific females was reported (Hennah et al., 2007), we could not replicate significant associations between seven NDE1 SNPs and SZ in our Japanese samples. Different results between our study and Hennah's study may be that (a) different end-state diagnosis subjects used; Hennah et al used a broad spectrum of SZ including SZ, schizoaffective disorder, schizophrenia spectrum conditions and mood disorder, (b) ethnic difference; different allele frequency and different LD patterns (Supplementary Table), (c) different sample size.

In conclusion, we failed to find the association between the NDE1 gene and SZ in the Japanese population. This gene may not play a major role in the etiology of SZ. However we can not rule out a possibility that DISC1-NDE1 interaction may be involved in the etiology of schizophrenia. Further studies will be needed to conclude whether DISC1-NDE1 interaction is associated with SZ.

Acknowledgement

This work was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology and a Grant-in-Aid for Scientific Research from the 21st Century COE program, Human Nutritional Science on Stress Control, Tokushima, Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2007.10.032.

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BRIEF REPORT

Abnormal microstructures of the basal ganglia in schizophrenia revealed by diffusion tensor imaging

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Abstract

There has been a hypothesis that deficits in the basal ganglia–thalamic system may play an important role in the dysfunctional goal-directed behaviour in schizophrenia. By using diffusion tensor imaging, we measured fractional anisotropy (FA) values in the basal ganglia–thalamic system in 42 schizophrenics and 42 matched controls to investigate microstructural tissue alterations in the basal ganglia–thalamic system in schizophrenia. Schizophrenics had significantly lower FA values in the bilateral globus pallidus and left thalamus compared to controls, suggesting that schizophrenics might have microstructural abnormalities in globus pallidus and thalamus. These data support the notion that myelination abnormalities in basal ganglia–thalamic system are related to the pathophysiology of schizophrenia.

Key words: *Schizophrenia, diffusion tensor imaging, basal ganglia, globus pallidus, MRI*

Introduction

Schizophrenia often demonstrated movement abnormalities, such as catatonia, pacing and other stereotyped behaviours considered to be associated with basal ganglia dysfunction. The basal ganglia regulates not only motor behaviours but also aspects of cognitive and limbic behaviours. There has been a hypothesis that deficits in the basal ganglia–thalamic system may play an important role in the dysfunctional goal-directed behaviour in schizophrenia (Andreasen 1999). In fact, several studies demonstrated abnormalities in the basal ganglia in schizophrenic brains, including the volume reductions of the pallidum internum of postmortem brains of patients with schizophrenia (Bogerts et al. 1985), higher volumes in the globus pallidus of previously treated patients with schizophrenia than the healthy comparison subjects and the neuroleptic-naive patients (Gur et al. 1998), fMRI evidence for basal ganglia dysfunction in subjects with schizophrenia (Menon et al. 2001), abnormality of oligodendroglial cells in caudate nucleus in schizophrenia (Uranova et al. 2001), and positive correlation between globus pallidus and the severity of global symptoms in neuroleptic-naive patients (Spinks et al. 2005).

Diffusion tensor imaging (DTI) is a relatively new technique, and it is useful for evaluating white matter abnormalities in schizophrenia. We have reported progressive changes of white matter integrity in schizophrenia using DTI (Mori et al. 2007).

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Recently, this technique was applied to investigate abnormalities of the subcortical regions in neurodegenerative diseases. Patients with Parkinson's disease had significantly decreased fractional anisotropy (FA) in the region of interest along a line between the substantia nigra and the lower part of the putamen/caudate complex, in which the nigrostriatal dopaminergic neurons are lost in Parkinson's disease, demonstrating its possibility to detect microstructural tissue alterations (Yoshikawa et al. 2004). To investigate possible microstructural abnormalities in the basal ganglia–thalamic system in schizophrenia, we measured FA values in the basal ganglia and the thalami in schizophrenics and in normal controls for comparison, as a sub-analysis of our previous study (Mori et al. 2007).

Material and methods

Subjects and clinical assessments

Forty-two patients with DSM-IV schizophrenia (26 male and 16 female, one left hander, mean age: 40.0 ± 9.3 years old, education: 13.0 ± 2.9 years, mean duration of illness; 16.8 ± 9.0 years, mean daily dose of antipsychotics (chlorpromazine equivalent): 1005.1 ± 735.3 mg/day) (Association 1994) and 42 controls (26 male and 16 female, one left hander, mean age: 39.2 ± 9.0 years old, education: 17.1 ± 3.5 years) were participated in our study. Written informed consent was obtained from all the subjects. This study has been approved by the local ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All the normal subjects were screened by a questionnaire on medical history and excluded if they had neurological, psychiatric or medical conditions that could potentially affect the central nervous system. We employed the Japanese version of National Adult Reading Test (JART) as a convenient tool to measure IQ for participants (premorbid IQ for schizophrenics). Patients had fewer years of education (two-sample t-test, $P < 0.0001$), lower scores of JART (controls: 78.8 ± 11.5, schizophrenics: 58.7 ± 25.3, two-sample t-test $P < 0.001$).

Neuroimaging analysis

MR studies were performed on a 1.5-Tesla Siemens Magnetom Vision Plus system. Axial DTI scans aligned to the plane containing anterior and posterior commissures were acquired with a pulsed-gradient, spin-echo, single-shot echo planar imaging (EPI) sequence (TR/TE = 4000/100 ms, 256 × 256 matrix, FOV 240 mm, $b = 1000$ s/mm^2, NEX = 4, 20 slices, 5 mm slice thickness, 1.5 mm gap). Diffusion was measured along six non-collinear directions, because six directions were maximum number of this Vision Plus system. For each of six gradient directions, four acquisitions were averaged. Four acquisitions without diffusion weighting ($b = 0$) were also averaged. Additionally, a three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence with a gapless series of thin sagittal sections using an MPRage sequence (TR/TE = 11.4/4.4 ms; flip angle, 15°; acquisition matrix, 256 × 256; NEX = 1, FOV 315 mm; slice thickness 1.23 mm) was acquired for evaluating the volume of grey matter (GM), WM and cerebrospinal fluid (CSF) space. Seven diffusion images acquired as above by an in-house script described previously (Mori et al. 2007) on Matlab 6.5 software (Mathworks, Inc., MA, USA). Then, the FA images were spatially normalized using high-dimensional-warping algorithm (Ashburner et al. 1999) and were matched to the FA template image (Figure 1, top). To make the FA template image, we warped FA images of four normal subjects (other than 42 control subjects) to the single-subject T1 template (skull stripped image) using spatial normalization function of SPM2 and averaged the four warped FA images. The transformed FA images were smoothed with a Gaussian kernel (the filter size, full-width half-maximum: 6 × 6 × 6 mm).

Since our interest was FA changes in the basal ganglia and thalamus, we excluded other brain areas by using an explicit mask (Figure 1, top). The resultant FA maps were analyzed using Statistical parametric mapping 2 (SPM2), which implements a 'general linear model'. To test hypotheses about regional population effects, data were analyzed by a two-sample t-test without global normalization. JART scores were treated as nuisance variables. Furthermore, we performed correlational analyses between duration of illness, age of onset, total daily dose of antipsychotic drugs (chlorpromazine equivalent) and FA value in the schizophrenics. Our a priori hypothesis is limited to the basal ganglia; however, investigation of the FA changes within this ROI is null hypothesis. Thus, we used $P < 0.05$, corrected for multiple comparisons with Family–Wise Error rate (FWE) within basal ganglia as a statistical threshold.

Results

In comparison with controls, schizophrenics had significantly lower FA values in the bilateral globus pallidus (GP) (Figure 1, bottom). Increased FA values in schizophrenics were not found in any regions (data not shown).

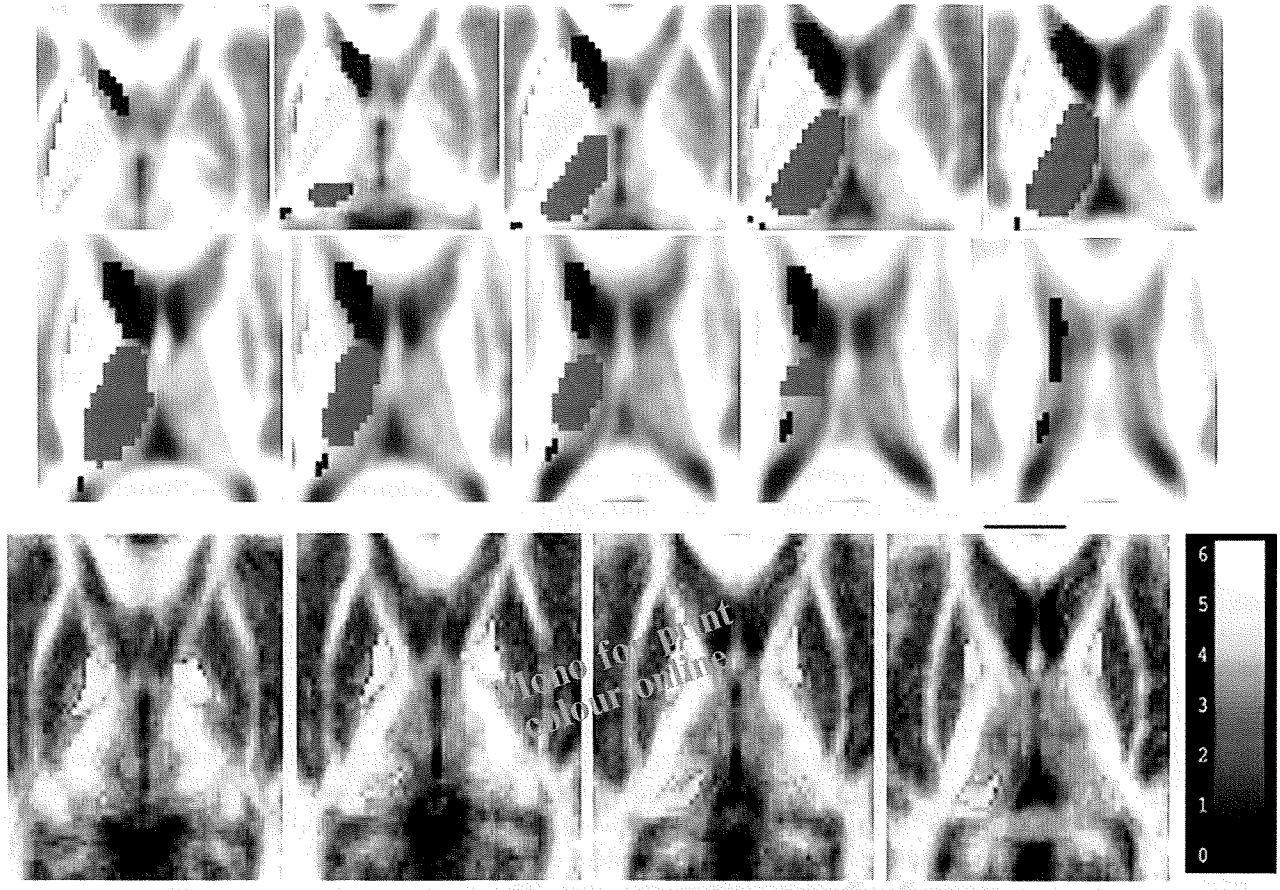

Figure 1. Top: A half of the explicit mask is displayed onto mean FA images of warped FA images obtained from 42 controls (dark blue: caudate nucleus; yellow: putamen; light blue: globus pallidus; red: thalamus). Even after averaging, the mean images are not blurred. Since globus pallidus is traversed by numerous myelinated nerve fibres, it shows higher FA value than other parts of basal ganglia. Bottom: The SPM {t} is displayed onto mean axial FA images of 42 schizophrenics. A significant reduction of FA value in schizophrenia was noted in the bilateral globus pallidus (right GP: t value = 6.52, Talairach coordinate x, y, z: 18, −2, −2, left GP: t value = 6.37, Talairach coordinate x, y, z: −18, −3, −2) and left thalamus (t value = 4.96, Talairach coordinate x, y, z: −18, −33, 10).

A correlational analysis in the schizophrenics demonstrated a significantly negative correlation between duration of illness and FA in the left head of the caudate nucleus (t value = 4.77, Talairach coordinate x, y, z: −11, −17, −6). However, there is no significant correlation between duration of illness and FA values in the GP and the thalamus. There was no significant correlation between FA values in the basal ganglia–thalamic system with age of onset or total daily dose of antipsychotic drugs.

Discussion

In this study, we found a significantly reduced FA value in the bilateral GP and left thalamus in schizophrenics compared to controls. We consider that reduced FA may reflect microstructural abnormalities in the basal ganglia–thalamic system in schizophrenia. A previous fMRI study suggested that GP itself may be the primary locus of the functional deficits in the basal ganglia and may be dysfunctional in schizophrenia (Menon et al. 2001). A postmortem study of basal ganglia morphology reported that only the GP were smaller in schizophrenics than in controls (Bogerts et al. 1985). These studies indicated functional and structural abnormalities in GP in schizophrenia. Our data, reduced FA in GP in schizophrenia, were obtained using a size-adjusted high-dimensional warping method (Ohnishi et al. 2006). Our results, microstructural abnormalities in the GP in schizophrenia, are consistent with previous reports.

Although the underlying mechanisms remain to be clarified, previous DTI studies in parkinsonism have well demonstrated ongoing pathological changes in neurodegenerative diseases, suggesting that this technique has the potential to detect microstructural alterations in the basal ganglia (Yoshikawa et al. 2004). Since pathological findings of schizophrenia are still ambiguous, the underlying pathological changes of reduced FA values in schizophrenia are unclear. However, multiple lines

of evidence now converge to implicate oligodendroglia and myelin in schizophrenia (Davis et al. 2003). We assume that damage of myelinated nerve fibres may contribute to FA reduction in the basal ganglia–thalamic system. The GP is traversed by numerous myelinated nerve fibres that give it the pale appearance for which it is named, and has rich connections to the putamen and the thalamus. These histological characteristics of the GP may contribute to its higher FA values. A qualitative electron microscopic study reported the density of concentric lamellar bodies (an indicator of damage of myelinated fibres) was dramatically increased in the caudate nucleus in schizophrenia, as compared to controls (Uranova et al. 2001). Such pathological changes seem to explain decreased FA values in the schizophrenic brain. However, there have been no data on whether GP also have alterations of myelinated fibres. Further pathological studies need to be conducted to draw a firm conclusion on this matter.

Although some studies demonstrated abnormalities of GP in neuroleptic-naïve schizophrenics (Spinks et al. 2005), abnormalities in the basal ganglia have been considered to relate to antipsychotic medication (Gur et al. 1998). In this study, FA changes in the GP and thalamus were not associated with the duration of illness or the daily dose of antipsychotic drugs, suggesting that FA changes in these regions might be independent of medication with neuroleptics. Guidelines for the biological treatment of schizophrenia developed by an international Task Force of the World Federation of Societies of Biological Psychiatry recommended atypical antipsychotics as first line drugs (Falkai et al. 2005, 2006). The differential treatment effects on brain morphology could be due to typical antipsychotics-associated toxicity or greater therapeutic effects of atypical antipsychotics (Lieberman et al. 2005). It would be interesting to compare patients treated with atypical antipsychotics to those with a history of typical antipsychotics treatment; however; the subgroup of patients that were only treated with atypical antipsychotics or the subgroup of patients that were only treated with typical antipsychotics were too small to investigate a possible difference between two groups in FA in our sample. To conclude whether observed change of FA value is a result of medication or relates to the pathophysiology of schizophrenia itself, longitudinal studies on treated schizophrenics, and studies on neuroleptic-naive schizophrenics should be conducted.

There is a limitation to our study: we used a 1.5-Tesla Siemens Magnetom Vision Plus system, which is a relatively old system. We chose six gradient directions, which is quite low, as this number is the maximum number of directions in this system. Slice thickness of 5 mm and 1.5-mm slice gaps are methodological drawbacks to this study. The reason why we used a slice thickness of 5 mm and 1.5-mm slice gaps is to cover the whole brain as in our previous study (Mori et al. 2007). There may be a partial volume effect in our mapping parameters, although we minimized the problem by using the high-dimensional warping algorithm.

Our data suggest that patients with schizophrenia might have microstructural abnormalities in globus pallidus and thalamus. The DTI study may be a promising method to investigate microstructural abnormalities in schizophrenia.

Acknowledgements/Statement of interest

We are grateful to Osamu Takizawa (Siemens) for supporting the development of a program for calculation of FA values. This work was supported in part by Grants-in-Aid from the Japanese Ministry of Health, Labor and Welfare (H17-kokoro-007 and H16-kokoro-002), the Japanese Ministry of Education, Culture, Sports, Science and Technology, and Core research for Evolutional Science and Technology of Japan Science and Technology Agency, Japan Foundation for Neuroscience and Mental Health.

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Manuscript Draft

Manuscript Number: PSY-D-07-00096R1

Title: Personality in schizophrenia assessed with the Temperament and Character Inventory (TCI)

Article Type: Research Article

Section/Category: Neurospychology

Keywords: schizophrenia; personality; temperament; character; gender difference

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Abstract: The Temperament and Character Inventory (TCI) is a well-established self-report questionnaire measuring 4 temperament and 3 character dimensions. However, surprisingly few studies have used it to examine the personality of patients with schizophrenia, and none in Japan. Moreover, possible gender differences in personality among patients with schizophrenia have not been well documented. We administered the TCI to 86 Japanese patients with schizophrenia and age- and gender-matched 115 healthy controls to characterize personality traits in patients with schizophrenia and to examine their relationships with clinical variables, particularly gender and symptoms. Compared to controls, patients demonstrated significantly lower novelty seeking, reward dependence, self-directedness and cooperativeness, and higher harm avoidance and self-transcendence. Male patients showed even more pronounced personality alteration than female patients when both of them were compared to healthy people. Personality dimensions were moderately correlated with symptom dimensions assessed by the Positive and Negative Syndrome Scale (PANSS). These results, together with prior findings in several other countries, suggest that

schizophrenia patients have a unique personality profile which appears to be present across cultures and that the greater alteration of personality in schizophrenia males might be related to their poorer social and community functioning.

Cover Letter

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May 11, 2007

Re: Personality in schizophrenia assessed with the Temperament and Character Inventory (TCI) (PSY-D-07-00096)

Dear Prof. Buchsbaum:

Thank you for providing us with an opportunity to resubmit our manuscript. We are also very grateful to the anonymous referee for valuable comments. According to the comments, we have revised our manuscript. Please refer to the answers to the referees on separate sheets. I hope that the revised version will be suitable for publication in *Psychiatry Research*.

We are looking forward to hearing from you in due course.

Sincerely,

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Answers to the editors' requirements:

Comment 1 In addition to the points raised by the reviewers, please carefully review the Guide to Authors on our website. It would be also be useful for you to review the articles contained in the sample issue on our website for examples of title page format, heading typography, and other style features.

Answer: According to the editors' comments, we have revised the manuscript as carefully as we can, based on the stylistic requirements of the *Journal*.

Comment 2 We also thought the ms needs to be shortened. You can easily combine Tables 1 and 2. It also appears that the figure duplicates the data in Table 3. Please chose one or the other.

Answer: According to the comment, we have combined the previous Tables 1 and 2 into a new table, Table 1, and removed the data on male vs. female comparisons from Table 3 since the similar data are, as pointed out by the editors, also presented in Fig. 1. Descriptions on the gender difference in the Results section have been modified accordingly (L4-19, P11).

Answers to the reviewer #1:

Comment 1 The introduction might say more about what exactly studies have previously found. What symptoms have been linked with what personality dimensions and what was made of that by previous authors? This should be spelled out as it is the context in which the authors should later interpret the results. The literature on this subject could also be more broadly noted and cited.

Answer: According to the reviewer's comment, we have increased the descriptions on what previous studies have found regarding the following issues: relationships between symptoms and personality dimensions (L14-20, P4), variability of personality as assessed with the TCI across cultures (L18-21, P3), and various aspects of gender difference unfavorable to male patients (L3-6, P5). Furthermore, several relevant references have been added to the Introduction section.

Comment 2 The introduction might say more about what specific previously unaddressed or under studied questions this study seeks to explore. What is the gap these finding intend to fill besides just using another instrument.

Answer: The questions we had considered unaddressed or under studied were gender difference and cross-cultural comparisons of personality in schizophrenia, and comparisons of TCI and NEO-FFI findings in schizophrenia. In addition, we thought that the relationships between symptoms and personality in schizophrenia remain to be further examined. According to the helpful comment, we have clearly stated the gaps this study intended to fill regarding these questions (L21, P3 to L1, P4; L9-13, P4; L20, P4 to L1, P5; L6-8, P5).

Comment 3 The introduction could include they study hypotheses. What was anticipated?

Answer: According to the helpful comments, we have added 3 hypotheses that correspond to the aims of this study (L13-19, P5).

Comment 4 I would not use the word deviant when referring to the personality of the sample. "Deviant" can have a very negative connotation, implying in some senses criminality or antisocial tendencies which certainly is not what the authors intend.

Answer: We have now realized that "deviant" has a very negative connotation which we were at first unaware of. Therefore, we have changed the word "deviant" into "altered" or "unique", and "deviance" into "alteration" throughout the manuscript.

Comment 5 The discussion could be condensed and re-organized around the study hypotheses once laid out in the introduction.

Answer: According to this comment, we have re-organized the discussion section, focusing on the 3 hypotheses laid out earlier. Furthermore, we have explicitly

described whether the hypotheses have been supported (L13, P15; L17-19, P15; L5-7, P17). The speculative discussion on causal relationships between personality and development of schizophrenia has been deleted.

Comment 6 Results could also be linked back more carefully to the results of studies using other methods of personality assessment as noted in the introduction.

Answer: According to the helpful comment, we have added the detailed descriptions on comparisons between the present TCI results and previous findings from NEO studies in the Discussion section (L19, P14 to L13, P15).

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Personality in schizophrenia assessed with the Temperament and Character Inventory (TCI)

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