found, and most deficits remained significant even after current IQ was controlled for, supporting that memory impairment is a core feature of schizophrenia (Saykin et al., 1991; Heinrichs and Zakzanis, 1998; Aleman et al., 1999). The marked impairment in verbal memory is consistent with numerous studies (e.g., Saykin et al., 1991; Heinrichs and Zakzanis, 1998). Although visual memory deficits in schizophrenia have attracted less attention from researchers than verbal memory, several studies have reported substantial impairment of visual memory (Saykin et al., 1991; Aleman et al., 1999), consistent with the present study. The pronounced impairment in delayed recall observed here is also in line with prior reports (Aleman et al., 1999; Dickinson et al., 2004). Deficits of verbal and spatial working memory in schizophrenia tapped by the Wechsler digit span backward and spatial span backward subtests, respectively, are fairly consistent findings (Conklin et al., 2000; Silver et al., 2003; Dickinson et al., 2004), which were replicated in the current study. Previous studies have reported that the performance on the forward digit span task of schizophrenia patients is significantly poorer than that of healthy people, indicating impaired attentional function in schizophrenia (Conklin et al., 2000; Silver et al., 2003). The findings of the present study, by contrast, suggest that auditory attention as measured by the forward digit span subtest is preserved in schizophrenia. The discrepant findings regarding auditory attention in the present study relative to previous ones might be due in part to the distinct matching status between patients and controls regarding education and premorbid IQ.

In conclusion, our results suggest that patients with chronic schizophrenia have substantially lower intellectual function relative to their presumed premorbid level and that their memory impairment is even more severe than the IQ decline. To definitively delineate the lifetime course of cognitive decline in schizophrenia, longitudinal studies that range from childhood to the chronic phase are needed.

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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.psychres.2007.11.002.

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Failure to replicate the association between *NRG1* and schizophrenia using Japanese large sample

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Abstract

Systematic linkage disequilibrium (LD) mapping of 8p12–21 in the Icelandic population identified neuregulin 1 (*NRG1*) as a prime candidate gene for schizophrenia. However, results of replication studies have been inconsistent, and no large sample analyses have been reported. Therefore, we designed this study with the aim of assessing this putative association between schizophrenia and *NRG1* (especially HAP_{ICE} region and exon region) using a gene-based association approach in the Japanese population.

This study was a two-stage association analysis with a different panel of samples, in which the significant association found in the first-set screening samples (1126 cases and 1022 controls) was further assessed in the confirmation samples (1262 cases and 1172 controls, and 166 trio samples). In the first-set scan, 60 SNPs (49 tagging SNPs from HapMap database, four SNPs from other papers, and seven SNPs detected in the mutation scan) were examined.

One haplotype showed a significant association in the first-set screening samples (Global *P*-value = 0.0244, uncorrected). However, we could not replicate this association in the following independent confirmation samples. Moreover, we could not find sufficient evidence for association of the haplotype identified as being significant in the first-set samples by imputing ungenotyped SNPs from HapMap database.

Abbreviations: NRG1, neuregulin 1; SNP, single nucleotide polymorphism; GGF2, glial growth factor 2; LD, linkage disequilibrium; dHPLC, denaturing high performance liquid chromatography; MAF, minor allele frequency; TDT, transmission disequilibrium test; UTR, untranslated region.

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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.

Keywords: Schizophrenia; Neuregulin 1; Association study; False positive; Linkage disequilibrium

1. Introduction

Schizophrenia is a common psychiatric disorder with a lifetime prevalence of 1% worldwide. Family, twin and adoption studies show conclusive evidence of a substantial genetic component in this disorder. Progress towards detecting these genetic elements is now being made (Harrison and Weinberger, 2005).

The neuregulin 1 gene (*NRG1*) was first reported to be a prime candidate gene for schizophrenia in the Icelandic population (Stefansson et al., 2002). The significant association of a haplotype was detected in the 5′-region of glial growth factor 2 (GGF2) isoforms, and this at-risk haplotype, consisting of five single nucleotide polymorphisms (SNPs) and two microsatellites, was named as HAP_{ICE}. Several subsequent studies provided the following evidence to support this association with schizophrenia.

Firstly, the location of this gene corresponds to the linkage regions for schizophrenia (8p12–21, OMIM: SCZ5), which were identified by recent meta-analyses of genome-wide linkage studies (Badner and Gershon 2002; Lewis et al., 2003). Secondly, recent evidence suggests that mutation within the *NRG1* region might give rise to functional alterations that are in line with the neurodevelopmental hypothesis and glutamate/GABA hypothesis of schizophrenia (Corfas et al., 2004).

Thirdly, several independent association studies have replicated the original significant association found by Stefansson et al. (2002). However, the results of replication studies using the identical number or fewer sets of markers have been inconsistent. Thus, while some research groups did not report any association (Iwata et al., 2004), other studies showed a positive association but showed different 'at-risk' haplotypes to be associated with schizophrenia (Harrison and Law, 2006).

These inconsistent results could stem from the possibility that *NRG1* is not involved in the etiology of schizophrenia in all populations. However, this inconsistency could be a consequence of the unique structure of the human genome. In other words, differences in linkage disequilibrium (LD) among populations may also be responsible for the differences in the results, and the negative findings may only indicate a failure to reflect the actual predisposing variants due to the differences in populations.

Therefore, gene-wide (or region-wide) replication analysis based on LD pattern within the *NRG1* region is essential to detect an association in a certain population setting (Neale and Sham, 2004). In such analyses, particular attention should be paid to selection of genetic variants which adequately reflect the LD background in the targeted population (e.g. tagging SNPs).

Although the above-mentioned LD-based association analysis is based on the common disease–common variant hypothesis, one study reported an association between *NRG1* and schizophrenia from the standpoint of the common disease–rare variant hypothesis (Walss-Bass et al., 2006). The authors scanned the whole exon region, detected a non-synonymous SNP in exon 11, and showed a significant association of this SNP with schizophrenia. Detection of rare but potent functional variants relies on large mutation scan samples; however, such rare variants may also differ among populations (Pritchard, 2001).

Thus, in this study, we first focused on two attractive regions: the 5′ regions of GGF2, where the original study showed the association (henceforth referred to as 'HAP_{ICE} region') and the exon region (henceforth referred to as 'exon region'). In the exon region, prior to association analysis of tagging SNPs, we performed a mutation scan in order to detect the existence of possible potent functional variants in the ethnic samples. In addition, this study was a two-stage association analysis with a different panel of samples, in which the significant association in the first-set screening samples (1126 cases and 1022 controls) was further assessed in confirmation samples (1262 cases, 1172 controls, and 166 trio samples). This approach was adopted in order to avoid the possibility of type I or type II error.

2. Methods and materials

2.1. Subjects

Two independent sample sets were used in this study. For the first-set screening analysis, 1126 patients with schizophrenia (627 male and 499 female; mean age± standard deviation (SD) 47.0±15.3 years) and 1022

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healthy controls (530 male and 492 female; 38.8± 14.5 years) were examined. Confirmation analysis was conducted with three samples consisting of: (a) 1262 patients with schizophrenia (662 male and 600 female; 49.1±14.5 years) (b) 1172 controls (576 male and 596 female; 41.7±14.3 years), and (c) 166 family trios samples (of the patients, 91 male and 75 female; 30.0± 8.3 years).

The subjects for mutation search were 96 patients with schizophrenia. These subjects were also included in the first-set samples. 385 cases and 336 controls in the first-set samples, and 349 cases (including 84 cases from family samples) and 424 controls in confirmation samples are identical to those in our previous report (Iwata et al., 2004) and Fukui et al.'s (2006) report, respectively.

Characterization details and psychiatric assessment of these subjects were as follows. The patients were diagnosed according to DSM-IV criteria consensus of at least two experienced psychiatrists on the basis of unstructured interviews and review of medical records. All healthy controls were also psychiatrically screened based on unstructured interviews. All subjects were ethnically Japanese.

After the study had been described to subjects, written informed consent was requested from each. This study was approved by the ethics committees at Fujita Health University, Teikyo University, Okayama University, Osaka University, Niigata University and Nagoya University Graduate School of Medicine.

2.2. Mutation scan

We performed denaturing high performance liquid chromatography (dHPLC) analysis, details of which can be seen in a previous paper (Ikeda et al., 2005). Primer sequences were designed in accordance with another report (Walss-Bass et al., 2006).

2.3. Tagging SNP selection

We included the three signal SNPs (SNP8NRG221533, SNP8NRG241930 and SNP8NRG243177) from the report of Stefansson et al. (2002) (we excluded SNP8NRG221132 and SNP8NRG433E1006 from the first-set analysis due to low minor allele frequencies (MAFs) in the Japanese population), one positive SNP from the report of Walss-Bass et al. (2006), and SNPs we detected in the mutation scan. Next we consulted the HapMap database (release#19, population: Japanese in Tokyo (JPT), MAF: more than 0.05). In this step, we determined the boundaries of the 'HAP_{ICE} regions' that cover 5′ regions including 19,425 bp and 155,564 bp downstream (3′) from the significant SNPs (SNP8NRG221132 and SNP8NRG433E1006, respectively) in Stefansson's report (Table 1 and Supplementary Fig. 1) (Stefansson et al., 2002), and of the 'exon regions' that cover 5′ regions including 120,576 bp from the first exon and 3510 bp downstream 3′ from the last exon (GenBank accession No. NT_007995: Table 2 and Supplementary Fig. 2). Then fifteen and thirty-four 'tagging SNPs' for the HAP_{ICE} regions and exon regions, respectively were selected with the criterion of an r^2 threshold greater than 0.8 in 'Aggressive tagging: use 2- and 3-markers haplotypes' mode of the 'Tagger' program (de Bakker et al., 2005), a function of HAPLOVIEW software (Barrett et al., 2005).

2.4. SNP genotyping

All SNPs were genotyped by TaqMan assay (Applied Biosystems Japan Ltd, Tokyo).

The genotyping of C#5, C#6, C#7 (which were positive SNPs in the first-set screening analysis) was done with 768 randomly selected samples (384 cases and 384 control subjects) with direct sequencing to check for genotyping error. Detailed information including primer sequences of custom TaqMan SNP genotyping assays can be seen in Supplementary Tables 1 and 2.

Table 1
First-set case control analysis of HAP_{ICE} region

Markers	SNP ID	*P*-values		
		1-window	2-windows	3-windows
HAP_{ICE}#1	rs12674974	.0794		
HAP_{ICE}#2	rs4513929	.846	.181	
HAP_{ICE}#3	SNP8NRG221533	.188	.384	.196
HAP_{ICE}#4	rs10096573	.200	.397	.620
HAP_{ICE}#5	rs4733263	.310	.414	.462
HAP_{ICE}#6	rs4733263	.274	.616	.267
HAP_{ICE}#7	SNP8NRG241930	.724	.399	.578
HAP_{ICE}#8	SNP8NRG243177	.288	.113	.326
HAP_{ICE}#9	rs4733267	.769	.520	.492
HAP_{ICE}#10	rs13277456	.862	.889	.190
HAP_{ICE}#11	rs13274954	.457	.736	.847
HAP_{ICE}#12	rs12677942	.312	.670	.255
HAP_{ICE}#13	rs4403369	.0803	.271	.128
HAP_{ICE}#14	rs4566990	.625	.268	.548
HAP_{ICE}#15	rs13270788	.541	.628	.525
HAP_{ICE}#16	rs1503491	.813	.730	.699
HAP_{ICE}#17	rs2202262	.704	.866	.0960
HAP_{ICE}#18	rs10087212	.682	.324	.0653
HAP_{ICE}#4–#5		.414		
HAP_{ICE}#14–#16		.247		
HAP_{ICE}#15–#16		.730		

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

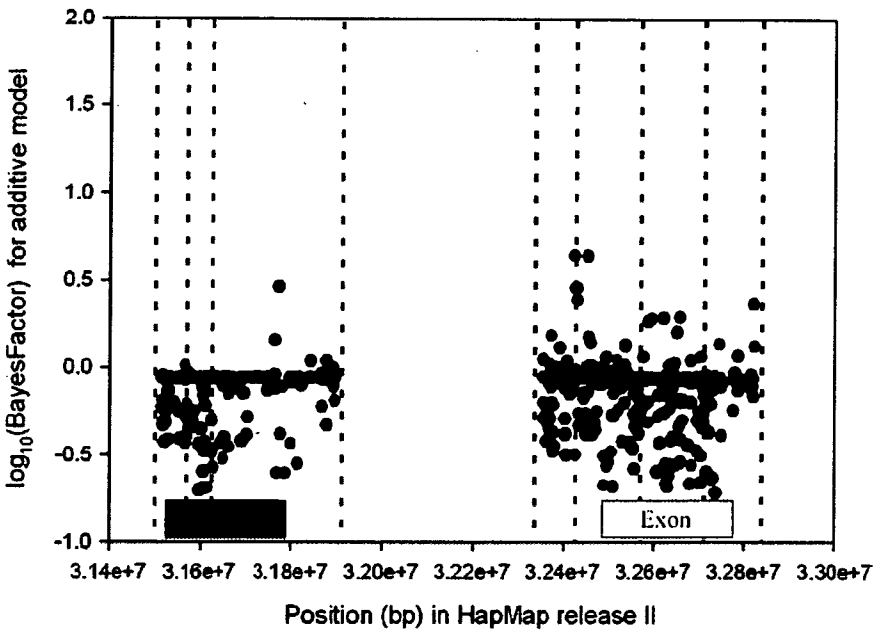

Fig. 1. Results of imputing SNP in the *NRG1* gene. The weights of evidence were calculated using imputed genotypes (red circles) and observed genotypes (black circles). Data from SNPs that constructed the significant haplotype in the first-set samples are shown in blue circles. Dotted lines indicate 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.

Acknowledgements

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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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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).

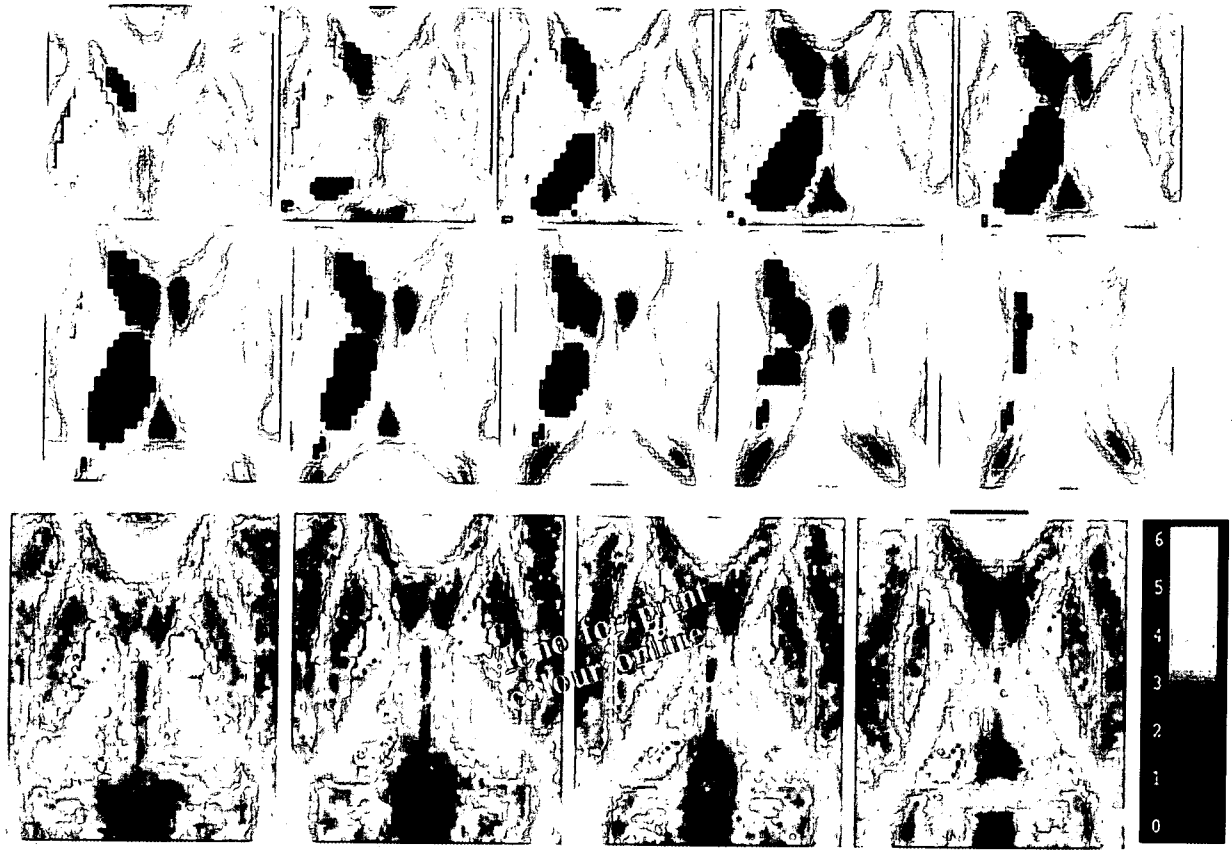

Figure 1. Top: A half of the explicit mask is displayed onto mean FA images of warped FA images obtained from 42 controls (dark blue: caudate nucleus; yellow: putamen; light blue: globus pallidus; red: thalamus). Even after averaging, the mean images are not blurred. Since globus pallidus is traversed 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

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