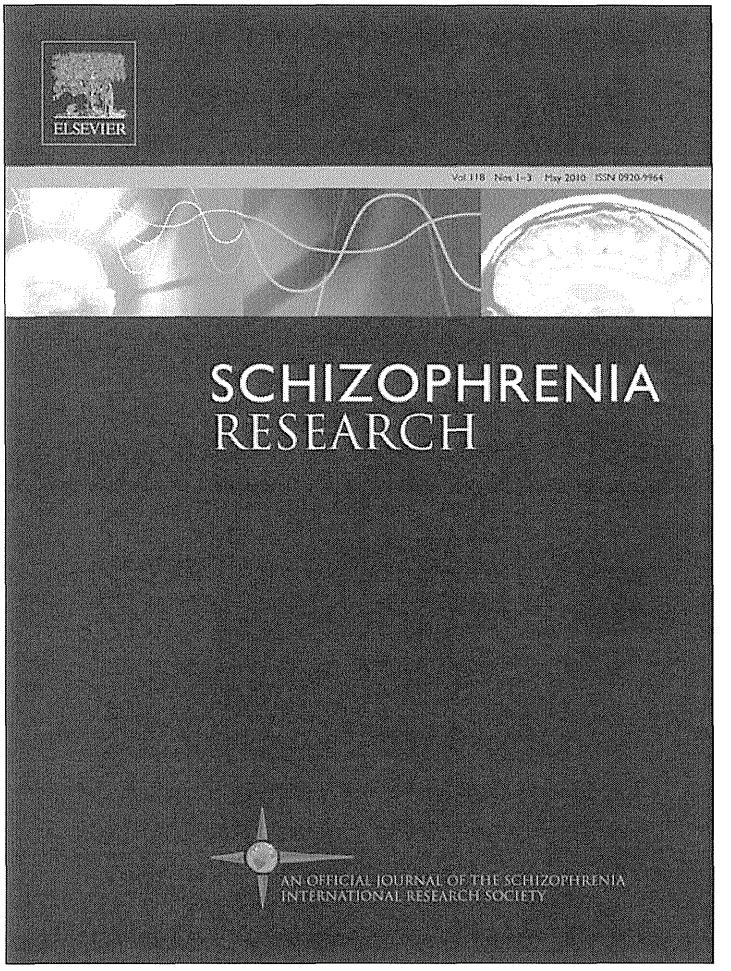

Schizophrenia Research 118 (2010) 106–112

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Failure to find an association between *myosin heavy chain 9, non-muscle* (*MYH9*) and schizophrenia: A three-stage case–control association study

Hideki Amagane [a,1], Yuichiro Watanabe [a,b,1], Naoshi Kaneko [a,*], Ayako Nunokawa [a], Tatsuyuki Muratake [c], Hiroki Ishiguro [d], Tadao Arinami [d], Hiroshi Ujike [e], Toshiya Inada [f], Nakao Iwata [g], Hiroshi Kunugi [h], Tsukasa Sasaki [i], Ryota Hashimoto [j], Masanari Itokawa [k], Norio Ozaki [l], Toshiyuki Someya [a]

[a] *Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, 757 Asahimachidori-ichibancho, Chuo-ku, Niigata 951-8510, Japan*
[b] *Health Administration Center, Niigata University, 8050 Ikarashi-ninocho, Nishi-ku, Niigata 950-2181, Japan*
[c] *Furumachi Mental Clinic, 608 Furumachidori-gobancho, Chuo-ku, Niigata 951-8063, Japan*
[d] *Department of Medical Genetics, Doctoral Program in Social and Environmental Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan*
[e] *Department of Neuropsychiatry, Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan*
[f] *Seiwa Hospital, Institute of Neuropsychiatry, 91 Bentencho, Shinjuku-ku, Tokyo 162-0851, Japan*
[g] *Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan*
[h] *Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan*
[i] *Health Service Center, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan*
[j] *The Osaka-Hamamatsu Joint Research Center for Child Mental Development, Osaka University Graduate School of Medicine, D3, 2-2, Yamadaoka, Suita, Osaka, 5650871, Japan*
[k] *Schizophrenia Research Project, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, Japan*
[l] *Department of Psychiatry, School of Medicine, Nagoya University, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan*

ARTICLE INFO

Article history:
Received 30 October 2009
Received in revised form 26 January 2010
Accepted 27 January 2010
Available online 26 February 2010

Keywords:
Chromosome 22
Case–control study
Japanese
MYH9
Schizophrenia

ABSTRACT

Several genome-wide linkage studies have suggested linkage between markers on the long arm of chromosome 22 and schizophrenia. It has also been reported that 22q11.2 deletions increase the risk of schizophrenia. Therefore, 22q is a candidate region for schizophrenia. To search for genetic susceptibility loci for schizophrenia on 22q, we conducted a three-stage case–control association study in Japanese individuals. In the first stage, we examined 13 microsatellite markers on 22q in 766 individuals (340 patients with schizophrenia and 426 control individuals) and found a potential association of AFM262VH5 (D22S283) with schizophrenia. In the second stage, we performed fine mapping of the *myosin heavy chain 9, non-muscle* (*MYH9*) gene, where AFM262VH5 is located, using 25 tagging single nucleotide polymorphisms (SNPs). We obtained potential associations between three SNPs in *MYH9* and schizophrenia in 1193 individuals (595 patients and 598 controls), which included the individuals analyzed in the first stage. In the third stage, however, we could not replicate these associations in 4694 independent individuals (2288 patients and 2406 controls). Our results suggest that *MYH9* does not confer increased susceptibility to schizophrenia in the Japanese population, although we could not exclude possible contributions of other genes on 22q to the pathogenesis of schizophrenia.

1. Introduction

Several genome-wide linkage studies have suggested linkage between markers on the long arm of chromosome 22 and schizophrenia (Blouin et al., 1998; DeLisi et al., 2002; Faraone et al., 2006; Williams et al., 2003). Two meta-analyses provided

* Corresponding author. Tel.: +81 25 227 2213; fax: +81 25 227-0777.
E-mail address: kane704@med.niigata-u.ac.jp (N. Kaneko).
[1] These authors contributed equally to this work.

0920-9964/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.schres.2010.01.023

supportive evidence for susceptibility loci for schizophrenia on 22q (Badner and Gershon, 2002; Lewis et al., 2003), whereas a multicenter study and the most recent meta-analysis conducted both failed to find linkage of 22q to schizophrenia (Mowry et al., 2004; Ng et al., 2009). There is a higher incidence of schizophrenia among patients with velocardiofacial syndrome (Murphy et al., 1999; Shprintzen et al., 1992), which is associated with a hemizygous interstitial deletion of 22q11.2. It has been reported that interstitial deletion of 22q11.2 increases the risk of schizophrenia (Arinami, 2006; Karayiorgou et al., 1995), and this was confirmed by recent genome-wide surveys of rare copy number variants (International Schizophrenia Consortium, 2008; Xu et al., 2008). In addition, there are some interesting candidate genes for schizophrenia in this region including *proline dehydrogenase 1* (*PRODH*) (Liu et al., 2002), *catechol-O-methyltransferase* (*COMT*) (Shifman et al., 2002) and *zinc finger, DHHC-type containing 8* (*ZDHHC8*) (Mukai et al., 2004). Therefore, 22q is a candidate region for schizophrenia, although the results of previous studies are not necessarily consistent.

To search for genetic susceptibility loci for schizophrenia on 22q, we conducted a three-stage case–control association study in Japanese individuals. In the first stage, we examined 13 microsatellite markers on 22q in 766 individuals (340 patients with schizophrenia and 426 control individuals) and found a potential association of AFM262VH5 (D22S283) with schizophrenia. In the second stage, we performed a fine mapping of the *myosin heavy chain 9, non-muscle* (*MYH9*) gene, where AFM262VH5 is located, using 25 tagging single nucleotide polymorphisms (SNPs) in 1193 individuals (595 patients and 598 controls), which included the individuals analyzed in the first stage. In the third stage, potential associations obtained in the second stage were further assessed in 4694 independent individuals (2288 patients and 2406 controls).

2. Materials and methods

2.1. Subjects

The present study was approved by the Ethics Committee of each participating institute, and written informed consent was obtained from each participant. All participants were unrelated Japanese individuals.

The screening population in the first stage consisted of 340 patients with schizophrenia (180 men and 160 women; mean age, 41.8 [SD 14.9] years) and 426 control individuals (219 men and 207 women: mean age, 38.3 [SD 10.4] years). The expanded screening population in the second stage consisted of 595 patients with schizophrenia (313 men and 282 women; mean age, 40.2 [SD 14.1] years) and 598 control individuals (311 men and 287 women; mean age, 38.1 [SD 10.5] years). The expanded screening population included the screening population. The confirmatory population in the third stage consisted of 2288 patients with schizophrenia (1213 men and 1075 women; mean age, 46.5 [SD 14.4] years) and 2406 control individuals (1270 men and 1136 women; mean age, 45.9 [SD 13.9] years), and this population did not overlap with the expanded screening population.

We conducted a psychiatric assessment of every participant, as described previously (Watanabe et al., 2006). In brief, the patients were diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* (DSM-IV) criteria by at least two experienced psychiatrists, on the basis of all available sources of information, including unstructured interviews, clinical observations and medical records. The control individuals were mentally healthy subjects with no self-reported history of psychiatric disorders; they showed good social and occupational skills, but were not assessed using a structured psychiatric interview.

2.2. Genotyping

Initially, we screened 13 microsatellite markers on 22q with an average inter-marker interval of 2.63 Mb. All microsatellite markers were genotyped using an ABI 377 genetic analyzer (Applied Biosystems, Foster City, CA) with the GeneScan program v2.1 (Applied Biosystems), as described previously (Kaneko et al., 2007). The sequences of primers used for amplification are available upon request.

Next, we examined 25 tagging SNPs for *MYH9*, covering gene region and the 5′ and 3′ flanking regions (chr22:34996074... 35125125). These tagging SNPs were selected from the HapMap database (release #22, population: Japanese in Tokyo [JPT], minor allele frequency [MAF]: more than 0.05). We applied the criterion of an r^2 threshold greater than 0.8 in 'aggressive tagging: use 2- and 3-marker haplotype' mode using the 'Tagger' program (de Bakker et al., 2005), as implemented in Haploview v3.32 (Barrett et al., 2005). All SNPs were genotyped using the TaqMan 5′-exonuclease assay, as described previously (Watanabe et al., 2006). The sequences of probes used for the TaqMan assay are available upon request.

2.3. Statistical analysis

Deviation from the Hardy–Weinberg equilibrium (HWE) of microsatellite markers was tested using the GENEPOP v4.0.9 program (Rousset, 2008). The allele frequencies of microsatellite markers between patients and control individuals were compared using CLUMP v2.3 (Sham and Curtis, 1995). The number of simulations was 10,000 in each test, and the T1 statistic was adopted.

Deviations from the HWE for any of the SNPs were tested using the likelihood ratio test. Linkage disequilibrium (LD) blocks defined in accordance with Gabriel's criteria (Gabriel et al., 2002) and haplotype frequencies were determined using Haploview v4.01. The genotype, allele and haplotype frequencies of SNPs in patients and control subjects were compared using χ^2 test or Fisher's exact test. A probability level of $p<0.05$ was considered to indicate statistical significance.

A power calculation was performed using the Genetic Power Calculator (Purcell et al., 2003). Power was estimated with an α of 0.05, assuming a disease prevalence of 0.01 and the risk allele frequencies to be the values observed in control individuals.

3. Results

Initially, we examined 13 microsatellite markers on chromosome 22q in the screening population (Table 1). However, the alleles of AFM268YG1 (D22S1170) could not be precisely assigned. Mean heterozygosity for 12 markers was 0.747. The genotype distribution of no marker deviated significantly from the HWE in either group. We observed a potential association of

Table 1
Case–control association study of 13 microsatellite markers on 22q in the screening population.

Marker	Patients		Controls		Heterozygosity	Allelic p
	n	HWE	n	HWE		
AFM217XF4 (D22S420)	338	0.445	424	0.186	0.730	0.593
AFMA037ZD1 (D22S539)	337	0.503	424	0.107	0.529	0.144
AFM309WD5 (D22S1174)	325	0.585	398	0.638	0.822	0.675
AFM183XE9 (D22S315)	338	0.110	426	0.525	0.821	0.704
AFMA298YB5 (D22S1154)	338	0.157	417	0.305	0.520	0.907
AFMB294ZC1 (D22S1163)	335	0.651	423	0.533	0.718	0.192
AFM225XF6 (D22S280)	337	0.872	423	0.635	0.806	0.803
AFM168XA1 (D22S277)	339	0.253	426	0.697	0.866	0.608
AFM262VH5 (D22S283)	332	0.077	417	0.296	0.795	0.047
AFM261XD9 (D22S423)	339	0.722	425	0.888	0.787	0.318
AFM164TH8 (D22S274)	337	0.319	424	0.172	0.823	0.728
AFM268YG1 (D22S1170)		NA		NA	NA	NA
AFMB337ZH9 (D22S1169)	338	0.751	424	0.758	0.748	0.674

HWE, Hardy–Weinberg equilibrium; NA, not analyzed.

AFM262VH5 (D22S283) with schizophrenia (allelic $p = 0.047$), suggesting that there may be susceptibility loci for schizophrenia near this marker.

Because AFM262VH5 is located in intron 1 of *MYH9*, we investigated 25 tagging SNPs for *MYH9* in the expanded screening population (Table 2). The genotype distribution of no SNP deviated significantly from the HWE in either group. We found potential associations of rs1557538 (SNP#11) in intron 11, rs5756154 (SNP#15) in intron 5, and rs739096 (SNP#17) in intron 2 with schizophrenia (allelic $p = 0.021$, 0.023 and 0.020, respectively). In *MYH9*, five LD blocks were defined (Table 3). The haplotype 2–1–2 of block 4, which contained the minor allele of rs5756154, the major allele of rs11704382 and the minor allele of rs739096, was potentially associated with schizophrenia ($p = 0.024$).

To confirm the potential associations of rs1557538, rs5756154 and rs739096 with schizophrenia, we examined these SNPs in the confirmatory population (Table 4). However, we were unable to replicate these associations in the confirmatory population or a combined population comprising the expanded screening and confirmatory populations. Because rs5756154 (SNP#15) and rs739096 (SNP#17) were in LD, we

Table 2
Genotype and allele frequencies of 25 tagging SNPs in *MYH9* in the expanded screening population.

SNP #	db SNP ID	Allele[a]	Patients					Controls					p	
			n	1/1[b]	1/2[b]	2/2[b]	MAF	n	1/1[b]	1/2[b]	2/2[b]	MAF	Genotype	Allele
1	rs4821475	T/C	594	282	249	63	0.316	598	294	252	52	0.298	0.520	0.341
2	rs767855	C/T	593	498	92	3	0.083	598	500	94	4	0.085	0.983[c]	0.815
3	rs11703176	A/C	593	252	264	77	0.352	591	249	271	71	0.349	0.840	0.877
4	rs735854	C/T	595	354	214	27	0.225	596	348	218	30	0.233	0.885	0.642
5	rs5756129	C/T	592	311	226	55	0.284	595	309	239	47	0.280	0.610	0.831
6	rs5756130	C/T	595	470	119	6	0.110	597	474	118	5	0.107	0.961[c]	0.821
7	rs2239788	A/G	593	537	55	1	0.048	598	542	55	1	0.048	1.000[c]	0.963
8	rs5756133	T/A	595	443	139	13	0.139	594	465	120	9	0.116	0.265	0.100
9	rs2239781	T/C	593	233	270	90	0.379	597	239	270	88	0.373	0.958	0.767
10	rs3830104	T/C	593	430	149	14	0.149	597	425	154	18	0.159	0.741	0.504
11	rs1557538	A/G	594	349	217	28	0.230	596	391	182	23	0.191	0.051	0.021
12	rs9610489	C/T	595	280	261	54	0.310	598	286	256	56	0.308	0.932	0.899
13	rs2239784	C/T	594	428	152	14	0.152	598	429	159	10	0.150	0.666	0.900
14	rs1005570	G/A	595	482	109	4	0.098	598	482	110	6	0.102	0.889[c]	0.764
15	rs5756154	C/T	594	420	165	9	0.154	595	458	129	8	0.122	0.047	0.023
16	rs11704382	C/A	595	473	117	5	0.107	597	470	120	7	0.112	0.845[c]	0.667
17	rs739096	G/C	592	426	157	9	0.148	597	467	122	8	0.116	0.043	0.020
18	rs11089788	C/A	592	538	53	1	0.046	595	555	38	2	0.035	0.205[c]	0.170
19	rs9306310	G/A	595	518	76	1	0.066	598	533	63	2	0.056	0.431[c]	0.330
20	rs933224	T/C	595	406	170	19	0.175	598	412	163	23	0.175	0.754	0.998
21	rs6000262	A/G	595	396	181	18	0.182	597	407	165	25	0.180	0.363	0.885
22	rs2294356	C/A	595	458	125	12	0.125	598	454	130	14	0.132	0.877	0.615
23	rs5756168	T/C	595	499	91	5	0.085	597	516	77	4	0.071	0.431[c]	0.213
24	rs9610498	G/A	592	527	64	1	0.056	598	543	51	4	0.049	0.174[c]	0.483
25	rs11703137	G/A	595	303	247	45	0.283	598	330	223	45	0.262	0.306	0.238

SNP, single nucleotide polymorphism; *MYH9*, *myosin, heavy chain 9, non-muscle*; MAF, minor allele frequency.
[a] Major/minor alleles.
[b] Genotypes, major and minor alleles are denoted by 1 and 2, respectively.
[c] Calculated using Fisher's exact test.

Table 3
Haplotype analyses of five LD blocks in *MYH9* in the expanded screening population.

Haplotype	Patients	Controls	*p*
Block 1 (SNP #2–3–4)			0.863 [a]
1–1–1	0.648	0.650	0.908
1–2–2	0.142	0.148	0.683
1–2–1	0.128	0.118	0.430
2–2–2	0.082	0.085	0.828
Block 2 (SNP #5–6)			0.121 [a]
1–1	0.606	0.613	0.736
2–1	0.284	0.280	0.831
1–2	0.110	0.107	0.826
Block 3 (SNP #8–9)			0.252 [a]
1–1	0.619	0.625	0.740
1–2	0.243	0.258	0.381
2–2	0.137	0.115	0.117
Block 4 (SNP #15–16–17)			0.073 [a]
1–1–1	0.738	0.766	0.112
2–1–2	0.146	0.115	0.024
1–2–1	0.107	0.112	0.665
Block 5 (SNP #21–22–23–24–25)			0.376 [a]
1–1–1–1–1	0.718	0.732	0.443
2–2–1–1–2	0.122	0.128	0.653
1–1–2–1–2	0.084	0.068	0.153
2–1–1–2–2	0.055	0.046	0.335

LD, linkage disequilibrium; *MYH9, myosin, heavy chain 9, non-muscle*; SNP, single nucleotide polymorphism.
Major and minor alleles are denoted by 1 and 2, respectively.
[a] Global *p* values.

performed haplotype analyses of these SNPs (Table 5). In the expanded screening population, the haplotype 1–1, which was constructed from the major alleles of rs5756154 and rs739096, was significantly less frequent in patients than in control individuals ($p=0.018$). By contrast, the haplotype 2–2, which was constructed from the minor alleles of these SNPs, was significantly more frequent in patients than in control individuals ($p=0.024$). However, these associations could not be replicated in either the confirmatory or combined populations.

4. Discussion

Our three-stage case–control association study failed to find an association between *MYH9* within the 22q region and schizophrenia in the Japanese population. In the first stage, we examined 13 microsatellite markers on 22q to pinpoint genes for association analysis. There was a potential association of the marker AFM262VH5 in *MYH9* with schizophrenia. *MYH9* encodes the heavy chain of non-muscle myosin IIA (NMHC II-A), one of three NMHC II isoforms (A, B and C). The biological functions of NMHC II-A in the brain are poorly understood. Blebbistatin, which inhibits both NMHC II-A and -B, altered the structure of dendritic spines and decreased excitatory synaptic transmission (Ryu et al., 2006). Inhibition of NMHC II-B most likely underlay the morphological and functional abnormalities of spines caused by blebbistatin because *NMHC II-B* mRNA is predominantly expressed in the human brain among the three *NMHC* isoforms (Golomb et al., 2004), and because RNAi of *NMHC II-B* altered the structure of dendritic spines similarly to blebbistatin (Ryu et al., 2006). However, it could not be excluded that NMHC II-A may be implicated in regulation of the structure and function of spines. Interestingly, it has been reported that dendritic spine density is decreased in the brains of patients with schizophrenia (Glantz and Lewis, 2000; Rosoklija et al., 2000). Although further investigation will be needed, the role of NMHC II-A in the development of dendritic spines has possible relevance to schizophrenia.

Application of corrections for multiple testing decreases the probability of type I error (false positive), but increases that of type II error (false negative). Although the sample size of the expanded screening population was moderate, the power was only 0.12–0.49 when the genotypic relative risk was set at 1.4 for homozygous risk allele carriers under the multiplicative model of inheritance. To avoid inflation of the type II error probability, we did not apply corrections for multiple testing. Replication is essential for establishing the credibility of genetic associations (NCI-NHGRI Working Group on Replication in Association Studies, 2007). Therefore, possible associations observed in the moderate-scale population were further assessed in the large-scale independent population. However, we were unable to replicate these associations. The nominally significant associations in the first and second stages were most likely the results of type I error. It is unlikely that the negative results in the third stage were caused by type II errors because the power was more than 0.8 in the confirmatory population. There is another possible explanation for the discrepancy between the results in the

Table 4
Genotype and allele frequencies of three SNPs in *MYH9* in the confirmatory and combined populations.

db SNP ID	Patients					Controls					*p*	
	n	1/1 [a]	1/2 [a]	2/2 [a]	MAF	*n*	1/1 [a]	1/2 [a]	2/2 [a]	MAF	Genotype	Allele
rs1557538												
Confirmatory	2233	1370	762	101	0.216	2375	1471	790	114	0.214	0.776	0.858
Combined	2827	1719	979	129	0.219	2971	1862	972	137	0.210	0.301	0.233
rs5756154												
Confirmatory	2257	1658	555	44	0.142	2359	1728	585	46	0.144	0.987	0.886
Combined	2851	2078	720	53	0.145	2954	2186	714	54	0.139	0.624	0.377
rs739096												
Confirmatory	2268	1706	521	41	0.133	2380	1783	555	42	0.134	0.957	0.853
Combined	2860	2132	678	50	0.136	2977	2250	677	50	0.131	0.659	0.381

SNP, single nucleotide polymorphism; *MYH9, myosin, heavy chain 9, non-muscle*; MAF, minor allele frequency.
[a] Genotypes, major and minor alleles are denoted by 1 and 2, respectively.

Table 5
Haplotype analyses for the SNPs rs5756154–rs739096 in *MYH9* in the screening, confirmatory and combined populations.

Haplotype	Patients	Controls	*p*
Expanded screening population			0.023 [a]
1–1	0.844	0.878	0.018
2–2	0.146	0.115	0.024
Confirmatory population			0.518 [a]
1–1	0.838	0.842	0.627
2–2	0.114	0.119	0.413
Combined population			0.619 [a]
1–1	0.840	0.849	0.149
2–2	0.120	0.118	0.735

Major and minor alleles are denoted by 1 and 2, respectively.
[a] Global *p* values.

second stage and those in the third stage. Allelic heterogeneity may exist for *MYH9*. In this case, it would be difficult to provide convincing evidence for an association. It might be noteworthy that a recent genome-wide association study (GWAS) of major depressive disorder suggested that there may be allelic heterogeneity for *glutamate receptor, metabotropic 7* (*GRM7*) (Muglia et al., 2008). Nevertheless, our results could not show sufficient evidence for an association of *MYH9* with schizophrenia in the Japanese population.

The genetic variants of *MYH9* have previously been tested for associations with schizophrenia. An initial study showed significantly distorted transmission of AFM262VH5 in 23 families multiply affected with schizophrenia (Vallada et al., 1995). However, subsequent case–control studies failed to replicate this association (Kitao et al., 2000; Williams et al., 1997). Our three-stage case–control study could not provide sufficient evidence for an association of *MYH9* with schizophrenia. It is noteworthy that although there were no significant associations between seven SNPs in *MYH9* and schizophrenia, four SNPs (rs3752463 in intron 9, rs1557540 in intron 3, rs713839 in intron 3, and rs739097 in intron 1) in *MYH9* were associated with a subgroup of schizophrenia patients without deficits in sustained attention (Liu et al., 2008). Considering these findings together, *MYH9* does not contribute to genetic susceptibility to schizophrenia, but may have effects on the neuropsychological endophenotypes for schizophrenia.

We recognize some limitations of the present study. First, we screened only 12 microsatellite markers with an average inter-marker interval of 2.87 Mb. Therefore, our results cannot exclude possible contributions of other genes on 22q to the pathogenesis of schizophrenia. Recent studies using large samples (more than 1000 cases and 1000 controls) have indicated that genes on 22q including *protein interacting with PRKCA 1* (*PICK1*), *claudin 5* (*CLDN5*), *DiGeorge syndrome critical region gene 2* (*DGCR2*), *armadillo repeat gene deletes in velocardiofacial syndrome* (*ARVCF*) and *COMT* are not associated with schizophrenia (Ishiguro et al., 2007, 2008a,b; Okochi et al., 2009; Sanders et al. 2008). However, to draw a definitive conclusion, further studies using large samples and sufficient markers should be carried out in various ethnic populations. Several GWAS of schizophrenia have been published (International Schizophrenia Consortium, 2009; Kirov et al., 2009; Lencz et al., 2007; Mah et al., 2006; Need et al., 2009; O'Donovan et al., 2008; Shi et al., 2009; Shifman et al., 2008; Stefansson et al., 2009; Sullivan et al., 2008). Interestingly, a polymorphism in intron 1 of *myosin XVIIIB* (*MYO18B*) on 22q12.1 was most significantly associated with schizophrenia in a large-scale GWAS (International Schizophrenia Consortium, 2009). A meta-analysis of GWAS for schizophrenia, which is currently being conducted (Psychiatric GWAS Consortium Coordinating Committee, 2009), will be useful for the process of narrowing down the region for fine mapping on 22q. Second, our individuals were not assessed using a standardized structured interview. However, the diagnosis of schizophrenia was made on the basis of all available sources of information. To the best of our knowledge, there were no control individuals who were likely to develop schizophrenia at their present stage of life. Thus, it is unlikely that our failure to find a significant association is attributable to misdiagnosis. Despite these caveats, our results suggest that *MYH9* does not confer increased susceptibility to schizophrenia in the Japanese population.

Role of funding source

Funding for this study was provided by a Grant for the Promotion of Niigata University Research Projects (to Y.W.), a Grant from the Niigata Medical Association (to Y.W.) and a Grant for the Promotion of Niigata University Research Projects (to T.M.). The funding sources had no involvement in the study design; collection, analysis and interpretation of the data; writing of the report and the decision to submit it for publication.

Contributors

Authors Amagane and Watanabe designed the study and performed genotyping. Authors Kaneko and Nunokawa undertook statistical analyses. Author Muratake designed the study. Author Ishiguro performed genotyping. Authors Arinami, Ujike, Inada, Iwata, Kunugi, Sasaki, Hashimoto, Itokawa, and Ozaki managed sample collection. Author Someya supervised the study. All authors contributed to and have approved the final manuscript.

Conflict of interest

None of the authors has a conflict of interest to declare.

Acknowledgments

The authors thank the patients, their families and the healthy volunteers for their participation; Mr. H. Kusano and Ms. N. Yamazaki for excellent technical assistance.

References

Arinami, T., 2006. Analyses of the associations between the genes of 22q11 deletion syndrome and schizophrenia. J. Hum. Genet. 51 (12), 1037–1045.

Badner, J.A., Gershon, E.S., 2002. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. Mol. Psychiatry 7 (4), 405–411.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21 (2), 263–265.

Blouin, J.L., Dombroski, B.A., Nath, S.K., Lasseter, V.K., Wolyniec, P.S., Nestadt, G., Thornquist, M., Ullrich, G., McGrath, J., Kasch, L., Lamacz, M., Thomas, M.G., Gehrig, C., Radhakrishna, U., Snyder, S.E., Balk, K.G., Neufeld, K., Swartz, K.L., DeMarchi, N., Papadimitriou, G.N., Dikeos, D.G., Stefanis, C.N., Chakravarti, A., Childs, B., Housman, D.E., Kazazian, H.H., Antonarakis, S., Pulver, A.E., 1998. Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. Nat. Genet. 20 (1), 70–73.

de Bakker, P.I., Yelensky, R., Pe'er, I., Gabriel, S.B., Daly, M.J., Altshuler, D., 2005. Efficiency and power in genetic association studies. Nat. Genet. 37 (1), 1217–1223.

DeLisi, L.E., Shaw, S.H., Crow, T.J., Shields, G., Smith, A.B., Larach, V.W., Wellman, N., Loftus, J., Nanthakumar, B., Razi, K., Stewart, J., Comazzi, M., Vita, A., Heffner, T., Sherrington, R., 2002. A genome-wide scan for linkage to chromosomal regions in 382 sibling pairs with schizophrenia or schizoaffective disorder. Am. J. Psychiatry 159 (5), 803–812.

Faraone, S.V., Hwu, H.G., Liu, C.M., Chen, W.J., Tsuang, M.M., Liu, S.K., Shieh, M.H., Hwang, T.J., Ou-Yang, W.C., Chen, C.Y., Chen, C.C., Lin, J.J., Chou, F.H., Chueh,

C.M., Liu, W.M., Hall, M.H., Su, J., Van Eerdewegh, P., Tsuang, M.T., 2006. Genome scan of Han Chinese schizophrenia families from Taiwan: confirmation of linkage to 10q22.3. Am. J. Psychiatry 163 (10), 1760–1766.

Gabriel, S.B., Schaffner, S.F., Nguyen, H., Moore, J.M., Roy, J., Blumenstiel, B., Higgins, J., DeFelice, M., Lochner, A., Faggart, M., Liu-Cordero, S.N., Rotimi, C., Adeyemo, A., Cooper, R., Ward, R., Lander, E.S., Daly, M.J., Altshuler, D., 2002. The structure of haplotype blocks in the human genome. Science 296 (5576), 2225–2229.

Glantz, L.A., Lewis, D.A., 2000. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. Arch. Gen. Psychiatry 57 (1), 65–73.

Golomb, E., Ma, X., Jana, S.S., Preston, Y.A., Kawamoto, S., Shoham, N.G., Goldin, E., Conti, M.A., Sellers, J.R., Adelstein, R.S., 2004. Identification and characterization of nonmuscle myosin II-C, a new member of the myosin II family. J. Biol. Chem. 279 (4), 2800–2808.

International Schizophrenia Consortium, 2008. Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature 455 (7210), 237–241.

International Schizophrenia Consortium, 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460 (7256), 748–752.

Ishiguro, H., Koga, M., Horiuchi, Y., Inada, T., Iwata, N., Ozaki, N., Ujike, H., Muratake, T., Someya, T., Arinami, T., 2007. *PICK1* is not a susceptibility gene for schizophrenia in a Japanese population: association study in a large case–control population. Neurosci. Res. 58 (2), 145–148.

Ishiguro, H., Imai, K., Koga, M., Horiuchi, Y., Inada, T., Iwata, N., Ozaki, N., Ujike, H., Itokawa, M., Kunugi, H., Sasaki, T., Watanabe, Y., Someya, T., Arinami, T., 2008a. Replication study for associations between polymorphisms in the CLDN5 and DGCR2 genes in the 22q11 deletion syndrome region and schizophrenia. Psychiatr. Genet. 18 (5), 255–256.

Ishiguro, H., Koga, M., Horiuchi, Y., Noguchi, E., Morikawa, M., Suzuki, Y., Arai, M., Niizato, K., Iritani, S., Itokawa, M., Inada, T., Iwata, N., Ozaki, N., Ujike, H., Kunugi, H., Sasaki, T., Takahashi, M., Watanabe, Y., Someya, T., Kakita, A., Takahashi, H., Nawa, H., Arinami, T., 2008b. Supportive evidence for reduced expression of GNB1L in schizophrenia. Schizophr Bull. 2008 Nov 14. doi:10.1093/schbul/sbn160 [Electronic publication ahead of print].

Kaneko, N., Muratake, T., Kuwabara, H., Kurosaki, T., Takei, M., Ohtsuki, T., Arinami, T., Tsuji, S., Someya, T., 2007. Autosomal linkage analysis of a Japanese single multiplex schizophrenia pedigree reveals two candidate loci on chromosomes 4q and 3q. Am. J. Med. Genet. B Neuropsychiatr. Genet. 144B (6), 735–742.

Karayiorgou, M., Morris, M.A., Morrow, B., Shprintzen, R.J., Goldberg, R., Borrow, J., Gos, A., Nestadt, G., Wolyniec, P.S., Lasseter, V.K., Eisen, H., Childs, B., Kazazian, H.H., Kucherlapati, R., Antonarakis, S.E., Pulver, A.E., Housman, D.E., 1995. Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11. Proc. Natl. Acad. Sci. U. S. A. 92 (17), 7612–7616.

Kirov, G., Zaharieva, I., Georgieva, L., Moskvina, V., Nikolov, I., Cichon, S., Hillmer, A., Toncheva, D., Owen, M.J., O'Donovan, M.C., 2009. A genome-wide association study in 574 schizophrenia trios using DNA pooling. Mol. Psychiatry 14 (8), 796–803.

Kitao, Y., Inada, T., Arinami, T., Hirotsu, C., Aoki, S., Iijima, Y., Yamauchi, T., Yagi, G., 2000. A contribution to genome-wide association studies: search for susceptibility loci for schizophrenia using DNA microsatellite markers on chromosomes 19, 20, 21 and 22. Psychiatr. Genet. 10 (3), 139–143.

Lencz, T., Morgan, T.V., Athanasiou, M., Dain, B., Reed, C.R., Kane, J.M., Kucherlapati, R., Malhotra, A.K., 2007. Converging evidence for a pseudoautosomal cytokine receptor gene locus in schizophrenia. Mol. Psychiatry 12 (6), 572–580.

Lewis, C.M., Levinson, D.F., Wise, L.H., DeLisi, L.E., Straub, R.E., Hovatta, I., Williams, N.M., Schwab, S.G., Pulver, A.E., Faraone, S.V., Brzustowicz, L.M., Kaufmann, C.A., Garver, D.L., Gurling, H.M.D., Lindholm, E., Coon, H., Moises, H.W., Byerley, W., Shaw, S.H., Mesen, A., Sherrington, R., O'Neill, F.A., Walsh, D., Kendler, K.S., Ekelund, J., Paunio, T., Lönnqvist, J., Peltonen, L., O'Donovan, M.C., Owen, M.J., Wildenauer, D.B., Maier, W., Nestadt, G., Blouin, J.-L., Antonarakis, S.E., Mowry, B.J., Silverman, J.M., Crowe, R.R., Cloninger, C.R., Tsuang, M.T., Malaspina, D., Harkavy-Friedman, J.M., Svrakic, D.M., Bassett, A.S., Holcomb, J., Kalsi, G., McQuillin, A., Brynjolfson, J., Sigmundsson, T., Petursson, H., Jazin, E., Zoëga, T., Helgason, T., 2003. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. Am. J. Hum. Genet. 73 (1), 34–48.

Liu, H., Heath, S.C., Sobin, C., Roos, J.L., Galke, B.L., Blundell, M.L., Lenane, M., Robertson, B., Wijsman, E.M., Rapoport, J.L., Gogos, J.A., Karayiorgou, M., 2002. Genetic variation at the 22q11 PRODH2/DGCR6 locus presents an unusual pattern and increases susceptibility to schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 99 (6), 3717–3722.

Liu, Y.L., Fann, C.S., Liu, C.M., Chen, W.J., Wu, J.Y., Hung, S.I., Chen, C.H., Jou, Y.S., Liu, S.K., Hwang, T.J., Hsieh, M.H., Chang, C.C., Yang, W.C., Lin, J.J., Chou, F.H., Faraone, S.V., Tsuang, M.T., Hwu, H.G., 2008. *RASD2*, *MYH9*, and *CACNG2* genes at chromosome 22q12 associated with the subgroup of schizophrenia with non-deficit in sustained attention and executive function. Biol. Psychiatry 64 (9), 789–796.

Mah, S., Nelson, M.R., DeLisi, L.E., Reneland, R.H., Markward, N., James, M.R., Nyholt, D.R., Hayward, N., Handoko, H., Mowry, B., Kammerer, S., Braun, A., 2006. Identification of the semaphorin receptor PLXNA2 as a candidate for susceptibility to schizophrenia. Mol. Psychiatry 11 (5), 471–478.

Mowry, B.J., Holmans, P.A., Pulver, A.E., Gejman, P.V., Riley, B., Williams, N.M., Laurent, C., Schwab, S.G., Wildenauer, D.B., Bauché, S., Owen, M.J., Wormley, B., Sanders, A.R., Nestadt, G., Liang, K.Y., Duan, J., Ribble, R., Norton, N., Soubigou, S., Maier, W., Ewen-White, K.R., DeMarchi, N., Carpenter, B., Walsh, D., Williams, H., Jay, M., Albus, M., Nertney, D.A., Papadimitriou, G., O'Neill, A., O'Donovan, M.C., Deleuze, J.F., Lerer, F.B., Dikeos, D., Kendler, K.S., Mallet, J., Silverman, J.M., Crowe, R.R., Levinson, D.F., 2004. Multicenter linkage study of schizophrenia loci on chromosome 22q. Mol. Psychiatry 9 (8), 784–795.

Muglia, P., Tozzi, F., Galwey, N.W., Francks, C., Upmanyu, R., Kong, X.Q., Antoniades, A., Domenici, E., Perry, J., Rothen, S., Vandeleur, C.L.,Mooser, V., Waeber, G., Vollenweider, P., Preisig, M., Lucae, S., Müller-Myhsok, B., Holsboer, F., Middleton, L.T., Roses, A.D., 2008. Genome-wide association study of recurrent major depressive disorder in two European case–control cohorts. Mol. Psychiatry 2008 Dec 23. doi:10.1038/mp.2008.131 [Electronic publication ahead of print].

Mukai, J., Liu, H., Burt, R.A., Swor, D.E., Lai, W.S., Karayiorgou, M., Gogos, J.A., 2004. Evidence that the gene encoding ZDHHC8 contributes to the risk of schizophrenia. Nat. Genet. 36 (7), 725–731.

Murphy, K.C., Jones, L.A., Owen, M.J., 1999. High rates of schizophrenia in adults with velo-cardio-facial syndrome. Arch. Gen. Psychiatry 56 (10), 940–945.

NCI-NHGRI Working Group on Replication in Association Studies, 2007. Replicating genotype–phenotype associations. Nature 447 (7145), 655–660.

Need, A.C., Ge, D., Weale, M.E., Maia, J., Feng, S., Heinzen, E.L., Shianna, K.V., Yoon, W., Kasperaviciūte, D., Gennarelli, M., Strittmatter, W.J., Bonvicini, C., Rossi, G., Jayathilake, K., Cola, P.A., McEvoy, J.P., Keefe, R.S., Fisher, E.M., St Jean, P.L., Giegling, I., Hartmann, A.M., Möller, H.J., Ruppert, A., Fraser, G., Crombie, C., Middleton, L.T., St Clair, D., Roses, A.D., Muglia, P., Francks, C., Rujescu, D., Meltzer, H.Y., Goldstein, D.B., 2009. A genome-wide investigation of SNPs and CNVs in schizophrenia. PLoS Genet. 5 (2), e1000373.

Ng, M.Y., Levinson, D.F., Faraone, S.V., Suarez, B.K., Delisi, L.E., Arinami, T., Riley, B., Paunio, T., Pulver, A.E., Irmansyah, Holmans, P.A., Escamilla, M., Wildenauer, D.B., Williams, N.M., Laurent, C., Mowry, B.J., Brzustowicz, L.M., Maziade, M., Sklar, P., Garver, D.L., Abecasis, G.R., Lerer, B., Fallin, M.D., Gurling, H.M., Gejman, P.V., Lindholm, E., Moises, H.W., Byerley, W., Wijsman, E.M., Forabosco, P., Tsuang, M.T., Hwu, H.G., Okazaki, Y., Kendler, K.S., Wormley, B., Fanous, A., Walsh, D., O'Neill, F.A., Peltonen, L., Nestadt, G., Lasseter, V.K., Liang, K.Y., Papadimitriou, G.M., Dikeos, D.G., Schwab, S.G., Owen, M.J., O'Donovan, M.C., Norton, N., Hare, E., Raventos, H., Nicolini, H., Albus, M., Maier, W., Nimgaonkar, V.L., Terenius, L., Mallet, J., Jay, M., Godard, S., Nertney, D., Alexander, M., Crowe, R.R., Silverman, J.M., Bassett, A.S., Roy, M.A., Mérette, C., Mérette, C., Pato, C.N., Pato, M.T., Louw, Roos, J., Mérette, C., Kohn, Y., Amann-Zalcenstein, D., Kalsi, G., McQuillin, A., Curtis, D., Brynjolfson, J., Sigmundsson, T., Petursson, H., Sanders, A.R., Duan, J., Jazin, E., Myles-Worsley, M., Karayiorgou, M., Lewis, C.M., 2009. Meta-analysis of 32 genome-wide linkase studies of schizophrenia. Mol. Psychiatry 14 (8), 774–785.

O'Donovan, M.C., Craddock, N., Norton, N., Williams, H., Peirce, T., Moskvina, V., Nikolov, I., Hamshere, M., Carroll, L., Georgieva, L., Dwyer, S., Holmans, P., Marchini, J.L., Spencer, C.C., Howie, B., Leung, H.T., Hartmann, A.M., Möller, H.J., Morris, D.W., Shi, Y., Feng, G., Hoffmann, P., Propping, P., Vasilescu, C., Maier, W., Rietschel, M., Zammit, S., Schumacher, J., Quinn, E.M., Schulze, T.G., Williams, N.M., Giegling, I., Iwata, N., Ikeda, M., Darvasi, A., Shifman, S., He, L., Duan, J., Sanders, A.R., Levinson, D.F., Gejman, P.V., Cichon, S., Nöthen, M.M., Gill, M., Corvin, A., Rujescu, D., Kirov, G., Owen, M.J., Buccola, N.G., Mowry, B.J., Freedman, R., Amin, F., Black, D.W., Silverman, J.M., Byerley, W.F., Cloninger, C.R., Molecular Genetics of Schizophrenia Collaboration, 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat. Genet. 40 (9), 1053–1055.

Okochi, T., Ikeda, M., Kishi, T., Kawashima, K., Kinoshita, Y., Kitajima, T., Yamanouchi, Y., Tomita, M., Inada, T., Ozaki, N., Iwata, N., 2009. Meta-analysis of association between genetic variants in *COMT* and schizophrenia: an update. Schizophr. Res. 110 (1–3), 140–148.

Psychiatric GWAS Consortium Coordinating Committee, 2009. Genomewide association studies: history, rationale, and prospects for psychiatric disorders. Am. J. Psychiatry 166 (5), 540–556.

Purcell, S., Cherny, S.S., Sham, P.C., 2003. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19 (1), 149–150.

Rosoklija, G., Toomayan, G., Ellis, S.P., Keilp, J., Mann, J.J., Latov, N., Hays, A.P., Dwork, A.J., 2000. Structural abnormalities of subicular dendrites in subjects with schizophrenia and mood disorders: preliminary findings. Arch. Gen. Psychiatry 57 (4), 349–356.

Rousset, F., 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Resour. 8 (1), 1103–1106.

Ryu, J., Liu, L., Wong, T.P., Wu, D.C., Burette, A., Weinberg, R., Wang, Y.T., Sheng, M., 2006. A critical role for myosin IIb in dendritic spine morphology and synaptic function. Neuron 49 (2), 175–182.

Sanders, A.R., Duan, J., Levinson, D.F., Shi, J., He, D., Hou, C., Burrell, G.J., Rice, J.P., Nertney, D.A., Olincy, A., Rozic, P., Vinogradov, S., Buccola, N.G., Mowry, B.J., Freedman, R., Amin, F., Black, D.W., Silverman, J.M., Byerley, W.F., Crowe, R.R., Cloninger, C.R., Martinez, M., Gejman, P.V., 2008. No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: implications for psychiatric genetics. Am. J. Psychiatry 165 (4), 497–506.

Sham, P.C., Curtis, D., 1995. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. Ann. Hum. Genet. 59 (1), 97–105.

Shi, J., Levinson, D.F., Duan, J., Sanders, A.R., Zheng, Y., Pe'er, I., Dudbridge, F., Holmans, P.A., Whittemore, A.S., Mowry, B.J., Olincy, A., Amin, F., Cloninger, C.R., Silverman, J.M., Buccola, N.G., Byerley, W.F., Black, D.W., Crowe, R.R., Oksenberg, J.R., Mirel, D.B., Kendler, K.S., Freedman, R., Gejman, P.V., 2009. Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature 460 (7256), 753–757.

Shifman, S., Bronstein, M., Sternfeld, M., Pisanté-Shalom, A., Lev-Lehman, E., Weizman, A., Reznik, I., Spivak, B., Grisaru, N., Karp, L., Schiffer, R., Kotler, M., Strous, R.D., Swartz-Vanetik, M., Knobler, H.Y., Shinar, E., Beckmann, J.S., Yakir, B., Risch, N., Zak, N.B., Darvasi, A., 2002. A highly significant association between a COMT haplotype and schizophrenia. Am. J. Hum. Genet. 71 (6), 1296–1302.

Shifman, S., Johannesson, M., Bronstein, M., Chen, S.X., Collier, D.A., Craddock, N.J., Kendler, K.S., Li, T., O'Donovan, M., O'Neill, F.A., Owen, M.J., Walsh, D., Weinberger, D.R., Sun, C., Flint, J., Darvasi, A., 2008. Genome-wide association identifies a common variant in the reelin gene that increases the risk of schizophrenia only in women. PLoS Genet. 4 (2), e28.

Shprintzen, R.J., Goldberg, R., Golding-Kushner, K.J., Marion, R.W., 1992. Late-onset psychosis in the velo-cardio-facial syndrome. Am. J. Med. Genet. 42 (1), 141–142.

Stefansson, H., Ophoff, R.A., Steinberg, S., Andreassen, O.A., Cichon, S., Rujescu, D., Werge, T., Pietiläinen, O.P., Mors, O., Mortensen, P.B., Sigurdsson, E., Gustafsson, O., Nyegaard, M., Tuulio-Henriksson, A., Ingason, A., Hansen, T., Suvisaari, J., Lonnqvist, J., Paunio, T., Børglum, A.D., Hartmann, A., Fink-Jensen, A., Nordentoft, M., Hougaard, D., Norgaard-Pedersen, B., Böttcher, Y., Olesen, J., Breuer, R., Möller, H.J., Giegling, I., Rasmussen, H.B., Timm, S., Mattheisen, M., Bitter, I., Réthelyi, J.M., Magnusdottir, B.B., Sigmundsson, T., Olason, P., Masson, G., Gulcher, J.R., Haraldsson, M., Fossdal, R., Thorgeirsson, T.E., Thorsteinsdottir, U., Ruggeri, M., Tosato, S., Franke, B., Strengman, E., Kiemeney, L.A., Genetic Risk and Outcome in Psychosis (GROUP), Melle, I., Djurovic, S., Abramova, L., Kaleda, V., Sanjuan, J., de Frutos, R., Bramon, E., Vassos, E., Fraser, G., Ettinger, U., Picchioni, M., Walker, N., Toulopoulou, T., Need, A.C., Ge, D., Yoon, J.L., Shianna, K.V., Freimer, N.B., Cantor, R.M., Murray, R., Kong, A., Golimbet, V., Carracedo, A., Arango, C., Costas, J., Jönsson, E.G., Terenius, L., Agartz, I., Petursson, H., Nöthen, M.M., Rietschel, M., Matthews, P.M., Muglia, P., Peltonen, L., St Clair, D., Goldstein, D.B., Stefansson, K., Collier, D.A., 2009. Common variants conferring risk of schizophrenia. Nature 460 (7256), 744–747.

Sullivan, P.F., Lin, D., Tzeng, J.Y., van den Oord, E., Perkins, D., Stroup, T.S., Wagner, M., Lee, S., Wright, F.A., Zou, F., Liu, W., Downing, A.M., Lieberman, J., Close, S.L., 2008. Genomewide association for schizophrenia in the CATIE study: results of stage 1. Mol. Psychiatry 13 (6), 570–584.

Vallada, H., Curtis, D., Sham, P.C., Murray, R.M., McGuffin, P., Nanko, S., Gill, M., Owen, M., Collier, D.A., 1995. Chromosome 22 markers demonstrate transmission disequilibrium with schizophrenia. Psychiatr. Genet. 5 (3), 127–130.

Watanabe, Y., Muratake, T., Kaneko, N., Nunokawa, A., Someya, T., 2006. No association between the *brain-derived neurotrophic factor* gene and schizophrenia in a Japanese population. Schizophr. Res. 84 (1), 29–35.

Williams, N.M., Jones, L.A., Murphy, K.C., Cardno, A.G., Asherson, P., Williams, J., McGuffin, P., Owen, M.J., 1997. No evidence for an allelic association between schizophrenia and markers D22S278 and D22S283. Am. J. Med. Genet. 74 (1), 37–39.

Williams, N.M., Norton, N., Williams, H., Ekholm, B., Hamshere, M.L., Lindblom, Y., Chowdari, K.V., Cardno, A.G., Zammit, S., Jones, L.A., Murphy, K.C., Sanders, R.D., McCarthy, G., Gray, M.Y., Jones, G., Holmans, P., Nimgaonkar, V., Adolfson, R., Osby, U., Terenius, L., Sedvall, G., O'Donovan, M.C., Owen, M.J., 2003. A systematic genomewide linkage study in 353 sib pairs with schizophrenia. Am. J. Hum. Genet. 73 (6), 1355–1367.

Xu, B., Roos, J.L., Levy, S., van Rensburg, E.J., Gogos, J.A., Karayiorgou, M., 2008. Strong association of de novo copy number mutations with sporadic schizophrenia. Nat. Genet. 40 (7), 880–885.

Neuropsychobiology

Neuropsychobiology 2010;62:72–78
DOI: 10.1159/000314708

Published online: May 8, 2010

The International Consortium on Lithium Genetics (ConLiGen): An Initiative by the NIMH and IGSLI to Study the Genetic Basis of Response to Lithium Treatment

Thomas G. Schulze[a, g, ε] Martin Alda[m, ε] Mazda Adli[h, ε] Nirmala Akula[a]
Raffaella Ardau[t] Elise T. Bui[a] Caterina Chillotti[t] Sven Cichon[i, ζ] Piotr Czerski[v]
Maria Del Zompo[t, u] Sevilla D. Detera-Wadleigh[a] Paul Grof[n, o, ε] Oliver Gruber[j]
Ryota Hashimoto[x, δ] Joanna Hauser[v] Rebecca Hoban[b, c] Nakao Iwata[y, δ] Layla Kassem[a]
Tadafumi Kato[z, δ] Sarah Kittel-Schneider[k] Sebastian Kliwicki[w] John R. Kelsoe[b, c]
Ichiro Kusumi[β, δ] Gonzalo Laje[a] Susan G. Leckband[b, d, e] Mirko Manchia[u] Glenda MacQueen[p]
Takuya Masui[β, δ] Norio Ozaki[γ, δ] Roy H. Perlis[f] Andrea Pfennig[l, ε] Paola Piccardi[u]
Sara Richardson[a] Guy Rouleau[q] Andreas Reif[k] Janusz K. Rybakowski[w, ε] Johanna Sasse[l, ε]
Johannes Schumacher[a, i] Giovanni Severino[u] Jordan W. Smoller[f] Alessio Squassina[u]
Gustavo Turecki[r] L. Trevor Young[s, ε] Takeo Yoshikawa[α, δ] Michael Bauer[l, ε]
Francis J. McMahon[a]

[a]Genetic Basis of Mood and Anxiety Disorders, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Md., [b]Department of Psychiatry, University of California San Diego, [c]Department of Psychiatry, VA San Diego Healthcare System, and [d]Department of Pharmacy, VA San Diego Healthcare System, La Jolla, Calif., [e]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, Calif., and [f]Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, Mass., USA; [g]Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, [h]Department of Psychiatry and Psychotherapy, Charité – Universitätsmedizin Berlin, Campus Charité Mitte, Berlin, [i]Department of Genomics, Life and Brain Center and Institute of Human Genetics, University of Bonn, Bonn, [j]Department of Psychiatry and Psychotherapy, Georg-August University, Göttingen, [k]Department of Psychiatry, Psychosomatics, and Psychotherapy, University of Würzburg, Würzburg, and [l]Department of Psychiatry and Psychotherapy, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany; [m]Department of Psychiatry, Dalhousie University, Halifax, N.S., [n]Mood Disorders Center of Ottawa, Ottawa, Ont., [o]Department of Psychiatry, University of Toronto, Toronto, Ont., [p]Department of Psychiatry, University of Calgary, Calgary, Alta., [q]CHU Sainte-Justine Research Center, Department of Medicine, University of Montreal, and [r]Department of Psychiatry, Douglas Hospital Research Institute, McGill University, Montreal, Que., and [s]Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada; [t]Unit of Clinical Pharmacology, Hospital University Agency, and [u]Department of Neuroscience 'B.B. Brodie', University of Cagliari, Cagliari, Italy; [v]Psychiatric Genetic Unit, and [w]Department of Adult Psychiatry, Poznan University of Medical Sciences, Poznan, Poland; [x]Osaka University Graduate School of Medicine, Osaka, [y]Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, [z]Laboratory for Molecular Dynamics of Mental Disorders, and [α]Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama, [β]Department of Psychiatry, Hokkaido University Graduate School of Medicine, Sapporo, [γ]Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan; [δ]The Japanese Collaborative Group on the Genetics of Lithium Response in Bipolar Disorder; [ε]The International Group for the Study of Lithium-Treated Patients (IGSLI); [ζ]Institute of Neurosciences and Medicine (INM-1), Research Center Juelich, Juelich, Germany

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/nps

Thomas G. Schulze, MD, Unit on the Genetic Basis of Mood and Anxiety Disorders
National Institute of Mental Health (NIMH), National Institutes of Health (NIH)
35 Convent Drive, Bldg. 35, Rm 1A205, MSC 3719
Bethesda, MD 20892-3719 (USA)
Tel. +1 301 451 7213, Fax +1 301 402 9081, E-Mail schulzet@mail.nih.gov

Key Words
Manic-depressive illness · Schizoaffective disorder · Mood stabilizer · Antidepressants · Suicidal behavior · Genome-wide association study · Neurogenesis · Neuroplasticity

Abstract
For more than half a decade, lithium has been successfully used to treat bipolar disorder. Worldwide, it is considered the first-line mood stabilizer. Apart from its proven antimanic and prophylactic effects, considerable evidence also suggests an antisuicidal effect in affective disorders. Lithium is also effectively used to augment antidepressant drugs in the treatment of refractory major depressive episodes and prevent relapses in recurrent unipolar depression. In contrast to many psychiatric drugs, lithium has outlasted various pharmacotherapeutic 'fashions', and remains an indispensable element in contemporary psychopharmacology. Nevertheless, data from pharmacogenetic studies of lithium are comparatively sparse, and these studies are generally characterized by small sample sizes and varying definitions of response. Here, we present an international effort to elucidate the genetic underpinnings of lithium response in bipolar disorder. Following an initiative by the International Group for the Study of Lithium-Treated Patients (www.IGSLI.org) and the Unit on the Genetic Basis of Mood and Anxiety Disorders at the National Institute of Mental Health, lithium researchers from around the world have formed the Consortium on Lithium Genetics (www.ConLiGen.org) to establish the largest sample to date for genome-wide studies of lithium response in bipolar disorder, currently comprising more than 1,200 patients characterized for response to lithium treatment. A stringent phenotype definition of response is one of the hallmarks of this collaboration. ConLiGen invites all lithium researchers to join its efforts.

Background

The articles in this special issue of *Neuropsychobiology* comprehensively review the use of lithium as a mood stabilizer in bipolar and unipolar affective disorders. They show that 60 years after Cade's discovery, lithium is still a first-line choice for prophylaxis in bipolar disorder. They furthermore discuss the evidence regarding lithium's antisuicidal effects, its use as an augmentation strategy in the treatment of unipolar depression, and provide novel insights into its neurobiological mechanisms of action. Finally, current pharmacogenetic knowledge about lithium treatment is reviewed. Taken together, however, these articles also highlight that, despite decades of lithium use in psychiatry and despite the current emphasis on the study of psychiatric genetics in modern biological psychiatry, pharmacogenetic data regarding lithium treatment have a tendency to be circumstantial and inconclusive.

Pharmacogenetics is a rapidly growing field that holds considerable promise for the development of medications that are more personalized and effective than those currently available. In all areas of medicine, pharmacogenetic studies of outcomes such as treatment response or characteristic side effects are on the rise; based on these findings, more and more pharmacogenetic tests are being offered and approved by the US Food and Drug Administration [1]. Pretreatment genetic testing has now even been added to the prescribing information for the anticoagulant warfarin [2]. Similarly, the Food and Drug Administration updated labeling for carbamazepine, recommending that patients of Asian ancestry be screened for the presence of the HLA allele B*1502 that has been implicated in carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Han Chinese people [3].

Sufficiently large, well-characterized samples as well as effective and efficient collaboration between academia and the pharmaceutical industry are among the critical prerequisites for success in the field of pharmacogenetics [4, 5]. Pharmacogenetic research in psychiatry has long been characterized by single lab efforts and small sample sizes. Only recently has our field witnessed large collaborative studies such as the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study (http://www.edc.pitt.edu/stard/) [6] in the United States, or the Genome-Based Therapeutic Drugs for Depression (GENDEP) project (http://gendep.iop.kcl.ac.uk) [7] in Europe, both of which study the pharmacogenetics of major depression. Indeed, the STAR*D and GENDEP projects have already generated several intriguing findings concerning the genetics of treatment response and side effects [8–14]. It is hoped that genome-wide association studies (GWAS) conducted in these and other samples will significantly increase our ability to guide the pharmacological treatment of psychiatric patients through the identification of genetic markers.

Notably, despite lithium's proven efficacy [15], to date there has been only one GWAS examining this 'pharmacological workhorse' of psychiatry [16]. In two cohorts encompassing more than 800 lithium-treated patients,

multiple regions of interest were identified but none met the threshold for genome-wide significance. While intriguing, no adequately powered cohort yet exists to replicate and extend these findings. Here, we present a worldwide effort to address this situation: the international Consortium on Lithium Genetics (ConLiGen), spearheaded by researchers from the International Group for the Study of Lithium-Treated Patients (IGSLI) and the National Institute of Mental Health (NIMH).

The International Group for the Study of Lithium-Treated Patients

The IGSLI is an international group of scientists dedicated to lithium-related research, and its use in mental illness and mood disorders in particular. Founded in 1988 by Mogens Schou (Risskov/Aarhus, Denmark), Bruno Müller-Oerlinghausen (Berlin, Germany), and Paul Grof (Ottawa, Canada), the IGSLI has significantly contributed to lithium research over the past 20 years (www.igsli.org). Other scientists and centers have since joined the group, which currently comprises 35 members from Austria, Canada, the Czech Republic, Denmark, Germany, Poland, Switzerland, and the United States. The main goal of this group has been to conduct systematic work on those key questions regarding lithium treatment that can only be resolved by joint international effort. Unified designs have been created and scientific data from the IGSLI member centers have been linked for the purpose of shared analysis. This approach allows investigators to work with large numbers of prospectively followed patients – something that could only be accomplished via a multicenter approach. Overall, IGSLI research is based on shared, standardized, computer-based documentation of patients' diagnoses, family histories, course of illness before and during treatment, and on comparable modalities of treatment. The group meets regularly at research conferences to plan and discuss joint projects and to prepare publications.

At the 21st IGSLI meeting, which took place in late September 2007 in Dresden, Germany, the group discussed the results from the first, newly released GWAS of bipolar disorder, performed by researchers from the NIMH and Germany [17]. The strongest findings identified and replicated in this study were those encoding diacylglycerol kinase eta, a key protein in the lithium-sensitive phosphatidylinositol pathway and several genes in the *Wnt*-signaling cascade. Given the absence of a hypothesis-driven selection of single nucleotide polymorphisms in GWAS – a method more typical of candidate gene association studies – the observation that these findings implicated pathways relevant to lithium's mechanism of action was particularly intriguing. Spurred on by these findings, the IGSLI researchers concluded that studying these genes in samples that included data on patient response to lithium treatment could improve our understanding of how these genes determine response to lithium treatment and impact susceptibility to bipolar disorder. The IGSLI collaborators thus agreed to explore a framework that would allow researchers to engage in genetic studies of lithium response that were sufficiently powered. It was stated that such an endeavor should allow for participation by all bona fide lithium researchers within and beyond the IGSLI, while maintaining the highest possible level of stringency regarding phenotype definition.

May 6, 2008: The Consortium on Lithium Genetics Is Born

Following an invitation by IGSLI member Thomas G. Schulze and Francis J. McMahon, both from the NIMH's Unit on the Genetic Basis of Mood and Anxiety Disorders, prominent scientists in the field of lithium and bipolar genetic research met at the NIMH to discuss the possibility of creating an international consortium dedicated to the study of lithium pharmacogenetics. In attendance were (in alphabetical order): Martin Alda (Halifax, N.S., Canada), Michael Bauer (Dresden, Germany), Maria Del Zompo (via phone from Cagliari, Italy), Gonzalo Laje (Bethesda, Md., USA), Francis J. McMahon (Bethesda, Md., USA), Mirko Manchia (Cagliari, Italy), Roy H. Perlis (Boston, Mass., USA), Janusz K. Rybakowski (Poznan, Poland), Thomas G. Schulze (Bethesda, Md., USA), Johannes Schumacher (Bethesda, Md., USA), and Jordan W. Smoller (Boston, Mass., USA).

Reviewing evidence from the literature, and based on their own observations, the group emphasized the evident familiality in lithium treatment response, raising the possibility that genetic variation may contribute to interindividual differences in treatment response. If such differences could be identified, they might facilitate the development of novel treatments for bipolar disorder, or allow for better matching between patients and treatments. Over the last decade, the quest for a 'personalized medicine' approach in psychiatry has propelled a host of pharmacogenetic studies. Because of the lengthy trial-and-error process that currently characterizes the search

for the most optimal treatment, pharmacogenetic studies in psychiatry have traditionally focused on treatment response or adverse effects associated with antidepressants or antipsychotic medications [18–22]. While initially limited by small sample sizes, pharmacogenetic studies in psychiatry have increasingly come to rely on large-scale collaborative efforts, such as STAR*D, GENDEP, or the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) project. While some pharmacogenetic studies performed with these collaborative samples have produced intriguing results, difficulties in defining stringent target phenotypes across the various subsamples remain an important challenge [23, 24].

The researchers gathered at the NIMH on May 6, 2008 noted that, despite considerable and well-documented worldwide experience with lithium as an effective antimanic agent, mood stabilizer, and putative antisuicidal agent, there is a surprising dearth of large-scale pharmacogenetic studies of lithium treatment. We thus decided to create an international initiative whose goal would be to facilitate high-quality, well-powered analyses of lithium treatment response data that would ultimately allow for robust conclusions. The Consortium on Lithium Genetics, hereafter referred to as ConLiGen, was born.

ConLiGen's Scientific Goals

ConLiGen aims to identify genetic determinants of response to lithium treatment in bipolar disorder, as well as genetic determinants of adverse events emerging during lithium treatment. In the long run, ConLiGen may also study response to lithium treatment in general (e.g. lithium augmentation in the treatment of major depression).

Membership in ConLiGen

Any bona fide researcher or research group with access to samples of lithium-treated patients for whom DNA is available can join ConLiGen. Any new admission request is voted upon by ConLiGen members.

Communication between the ConLiGen Members

To ensure a constant exchange of ideas between members and allow for a straightforward realization of ConLiGen's goals, a monthly conference call is conducted. Furthermore, members meet once or twice a year at international meetings of various biological psychiatric organizations.

ConLiGen Advisory Board

An Advisory Board comprising international experts in the field of mood disorders research, and lithium research in particular, was established to offer ConLiGen an outside perspective as well as guidance on broad scientific directions, to serve as a liaison to nonacademic communities such as funding institutions, or industry, and finally, to act as one of ConLiGen's publicly visible faces. Currently, the following researchers are members of the Advisory Board (in alphabetical order): Robert H. Belmaker (Division of Psychiatry, Ben Gurion University of the Negev, Beersheva, Israel), Gian Luigi Gessa (Department of Neuroscience 'B.B. Brodie', University of Cagliari, Cagliari, Italy), Paul Greengard (Laboratory of Molecular and Cellular Neuroscience, Rockefeller University, New York, N.Y., USA), Kay R. Jamison (Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Md., USA), Richard S. Jope (Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, Ala., USA), Husseini K. Manji (CNS & Pain, Johnson and Johnson Pharmaceutical Research and Development, Titusville, N.J., USA), and Leon E. Rosenberg (Department of Molecular Biology and the Woodrow Wilson School of Public and International Affairs, Princeton University, Princeton, N.J., USA).

Phenotype Definition of Lithium Response: A Major Prerequisite for Pharmacogenetic Studies of Lithium

ConLiGen's first and most crucial goal is to define the phenotype of lithium response. Treatment response is a complex construct that requires researchers to make judgments about adequacy of treatment and tolerability as well as assess changes in episode frequency or symptom severity. In many cases this information must be assessed retrospectively, with the inherent limitations associated with recall bias, missing information, or the fact that the treatment has not followed a strict research protocol. One scale that incorporates such data is an 11-point scale developed by Martin Alda and colleagues [25]

Name: _____ **Date:** _____

Drug: _____ **Evaluated By:** _____

Criterion A

The criterion A is used to determine an association between clinical improvement and the treatment. The rating should apply to the period of treatment considered adequate in duration and dosage. The illness activity should be judged by frequency, severity, and duration of episodes.

10 = Complete response, no recurrences in the course of adequate treatment, no residual symptoms, and full functional recovery
9 = Very good response, no recurrences, but the patient may have minimal residual symptoms (transient anxiety, sleep disturbance, dysphoria, irritability) not requiring any intervention
8 = Very good response. Illness activity reduced by more than 90%
7 = Good response. Illness activity reduced by 80 - 90 %
6 = Good response. Reduction in activity of illness by 65 - 80%
5 = Moderate response. Reduction in illness activity by 50 - 65%
4 = Moderate improvement. Reduction in illness activity by 35 - 50%
3 = Mild improvement. Reduction of illness activity by 20 - 35%
2 = Mild improvement. Reduction of illness activity by 10 - 20%
1 = Minimal improvement. Reduction of illness activity by 0 - 10%
0 = No change or worsening

A Criterion Score: _____

Criteria B

The criteria B are used to establish whether there is a causal relationship between clinical improvement and the treatment. Score 0, 1 or 2 points for each item:

B1: Number of episodes off the treatment.

0 = 4 or more episodes
1 = 2 or 3 episodes
2 = 1 episode

B1: _____

B2: Frequency of episodes off the treatment.

0 = Average to high, including rapid cycling
1 = Low, spontaneous remissions of 3 or more years on average
2 = 1 episode only, risk of recurrence cannot be established

B2: _____

B3: Duration of the treatment.

0 = 2 or more years
1 = 1 - 2 years
2 = Less than 1 year

B3: _____

B4: Compliance during period(s) of stability.

0 = Excellent, e.g. documented by drug levels in the therapeutic range
1 = Good, more than 80% levels in the therapeutic range
2 = Poor, repeatedly off treatment, less than 80% levels in the therapeutic range

B4: _____

B5: Use of additional medication during the period of stability.

0 = None except infrequent sleep medication (1 per week or less); no other mood stabilizers, antidepressants or antipsychotics for control of mood symptoms
1 = Low-dose antidepressants or antipsychotics as an "insurance", or prolonged use of sleep medication
2 = Prolonged or systematic use of an antidepressant or antipsychotic

B5: _____

B Criteria Score: _____

Total Scale Score: _____
(Subtract B from A)

Fig. 1. Retrospective criteria of long-term treatment response in research subjects with bipolar disorder.

(fig. 1); other approaches include longitudinal outcome measures that consider time to recurrence or symptom burden during treatment [16, 26].

The 11-point scale measures the extent of improvement during long-term treatment. The scale's A score is a composite measure of change in frequency, duration, and severity of illness episodes in the course of lithium treatment. It is weighted by factors that influence the degree to which the observed clinical change is considered to be due to lithium (B1–B5 scores in the scale). The scale has been developed in the context of a study assessing response to treatment in subjects not followed according to a research protocol, namely relatives of probands in our genetic studies [25]. Subsequently, it has been widely used in several other studies at IGSLI centers [27–29] and at other centers involved in lithium research [pers. commun. from John Kelsoe, San Diego, Calif., USA and Maria del Zompo, Cagliari, Italy], which imparts face validity. Within ConLiGen, phenotypic assessment will be based on any available information in-

cluding life charts when available and quantified using the scale; interrater reliability meetings will be organized, facilitated by ConLiGen member Martin Alda, and case vignettes will also be reviewed to establish between-center reliability.

Variables describing treatment tolerability or side effects may be studied in subsequent projects. Because the issue of 'best response phenotype' is far from trivial, ConLiGen will strive to continuously weigh evidence from future clinical and biological studies of lithium in an effort to refine the definition of phenotype response. Evaluating response to long-term treatment in an illness with a highly variable natural course presents a challenge. Many patients with bipolar disorder experience spontaneous remissions of variable timing and duration. Moreover, in a pharmacogenetic study we need to evaluate the quality of response not for groups of subjects as in clinical trials but individually for each patient. While prospective studies will be able to implement more precise measures, our approach is a practical way to assess the quality of response in a variety of patients treated in diverse settings.

ConLiGen's Current Project and Long-Term Mission

ConLiGen is poised to assess all aspects of the pharmacogenetics of lithium treatment in psychiatric disorders, including the study of genetic susceptibility to potential treatment-emergent adverse events (e.g. weight gain, hypothyroidism, tremor). As its first project, ConLiGen intends to conduct a GWAS of stringently defined response to lithium treatment in bipolar disorder. ConLiGen members and the various research centers which they are affiliated with are joining their samples for a centralized genotyping effort to be performed at the Unit on the Genetic Basis of Mood and Anxiety Disorders of the NIMH and the Department of Genomics of the Life and Brain Center at the University of Bonn, Germany. For the primary projects, a previously validated scale will be used to define response to lithium treatment, as described above. Individuals scoring between 7 and 10 will be considered lithium 'responders', while individuals with scores between 0 and 6 will be considered 'nonresponders'. Presently, the total sample comprises more than 1,200 bipolar patients for whom response to lithium treatment has been or is currently being assessed by means of the scale. From preliminary analyses conducted in select IGSLI samples (data not shown), we can assume that about 35–40% of patients will qualify as responders. Previous studies [8, 9] suggest larger genetic effect sizes (e.g. allelic odds ratios between 1.5 and 2) for a narrowly defined pharmacogenetic phenotype than for a categorically defined clinical diagnosis. Thus, assuming a minor allele frequency of 0.3 and genotype relative risks of 1.4 for individuals heterozygous, and of 1.96 for individuals homozygous for the risk allele, the combined ConLiGen sample will have a power of 83% to detect an effect at a significance level of 1×10^{-8} [30].

Although the combined ConLiGen sample will be the largest sample to date to investigate lithium response on a genome-wide scale, we are aware that any finding, regardless of whether it reaches levels of genome-wide significance, will ultimately have to be confirmed in independent samples. Thus, ConLiGen's mission will not be finished after the completion of its GWAS. On the contrary, ConLiGen will continue to invite researchers to join its efforts in order to increase the available sample size of patients adequately characterized for lithium response. In collaboration with both IGSLI centers and large, long-standing multicenter projects such as the NIMH Bipolar Disorder Genetics Initiative, ConLiGen will be actively engaged in supporting and organizing urgently needed prospective studies of lithium response in bipolar disorder and other conditions.

Since Cade discovered lithium's beneficial effects in the treatment of bipolar disorder 60 years ago, this agent has become almost synonymous with the treatment of bipolar disorder worldwide [15]. Yet, little is known about the genetic underpinnings of lithium response or the development of side effects associated with its use. In a scientific environment characterized by calls for personalized medicine and the growth of large-scale pharmacogenetic studies in many fields of medicine, ConLiGen's goal is to put lithium at the forefront of pharmacogenetic studies in psychiatry.

Acknowledgments

This research was in part supported by the Intramural Program of the NIMH. Dr. Thomas G. Schulze's work is furthermore supported by NARSAD. Dr. Martin Alda's work on the phenotype of lithium response has been supported by grants from the Canadian Institutes of Health Research (CIHR, grant 64410) and by NARSAD. Dr. Smoller's work is supported in part by a grant from the NIMH (MH079799). Dr. Reif is supported by the Deutsche Forschungsgemeinschaft (DFG) (KFO 125 and SFB TRR 58) and the German Ministry for Education and Research (BMBF) (IZKF N-4). The work of the Cagliari group was partly supported by the Regional Councillorship of Health, 'Regione Autonoma della Sardegna'.

References

1 Swen JJ, Huizinga TW, Gelderblom H, de Vries EG, Assendelft WJ, Kirchheiner J, Guchelaar HJ: Translating pharmacogenomics: challenges on the road to the clinic. PLoS Med 2007;4:e209.

2 Wadelius M, Sörlin K, Wallerman O, Karlsson J, Yue QY, Magnusson PK, Wadelius C, Melhus H: Warfarin sensitivity related to CYP2C9, CYP3A5, ABCB1 (MDR1) and other factors. Pharmacogenomics J 2004;4:40–48.

3 Chung WH, Hung SI, Hong HS, Hsih MS, Yang LC, Ho HC, Wu JY, Chen YT: Medical genetics: a marker for Stevens-Johnson syndrome. Nature 2004;428:486.

4 Penny MA, McHale D: Pharmacogenomics and the drug discovery pipeline: when should it be implemented? Am J Pharmacogenomics 2005;5:53–62.

5 Roses AD: Pharmacogenetics in drug discovery and development: a translational perspective. Nat Rev Drug Discov 2008;7:807–817.

6 Fava M, Rush AJ, Trivedi MH, Nierenberg AA, Thase ME, Sackeim HA, Quitkin FM, Wisniewski S, Lavori PW, Rosenbaum JF, Kupfer DJ: Background and rationale for the sequenced treatment alternatives to relieve depression (STAR*D) study. Psychiatr Clin North Am 2003;26:457–494.

7 Uher R, Farmer A, Maier W, Rietschel M, Hauser J, Marusic A, Mors O, Elkin A, Williamson RJ, Schmael C, Henigsberg N, Perez J, Mendlewicz J, Janzing JG, Zobel A, Skibinska M, Kozel D, Stamp AS, Bajs M, Placentino A, Barreto M, McGuffin P, Aitchison KJ: Measuring depression: comparison and integration of three scales in the GENDEP study. Psychol Med 2008;38:289–300.

8 McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, Sorant AJ, Papanicolaou GJ, Laje G, Fava M, Trivedi MH, Wisniewski SR, Manji H: Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. Am J Hum Genet 2006;78:804–814.

9 Laje G, Paddock S, Manji H, Rush AJ, Wilson AF, Charney D, McMahon FJ: Genetic markers of suicidal ideation emerging during citalopram treatment of major depression. Am J Psychiatry 2007;164:1530–1538.

10 Perlis RH, Purcell S, Fava M, Fagerness J, Rush AJ, Trivedi MH, Smoller JW: Association between treatment-emergent suicidal ideation with citalopram and polymorphisms near cyclic adenosine monophosphate response element binding protein in the STAR*D study. Arch Gen Psychiatry 2007;64:689–697.

11 Paddock S, Laje G, Charney D, Rush AJ, Wilson AF, Sorant AJ, Lipsky R, Wisniewski SR, Manji H, McMahon FJ: Association of GRIK4 with outcome of antidepressant treatment in the STAR*D cohort. Am J Psychiatry 2007;164:1181–1188.

12 Perlis RH, Moorjani P, Fagerness J, Purcell S, Trivedi MH, Fava M, Rush AJ, Smoller JW: Pharmacogenetic analysis of genes implicated in rodent models of antidepressant response: association of TREK1 and treatment resistance in the STAR(*)D study. Neuropsychopharmacology 2008;33:2810–2819.

13 Lekman M, Laje G, Charney D, Rush AJ, Wilson AF, Sorant AJ, Lipsky R, Wisniewski SR, Manji H, McMahon FJ, Paddock S: The FKBP5-gene in depression and treatment response – an association study in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Cohort. Biol Psychiatry 2008;63:1103–1110.

14 Uher R, Huezo-Diaz P, Perroud N, Smith R, Rietschel M, Mors O, Hauser J, Maier W, Kozel D, Henigsberg N, Barreto M, Placentino A, Dernovsek MZ, Schulze TG, Kalember P, Zobel A, Czerski PM, Larsen ER, Sourey D, Giovannini C, Gray JM, Lewis CM, Farmer A, Aitchison KJ, McGuffin P, Craig I: Genetic predictors of response to antidepressants in the GENDEP project. Pharmacogenomics J 2009;9:225–233.

15 Bauer M, Grof P, Müller-Oerlinghausen B (eds): Lithium in Neuropsychiatry: The Comprehensive Guide. London, Informa Healthcare, 2006.

16 Perlis RH, Smoller JW, Ferreira MAR, McQuillin A, Bass N, Chir MB, Lawrence J, Sachs GS, Nimgaonkar V, Scolnick EM, Gurling H, Sklar P, Purcell S: A genomewide association study of response to lithium for prevention of recurrence in bipolar disorder. Am J Psychiatry 2009;166:718–725.

17 Baum AE, Akula N, Cabanero M, Cardona I, Corona W, Klemens B, Schulze TG, Cichon S, Rietschel M, Nöthen MM, Georgi A, Schumacher J, Schwarz M, Abou Jamra R, Höfels S, Propping P, Satagopan J, Detera-Wadleigh SD, Hardy J, McMahon FJ: A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. Mol Psychiatry 2008;13:197–207.

18 Rietschel M, Kennedy JL, Macciardi F, Meltzer HY: Application of pharmacogenetics to psychotic disorders: the first consensus conference. The Consensus Group for Outcome Measures in Psychoses for Pharmacological Studies. Schizophr Res 1999;37:191–196.

19 Lerer B, Segman RH, Fangerau H, Daly AK, Basile VS, Cavallaro R, Aschauer HN, McCreadie RG, Ohlraun S, Ferrier N, Masellis M, Verga M, Scharfetter J, Rietschel M, Lovlie R, Levy UH, Meltzer HY, Kennedy JL, Steen VM, Macciardi F: Pharmacogenetics of tardive dyskinesia: combined analysis of 780 patients supports association with dopamine D_3 receptor gene Ser9Gly polymorphism. Neuropsychopharmacology 2002;27:105–119.

20 Malhotra AK, Murphy GM Jr, Kennedy JL: Pharmacogenetics of psychotropic drug response. Am J Psychiatry 2004;161:780–796.

21 Correll CU, Malhotra AK: Pharmacogenetics of antipsychotic-induced weight gain. Psychopharmacology (Berl) 2004:477–489.

22 Malhotra AK, Lencz T, Correll CU, Kane JM: Genomics and the future of pharmacotherapy in psychiatry. Int Rev Psychiatry 2007;19:523–530.

23 van den Oord EJ, Adkins DE, McClay J, Lieberman J, Sullivan PF: A systematic method for estimating individual responses to treatment with antipsychotics in CATIE. Schizophr Res 2009;107:13–21.

24 Need AC, Keefe RS, Ge D, Grossman I, Dickson S, McEvoy JP, Goldstein DB: Pharmacogenetics of antipsychotic response in the CATIE trial: a candidate gene analysis. Eur J Hum Genet 2009;17:946–957.

25 Grof P, Duffy A, Cavazzoni P, Grof E, Garnham J, MacDougall M, O'Donovan C, Alda M: Is response to prophylactic lithium a familial trait? J Clin Psychiatry 2002;63:942–947.

26 Perlis RH, Ostacher MJ, Patel JK, Marangell LB, Zhang H, Wisniewski SR, Ketter TA, Miklowitz DJ, Otto MW, Gyulai L, Reilly-Harrington NA, Nierenberg AA, Sachs GS, Thase ME: Predictors of recurrence in bipolar disorder: primary outcomes from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). Am J Psychiatry 2006;163:217–224.

27 Garnham J, Munro A, Slaney C, Macdougall M, Passmore M, Duffy A, O'Donovan C, Teehan A, Alda M: Prophylactic treatment response in bipolar disorder: results of a naturalistic observation study. J Affect Disord 2007;104:185–190.

28 Passmore MJ, Garnham J, Duffy A, MacDougall M, Munro A, Slaney C, Teehan A, Alda M: Phenotypic spectra of bipolar disorder in responders to lithium versus lamotrigine. Bipolar Disord 2003;5:110–114.

29 Hajek T, Hahn M, Slaney C, Garnham J, Green J, Ruzickova M, Zvolsky P, Alda M: Rapid cycling bipolar disorders in primary and tertiary-care treated patients. Bipolar Disord 2008;10:495–502.

30 Purcell S, Cherny SS, Sham PC: Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19:149–150.

Schizophrenia Research 118 (2010) 300–302

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Letter to the Editors

No association between *DAO* and schizophrenia in a Japanese patient population: A multicenter replication study

Dear Editors

D-serine, a co-agonist that enhances N-methyl-D-aspartate (NMDA) glutamate receptors, is hypothesized to be involved in the pathophysiology of schizophrenia because treatment with D-serine improves some schizophrenic symptoms (Tsai et al., 1998). Thus, genes that are related to D-serine metabolism have arisen as candidate genes that may play a role in schizophrenia. Genomic case-control studies of one such candidate gene, D-amino acid-oxidase (*DAO*; 12q24), have shown relatively consistent positive results in which *DAO* is a susceptibility locus for patients with schizophrenia (Chumakov et al., 2002; Corvin et al., 2007; Schumacher et al., 2004; Wood et al., 2007). A previous well-explained report about the role of *DAO* in schizophrenia (Verrall et al., 2009) and two representative meta-analyses showed an association between *DAO* and schizophrenia, specifically with the SNP, rs4623951 (Allen et al., 2008; Shi et al., 2008). Previously, we performed stage 1 genomic case-control studies (340 schizophrenia patients, 340 healthy controls) for genes (*PHGDH*, *SHMT1*, *SRR*, *DAO*) related to D-serine synthesis/degradation. Our results from a haplotype case-control analysis showed that only three SNPs (rs3825251, rs3918347, and rs4964770) in *DAO* which are in strong linkage disequilibrium (LD) and show an association with schizophrenia, even after correction for multiple testing (Ohnuma et al., 2009). Some negative results have also been reported regarding a possible association between *DAO* and schizophrenia (Fallin et al., 2005; Jonsson et al., 2009; Liu et al., 2006; Shinkai et al., 2007; Vilella et al., 2008; Yamada et al., 2005). Thus, we performed a stage 2 replication study to clarify the conclusions of a possible role for *DAO* in schizophrenia. We reinvestigated the association between the above *DAO* haplotype (rs3825251–rs3918347–rs4964770) and schizophrenia using adequate statistical power.

The case-control genetic association studies were performed using 1656 unrelated Japanese patients with schizophrenia (863 males, 793 females; mean age 45.2 years, S.D. ± 15.4) who met the DSM-IV diagnosis of schizophrenia. We also used 1842 unrelated healthy controls (784 males, 1058 females; mean age 47.1 years, S.D. ± 19.1). Patients and controls were recruited from four geographic regions of Japan: Osaka, Aichi, Saitama, and Tokyo. The criteria for enrolling both patients and controls have been previously described (Ohnuma et al., 2009). Of these participants, 340 patients with schizophrenia and 340 healthy controls had been analyzed in our previous stage 1 study (Ohnuma et al., 2009); the remaining patients and controls were new to the present study. The mean age of the schizophrenic group was significantly different from that of the control group (Student's *t*-test, $t = 3.27$, $P = 0.001$), and the gender distribution of the two groups was significantly different ($\chi^2 = 31.9$, $P < 0.001$). The Ethics Committee of the Juntendo University School of Medicine approved this study. All participants gave their written informed consent prior to participating in the study.

Genomic DNA was extracted from peripheral white blood cells using a QIAamp® DNA Blood Maxi kit (Qiagen, Courtaboeuf, France). Three SNPs (rs3825251, rs3918347, and rs4964770) were typed using TaqMan® technology as previously described in detail (Ohnuma et al., 2009). SNP information, including position and distance between SNPs, has also been described (Ohnuma et al., 2009).

For the case-control association study, genetic statistical analysis, Hardy–Weinberg Equilibrium (HWE) testing, differences in genotypic/allelic frequencies, LD, and case-control haplotype analysis were all done using SNPAlyse V7.0 Pro (Ohnuma et al., 2009). All *P*-values reported are two-tailed and were considered statistically significant when <0.05. We performed power calculations using the Power Calculator for Two Stage Association Studies (http://www.sph.umich.edu/csg/abecasis/CaTS/). Power was calculated under the prevalence of 0.01 using an additive or a multiplicative model, which was based on allelic frequencies of the associated markers that ranged from 0.49 (rs3918347) to 0.50 (rs4964770). The odds ratio ranged from 1.152 (rs3918347) to 1.166 (rs4964770) for the SNPs investigated in our current study (Ohnuma et al., 2009), with an alpha level of 0.05. Results of power analysis showed that the power ranged from 80% (rs3918347) to 90% (rs4946770).

The three SNPs in *DAO* were genotyped in 1656 patients with schizophrenia and in 1842 controls. No deviation from HWE in either patients or controls was observed, and no single SNP showed a significant association between their allelic or genotypic frequencies and schizophrenia (Supplementary Table 1). Further investigation of the three SNPs showed a strong LD (Table 1), but case-control haplotype association analysis (minor haplotypes with frequencies less than 3% in either group were omitted) using windows of two or three SNPs failed to show a significant association with Japanese schizophrenia (Table 1).

0920-9964/$ – see front matter
doi:10.1016/j.schres.2010.01.028

Table 1
Results of linkage disequilibrium (D'/r^2 value) between the SNPs and two- and three-SNP-based haplotype analyses.

SNPs		D'/r^2 [a]		Haplotypic global *P*-value	
				2 windows	3 windows
rs3825251		0.91/0.82	0.93/0.86	0.85	0.84
rs3918347	0.96/0.89		0.95/0.88	0.87	
rs4964770	0.94/0.87	0.98/0.97			

[a] For D'/r^2 value, right upper diagonal: schizophrenia, left lower diagonal: controls.

In our previous stage 1 study, the 3′ regions of *DAO* showed a strong LD, including three SNPs (rs3825251–rs3918347–rs4964770) that showed an association with Japanese schizophrenia. In particular, haplotype GGC may be a protective haplotype in schizophrenia (Ohnuma et al., 2009). But in our current stage 2 study that had adequate statistical power, our results did not show any significant association between schizophrenia and single SNPs or two- and three-SNP-based haplotypes. Thus, the results from our previous study should be considered a type I error (false positive), because they did not have adequate statistical power to produce reliable results. Previously, two representative meta-analyses showed an association between rs4623951 in the 5′ region and schizophrenia; however, none of the studies that only used Japanese subjects showed positive findings for rs4623951, whereas the studies with only Japanese subjects showed marginal positive findings in the 3′ region (Ohnuma et al., 2009; Yamada et al., 2005). In addition, Japanese subjects were not included in all studies that reported positive associations between schizophrenia and SNPs situated in the 5′ region of *DAO* (Chumakov et al., 2002; Corvin et al., 2007; Schumacher et al., 2004; Wood et al., 2007). Taken together, these results suggest that there may be some ethnic differences in *DAO*, and that a schizophrenia susceptibility locus in Japanese patients may have a nominal effect and may be situated in the 3′ region. Indeed, the minor allelic frequencies of the three investigated SNPs, especially rs3825251, were different among different ethnic populations, and the LD pattern in the 3′ region was also different between Japanese and Caucasian individuals (http://www.hapmap.org/index.html.ja). In addition, symptomatic differences and schizophrenia subtypes were not considered in the current study, and thus the heterogeneity of this disease may also lead to inconsistent results in genetic studies of schizophrenia.

Thus, further genetic case-control studies of *DAO* with careful consideration of schizophrenia heterogeneities (i.e., differences in ethnicity, clinical subtypes, and symptoms) and investigation of gene–gene interactions among D-serine-related genes may be needed to determine a possible association with schizophrenia. Thus, we currently conclude that *DAO* does not have an apparent degree of association with Japanese schizophrenia.

Funding for this study was provided by the Juntendo Institute of Mental Health (2008).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2010.01.028.

References

Allen, N.C., Bagade, S., McQueen, M.B., Ioannidis, J.P., Kavvoura, F.K., Khoury, M.J., et al., 2008. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat. Genet. 40, 827–834.
Chumakov, I., Blumenfeld, M., Guerassimenko, O., Cavarec, L., Palicio, M., Abderrahim, H., et al., 2002. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci U S A 99, 13675–13680.
Corvin, A., McGhee, K.A., Murphy, K., Donohoe, G., Nangle, J.M., Schwaiger, S., et al., 2007. Evidence for association and epistasis at the DAOA/G30 and D-amino acid oxidase loci in an Irish schizophrenia sample. Am J Med Genet B Neuropsychiatr Genet 144, 949–953.
Fallin, M.D., Lasseter, V.K., Avramopoulos, D., Nicodemus, K.K., Wolyniec, P.S., McGrath, J.A., et al., 2005. Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios. Am. J. Hum. Genet. 77, 918–936.
Jonsson, E.G., Saetre, P., Vares, M., Andreou, D., Larsson, K., Timm, S., et al., 2009. DTNBP1, NRG1, DAOA, DAO and GRM3 polymorphisms and schizophrenia: an association study. Neuropsychobiology 59, 142–150.
Liu, Y.L., Fann, C.S., Liu, C.M., Chang, C.C., Wu, J.Y., Hung, S.I., et al., 2006. No association of G72 and D-amino acid oxidase genes with schizophrenia. Schizophr. Res. 87, 15–20.
Ohnuma, T., Shibata, N., Maeshima, H., Baba, H., Hatano, T., Hanzawa, R., Arai, H., 2009. Association analysis of glycine- and serine-related genes in a Japanese population of patients with schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 511–518.
Schumacher, J., Jamra, R.A., Freudenberg, J., Becker, T., Ohlraun, S., Otte, A.C., et al., 2004. Examination of G72 and D-amino-acid oxidase as genetic risk factors for schizophrenia and bipolar affective disorder. Mol Psychiatry 9, 203–207.
Shi, J., Gershon, E.S., Liu, C., 2008. Genetic associations with schizophrenia: meta-analyses of 12 candidate genes. Schizophr. Res. 104, 96–107.
Shinkai, T., De Luca, V., Hwang, R., Muller, D.J., Lanktree, M., Zai, G., et al., 2007. Association analyses of the DAOA/G30 and D-amino-acid oxidase genes in schizophrenia: further evidence for a role in schizophrenia. Neuromolecular Med 9, 169–177.
Tsai, G., Yang, P., Chung, L.C., Lange, N., Coyle, J.T., 1998. D-serine added to antipsychotics for the treatment of schizophrenia. Biol. Psychiatry 44, 1081–1089.
Verrall, L., Burnet, P.W., Betts, J.F., Harrison, P.J., 2009. The neurobiology of D-amino acid oxidase and its involvement in schizophrenia. Mol Psychiatry 29, 1–16.
Vilella, E., Costas, J., Sanjuan, J., Guitart, M., De Diego, Y., Carracedo, A., et al., 2008. Association of schizophrenia with DTNBP1 but not with DAO, DAOA, NRG1 and RGS4 nor their genetic interaction. J Psychiatr Res 42, 278–288.
Wood, L.S., Pickering, E.H., Dechairo, B.M., 2007. Significant support for DAO as a schizophrenia susceptibility locus: examination of five genes putatively associated with schizophrenia. Biol. Psychiatry 61, 1195–1199.
Yamada, K., Ohnishi, T., Hashimoto, K., Ohba, H., Iwayama-Shigeno, Y., Toyoshima, M., et al., 2005. Identification of multiple serine racemase (SRR) mRNA isoforms and genetic analyses of SRR and DAO in schizophrenia and D-serine levels. Biol. Psychiatry 57, 1493–1503.

Tohru Ohnuma*
Nobuto Shibata
Hajime Baba
Juntendo University Schizophrenia Projects (JUSP), Department of Psychiatry, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
*Corresponding author. Tel./fax: +81 35802 1071.
E-mail address: tohru.oonuma@nifty.ne.jp (T. Ohnuma).

Kazutaka Ohi
Department of Psychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Yuka Yasuda
Department of Psychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan
The Osaka-Hamamatsu Joint Research Center for Child Mental Development, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Yukako Nakamura
Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan

Tomo Okochi
Hiroshi Naitoh
Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi, Japan

Ryota Hashimoto
Department of Psychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan
The Osaka-Hamamatsu Joint Research Center for Child Mental Development, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Nakao Iwata
Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi, Japan

Norio Ozaki
Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan

Masatoshi Takeda
Department of Psychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan
The Osaka-Hamamatsu Joint Research Center for Child Mental Development, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Heii Arai
Juntendo University Schizophrenia Projects (JUSP), Department of Psychiatry, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

6 October 2009

Schizophrenia Research 118 (2010) 113–117

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Genetic association study of KREMEN1 and DKK1 and schizophrenia in a Japanese population

Branko Aleksic[a,*], Itaru Kushima[a], Yoshihito Ito[a], Yukako Nakamura[a], Hiroshi Ujike[b], Michio Suzuki[c,d], Toshiya Inada[e], Ryota Hashimoto[f,g], Masatoshi Takeda[f,g], Nakao Iwata[h], Norio Ozaki[a]

[a] Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan
[b] Department of Neuropsychiatry, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan
[c] Department of Neuropsychiatry, University of Toyama, Toyama, Japan
[d] Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo, Japan
[e] Seiwa Hospital, Institute of Neuropsychiatry, Tokyo, Japan
[f] The Osaka-Hamamatsu Joint Research Center For Child Mental Development, Japan
[g] Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan
[h] Department of Psychiatry, Fujita Health University School of Medicine, Aichi, Japan

ARTICLE INFO

Article history:
Received 2 October 2009
Received in revised form 13 January 2010
Accepted 23 January 2010
Available online 11 February 2010

Keywords:
Schizophrenia
Japanese population
Epistasis
KREMEN1
DKK1
wnt pathway

ABSTRACT

The aim of the current study was to examine the association of KREMEN1 and DKK1, two wnt pathway-related genes with schizophrenia in Japanese subjects. We genotyped 16 common genetic variants within the aforementioned genes and examined their associations with schizophrenia. Results demonstrated that a common variant in the promoter region of KREMEN1 might modulate the risk of schizophrenia in the Japanese. However, further replication will be needed for conclusive interpretation of the effect of this locus on the pathogenesis of schizophrenia.

1. Introduction

Irregularities consistent with abnormal brain development, including faulty neuronal migration, altered spatial neuronal arrangement, and absence of significant gliosis, have been reported in schizophrenia. Together with behavioral, neuromotor, and other functional abnormalities that occur in childhood and predict schizophrenia, such as low IQ, poor motor skills, and poor development of language and social skills, these morphological findings indicate a developmental origin for schizophrenia (Crow 1995, Benes et al., 1986).

Recent advances in the understanding of genes that regulate brain development offer insights into the mechanisms of developmental brain changes. One important issue is the identification of signaling pathways that coordinate changes in gene expression with dynamic changes in cell adhesion and migration, events that are important for the complex cellular architecture of the human brain. Although several growth factors affect both gene expression and cell migration, recent focus has been on the wnt signaling pathway (Clevers, 2006). wnts are powerful regulators of cell proliferation and differentiation, and their signaling pathway involves proteins that directly participate in both gene transcription and cell adhesion

Abbreviations: SNP, single nucleotide polymorphism;tSNP, tagging SNP; HWE, Hardy–Weinberg equilibrium;MDR, multifactor dimensionality reduction method;TA, testing accuracy;CVC, cross-validation consistency.

* Corresponding author. Department of Psychiatry Nagoya University Graduate School of Medicine 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Tel.: +81 52 744 2282; fax: +81 52 744 2293.
E-mail address: branko@med.nagoya-u.ac.jp (B. Aleksic).

0920-9964/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.schres.2010.01.014

(currently >150 genes (Li et al., 2006). Because schizophrenia involves developmental brain changes and abnormal neuronal and synaptic organization, alterations in the transduction of wnt signaling pathway may represent such a mechanism (Clevers, 2006; Emamian et al., 2004). The genetic associations of several wnt pathway genes and schizophrenia have been reported (Proitsi et al., 2008). As coordinate changes in gene expression are important for normal brain development, the present study focused on negative modulators of the wnt signaling pathway, KREMEN1 and DKK1.

wnt/beta-catenin signaling is inhibited by the secreted protein Dickkopf1, a member of a multigene family, which induces head formation in amphibian embryos (Niehrs, 2006). Dickkopf1 inhibits wnt signaling by triggering LRP5/6 internalization through formation of a ternary complex with Kremen receptors (Mao et al., 2002). DKK1 encodes a protein that is a member of the dickkopf family. This gene maps on chromosome 10 region q11.2, a region with suggestive linkage evidence for schizophrenia (Faraone et al., 1998). *KREMEN1* encodes a high-affinity dickkopf homolog 1, a transmembrane receptor that functionally cooperates with dickkopf1 to block wnt/beta-catenin signaling. KREMEN1 maps to chromosome 22q12.1, relatively close to the region with weak, but suggestive linkage evidence for schizophrenia (Pulver et al., 1994).

We genotyped 16 common genetic variants within the aforementioned genes and examined their associations with schizophrenia. Additionally, we examined interactions between single nucleotide polymorphisms (SNPs) within KREMEN1 and DKK1.

2. Materials and methods

Subjects comprised 1624 patients with schizophrenia (mean age, 46.5 ± 14.5 years) and 1621 volunteers with no personal or family history of psychiatric illness (mean age, 45.1 ± 14.0 years). Subjects were divided into a screening sample ($n = 1681$) and a confirmation sample ($n = 1564$). A general characterization and psychiatric assessment of subjects is available elsewhere (Ikeda et al., 2008). The screening and confirmation sample were collected independently at each university hospital. All subjects provided written informed consent. This study was approved by the ethics committee at Nagoya University.

DNA was extracted from peripheral blood. Genotyping was performed using a fluorescence-based allelic discrimination assay (Taqman, Applied Biosystems, Foster City, CA, USA). Power was calculated according to the methods of Skol et al (Skol et al., 2006).

SNP tagging criteria were based on minor allele frequency (>5%) and correlation coefficient between loci (>0.8) as reported in the HAPMAP database (rel #24, Japanese population). Based on these criteria, 16 tagging SNPs (tSNP) were selected for genotyping. To exclude low-quality DNA sample or genotyping probes, data sets were filtered on the basis of tSNP genotype call rates (95% completeness) or deviation from the Hardy-Weinberg equilibrium (HWE) ($P = 0.05$) in the control sample. Subjects whose percentage of missing genotypes was >10% or who had evidence of possible DNA contamination were excluded from subsequent analyses.

All allele-wise association analyses (screening or confirmation sample) were carried out by calculating the *P* values for each tSNP. Significance was determined at the 0.05 level using Fisher's exact test. All *P* values were two-sided. Log likelihood ratio tests for assessing haplotype-wise associations between schizophrenia and combination of tagging SNPs was performed using UNPHASED software v3.04 (Dudbridge, 2008). The rare haplotype frequency threshold was set at 5%.

To reduce the total number of tests, clearly unassociated markers were removed in the first stage involving the screening sample of the present study. Next, conditional on the findings of the first stage, which used a less stringent nominal level, was tested in the second stage involving the confirmation sample using the augmented data and data from the first stage. In this joint sample analysis, *P* values were generated by Cochran–Mantel–Haenszel stratified analysis, and the Breslow–Day test was performed for evaluation of heterogeneous associations as implemented in PLINK v1.06 (Purcell et al., 2007).

To determine high- and low-risk genotype combinations, the multifactor dimensionality reduction method (MDR) was used (Moore et al., 2006). This procedure was developed to detect higher-order interactions between polymorphisms to predict a dichotomous trait variation, even when marginal effects are small. A 10-fold cross-validation test was conducted. Accordingly, a model was constructed based on 9/10 of the data (training data) and then evaluated using the remaining 1/10 of the data (testing data). The fitness of the MDR model was assessed by estimating the testing accuracy (TA). Models that were true positive would have an estimated TA of >0.5. The cross-validation consistency (CVC) was a measure of how many times among 10 divisions of the data the MDR found the same best model. We used the Tuned ReliefF algorithm (Moore and White, 2007) to removed noisy SNPs from the pool of possible candidates. Statistical significance was evaluated using a 1000-fold permutation test to compare observed testing accuracies with those expected under the null hypothesis. Permutation testing corrects for multiple testing by repeating the entire analysis on 1,000 data sets that are consistent with the null hypothesis. Models were significant at the 0.01 level.

3. Results

The results of the power analysis are presented in Table 1. We genotyped 16 tSNPs using the screening sample All tSNPs were in HWE, and the SNP-wise call rate was >95%. The association signal was detected in allele-wise analysis at rs713526 as well as in the haplotype-wise analysis (Table 2). To deal with multiple testing issues and to keep the statistical power uncompromised, we performed additional genotyping of rs713526 using the confirmation sample. Although the direction of the association was the same, no statistically significant results were obtained (Table 3). To maximize statistical power, we performed joint association analysis combining the data from the screening and confirmation sample and obtained a positive association signal.

We performed an exploratory SNP-SNP interaction analysis. For a specific number of SNPs we reported a single best model that maximized the TA. This is the model that is most likely to generalize to independent data sets. We also reported the CVC, which represents the number of times in a particular cross validated run that a given SNP combination was selected as the best model. The strongest evidence for SNP interaction was between rs1896368 (DKK1), rs2288335 (DKK1), and rs5752866