extremely powerful. Smiling to devalue something is often used in daily life; for example, when the train door shuts in our face, we often give a wry smile to cancel out the impact of the event.

Laughter in dementia patients

Laughter is usually provoked or accompanied by positive emotions. In clinical settings, it is always desirable for patients, their families, and staff to share relaxed and happy feelings, because patients are often under continuous strain and enormous pressure as a result of their illness. The more serious the illness, the more overwhelming the strain to the patients and their families. Dementia patients are usually under considerable strain, at least at the beginning of their illness. Patients' families are placed under even more stress because of the burden of care [41]. A positive emotion, together with laughter, may enable dementia patients to cope with their illness better, improve immune function, increase pain tolerance, and decrease the stress response. When a positive attitude is shared by patients and staff, it can have a positive effect on the emotional-affective and cognitive functioning of the patients [42,43].

Because the social life of dementia patients is impaired by their illness, they can easily feel isolated. Thus, a feeling that unites them, or provides some sort of bond, with their family and the community can be very beneficial. Dementia patients are often encouraged to participate in daily activities with other people and the positive emotions that are shared by the patients and the care staff help the patients maintain social contact.

Several psychosocial interventions are applied to dementia patients in clinical settings [44]. Examples include cognitive rehabilitation, reminiscence therapy, art therapy, drama therapy, and aerobic exercise [45]. In these activities, a positive attitude of patients is essential and it is always true that a greater effect can be expected when patients participate willingly with a positive outlook. In the case of cognitive rehabilitation, active participation is the condition under which good outcomes can be expected. If the patients are reluctant to participate in the activities, it is unlikely that the program will have any beneficial effects.

Dementia patients become anxious and irritated because they are unable to glean sufficient information from their surroundings due to their impaired cognitive functioning [46]. They are easily trapped in a state in which they feel unsafe, alarmed, and insecure, which, in turn, reduces their ability to process information from their surroundings. With even less secure information, they become more alarmed, leading to negative emotional behavior.

Dementia patients often show various types of BPSD during the course of their illness. Aggression, refusal to cooperate, negativity, and apathy are common, all of which contribute to the further isolation of these patients. In this sense, it is important to keep patients with BPSD within the community.

Because BPSD can often be the most formidable barrier to the care of dementia patients, it is highly recommended that the occurrence of BPSD is prevented. To reduce the occurrence of BPSD in dementia patients, patients should be kept in a stable and safe environment, efforts should be made to ensure good communication with the patients, and patients should be kept feeling relaxed and safe. By doing so, the patients are more likely to laugh and smile.

It is true that laughter and smiling decrease over time in most dementia patients, but it is important to note that not all forms of laughter and smiling are equally reduced. The ability to laugh for social communication is readily lost by dementia patients at the onset of their illness, concomitant with the loss of a social life and their ability to process information, but laughter in response to the release of tension is preserved until the advanced stages of the disease. When dementia patients are released from either physical or mental strain, they always smile. Laughter caused by feelings of disharmony is not usually preserved in dementia patients because of impaired cognitive functioning and because these patients are no longer able to understand the meaning of complicated situations, which means they often cannot understand the punch lines of jokes or appreciate humor.

As discussed above, laughter associated with pleasant feelings can be further subdivided into four types, fulfillment of instinctive needs, fulfillment of expectations, a feeling of superiority, and recognition of mix-ups. Most laughter associated with pleasant feelings is preserved in dementia patients, with observations indicating that these patients laugh and smile when they are exposed to pleasant stimuli. They smile when they are well fed and when they have had a good sleep. They also smile and laugh when they have attained self-set goals. Laughter associated with feelings of superiority is clearly preserved in most dementia patients; they become happy and pleasant when their superiority is recognized. Conversely, when these patients feel humiliated, they become angry and insulted.

Thus, the basic form of laughter is preserved in dementia patients, but the social form of laughter is sometimes lost in the advanced stages of the disease. It is important to ensure that dementia patients are kept in a safe and relaxed environment (and not in alarmed and tensioned),

which will make it more likely that these patients will be able to laugh and smile.

Humor in dementia patients

Humor has positive physiological and psychological effects in a variety of situations. The psychiatric literature purports humor as an effective tool in psychiatric illness and psychotherapy. Benefits of humor in business, management, education, and clinical settings are widely recognized because the right perspective facilitates problem solving both interpersonally and in a group setting. Furthermore, humor puts people at ease, promoting the expression and exchange of ideas. Not only can humor benefit patients, but the use of humor can facilitate the effective management of staff and others in the health care setting [22].

Humor is delicate and sensitive by nature. Humor can be properly appreciated when it is expressed in the right time, right place, and on the right occasion. Confidence, or trust, between the sender and receiver is an important aspect of humor. Establishing this trust is a prerequisite for the introduction of appropriately timed humor. No humor can be appreciated by patients when there is no trust between the patient and care staff. If one side is defensive or angry, he/she may find that the use of humor by the other party is offensive or insulting [47,48]. Patients may also become upset about jokes made at their expense, fearing humiliation and stigmatization [49]. The appropriateness of humor depends on the culture, education, and cognitive function of the receiver. Therefore, the use of humor must be timed wisely and it must be used carefully.

Dementia patients may be more sensitive to jokes or humor than healthy people because patients in the early stages of the disease know that they have difficulties understanding complicated things. Dementia patients with cognitive impairment have difficulty appreciating the disharmony in information sent as humor. Humor should be presented to dementia patients after close evaluation. There are no definitive rules, but humor should generally be introduced slowly; if there is no response or the response is negative, it may be a good idea to abandon all attempts to introduce humor, at least during that clinical encounter [50]. Humor can be used as a defense mechanism in an adverse setting and has obvious value for dementia patients if it is properly addressed and accepted. But the impaired cognitive function of dementia patients must be kept in mind so that humor is presented at the right time, in the right place, and on the right occasion. Everyone enjoys laughing, but a misjudged humorous comment can cause offense, so although laughter is almost always positive, humor itself can provoke mixed emotional responses.

> The other reactions--anger, depression, suppression, denial--took a little piece of me with them. Each made me feel just a little less human. Laughter made me more open to ideas, more inviting to others, and even a little stronger inside. It proved to me that, even as my body was devastated and my spirit challenged, I was still a vital human being. Scott Burton [51]

Summary

Dementia patients should be cared for taking into consideration their individual capacities, which differ from patient to patient. Most laughter and smiling is preserved in dementia patients until the end of the clinical course, even though laughter and smiling as a means of communication is lost during the early stages of the disease. Laughter and smiling associated with pleasant feelings, with the exception of laughing in response to feelings of disharmony, and laughter induced by the release of tension can be used in the treatment of dementia patients. The use of humor, covering issues of the fulfillment of instinctive needs and expectations, as well as feelings of superiority (Table 1), can be a good and effective complementary and alternative intervention in the treatment of dementia patients.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
MT, TK, and TT discussed the importance of laughter and humor to dementia patients and drafted the manuscript. MO, ST, and TM searched for the data on the topics in the literatures. MT, RH, and GS devised the table. All authors have read and approved the final manuscript.

Author Details
Department of Psychiatry, Osaka University Graduate School of Medicine, Suita, Japan

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A functional polymorphism in the disrupted-in schizophrenia 1 gene is associated with chronic fatigue syndrome

Sanae Fukuda [a,b,1], Ryota Hashimoto [b,c,d,e,1], Kazutaka Ohi [d,e], Kouzi Yamaguti [f,g], Yasuhito Nakatomi [g,j], Yuka Yasuda [c,d], Kouzin Kamino [h], Masatoshi Takeda [c,d], Seiki Tajima [g,i], Hirohiko Kuratsune [g,i], Yoshiki Nishizawa [g,j], Yasuyoshi Watanabe [b,f,k,*]

[a] *Department of Biomarker and Molecular Biophysics, Osaka City University Graduate School of Medicine, Osaka, Japan*
[b] *RISTEX/JST, Tokyo, Japan*
[c] *The Osaka–Hamamatsu Joint Research Center for Child Mental Development, Osaka, Japan*
[d] *Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan*
[e] *CREST/JST, Kawaguchi, Japan*
[f] *Department of Physiology, Osaka City University Graduate School of Medicine, Osaka, Japan*
[g] *Fatigue Clinical Center, Osaka City University Hospital, Osaka, Japan*
[h] *Shoraiso National Hospital, Yamatokoriyama, Japan*
[i] *Department of Health Science, Kansai University of Welfare Sciences, Osaka, Japan*
[j] *Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan*
[k] *Center for Molecular Imaging Science, RIKEN, Kobe, Hyogo, Japan*

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ABSTRACT

Aims: Disrupted-in schizophrenia 1 (DISC1), identified in a pedigree with a familial psychosis with the chromosome translocation (1:11), is a putative susceptibility gene for psychoses such as schizophrenia and major depressive disorder (MDD). Patients with chronic fatigue syndrome (CFS) report having continuous severe fatigue and many overlapping symptoms with MDD; however, the mechanism and effective treatment of CFS are still unclear. We focused on the overlapping symptoms between CFS and MDD and performed an association study of the functional single-nucleotide polymorphism (SNP) in the DISC1 gene with CFS.

Main methods: Venous blood was drawn from CFS patients and controls and genomic DNA was extracted from the whole blood according to standard procedures. Ser704Cys DISC1 SNP was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay.

Key findings: We found that the Cys704 allele of Ser704Cys SNP was associated with an increased risk of CFS development compared with the Ser704 allele.

Significance: DISC1 Ser704Cys might be a functional variant that affects one of the mechanisms implicated in the biology of CFS. Some patients with CFS showed a phenotype similar to that of patients with MDD, but further studies are needed to clarify the biological mechanism, because this study is of a rather preliminary nature. Despite the variety of patients with CFS, DISC1 Ser704Cys has an association with CFS, which may also suggest that *DISC1* plays a central role in the induction of various psychiatric diseases.

Introduction

The disrupted-in schizophrenia 1 (DISC1) gene was initially identified at the breakpoint of a balanced translocation (1,11)(q42.1;q14.3), which segregated it from major mental disorders in a large Scottish family (Millar et al. 2000). In this family, patients with schizophrenia, bipolar disorder, and recurrent major depressive disorder (MDD) were identified as carriers of the translocation (Millar et al. 2000; Blackwood et al. 2001). Subsequent genetic studies in several independent populations, including association and linkage studies, have also suggested that the DISC1 gene may be implicated in schizophrenia, bipolar disorder, and MDD (Ekelund et al. 2001, 2004; Hennah et al. 2003; Hodgkinson et al. 2004; Sachs et al. 2005; Thomson et al. 2005; Hashimoto et al. 2006). Chronic fatigue syndrome (CFS) is a disorder diagnosed following at least 6 months of disabling, unexplained mental and physical fatigue accompanied by other physical and psychological

* Corresponding author. Center for Molecular Imaging Science, RIKEN, 6-7-3 Minatojima-minamimachi, Chuo-ku, Kobe City, Hyogo 650-0047, Japan. Tel.: +81 78 304 7101; fax: +81 78 304 7112.
E-mail address: yywata@riken.jp (Y. Watanabe).
[1] These two authors contributed equally to this work.

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symptoms (Fukuda et al. 1994; Prins et al. 2006). Patients with CFS have an inordinately high rate of depression symptoms or MDD. Clinical samples indicate that between 35% and 46% exhibit depressive symptoms (Johnson et al. 1996; Ciccone et al. 2003) and 31% have MDD (Henderson and Tannock 2005). Among a clinical sample of individuals with CFS, patients concurrently experiencing depressive symptoms were found to have significantly worse outcomes, such as more persistent symptoms and unemployment, than those without depressive symptoms (Bombardier and Buchwald 1995). However, no association study between the DISC1 gene and CFS has been reported, despite the observations that some of the symptoms typically observed in CFS were also common in MDD and that patients with CFS had high rates of major current and lifetime depressive episodes (Afari and Buchwald 2003). Here, we report an association between the functional single-nucleotide polymorphism (SNP) Ser704Cys of DISC1 and CFS.

Materials and methods

Subjects

Subjects for the clinical association study were recruited at Fatigue Clinical Center in Osaka City University Hospital, Osaka, Japan, and the Osaka University Hospital Department of Psychiatry. Enrolled in the study were 155 patients with CFS [55 men and 100 women with a mean age of 36.0 years (SD: 8.51 years)] and 502 healthy control subjects [236 men and 266 women with a mean age of 65.3 years (SD: 19.0 years)]. All of the subjects were Japanese. Of the patients with CFS, 59% also had psychiatric diseases. We classified psychiatric disorders based on ICD-10 (World Health Organization 1992): F3 (Mood Disorders including MDD), $N=7$; F4 (Neurotic, Stress-related and Somatoform Disorder), $N=69$; F3 and F4, $N=3$; F51 (Nonorganic Sleep Disorder), $N=3$; and Unknown, $N=9$. Individual diagnoses were made according to the criteria for CFS of the Centers for Disease Control and Prevention (Fukuda et al. 1994). Control subjects were healthy volunteers who had no current or past connection with psychiatric services. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki and all procedures were carried out with the adequate understanding and written consent of the subjects. The study protocol was approved by institutional ethics committees.

Detection of SNP

Venous blood was drawn from subjects and genomic DNA was extracted from the whole blood according to standard procedures. DISC1 has several SNPs but only the Ser704Cys DISC1 SNP shows a significant association with brain function and it might be a functional variant that affects neural mechanisms (Hashimoto et al. 2006). In addition, robust effects of DISC1 on ERK and Akt signaling and evidence that the Cys704 DISC1 (the risk allele for MDD) might exert a weaker effect on the ERK activation than Ser704 DISC1 have been shown (Hashimoto et al. 2006). One related SNP was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay as described previously (Hashimoto et al. 2005a,b). Primers and probes for detection of the SNPs are available upon request. Statistical analyses were performed using SNPAlyze Pro software, version 5.1.1 (DYNACOM, Yokohama, Japan) and SPSS 16.0 J software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls were analyzed using χ^2 tests for sex and the t-test for age. Genotypic and allelic distributions between patients and controls were analyzed by the χ^2 test. All P-values reported are two-tailed. Hardy–Weinberg equilibrium for the SNP (Ser740Cys) in controls and patients was examined to test the genotype distribution. Statistical significance was defined as $P<0.05$.

Results

The genotype distribution was in Hardy–Weinberg equilibrium for the SNP (Ser740Cys) in the group of patients with CFS ($P=0.12$) and controls ($P=0.21$). We examined the association between the genetic variant Ser704Cys SNP and CFS (Table 1). The Cys allele frequency of Ser704Cys was significantly greater in patients with CFS when compared with controls [$\chi^2=4.50$, df$=1$, $P=0.037$, odds ratio$=$ 1.50, 95%CI (confidence interval): 1.12–2.19]. There were no differences among genotype groups (A/A, T/T, and A/T) between CFS patients and the controls ($\chi^2=4.47$, df$=2$, $P=0.10$).

There was no significant difference among genotype groups between male CFS patients and controls ($\chi^2=1.24$, df$=2$, $P=0.54$) but there were significant differences between female CFS patients and controls ($\chi^2=8.81$, df$=2$, $P=0.012$). The Cys allele frequency of Ser704Cys was significantly greater in female patients with CFS when compared with controls ($\chi^2=10.37$, df$=1$, $P=0.0013$, odds ratio$=2.12$, 95% CI: 1.33–3.36) but not in male patients, consistent with previous association study in schizophrenia (Hennah et al. 2003).

Discussion

We initially found evidence for an association between CFS and the functional Ser704Cys SNP in the DISC1 gene. The association level was slightly weak because CFS includes various types of patients, some of whom also have psychiatric diseases or symptoms, such as depressive symptoms and MDD, creating a syndrome. False-positive associations due to population stratification cannot be excluded in our case–control study, despite the precaution of ethnic matching. The mean age in patients with CFS was significantly younger than that of the controls ($t=26.9$, df$=575.4$, $P<0.001$) and the frequency of females in the patients with CFS was significantly higher than that of the controls ($\chi^2=6.38$, df$=1$, $P=0.012$). This is a limitation of the study and differences in sex ratio and ages between groups could be potential confounding factors. Therefore, it is necessary to carry out further investigations to confirm our findings in other samples. The other limitation of the study was that we have not done stratified analysis by looking at CFS only, CFS with MDD, MDD, and healthy controls. Physicians recommend CFS patients who are suspected to be comorbid with psychiatric diseases to visit psychiatrists, however some of them refuse to visit psychiatrists, and thereafter it is difficult to discriminate patients with MDD and other psychiatric disorders exactly. Schrijvers et al. (2009) reported that the relationship between CFS and MDD (and other psychiatric disorders) remains an area of controversy and they mentioned these two reasons. First, the fundamental issue is one of diagnostic labeling for symptom-based disorders in the absence of biological markers or a clear aetiology (Afari and Buchwald 2003). Second, MDD and CFS are heterogeneous conditions. As to this heterogeneity, CFS shares some clinical and

Table 1
Genotype and allele distributions of single-nucleotide polymorphisms in the DISC1 gene between patients with chronic fatigue syndrome and healthy controls.

Marker dbSNP IDs rs821616	Amino acid substitution Ser704Cys	*N*	Genotype (A2110T)			Genotype *P*-value (χ^2) df$=2$	MAF *T*	Allelic *P*-value (χ^2) df$=1$	OR (95%CI)
			A/A	A/T	T/T				
CFS		155	177	32	6	0.10 (4.47)	**0.14**	**0.037 (4.50)**	**1.50 (1.12–2.19)**
CON		502	410	84	8		**0.10**		

CFS, patients with chronic fatigue syndrome; CON, healthy controls; MAF, minor allele frequency; OR, odds ratio; 95%CI, 95% confidence interval.

neurobiological characteristics with the atypical subtype of MDD (American Psychiatric Association 1994). It is the limitation of clinical research. The study is of a rather preliminary nature, because the difference in the DISC1 Ser740Cys allele frequencies between CFS patients and controls is very small.

DISC1 is a multi-functional protein. Several research groups have identified DISC1-interacting proteins that are associated with the components of the cytoskeleton and centrosomes, such as dynein, Nudel, and elongation protein zeta-1 (Kamiya et al. 2005; Millar et al. 2003; Morris et al. 2003; Miyoshi et al. 2003, 2004; Ozeki et al. 2003). DISC1 plays critical roles in the cerebral cortex development via microtubular dynamics and the DISC1–dynein complex (Kamiya et al. 2005). Another function of DISC1 may be the modulation of cAMP signaling via an interaction with phosphodiesterase 4B, which also has been found to be disrupted by a balanced translocation in a patient with schizophrenia (Millar et al. 2005). Phosphodiesterase inhibitors may have a role in the treatment of certain neuropsychiatric fatigue-related conditions, such as CFS (Staines et al. 2009). Other functions of DISC1, including mitochondrial and nuclear functions, have also been suggested (Morris et al. 2003; Sawamura et al. 2005; James et al. 2004). Mitochondrial ability was significantly associated with CFS severity (Myhill et al. 2009). Hashimoto et al. (2006) revealed that healthy subjects who carried the risk allele for MDD (Cys704 DISC1) had relatively reduced gray matter volumes in their cingulate cortex. Previous studies revealed a reduction in gray matter volume in the bilateral prefrontal cortex in patients with CFS (Okada et al. 2004). CFS shows results that were reportedly generated by DISC1 (Kamiya et al. 2005; Millar et al. 2003, 2005; Morris et al. 2003; Miyoshi et al. 2003, 2004; Ozeki et al. 2003; Staines et al. 2009; Sawamura et al. 2005; James et al. 2004; Myhill et al. 2009). DISC1 Ser704Cys might be a functional variant that affects one of the mechanisms implicated in the biology of CFS. Despite the variety of patients with CFS, DISC1 Ser704Cys has an association with CFS, which may also suggest that *DISC1* plays a central role in the induction of various psychiatric diseases. *DISC1* might be more central to human psychological functioning than previously thought (Tomppo et al. 2009). However, the mechanism or causes of CFS are still unclear. Further studies are needed to clarify the neural mechanism of the Cys allele in patients with CFS.

Conclusion

In summary, the Cys704 allele of Ser704Cys SNP was associated with an increased risk of CFS development compared with the Ser704 allele. DISC1 Ser704Cys might be a functional variant that affects one of the mechanisms implicated in the biology of CFS. Some patients with CFS showed a phenotype similar to that of patients with MDD, but further studies are needed to clarify the mechanism and the cause of CFS, because this study is of a rather preliminary nature.

Conflict of interest statement

We have no conflict of interest to declare in all of the above categories.

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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*

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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.

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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.

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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.

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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

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Fax +41 61 306 12 34
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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

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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,

The Consortium on Lithium Genetics

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.

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