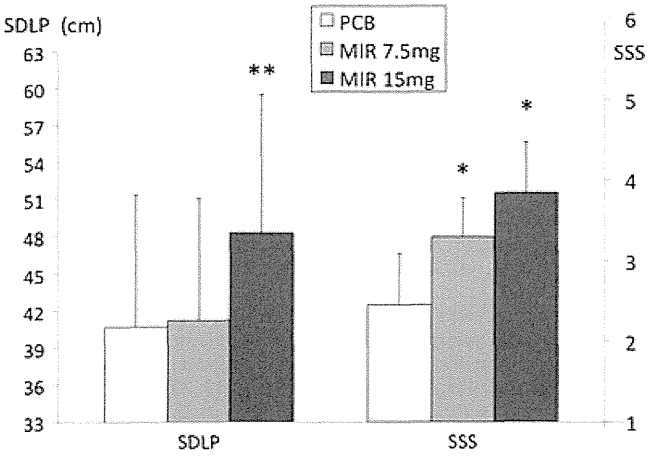

Figure 2. Mean standard deviation of lateral position (SDLP) (left) and Stanford Sleepiness Scale (SSS) (right) on days 2 of the crossover treatment with mirtazapine (MIR) 7.5 mg, MIR 15 mg, or placebo (PCB). **Post-hoc Bonferroni test demonstrated that SDLP under the MIR 15 mg series was significantly greater than that observed under the MIR 7.5 mg series or PCB series ($p < 0.01$, both). *Post-hoc Bonferroni test demonstrated that SSS in the MIR 7.5 and 15 mg series were significantly greater than that observed in the PCB series ($p < 0.05$, both). All statistics were corrected for baseline values

histamine$_1$ receptor occupancy have been confirmed using positron emission tomography (Tashiro *et al.*, 2009). The same effect may also be applicable to mirtazapine only at single low dose. In fact, the present study showed that SDLP after single dose of mirtazapine 7.5 mg was significantly lower than that of 15 mg and SSS after single dose of mirtazapine 7.5 mg was nonsignificantly lower than that of 15 mg. In addition, this impairing effect of mirtazapine disappeared after repeated dosing because of tolerance (Ramaekers, 2003) as with antihistamines. Moreover, the sensitivity of road-tracking test for histamine$_1$ antagonism may be related to the difference in driving impairment between mirtazapine 7.5 and 15 mg doses. Further studies should investigate the dose-dependene of mirtazapine effects using subjective and objective measures of sedation, including neuroimaging.

Mirtazapine 7.5 mg did not impair road-tracking performance in acute dosing, but significantly increased subjective sleepiness. This discrepancy between objective performance and subjective sedation may be attributable to different level and mechanisms of sedation (Hindmarch, 1998). Wezenberg *et al.* (2007) showed that objective sedation tests helped uncover differences in sedative effects, whereas subjective testing or use of a visual analogue scale could not discriminate between drugs and dosages. In the present study, mirtazapine 7.5 mg may result in less sedation as measured by driving performance than mirtazapine 15 mg, whereas SSS did not discriminate between sedation with different dosage regimens. Furthermore, evening dose of mirtazapine produced somnolence, but its effect on driving performance was mild in the next day (Ramaekers, 2003). On the contrary, the predictive validity of the alertness for driving performance was low (Verster and Roth, 2012). Thus, the examinations of both objective and subjective measures are important when considering psychotrophics' effects on driving performance.

Previous study examined low-dose effects of esmirtazapine on actual driving (Ramaekers *et al.*, 2011). Esmirtazapine 4.5 mg, unlike 1.5 mg, impaired actual road-tracking performance, and its' acute effect on driving impairment is suggested to be dose-dependent. It is difficult to clearly explain that esmirtazapine 4.5 mg caused significant driving impairment and mirtazapine 7.5 mg did not. Esmirtazapine has approximately the same affinity to histamine$_1$ receptors as mirtazapine and is believed to be responsible for alpha$_2$ heteroreceptor blockade and the 5-HT$_3$ receptor antagonism (de Boer *et al.*, 1988; Kooyman *et al.*, 1994; Haddjeri *et al.*, 1996). This discrepancy in driving impairment cannot be accounted for by the difference in receptor binding profiles. Instead, sample size, sex of subjects (Timmer *et al.*, 2000; Borobia *et al.*, 2009), CYP2D6 genotype (Timmer *et al.*, 2000; Brockmoller *et al.*, 2007; Borobia *et al.*, 2009; Ramaekers *et al.*, 2011), and the sensitivity of driving test may explain the different results. Future study needs to draw a comparison between mirtazapine and esmirtazapine in the same low dose. Meanwhile, dose-dependent influence of mirtazapine may be consistent with that of esmirtazapine at low dosage.

The present study has several limitations. First, participation was restricted to a small number of healthy adult male volunteers. Female, elderly, and depressed patients were not included. Mirtazapine could both impair driving performance (Wingen *et al.*, 2005) and improve driving ability in depressed patients (Brunnauer *et al.*, 2008; Shen *et al.*, 2009). Meanwhile, depressed patients' psychomotor impairments related to driving abilities were influenced by different classes of antidepressants (Brunnauer *et al.*, 2006). Because of many confounding factors such as antidepressant treatment and the depression itself, it is important to examine the effect of antidepressant on driving performance in healthy subject to find the inherent influences of antidepressants for driving impairment. Future study needs to elucidate the impact of same antidepressants in depressed patients in same experimental line and make a comparison with depressed patients. Second, the validity and sensitivity of DS need to be considered. This

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DS has not been validated against real car driving; however, our past results using same DS are roughly consistent with preceding results using actual driving test (Iwamoto *et al.*, 2008; Takahashi *et al.*, 2010). In future studies, we are aiming to verify the validity of DS for real car driving in cooperation with Toyota Central R&D Labs to return the results of research to society. Third, we need to evaluate dose–response relationships within the range of up to 30 mg, to clarify the impact of a lower initial dose of mirtazapine on driving performance.

Finally, mirtazapine 7.5 mg did not impair road-tracking performance compared with mirtazapine 15 mg. An initial lower dose of mirtazapine may have less harmful effect on driving performance and be more suitable for some patients as a starting dosage.

CONFLICT OF INTEREST

There is no conflict of interest that is directly relevant to the content of this study.

ACKNOWLEDGEMENTS

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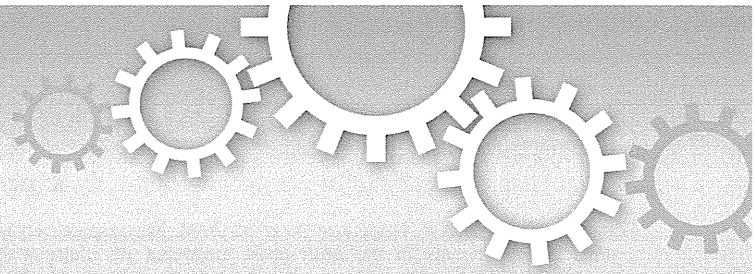

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Definition and refinement of the 7q36.3 duplication region associated with schizophrenia

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Using a very high-resolution oligonucleotide array for copy number variant (CNV) screening of samples comprising schizophrenic patients, we detected a novel CNV within the critical region (NCBI36/hg18, Chr7: 158,630,410–158,719,410) previously shown to be associated with schizophrenia. We investigated the association between the novel CNV identified in the current study and schizophrenia. Three independent samples were used: (1) *Screening set*, 300 Japanese schizophrenic patients (53.28 ± 14.66 years); (2) *Confirmation set*, 531 schizophrenic patients (46.03 ± 12.15 years); and (3) 711 healthy controls (47.12 ± 11.03 years). All subjects enrolled in the study were Japanese. Chromosomal position was determined using fluorescence in situ hybridization. We identified a novel duplication within the region associated with schizophrenia identified on 7q36.3 that is adjacent to *VIPR2* and is not associated with schizophrenia. In the Japanese population, the 35-kb region that harbors the common, novel CNV should be excluded from the region associated with schizophrenia on 7q36.3.

Schizophrenia is a chronic, debilitating illness characterized by impairments in cognition, affect and behaviour[1]. The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM IV-TR)[2] defines the essential features of schizophrenia as a mixture of characteristic signs and symptoms (both positive and negative) that have been present for a significant portion of time during a 1-month period (or for a shorter time if successfully treated), with some signs of the disorder persisting for at least 6 months. In this regard, positive refers to the presence of active symptoms including delusions and hallucinations. Negative symptoms refer to a loss, typically of emotions, speech, or motivation. Schizophrenic disorders exist on a continuum from mild to severe. The DSM IV-TR[2] recognizes a number of different types, which include disorganized, catatonic, paranoid, schizophreniform, residual, schizoaffective, undifferentiated and not otherwise specified[2]. Schizophrenia is a relatively common disorder, with a lifetime prevalence of about 1%[1]. Although the overall sex ratio is almost equal, males tend to have an earlier onset than females, a finding accounted for by the later age of onset in those females who lack a family history of the disease[3]. Family history is the most important risk factor for schizophrenia, consistent with a genetic contribution to its etiology[4]. However, as with most mental disorders, the origins and mechanisms of schizophrenia are not fully understood.

Genetic factors influence human disorders by determining disease susceptibility or resistance[5]. Therefore, genetic studies can help pinpoint the exact molecular mechanism of a disease. Recent successes in the genetic mapping and molecular mechanism of the Mendelian traits have been remarkable, owning to the development of genome wide screening techniques[6]. As such, attention has been gradually shifting towards more complex, common, genetic disorders and traits that involve multiple genes and environmental effects, such as celiac disease[7], diabetes[8], rheumatoid arthritis[9] and psychiatric disorders[10]. In this context, recurrent microdeletions at 1q21.1[11], 15q13.3[12], and 15q11.2[12], microduplications at 16p11.2[13], and copy number variations (CNVs) at other genomic loci[14] have been shown to be associated with schizophrenia in large cohorts examined by CNV analyses and other molecular studies. Furthermore, duplication at chromosome 7q36.3, encompassing *VIPR2*, was implicated in schizophrenia for the first time in a recent report[15]. In a specific genome-wide association study of 8,290 patients with schizophrenia performed by Vacic et al.[15], the authors found that 0.35% of these patients carry rare CNVs in the chromosomal locus 7q36.3. In contrast, these microduplications were much less frequent

(0.03%) among the 7,431 healthy controls. All variants overlap with *VIPR2* or lie within the noncoding subtelomeric region, <89 kb (NCBI36/hg18, Chr7: 158,630,410–158,719,410) from the transcriptional start site of *VIPR2*. This gene encodes the vasoactive intestinal peptide (VIP) receptor VPAC2, which is a G-protein-coupled receptor that is expressed in the suprachiasmatic nucleus, hippocampus, amygdala and hypothalamus[16]. VPAC2 binds VIP, activates cyclic AMP (cAMP)-signalling and PKA, regulates synaptic transmission in the hippocampus, and promotes the proliferation of neural progenitor cells in the dentate gyrus[17]. Moreover, it has been shown that alteration in synaptic plasticity of hippocampal neurons may contribute to the symptoms observed in schizophrenic patients[18]. The aforementioned lines of evidence provide support for the role of *VIPR2* as a candidate gene for schizophrenia from a biological point of view.

In the present study, by using a very high-resolution oligonucleotide array for CNV screening of samples from schizophrenic patients, we were able to detect CNV within the critical region (NCBI36/hg18, Chr7: 158,630,410–158,719,410) on 7q36.3 that was shown to be associated with schizophrenia by the Vacic et al. study[15]. Thus, the goal of the present study was to follow-up on the novel CNV that was previously detected in schizophrenic patients and further investigate any association between this CNV and schizophrenia.

Results

In the present study, we detected a smaller (35 kb) duplication (NCBI36/hg18, Chr7: 158,658,128–158,693,128) within the critical region identified by Vacic et al.[15] (Figure 1). The observed frequency of the CNVs was ~2% and we did not detect any statistically significant difference between the patients and controls (Table 1). There was a 100% concordance rate between the custom NimbleGen 12 × 135,000 CGH arrays and the NimbleGen 3 × 720,000 CGH arrays or custom TaqMan copy number assay for the detection of the smaller (35 kb) duplication (NCBI36/hg18, Chr7: 158,658,128–158,693,128) within the critical region (NCBI36/hg18, Chr7: 158,630,410–158,719,410) previously identified by Vacic et al.[15].

In metaphase cells, all duplication-specific FISH signals localize to the subtelomeric region of 7q, confirming that the duplications lie adjacent to each other in the 7q36.3 region (Figure 2). In addition, NS102 exhibited two signals, one of which had a higher intensity compared to that of the other. This suggests that there is unilocus duplication in the *VIPR2* promoter region. During the orientation analysis, an amplicon was detected by electrophoresis only in samples with duplication, which indicates that there is a head-to-tail orientation of the repeated DNA fragment (Supplementary Figure 1). Additionally, sequence analysis of the repeat junction revealed that all samples with duplication shared exactly the same sequence within the junction region (Supplementary Figure 2). Based on the

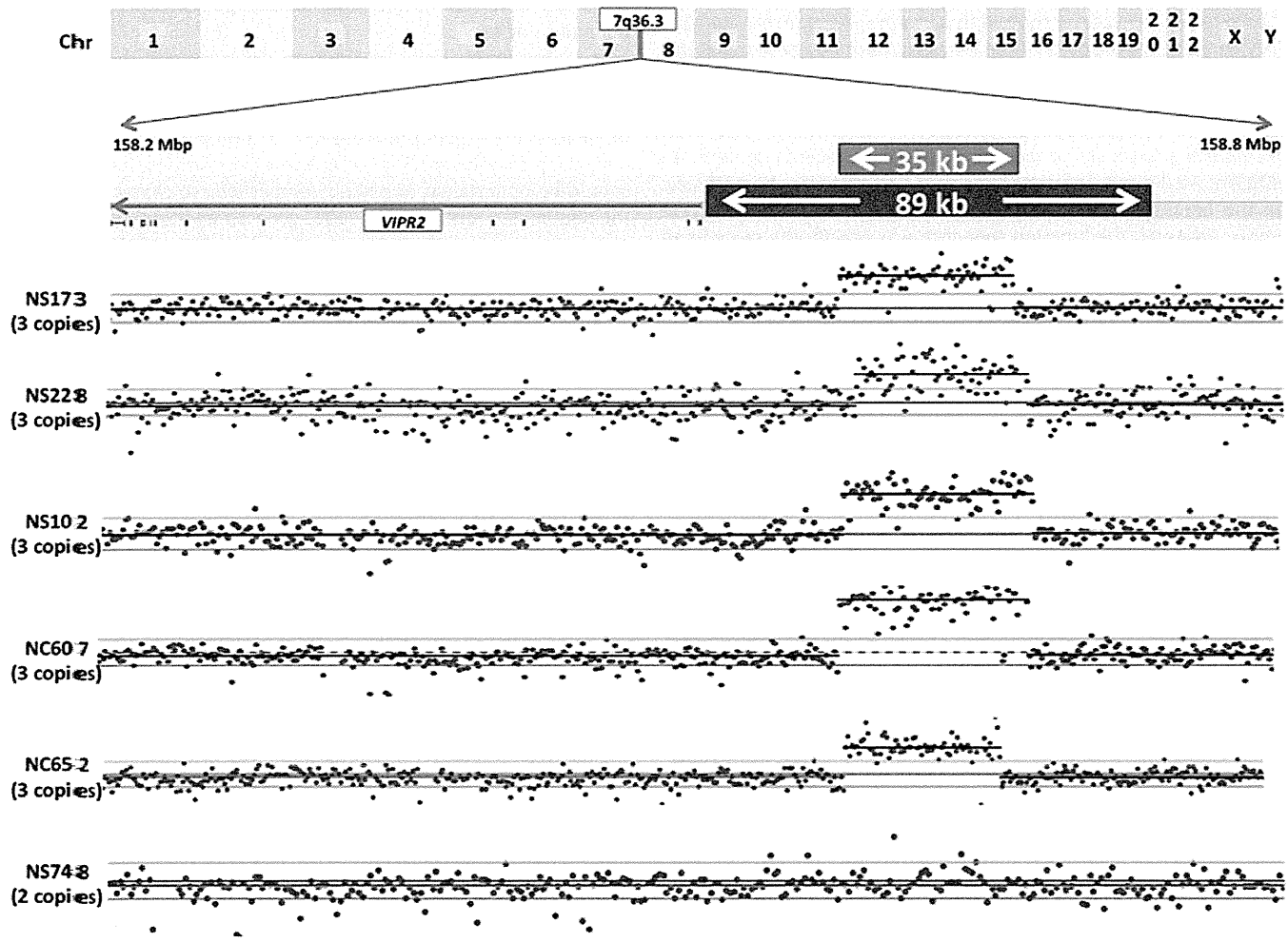

Figure 1 | High-resolution aCGH data. Probe intensity ratios. The orange box (NCBI36/hg18, Chr7: 158,658,128–158,693,128) represents CNVs detected in the present study and the purple box (NCBI36/hg18, Chr7: 158,630,410–158,719,410) represents the region revealed by Vacic et al. to show an association peak in a schizophrenia group. Coordinates are based on the NCBI36 build.

Table 1 | Frequency distribution (confirmation set)

		Number of copies										Aggregated data		
		0		1		2		3		4		>2 copies		P-Value
Set	Sample size	N	%	N	%	N	%	N	%	N	%	N	%	(Fisher exact test)
Cases	531	0	0.0%	0	0.0%	520	97.9%	10	1.9%	1	0.2%	11	2.1%	0.96
Controls	711	0	0.0%	0	0.0%	696	97.9%	13	1.8%	2	0.3%	15	2.1%	

panel of 8 SNPs in the CNV region that were detected in the current study, we did not observe the existence of any common haplotype (Supplementary Table 2).

Discussion

Using samples from only Japanese subjects, we identified a smaller 35-kb (NCBI36/hg18, Chr7: 158,658,128–158,693,128) common (>1%) duplication within the region that Vacic et al.[15] previously showed was associated with schizophrenia (NCBI36/hg18, Chr7: 158,630,410–158,719,410). However, the common duplication that we detected in the present study was not found to be associated with schizophrenia. It is of note that these results may be specific to Japanese subjects, and therefore further studies involving other population groups will need to be undertaken. We have experimentally confirmed that the common CNV detected in the current study was located adjacent to *VIPR2*. Our analysis of the breakpoint junctions at the sequence level showed there was no difference among the CNV carriers. On the basis of the 2-bp microhomology found at this junction (Supplementary Figure 2), we speculate that duplication formation occurs by the FoSTeS (fork stalling and template switching)/MMBIR (microhomology mediated break-induced replication) mechanism previously proposed by Lupski's research group[23]. In addition, we did not find any sequence motif that was characteristic for the breakpoint of recurrent rearrangements at the junction region. Although the junction sequence was exactly the same in all of the subjects in whom the 35-kb CNV was detected, we could not confirm that these subjects shared any common haplotype. Differences in the haplotype among the duplication carriers were likely due to the high recombination rate that occurs at the subtelomeric region[24]. Regarding the origin of the CNV that was identified in the current study, our observation of the same breakpoint junction sequence that was observed in the CNV carriers is highly suggestive of a common ancestral founder.

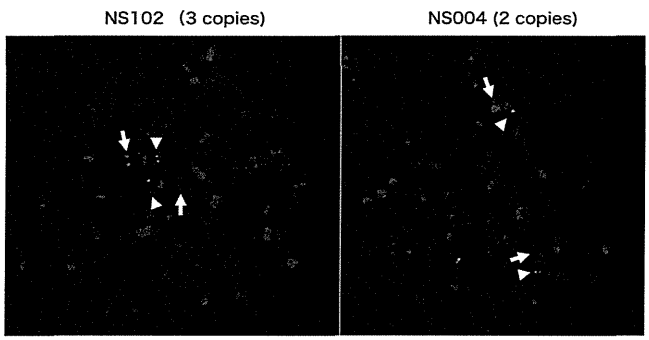

Figure 2 | Tandem duplications of 7q36.3 confirmed in two patients by fluorescence in situ hybridization (FISH). 7p-green (arrowheads) and 7q-red (arrows, CNV specific). Left NS102 (3 copies); Right NS004 (2 copies). Cytogenetic confirmation was obtained for two samples with and without duplication of VIPR2. Probes for duplicated region were produced by long range PCR. The subtelomeric probe, 7p-green (Abbott Molecular), was used as a reference. Hybridizations were performed according to the manufacturer's protocols.

The main limitation of the current study was that we examined a much smaller number of samples as compared to the Vacic et al. study[15]. It is of note that the frequency of the common 35-kb CNV detected in the current study was 2%, and thus with our current sample size of 300 schizophrenic patients, it was large enough to capture the variation. Regarding the individual with 4 copies, we do not have any data indicating whether the individual is a homozygote of duplication or is a carrier of triplication. This point should be considered as another limitation of the current study. The results of the current study do suggest that in case of a duplication event on 7q36.3, the relevant region is not the *VIPR2* promoter (as has been suggested by Vacic et al.[15]), but rather suggest that it is the *VIPR2* gene region. In addition, the 2-bp microhomology in the promoter region of VIPR2 may be associated with the relative meiotic instability of the region harboring the common CNV that is adjacent to the *VIPR2* gene[25]. This in turn may give rise to the larger *VIPR2* duplications that were shown to be associated with schizophrenia with an odds ratio of 4.0[14].

It is interesting that our findings demonstrated that CNV was detected in our study in contrast to the previous reports by both Vacic et al.[15] and Beri et al.[26]. Moreover, CNV has not been listed in the database of genomic variants[27]. Although the CNV detected by our group may be specific to Japanese populations, further studies should be undertaken to ensure comprehensive characterization of the region surrounding the *VIPR2* gene. In addition, to address the question regarding the origin of the CNV detected in the current study, it is necessary to perform family analysis of the carriers and determine whether CNV is a *de novo* event, or if it is transmitted from the parents. In conclusion, the 35-kb region that harbors the common CNV in the Japanese population should be excluded from the region of the association peak in the schizophrenia group reported in the Vacic et al. study[15].

Methods

Three independent samples were used in the current study: (1) *Screening set*, 300 Japanese patients suffering from schizophrenia (53.28 ± 14.66 years); (2) *Confirmation set*, 531 patients suffering from schizophrenia (46.03 ± 12.15 years); and (3) 711 healthy control subjects (47.12 ± 11.03 years). All schizophrenic patients met the current DSM IV-TR criteria[2], which was reflected by consensual diagnosis of two experienced psychiatrists. Prior to inclusion in the control set, subjects were screened on the basis of a brief diagnostic interview. Detailed characterization and psychiatric assessment of the subjects is available elsewhere[19]. All subjects enrolled in the study were Japanese and provided written informed consent prior to the study. Venous blood was drawn from each subject and genomic DNA was extracted according to the standard phenol/chloroform method. Comparative genomic hybridization of DNA was performed using the high-resolution NimbleGen (Roche NimbleGen, Inc., U.S.) CGH array (3 × 720,000 or 12 × 135,000). Labeling and hybridization of patient (test) and sex-matched commercial (Promega Corporation, U.S.) reference DNA was performed according to the manufacturer's protocols. Test and reference DNA were labeled by Cy3- and Cy5-labeled random primers, respectively, and were combined and hybridized to the array for 40–72 h. Arrays were washed in four steps, as indicated in the protocol. Two-color scanning was performed using a NimbleGen MS 200 microarray scanner. Acquisition of the microarray images was performed with NimbleGen MS 200 software. Data extraction, analysis and visualization were done using NimbleScan version 2.4 software. CNV calling was performed using NEXUS software. The FASST2 Segmentation Algorithm, a Hidden Markov Model (HMM) based approach, was used to make copy number calls. The FASST2 algorithm, unlike other common HMM methods for copy number estimation, does not aim to estimate the copy number state at each probe, but uses many states to cover more possibilities, such as mosaic events. These state values are then used to make calls based on a log ratio threshold. The significance threshold for

segmentation was set at 10^{-6} and also required a minimum of three probes per segment. The log ratio thresholds for single copy gain and single copy loss were set at 0.3 and −0.3, respectively. The log ratio thresholds for the gain of two or more copies and homozygous loss were set at 0.9 and −0.9 respectively.

Custom TaqMan copy number assay was specifically designed to interrogate a duplication region (NCBI36/hg18, Chr7: 158,630,410–158,719,410) without interspersed repeats, low complexity or a homologous DNA sequence. A TaqMan copy number assay for RNase P was used as a reference. Experiments were carried out on four technical replicates according to the manufacturer's protocol. CNV typing of the screening sample was performed using Roche NimbleGen, Inc. CGH array 3 × 720,000, while confirmation of the sample was performed using the TaqMan copy number assay. Sixteen randomly selected duplication events (both in the screening and confirmation samples) were validated using custom NimbleGen 12 × 135,000 CGH arrays (Roche NimbleGen, Inc., U.S.) covering the region (NCBI36/hg18, Chr7: 158,630,410–158,719,410) implicated in the Vacic et al. study[15], with an average of one probe per 500 bp. P values derived from association analysis were based on Fisher's exact test.

We performed PCR based analysis to determine the orientation of the detected duplications. We designed forward and reversed primers to align with the region of the duplication junction (F: 5′-TGTGGATTCCCTTCAGAGGCGAC-3′, R: 5′-CATTCTTCAGCCCATGGAGTCATC-3′) (Supplementary Figure 1). Cytogenetic confirmation was obtained for two samples with and without duplication of VIPR2. Probes for duplicated region were produced by long range PCR (NCBI36/hg18, Chr7: 158,658,128–158,693,128). Subtelomeric probe, 7p-green (Abbott Molecular, U.S.), was used as a reference. Hybridizations were performed according to the manufacturer's protocols.

Haplotypes were estimated using the statistical software package PHASE version 3.4.1 (http://www.stat.washington.edu/stephens/)[20–22]. This program is based on a Bayesian statistical method using coalescent-based models that infers phases at loci from unphased genotype data for a sample of unrelated individuals[20]. The algorithm uses a flexible model for the decay of linkage disequilibrium with distance and explicitly incorporates an assumption about the recombination rate variation. PHASE uses Gibbs sampling, a Markov-Chain Monte Carlo algorithm for the estimation of the posterior distribution. Hence, the individual haplotype can be estimated from the posterior distribution by choosing the most likely haplotype reconstruction for each individual. Using the extension for unrelated individuals, we used the default settings to infer the haplotypes from the genotype data[19] of the 8 SNPs (Supplementary Table 1) surrounding the duplication in sample that comprised 517 subjects (7 with a structural variant detected in the current study). Estimates of the sample haplotype frequencies together with their standard deviation, a list of the most likely pairs of haplotypes for each individual together with their probability, and the estimates of recombination parameters in the region, were calculated using the same software.

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Author contributions

B.A., I.K., T.O. and N.O. designed the study and wrote the protocol. B.A., I.K., T.O., N.I., H.K. and N.O. performed the literature review. B.A., I.K. and T.O. made and managed the sample database. B.A., I.K., T.O., M.I., S.K., Y.N., A.Y., T.K., S.I., H.K., N.I. and N.O. collected and managed the genome samples. B.A., I.K., T.O., M.I., S.K., Y.N., A.Y., T.K. and S.I. conducted the statistical analysis. B.A., I.K., T.O., M.I., S.K., Y.N., A.Y., T.K., S.I., H.K., N.I. and N.O. interpreted and discussed the results. B.A., I.K., T.O., M.I., H.K., N.I. and N.O. wrote the manuscript and edited the final manuscript.

Additional information

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Analysis of the *VAV3* as Candidate Gene for Schizophrenia: Evidences From Voxel-Based Morphometry and Mutation Screening

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In recently completed Japanese genome-wide association studies (GWAS) of schizophrenia (JPN_GWAS) one of the top association signals was detected in the region of *VAV3*, a gene that maps to the chromosome 1p13.3. In order to complement JPN_GWAS findings, we tested the association of rs1410403 with brain structure in healthy individuals and schizophrenic patients and performed exon resequencing of *VAV3*. We performed voxel-based morphometry (VBM) and mutation screening of *VAV3*. Four independent samples were used in the present study: (1) for VBM analysis, we used case-control sample comprising 100 patients with schizophrenia and 264 healthy controls, (2) mutation analysis was performed on a total of 321 patients suffering from schizophrenia, and 2 case-control samples (3) 729 unrelated patients with schizophrenia and 564 healthy comparison subjects, and (4) sample comprising 1511 cases and 1517 healthy comparison subjects and were used for genetic association analysis of novel coding variants with schizophrenia. The VBM analysis suggests that rs1410403 might affect the volume of the left superior and middle temporal gyri ($P = .011$ and $P = .013$, respectively), which were reduced in patients with schizophrenia compared with healthy subjects. Moreover, 4 rare novel missense variants were detected. The mutations were followed-up in large independent sample, and one of the novel variants (Glu741Gly) was associated with schizophrenia ($P = .02$). These findings demonstrate that *VAV3* can be seen as novel candidate gene for schizophrenia in which both rare and common variants may be related to increased genetic risk for schizophrenia in Japanese population.

Key words: resequencing/MRI/Japanese population/axon guidance/rare variant/GWAS

Introduction

Schizophrenia is a severe mental disorder with a lifetime risk of about 1%, characterized by hallucinations, delusions, and cognitive deficits, with heritability estimated at up to 80%. Recently, there have been a few major advances in identifying common variants associated with schizophrenia by genome-wide association studies (GWAS). The GWAS approach has both highlighted genes previously identified by the candidate gene approach studies or by basic biological investigation and illuminated novel genomic loci clearly associated with schizophrenia that were previously unsuspected.[1–3]

In recently completed Japanese GWAS of schizophrenia (JPN_GWAS),[4] one of the top association signals based on the meta-analysis for Japanese sample (rs1410403, $P_{CMH} = 9.3 \times 10^{-4}$, OR = 0.86) was detected in the region of VAV3 (see online supplementary table 1). VAV3 is closely related to the axon guidance pathways, which are implicated in etiology of schizophrenia.[5] During axon guidance, ephrin binding to Ephs triggers VAV-dependent endocytosis of the ligand-receptor

complex, converting an initially adhesive interaction into a repulsive event. In the absence of VAVs, ephrin-Eph endocytosis is blocked, leading to defects in growth cone collapse in vitro and significant defects in the ipsilateral retinogeniculate projections in vivo.[6] Therefore, VAV family guanine nucleotide exchange factors (GEFs) may play an important role as regulators of ligand-receptor endocytosis and determinants of repulsive signaling during axon guidance. The additional findings implicating the relevance of this locus for pathogenesis of schizophrenia came from genomewide linkage analysis of 236 Japanese families.[7] Specifically, they located the strongest evidence of linkage at rs2048839 (LOD [logarithm (base 10) of odds] = 3.39) and 95% CI comprises *VAV3* locus (Chr1: 102.0–111.9 Mbp, based on NCBI36 annotation).

Based on genetic evidence from the JPN_GWAS, the meta-analysis for Japanese sample, biological studies, and linkage evidence *VAV3* can be seen as novel candidate gene for schizophrenia. Therefore, to follow-up JPN_GWAS findings, we tested the association of rs1410403 with brain structure in healthy individuals and schizophrenic patients. Because biological phenotypes (eg, brain structure and function) are thought to more closely reflect the effects of genetic variation as compared with manifest psychiatric illness, endophenotype studies have proven to be more robust and require vastly smaller sample sizes than purely diagnosis-based studies.[8] Furthermore, statistical genetic association studies can provide a link between genes and complex polygenetic constructs like schizophrenia, but this approach does not illuminate the possible underlying pathophysiology impacted or the mechanisms of association. Here, we used imaging approach to examine the impact of variation in *VAV3* on risk for schizophrenia and function and structure in human brain of neural circuitries implicated in the pathophysiology of schizophrenia. Furthermore, in terms of genetic architecture, liability to schizophrenia is related to the number of loci involved and the effect size of each risk variant, and on the population level, these 2 factors combine to form an "allelic spectrum" which is bounded by "common disease/common variant" and "multiple rare variant" models.[9] Based on the results of recent schizophrenia GWAS, it was suggested that common variants can explain at least one-third of the total variation in liability, and genetic transmission patterns in schizophrenia may be a complex hybrid of common, low-penetrant alleles and rare, highly penetrant variants.[1] Therefore, in order to complement JPN_GWAS findings and search for novel rare variants with larger effect, we performed exon resequencing of *VAV3*.

Methods

Sample

Four independent samples were used in the present study: (1) for the voxel-based morphometry (VBM) analysis, we used case-control sample comprising 100 patients with schizophrenia (38.3 ± 13.0 y) and 264 healthy comparison subjects (36.7 ± 11.9 y), (2) mutation analysis was performed on a total of 321 patients suffering from schizophrenia (54.3 ± 14.1 y) (JMut sample) (3) JPN_GWAS comprised of 729 unrelated patients with schizophrenia (45.4 ± 15.1 y) and 564 healthy comparison subjects (44.0 ± 14.4 y) and (4) Rep_JPN comprising 1511 cases (45.9 ± 14.0 y) and 1517 healthy comparison subjects (46.0 ± 14.6 y). JPN_GWAS and Rep_JPN were used for genetic association analysis of novel coding variants with schizophrenia. The individuals with personal or family history of psychiatric disorders (first-degree relatives only based on the subject's interview) were not included in the healthy comparison group. After complete description of the study to the subjects, written informed consent was obtained. A general characterization and psychiatric assessment of subjects are available elsewhere.[10]

Voxel-Based Morphometry

All magnetic resonance (MR) studies were performed on a 1.5T GE Sigma EXCITE system. A 3-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of 124 sagittal sections using a spoiled gradient recalled acquisition in the steady state sequence (TE [Eecho time]/TR [repetition time], 4.2/12.6 ms; flip angle, 15°; acquisition matrix, 256 × 256; 1NEX [number of excitations], FOV [Field of view], 24 × 24 cm; and slice thickness, 1.4 mm). Statistical analyses were performed with Statistical Parametric Mapping 5 (SPM5) software (http//www.fil.ion.ucl.ac.uk/spm) running on MATLAB R2007a (MathWorks, Natick, MA). MR images were processed using optimized VBM in SPM5 according to VBM5.1-Manual (http://dbm.neuro.uni-jena.de/vbm/vbm5-for-spm5/manual/) as described in detail previously.[11,12] Each image was confirmed to eliminate images with artifacts and then anterior commissure-posterior commissure line was adjusted. The normalized segmented images were modulated by multiplication with Jacobian determinants of the spatial normalization function to encode the deformation field for each subject as tissue density changes in the normal space. Finally, images were smoothed with a 12-mm full-width half-maximum of isotropic Gaussian kernel.

In first stage of the analysis, we performed whole brain search to explore the effects of diagnosis, genotype, and their interaction on gray matter (GM) volume in patients with schizophrenia and controls. These effects on GM volume were assessed statistically using the full factorial model for a 2 × 2 ANOVA in SPM5. We contrasted GM volumes between the genotype groups (individuals with A/A genotype and G-carriers), the diagnosis groups (smaller volume region in patients with schizophrenia relative to controls), and the diagnosis-genotype interaction. Age, sex, and education years were included to

control for confounding in all analyses. Because it is desirable to adjust for each subject's global GM volume,[11] adjustment was performed by entering the global GM values as a covariate. Nonsphericity estimation was used. These analyses yielded statistical parametric maps (SPM (t)) based on a voxel-level height threshold of $P < .001$ (uncorrected for multiple comparisons). Only the clusters of more than 100 contiguous voxels were considered in the analyses. Additionally, small volume correction (SVC) was applied in order to protect against type I error using family wise error (FWE). The significance level was set $P < 0.05$ (FWE corrected) after SVC, spheres with radius 10 mm around the peak.

In second stage of the analysis, we extracted a sphere of 10 mm volume of interest (VOI)-radius on left superior temporal gyrus and left middle temporal gyrus to compare regions of the genotype effects. Anatomic localization was according to both MNI coordinates and Talairach coordinates, obtained from M. Brett's transformations (http://www.mrccbu.cam.ac.uk/Imaging/Common/mnispace.shtml) and presented as Talairach coordinates.

Statistical analyses were performed using PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls or between genotypes were analyzed using χ^2 tests for categorical variables and the Mann-Whitney U test for continuous variables. We extracted the "y" values from the left superior temporal gyrus and left middle temporal gyrus maxima voxel and used these values in the VOI analysis using PASW. The effects of the variant in *VAV3* on extracted VOI were tested using the 2-way ANOVA without covariates because the extraction of VOI was performed after confounding factors, including age, sex, education years, and total GM volumes, were included in the whole-brain search analyses. Statistical significance was defined as $P < .05$.

Mutation Screening

For the purpose of mutation screening, we have designed a custom resequencing microarray, based on NCBI36 build (Affymetrix, Santa Clara, CA), NAGOYA_DESIGN, which primarily focuses on the genes selected, based on the JPN_GWAS findings. The sequences tiled on the microarray included the sequences of all *VAV3* exons totaling 4933 bps (consensus CDS transcripts ENST00000370056 and ENST00000343258). Because the principle of the resequencing microarray is based on sequencing by hybridization, it was crucially important to avoid cross-hybridization to increase the accuracy of resequencing. For this purpose, we conducted an in-silico screening to compare the tiled sequences with a sliding 25-nucleotide window to detect the sequences with an identity exceeding 22 bases in the tiled sequences and optimized the design of the microarrays and polymerase chain reaction (PCR) primers. Initially, the arrays were run according to the manufacturer's protocol. Briefly, long-range PCR conditions for the LA TaKaRa Polymerase (Takara, Japan) were: TaKaRa LA Taq 0.05 U/μl, 1X LA PCR Buffer II, 400 μM (each) deoxynucleotide triphosphate, 0.3 μM (each) primers, 4 ng/μl genomic DNA in a 25 μl reaction volume. Modifications using standard approaches to PCR optimization were made for some difficult reactions. All PCR assays were quantified using PicoGreen (Molecular Probes, Eugene, OR) and then pooled in equimolar amounts. The PCR products were then purified, fragmented, labeled, and hybridized to the array. Finally, affymetrix GSEQ 4.0 Software (default settings) was used to process raw data and analyze the nucleotide sequences. SeqC Ver. 3.2.1.5 (JSI-medisys, www.jsi-medisys.de) was used to reanalyze the acquired datasets and assign annotation (based on NCBI 36 build). Novel variants with frequency of less than 5% were validated by cycle sequencing on ABI 3130xl DNA Analyzer (Applied Biosystems) according to standard manufacturer protocol. Allelic discrimination was performed using Taqman (Applied Biosystems) custom probes (details about DNA sequences, and PCR conditions are available upon request). Each 384 microtiter plate contained at least 3 nontemplate controls and the sample(s) in which novel variant was observed. Analysis was performed on HT7500 instrument (Applied Biosystems) according to the standard protocol. In-silico analysis of deleterious effect of amino acid substitution was done by algorithms implemented in LRT,[13] PMUT,[14] and PANTHER.[15] All these tools operate using approximately the same principles, that is, they are all supervised and employ features based on protein sequence, sequence conservation, and/or protein structure. The interpretation of the results was done based on score of the likelihood that missense variants, which cause a single amino acid substitution within a protein sequence, may or may not lead to altered protein function.

As basis for a more detailed functional interpretation of the novel rare variants, we performed ab-initio structure predictions based on I-TASSER algorithm.[16] This automated pipeline predicts secondary and tertiary protein structure based on sequence homology between protein/domain of interest and the proteins/domains with experimentally determined structures. The output of I-TASSER is analyzed and visualized using UCSF Chimera.[17]

All allele-wise association analyses were carried out by calculating the P values for each SNP using Fisher's exact test. In meta-analysis (JPN_GWAS and Rep_JPN sample), P values were generated by Cochran-Mantel-Haenszel stratified analysis. Two-tailed P values of less than 0.05 were considered significant. Calculations were done using Plink v1.07.[18]

Table 1. Demographic Information for Patients With Schizophrenia and Healthy Controls Included in the VBM Analysis

	Schizophrenia (N = 100)					Control (N = 264)					Group Difference
	A/A		G-carrier			A/A		G-carrier			
	(N = 52)		(N = 48)			(N = 131)		(N = 133)			
Variables[a]	Mean	SD	Mean	SD	P values (z)[b]	Mean	SD	Mean	SD	P values (z)[b]	P values (z)[b]
Age (years)	37.7	12.5	39	13.5	0.91 (−0.12)	36.2	11.9	37.2	11.3	0.35 (−0.93)	0.37 (−0.89)
Sex (male/female)	25/27		28/20		0.30 (1.05)	51/80		69/64		**0.035** (**4.46**)	0.20 (1.66)
Education (years)	14.1	2.5	13.7	2.2	0.38 (−0.87)	14.9	2.1	15.3	2.4	0.21 (−1.26)	**<0.001** (**−4.13**)
Estimated premorbid IQ	102.7	10.8	99.9	9.2	0.23 (−1.20)	107.4	8.6	106.9	7.6	0.58 (−0.55)	**<0.001** (**−5.07**)
Gray matter volume (mm^3)	666.2	73.1	688	82.6	0.23 (−1.20)	702.4	71.7	706.2	81.3	0.79 (−0.26)	**0.003** (**−2.96**)
CPZ-eq (mg/day)[c]	584.4	510.2	639.5	610.7	0.75 (−0.32)	—	—	—	—	—	—
Age at onset (years)	25.5	10.6	25.1	10.9	0.91 (−0.11)	—	—	—	—	—	—
Duration of illness (years)	12.2	10	13.9	10.7	0.37 (−0.90)	—	—	—	—	—	—
PANSS positive symptoms	20.1	5.7	17.1	5.9	**0.009** (**−2.61**)	—	—	—	—	—	—
PANSS negative symptoms	19.8	6.4	18.3	6.2	0.13 (−1.50)	—	—	—	—	—	—

Note: PANSS, Positive and Negative Syndrome Scale.
[a]Some demographic information was obtained in part of subjects (estimated premorbid IQ and PANSS in patients: A/A, N = 49; G-carriers, N = 46, Estimated premorbid IQ in controls: A/A, N = 130).
[b]Significant results are bolded and underlined.
[c]CPZ-eq: chlorpromazine equivalent of total antipsychotics.

Results

Voxel-Based Morphometry

We investigated effects of diagnosis, genotype, and their interaction on GM volumes in the whole brain analyses. There was no difference in demographic variables between *VAV3* genotype groups, except for scores of positive symptom between the genotype groups in patients with schizophrenia and sex between the genotype groups in healthy controls (table 1). Patients with schizophrenia showed reduced GM volumes compared with controls mainly in the frontal lobe and temporal lobe (data not shown), consistent with previous studies.[19,20] Genotype effects on GM volume in several brain regions were found in all subjects (uncorrected $P < .001$, table 2). Individuals with A/A genotype had reduced GM volumes of left superior and middle temporal gyri than G-carriers, while individuals with A/A genotype had larger GM volumes of cerebellum anterior lobe (culmen) and right medial frontal gyrus than G-carriers (uncorrected $P < .001$, table 2). Additionally, we found significant diagnosis-genotype interaction of GM volume in the right medial frontal

Table 2. Effects of the VAV3 Genotype on Brain Morphology in All Subjects

						Talairach Coordinates		
Brain regions	R/L	BA	Cluster Size	T_{356}	P values[a]	x	y	z
A/A < G-carriers								
Superior temporal gyrus	L	13	194	3.8	**0.011**	−50	−48	18
Middle temporal gyrus	L	22	124	3.8	**0.013**	−53	−42	0
A/A > G-carriers								
Cerebellum anterior lobe (culmen)	R	NA	1533	4.1	**0.004**	7	−40	−12
Medial frontal gyrus	R	25	286	3.8	**0.012**	6	6	−17
VAV3 genotype × diagnosis interaction								
Medial frontal gyrus	R	25	271	3.9	**0.009**	6	7	−18

Note: R, right; L, left; BA, brodmann area.
[a]Significant results ($P < .05$ [FWE corrected]) are indicated with bold and underline.

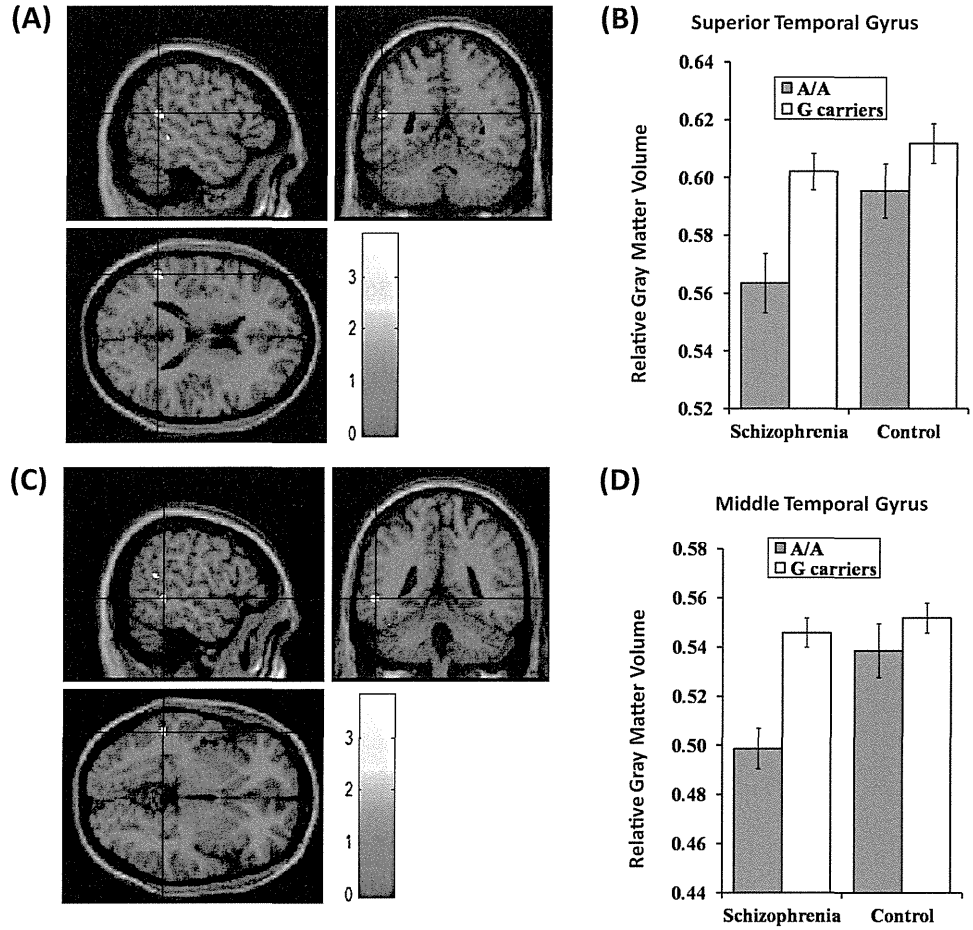

Fig. 1. Voxel-based morphometry. Impacts of VAV3 genotype on gray matter (GM) volumes of left superior temporal gyrus and left middle temporal gyrus. (A and C) Anatomical localizations were displayed on coronal, sagittal, and axial sections of a normal magnetic resonance imaging spatially normalized into the Montreal Neurological Institute template (uncorrected $P < .001$, cluster size >100). Significant clusters of genotype effect were in the left superior temporal gyrus (Talairach cordinate; −50, −48, 18) (A) and in the left middle temporal gyrus (−53, −42, 0) (C). These regions were showed as cross-hairline. The color bars showed t values corresponding to color in the figure. (B and D) Each column showed relative GM volumes extracted from left superior temporal gyrus (−50, −48, 18) (B) and left middle temporal gyrus (−53, −42, 0) (D). Data represent means ± SEM.

gyrus (uncorrected $P < .001$, table 2). These results remained positive after SVC for multiple tests (FWE corrected $P < .05$ after SVC).

Systematic searches for VBM studies have reported that smaller volumes of the left temporal gyrus were found in patients with schizophrenia than those in healthy subjects. Based on the JPN_GWAS data, individuals with A/A genotype of the rs1410403 were enriched in patients with schizophrenia. Therefore, we focused on the individuals with A/A genotype of the rs1410403 in *VAV3* as they may have a reduction of GM in the left superior temporal and left middle temporal gyri. Two-way ANOVA revealed significant effects of diagnosis ($F_{1,360} = 5.77$, $\eta^2 = 0.016$, $P = .017$) and genotype ($F_{1,360} = 10.04$, $\eta^2 = 0.027$, $P = .0017$) in the extracted region centering the left superior temporal gyrus (−50, −48, 18) (figure 1A and B). No interaction was found in the left superior temporal gyrus ($F_{1,360} = 1.65$, $\eta^2 = 0.0046$, $P = .20$). Individuals with homozygous A had smaller GM volumes of the left superior temporal gyrus than G-carriers. We also found significant effects of diagnosis ($F_{1,360} = 8.03$, $\eta^2 = 0.022$, $P = .0049$) and genotype ($F_{1,360} = 14.04$, $\eta^2 = 0.038$, $P < .001$) and their interaction ($F_{1,360} = 4.40$, $\eta^2 = 0.012$, $P = .037$) in the extracted region centering the left middle temporal gyrus (−53, −42, 0) (figure 1C and D). As the genotype-diagnosis interaction was found in the left middle temporal gyrus, we analyzed the effects of genotype on the region in patients and controls separately. Patients with schizophrenia showed that patients with A/A genotype had smaller GM volumes of the region than G-carriers ($F_{1,98} = 12.00$, $\eta^2 = 0.11$, $P < .001$). In contrast, controls showed no genotype effect on GM volumes of the region ($F_{1,262} = 2.46$, $\eta^2 = 0.0093$, $P = .12$).

Mutation Screening

We detected 4 novel nonsynonymous heterozygous variants within the JMut sample (321 schizophrenic

patients). Protein homology analysis showed that VAV3 is highly conserved between species (~95% identical amino acids between human and mouse), accordingly the identified point mutations affected conserved residues (see online supplementary table 2). All the variants detected were located in the C-terminal region of VAV3 (see online supplementary figure 1).

The identified novel variants were reconfirmed by cycle sequencing and followed-up in 2 large independent schizophrenia case-control samples (Rep_JPN and JPN_GWAS sample). Only one rare variant (Glu741Gly) showed statistical evidence for association in meta-analysis (P_{CMH} = .020, OR = 0.58), while the others were observed at similar frequencies both in case and control samples (table 3). In-silico analysis of the missense variants applying 3 different algorithms predicted Glu741Gly as variant of functional relevance (table 4). Detailed 3-dimensional structural analysis of SH2 domain (wild type—figure 2a) indicated specific interaction (hydrogen bond) between Glu741 (side chain) and Lys735 (main chain). Point mutation at position 741 (Glu→Gly) would abolish hydrogen bonding because glycine does not contain a side chain (figure 2b). Moreover, beta strand extending into Lys735 is lost in the model of VAV3 mutant. The functional consequence of the associated point mutation is disappearance of casein kinase 2 phosphorylation site (SXXE)[21] as shown on figure 2c and d.

Discussion

Our study reports the systematic genetic evaluation of *VAV3*, as a candidate gene for schizophrenia based on our genome wide screening.[4] Specifically, meta-analysis of the JPN_GWAS and follow-up sample provided genetic evidence for the involvement of *VAV3* locus in schizophrenia in the Japanese population. Mutation screening of all *VAV3* coding exons did not reveal evidence for the existence of a common (minor allele frequency >5%) nonsynonymous variant that explains the association signal in JPN_GWAS.

The VBM analysis showed that the associated common SNP (rs1410403) might affect the volume of the left superior and middle temporal gyri, which were reduced in patients with schizophrenia compared with healthy subjects. VBM analysis suggests that *VAV3*'s influence is focal to the aforementioned regions. Furthermore, the Allen Brain Atlas (http://human.brain-map.org) records relatively high levels of expression of the human *VAV3* gene and lower expression levels of other VAV family GEFs (*VAV1* or *VAV2*) in left superior and middle temporal gyri. Considering VAV3 biological function (developmental processes in particular), we speculate that our macroscopic observation using VBM approach is result of neuronal distribution and differential activity of VAV3 protein associated with rs1410403 genotypes.

Table 3. Resequencing Results

Chr	Variant	Physical Position[a]	Protein Domain	M	JMut (Minor Allele Count)	m	JPN_GWAS (MAF)				Rep_JPN (MAF)				Meta-Analysis	
							Cases	Control	P_{allele}	OR	Cases	Control	P_{allele}	OR	P_{CMH}	OR
1	p.Asp623Val	107.986.810	N-SH3	A	2	T	0.0006964	0.0008993	0.8561	0.7742	0.0003344	0	0.3171	NA	0.6649	1.662
1	p.Glu685Lys	107.947.271	SH2	G	1	A	0.0006974	0.001821	0.4151	0.3824	0.0003336	0	0.3168	NA	0.8415	0.8246
1	p.Glu705Lys	107.947.211	SH2	G	3	A	0.0007022	0.0009074	0.8557	0.7737	0.0006658	0.0003311	0.5605	2.011	0.7354	1.355
1	P.Glu741Gly	107.940.485	SH2	A	7	G	0.004972	0.01087	0.09038	0.4547	0.0074480	0.0117400	0.0897	0.6314	0.02065	0.5821

Note: M, major allele; m, minor allele; MAF, minor allele frequency; OR, odds ratio; P_{CMH}, Cochran-Mantel-Hentzel test.
[a]NCBI 36 build.

Table 4. In-Silico Analysis

		Genomic Data			Impact on Protein Structure/Function		
Variant	Protein Domain	Physical Position[a]	Strand	Alleles M/m	PMUT (Prediction Score)[b]	Panther (subPSEC Score)[c]	LRT (P value)[d]
p.Asp623Val	N-SH3	Chr1: 107986810	−1	A/T	Yes (0.97)	Yes (−3.99)	Yes (4.29×10^{-8})
p.Glu685Lys	SH2	Chr1: 107947271	−1	G/A	Yes (0.93)	No (−2.04)	Yes (7.08×10^{-10})
p.Glu705Lys	SH2	Chr1: 107947211	−1	G/A	Yes (0.75)	No (−1.74)	Yes (1.83×10^{-7})
p.Glu741Gly	SH2	Chr1: 107940485	−1	A/G	Yes (0.89)	Yes (−3.24)	Yes (1.64×10^{-6})

Note: M/m, major/minor allele.
[a]NCBI36 build.
[b] >0.5 is interpreted as nonneutral substitution.
[c]Less than −3 is interpreted as nonneutral substitution.
[d]<0.001 interpreted as nonneutral substitution.

Systematic searches for VBM studies have reported that reduced volumes of the left temporal gyrus were found in patients with schizophrenia.[19] Furthermore, the data from previous study of the superior temporal gyrus[22] and a study of the middle temporal gyrus in which patients with schizophrenia who were predisposed to auditory hallucinations showed reduced activation of the left middle temporal gyrus when imagining sentences in another person's voice.[23] Several other functional magnetic resonance imaging studies have reported decreased left and increased right middle temporal gyrus activation in schizophrenia during auditory verbal hallucinations.[24,25] In our exploratory analysis (see online supplementary material), positive symptoms scores of Positive and Negative Syndrome Scale (PANSS) were not able to predict the VOIs in left superior and left middle temporal gyri ($P > .05$). It is of note that VBM sample size in the current study might not be sufficient to detect the effect of positive symptoms score of PANSS on VOIs in left superior and left middle temporal gyri because positive symptoms score of PANSS is derived from symptomatology that is characterized by excess or distortion

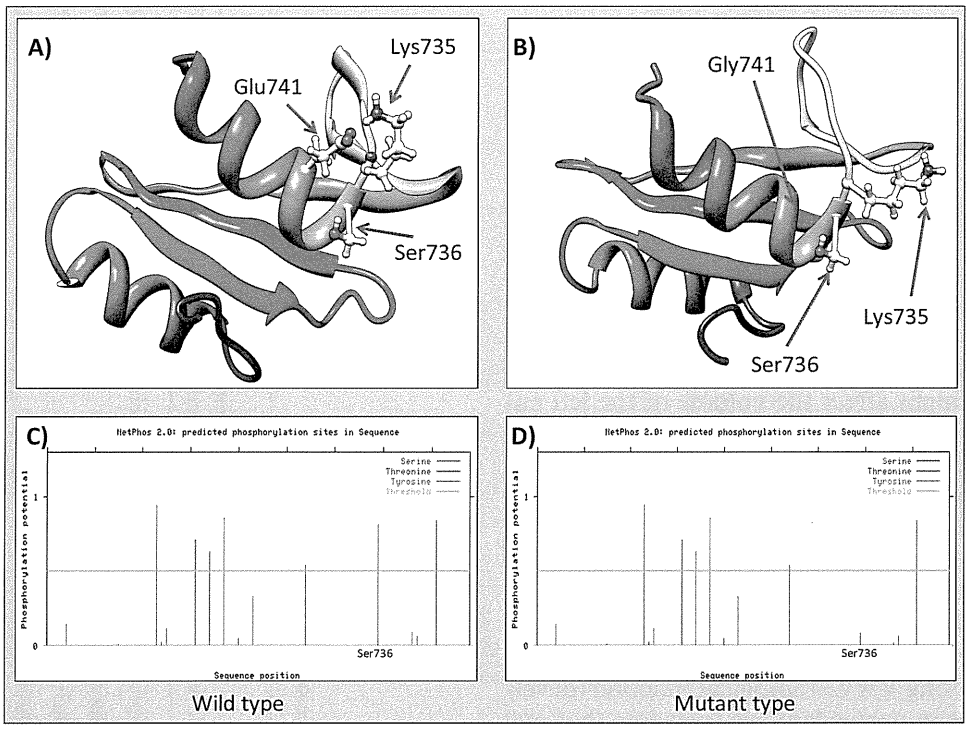

Fig. 2. Ab-initio 3D modeling of SH2 domain (VAV3). (a) Wild type, note hydrogen bond between Glu741 and Lys735 (blue line); (b) Mutant type, hydrogen bond between Gly741 and Lys735 cannot be formed; (c) and (d) posphorylation potential of serine (blue), threonine (green), and tyrosine (red). Threshold is marked by horizontal gray line (phosphorylation analysis performed by NetPhos 2.0).

of the individual's normal functioning and therefore may not reflect only auditory hallucinations.

We detected several rare variants close to original association signal and in case of one rare variant (Glu741Gly), we observed nonsignificant association trend. When the evidence was combined across the 2 samples (JPN_GWAS and Rep_JPN sample), we found that the *P* values had strengthen. VAV3 is composed of 8 domains: calponin homology (CH), Acidic (Ac), Dbl homology (DH), pleckstrin homology (PH), zinc finger (ZF), Src homology 3 (SH3), Src homology 2 (SH2), and a second SH3 (see online supplementary figure 1).[26] Interestingly, the associated common variant (rs1410403) is located in LD block that encompass coding exons of SH2 domain of vav3 (see online supplementary figure 2), and the associated rare variant (Glu741Gly) is located within the exon that is translated into the SH2 domain of vav3 (see online supplementary figure 1).

VAV family GEFs have been implicated as regulators of Eph receptor endocytosis, the event which is required for efficient cell detachment.[27] This can provide a complex and dynamic set of cues that either repel or attract axons toward their synaptic targets, converting initially adhesive interaction into a repulsive force. Several studies had shown that SH2 domain of the VAV3 binds to phosphorylated tyrosine residue(s) on the EphA receptors,[6,28] which triggers endocytosis of EphA receptors and growth cone collapse.[27] Although the mechanisms by which Glu741Gly contributes to schizophrenia pathogenesis remain to be explored, we note that 3 different bioinformatics algorithms had predicted functional effect, and Glu741Gly have stronger protective effects on schizophrenia risk (OR = 0.58) than does the associated common SNP (OR = 0.81). Moreover, analysis of phosphorylation sites showed that point mutation at position 741 (Glu→Gly) would abolish phosphorylation of Ser736 by casein kinase II. The substitution of glutamic acid by Glycine at position 741 might have as a consequence alteration of the biological function of VAV3 because a substrate with *n* phosphorylation sites has an exponential number (2^n) of phosphoforms and each phosphoform may have distinct properties.[29] Our genetic association results suggested that the rare variant, which is predicted to alter function of the VAV3, would decrease the risk of schizophrenia, whereas normal function is associated with schizophrenia. Same protective allelic effect was observed for common variant identified by JPN_GWAS.

As the conclusion, our results showed that in case of schizophrenia, the "rare high-risk variant" vs the "common variant with low effect" hypotheses should not be viewed as 2 mutually exclusive hypotheses. Therefore, direct resequencing of candidate genes and copy number variants on the one side and GWAS analyses on the other side could be viewed as complementary approaches to analyze the genetic susceptibilities to schizophrenia.

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Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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The authors have no conflicts to declare.

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特集　女子刑務所のあり方を考える

摂食障害のこころとからだ

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はじめに

拒食症や過食症などの摂食障害は若い女性のありふれた病気になりました。厚生労働省調査研究班の最新の疫学調査では、拒食症の有病率は女子高校生の〇・一～〇・四％で、多発国である米国の有病率に匹敵します。[1] 刑務所や拘置所などの矯正施設でも摂食障害患者の数が年々増加し、[2] 二〇一三年一一月の新聞報道では女子服役者の三％が摂食障害であると報告されました。摂食障害はマスコミで取り上げられる機会は増えましたが、その異常な心理や行動は健康人には簡単に理解できません。本稿では摂食障害の心理と行動についてわかりやすく概説します。

一　過食・嘔吐に苦しむ過食症のK子さん

K子さんは二〇歳の女子大生です。父親の勧めで経済学部に入学しましたが、勉強に興味がわかず、入部したテニス部では友人関係にも不満があります。失恋も経験

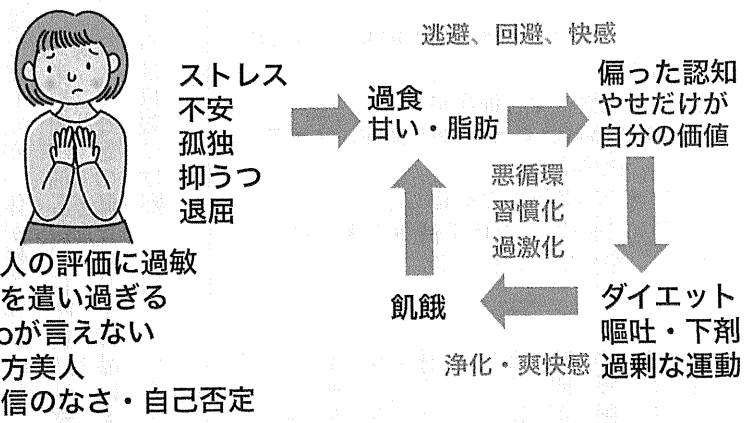
し、やせて綺麗になれば自信も取り戻せると思い、大学二年生の七月にダイエットを開始しました。身長一六一センチメートル、体重四七キログラムでしたが、九月には四二キログラムになりました。ところが、翌年一月の試験前に急に猛烈な食欲が出て、今まで我慢していたクッキーを一枚食べたところ、止まらなくなり一缶全部食べてしまいました。それをきっかけに、二時間で四食分くらいの量をむちゃ食いする発作が一週間に二回くらい起こるようになりました。体重は一気に元に戻りました。四〇キログラム以下の体重にならないと自分の価値はないと思い込み、指をのどにつっこんで嘔吐するようになりました。過食が始まると自分で抑えることができず、自宅の食品を食べ尽くし、コンビニに何回も買い物に行き、食べては吐くことを繰り返します。母親は食材を食べ尽くされて翌朝の食事の用意ができず、トイレを吐物で詰まらせることがあり、本人と言い争いが絶えません。体重増加を防ぐために、朝と昼食は抜き、下剤も規定の倍量を常用するようになりました。朝は胃のもたれがひどく、体はぐったりし、気分も落ち込み、通学が困難になり、三年生からまったく通学できずに留年が確定したため退学しました。

過食するのは甘く脂っこい食品です。甘くて脂肪に富む高カロリー食品でしか快感は得られないことが脳機能の研究で明らかにされています。大量の水分を一緒に飲むのは吐きやすくするためです。過食発作は夜が多く、不満や心配ごとがあるとき、家族が不在、暇なときに起こりやすく、習慣になると毎食後にも起こります。K子さんは過食さえなければ人生はうまくいくと考えています。一方で、過食はアルコールに似て、食べている最中は何も考えないという解放感があります[3]。食べることが一番のストレス発散方法でありながら、自分の価値は体型や体重で決まるという認知のゆがみがあり、希望する体重は達成困難なほど低いので、その体重をめざしてやせようとすればするほど過食衝動は強まります（図1）。

過食後は後悔や自責の念にさいなまれ、強い抑うつに襲われ、リストカットなどの自傷行為をすることがあります。約束を急にキャンセルしたり、就学・就労できなくなったり、社会生活に支障が出ます。過食する食費が一日一万円に及ぶ例もあります。

過食症はやせていないので、栄養失調による体の障害

図1　神経性大食症の症状と悪循環



患者は「過食中は何も考えなくて良いので楽」と言い、過食は最高のストレス解消方法になっている。その一方で、自分の価値は痩身や良い体型にしか見いだせず、過食後、絶食や運動、自己嘔吐や下剤乱用で体重の増加を抑え、その身体的飢餓が再び過食を誘発するという悪循環を繰り返して、習慣化している。この悪循環を断つには、体重の是正化とストレスを減らすこと、そして、ストレスを過食・嘔吐で紛らわさなくて済むような対処能力を向上させることである。

はありません。月経もあり、骨粗鬆症の心配もありません。ただ、嘔吐や下剤の乱用による弊害はあります。脱水で腎機能が悪化したり、血液のナトリウムやカリウムが低くなったりします。

二　やせと栄養失調のN美さん

N美さんは一六歳の女子高生です。公立中学ではクラブの部長を務め、教員からの信頼も厚く、友人関係も良好でした。女子高校では、表面は友好的な友人が陰で自分の悪口を言っていると漏れ聞いたり、インターネットで根拠のない悪い評判を書き込まれたりして、人間不信に陥りました。担任や学校カウンセラーからは、「相手にしないように」と言われましたが、辛い気持ちは解決しませんでした。入学時、身長一五八センチメートル、体重四七キログラムでした。通学時間は一時間半もかかり、吹奏楽部の練習で朝早く登校していました。英語の定期試験の成績が良く、担任の教師に期待されるような言葉かけをされたので、成績を落とせないと思い勉強に励んでいました。夏休みもクラブの合宿や塾で忙しくし

ていたところ、風邪をひいて食欲が落ちました。その後、食欲が回復せず、一〇月には四〇キログラムになり月経が止まりましたが、体が軽く感じられ、勉強に集中できました。米や肉、油もの、甘味食品は避け、野菜や海草やこんにゃくなどの低カロリー食品は大量に食べます。昼の弁当は母親には黙って捨てています。夕食後、縄跳びを一時間しています。二年生の四月には三二キログラム（標準体重五三・二キログラムの六〇％）になり、体力と記憶力が低下してクラリネットが思うように吹けず、勉強に時間がかかり、ますます睡眠時間が短くなりました。心配した養護の先生と家族の勧めでしぶしぶ病院を受診したところ、体育は見学、標準体重の五五％以下になったら緊急入院と言われました（表1）。栄養士から、「食べることが苦手な病気なので、最初は楽に食べられる食品でなんとかカロリーを確保して体重をこれ以上下げないようにしましょう」と言われ、少しほっとしました。でも、少し多く食べると胃がもたれ、一口食べるとすぐに一〇〇キログラムになるような恐怖に襲われ、顔やお腹はまだ太く見えてもっとやせたいと思います。自分でもおかしいと思いますが、どうにもできません。

表1　緊急入院が必要なからだの基準

1　全身衰弱（起立、階段昇降が困難）や意識障害がある
2　重篤な検査異常や合併症がある（低血糖性昏睡、低カリウム血症、不整脈、腎不全、感染症など）
3　標準体重の55％以下のやせ
4　1か月に5kg以上の体重減少があり消耗が激しく、絶食に近い食事量

拒食症は、標準体重の八〇％以下の著しいやせが一番の特徴です。ダイエットではなく、半数が無理をしているうちに知らず知らずにやせます。ずっと少食タイプと、途中から過食症と同じような過食が起こり、嘔吐や下剤を使用するタイプがあります。過食症との違いはやせの有無です。全身の筋肉が落ち、血圧や脈拍数や体温も低くなる。低栄養による低血糖、肝機能障害、不整脈、感染症などの重症の合併症が起こります。[4] 入院歴のある拒食症患者の六年間の死亡率は六～一一％と精神疾患の中では異常に高いと言えます。死因は飢餓、心不全、感染症、自殺です。成長期に発病すると身長の伸びが止まり、

栄養失調とやせによる女性ホルモンの低下で若いにもかかわらず骨粗鬆症になります。[5]

やせ始めは飢餓による高揚状態で成績が上がり、運動の記録も一時的に良くなります。その後、やせる途中のことは覚えておらず、気が付いた時には胃腸機能も低下して、肥満に対する恐怖が強く、食べようと思っても食べられない状況になっています。やせの最大のメリットは、辛いことに対する感受性が鈍麻することです。以前は億劫だった宿題やクラブの練習に励んで成績が上がり、過酷な環境に耐えられます。また、これまで「手のかからない良い子」を無理して演じてきた場合、やせてからの周囲の気遣いや年齢相応の義務の免除は嬉しい利得です（表2）。反対に、体重を増やすことは嫌な現実に近づくことを意味しているので受診・治療を拒むのです。[6]

表2　やせたい気持ちのなぞ
―回復した患者さんのアンケートから

○体重や食べ物のことだけ考えていると楽だった
○やせていると、つらい現実や見たくない現実から離れられるような気分になれた（現実逃避）
○辛いこと、不愉快なことへの感受性が鈍磨して、他人事になる
○やせていると年相応の義務を果たさなくてもいいと思った
○健康体重になると、また以前のように頑張ってしまうことが辛かった
○やせていると、できないことの言い訳になる
　食べているのにできないのは許せない（失敗の言い訳）
○やせは自分をコントロールできる証拠（達成感）
○やせだけがとりえで、ほかに誇れるものがない気がした
○やせていなかったら、自殺したかもしれない
○やせていると周りの人が心配してくれたり、親切になったりする。
　太ると見捨てられてしまいそう（周囲の関心）
○理由はわからないが、助けて欲しかった（SOS）
○やせている頃の記憶がはっきりしない

三　摂食障害の原因は何か
―挫折体験とコーピングスキル―

「拒食症はダイエットの失敗」、「過食症は意志が弱い」と誤って認識されています。レイプ、いじめ、虐待などの過酷な事件の場合もありますが、多くは思春期・青年期にありがちな進路や人間関係での挫折体験を契機に発症しています。それらのストレスが本人のストレス対処能力（コーピングスキル）を超えて、適切に対処できないときに、拒食症では急にやせたい気持ちになり、過食症では過食発作が始まります。拒食も過食もストレスを

回避する手段なのです。

発病を増長しやすい要因として、患者の性格傾向、養育環境や家族関係、教育や文化の影響が指摘されています。一般に、怖がりで不安症で、他人から叱責されることを極度に恐れ、本音を出せない、弱音を吐けないようです。完璧主義と強迫性も指摘されており、物事の完全性を求めるあまり挫折感を経験しやすく、他人の評価に敏感であるが自己評価は低いため、物事をストレスと受け取りやすく、ためやすいと言われています。

家族関係が摂食障害の発症因子であるという科学的根拠はありません。ただ、親が過保護・過干渉で患者の自主性の発達を妨げているか、あるいは、家庭が安らぎの場にならないような家族内葛藤があることは経験されます。摂食障害はしばしば同一家族内に複数例の発症があるので、遺伝素因があると考えられています。食欲と情動に関わるホルモンの受容体遺伝子の解析が行われていますが、民族によって一定の結果は得られていません。

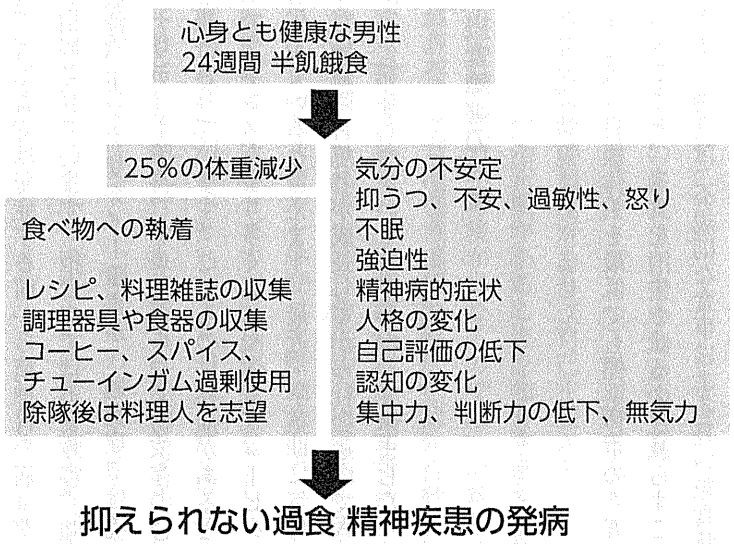
四　摂食障害の何が治療者・管理者を困らせるのか

(一)　疾患特有の心理と行動異常

過食症患者は過食を止めたいと言いながら、過食したいのです。医療には憎い過食を止めることだけを期待しています。絶食、運動、自己嘔吐や下剤・利尿剤の乱用による飢餓が次の過食を誘発するという悪循環を繰り返しています。原因であるストレスへの対処能力を向上させないで、過食だけを止めることはできません。たとえ過食を止めても、アルコールや薬物や自傷行為などの他の問題行動に移行することも多く、過食は防波堤になっているとも言えます。

拒食症患者にとって、体重を増やそうとする医療者や家族は敵になり、体重を増やさないためならばどんなことでもするような心理に陥っています。病気であることを否定し、体重測定時に密かに錘をつけて体重をごまかしたり、全部食べたという虚偽の申告をして食事はゴミ箱に捨てたりします。さらに、飢餓そのものが食に執着

図2　ヒトの半飢餓臨床試験（ミネソタ・スタディ）



した行動を惹起します。思考も行動も生活すべてが食に振り回されます。調理して家族に摂食を強要し、大量の食品を隠し持ち、進路も栄養士や調理師をめざします。飢餓の反動の過食や嘔吐、下剤乱用も始まります。もっと悪いことに飢餓は深刻な精神症状も起こします[7]。一九四〇年代にKeysらが心身ともに強健な男性に徴兵免除の代わりに、約五〇％のカロリー制限食を六か月摂取させる臨床試験（ミネソタ・スタディ）を行いました（図2）。被験者の興味は食事のことばかりで、献立のレシピや調理器具を収集し、食べ方も異常になって食品を切り刻んで食べ、試験終了後はみな調理人になりたいと言い出しました。同時に、気分が不安定になり、抑うつ、不安、過敏性、不眠、強迫性が増強して、人柄が変わりました。試験終了後、全員が抑えられない過食になり、試験終了後に精神科治療を必要とした人も出ました。これらの異常な行動を改善させるのに必要なのは、叱責や説得、閉鎖病棟への収容ではなく、適切な栄養療法です。低栄養状態では思考力や認知能力も低下しており、精神療法を有効にするためにも飢餓の改善が優先されます。