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Genetic analysis of a functional *GRIN2A* promoter (GT)_n repeat in bipolar disorder pedigrees in humans

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Abstract

Hypofunction of glutamatergic neurotransmission has been hypothesized to underlie the pathophysiology of bipolar affective disorder, as well as schizophrenia. We examined the role of the *N*-methyl-D-aspartate receptor 2A subunit (*GRIN2A*) gene on 16p13.3, a region thought to be linked to bipolar disorder, (1) because in a prior study we identified a functional and polymorphic (GT)_n repeat in the 5' regulatory region of the gene, with longer alleles showing lower transcriptional activity and an over representation in schizophrenia, and (2) because of the suggestion of a genetic overlap between affective disorder and schizophrenia. Family-based association tests detected a nominally significant preferential transmission of longer alleles in a panel of 96 multiplex bipolar pedigrees. These results support the hypothesis that a hypoglutamatergic state is involved in the pathogenesis of bipolar affective disorder.

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Keywords: *N*-Methyl-D-aspartate receptor 2A subunit; Short tandem repeat; Pedigree disequilibrium test; Family-based association; Linkage; Schizophrenia

Bipolar affective disorder is a severe psychiatric disease that affects ~1% of the population worldwide and is characterized by recurrent episodes of mania and depression. Once the disease develops, episodes tend to recur throughout life, and in some cases prophylaxis is difficult to achieve by using presently available therapeutics. The etiologic basis is unknown however; twin, family and adoption studies have provided evidence for the involvement of heritable risk factors [15].

Several lines of evidence implicate glutamatergic dysfunction in the pathophysiology of affective disorder. Antidepressant administration has been shown to affect *N*-methyl-D-aspartate (NMDA) receptor function [14], receptor binding profile [17], and expression of mRNAs of multiple NMDA receptor subunits [2]. Magnetic resonance spectroscopy has revealed a reduced glutamate level in the anterior cingulate cortex in depressed patients [1].

A number of genetic linkage studies have shown modest linkage signals for mood disorder on the short arm of

chromosome 16 [5,6,13]. A compelling candidate gene in this region that has relevance to the 'hypoglutamatergic hypothesis' of mood disorders is the NMDA receptor 2A subunit (*GRIN2A*) gene on 16p13.3. In a prior study, we identified a variable (GT)_n repeat in the promoter region of this gene that elicited repression of transcriptional activity in a length-dependent manner [7]. NMDA receptor binding sites in postmortem brains were also negatively correlated with the length of (GT)_n of samples. Our case-control study showed evidence of an association between the repeat polymorphism and schizophrenia, with longer alleles overrepresented in patients with severe outcome. These findings led to the conclusion that the longer (GT)_n stretch could act as a risk-conferring factor for schizophrenia, especially with malignant chronic outcome, by reducing *GRIN2A* levels in the brain [7].

It has been suggested that schizophrenia may share the susceptibility genes with mood disorder, since nearly half of the candidate regions on the human genome linked to schizophrenia overlap with those of bipolar disorder [10], and several controlled family studies have revealed a co-aggregation of schizophrenia and affective disorder in the

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same families [8]. Therefore, in this study, we set out to investigate a potential role of the (GT)_n polymorphism of the *GRIN2A* gene in mood disorder, using family-based association analysis to reduce a chance of false positive findings due to population stratification.

We examined the National Institute of Mental Health (NIMH) Genetic Initiative bipolar pedigrees containing 96 families with 536 individuals [16]. In this panel of families Edenberg et al. [5] have reported evidence of linkage to mood disorders with markers on 16p12-13 including *D16S2619*, *D16S2618* and *D16S749*. The primers used to amplify the repeat-containing genomic fragment were: a FAM-labeled upstream primer, 5'-GAAGGAAG-CATGTGGGAAATGCAG-3' (the 3' end is 88 bp upstream of the 5' end of the GT repeat; see GenBank accession No. AF443855), and a non-labeled downstream primer, 5'-gtttctGCTGGGTACAGTTATCCCCCT-3' (the 3' end is 19 bp downstream of the 3' end of the GT repeat). The underlined tail sequence was added because Taq DNA polymerase catalyzes the non-templated addition of adenosine to the 3' end of PCR products affecting the degree of product adenylation. This phenomenon is primer-specific and presents a potential source of genotyping error. Brownstein et al. [3] have shown that by placing the sequence GTTCTT at the 5' end of reverse primers, nearly 100% adenylation of the 3' end of the forward strand is achieved, facilitating accurate genotyping. PCR was performed with an initial denaturation at 95 °C for 12 min, followed by 35 cycles of 94 °C for 15 s, 58 °C for 15 s and 72 °C for 30 s, and a final extension at 72 °C for 30 min, using AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). PCR products were analyzed using an ABI 3700 sequencer equipped with GeneScan software (Applied Biosystems). We performed a family-based

linkage/association analysis by the pedigree disequilibrium test (PDT) (program version 3.12 available from <http://www.chg.mc.duke.edu/software/pdt.html>) [11,12]. The PDT can take advantage of using all informative individuals in the families, and is reported to be a valid test of association even when multiple related nuclear families from an extended pedigree are used [11]. The program computes the two statistical measures PDT-sum and PDT-ave. Briefly, PDT-sum gives more weight to larger families, whereas PDT-ave places equal weight on all families. The suitability of either statistical method depends on family structure and genetic models [12].

Given the uncertainty regarding the spectrum of inherited mood disorder illnesses, we used three affection status models (ASMs) [16]. ASM I included bipolar I and schizoaffective disorder. ASM II was composed of ASM I phenotype plus bipolar II. ASM III contained unipolar recurrent depression in addition to ASM II phenotype. Under model I, there were 121 affected sib pairs, and 227 affected relative pairs. Model II contained 197 affected sib pairs and 324 affected relative pairs, and model III included 282 affected sib pairs and 412 affected relative pairs [16]. Table 1 shows the results of PDT analysis. Note that the transmissions from uninformative parents (i.e. those who had homozygous repeat alleles) were not counted in the table. We detected the repeat numbers ranging from 18 to 33 in the present Caucasian samples, while in Japanese cohorts they ranged from 12 to 42 [7]. The allele with 24 repeats was significantly transmitted to affected offspring from parents using the PDT-ave statistic ($P = 0.005$ under ASM I; $P = 0.020$ under ASM II; $P = 0.027$ under ASM III) and the PDT-sum statistic ($P = 0.020$ under ASM I) (Table 2). The allele with 25 repeats showed a significant excess of transmission under ASM III ($P = 0.033$) in the PDT-sum

Table 1
Transmission status of alleles in the NIMH bipolar pedigrees analyzed by PDT

Repeat number	Transmission state					
	ASM I		ASM II		ASM III	
	Transmitted	Non-transmitted	Transmitted	Non-transmitted	Transmitted	Non-transmitted
19	1	1	1	1	1	2
20	28	27	34	34	45	42
21	12	14	15	17	17	22
22	2	1	2	1	2	1
23	4	4	5	5	6	5
24	13	4	16	6	19	9
25	20	14	22	15	26	16
26	13	14	14	17	15	20
27	15	21	18	26	20	29
28	13	17	19	18	22	21
29	6	5	7	5	8	5
30	2	4	2	5	4	6
31	1	2	1	3	1	3
32	2	4	2	5	2	7

ASM, affection status model. Under ASM I, an affected individual includes either bipolar I or schizoaffective, bipolar type. ASM II is ASM I plus bipolar II, and ASM III contains unipolar recurrent depression in addition to ASM II.

Table 2
Results of the PDT analysis of NIMH bipolar pedigrees

Repeat number	P value (PDT-sum)			P value (PDT-ave)		
	ASM I	ASM II	ASM III	ASM I	ASM II	ASM III
19	0.655	0.654	0.317	0.527	0.527	0.317
20	0.482	0.939	0.625	0.465	0.900	0.681
21	1.000	1.000	0.892	0.431	0.787	0.941
22	1.000	1.000	0.317	0.857	0.781	0.317
23	0.405	0.563	1.000	0.384	0.420	0.705
24	0.020	0.066	0.138	0.005	0.020	0.027
25	0.144	0.465	0.033	0.434	0.526	0.059
26	0.329	0.537	0.262	0.230	0.713	0.930
27	0.833	0.277	0.205	0.849	0.591	0.227
28	0.220	0.705	0.650	0.176	0.648	0.627
29	0.483	0.738	0.865	0.756	0.988	0.481
30	0.777	0.593	0.179	0.143	0.126	0.172
31	0.317	0.317	0.317	0.317	0.317	0.317
32	0.705	0.256	0.179	0.726	0.890	0.587

statistic and marginal significance under ASM III ($P = 0.059$) in the PDT-ave statistic. The significance in distortion of transmission disappeared after correcting for multiple tests (global PDT $P > 0.05$).

We calculated an average repeat number of alleles using those of unrelated members of the pedigrees (parents and married-in individuals) as 23.9. Since the prior *in vitro* promoter assay revealed a length-dependent inhibition of transcriptional activity by the (GT)_n repeat [7] and decreased NR2A receptor expression, the present results that show the preferential transmission of longer than average alleles to affected offspring suggest a role for hypoglutamatergic neurotransmission via reduced NR2A receptors in bipolar susceptibility. The detection of a limited number of alleles showing significant transmission disequilibrium and only modest P values could be partly due to the existence of multiple alleles and the loss of statistical power due to the reduced frequency of each allele. Other well-known examples of repeat polymorphisms affecting complex traits include the variable 20–23 bp long tandem repeats located in the promoter region of the serotonin transporter gene. The short alleles comprising six through to eight repeat units were shown to be associated with lower transcriptional activity, reduced serotonin uptake, and anxiety-related personality traits [9].

Involvement of a hypoglutamatergic state in the pathophysiology of bipolar disorder is postulated also from the fact that valproic acid and lithium, both of which are well recognized anti-manic and prophylactic drugs, stimulate glutamate release [4]. Our current finding that shows the over transmission of (GT)₂₄ and (GT)₂₅ alleles to affected offspring may support the hypoglutamatergic hypothesis for bipolar disorder. Independent confirmation from studies using larger samples is warranted.

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Evidence of association between gamma-aminobutyric acid type A receptor genes located on 5q34 and female patients with mood disorders

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Abstract

Pharmacological evidence suggests the involvement of gamma-aminobutyric acid (GABA) perturbation in the etiology of mood disorders. A linkage study has detected chromosomal area 5q34, where GABA type A (GABA_A) receptor subunit genes are mapped, as a susceptibility region for mood disorders, making these genes compelling candidates for such diseases. Our prior quantitative trait loci (QTL) analysis of mouse depression models identified a QTL on mouse chromosome 11, a genomic region whose human synteny includes 5q34. This further supports a contribution from GABA_A receptors to a predisposition towards mood disorder. In the present study, we examined GABA_A receptor α 1 (*GABRA1*), α 6 (*GABRA6*) and γ 2 (*GABRG2*) subunit genes on 5q34. Polymorphisms on *GABRA1* and *GABRA6* genes displayed significant associations with mood disorders in female patients. These data offer genetic support for a role of GABA_A receptor genes in susceptibility to mood disorders.

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Keywords: Bipolar disorder; Unipolar disorder; *GABRA1*; *GABRA6*; *GABRG2*; Quantitative trait loci

Mood disorder comprises one of the major categories of psychiatric disorder, with a lifetime prevalence of about 1% for bipolar patients and 4–7% for unipolar patients in the global population. The high rates of suicidal behavior and psychosocial impairment attributable to mood disorder represent major public health problems [10]. Heredity components of the disease are strongly supported by family, twin and adoption studies [7]. Numerous linkage analyses and association studies have been performed to identify genes conferring susceptibility to the disease. Nevertheless, promising findings have yet to be conclusively reported.

We recently performed quantitative trait loci (QTL) analysis of two mouse depression models, forced swim test (FST) and tail suspension test (TST), to identify chromosomal regions containing susceptibility genes for behavioral despair (longer immobility time). We found that mouse chromosome 11 shared a common QTL between FST and TST immobilities [13]. The region of synteny for this mouse genomic interval on chromosome 11 corresponds to 5q32–35 in humans. Edenberg et al. [3] reported evidence of linkage to mood disorder with markers on 5q33–35, an area

encoding a cluster of gamma-aminobutyric acid (GABA) type A receptor (GABA_A) genes, including the α 1 (*GABRA1*), α 6 (*GABRA6*) and γ 2 (*GABRG2*) subunits.

GABAergic dysfunction is thought to play a role in mood disorder [6], and low cerebrospinal fluid and plasma levels of GABA have been reported in mood disorders [2,8]. A recent study using proton magnetic resonance spectroscopy displayed a highly significant reduction of GABA concentrations in the brains of depressive patients [11]. Dysfunction in GABAergic neurotransmission has also been reported in postmortem brain studies of bipolar disorder [1]. Such evidence suggests the involvement of GABAergic systems in the pathogenesis of mood disorders, and prompted us to screen for associations between mood disorder patients and single nucleotide polymorphisms (SNPs) on GABA receptor genes at 5q34.

We analyzed 203 mood disorder patients (average age 49.9 ± 11.0 years; 101 bipolar patients, 102 unipolar patients), and age- and gender-matched mentally healthy controls (average age 48.3 ± 9.4 years; 202 individuals). Subjects were recruited from a geographic area located in central Japan and patients were diagnosed according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, 1994) criteria on the consensus of at least two experienced psychiatrists. After receiving an

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explanation of the protocol and purposes of the study, written informed consent was obtained from all participants. The present study was approved by the ethics committee of RIKEN.

DNA was extracted from whole blood according to a standard protocol. We examined one SNP each from the *GABRA1* (IVS10 + 15A > G: rs2279020/IMS-JST026551) and *GABRA6* (c.1497C > T: rs3219151) genes, and two SNPs from the *GABRG2* gene (IVS8 – 658A > G: rs211013; IVS8 + 99A > C: rs211014/TSC0507852/IMS-JST034730). These SNPs were selected on the criteria that: (1) they had been examined for association with a psychiatric disease in a prior study [4]; and/or (2) they showed sufficient heterozygosity (a frequency of minor allele > 0.2) in our preliminary examination. These SNPs have also been registered in The National Center for Biotechnology Information database (rs number) (<http://www.ncbi.nih.gov>), the SNP consortium database (TSC number) (<http://snp.cshl.org/>) and/or the JSNP database (IMS-JST number) (<http://snp.ims.u-tokyo.ac.jp/>). We used Assays-by-Design™ SNP genotyping products to score SNPs (Applied Biosystems, <http://www.appliedbiosystems.com/>), based on TaqMan assay methods [9]. Genotypes were determined using an ABI7900 sequence detection system instrument (Applied Biosystems) and the SDS v2.0 software package (Applied Biosystems). To avoid genotyping errors, ambiguous genotypes were re-analyzed by PCR-direct sequencing using the BigDye terminator cycle sequencing kit (Applied Biosystems) and an ABI 3700 DNA analyzer (Applied Biosystems). Detailed information on markers and experimental procedures is available upon request.

Haplotype frequencies were estimated using the expectation-maximization algorithm implemented in the Arlequin 2000 [12]. Deviations from Hardy–Weinberg equilibrium,

the normalized linkage disequilibrium coefficient D' , squared correlation coefficient r^2 and P values were also calculated using the Arlequin program. Allelic, genotypic and haplotypic distributions between patients and controls were assessed using Fisher's exact test.

Genotypic and allelic frequencies of the SNPs on *GABRA1* and *GABRA6* genes are summarized in Tables 1 and 2, respectively. The IVS10 + 15A > G on the *GABRA1* gene showed genotypic associations with unipolar patients of both sexes ($P = 0.029$) and female bipolar patients ($P = 0.044$), but not with male mood disorder patients (Table 1). The c.1497C > T on the *GABRA6* gene were genotypically associated with female total mood disorder patients (unipolar + bipolar) ($P = 0.041$), but again, not with male mood disorder patients (Table 1). The A allele of IVS10 + 15A > G on the *GABRA1* gene was over-represented among female total mood disorder patients ($P = 0.021$) and female bipolar subjects ($P = 0.014$), compared with female controls (Table 2). The T allele of c.1497C > T on the *GABRA6* gene was more frequent in the female total mood disorder group than in female controls ($P = 0.020$) (Table 2). A significant deviation from Hardy–Weinberg equilibrium was found in the genotypic distribution of the *GABRA1* gene in patients ($P = 0.0061$ for female unipolar), but not controls. This might imply that the SNP was in close vicinity to disease-predisposing mutation(s).

The two SNPs of *GABRA1* and *GABRA6* genes were in linkage disequilibrium to each other (control: $D' = 0.450$, $r^2 = 0.0843$, $P < 10^{-8}$; mood disorder: $D' = 0.467$, $r^2 = 0.0906$, $P < 10^{-8}$). Haplotypic analysis revealed a significantly increased rate of AT haplotype [IVS10 + 15A (*GABRA1*) – c.1497T (*GABRA6*)], and a decreased rate of GC haplotype [IVS10 + 15G (*GABRA1*) – c.1497C

Table 1
Genotypic frequencies (%) of polymorphisms in GABA_A receptor genes at 5q34

Gene and polymorphism (<i>n</i>)	<i>GABRA1</i> : IVS10 + 15A > G*				<i>GABRA6</i> : c.1497C > T**			
	A/A	A/G	G/G	<i>P</i> value	C/C	C/T	T/T	<i>P</i> value
Mood disorders (203)	36	40	24	0.120	44	42	14	0.347
Bipolar (101)	36	47	17	0.180	40	44	16	0.115
Unipolar (102)	37	33	30	0.029	48	41	11	0.922
Controls (202)	27	48	25		49	42	9	
Male patients (100)	28	43	29	0.547	51	35	14	0.176
Bipolar (58)	29	48	23	0.908	43	38	19	0.225
Unipolar (42)	26	36	38	0.168	62	31	7	0.103
Male controls (101)	26	50	24		42	48	10	
Female patients (103)	45	37	18	0.060	36	50	14	0.041
Bipolar (43)	44	47	9	0.044	35	51	14	0.084
Unipolar (60)	45	30	25	0.079	39	48	13	0.131
Female controls (101)	29	46	25		54	37	9	

*A to G substitution at nucleotide 15 from the 5' end of intron 10 of the *GABRA1* gene. **C to T substitution at nucleotide 1497 in the cDNA sequence of the *GABRA6* gene. A/A, homozygote of A allele; A/G, heterozygote of A and G alleles; G/G, homozygote of G allele; C/C, homozygote of C allele; C/T, heterozygote of C and T alleles; T/T, homozygote of T allele.

Table 2
Allelic frequencies (%) of polymorphisms in GABA_A receptor genes at 5q34

Gene and polymorphism (n)	GABRA1: IVS10 + 15A > G*			GABRA6: c.1497C > T**		
	A	G	P value	C	T	P value
Mood disorders (406)	56	44	0.159	65	35	0.178
Bipolar (202)	59	41	0.058	61	39	0.054
Unipolar (204)	53	47	0.667	69	31	0.852
Controls (404)	51	49		70	30	
Male patients (200)	50	50	0.842	68	32	0.671
Bipolar (116)	53	47	0.727	62	38	0.466
Unipolar (84)	44	56	0.301	77	23	0.068
Male controls (202)	51	49		66	34	
Female patients (206)	63	37	0.021	62	38	0.020
Bipolar (86)	67	33	0.014	60	40	0.051
Unipolar (120)	60	40	0.165	62	38	0.062
Female controls (202)	51	49		73	27	

(GABRA6)] in female mood disorder patients compared to female controls (control: AT/AC/GT/GC = 0.1977/0.3171/0.0746/0.4106; mood disorder: AT/AC/GT/GC = 0.3192/0.3119/0.0643/0.3046; $P = 0.0256$). The odds ratio of AT haplotype over GC was 2.174 (95% CI = 1.303–3.625, $P = 0.0033$).

The distributions of the two SNPs in the *GABRG2* gene did not differ between controls and mood disorders in both allele ($P = 0.879$ for IVS8 – 658A > G; $P = 0.888$ for IVS8 + 99A > G) and genotype ($P = 0.957$ for IVS8 – 658A > G; $P = 0.983$ for IVS8 + 99A > G) frequencies. Nor were they associated with any subtype of mood disorder or gender. We have not applied correction for multiple testing in order to avoid deriving false negative findings due to the relatively small number of subjects in the subcategories of mood disorder patients.

In the previous QTL study, we conducted candidate gene analysis on mouse chromosome 11 QTL, revealing that one or both of *Gabra1* and *Gabra6* genes might represent real susceptibility genes. This was because of the significant difference in the expression level of *Gabra1* gene between C57BL/6 (B6) and C3H/He (C3), the progenitor mouse strains used in QTL mapping, and because of an amino acid substitution in *Gabra6* protein between B6 and C3 mice [13]. In addition, we revealed that the QTL on mouse chromosome 11 could epistatically interact with a locus on the X chromosome to influence TST immobility time. In this context, it is interesting that the present results showed evidence of association for both human homologue genes with female mood disorders. The epistatic QTL on mouse chromosome X contains the *Gabra3* gene. Recently, Massat et al. [5] reported that the human *GABRA3* gene displays a highly significant association with female bipolar disorder patients. Examination of genetic interactions between GABA_A receptor genes on human 5q34 and the *GABRA3* gene might therefore be important. However, testing the genetic effects of combinations of multiple loci requires

much larger samples. We are continuing to recruit mood disorder samples in order to perform epistatic analysis.

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Original Article

***C18orf1* located on chromosome 18p11.2 may confer susceptibility to schizophrenia**

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The pericentromeric region of chromosome 18, especially 18p11.2, is described as a schizophrenia susceptibility locus. We had previously cloned two novel brain-derived transcripts from this region: the gene for a second human *myo*-inositol monophosphatase (*IMPA2*) and a gene of unknown function, *C18orf1*. Recently, we reported a distortion of transmission of the tandem repeat marker D18S852, embedded in the 3'-untranslated region of *C18orf1*, in schizophrenia, using a family-based association test. A subsequent case-control study also revealed a significant association between the haplotype constructed from D18S852 and the 6409T>C polymorphism located in *C18orf1* and schizophrenia. In the present study, we screened the *C18orf1* gene for mutations and identified a novel single nucleotide polymorphism (SNP), -96T>C in exon 2. This SNP showed significant genotypic ($P = 0.048$) and allelic association ($P = 0.005$) with schizophrenia in a case-control study. The distributions of haplotypes defined by D18S852 and -96T>C were different between control and schizophrenia groups ($P = 0.021$). These findings suggest that *C18orf1* or a gene nearby

may contribute to the overall genetic risk for schizophrenia.

Key words: association, chromosome 18, haplotype, linkage disequilibrium

Introduction

Schizophrenia is a common and devastating mental disorder of unknown etiology. Multiple factors including risk-conferring genes and undefined environmental variables may contribute to overall susceptibility.¹ Several chromosomal loci have been reported to show linked with schizophrenia,² one of which is the pericentromeric region of chromosome 18. Schwab *et al.*³ demonstrated linkage and association of schizophrenia to genetic markers within and near the G-olf α (*GNAL*) gene on 18p11.2. Williams *et al.*⁴ detected 18p as a locus exerting a nominal significance in their first-stage genome scan of schizophrenic sibling pairs. Cytogenetic studies have also identified schizophrenic patients who carried a pericentric inversion of chromosome 18⁵ and translocations between 2p11.2 and 18p11.2,⁶ and between 18p11.1 and 21p11.1,⁷ supporting the notion that the short arm/pericentromeric region of chromosome 18 is involved in the manifestation of schizophrenia.

To identify susceptibility gene(s) for functional psychoses on chromosome 18, we isolated 25 novel brain-derived transcripts by employing a cDNA selec-

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tion strategy.⁸ Among these, was the gene for a second human *myo*-inositol monophosphatase (*IMPA2*)^{9,10} and a gene of unknown function, *C18orf1*,¹¹ both mapping to 18p11.2. Our genetic analysis of *C18orf1* showed evidence for a distortion of transmission of the D18S852 triplet repeat, present in the 3'-untranslated region of *C18orf1*, in schizophrenic trios (patients and their parents). A subsequent case-control study confirmed a significant association between the haplotype constructed from D18S852 and 6409T>C polymorphism located in *C18orf1*, and schizophrenia.¹² In this study, we performed mutation screening of the *C18orf1* gene, and conducted association tests using the newly detected polymorphism in a case-control paradigm.

Material and Methods

Subjects

Schizophrenic samples were composed of 49 females (mean age, 50±12 years) and 59 males (mean age, 48±12 years). The controls were 48 females (mean age, 45±10 years) and 58 males (mean 42±10 years). All the samples were derived from the same region of central Japan. Best-estimate lifetime diagnoses of schizophrenia were made according to criteria of the Diagnostic and Statistical Manual of Medical Disorders, 4th edition (DSM-IV) and by the consensus of at least two experienced psychiatrists. In addition to direct interviews, all available medical records and information from relatives and hos-

pital staff were considered. The present protocol was approved by the ethics committees of both RIKEN and Tokyo Medical and Dental University. Written informed consent was obtained from all participants after receiving an explanation of the protocol and purpose of the study. All samples were taken in accordance with the Helsinki declaration.

Mutation screening and genotyping of the *C18orf1* gene

We screened for polymorphisms in the protein coding regions and exon-intron junctions of the *C18orf1* α and β isoforms by PCR followed by sequencing. The primers used for amplification are listed in Table 1. All exons except 2 and 5 were amplified using rTaq (Takara, Tokyo, Japan). To amplify exons 2 and 5, MasterAmp DN buffer (Epicentre, Madison, WI, USA) and rTaq were used. The PCR reactions were performed starting at 96 °C for 3 min, followed by 35 cycle of 95 °C for 50 s, 59 °C for 50 s, 72 °C for 1 min, and a final extension period at 72 °C for 10 min. Sequencing of the PCR product was conducted using the dGTP BigDye Terminator Ready Reaction Kit (PE Applied Biosystems, Foster City, CA, USA) on an ABI 377 DNA sequencer (PE Applied Biosystems).

Statistics analysis

Deviations from Hardy-Weinberg equilibrium, linkage disequilibrium statistics and estimated haplotype frequencies were computed using Arlequin software¹³ (<http://lgb.unige.ch/arlequin/>). Genotypic, allelic and haplotypic associations were assessed using the

Table 1. PCR primers and conditions used to screen for nucleotide variants in the *C18orf1* gene

Region ¹	Primers (F,forward; R,reverse)	Product		Polymerase and buffer
		size (bp)	5' end of primer	
exon 2	(F) 5'-AATGAGTTTCTCCCGAGGTG-3'	955	250 bp upstream of exon 2	Taq and MasterAmp DN ^{a,b}
	(R) 5'-ACTTGGGAGACGCTGCGAATACTG-3'		282 bp downstream of exon 2	
exon 3	(F) 5'-ATGCAAATGGGGGAAAGAAA-3'	479	94 bp upstream of exon 3	Taq ^a
	(R) 5'-TCAGACAACAGCAAAGCCTATT-3'		244 bp downstream of exon 3	
exon 4	(F) 5'-CAGCTGCCGGCCCAATGTGC-3'	1150	350 bp upstream of exon 4a	Taq ^a
	(R) 5'-CCAGGACCCAGTTCAGGA-3'		396 bp downstream of exon 4b	
exon 5	(F) 5'-ACCTCGTGATCCATCCGCTCTGC-3'	413	194 bp upstream of exon 5	Taq and MasterAmp DN ^{a,b}
	(R) 5'-TCCCTCCCGCTTTTGACCA-3'		165 bp downstream of exon 5	
exon 6	(F) 5'-GCCAAGCTTCCATTACC-3'	1097	283 bp upstream of exon 6	Taq ^a
	(R) 5'-CTTCAAGCATCCCGTTCTGTGTT-3'		283 bp downstream of the stop codon	

^aTaq polymerase was rTaq (TAKARA, Tokyo, Japan).

^bMasterAmp buffer was from Epicentre Technologies (Madison, WI, USA).

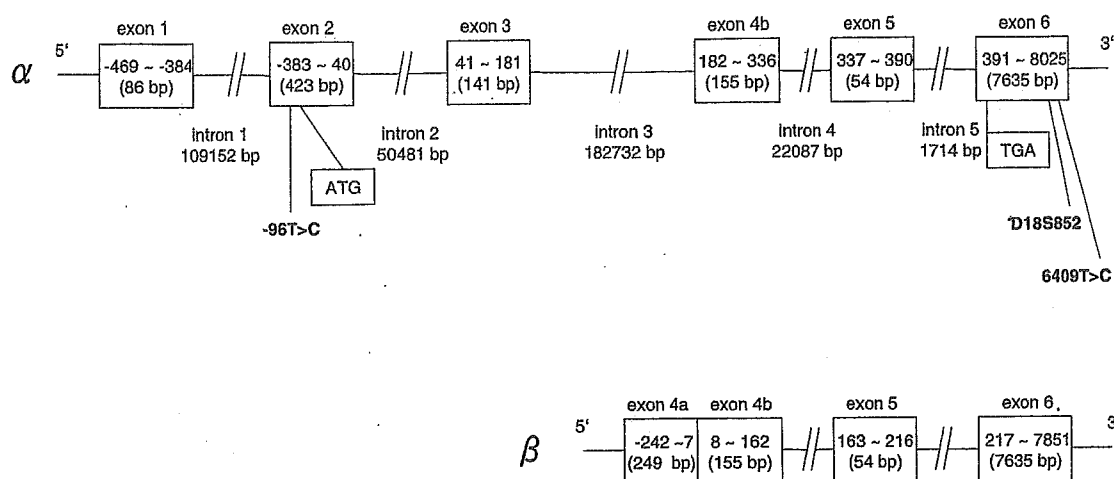


Fig. 1. Genomic structure and cDNA isoforms (α and β) of the *C18orf1* gene. The numbers in the exon boxes denote nucleotide positions (A of the initiation codon ATG is counted as +1). The locations of three polymorphisms, -96T>C, D18S852 and 6409T>C are also shown.

Table 2. Allelic and genotypic distributions of -96T>C in *C18orf1*

Sample	Genotype counts (% frequency)				Allele counts (% frequency)		
	T/T	T/C	C/C	<i>P</i> value	T	C	<i>P</i> value
Schizophrenia (n = 108)	84 (77.8)	21 (19.4)	3 (2.8)	0.048	189 (87.5)	27 (12.5)	0.005
Control (n = 106)	66 (62.3)	33 (31.1)	7 (6.6)		165 (77.8)	47 (22.2)	

CLUMP program¹⁴ (<http://www.mds.qmw.ac.uk/statgen/dcurtis/software.html>) with 10,000 simulations, which calculates empirical *P*-values using a Monte Carlo permutation procedure.

Results

Polymorphism identified

The initiation ATG codon of the *C18orf1* α variant is located in exon 2, while that of the β form is in exon 4a (Fig. 1).¹¹ The stop codon for both isoforms is encoded by exon 6. To find functional polymorphisms in the protein-coding sequences of the *C18orf1* gene, the region spanning from the translation start site to the stop codon including splice boundaries were sequenced from 30 schizophrenic patients. No unsynonymous mutation was detected, but we found a novel T>C SNP located 96bp upstream from the ATG codon in exon 2 (Fig. 1).

Genetic analysis of the -96T>C polymorphism

We typed the -96T>C SNP in 108 schizophrenic patients and 106 age- and sex-matched controls. This SNP showed a modest genotypic association (*P* = 0.048), and significant allelic association (*P* = 0.005) with schizophrenia (Table 2). The T allele was overly represented in the disease group [odds ratio and 95% C.I. = 1.99 (1.19 - 3.30)]. The genotypic frequencies conformed to Hardy-Weinberg equilibrium in both the control (*P* = 0.314) and schizophrenia (*P* = 0.248) samples.

Linkage disequilibrium (LD) analysis among polymorphisms in the *IMPA2* and *C18orf1* genes, and D18S40

In prior genetic studies of the chromosome 18 short arm, we detected evidence of associations in schizophrenia with the following genes/markers: *D18S852* and 6409T>C within the *C18orf1* gene,¹² IVS1-15G>A and 800C>T within *IMPA2*,¹⁵ and D18S40.¹² Therefore, we examined LD among the markers including the newly identified -96T>C polymorphism of the *C18orf1* gene (Table 3). The -96T>C was in signif-

Table 3. Pairwise linkage disequilibrium among polymorphisms in the *IMPA2* and *C18orf1* genes, and D18S40

	IVS1-15G>A (<i>IMPA2</i>)	800C>T (<i>IMPA2</i>)	-96T>C (<i>C18orf1</i>)	D18S852 (<i>C18orf1</i>)	6409T>C (<i>C18orf1</i>)	D18S40
IVS1-15G>A (<i>IMPA2</i>)		$D = 0.020$ $D' = 0.251$ $P = 0.141$	$D = 0.032$ $D' = 0.500$ $P = 0.051$	$D = 0.009$ $D' = 0.404$ $P = 0.499$	$D = 0.006$ $D' = 0.112$ $P = 0.767$	$D = 0.033$ $D' = 1.000$ $P = 0.154$
800C>T (<i>IMPA2</i>)	$r^2 = 0.008$		$D = 0.008$ $D' = 0.404$ $P = 0.451$	$D = 0.014$ $D' = 0.204$ $P = 0.529$	$D = 0.011$ $D' = 0.110$ $P = 0.461$	$D = 0.014$ $D' = 1.000$ $P = 0.155$
-96T>C (<i>C18orf1</i>)	$r^2 = 0.026$	$r^2 = 0.003$		$D = 0.028$ $D' = 1.000$ $P = 0.039$	$D = 0.011$ $D' = 0.237$ $P = 0.493$	$D = 0.011$ $D' = 1.000$ $P = 0.123$
D18S852 (<i>C18orf1</i>)	$r^2 = 0.005$	$r^2 = 0.005$	$r^2 = 0.034$		$D = 0.096$ $D' = 1.000$ $P < 0.001$	$D = 0.054$ $D' = 1.000$ $P < 0.001$
6409T>C (<i>C18orf1</i>)	$r^2 = 0.000$	$r^2 = 0.002$	$r^2 = 0.002$	$r^2 = 0.326$		$D = 0.044$ $D' = 1.000$ $P < 0.001$
D18S40	$r^2 = 0.049$	$r^2 = 0.043$	$r^2 = 0.088$	$r^2 = 0.665$	$r^2 = 0.339$	

D values indicate deviation from linkage equilibrium ($D = h-p_1p_2$; h , haplotype frequency; p_1, p_2 , frequencies of two alleles at two loci). D' ($= D/D_{max}$) is the normalized linkage disequilibrium statistic, which lies in the range {0,1} with the greater value indicating stronger linkage disequilibrium. r^2 shows the squared correlation coefficient.

Table 4. Haplotype frequencies of the *C18orf1* gene

Haplotype (-96T>C -D18S852)	Estimated haplotype frequency (%)	
	Schizophrenia ($n = 104$)	Control ($n = 102$)
T - (GCT) ₁₀	0.713	0.657
C - (GCT) ₁₀	0.114	0.211
T - (GCT) ₁₁	0.114	0.113
C - (GCT) ₁₁	0.016	0.000
T - (GCT) ₁₂	0.038	0.020
T - (GCT) ₁₃	0.005	0.000
Significance	$P = 0.021$	

icant LD with D18S852 ($P = 0.039$) (Table 3).

Haplotype analysis

The -96T>C SNP and D18S852 marker from *C18orf1* gene, were in LD with each other, allowing us to construct haplotypes and calculate haplotype frequencies. The distributions of haplotypes were significantly different between control and schizophrenia groups ($P = 0.021$) (Table 4).

Discussion

The *C18orf1* gene was originally cloned from brain expressed transcripts that had been selected through hybridization to chromosome 18-specific cosmid clones.^{8,11} The gene was predicted to have at least four splice variants and to encode 306 ($\alpha 1$ form), 288 ($\alpha 2$), 248 ($\beta 1$) or 230 ($\beta 2$) amino-acid proteins, and a 7.1 kb-long 3'-UTR (Fig. 1).¹¹ A protein motif search suggested the presence of a putative type Ib transmembrane domain in both α and β isoforms and a low-density lipoprotein receptor class A domain in the α -specific N-terminus.¹¹ We have also shown evidence of RNA editing in the 5'-UTR of β form,¹¹ but the precise function of the gene remains unknown. Recently, Xu *et al.*¹⁶ reported a novel androgen-induced gene called *PMEPA1* located on chromosome 20q13, of which the predicted protein is similar in size to the $\beta 1$ isoform of *C18orf1* (67% sequence identity at the protein level). They suggested that *C18orf1* and *PMEPA1* belong to a novel gene family. More recently, Rae *et al.*¹⁷ described *PMEPA1* as a solid tumor-associated gene and named it *STAG1/PMEPA1*. They also indicated

that both *C18orf1* and *STAG1/PMEPA1* have potential binding sites for src homology 3 (SH3) and tryptophan tryptophan (WW) domains. These genes may function by interacting with signaling molecules.

Including the present results, the three genes (*GNAL*, *IMPA2*, *C18orf1*) on the short arm of chromosome 18 show association with schizophrenia or functional psychoses.^{3,12,15} These genes are localized to a limited interval of approximately 1.5 Mb. Association/linkage disequilibrium behavior over a relatively short sequence stretch is extremely variable,¹⁸ partly because LD is determined by the heterozygosity and history of markers and other confounding factors. In some cases the association of markers with a disease and the measure of LD between the markers, are not consistent in the genomic vicinity of the disease locus.¹⁹ This situation makes the precise identification of susceptibility variants formidable. However, evidence for the association of *C18orf1* gene polymorphisms with schizophrenia in addition to evidence implicating *GNAL*³ and *IMPA2*¹⁵ provides cogent support for the existence of a causal susceptibility gene(s) at 18p11.2. Excluding *C18orf1* a transcript search using the UCSC Human Genome Browser v6 (<http://genome.ucsc.edu/goldenPath/hgTracks.html>) failed to detect any other genes within the radius of 50 kb from -96T>C and D18S852. In the present study, we screened only the protein-coding region of *C18orf1*. The genomic interval surrounding *C18orf1*, especially the promoter region, warrants further scrutiny.

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気分障害(うつ病)の遺伝的基盤 動物モデルのQTL解析

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うつ病は、その発症に複数の感受性遺伝子および環境要因が関係し、病的にも異質な疾患の集まりと考えられている。このような複雑遺伝疾患の感受性遺伝子同定には、動物モデルも含めた多面的アプローチが必要である。筆者らはマウス強制水泳テストおよび尾懸垂テストにおける無動時間が、ヒトでのうつ感受性に相関すると考え、それら無動時間を制御している遺伝子座をQTL (quantitative trait loci) 解析で求めた。全ゲノム上で、強制水泳テストのQTLが5か所、尾懸垂テストのそれが4か所検出されたが、第8染色体と第11染色体のQTLが両テストで重なっていた。第11染色体のQTLには候補遺伝子としてGABA_Aレセプター遺伝子がコードされている。ヒトうつ病でのこれら遺伝子の解析に興味もたれる。

Key words depression, forced swim test, tail suspension test, quantitative trait loci (QTL), GABA

気分障害と統合失調症(以前の精神分裂病)を合わせて、2大精神疾患あるいは機能的な精神疾患という。気分障害は、気分の変化が単極性のうつ病と、双極性(うつ病相, 躁病相)の躁うつ病から成る。経過で見ると、統合失調症が慢性の経過をたどるのに対して、気分障害は病相間に正常気分の時期が挟まるのが特徴である。気分障害のうち、躁うつ病でも生涯発症率は1%弱と高いが、うつ病は先進国で20%を超えるとされているほど頻度の高い疾患である¹⁾。気分障害全般の原因、病態生理、分子機序などが不明な現在では、うつ病と躁うつ病の生物学的知見に基づいた区分は不可能であるが、臨床的には当初うつ病相を繰り返すうつ病として治療されていた患者が、途中から躁病相が出現して躁うつ病の診断に変わることがしばしば経験され、病因病態においてある程度の連続性が想定されている。本稿で

はうつ病に主眼を置いて、筆者らの動物モデルを用いた分子遺伝学的アプローチを紹介する。なお、表1にうつ病の典型的な症状をあげる²⁾。

うつ病は複数の因子によって発症が規定される複雑遺伝疾患である

うつ病の発症に遺伝的基盤が関係していることは、これまでの膨大な疫学的研究が示しているが³⁾、実際の病因遺伝子(感受性遺伝子)の同定は難航している。うつ病は多因子—多遺伝子疾患と考えられており、感受性遺伝子の解明が困難な理由は他のありふれた疾患と共通する。うつ病を含めた多因子—複雑遺伝疾患^{*1)}と、ポジショナルクローニングで原因遺伝子同定の手順が確立したメンデル疾患の対比を表2に示す。しかし、多因子疾患のなかでもうつ病を含めた精神疾患の原因解明が難しいのは、たと

Approach to genetic basis of depressive disorder — QTL study of animal models

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よしかわ・たけお 1984年大阪大学医学部卒業。93~97年まで米国国立精神衛生研究所で躁うつ病の遺伝学的研究に従事。東京医科歯科大学助手、講師を経て99年より現所属。現在、精神疾患の感受性遺伝子の同定に関する仕事を行っている。

表1 うつ病の症状

- 1日の大半が沈んだ気分で見られる(思春期あるいはそれ以前では不機嫌が前景にみられることがある)
- 興味や楽しいという感情の喪失
- 食欲の低下,あるいは逆に亢進
- 不眠あるいは過剰睡眠
- いらいら, 焦燥感
- 倦怠感やエネルギーの喪失
- 思考の抑制, 判断力の低下, 集中力の低下
- 自分が無価値と感じたり, 自責的となる
- 希死念慮や自殺企図

(Cryan JF et al.: Trends Pharmacol Sci 23, 238-45, 2002²⁾ より)

例えばアルツハイマー病と比較してみると参考になる。①うつ病は, その少数例においても, アルツハイマー病にみられるAPP (アミロイド前駆体タンパク質), PS (プレセニリン) 1, PS2 のような1遺伝子で完全浸透率をもった遺伝子は存在しない可能性が高い, ②不完全浸透率の危険因子に分類される遺伝子多型でも, アルツハイマー病におけるAPOE (アポリポタンパク質E) ε4のように高いオッズ比を示す多型は一般集団には存在しない可能性がある, ③脳内アミロイド沈着, 画像検査での脳萎縮の所見など, 信頼性のある生物学的マーカーがないため客観的診断基準がなく, 個々の患者の原因には異質性が大きい, などである。

このような状況のなか, 遺伝的にはうつ病より均一であろうと思われる躁うつ病の家系を用いて数多くの連鎖解析がなされてきたが, すべての報告に共通する領域あるいはコンセンサスの得られた感受性遺伝子は今のところない⁴⁾。

うつ病のモデル動物について

— 利用価値と限界

うつ病の診断が患者の主観的体験に基づいている以上, 厳密な動物モデルは存在しない。たとえばヒトでみられる希死念慮, 自殺企図, 罪業感などは動

*1 複雑遺伝疾患 メンデル疾患と対比される用語で, 複数の遺伝子, 環境要因によって発症が規定される疾患である。通常罹患率が高いため common disease となる。また, 複雑遺伝疾患の形質は連続分布を示し, 量的 (quantitative) である。

表2 メンデル疾患と複雑遺伝疾患の対比

	メンデル疾患	複雑遺伝疾患
頻度	まれ	多い
責任遺伝子	単一	複数
浸透率	高い (~100%)	低い
遺伝子間相互作用	(-)	(+)
環境要因 (生活習慣)	(-)	(+)
病因	均一	異質
表現型	質的	量的
社会的コスト	+	+++

物に求めようがない。現在までに用いられている代表的なモデルを表3に示す⁵⁾。これらは以下の3つの視点から評価できる⁶⁾。

- ① face validity (症状の妥当性): モデルの行動, 状態がうつ病の症状に類似しているか。
- ② construct validity (構成概念の妥当性): 従来提唱されているうつ病の理論にあてはまるか。
- ③ predictive validity (予測の妥当性): うつ病の治療薬でモデルの状態が改善されるか。

①に関しては, ヒトでみられる意欲, 活動力の低下を動物の行動量に置き換えて考える。②については, うつ病の大きな誘因の一つにストレスがあるため, 動物を種々のストレス環境下において作製するモデルが多い。③はモデルとして受け入れられるためにほぼ必須のものである。うつ病の治療薬は, 抗結核薬のイプロニアジド, 抗ヒスタミン薬から派生した三環系化合物イミプラミンの2種がセレンディピタスに発見され, その後それらに共通する薬理作用としてモノアミン (主としてノルアドレナリン, セロトニン) 神経伝達の増強が発見された経緯がある⁷⁾。ただ現在に至っても, 薬物開発においてはこの“モノアミン仮説”以外によりどころとするパラダイムがなく, まったく新機序の抗うつ薬が, 現在用いられているモデルでその抗うつ作用を検出できるのかどうかについて疑問は残る。

以上のようにモデルには限界があるものの, 遺伝的に種々雑多なヒトを対象とした研究ではノイズに隠れてしまっている遺伝子あるいは遺伝子間相互作用も, (純系の) 動物を使った研究では抽出できる可能性があり, 動物で抽出した遺伝子をヒトに戻し

表3 うつ病研究で用いられる動物モデル実験

	利点	欠点
強制水泳テスト	抗うつ薬に反応する 実験が比較的容易	抗うつ薬の急性投与にも反応する
尾懸垂テスト	上記に同じ	上記に同じ
学習性無力	ヒトうつ病類似の症候を引き起こす 抗うつ薬に反応する goal-directed behaviorの理論に基づいている	作製するのに強いストレスを必要とする PTSD(心的外傷後ストレス障害)のモデルに近い可能性はある
慢性ストレス	ヒトうつ病類似の症候を引き起こす	再現性に難点がある 抗うつ薬が効きにくい
幼弱期ストレス	ヒトうつ病類似の症候を引き起こす 齧歯類ばかりでなく、霊長類にも適用できる	社会的行動の変化をきたすが齧歯類ではよく調べられていない 抗うつ薬の効果はまだ調べる余地がある
選択的飼育	うつ病のなりやすさに関して、個体ごとの差に焦点をあてる	今のところうつ感受性の系統が確立していない
報酬モデル	情動面のパラメータやうつ病の欲動低下を反映できる可能性がある 抗うつ薬に反応する	より古典的なうつ病モデルとの比較検討が不十分

(Nestler EJ et al : *Biol Psychiatry* 52, 503-28, 2002⁵⁾ より)

て解析することを視野に入れたのが筆者らのアプローチである。

うつ病モデルの量的形質遺伝子座 (QTL)^{*2}

1. うつ病は症状の重症度も発症のしやすさも量的である

量的形質 (quantitative trait) とは、連続分布 (正規分布) を示す表現形質のことであり、ごくありふれたものでは身長、体重があるが、知能、性格の諸側面など多くの精神機能も量的なものである。これらはメンデル疾患にみられるほぼ悉無律に従う質的形質と対照的である (表2)。うつ病は、症状の重症度でも発症のしやすさ (脆弱性) の点でも量的といえる。前者は、うつ病と診断された患者のなかに症状評価尺度でさまざまな程度のものが含まれると同時に、正常と明確なうつ病との間に“気分変調性障害”という診断名を DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-IV)⁶⁾ が用意していることでもわかる。後者については、同じストレスを受けてもうつ病になる人とそうでない人がいることを考えればよい。「量的形質 ↔ 複数の遺伝子で規定される」という関係があるが、この点でもうつ病は多遺伝子によって規定されていると考えられる。

2. マウスの強制水泳テスト、尾懸垂テストでの無動時間がヒトのうつ感受性に対応

筆者らは、マウスを使った強制水泳テストと尾懸垂テストをモデルとして選んだ。前者では、マウスを水を張った円筒容器に入れ一定時間行動を観察する (図1A)。当初は逃げ道を探して泳ぐが、途中から泳ぐのをやめ浮いた状態にいる時間が長くなる。この泳がないで浮いた状態にいる時間 (無動時間) をパラメータとして測定する。尾懸垂テストも同様で、マウスの尾を固定して一定時間吊り下げたときの無動時間を測定するものである (図1B)。両テストにおける無動時間は抗うつ薬で比較的特異的に短縮し、抗うつ薬開発のスクリーニングに用いられている。無動は動物行動学上“絶望”と考えられている。また、無動時間にはマウスの系統差があり、したがって無動時間が長いマウスほどストレス下で絶望しやすい、ヒトに当てはめるとうつ状態になりやすいと考えられる。以上の推論から、マウスで無動時間を規定している遺伝子が判明すれば、それらの遺伝子をヒトのうつ病で候補遺伝子として解析することが可能となる。

*2 QTL: quantitative trait loci の略で、量的形質を支配している遺伝子座を指す。QTLを求める方法をQTL解析という。

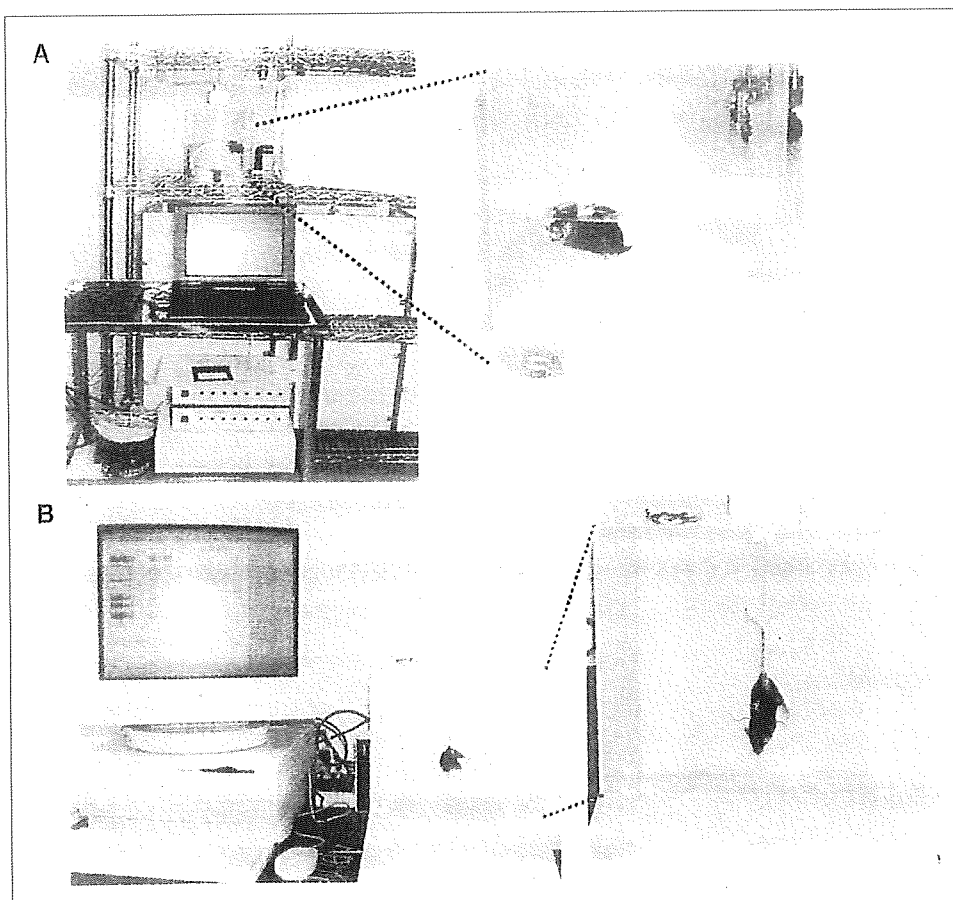


図1 強制水泳テストの測定装置 (A) と尾懸垂テストの測定装置 (B)

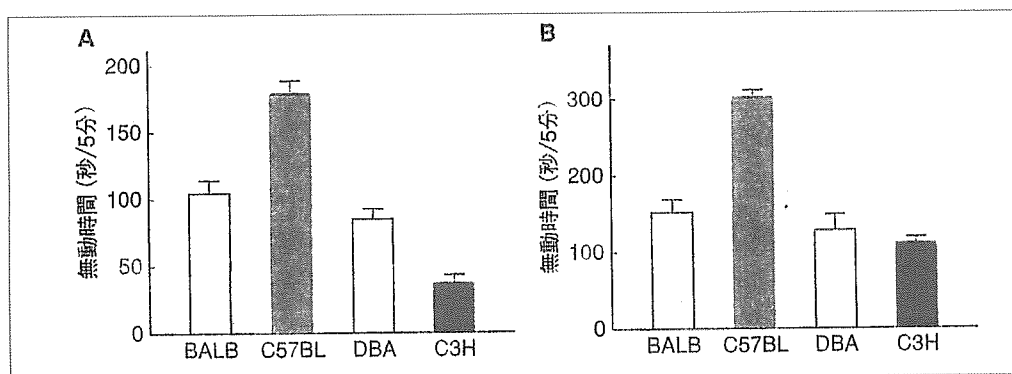


図2 無動時間のマウス系統による差
強制水泳テスト (A) と尾懸垂テスト (B) における無動時間を4系統のマウス (BALB, C57BL, DBA, C3H) で比較検討した。

3. 無動時間のQTL解析

1) 強制水泳テスト, 尾懸垂テストにおける無動時間の遺伝率はヒトうつ病のデータと類似

QTLを求めるには, 調べている表現形質に関するべく離れた計測値を示す2つの親系統 (F_0) をみつける必要がある. 筆者らは, 図2に示すように4系統のマウスを検討した結果, 両テストでC57BL/6 (B6) が一番無動時間が長く, C3H/He (C3) が一番短いことを見出した⁹⁾. そこでB6とC3から F_1 個体を作製し, さらに F_1 世代の兄妹交配

から560匹の F_2 個体を作製した. F_1 世代はどの個体も両親から半分ずつ遺伝子を引き継ぎ, 遺伝的にはまったく同一である. F_2 世代になると, 個体ごとに F_0 由来 (B6またはC3) の遺伝子の割合が多様化する. 図3に F_0 , F_1 , F_2 世代の無動時間の分布を示す. F_1 , F_2 の分布は, 両系統の F_0 の分布をカバーするように広がっている. いずれにしても分布は正規性に合致し, 正規分布=多遺伝子によってコントロールされる形質という関係がある.

ある形質について, 遺伝子によって決まる割合

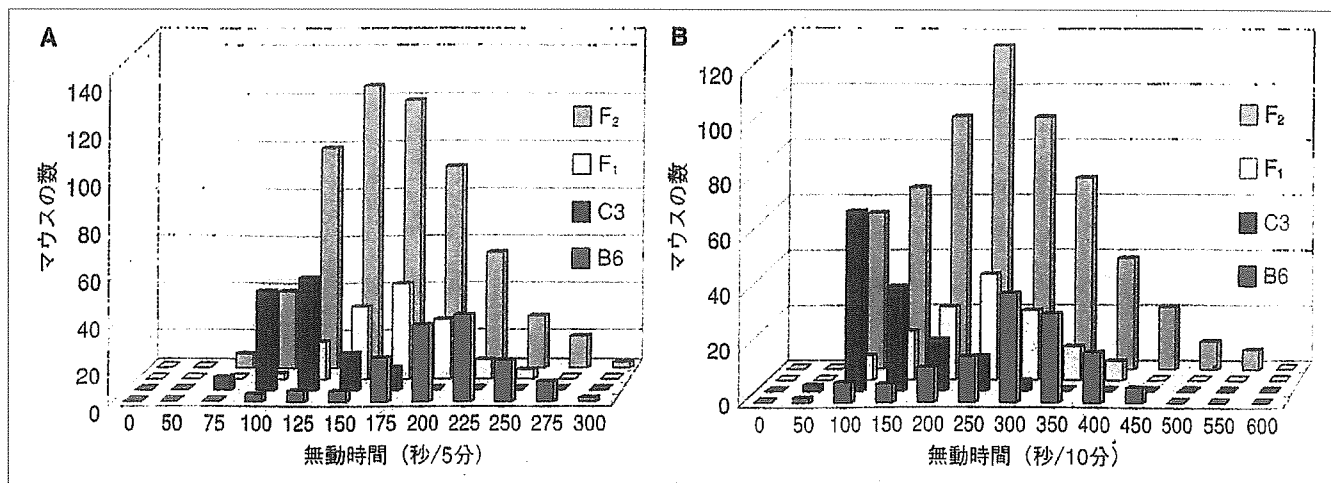


図3 マウス各世代の表現形質のヒストグラム

A: 強制水泳テストにおける無動時間の分布. 計測した動物の数は B6 が 126 匹, C3 が 124 匹, F₁ が 126 匹, F₂ が 560 匹である.
 B: 尾懸垂テストにおける無動時間の分布. 計測した動物の数は B6 が 122 匹, C3 が 121 匹, F₁ が 124 匹, F₂ が 560 匹である.
 (Yoshikawa T et al : *Genome Res* 12, 357-66, 2002⁹⁾ より)

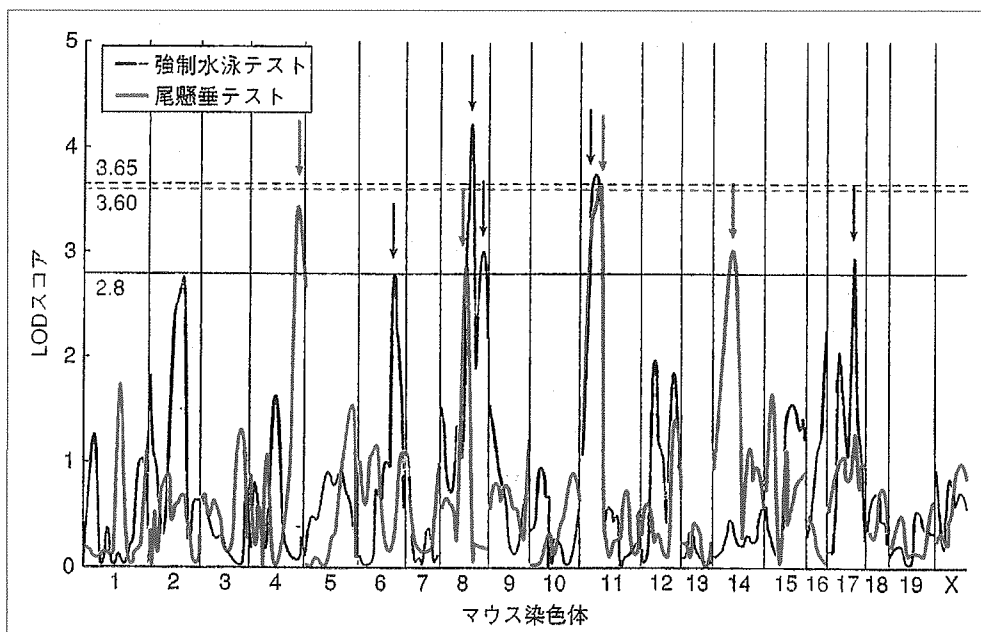


図4 強制水泳テストと尾懸垂テストにおける無動時間のゲノムスキャン
 矢印をつけた部位が、ゲノムワイドで $P < 0.05$ を満たす QTL である。
 (Yoshikawa T et al : *Genome Res* 12, 357-66, 2002⁹⁾ より)

(残りは環境要因) を遺伝率 (h_B^2) といい、次の式で求められる¹⁰⁾。

$$h_B = \frac{c_1A + c_2D}{c_1A + c_2D + E}$$

A: 相加分散, D: 優性分散, E: 環境分散

強制水泳テストにおける無動時間の遺伝率は 0.53, 尾懸垂テストにおける無動時間のそれは 0.45 であった⁹⁾。実験動物における各種心理学的形質の遺伝率は約 50% といわれており、またヒトの双極性

障害の遺伝率は 70% 前後、うつ病では 30~40%¹¹⁾ といわれているので、妥当な数字と思われる。

2) 単一遺伝子座解析—ゲノムワイド危険率 $P < 0.05$ を満たす QTL を探索する

F₂ 個体すべてから DNA を抽出し、ゲノムワイドな 120 個のマイクロサテライトマーカーを用いて遺伝子型をタイピングした。その後、F₂ の個体ごとの表現型 (無動時間) と遺伝子型を対応させ連鎖解析をして QTL を求めたのが図 4 である⁹⁾。LOD スコ