Table 2. Genotypic and Allelic Distributions of the *NCAM1* Gene Polymorphism, IVS0–30 $(GTTTT)_{4-7}$

IVS0–30$(GTTTT)_{4-7}$ (SNP3)	Bipolar Disorder (n = 151)	Unipolar Disorder (n = 78)	Control Subjects (n = 357)
Genotype Counts (Frequency)			
4/4	64 (.42)	25 (.32)	122 (.34)
4/5	24 (.16)	12 (.15)	68 (.19)
4/6	35 (.23)	23 (.29)	92 (.26)
4/7	10 (.07)	2 (.03)	18 (.05)
5/5	3 (.02)	1 (.01)	4 (.01)
5/6	4 (.03)	5 (.06)	18 (.05)
5/7	3 (.02)	3 (.04)	8 (.02)
6/6	4 (.03)	7 (.09)	14 (.04)
6/7	3 (.02)	0 (0)	13 (.04)
7/7	1 (.01)	0 (0)	0 (0)
p^a	.44	.36	
Allele Counts (Frequency)			
4	197 (.65)	87 (.56)	422 (.59)
5	37 (.12)	22 (.14)	102 (.14)
6	50 (.17)	42 (.27)	151 (.21)
7	18 (.06)	5 (.03)	39 (.05)
p^a	.25	.33	

NCAM1, neural cell adhesion molecule 1.
[a]Differences in genotypic and allelic distributions were evaluated by the Monte Carlo method.

Allelic distributions in the above three polymorphisms all displayed significant deviations in bipolar samples compared with control subjects: IVS6+32T>C, nominal p = .04, odds ratio (OR) = 1.47, 95% confidence interval (CI) = 1.03–2.10; IVS7+11G>C, nominal p = .02, OR = 1.37, 95% CI = 1.05–1.80; IVS12+21C>A, nominal p = .004, OR = 1.64, 95% CI = 1.18–2.28 (Table 3). After Bonferroni correction for multiple testing of 10 SNPs and two disease classifications, these deviations were not significant. For unipolar disorder, none of these polymorphisms displayed nominally significant genotypic or allelic associations with the disease.

Power calculations were performed on the basis of an arbitrary assumption of relative risk and frequency of risk allele. When a relative risk of 2.0 was assumed, the bipolar sample in the present study displayed ≥93% power to detect significant association (α < .05, frequency of risk allele = .3). The unipolar samples had ≥76% power. With a relative risk of 1.5, our bipolar samples had 51% power to detect significant association (α < .05, frequency of risk allele = .3). The unipolar samples retained 34% power.

Common (frequency of minor allele > .03) variants in the gene were selected for pairwise LD testing: IVS4+80G>C (SNP4); IVS6+32T>C (SNP5); IVS7+11G>C (SNP6); IVS7+155G>T (SNP7); 1621G>T (SNP8); IVS12+21C>A (SNP9); and 2208T>G (SNP10) (Figure 1, Table 4). IVS0–49delG (SNP1) and IVS0–30$(GTTTT)_{4-7}$ (SNP3) were also included for LD calculations to examine the 5′ upstream genomic structure of *NCAM1*. D' (normalized D) and r^2 (squared correlation coefficient) values were computed in patients with bipolar disorder and control subjects. Both LD measures take values between 0 (lack of LD) and 1 (complete LD). Abecasis et al (2001) suggested a D' value of >.33 as a useful measure of LD. Nakajima et al (2002) proposed r^2 > .1 as a criterion for useful LD. Linkage disequilibrium relationships between markers are shown graphically in Figure 2. Linkage disequilibrium structure was similar in the two measures (also see Table 4). These data revealed that the region spanning SNP1 through SNP9 was in a block of moderate-to-strong LD, and there was an overt LD gap between SNP9 and SNP10. Polymorphisms associated with bipolar disorder displayed relatively strong LD between IVS6+32T>C (SNP5) and IVS7+11G>C (SNP6) (D' = 1.00, r^2 = .181) and between IVS6+32T>C (SNP5) and IVS12+21C>A (SNP9) (D' = .728, r^2 = .397), but not between IVS7+11G>C (SNP6) and IVS12+21C>A (SNP9) (D' = .277, r^2 = .019) (Table 4).

Next, we examined three SNP-based haplotypic associations in a sliding manner in the bipolar group, with the polymorphisms that spanned the LD block (SNP1–9) (Figure 3, Table 5). All the three SNP combinations except for SNP5-6-7 and SNP6-7-8 showed significant association with bipolar disorder in terms of both global p values and p values for individual risk haplotypes. The haplotypes defined by SNP5-6-7 displayed significant individual haplotypic association (p = .034) and a trend of global association (p = .097), whereas those constructed by SNP6-7-8 showed marginal individual haplotypic association (p = .067) (Figure 3). These haplotype analyses demonstrated that the risk haplotype consisting of SNP1–9 for bipolar disorder was Ins-$(GTTTT)_4$-G-C-C-G-G-A (global p = .033, individual haplotype p = .009) (Figure 3, Table 5).

Discussion

Neural cell adhesion molecule 1 is essential for cell adhesion, cell migration, axonal guidance, signal transduction, and synaptic plasticity during brain development. Bouras et al (2001) reported decreased neuron densities in layers III, V, and VI of Brodmann's area 24 (anterior cingulate cortex) in patients with bipolar disorder. Densities of neurons and pyramidal and glial cells were reduced in the prefrontal cortex of bipolar patients (Rajkowaska et al 2001). Animal studies have also suggested that disruption of NCAM1 function might underlie the pathophysiology of affective disorder through dysregulation of the cytoarchitecture (Cremer et al 1994; Tomasiewicz et al 1993). *NCAM1* is therefore deemed to possess compelling functional relevance to affective disorders.

Our case–control analysis revealed that the IVS6+32T>C (SNP5), IVS7+11G>C (SNP6), and IVS12+21C>A (SNP9) polymorphisms of *NCAM1* are nominally significantly associated with bipolar disorder, with the IVS12+21A (SNP9) allele displaying the strongest association (allelic p = .08 after correction for 10 SNPs and two disease category examinations). Sixty-six percent of our bipolar subjects suffered from bipolar disorder type I. It might be possible that bipolar I and bipolar II disorders are separate entities; however, there seems to be no difference in genetic association with *NCAM1* between bipolar I and II groups in the present study: allele frequencies of IVS12+21A (SNP9), which showed the strongest p value, were similar in bipolar I (.23, allelic p = .067) and bipolar II (.26, allelic p = .058) cohorts. Linkage disequilibrium analysis revealed that IVS12+21C>A (SNP9) was located at the 3′ edge of the LD block, and a gap existed between SNP9 and the neighboring SNP10. These results suggest that the real disease-causing variant(s), if one exists, might reside in the 3′ portion of the haplotype block spanning SNP1 to SNP10. The association of IVS6+32T>C (SNP5) and IVS7+11G>C (SNP6) polymorphisms with bipolar disorder might reflect tapering but remnant LD between these polymorphisms and the neighboring risk variant(s); however, more thorough genetic analyses are needed to precisely locate the genomic boundaries contributing to the development of bipolar disorder.

Table 3. Genotypic and Allelic Distributions of Nine *NCAM1* Gene Polymorphisms

Polymorphism	*n*	Genotype Counts (Frequency)			*p*[a]	Allele Counts (Frequency)		*p*[a]
IVS0-49de IG (SNP1)		I/I	I/D	D/D		I	D	
Bipolar disorder	151	128 (.85)	22 (.15)	1 (.01)	.89	278 (.92)	24 (.08)	.55
Unipolar disorder	78	64 (.82)	13 (.17)	1 (.01)	.95	141 (.90)	15 (.10)	.89
Control subjects	357	295 (.83)	58 (.16)	4 (.01)		648 (.91)	66 (.09)	
IVS4 + 80G > C (SNP4)		G/G	G/C	C/C		G	C	
Bipolar disorder	151	63 (.42)	68 (.45)	20 (.13)	.17	194 (.64)	108 (.36)	.08
Unipolar disorder	78	25 (.32)	38 (.49)	15 (.19)	.85	88 (.56)	68 (.44)	.72
Control subjects	357	118 (.33)	180 (.50)	59 (.17)		416 (.58)	298 (.42)	
IVS6 + 32T > C (SNP5)		T/T	T/C	C/C		T	C	
Bipolar disorder	151	96 (.64)	51 (.34)	4 (.03)	.06	243 (.80)	59 (.20)	.04
Unipolar disorder	78	59 (.76)	17 (.22)	2 (.03)	.37	135 (.87)	21 (.13)	.90
Control subjects	357	260 (.73)	93 (.26)	4 (.01)		613 (.86)	101 (.14)	
IVS7 + 11G > C (SNP6)		G/G	G/C	C/C		G	C	
Bipolar disorder	151	25 (.17)	79 (.52)	47 (.31)	.06	129 (.43)	173 (.57)	.02
Unipolar disorder	78	25 (.32)	34 (.44)	19 (.24)	.45	84 (.54)	72 (.46)	.48
Control subjects	357	91 (.25)	179 (.50)	87 (.24)		361 (.51)	353 (0.49)	
IVS7 + 155G > T (SNP7)		G/G	G/T	T/T		G	T	
Bipolar disorder	151	127 (.84)	23 (.15)	1 (.01)	1.00	277 (.92)	25 (.08)	.90
Unipolar disorder	78	64 (.82)	13 (.17)	1 (.01)	.89	141 (.90)	15 (.10)	.76
Control subjects	357	298 (.83)	55 (.15)	4 (.01)		651 (.91)	63 (.09)	
I621G > T (SNP8)		G/G	G/T	T/T		G	T	
Bipolar disorder	151	61 (.40)	74 (.49)	16 (.11)	.64	196 (.65)	106 (.35)	.38
Unipolar disorder	78	42 (.54)	29 (.37)	7 (.09)	.28	113 (.72)	43 (.28)	.29
Control subjects	357	159 (.45)	166 (.46)	32 (.09)		484 (.68)	230 (.32)	
IVS12 + 21 C > A (SNP9)		C/C	C/A	A/A		C	A	
Bipolar disorder	151	87 (.58)	54 (.36)	10 (.07)	.01	228 (.75)	74 (.25)	.004
Unipolar disorder	78	51 (.65)	26 (.33)	1 (.01)	.51	128 (.82)	28 (.18)	.64
Control subjects	357	249 (.70)	98 (.27)	10 (.03)		596 (.83)	118 (.17)	
2208T > G (SNP10)		T/T	T/G	G/G		T	G	
Bipolar disorder	151	79 (.52)	64 (.42)	8 (.05)	.15	222 (.74)	80 (.26)	.23
Unipolar disorder	78	36 (.46)	36 (.46)	6 (.08)	.54	108 (.69)	48 (.31)	.92
Control subjects	357	178 (.50)	141 (.39)	38 (.11)		497 (.70)	217 (.30)	
2256_2257 insATGG (SNP11)		D/D	D/I	I/I		D	I	
Bipolar disorder	151	143 (.95)	8 (.05)	0 (.00)	.32	294 (.97)	8 (.03)	.33
Unipolar disorder	78	78 (1.00)	0 (.00)	0 (.00)	.14	156 (1.00)	0 (0.00)	.14
Control subjects	357	345 (.97)	12 (.03)	0 (.00)		702 (.98)	12 (.02)	

NCAM1, neural cell adhesion molecule 1; I, insertion; D, deletion; SNP, single nucleotide polymorphism.
[a]Differences in genotypic and allelic distributions were evaluated by Fisher's exact test.

Interestingly, IVS6+32T>C, IVS7+11G>C, and IVS12+21C>A were all located in close proximity to the intron–exon boundaries. Mutations located near splicing donor and acceptor sites were found in patients with frontotemporal dementia, FTDP-17, and affected splicing regulation of τ-protein by causing distortion of the stem-loop structure (Hutton et al 1998). Previous postmortem studies have shown that the VASE- and SEC-NCAM isoforms are increased in the brains of patients with bipolar disorder,

Table 4. Pairwise Linkage Disequilibrium Estimations Between Polymorphisms in the *NCAM1* Gene

Polymorphism	IVS0-49delG (SNP1)	IVS0-30(GTTTT)$_{4-7}$ (SNP3)	IVS4 + 80 G > C (SNP4)	IVS6 + 32 T > C (SNP5)	IVS7 + 11 G > C (SNP6)	IVS7 + 155 G > T (SNP7)	1621 G > T (SNP8)	IVS12 + 21 C > A (SNP9)	2208 T > G (SNP10)
SNP1		1.000 (1.000)	1.000 (1.000)	1.000 (1.000)	1.000 (1.000)	1.000 (1.000)	1.000 (1.000)	.351 (.450)	.277 (.150)
SNP3	.070 (.046)		.944 (1.000)	1.000 (1.000)	.923 (1.000)	1.000 (1.000)	.882 (.792)	.699 (.670)	.112 (.088)
SNP4	.071 (.048)	.838 (.957)		1.000 (1.000)	.885 (.947)	1.000 (1.000)	.896 (.770)	.469 (.601)	.125 (.065)
SNP5	.017 (.021)	.112 (.129)	.114 (.131)		1.000 (1.000)	1.000 (1.000)	1.000 (1.000)	.534 (.728)	.141 (.015)
SNP6	.098 (.116)	.547 (.715)	.548 (.670)	.169 (.181)		.900 (.756)	.847 (.818)	.005 (.277)	.136 (.087)
SNP7	1.000 (1.000)	.067 (.048)	.068 (.050)	.016 (.022)	.076 (.069)		1.000 (.561)	.363 (.483)	.104 (.180)
SNP8	.048 (.047)	.249 (.196)	.273 (.178)	.077 (.126)	.349 (.270)	.046 (.015)		.803 (.862)	.290 (.088)
SNP9	.063 (.054)	.070 (.091)	.031 (.065)	.238 (.397)	.000 (.019)	.065 (.065)	.061 (.130)		.283 (.443)
SNP10	.003 (.005)	.013 (.018)	.009 (.003)	.001 (.000)	.008 (.004)	.005 (.008)	.017 (.001)	.007 (.005)	

The diagonal upper right part shows standardized D′ in control (bipolar) group between two markers calculated by the COCAPHASE program. The lower left part of diagonal shows r^2 (squared correlation coefficient) in control (bipolar) for bi-allelic marker pairs, and squared values of Cramer's coefficient for pairs with the multi-allelic marker, IVS0-30(GTTTT)$_{4-7}$. NCAM1, neural cell adhesion marker 1; SNP, single nucleotide polymorphism.

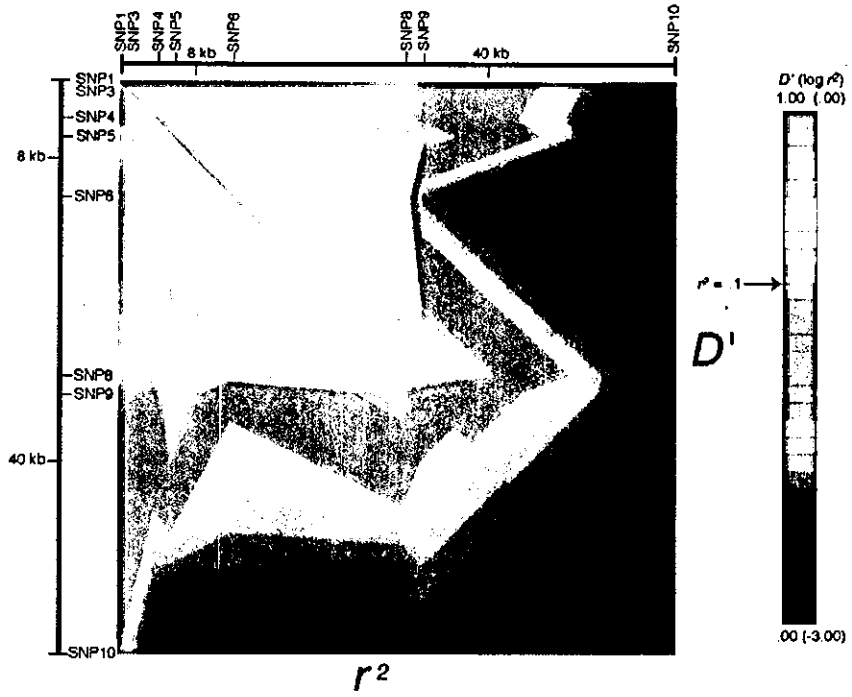


Figure 2. Linkage disequilibrium (LD) map of the *NCAM1* locus. Gold plot of color-coded, pairwise disequilibrium statistics (r^2 in diagonal bottom left and D' in diagonal upper right) is shown. Red and yellow indicate areas of strong LD. For single nucleotide polymorphism (SNP) numbers, see Figure 1.

compared with those of control subjects (Vawter et al 1998, 1999). The VASE exon is thought to play functional roles in the modulation of neurite growth activity (Doherty et al 1992). Use of the SEC exon resulted in premature termination of the coding sequence and production of a truncated NCAM polypeptide in brains (Gower et al 1988). IVS7+11G>C is upstream of the VASE exon, and the IVS12+21C>A variant is upstream of the alternatively spliced small exons and SEC exon. Examination of the correlation between NCAM1 genotypes and the content of alternatively spliced exons in bipolar brains would therefore be intriguing. We recently demonstrated just such a genotype (polymorphic repeats in a gene promoter region)–phenotype (expression level of gene product in postmortem brains) in a study of the *N*-methyl-D-aspartate receptor NR2A subunit gene (Itokawa et al 2003).

NCAM1 displayed a significant association with bipolar disorder but not with unipolar disorder. Power analysis showed that the size of our unipolar sample had adequate power to detect a relative risk of more than 2.0 but might miss small gene effects (relative risk < 1.5). Nevertheless, the failure to discern an

	SNP1	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9
SNP1	Ins							
SNP3								
SNP4	G	G	G					
SNP5		C	C	C				
SNP6			C	C	C			
SNP7				G	G	G		
SNP8					G	G	G	
SNP9						A	A	A
SNP10							T	T
SNP11								Del
Global (*P* value)	.014	.034	.038	.097	.136	.006	.015	.024
Individual Haplotype (*P* value)	.010	.031	.029	.034	.067	.005	.005	.018
Risk Allele	Ins	$(GTTTT)_4$	G	C	C	G	G	A
Global (*P* value)	.033							
Individual Haplotype (*P* value)	.009							

Figure 3. Results of three-marker and nine-marker haplotype analyses in bipolar samples. For three-marker analysis, a sliding window of three markers was tested, with two-marker overlaps. Below each over-represented haplotype is the *p* value for that haplotype. "Global (*P* value)" represents the overall significance when the observed versus expected frequencies of all of the haplotypes are considered together. "Individual Haplotype (*P* value)" represents significance of the deviated distribution of the risk haplotype in the bipolar group compared with control subjects. The *p* values were calculated with COCAPHASE (Dudbridge 2002; http://www.hgmp.mrc.ac.uk/~fdudbrid/software/).

Table 5. Estimated Haplotype Frequencies of the *NCAM1* Gene

Haplotype SNP1-3-4-5-6-7-8-9[a]	Frequency[b] Bipolar Subjects (n = 151)	Control Subjects (n = 357)	p[c]
Del-1-G-T-G-T-G-A	.0501	.0466	.8044
Del-1-G-T-G-T-G-C	.0268	.0456	.1519
Ins-1-G-T-C-G-T-C	.3241	.2916	.3202
Ins-1-G-T-C-G-G-C	.0570	.0547	.8936
Ins-1-G-C-C-G-G-A	.1472	.0883	.0086
Ins-1-G-C-C-G-G-C	.0381	.0493	.4416
Ins-1-C-T-G-G-G-C	.0000	.0146	.0087
Ins-2-C-T-G-G-T-C	.0255	.0240	.8938
Ins-2-C-T-G-G-G-C	.1004	.1136	.5520
Ins-3-C-T-G-G-G-A	.0296	.0158	.2011
Ins-3-C-T-G-G-G-C	.1417	.2043	.0229
Ins-4-C-T-G-G-G-A	.0109	.0112	.9128
Ins-4-C-T-G-G-G-C	.0486	.0406	.6353
Global *p* value[c]			.0326

NCAM1, neural cell adhesion molecule 1; SNP, single nucleotide polymorphism.

[a]SNP1, allele Del = deletion, allele Ins = insertion; SNP3, allele 1 = $(GTTTT)_4$, allele 2 = $(GTTTT)_5$, allele 3 = $(GTTTT)_6$, allele 4 = $(GTTTT)_7$.

[b]Haplotpe frequencies were estimated by COCAPHASE.

[c]Calculated by COCAPHASE

association with unipolar disorder might not be due to the smaller statistical power of the analysis compared with bipolar disorder, because the genotypic and allelic frequencies in unipolar disorder resembled those of control subjects, not subjects with bipolar disorder. The present genetic findings suggest that the role of NCAM1 is pathophysiologically more relevant to bipolar disorder than to unipolar disorder. Such a result is in line with the aforementioned reports on NCAM1 perturbation in bipolar disorder (Vawter et al 1998, 1999) and might be in line with reports on disturbed brain histopathology in bipolar disorder (Bouras et al 2001; Rajkowska et al 2001). Other recent studies have also demonstrated pathophysiologic distinctions between bipolar and unipolar depression (Beyer and Krishnan 2002; Cotter et al 2001; Ongur et al 1998; Vawter et al 2000b).

In conclusion, our data suggest the possible involvement of human *NCAM1* or a nearby gene in vulnerability to bipolar affective disorder.

We thank Dr. Meerabux for her critical reading of the manuscript and Ms. Iwayama-Shigeno for her technical assistance.

This study was supported in part by a Grant-in-Aid from the Japan Society for the Promotion of Science (KAKENHI 14570953) and The Ministry of Education, Culture, Sports, Science and Technology (KAKENHI 15790645) in Japan.

Abecasis GR, Cookson WO (2000): GOLD-graphical overview of linkage disequilibrium. *Bioinformatics* 16:182–183.

Abecasis GR, Noguchi E, Heinzmann A, Traherne JA, Bhattacharyya S, Leaves NI, et al (2001): Extent and distribution of linkage disequilibrium in three genomic regions. *Am J Hum Genet* 68:191–197.

American Psychiatric Association (1994): *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* Washington, DC: American Psychiatric Press.

Barton CH, Dickson G, Gower HJ, Rowett LH, Putt W, Elsom V, et al (1988): Complete sequence and in vitro expression of a tissue-specific phosphatidylinositol-linked N-CAM isoform from skeletal muscle. *Development* 104:165–173.

Berrettini WH (2000): Are schizophrenic and bipolar disorders related? A review of family and molecular studies. *Biol Psychiatry* 48:531–538.

Berrettini WH (2001): Molecular linkage studies of bipolar disorders. *Bipolar Disord* 3:276–283.

Berrettini W (2002): Review of bipolar molecular linkage and association studies. *Curr Psychiatry Rep* 4:124–129.

Beyer JL, Krishnan KR (2002): Volumetric brain imaging findings in mood disorders. *Bipolar Disord* 4:89–104.

Bouras C, Kovari E, Hof PR, Riederer BM, Giannakopoulos P (2001): Anterior cingulate cortex pathology in schizophrenia and bipolar disorder. *Acta Neuropathol* 102:373–379.

Cardno AG, Marshall EJ, Coid B, Macdonald AM, Ribchester TR, Davies NJ, et al (1999): Heritability estimates for psychotic disorders: The Maudsley twin psychosis series. *Arch Gen Psychiatry* 56:162–168.

Cotter D, Mackay D, Landau S, Kerwin R, Everall I (2001): Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. *Arch Gen Psychiatry* 6:545–553.

Craddock N, Dave S, Greening J (2001): Association studies of bipolar disorder. *Bipolar Disord* 3:284–298.

Craddock N, Jones I (1999): Genetics of bipolar disorder. *J Med Genet* 36:585–594.

Cremer H, Lange R, Christoph A, Plomann M, Vopper G, Roes J, et al (1994): Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. *Nature* 367:455–459.

Doherty P, Moolenaar CE, Ashton SV, Michalides RJ, Walsh FS (1992): The VASE exon downregulates the neurite growth-promoting activity of NCAM 140. *Nature* 356:791–793.

Dudbridge F (2002): Methods and software for association tests of uncertain haplotypes in case-parent trios. *Am J Hum Genet* 71(suppl):A2338.

First MB, Spitzer RL, Gibbon M (1997): *Structured Clinical Interview for DSM-IV Axis I Disorders (Clinician Version).* Washington, DC: American Psychiatric Press.

Goridis C, Brunet JF (1992): NCAM: Structural diversity, function and regulation of expression. *Semin Cell Biol* 3:189–197.

Gower HJ, Barton CH, Elsom VL, Thompson J, Moore SE, Dickson G, et al (1988): Alternative splicing generates a secreted form of N-CAM in muscle and brain. *Cell* 55:955–964.

Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al (1998): Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* 393:702–705.

Itokawa M, Yamada K, Yoshitsugu K, Toyota T, Suga T, Ohba H, et al (2003): A microsatellite repeat in the promoter of the N-methyl-D-aspartate receptor 2A subunit (GRIN2A) gene suppresses transcriptional activity and correlates with chronic outcome in schizophrenia. *Pharmacogenetics* 13:271–278.

Kato T (2001): Molecular genetics of bipolar disorder. *Neurosci Res* 40:105–113.

Kiss JZ, Muller D (2001): Contribution of the neural cell adhesion molecule and synaptic plasticity. *Rev Neurosci* 12:297–310.

Mendlewicz J, Rainer JD (1977): Adoption study supporting genetic transmission in manic-depressive illness. *Nature* 268:327–329.

Nakajima T, Jorde LB, Ishigami T, Uemura S, Emi M, Lalouel J-M, et al (2002): Nucleotide diversity and haplotype structure of the human angiotensin gene in two populations. *Am J Hum Genet* 70:108–123.

Ongur D, Drevets WC, Price JL (1998): Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc Natl Acad Sci U S A* 95:13290–13295.

Poltorak M, Frye MA, Wright R, Hemperly JJ, George MS, Pazzaglia PJ, et al (1996): Increased neural cell adhesion molecule in the CSF of patients with mood disorder. *J Neurochem* 66:1532–1538.

Rajkowska G, Halaris A, Selemon LD (2001): Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biol Psychiatry* 49:741–752.

Ronn LC, Hartz BP, Bock E (1998): The neural cell adhesion molecule (NCAM) in development and plasticity of the nervous system. *Exp Gerontol* 33:853–864.

Saito S, Tanio Y, Tachibana I, Hayashi S, Kishimoto T, Kawase T (1994): Complementary DNA sequence encoding the major neural cell adhesion molecule isoform in a human small cell lung cancer cell line. *Lung Cancer* 10:307–318.

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

Taylor L, Faraone SV, Tsuang MT (2002): Family, twin, and adoption studies of bipolar disease. *Curr Psychiatry Rep* 4:130–133.

Tomasiewicz H, Ono K, Yee D, Thompson C, Goridis C, Rutishauser U, et al (1993): Genetic deletion of a neural cell adhesion molecule variant (N-CAM-180) produces distinct defects in the central nervous system. *Neuron* 11:1163–1174.

van Duijnhoven HL, Helfrich W, de Leij L, Roebroek AJ, van de Ven WJ, Healey K, et al (1992): Splicing of the VASE exon of neural cell adhesion molecule (NCAM) in human small-cell lung carcinoma (SCLC). *Int J Cancer* 50:118–123.

Vawter MP (2000a): Dysregulation of the neural cell adhesion molecule and neuropsychiatric disorders. *Eur J Pharmacol* 405:385–395.

Vawter MP, Freed WJ, Kleinman JE (2000b): Neuropathology of bipolar disorder. *Biol Psychiatry* 48:486–504.

Vawter MP, Hemperly JJ, Hyde TM, Bachus SE, VanderPutten DM, Howard AL, et al (1998): VASE-containing N-CAM isoforms are increased in the hippocampus in bipolar disorder but not schizophrenia. *Exp Neurol* 154:1–11.

Vawter MP, Howard AL, Hyde TM, Kleinman JE, Freed WJ (1999): Alterations of hippocampal secreted N-CAM in bipolar disorder and synaptophysin in schizophrenia. *Mol Psychiatry* 4:467–475.

Psychiatry and Clinical Neurosciences (2004), **58**, 333–337

Short Communication

Identification of a male schizophrenic patient carrying a de novo balanced translocation, t(4; 13)(p16.1; q21.31)

MASANARI ITOKAWA, MD,[1,2] TAKEHIKO KASUGA, MD,[3] TAKEO YOSHIKAWA, MD[2] AND MASAAKI MATSUSHITA, MD[3,4]

[1]*Department of Schizophrenia Research,* [4]*Tokyo Institute of Psychiatry,* [3]*Department of Psychiatry, Tokyo Metropolitan Matsuzawa Hospital, Setagaya, Tokyo and* [2]*Laboratory for molecular Psychiatry, RIKEN Brain Science Institute, Wako, Saitama, Japan*

Abstract Herein is reported the case of a male patient with schizophrenia who displayed a de novo balanced translocation between the short arm of chromosome 4 and the long arm of chromosome 13, t(4; 13)(p16.1; q21.31). The 4p16.1 region is where the causative gene (*WFS1*) for Wolfram syndrome has been mapped. In Wolfram syndrome, approximately 60% of patients suffer from major mental illness. The other breakpoint, chromosome 13q21.31, is another region where previous linkage studies have repeatedly detected linkage to schizophrenia. The documentation of the present case could therefore provide a valuable resource for identifying disease susceptibility genes by localizing the breakpoints.

Key words: chromosome 4, chromosome 13, cytogenetic abnormality, karyotype, GTG-banding, Wolfram syndrome.

INTRODUCTION

Evidence from family, twin and adoption studies has suggested the existence of important genetic contributions to the etiology of schizophrenia.[1] Linkage and association studies often yield valuable, but sometimes conflicting, results for schizophrenia, and the responsible genes have proven difficult to isolate.[2] An alternative to these genetic approaches would be the identification and precise genomic analysis of chromosomal abnormalities that are cosegregated with the disease. MacIntyre *et al.* compiled a list of translocations coexisting with mental illnesses and tried to assess the relevance of these to diseases based on three criteria.[3] These criteria were (i) rarity of the translocation and independent reports of coexistence of the translocation with psychiatric illness; (ii) colocalization of the abnormality with suggestive linkage findings; and (iii) cosegregation of the abnormality with illness within a family. Analysis of a chromosomal aberration fulfilling all three criteria, a t(1; 11)(q42.2; q14.3) translocation identified in a Scottish family suffering from major psychiatric illnesses, led to the isolation of genes *disrupted-in-schizophrenia-1* and -2 (*DISC1* and *DISC2*).[4]

We report herein the case of a male patient with schizophrenia who displayed a de novo balanced translocation between chromosomes 4 and 13, and discuss the usefulness of this case for molecular dissection of schizophrenia susceptibility genes.

CASE REPORT

Clinical course

The proband was a 42-year-old man who was the second child of unrelated parents. He was born at term after an uneventful pregnancy and normal delivery. Psychomotor development was normal. After graduating from high school at 18 years of age, he was employed by a company. Thereafter, he changed jobs every 1–2 years. From around 30 years of age he began to manifest alcohol-related problems, such as violence against his parents while intoxicated. At 37 years of age

Correspondence address: Dr Masanari Itokawa, Department of Schizophrenia Research, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya, Tokyo 156-8585, Japan. Email: mitokawa@prit.go.jp

Received 1 September 2003; revised 3 December 2003; accepted 14 December 2003.

he borrowed 2000 000 yen from his father and went to the USA in an attempt to set up a business. However, 3 months later he returned to Japan without any business success and started to live alone in an apartment. At 38 years of age he began to exhibit insomnia, psychomotor excitement, and persecutory delusion, and was admitted to a psychiatric hospital.

He was 170 cm tall and weighed 60 kg. Neurological examination and computed tomography of the brain revealed no abnormalities. Laboratory tests identified renal abnormality, and ultrasonography detected polycysts in the left kidney and stones in the right. He suffered from bizarre delusions of being influenced by external forces ('Government has put a microchip in my brain to control all of my behavior, read my thought and inform everybody of my private thoughts'). Although not prominent, disorganized speech was also noted, with the patient moving quickly from one topic to another. Based on the consensus of two experienced psychiatrists in non-structural psychiatric interviews, family information and all available medical records, a diagnosis of paranoid schizophrenia was made according to *Diagnostic and Statistical Manual of Mental Disorders* (4th edn; DSM-IV) criteria. Psychotic symptoms responded well to treatment with resperidon. The Positive and Negative Symptom Scale (PANSS)[5] was examined in the remission state after treatment, giving scores of 9 on positive symptoms, 13 on negative symptoms, and 29 on general non-psychotic psychiatric symptoms.

Family history

Non-structural detailed interviews of family members by two experienced psychiatrists, revealed no suggestion of psychiatric illness among individuals within the second-degree relatives of the proband (Fig. 1). Of the seven siblings of the proband's father, three sisters (05, 06 and 11) suffered from diabetes mellitus, and a brother (09) had been blind since 6 years of age, although the cause was unknown. The mother (18) suffered from hypertension and diabetes mellitus, and both her brother (16) and mother (04) displayed histories of renal disease (precise diagnoses unknown).

Cytogenetic findings

High-resolution karyotyping of GTG-banded chromosomes (850 band level) in the patient demonstrated a balanced translocation between band p16.1 of chromosome 4 and band q21.31 of chromosome 13 in each of the 30 lymphocytes examined (Figs 2,3). Karyotype was thus considered to be 46,XY,t(4;13)(p16.1;q21.31). Neither parent displayed any cytogenetic abnormalities, with karyotypes of 46,XY and 46,XX, respectively.

The present study was approved by the Ethics Committees of the Tokyo Institute of Psychiatry and Matsuzawa Hospital, and all participants provided written informed consent.

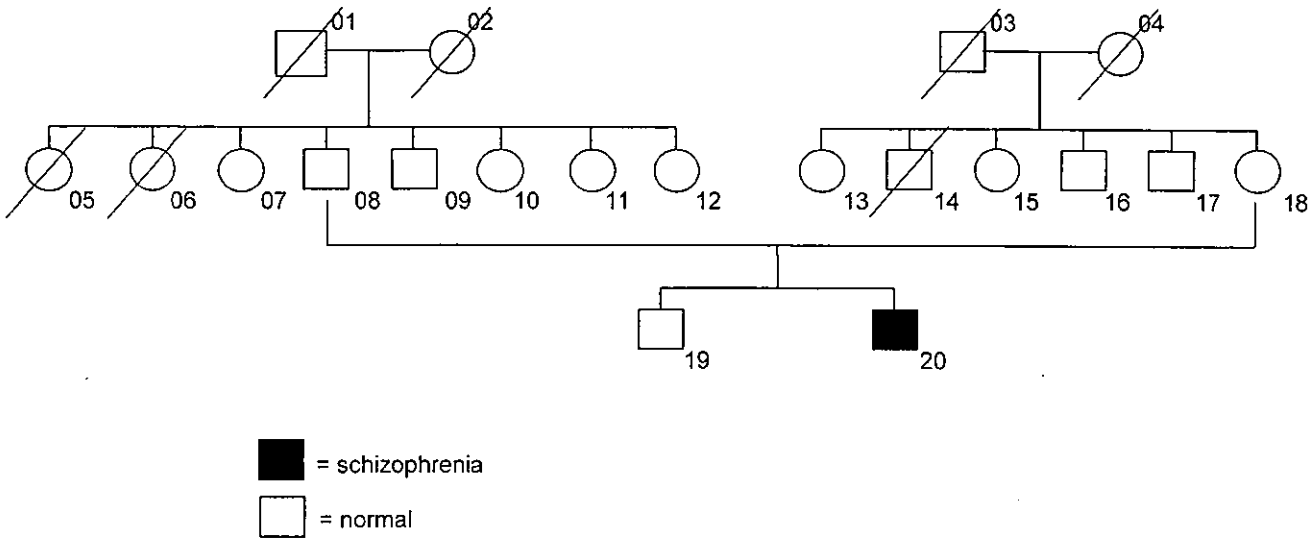


Figure 1. Family structure and psychiatric phenotype. Individual 20 displayed a chromosomal abnormality, while both parents demonstrated normal karyotypes.

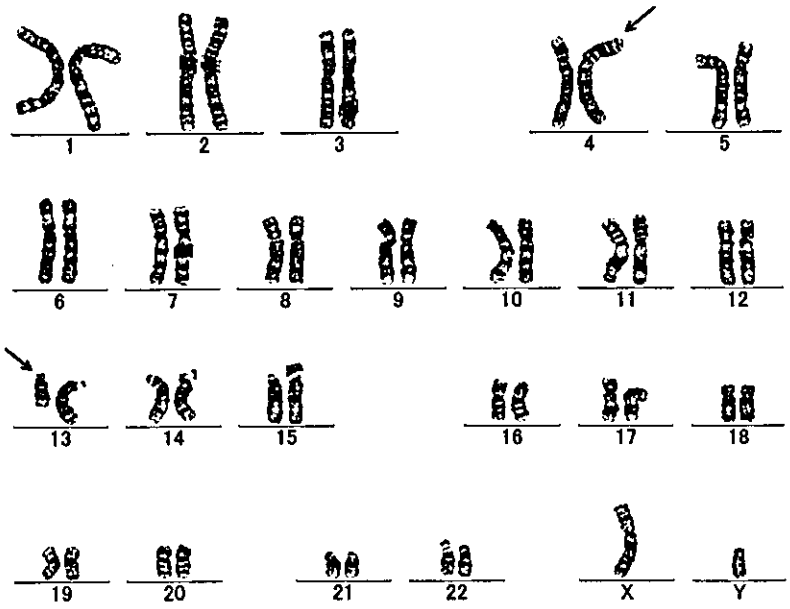


Figure 2. Karyotype of individual 20. Balanced translocation between 4p and 13q is illustrated, 46,XY,t (4;13) (p16.1;q21.31).

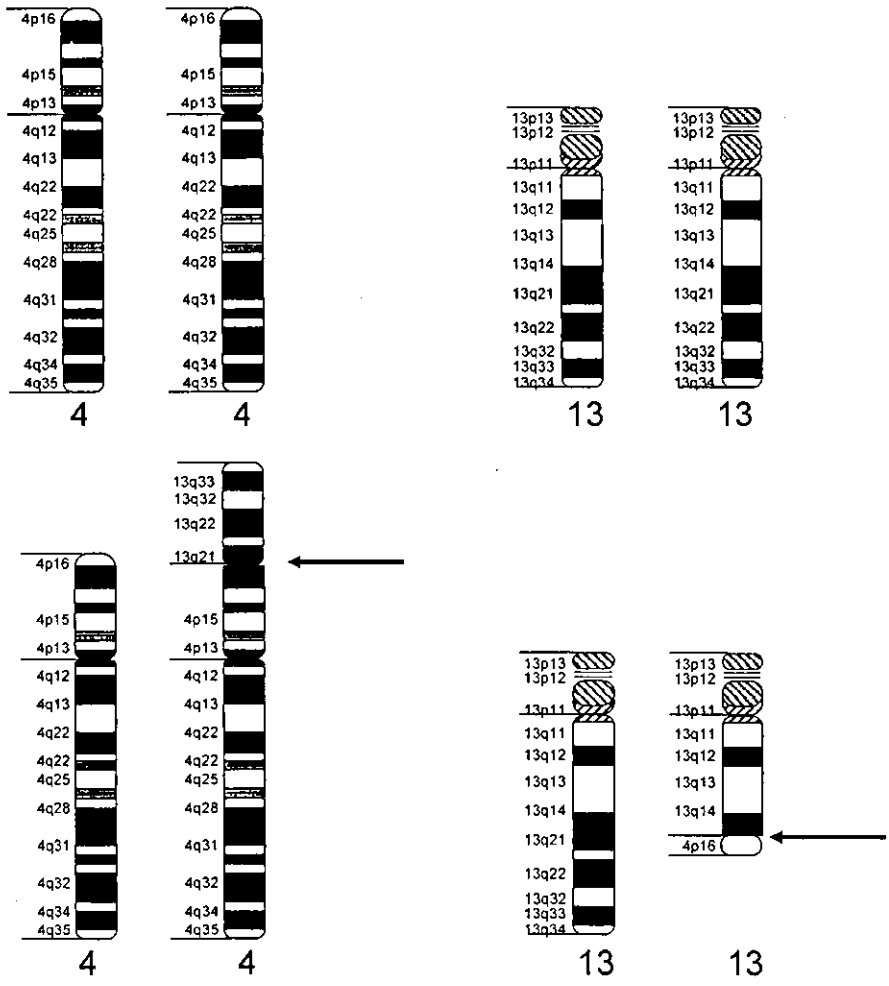


Figure 3. Diagram showing normal chromosomes 4 and 13 (top) and cytogenetic interpretation of the breakpoints (arrow) involved in the translocation (below).

DISCUSSION

We have reported the case of a male schizophrenic patient carrying a de novo balanced translocation between 4p16.1 and 13q21.31. Among the three criteria that MacIntyre *et al.* recently proposed for determining the relevance of chromosomal abnormalities and diseases,[3] the present case fulfills those of (i) rarity of the translocation and independent reports of coexistence of the translocation with psychiatric illness (a link between chromosomal break at 13q21 and psychosis was reported by Roberts *et al.*[6]); and (ii) colocalization of the abnormality with suggestive linkage findings. Cosegregation of the abnormality with illness within a family was not validated by the present case, because the abnormality was de novo. Apparently, no other family members had manifested psychiatric problems.

Chromosome region 13q14-q32 has previously been reported as a linkage locus for schizophrenia.[7,8] A recent meta-analysis has also provided evidence of linkage on the long arm of chromosome 13.[9] The gene for spinocerebellar ataxia type 8 (*SCA8*) maps to 13q21, and a case–control study reported increased occurrence of large triplet repeats in *SCA8* associated with major psychosis.[10] The gene encoding Wolframin (*WFS1*) maps to 4p16.[11] Mutation of *WFS1* causes Wolfram syndrome, which frequently (approx. 60%) accompanies episodes of severe mental illness with psychotic symptoms, in addition to diabetes insipidus, diabetes mellitus, optic atrophy and deafness.[12] The genes *SCA8* and *WFS1* were among candidates to be analyzed in the present translocation case.

The incidence of alcoholism was found to be three times higher for schizophrenia patients compared to the rest of the population.[13] Schizophrenia patients who abused alcohol were seven times more likely to manifest violent behavior relative to non-alcoholic patients with schizophrenia.[14] The present patient also displayed alcohol-related problems including violent behavior. Previous linkage studies of alcoholism have provided suggestive evidence for linkage on chromosomes 4p and 13q.[15,16]

The patient had a polycystic kidney. Family history suggested heritability of renal disease because the uncle (16 in Fig. 1) and grandmother (04 in Fig. 1) of the patient also suffered from renal dysfunction (details were unclear from family information and medical records). However, no studies have yet reported chromosomes 4p or 13q as being susceptibility regions for polycystic kidney. The current t(4; 13)(p16.1; q21.31) translocation was therefore not deemed to represent an underlying mechanism for polycystic kidney in the present case.

The patient had lived with his parents until he was 38 years old, and the parents never recognized any psychotic symptoms before he was 39 years old. However, the patient had exhibited social and occupational dysfunctions prior to the evident manifestation of psychotic features, with frequent changes in jobs, alcohol-related problems and so on. The real onset of schizophrenia may have occurred earlier than 39 years of age.

In summary, the present case could provide a valuable resource for localizing the breakpoints on chromosomes 4 and 13, which may lead to the cloning of susceptibility genes for schizophrenia.

ACKNOWLEDGMENTS

This work was partly funded by a Grant-in-Aid for Scientific Research (C) from the JSPS, Japan (No. 14570953). We thank Dr Meerabux for her critical reading of the manuscript.

REFERENCES

1. Gottesmann II. *Schizophrenia Genetics: The Origin of Madness*. Freeman, New York, 1991.
2. Sawa A, Snyder SH. Schizophrenia: diverse approaches to a complex disease. *Science* 2002; **296**: 692–695.
3. MacIntyre DJ, Blackwood DH, Porteous DJ *et al.* Chromosomal abnormalities and mental illness. *Mol. Psychiatry* 2003; **8**: 275–287.
4. Millar JK, Wilson-Annan JC, Anderson S *et al.* Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum. Mol. Genet.* 2000; **22**: 1415–1423.
5. Kay SR. Positive–negative symptom assessment in schizophrenia: psychometric issues and scale comparison. *Psychiatr. Q.* 1990; **61**: 163–178.
6. Roberts SH, Cowie VA, Singh KR. Intrachromosomal insertion of chromosome 13 in a family with psychosis and mental subnormality. *J. Ment. Defic. Res.* 1986; **30**: 227–232.
7. Blouin JL, Dombroski BA, Nath SK *et al.* Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. *Nat. Genet.* 1998; **20**: 70–73.
8. Riley BP, Lin MW, Mogudi-Carter M *et al.* Failure to exclude a possible schizophrenia susceptibility locus on chromosome 13q14.1-q32 in southern African Bantu-speaking families. *Psychiatr. Genet.* 1998; **8**: 155–162.
9. Badner JA, Gershon ES. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol. Psychiatry* 2002; **7**: 405–411.
10. Vincent JB, Yuan QP, Schalling M *et al.* Long repeat tracts at SCA8 in major psychosis. *Am. J. Med. Genet.* 2000; **96**: 873–876.
11. Strom TM, Hortnagel K, Hofmann S *et al.* Diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) caused by mutations in a novel gene (wol-

framin) coding for a predicted transmembrane protein. *Hum. Mol. Genet.* 1998; **7**: 2021–2028.
12. Swift RG, Sadler DB, Swift M. Psychiatric findings in Wolfram syndrome homozygotes. *Lancet* 1990; **336**: 667–669.
13. Dixon L. Dual diagnosis of substance abuse in schizophrenia: prevalence and impact on outcomes. *Schizophr. Res.* 1999; **35**: S93–S100.
14. Rasanen P, Tiihonen J, Isohanni M, Rantakallio P, Lehtonen J, Moring J. Schizophrenia, alcohol abuse, and violent behavior: a 26-year followup study of an unselected birth cohort. *Schizophr. Bull.* 1998; **24**: 437–441.
15. Long JC, Knowler WC, Hanson RL *et al.* Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. *Am. J. Med. Genet.* 1998; **81**: 216–221.
16. Tanna VL, Wilson AF, Winokur G, Elston RC. Possible linkage between alcoholism and esterase-D. *J. Stud. Alcohol* 1988; **49**: 472–476.

American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 127B:11–19 (2004)

Family-Based Association Study of Schizophrenia With 444 Markers and Analysis of a New Susceptibility Locus Mapped to 11q13.3

K. Yamada,[1] Y. Iwayama-Shigeno,[1] Y. Yoshida,[1] T. Toyota,[1,2] M. Itokawa,[1,3] E. Hattori,[1] H. Shimizu,[4] and T. Yoshikawa[1]*

[1]*Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama, Japan*
[2]*Tokyo Medical and Dental University, Tokyo, Japan*
[3]*Tokyo Institute of Psychiatry, Tokyo Metropolitan Organization for Medical Research, Tokyo, Japan*
[4]*Hokushin General Hospital, Nagano, Japan*

Family-based linkage disequilibrium (LD) mapping has been suggested as a powerful and practical alternative to linkage analysis. We have performed a genome-wide LD survey of susceptibility loci for schizophrenia in a Japanese population. We first typed 119 schizophrenic pedigrees (357 individuals) using 444 microsatellite markers, and analyzed the data using the pedigree disequilibrium test. This analysis revealed 14 markers demonstrating significant transmission distortion. To corroborate these findings, the statistical methods were changed to the extended transmission disequilibrium test (ETDT), using 80 independent complete trios (schizophrenic proband and both parents), with 68 derived from initial pedigrees and 12 newly recruited trios. ETDT supported two markers for continued association, *D11S987* on 11q13.3 ($P = 0.00009$) and *D16S423* on 16p13.3 ($P = 0.002$). We scrutinized the most significant genomic locus on 11q11-13 by adding 26 new markers for analysis. Results of three-marker haplotype analysis in the region showed evidence of association with schizophrenia (most significant haplotype $P = 0.0005$, global $P = 0.022$). Although the present study may have missed other potential genomic intervals because of the sparse mapping density, we hope that it has identified promising anchor points for further studies to identify risk-conferring genes for schizophrenia in the Japanese population. In addition, we provide useful information on genomic LD structures in Japanese populations, which can be used for LD mapping of complex diseases.

KEY WORDS: **linkage disequilibrium structure; Japanese; pedigree disequilibrium test; extended transmission disequilibrium test; chromosome 11; chromosome 16**

INTRODUCTION

Schizophrenia is a complex psychiatric illness, possibly precipitated by multiple disease-associated genes with weak-to-moderate effects. Despite intensive efforts to identify loci harboring predisposing genes, linkage studies of schizophrenia have yet to yield decisive results [Owen et al., 2000; Baron, 2001]. This may be due to the limited mapping resolution of linkage analysis for complex diseases [Boehke, 1994], disease heterogeneity and/or the absence of shared genes with substantial contributions among individual families. In contrast, increased interest has been displayed in searching for allelic associations based on linkage disequilibrium (LD) as a strategy for systematic genome-wide mapping and refinement of candidate gene locations [Risch and Merikangas, 1996]. The narrow genomic distance of LD between a marker allele and a trait locus simplifies the task of gene hunting. Family-based association/LD analyses, which generally test transmission disequilibrium, can avoid spurious results arising from population stratification. These methods are thus considered more reliable than population-based association approaches. The merits of family-based type studies have also been noted by Schaid [1998], who stated that this type of analysis might prove to be the most fruitful in isolating genes contributing small effects to complex disorders.

To identify susceptibility loci for schizophrenia, we performed a genome-wide search in a Japanese population using family-based association studies. We recognize the limitations of this approach with respect to analyzing sufficient markers to provide high resolution. However, in complex diseases, even though a complete set of susceptibility loci were identified, isolation of real gene(s) from each locus is still a mammoth task. Therefore, our aim is to obtain a few meaningful potential genomic regions from which we can clone disease-causing genes.

SUBJECTS AND METHODS

Subjects

All the families examined in this study were recruited from a geographic area located in central Japan. Schizophrenia was diagnosed according to the criteria of DSM-IV [American Psychiatric Association, 1994], by the consensus of at least two experienced psychiatrists who provided best-estimate lifetime diagnoses and were blind to family pedigrees. In addition to direct interviews, all available medical records and information from relatives and hospital staff were considered. The first screening comprised 119 families with 357 members, of whom 169 displayed schizophrenia. Families included both nuclear and extended families, containing 68 independent complete trios (schizophrenic probands and both parents), 22 probands with one parent, 21 probands with affected siblings, and 36

Grant sponsor: JSPS, Japan (Grant-in-Aid for Scientific Research); Grant number: 12307020.

*Correspondence to: T. Yoshikawa, M.D., Ph.D., 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan.
E-mail: takeo@brain.riken.go.jp

Received 28 February 2003; Accepted 20 October 2003
DOI 10.1002/ajmg.b.20166

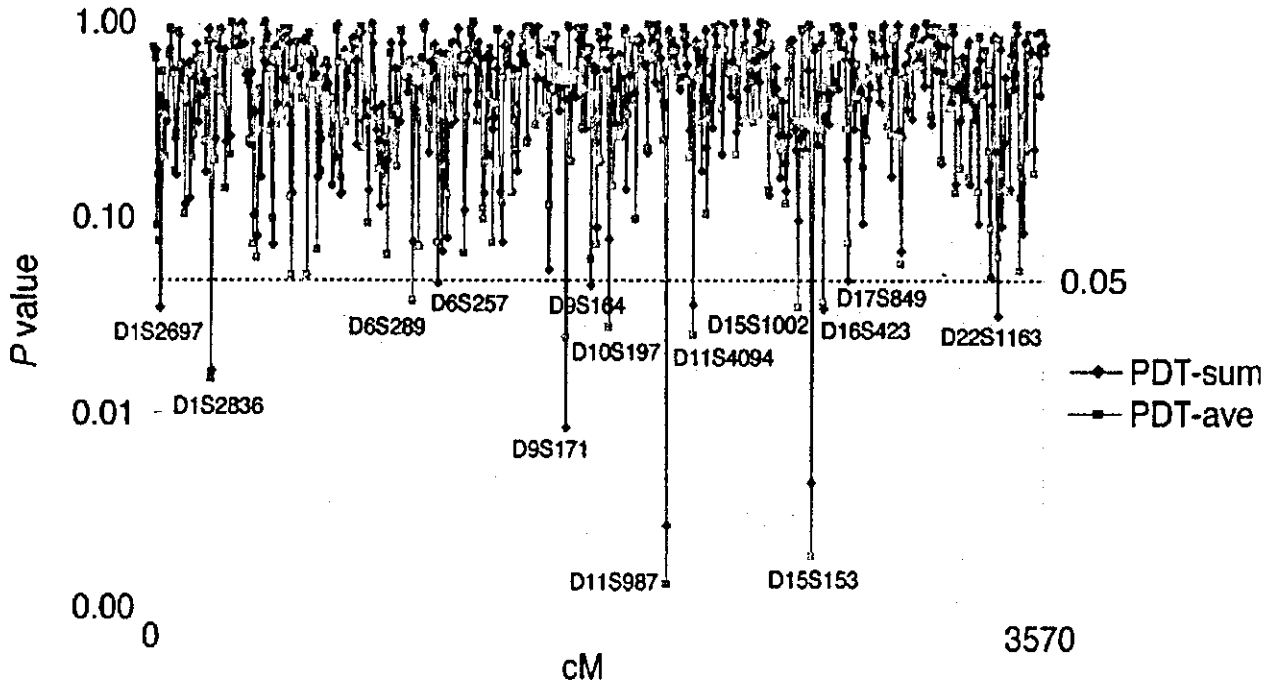


Fig. 1. Graphic representation of significance of transmission distortions. The $-\log_{10} P$ values for all 444 microsatellite markers calculated using pedigree disequilibrium test (PDT) statistics are plotted. Blue lines indicate results computed by PDT-sum statistic, and red lines by PDT-ave statistic. Fourteen markers showed significant transmission distortions (indicated).

probands with discordant siblings. Twelve additional families of complete trios were separately recruited and combined with the 68 trios from the initial sample panel for follow-up extended transmission disequilibrium test (ETDT) analysis and dense mapping of the chromosome 11q region. The present study was approved by the ethics committee of RIKEN. After receiving an explanation of the protocols and purpose of the study, written informed consent was obtained from all participants.

TABLE I. Summary of Markers Showing Significant Results From PDT and ETDT Searches

			PDT		MCETDT[b]	
Marker	cM[a]	Locus	Sum	Mean	Allele-wise	Genotype-wise
Chromosome 1						
D1S2697	33	1p36.13	0.0347	0.0752	0.051	0.085
D1S2836	284.5	1q44	0.0164	0.0149	0.396	0.117
Chromosome 6						
D6S289	22.8	6p25.1	0.0747	0.0373	0.209	0.279
D6S257	71.6	6p12.1	0.046	0.0742	0.925	0.808
Chromosome 9						
D9S171	33.5	9p21.3	0.0084	0.0241	0.079	0.099
D9S164	142	9p34.2	0.0445	0.0611	0.626	0.127
Chromosome 10						
D10S197	52.4	10p12.1	0.0776	0.0272	0.312	0.535
Chromosome 11						
D11S987	66.4	11q13.3	0.0026	0.0013	0.00009	0.002
D11S4094	122	11q24.1	0.0354	0.0247	0.14	0.31
Chromosome 15						
D15S1002	9.1	15q12	0.0964	0.0346	0.358	0.634
D15S153	62.4	15q22.31	0.0044	0.0018	0.279	0.264
Chromosome 16						
D16S423	0.1	16p13.3	0.0338	0.0365	0.002	0.113
Chromosome 17						
D17S849	0.1	17p13.3	0.0474	0.0733	0.2	0.413
Chromosome 22						
D22S1163	23.3	22q12.1	0.0316	0.0635	0.474	0.464

[a]Order and genetic distance (cM) of markers based on data from Genethon database (http://www/genethon.fr/).
[b]ETDT algorithm implementing Monte Carlo method.
Values of $P < 0.05$ are underlined.

TABLE II. ETDT Analysis of Single Markers and TRANSMIT Analysis of Three-Marker Haplotypes on 11q

Marker name[a]	Marker number[b]	Cytogenetic band	GoldenPath (bp)[c]	MCETDT[d]			Three-marker haplotypes[f]			
				Allele	Genotype	Single[e]	Global P (haplotypic P)			
AFM211XE1	1	11q11	58,627,631	0.603	0.548	0.433	0.865 (0.013)			
AFM344ZG1	2	11q11	59,016,817	0.979	0.972	0.980		0.200 (0.007)		
GATA116F08	3	11q12.1	60,347,642	0.907	0.549	0.645			0.409 (0.021)	
hCV3185821 (rs1938596)	4	11q12.1	60,775,637	0.189	0.189	0.189				0.341 (0.009)
hCV1000303 (rs550942)	5	11q12.1	60,788,107	0.325	0.325	0.325	0.253 (0.026)			
AFM165ZC3	6	11q12.2	63,171,642	0.286	0.320	0.720		<u>0.045</u> (0.010)		
AFMA085YC9	7	11q12.3	65,695,316	0.881	1.000	0.824			0.058 (0.004)	
AFM039XG3	8	11q13.1	65,922,400	0.109	0.169	<u>0.007</u>				0.773 (0.119)
hCV11661916 (rs613924)	9	11q13.2	68,303,357	0.754	0.829	0.829	0.740 (0.102)			
AFM164ZF12	10	11q13.3	68,470,236	0.990	0.488	1.000		0.108 (0.047)		
AFM147XD10	11	11q13.3	69,836,060	0.484	0.848	0.672			0.664 (0.055)	
AFMB304YH9	12	11q13.3	69,934,923	0.541	0.562	0.433				0.303 (0.062)
hCV2623961 (rs308328)	13	11q13.3	70,287,989	0.657	0.657	0.657	0.244 (0.0005)			
hCV1562388	14	11q13.3	70,356,483	0.358	0.358	0.358		<u>0.045</u> (0.003)		
D11S987	15	11q13.3	70,416,186	<u>0.00009</u>	<u>0.0020</u>	<u>0.0001</u>			<u>0.022</u> (0.014)	
hCV388085 (rs1894204)	16	11q13.3	70,452,596	0.392	0.437	0.437				0.899 (0.332)
hCV3161889 (rs624210)	17	11q13.3	70,560,253	0.736	0.736	0.736	0.738 (0.184)			
AFMA152YH1	18	11q13.3	70,621,980	0.296	0.296	0.701		0.645 (0.095)		
hCV780035 (rs667126)	19	11q13.3	70,697,026	1.000	1.000	1.000			0.404 (0.033)	
AFMB358XA9	20	11q13.3	70,712,037	0.810	0.695	0.966				0.811 (0.040)
hCV3043340	21	11q13.3	70,812,316	0.320	0.379	0.379	0.181 (0.002)			
hCV1514997	22	11q13.3	70,976,217	0.235	0.235	0.235		0.302 (0.019)		
AFMA272YB5	23	11q13.3	71,289,018	0.129	0.251	0.097			0.678 (0.045)	
AFMA190XD9	24	11q13.3	71,762,991	0.982	0.997	0.990				0.393 (0.064)
AFMB032ZG5	25	11q13.3	72,110,513	0.519	0.071	0.378	0.180 (0.034)			
AFMB038YB9	26	11q13.4	72,835,868	0.990	0.927	0.868				
AFMB331ZH5	27	11q13.4	73,307,947	0.765	0.491	0.765				

[a]hCV number: Celera ID from Assays-on-Demand™ Products Database, rs number: dbSNP ID from NCBI database (http://www.ncbi.nlm.nih.gov/). Microsatellite markers are italicized. Others represent SNPs.
[b]Marker numbers are used in Table III.
[c]Goldenpath: http://genome.ucsc.edu/cgi-bin/hgGateway, as of June 2002.
[d]Values of $P < 0.05$ are underlined.
[e]Allele-wise ETDT result without correction for multi-allele testing.
[f]Global values of $P < 0.05$ are underlined.

Markers and Genotyping

DNA was extracted from whole blood according to a standard protocol. Microsatellite marker loci were amplified by PCR using fluorescently labeled primers. PCR fragments were analyzed on an ABI PRISM 3700 Genetic Analyzer (Applied Biosystems, Foster City, CA). Genotypes were determined using GeneScan 3.5.2 and Genotyper 3.6 software (Applied Biosystems). Markers from the ABI PRISM Linkage Mapping Set ver2.0 (Applied Biosystems) were examined for heterozygosity using 100 unrelated Japanese individuals. A total of 431 markers displayed a heterozygosity value ≥0.5. Thirteen dinucleotide repeat markers with sufficient heterozygosity were selected from the Genome Database (http://gdbwww.gdb.org/), the Unified Database for Human Genome Mapping (http://bioinformatics.weizmann.ac.il/udb/), and the UCSC Human Genome Project Working Draft (http://genome.ucsc.edu/). In total, 444 markers were used to screen all chromosomes, at an average distance of 4.6 cM for chromosomes 6, 18, and 22, and 9.2 cM for the remaining 19 autosomes and the X chromosome. Mean heterozygosity for the 444 markers was 0.73. Detailed information on markers is available on request.

We selected 26 markers from the above database and Assays-on-Demand™ SNP genotyping products (Applied Biosystems;http://www.appliedbiosystems.com/) for dense mapping of the11q11-13.4 region. TaqMan assay was used to type single nucleotide polymorphism (SNP) markers [Ranade et al., 2001]. SNPs were scored using an ABI 7900 instrument and SDS v2.0 software (Applied Biosystems).

Each marker was checked for allele-inheritance inconsistency within a pedigree using PEDCHECK software [O'Connell and Weeks, 1998], and conflicts or flagged alleles were resolved by re-genotyping.

Analytical Procedures

The initial screening panel of the 119 families was analyzed using the pedigree disequilibrium test (PDT) program, version 3.12 (http://www.chg.duke.edu/software/pdt.html) [Martin et al., 2000, 2001]. The subsequent statistical follow-up study and inspection of chromosome 11q were performed using the extended 80-trio set and the ETDT algorithm, version 2.2 [Sham and Curtis, 1995]. Empirical significance levels of the ETDT results were simulated from 10,000 Monte Carlo permutations using the MCETDT program, version 1.3 (http://www.mds.qmw.ac.uk/statgen/dcurtis/software.html) [Zhao et al., 1999]. For the markers showing transmission disequilibrium by both PDT and ETDT (*D11S987* and *D16S423*), up to 32,767 permutations were performed to confirm the *P* values (since the data type for the number of simulations is described as an integer of 16-bit variables in the MCETDT program, 32,767 is the maximum value that can be set).

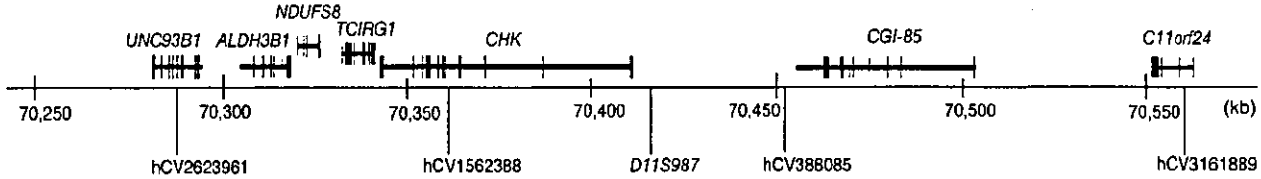
The TRANSMIT program, version 2.5.4 [Clayton, 1999; Clayton and Jones, 1999] was used for haplotype-based transmission disequilibrium testing on 11q. Three-locus analyses were performed for each successive set of adjacent markers. As rare alleles or haplotypes would generate misleading data, the command-line flags "-agg3" and "-c3" were used to aggregate all alleles or haplotypes with frequencies <0.03 before haplotype construction and calculation. Uncorrected *P* values for individual haplotype (haplotypic *P*) and corrected *P* values for multi-allele testing (global *P*) were calculated using the TRANSMIT program.

For the assessment of genomic LD patterns retained in the Japanese population, pair-wise LD measures (those evaluated by *P* values) were examined between markers in the four chromosomes (chromosomes 6, 18, 22, and 11q11-13.4), using 186 unrelated individuals from our schizophrenic pedigree panel. *P* values for LD were computed using the Arlequin program [Schneider et al., 2000]. Disequilibrium across each locus was plotted using the GOLD program [Abecasis and Cookson, 2000].

RESULTS

PDT Analysis

Figure 1 shows the results of PDT analysis in the initial pedigree panel. The PDT program computes 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 structures and genetic models



Genes located around *D11S987*

Symbol	Start position (bp)	End position (bp)	Genomic size (bp)	Full gene name
UNC93B1	70,281,508	70,294,487	12,980	unc-93 homolog B1 (*C. elegans*)
ALDH3B1	70,305,697	70,319,674	13,978	aldehyde dehydrogenase 3 family, member B1
NDUFS8	70,321,042	70,327,047	6,006	NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23 kDa (NADH-coenzyme Q reductase)
TCIRG1	70,333,380	70,341,299	11,884	T-cell, Immune regulator 1, ATPase, H+ transporting, lysosomal V0 protein a isoform 3
CHK	70,343,379	70,411,597	68,219	choline kinase
CGI-85	70,456,111	70,503,669	47,559	CGI-85 protein
C11orf24	70,551,746	70,562,360	10,615	chromosome 11 open reading frame 24
LPR5	70,603,074	70,739,678	136,605	low density lipoprotein receptor-related protein 5

Fig. 2. Genes and markers around *D11S987*. Position and genomic span of each gene are shown by horizontal lines, with exons represented by vertical lines (**upper panel**). Nucleotide positions in the genome are based on goldenPath (http://genome.ucsc.edu/cgi-bin/hgGateway), as of June 2002.

[Martin et al., 2001]. Fourteen chromosomal loci spread over 9 chromosomes showed significant associations with schizophrenia ($P < 0.05$), by either PDT-sum, PDT-ave or both. These loci comprised 1p36.13, 1q44, 6p25.1, 6p12.1, 9p21.3, 9q34.2, 10p12.1, 11q13.3, 11q24.1, 15q12, 15q22.31, 16p13.3, 17p13.3, and 22q12.1 (Fig. 1 and Table I). The most significant P value was obtained from marker *D11S987* on 11q13.3 ($P = 0.0013$), followed by markers *D15S153* on 15q22.31 ($P = 0.0018$) and *D9S171* on 9p21.3 ($P = 0.0084$) (Table I).

ETDT Analysis

To confirm the above 14 loci in a statistically different and more conservative way, we performed ETDT analysis in the extended 80 triad families. ETDT is based on a conditional logistic regression test and can be applied to markers with multiple alleles. The program calculates an allele-wise TDT statistic that attempts to determine the preferential transmission of specific alleles, and a genotype-wise TDT statistic that separately evaluates the deviation of allele transmission from each parental genotype. These ETDT analyses detected two marker loci, *D11S987* on 11q13.3 (empirical $P = 0.00009$ by allele-wise ETDT) and *D16S423* on 16p13.3 (empirical $P = 0.002$ by allele-wise) as significantly associated with schizophrenia (Table I).

A number of genetic studies on schizophrenia have shown repeated linkage to regions on chromosomes 6, 18, and 22 [Schwab et al., 1998, 2000; Owen et al., 2000; Baron, 2001; Badner and Gershon, 2002; Lewis et al., 2003]. Therefore, we tested these chromosomes using a higher density of markers compared to the remaining chromosomes. Although PDT detected two weak signals on chromosome 6p and one weak signal on chromosome 22q (Table I), further support for these loci was not obtained by subsequent ETDT analysis.

Analysis of 11q

As the marker *D11S987* on 11q13.3 yielded the most significant P values in both PDT and EDTD analyses, this chromosomal region was examined more closely by conducting single-marker and haplotype-based association analyses, using an additional 26 polymorphisms (16 microsatellites and 10 SNPs) selected from 11q11-13.4, spanning ~14 Mb (Table II). ETDT analyses detected a significant association with *AFM039XG3* by single allele analysis (allele-wise analysis that does not correct for multiple alleles), but this failed to reach significance after correction for multi-allele testing.

Both the transmission of an individual haplotype (haplotypic P) defined by three consecutive loci and all individual haplotypes for each three-locus combination as a whole (global P) were assessed using the TRANSMIT program. Significant distortions of haplotype transmission were observed for 18

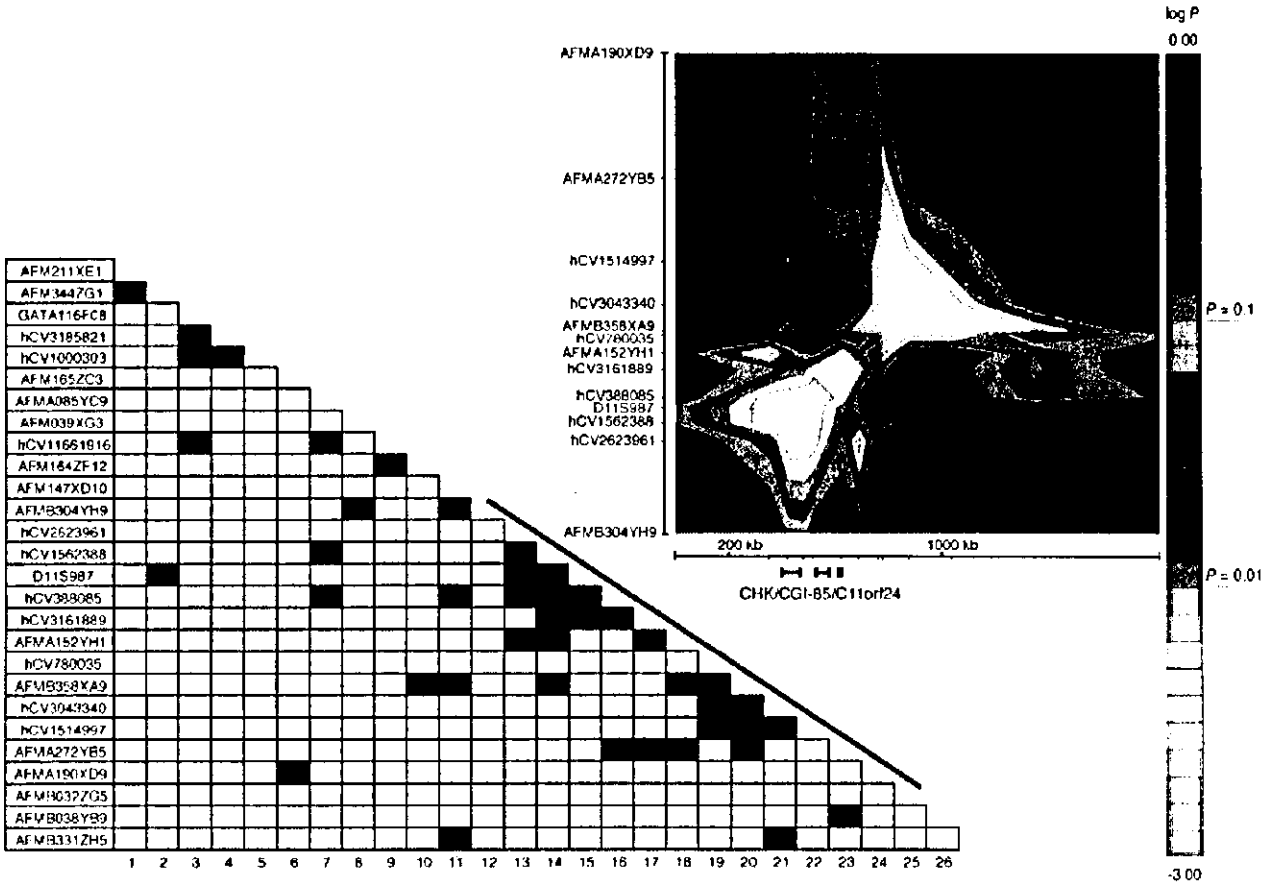


Fig. 3. Pairwise linkage disequilibrium (LD) in 11q11-13.4 and haplotype block structures around *D11S987*. P values for LD between all 351 marker pairs were calculated by examining 372 chromosomes from unrelated samples in a Japanese population, and those with P of < 0.05 are indicated in solid squares (**left lower panel**). Disequilibrium across the locus around *D11S987*, indicated by the slanted bar in left lower panel, was plotted using the GOLD program (**right upper part**). The color-coded plot by GOLD implies that the locus is divided into two haplotype blocks, each extending to 500~1,000 kb.

haplotype blocks, but only three of these that contained either *AFMA085YC9* or *D11S987* markers still displayed significant after correction for multi-haplotype testing (Table II). Detailed information including the size and frequency of each marker allele and haplotype components associated with the disease are available on request. According to the UCSC database, genes encoding choline kinase (*CHK*), CGI-85 protein (*CGI-85*), and *C11orf24*, map close to D11S987 (Fig. 2). LD analysis demonstrated that these genes are located in the same haplotype block in Japanese populations (Fig. 3).

LD Structure in Japanese Population

Long-range LD (for chromosomes 6, 18, and 22) and short-range LD (chromosome 11q11-13.4) were assessed in a Japanese population. Pair-wise *P* values for LD between markers in ~1,000 kb intervals on 11q11–13.4 are shown according to inter-marker distances in Table III. In the left lower part of Figure 3, marker pairs displaying significant *P* values (<0.05) on the chromosome 11q11–13.4 region are marked, and the right upper part of Figure 3 illustrates short-range LD structure in the limited interval encompassing *D11S987*. This data from short-range LD analysis suggests that LD was strongly maintained within ~400 kb and occasionally detectable up to 800–900 kb.

Patterns of long-range LD structures in chromosomes 6, 8, and 22 are graphically represented in Figure 4. The structures of LD were highly variable in individual chromosomal regions: disequilibrium occasionally extended as far as several centimorgans in some genomic regions (e.g., chromosome 18). Conversely, on chromosome 22, very little LD was detected in long-range marker pairs.

DISCUSSION

Significance of Evaluation Using Two Different Statistical Methods

Our goal in this study was to search for potential susceptibility loci on all chromosomes while attempting to minimize type I errors (false positives), even at the risk of generating type II errors (false negatives). The rationale behind this was that the detection of even one true locus would have great scientific impact, since LD regions are smaller than linkage regions, reducing the difficulty in identifying pathogenic alleles. PDT analysis was first performed in 119 mixed nuclear and large families. PDT can utilize data from all informative individuals, and is a valid test of association even when several nuclear families from a large pedigree are related [Martin et al., 2000]. PDT is therefore expected to be more powerful than other algorithms for family-based association tests such as ours. In the next step, confirmation of initial results in a statistically more stringent way was attempted by applying ETDT to the extended trio samples. This revealed two candidate loci on chromosomes 11 and 16 that could harbor risk-conferring genes for schizophrenia. Although decreasing the rate of false-positive findings is important, no widely approved guidelines for correcting *P* values from genome-wide association scans have been determined. Risch and Merikangas [1996] assumed a significance level of $\alpha = 5 \times 10^{-8}$ for independent association tests of whole human genes (100,000 in total) based on Bonferroni correction. However, correction for multiple testing should be applied based on the number of statistical tests performed and genome-wide significance levels in association mapping depend on a combination of

TABLE III. Short-Range Linkage Disequilibrium on 11q11–13.4

Marker-pairs[a]	Distance (kb)	*P*	Marker-pairs[a]	Distance (kb)	*P*	Marker-pairs[a]	Distance (kb)	*P*
4–5	11	<0.0001	14–18	265	0.04634	16–22	524	0.59512
19–20	15	<0.0001	13–17	272	0.09756	13–21	524	0.5061
15–16	36	0.0006	19–22	279	<0.0001	15–22	560	0.61524
14–15	60	0.0006	15–19	281	0.62683	20–23	577	0.00122
17–18	62	0.0031	15–20	296	0.60122	11–15	580	0.11341
13–14	68	<0.0001	22–23	313	0.91707	19–23	592	0.77195
18–19	75	0.5287	13–18	334	0.00061	11–16	617	0.03598
18–20	90	<0.0001	14–19	341	0.90488	14–22	620	0.11402
14–16	96	<0.0001	24–25	348	0.90793	12–17	625	0.28537
11–12	99	<0.0001	12–13	353	0.70976	18–23	667	0.0189
20–21	100	<0.0001	18–22	354	0.88598	12–18	687	0.49085
16–17	108	0.0031	14–20	356	0.02744	13–22	688	0.65976
19–21	115	<0.0001	16–21	360	0.98598	11–17	724	0.48476
13–15	128	<0.0001	1–2	389	<0.0001	25–26	725	0.88902
17–19	137	0.3909	15–21	396	0.32622	17–23	729	0.01463
15–17	144	0.0128	13–19	409	0.94085	12–19	762	0.64329
17–20	152	0.0872	17–22	416	0.26341	12–20	777	0.61829
21–22	164	<0.0001	12–14	422	0.125	11–18	786	0.1939
13–16	165	<0.0001	13–20	424	0.35061	22–24	787	0.25915
9–10	167	<0.0001	3–4	428	<0.0001	23–25	821	0.23476
16–18	169	0.2049	3–5	440	0.0372	16–23	836	0.02927
18–21	190	0.9146	11–13	452	0.26402	11–19	861	0.77378
14–17	204	0.0201	14–21	456	0.61768	15–23	873	0.85671
15–18	206	0.1396	26–27	472	0.08841	11–20	876	0.0311
7–8	227	0.7250	23–24	474	0.59939	12–21	877	0.53415
16–19	244	0.5092	21–23	477	0.65732	14–23	933	0.6689
17–21	252	0.7787	12–15	481	0.09817	21–24	951	0.6872
16–20	259	0.3793	12–16	518	0.81585	11–21	976	0.87073
20–22	264	<0.0001	11–14	520	0.1128	13–23	1,001	0.52195

[a]Marker numbers are shown in Table II. Marker-pairs are ordered according to inter-marker distance.
Values of $P < 0.05$ are underlined.

parameters, making correction by Bonferroni procedure inappropriate [Ophoff et al., 2002]. Applying Lander and Kruglyak's guidelines for genome-wide linkage analysis, which are based on the number of times that one would expect to see a significant result at random in a genome scan, the *P* value for transmission distortion observed with *D11S987* (ETDT $P = 0.00009$) corresponded to an LOD score of 3.33 [Schaid, 1998]. This satisfies the criteria for genome-wide significance, while the significance value for *D16S423* does not.

To test the possibility that the present transmission distortion resulted from selection due to meiotic drive, we examined transmission disequilibrium for *D11S987* using 36 unaffected sibs in our panel as non-disease trios or discordant sibs. PDT analysis provided no evidence of segregation distortion (PDT-sum, $P = 0.4569$; PDT-ave, $P = 0.1447$), although this sample size was not large.

Consideration of Marker Type and Density

To determine the appropriate marker density for an LD scan of the whole genome, it is essential to know the degree of LD maintained in a specific region of the human genome in specific populations [Kruglyak, 1999]. However, these parameters remain largely undetermined. Some debate also exists over the effectiveness of different types of markers. SNPs display several advantages over other types of polymorphisms, due to their abundance in the genome and the ability of some SNPs to directly confer disease susceptibility by affecting protein function and gene regulation. However, information derived from bi-allelic markers is limited by low heterozygosity, producing few detectable LD intervals [Goldstein, 2001; Pritchard and Przeworski, 2001]. In contrast, microsatellite markers can detect LD over longer distances [Zavattari et al., 2000]. Highly polymorphic microsatellites are therefore desirable in a primary LD screening followed by SNPs in fine mapping. The Japanese population is relatively homogeneous and displays longer LD stretches than populations that have undergone recent admixture. Recent reports have shown that strong LD is maintained over stretches of 400 ~ 500 kb in the Japanese population, and substantial LD can be detectable in 2.5 cM intervals using microsatellite markers in the X chromosome [Koch et al., 2000] and the ALDH2 locus on chromosome 12q24.2 [Katoh et al., 2002]. Theoretically, markers spaced at 5 cM intervals (each maker flanked by 2.5 cM) should therefore enable a reasonable LD genome scan in Japanese samples. According to this simulation, the design of the present study

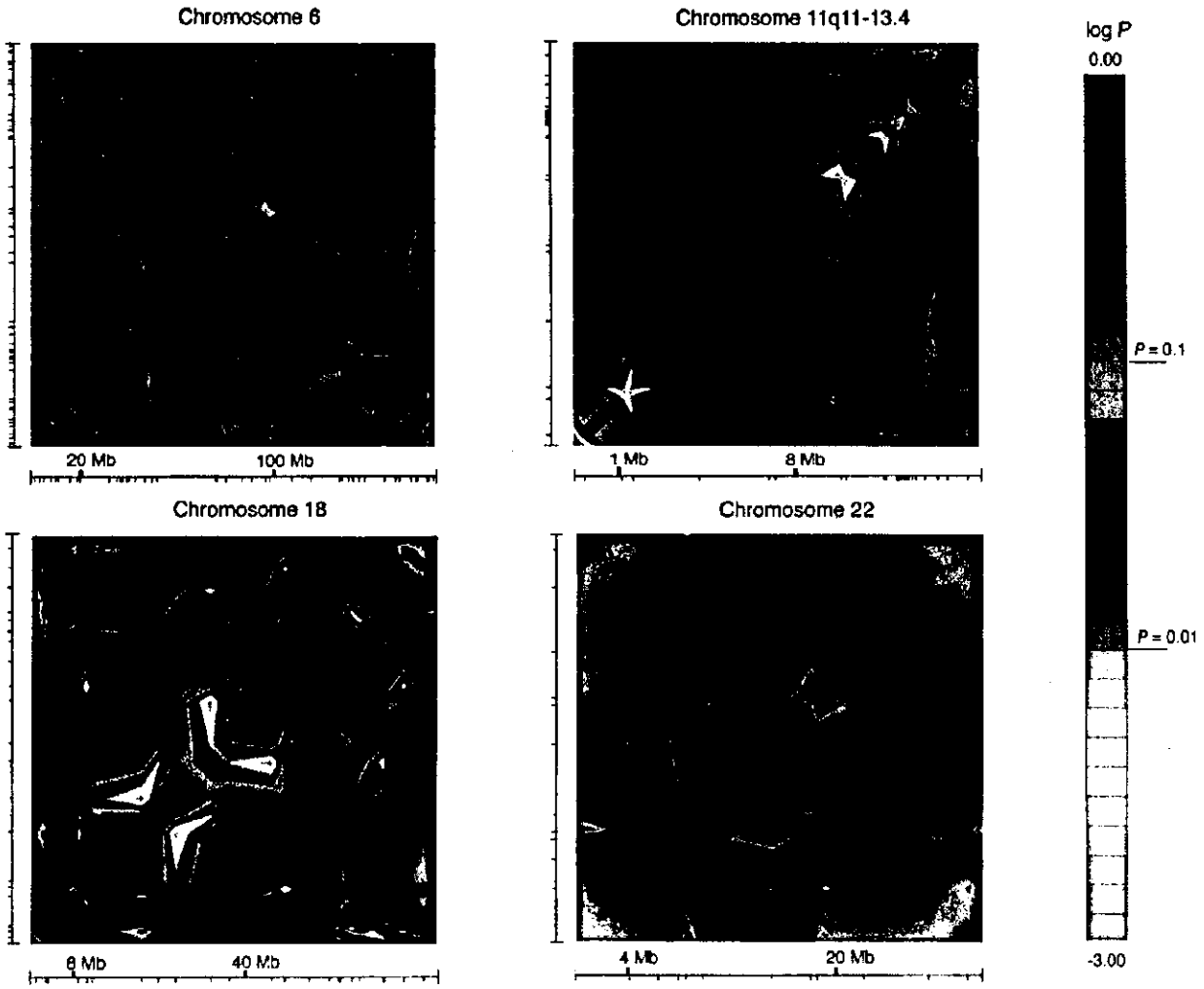


Fig. 4. Graphical representation of LD patterns observed in three chromosomes. Red, yellow, and green indicate strong, moderate, and weak disequilibrium, respectively.

assumed that more than half of the genome could be surveyed. Overall, our LD analysis of portions of the genome yielded similar results to previous studies of the Japanese population [Koch et al., 2000; Katoh et al., 2002] and suggested that genome-wide LD mapping would be feasible using microsatellite markers spacing at 1–2 cM intervals (distances between disease alleles and nearest markers within 0.5–1 cM). Information on LD structures in a population is crucial for successful LD mapping to localize the genes responsible for complex disorders. Our results should provide useful indications for LD mapping in Japanese populations.

Genes on Chromosome 11q

Our data from the initial PDT and ETDT surveys and subsequent local inspection suggest that a limited interval encompassing *D11S987* on 11q13.3 could represent a potential susceptibility locus, warranting further examination. On chromosome 11, Gurling et al. [2001] reported LOD scores ≥3.0 in schizophrenia pedigrees comprising British and Icelandic cohorts, with markers located on 11q22.1-24.3. A linkage study using a Japanese population [Nanko et al., 1992] revealed LOD scores of 1.00–1.50 with markers at 11q22.1. A recent meta-analysis of schizophrenia studies revealed chromosome q22.3-24.1 as one of the strongest candidate regions [Lewis et al., 2003]. However, these reported linkage loci are ~30 Mb or more apart from the susceptibility interval detected in the present study.

Overlapping genetic bases for schizophrenia and bipolar disorder have been proposed [Wildenauer et al., 1999; Berrettini, 2000]. Our data supports this theory with the identification of a schizophrenia susceptibility locus at 11q13.3, a region previously reported to display linkage with bipolar disorder [Detera-Wadleigh et al., 1999].

In silico analysis located three genes, Choline kinase *(CHK),CGI-85,* and *C11orf24* within the boundaries of the at-risk haplotypes around marker *D11S987. CHK,* an early enzyme in the cytidine(5′)-diphosphocholine (CDP-choline) pathway, mediates the conversion of choline to phosphorylcholine [Ishidate, 1997; Yamashita and Hosaka, 1997]. The *CHK* gene is regulated by myo-inositol and choline. CDP-choline elevates the rate of brain phospholipid synthesis. Involvement of the brain membrane phospholipid system has been suggested for a variety of neurodegenerative and psychiatric conditions [Ross et al., 1997a,b]. *CGI-85* was identified as an evolutionally conserved gene in *Caenorhabditis elegans* [Lai et al., 2000]. *C11orf24* is predicted to represent a novel membrane protein of 449 amino acids with no homology to any known proteins.

Interestingly, the gene for ciliary neurotrophic factor (*CNTF*) is located ~2 Mb centromeric to the *AFM165ZC3* marker, since haplotypes containing this marker have shown significant transmission distortion. Several association studies have examined relationships between *CNTF* and schizophrenia, but the results have been inconclusive [Thome et al., 1996; Virgos et al., 2001].

CONCLUSIONS

In summary, we conducted a family-based association scan of Japanese schizophrenic samples at mean intervals of 6–7 cM, using statistical methods to minimize type I errors. A number of potential anchor genomic loci were identified and are available for further study, particularly in the 11q region. Ophoff et al. [2002] recently suggested that LD mapping with markers spaced at a few centimorgens would be appropriate for an initial genome-wide screen of complex traits in a young population isolate. The levels of LD observed in this study conformed to their predictions. The present study should be expanded upon at increased marker resolution to pinpoint missed loci, followed by replication and confirmation tests in different sets of larger samples.

ACKNOWLEDGMENTS

We are thankful to all the patients and their families who participated in this study. We also wish to thank Dr. Joanne Meerabux for her critical review of the article.

REFERENCES

Abecasis GR, Cookson WO. 2000. GOLD—Graphical overview of linkage disequilibrium. Bioinformatics 16:182–183.

American Psychiatric Association. 1994. Diagnostic and statistical manual of mental disorders, 4th edn. Washington, DC: American Psychiatric Association Press.

Badner JA, Gershon ES. 2002. Meta-analysis of whole-genome linkage scans of bipolar and schizophrenia. Mol Psychiatry 7:405–411.

Baron M. 2001. Genetics of schizophrenia and the new millennium: Progress and pitfalls. Am J Hum Genet 68:299–312.

Berrettini WH. 2000. Susceptibility loci for bipolar disorder: Overlap with inherited vulnerability to schizophrenia. Biol Psychiatry 47:245–251.

Boehke M. 1994. Limits of resolution of genetic linkage studies: Implications for the positional cloning of human disease genes. Am J Hum Genet 55:379–390.

Clayton D. 1999. A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. Am J Hum Genet 65:1170–1177.

Clayton D, Jones H. 1999. Transmission/disequilibrium tests for extended marker haplotypes. Am J Hum Genet 65:1161–1169.

Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G, Rollins DY, Moses T, Sanders AR, Karkera JD, Esterling LE, Zeng J, Ferraro TN, Guroff JJ, Kazuba D, Maxwell ME, Nurnberger JI Jr, Gershon ES. 1999. A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. Proc Natl Acad Sci USA 96:5604–5609.

Goldstein DB. 2001. Islands of linkage disequilibrium. Nat Genet 29:109–111.

Gurling HM, Kalsi G, Brynjolfson J, Sigmundsson T, Sherrington R, Mankoo BS, Read T, Murphy P, Blaveri E, McQuillin A, Petursson H, Curtis D. 2001. Genomewide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21-22 and provides support for linkage to schizophrenia, on chromosomes 11q23.3-24 and 20q12.1-11.23. Am J Hum Genet 68:661–673.

Ishidate K. 1997. Choline/ethanolamine kinase from mammalian tissues. Biochim Biophys Acta 1348:70–78.

Katoh T, Mano S, Ikuta T, Munkhbat B, Tounai K, Ando H, Munkhtuvshin N, Imanishi T, Inoko H, Tamiya G. 2002. Genetic isolates in East Asia: A study of linkage disequilibrium in the X chromosome. Am J Hum Genet 71:395–400.

Koch HG, McClay J, Loh EW, Higuchi S, Zhao JH, Sham P, Ball D, Craig IW. 2000. Allele association studies with SSR and SNP markers at known physical distances within a 1 Mb region embracing the ALDH2 locus in the Japanese, demonstrates linkage disequilibrium extending up to 400 kb. Hum Mol Genet 9:2993–2999.

Kruglyak L. 1999. Genetic isolates: Separate but equal? Proc Natl Acad Sci USA 96:1170–1172.

Lai CH, Chou CY, Ch'ang LY, Liu CS, Lin W. 2000. Identification of novel human genes evolutionarily conserved in *Caenorhabditis elegans* by comparative proteomics. Genome Res 10:703–713.

Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, Williams NM, Schwab SG, Pulver AE, Faraone SV, Brzustowicz LM, Kaufmann CA, Garver DL, Gurling HM, Lindholm E, Coon H, Moises HW, Byerley W, Shaw SH, Mesen A, Sherrington R, O'Neill FA, Walsh D, Kendler KS, Ekelund J, Paunio T, Lonnqvist J, Peltonen L, O'Donovan MC, Owen MJ, Wildenauer DB, Maier W, Nestadt G, Blouin JL, Antonarakis SE, Mowry BJ, Silverman JM, Crowe RR, Cloninger CR, Tsuang MT, Malaspina D, Harkavy-Friedman JM, Svrakic DM, Bassett AS, Holcomb J, Kalsi G, McQuillin A, Brynjolfson J, Sigmundsson T, Petursson H, Jazin E, Zoëga T, Helgason T. 2003. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. Am J Hum Genet 73:34–48.

Martin ER, Monks SA, Warren LL, Kaplan NL. 2000. A test for linkage and association in general pedigrees: The pedigree disequilibrium test. Am J Hum Genet 67:146–154.

Martin ER, Bass MP, Kaplan NL. 2001. Correcting for a potential bias in the pedigree disequilibrium test. Am J Hum Genet 68:1065–1067.

Nanko S, Gill M, Owen M, Takazawa N, Moridaira J, Kazamatsuri H. 1992. Linkage study of schizophrenia with markers on chromosome 11 in two Japanese pedigrees. Jpn J Psychiatry Neurol 46:155–159.

O'Connell JR, Weeks DE. 1998. PedCheck: A program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63: 259–266.

Ophoff RA, Escamilla MA, Service SK, Spesny M, Meshi DB, Poon W, Molina J, Fournier E, Gallegos A, Mathews C, Neylan T, Batki SL, Roche E, Ramirez M, Silva S, De Mille MC, Dong P, Leon PE, Reus VI, Sandkuijl LA, Freimer NB. 2002. Genomewide linkage disequilibrium mapping of severe bipolar disorder in a population isolate. Am J Hum Genet 71:565–574.

Owen MJ, Cardno AG, O'Donovan MC. 2000. Psychiatric genetics: Back to the future. Mol Psychiatry 5:22–31.

Pritchard JK, Przeworski M. 2001. Linkage disequilibrium in Humans: Models and Data. Am J Hum Genet 69:1–14.

Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, Pesich R, Hebert J, Chen YD, Dzau VJ, Curb D, Olshen R, Risch N, Cox DR, Botstein D. 2001. High-throughput genotyping with single nucleotide polymorphisms. Genome Res 11:1262–1268.

Risch N, Merikangas K. 1996. The future of genetic studies of complex human diseases. Science 273:1516–1517.

Ross BM, Moszczynska A, Blusztajn JK, Sherwin A, Lozano A, Kish SJ. 1997a. Phospholipid biosynthetic enzymes in human brain. Lipids 32:351–358.

Ross BM, Hudson C, Erlich J, Warsh JJ, Kish SJ. 1997b. Increased phospholipid breakdown in schizophrenia. Evidence for the involvement of a calcium-independent phospholipase A2. Arch Gen Psychiatry 54: 487–494.

Schaid DJ. 1998. Transmission disequilibrium, family controls, and great expectations. Am J Hum Genet 63:935–941.

Schneider S, Roessli D, Excoffier L. 2000. Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Schwab SG, Hallmayer J, Lerer B, Albus M, Borrmann M, Honig S, Strauss M, Segman R, Lichtermann D, Knapp M, Trixler M, Maier W, Wildenauer DB. 1998. Support for a chromosome 18p locus conferring susceptibility to functional psychoses in families with schizophrenia, by association and linkage analysis. Am J Hum Genet 63: 1139–1152.

Schwab SG, Hallmayer J, Albus M, Lerer B, Eckstein GN, Borrmann M, Segman RH, Hanses C, Freymann J, Yakir A, Trixler M, Falkai P, Rietschel M, Maier W, Wildenauer DB. 2000. A genome-wide autosomal screen for schizophrenia susceptibility loci in 71 families with affected siblings: Support for loci on chromosome 10p and 6. Mol Psychiatry 5:638–649.

Sham PC, Curtis D. 1995. An extended transmission/disequilibrium test (TDT) for multi-allele marker loci. Ann Hum Genet 59:323–336.

Thome J, Kornhuber J, Baumer A, Rosler M, Beckmann H, Riederer P. 1996. CNTF and endogenous psychoses? Nat Genet 12:123.

Virgos C, Martorell L, Valero J, Figuera L, Civeira F, Joven J, Labad A, Vilella E. 2001. Association study of schizophrenia with polymorphisms at six candidate genes. Schizophr Res 49:65–71.

Wildenauer DB, Schwab SG, Maier W, Detera-Wadleigh SD. 1999. Do schizophrenia and affective disorder share susceptibility genes? Schizophr Res 39:107–111.

Yamashita S, Hosaka K. 1997. Choline kinase from yeast. Biochim Biophys Acta 1348:63–69.

Zavattari P, Deidda E, Whalen M, Lampis R, Mulargia A, Loddo M, Eaves I, Mastio G, Todd JA, Cucca F. 2000. Major factors influencing linkage disequilibrium by analysis of different chromosome regions in distinct populations: Demography, chromosome recombination frequency and selection. Hum Mol Genet 9:2947–2957.

Zhao JH, Sham PC, Curtis D. 1999. A program for the Monte Carlo evaluation of significance of the extended transmission/disequilibrium test. Am J Hum Genet 64:1484–1485.

BRIEF REPORT

Genetic and Expression Analyses of *FZD3* in Schizophrenia

Masayuki Ide, Tatsuyuki Muratake, Kazuo Yamada, Yoshimi Iwayama-Shigeno, Kazuya Iwamoto, Hitomi Takao, Tomoko Toyota, Naoshi Kaneko, Yoshio Minabe, Kazuhiko Nakamura, Tadafumi Kato, Norio Mori, Takashi Asada, Toshiyuki Someya, and Takeo Yoshikawa

Background: *Wnt signaling plays important roles in neurodevelopmental processes. Frizzled is a receptor of Wnt protein, and the Frizzled 3* (FZD3) *gene was recently reported to be associated with schizophrenia. Our study attempted to confirm associations between* FZD3 *and schizophrenia in Japanese family and case–control samples.*

Methods: *Genetic associations were evaluated using family-based transmission tests (212 families, 643 subjects) and case–control analysis (540 schizophrenia patients, 540 control sample). Six single nucleotide polymorphisms (SNPs) on the* FZD3 *locus were genotyped, and levels of* FZD3 *mRNA expression in postmortem brains were examined.*

Results: *Neither family- nor population-based studies supported associations between* FZD3 *and schizophrenia.* FZD3 *expression was unaltered in schizophrenic brains.*

Conclusions: *Although two prior studies have reported associations using limited numbers of SNPs on* FZD3, *our intensive study failed to support any major contribution of* FZD3 *to schizophrenia susceptibility.*

Key Words: Case–control study, family-based association study, neurodevelopment, postmortem brain, real-time quantitative RT-PCR, Wnt signaling

The pathogenesis of schizophrenia remains largely unknown, but it is hypothesized that causal events may begin in early neurodevelopment (Weinberger 1995). Wnt signaling plays important roles in neural development (Pleasure 2001), and this cascade is divided into two major pathways, canonical and noncanonical. In the canonical pathway, binding of Wnt to Frizzled receptors activates Disheveled, inhibiting glycogen synthase kinase-3β and stabilizing β-catenin. The noncanonical pathway does not depend on β-catenin, involving instead Wnt/Ca^{2+} signaling. Murine Frizzled 3 is involved in Wnt/Ca^{2+} signaling and weakly involved in the canonical pathway (Sheldahl et al 1999). The human Frizzled 3 gene (*FZD3*) is located on chromosome 8p21, a potential susceptibility region for schizophrenia (Blouin et al 1998; Gurling et al 2001).

Given the compelling functional and positional interests of *FZD3*, Yang et al (2003a) recently studied the role of the gene in schizophrenia, using three single nucleotide polymorphisms (SNPs) on *FZD3* in 246 Chinese family trios and reported preferential transmissions with all three SNPs ($p = .003–.00007$). Katsu et al (2003) reported significant case–control associations with one SNP on the gene; however, these two studies examined only limited numbers of SNPs, leaving both linkage disequilibrium (LD) structures of the locus and positions of causal genomic boundaries unknown. Our study reexamined associations of *FZD3* with schizophrenia, using denser markers and two sample panels comprising families (comparable in size to that of Yang et al [2003a]) and case–control samples (over twofold larger than those of Katsu et al [2003]). The possibility of disease-related aberrant gene expression was also examined using postmortem brains.

From the Laboratories for Molecular Psychiatry (MI, KY, YI-S, HT, TT, TY) and Molecular Dynamics of Mental Disorders (KI, TK), RIKEN Brain Science Institute, Wako, Saitama; Department of Psychiatry (MI), University of Tsukuba School of Medicine, Ibaraki; Department of Psychiatry (TM, NK, TS), Niigata University Graduate School of Medical and Dental Sciences, Niigata; Department of Psychiatry and Neurology (YM, KN, NM), Hamamatsu University School of Medicine, Hamamatsu, Japan.

Address reprint requests to Takeo Yoshikawa, M.D., Ph.D., Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan; E-mail: takeo@brain.riken.go.jp.

Methods and Materials

Subjects

Family samples comprised 212 schizophrenia families (643 members), including 168 independent and complete trios (Yamada et al 2004). Case–control samples comprised 540 unrelated schizophrenia patients (270 men, 270 women; mean age, 45.6 ± 11.0 years) and 540 age- and gender-matched control subjects who showed no history of mental illness in a brief psychiatric interview (270 men, 270 women; mean age, 45.1 ± 11.7 years). All subjects resided in central Japan. Consensus diagnosis was made by at least two psychiatrists according to the criteria of the DSM-IV. Written informed consent was obtained from all participants after explanation of study protocols and purposes. The study protocol was approved by the Ethics Committees of RIKEN and Niigata University.

Genetic Markers and Genotyping

Analyzed SNPs included the three reported by Yang et al (2003a): 435G>A (rs2241802), IVS5+5289A>G (rs2323019), and IVS5+9020*T*>C (rs352203) (Figure 1). In addition, five more SNPs were selected to cover the entire *FZD3* locus, based on the following criteria: 1) SNP on the *FZD3* genomic structure as defined by UCSC Genome Bioinformatics version July 2003 (http://genome.ucsc.edu/); 2) SNP in the National Center for Biotechnology Information database (http://www.ncbi.nih.gov), SNP consortium database (http://snp.cshl.org/), or JSNP database (http://snp.ims.u-tokyo.ac.jp/); and 3) SNP showing sufficient heterozygosity (frequency of minor allele>.1). Suitable SNPs included IVS2+595*T*>A (rs3757884), IVS3+258*T*>C (rs960914), IVS4–2244*T*>G (rs352210), IVS6+771A>G (rs352226), and Stop+1252C>A (rs352222). Assays-by-Design SNP genotyping products and TaqMan assay methods (Applied Biosystems, Foster City, California) were used to score SNPs. Genotypes were determined using an ABI7900 sequence detection system (Applied Biosystems). No TaqMan probe could be successfully designed for IVS2+595*T*>A, and Stop+1252C>A

0006-3223/04/$30.00
doi:10.1016/j.biopsych.2004.06.039

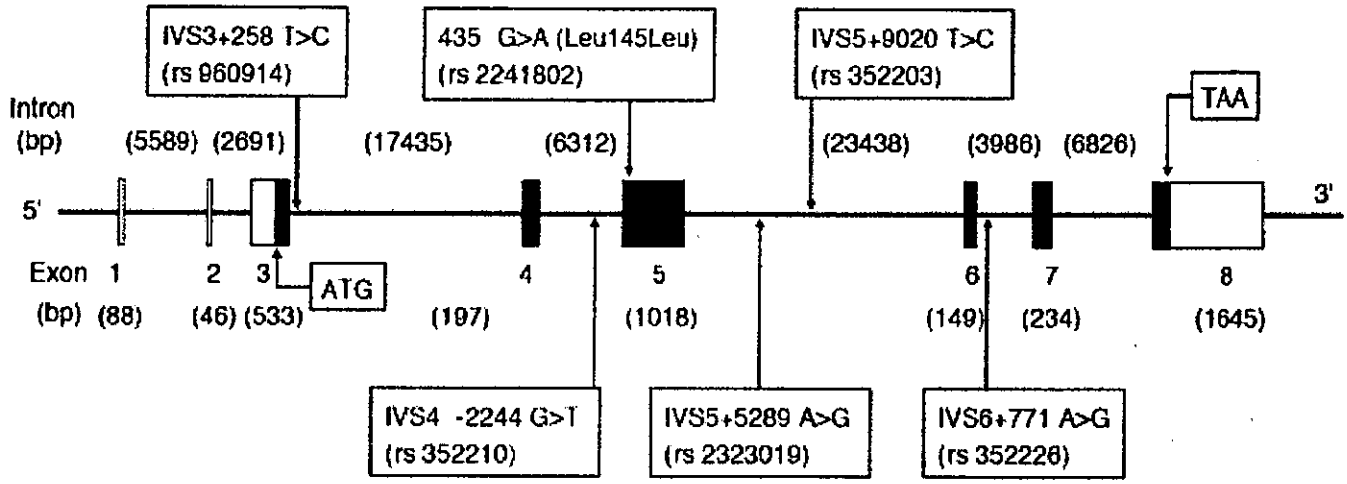


Figure 1. Genomic structure of human *FZD3* gene and locations of the six SNPs analyzed. Black boxes represent protein-coding regions, and white boxes represent untranslated regions. The rs numbers show National Center for Biotechnology Information single nucleotide polymorphism cluster IDs from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/). Locations of initiation (ATG) and stop (TAA) codons and sizes of introns and exons are also provided.

could not be genotyped unambiguously. These two SNPs were therefore removed from the study, leaving six SNPs for analysis (Figure 1).

Assessment of Sample Stratification

To assess population stratification in case–control samples, 20 SNPs and 30 microsatellite markers (marker information is available upon request) were selected from across the genome. We used Structure software (http://pritch.bsd.uchicago.edu/software.html; Pritchard et al 2000) to attempt to identify genetically similar diploid subpopulations by grouping individuals and simulating 1,000,000 replications for parameter estimation. The number of populations present in the sample (K) is unknown, so analyses were run at K = 1–5. From these results, the best estimate of K was found by calculating posterior probabilities, Pr (K = 1, 2, 3, 4, 5).

Brain Tissues and Quantitative Reverse Transcriptase Polymerase Chain Reaction

RNA from the dorsolateral prefrontal cortex (DLPFC; Brodmann's area 46) was obtained from Stanley Medical Research Institute (http://www.stanleyresearch.org/programs/brain_collection.asp). Samples were taken from 27 schizophrenia patients (20 men, 7 women; aged 42.3 ± 1.8 years; postmortem interval (PMI) 32.2 ± 3.1 hour; brain pH 6.5 ± .04) and 27 control subjects (20 men, 7 women; aged 44.6 ± 1.5 years; PMI 29.7 ± 2.6 hours; brain pH, 6.6 ± .05). Age, PMI, and brain pH were not significantly different between the two groups (p = .436, .592, and .159, respectively). All schizophrenia patients were medicated with antipsychotics. This study was unblinded.

Real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis was conducted using an ABI7900 according to the manufacturer's instructions. TaqMan probes and primers for *FZD3* and β2-microglobulin (internal control) were from Assay-on-Demand gene expression products (Applied Biosystems). All qRT-PCR reactions were performed in triplicate, based on standard curve methods described by the manufacturer.

Statistical Analyses

Transmission distortions in the family panel were evaluated using the pedigree disequilibrium test (Martin et al 2000; http://www.chg.duke.edu/software/pdt.html) and extended transmission disequilibrium test (Sham and Curtis 1995; http://www.mds.qmw.ac.uk/statgen/dcurtis/software.html). Transmit software (Clayton 1999; http://watson.hgen.pitt.edu/docs/transmit.html) was run as a global test of haplotype transmission. Genetic Power Calculator (Purcell et al 2003; http://statgen.iop.kcl.ac.uk/gpc/) was used to compute statistical power. In the case-control study, haplotype frequencies, normalized LD coefficient D' and squared correlation coefficient r^2 were calculated using Cocaphase software (Dudbridge 2003; http://www.hgmp.mrc.ac.uk/~fdudbrid/software/). Allelic and genotypic frequencies of markers between patients and control subjects were assessed

Table 1. Results of Family-Based and Case–Control Association Studies Between *FZD3* and Schizophrenia

	Minor Allele Frequency		PDT p Value		ETDT	Case–Control p Value		3 SNP-Based Haplotype p Value			
SNP	Control	Schizophrenia	AVE	SUM	p Value	Allele	Genotype	Family-Based/Case–Control			
IVS3+258T>C	.39	.41	.985	.949	.632	.568	.096				
IVS4−2244G>T	.39	.40	.864	.899	.687	.659	.101	.758/.888			
435G > A (Leu145Leu)	.45	.47	.804	.756	.399	.388	.133		.879/.865		
IVS5+5289A>G	.41	.42	.969	1.00	.605	.597	.263			.914/.884	
IVS5+9020T>C	.39	.40	.909	.843	.544	.723	.103				.659/.901
IVS6+771A>G	.39	.40	.985	.949	.670	.536	.064				

EDT, extended transmission disequilibrium test; PDT, pedigree disequilibrium test; SNP, single nucleotide polymorphism.