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A Promoter Haplotype of the Inositol Monophosphatase 2 Gene (*IMPA2*) at 18p11.2 Confers a Possible Risk for Bipolar Disorder by Enhancing Transcription

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Lithium is an effective mood stabilizer for bipolar disorder patients and its therapeutic effect may involve inhibition of inositol monophosphatase activity. In humans, the enzyme is encoded by two genes, *IMPA1* and *IMPA2*. *IMPA2* maps to 18p11.2, a genomic interval for which evidence of linkage to bipolar disorder has been supported by several reports. We performed a genetic association study in Japanese cohorts (496 patients with bipolar disorder and 543 control subjects). Interestingly, we observed association of *IMPA2* promoter single nucleotide polymorphisms (SNPs) (−461C and −207T) with bipolar disorder, the identical SNPs reported previously in a different population. *In vitro* promoter assay and genetic haplotype analysis showed that the combination of (−461C)–(−207T)–(−185A) drove enhanced transcription and the haplotypes containing (−461C)–(−207T)–(−185A) contributed to risk for bipolar disorder. Expression study on post-mortem brains revealed increased transcription from the *IMPA2* allele that harbored (−461C)–(−207T)–(−185A) in the frontal cortex of bipolar disorder patients. The examination of allele-specific expressions in post-mortem brains did not support genomic imprinting of *IMPA2*, which was suggested nearby genomic locus. Contrasting to a prior report, therapeutic concentrations of lithium could not suppress the transcription of *IMPA2* mRNA, and the mood-stabilizing effect of lithium is, if IMPA2 was one of the targets of lithium, deemed to be generated via inhibition of enzymatic reaction rather than transcriptional suppression. In conclusion, the present study suggests that a promoter haplotype of *IMPA2* possibly contributes to risk for bipolar disorder by elevating *IMPA2* levels in the brain, albeit the genetic effect varies among populations.
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INTRODUCTION

Bipolar disorder is a major functional psychiatric illness along with schizophrenia, with a lifetime prevalence of ~1%. It is characterized by recurrent episodes of mania and depression with chronic course (Evans *et al*, 2005). Although pathological mechanisms for bipolar disorder remain largely elusive, the role of genetic factors in the etiology and the therapeutic efficacy of lithium have been established (Dinan, 2002; Gould *et al*, 2004). Thus, genetic analysis of molecular components of pathways perturbed by lithium is a plausible strategy to gain insight into the pathophysiology of bipolar disorder.

The 'inositol-depletion hypothesis' has been proposed to explain the cellular action of lithium (Berridge *et al*, 1989; Gould *et al*, 2004; Gurvich and Klein, 2002; Harwood, 2005; Williams *et al*, 2002). Recently identified neuroprotective

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effect of lithium may be mediated also through inositol depletion (Williams *et al*, 2002). Several lines of evidence show that altered activity of inositol monophosphatase (IMPase) may be involved in the pathophysiology of bipolar disorder. IMPase (EC 3.1.3.25) catalyzes the release of the phosphate group from inositol monophosphate, an important step for the regeneration of free inositol. Reduced mRNA expression of *IMPA2* in lymphocytes (Nemanov *et al*, 1999) or lymphoblastoid cells (Yoon *et al*, 2001), and decreased concentration of inositol (Belmaker *et al*, 2002) or reduced IMPase activity (Shamir *et al*, 1998; Shaltiel *et al*, 2001) in lymphoblastoid cells from bipolar disorder patients have been reported.

We previously cloned the inositol(*myo*)-1(or 4)-monophosphatase 2 gene (*IMPA2*) (Yoshikawa *et al*, 1997, 2000). *IMPA2* has been targeted for genetic studies of bipolar disorder (Dimitrova *et al*, 2005; Sjøholt *et al*, 2004; Yoshikawa *et al*, 2001), because the gene is located on 18p11.2 (Yoshikawa *et al*, 1997), a region that has been highlighted in several linkage studies (Berrettini *et al*, 1994; Detera-Wadleigh *et al*, 1999) and in a meta-analysis of genome scans for bipolar disorder (Segurado *et al*, 2003).

Sjøholt *et al* (2004) detected association between *IMPA2* promoter single nucleotide polymorphisms (SNPs) and bipolar disorder in 92 Palestinian Arab trios. *IMPA2* is among tempting candidate genes in bipolar disorder based on convergent evidence from pharmacological, biochemical, linkage, and association analyses.

The major aims of present study are to (1) test whether previously reported association between promoter polymorphisms of *IMPA2* and bipolar disorder can be replicated in large Japanese case–control cohorts, (2) examine which promoter haplotypes of *IMPA2* are relevant to genetic predisposition and transcriptional regulation, and (3) assess whether the expression level of *IMPA2* is altered in postmortem brains of bipolar disorder patients.

Here, we report support for previous findings on association between bipolar disorder and the promoter SNPs of *IMPA2* in a different ethnic population, and that these SNPs exert effect on transcriptional activity both *in vitro* and *IMPA2* transcript levels in bipolar disorder postmortem brains. These results warrant further inspection of the inositol hypothesis of bipolar disorder.

MATERIALS AND METHODS

Patients and Controls

A group of unrelated Japanese bipolar patients (242 men and 254 women) consisting of both bipolar I (162 men and 170 women) and bipolar II (80 men and 84 women), and age- and gender-matched controls (259 men and 284 women) were recruited through the COSMO (Collaborative Study of Mood Disorders) consortium in Japan (Kunugi *et al*, 2004; Munakata *et al*, 2004). Each institute provided both case and control samples matched for gender, age, and geographic area. The mean ($\pm$SD) age of patients was 49.9 ± 14.6 years, and that of controls was 49.9 ± 11.7 years. Included in the analysis were 570 schizophrenics and the same number of age- and gender-matched controls (Yamada *et al*, 2005). All subjects in this panel resided in central Japan. None of the schizophrenia patients had additional Axis-I disorders as defined by DSM-IV. Consensus diagnosis of bipolar disorder and schizophrenia was made according to criteria from DSM-IV by at least two experienced psychiatrists, on the basis of unstructured interviews, available medical records, and information from hospital staff and relatives. Control subjects were healthy volunteers who had neither current nor past contact with psychiatric services, and showed good social functioning. The control subjects were recruited from hospital staff, their associates, and company employees. They underwent psychiatric interviews at each institute, either in an unstructured manner or using a structured instrument (SCID). The present study was approved by the ethics committees of all participating institutes. All controls and patients gave informed written consent to participate in the study, after provision and explanation of study protocols and objectives.

SNP Genotyping of *IMPA2*

Genomic DNA was isolated from blood samples using standard methods. *IMPA2* has a TATA-less and GC-rich promoter and the initiation codon is located in exon 1 (Yoshikawa *et al*, 2000). SNPs that were analyzed were chosen from those detected in our prior genomic screening (Yoshikawa *et al*, 2001) and in the Sjøholt *et al* (2004) report, and from databases, for example, NCBI (http://www.ncbi.nlm.nih.gov/) and The International HapMap Project database (http://www.hapmap.org/index.html.ja) (Altshuler *et al*, 2005). Genotyping was performed using the TaqMan system (Applied Biosystems, Foster City, CA), except for $-461C>T$, $-241\_237InsGGGCT$, $-207T>C$, and $-185A>G$. Probes and primers were designed using Assays-by-Design™ SNP genotyping (Applied Biosystems, Foster City, CA). PCR were carried out in an ABI 9700 thermocycler, and fluorescence was determined using an ABI 7900 sequence detector single point measurement and SDS v2.2 software (Applied Biosystems). The polymorphisms, $-461C>T$, $-241\_237InsGGGCT$, $-207T>C$, and $-185A>G$ were genotyped by direct sequencing using the BigDye Terminator Cycle Sequencing FS Ready Reaction kit (Applied Biosystems) and the ABI PRISM 3730 Genetic Analyzer (Applied Biosystems).

Statistical Analyses for Genetic Association

Deviations from Hardy–Weinberg equilibrium were computed using the Arlequin program (http://lgb.unige.ch/arlequin/) (Schneider *et al*, 2000). Allelic and genotypic frequencies of markers between patients and controls were assessed using Fisher's exact test. To determine the haplotype block structure in the region, we used the genotype data from all the samples ($n = 1039$) and Haploview program (http://www.broad.mit.edu/mpg/haploview/) (Barrett *et al*, 2005). Haplotype frequencies were computed using the expectation-maximization algorithm implemented in COCAPHASE ver2.35 (http://www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/) (Dudbridge, 2003). We used the program option, -zero 0.01. Haplotypes with a frequency below 1% were trimmed to zero, and assumed to be absent in the population when calculating degrees of freedom. Haplotype distributions were also evaluated using

the COCAPHASE program. Empirical significance levels were simulated from 10 000 Monte Carlo permutations using the COCAPHASE program.

For population homogeneity assessment of the bipolar and control samples, 20 genome-wide SNPs were selected randomly from the databases described above (Supplementary Table S1). The structure software (http://pritch.bsd.uchicago.edu/software.html) (Pritchard *et al*, 2000) was used to attempt to identify genetically similar diploid subpopulations by grouping individuals. In the application of this Markov chain Monte Carlo method, 1 000 000 replications were used for the burn-in period of the chain and for parameter estimation. The number of populations present in the sample (K) was unknown, so analysis was run at $K = 1$, 2, 3, 4, and 5. From these results, best estimate of K was found by calculating posterior probabilities, Pr ($K = 1$, 2, 3, 4, or 5), as described by Pritchard *et al* (2000).

Plasmid Constructs for *IMPA2* Promoter Assay

For *IMPA2* promoter assay, we prepared constructs in pGL3-basic (Promega, Madison, WI) by amplifying genomic DNA with the following primers: FW, 5′-AGT GACGCGTGTGAAGAGTTTATAAAGTCCAGCC (3′ end at nt −1185, A of the initiation codon ATG as +1); and RV, 5′-AGTGAAGATCTTGCGCTGGCGGGAAGGGCA (3′ end at nt −163). The amplicons from genomic DNA from subjects harboring appropriate haplotypes were directly cloned into the *Ml*uI/*Bg*lII site of pGL3-basic vector. The structure of each construct was verified by sequencing. pRL-TK (Promega) was used as an internal control reporter vector. The vector pGL3-control containing the SV40 promoter was used as a positive control.

Cell Culture, Transfection, and Luciferase Assay

The neuroblastoma cell line NB-1 was purchased from the Japanese Collection of Research Bioresources (Tokyo, Japan). HeLa TetOff cell line was from Clontech (Mountain View, CA). NB-1 was cultured in Eagle's minimum essential medium (Sigma, St Louis, MO) and RPMI1640 (Sigma) (1:1), supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA). HeLa TetOff was cultured in Dulbecco's modified Eagle's medium (Sigma), supplemented with 10% fetal bovine serum (Invitrogen). These cell lines were grown in 10 cm culture dishes and passaged at 60–70% confluence ($1–3 \times 10^5$ cells/well) onto a 24-well plate, 1 day before transfection. Transfections were performed using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. One microgram of plasmid DNA (reporter: internal reporter = 9:1) and 2 ml of Lipofectamine 2000 were mixed in 100 μl of OPTI-MEM (Sigma). After a 20-min incubation, 500 μl of OPTI-MEM was added to individual wells and the Lipofectamine 2000/plasmid mixture was then added to each well containing cells. The plate was placed in a CO_2 incubator at 37°C. Five hours after transfection, medium was replaced with fresh medium with or without the indicated concentration of lithium chloride. The transcriptional assay was performed 48 h after transfection using the PicaGene Dual SeaPansy kit (Toyo Ink, Tokyo, Japan). Transfected cells were washed with PBS and incubated in 100 μl of cell lysis buffer for 15 min at room temperature with shaking. Next, 100 μl of luciferase assay reagent was added to each cell lysate. The dual luciferase assay was carried out using a Lumat LB 9507 (EG&G Berthold, Bad Wildbad, Germany).

To examine the effect of lithium on the promoter activity of *IMPA2*, experiments were performed as follows: 5 h after transfection with one of test constructs in combination with an internal control vector, cells were treated with or without indicated concentration of lithium chloride in normal growth medium. The dual luciferase assay was performed 48 h after transfection as described above.

All luciferase assays were performed as three independent transfections, with each transfection performed in duplicate. We used the Fisher's PSLD test to evaluate transcriptional differences among multiple groups.

cDNA Preparation from Post-Mortem Brains

Post-mortem brain total RNA samples extracted from Brodmann's area (BA) 46 (dorsolateral prefrontal cortex: DLPFC) was obtained from the Stanley Foundation brain collection (http://www.stanleyresearch.org/programs/brain_collection.asp). Samples were taken from 33 bipolar disorder patients (16 men, 17 women; mean ± SD age, 45.5 ± 10.8 years; PMI (post-mortem interval) ± SD, 36.4 ± 17.7 h; brain pH ± SD, 6.4 ± 0.3), and 34 controls (25 men, nine women; mean ± SD age, 44.1 ± 7.7 years; PMI ± SD, 29.6 ± 13.0 h; brain pH ± SD, 6.6 ± 0.3) (Supplementary Table S2). Diagnoses were made according to the DSM-IV. There were no significant demographic differences between the bipolar and control brains (Torrey *et al*, 2000). This study was performed unblinded. Single-stranded cDNA was synthesized from 3 μg of total RNA samples by Reverscript II (Wako Pure Chemical Industries, Tokyo, Japan) and oligo(dT) primers (Roche Applied Science, Indianapolis, IN).

Real-Time Quantitative RT-PCR

mRNA levels were determined by real-time quantitative RT-PCR, using TaqMan universal PCR mastermix, transcript-specific minor groove binding (MGB) probes (Assays-on-Demand, Applied Biosystems) and an ABI 7900 sequence detection system, as described before (Aoki-Suzuki *et al*, 2005). The MGB probe for *IMPA1* was derived from exons 7 and 8, and that for *IMPA2* from exons 3 and 4. We used three different internal control probes for the assessment of *IMPA1* and *IMPA2* expression in the brain: glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), beta-actin (*ACTB*), and phosphoglycerate kinase 1 (*PGK1*). The PCR assay were performed simultaneously with test and standard samples and no template controls in the same plate. A standard curve plotting the cycle of threshold values against input quantity (log scale) was constructed for both the control genes and the target molecules (*IMPA1* and *IMPA2*) for each PCR assay. All real-time quantitative PCR data was captured using the SDS v2.2 (Applied Biosystems). The ratio of the relative concentration of the target molecule to each internal control gene was calculated. We used the Mann–Whitney *U*-test (two-tailed) to detect significant changes in the target gene expression levels.

Detection of Allele-Specific Expression of *IMPA2*

To deduce the effects of promoter SNPs on *in vivo* mRNA expression of *IMPA2*, and also to evaluate the possibility of allele-specific expression, we selected the brain samples whose genotypes were heterozygous on −185A > G SNP site. This SNP had the most upstream location on the cDNA for which genotypes could be determined. The more upstream SNP −207T > C SNP could not be genotyped in cDNA samples because of inability to design suitable primers for amplifying cDNA. cDNA from the heterozygous brain samples were amplified using a forward primer, 5′-GGGGAGCGGAAAGCAGGACG (3′ end at nt −187), a reverse primer, 5′-GCCTGGAAGCACTCCTCCCAG (3′ end at nt +65 from the A), and Pwo SuperYield DNA polymerase (Roche Applied Science). PCR conditions were as follows: initial denaturation at 98°C for 5 min, then 35 cycles at 98°C for 45 s and 68°C for 1 min, and a final extension at 68°C for 10 min. PCR products were cloned into pCRII TOPO vector (Invitrogen), and 80 clones on average from each brain sample were picked up and sequenced as described above. The expression ratio of −185A:−185G alleles was evaluated by the Mann–Whitney *U*-test (two-tailed). The exact haplotypes constructed by (−461C > T)–(−207T > C)–(−185A > G) in the '(−185)-heterozygous' brain samples were determined by PCR on genomic DNA using a forward primer, 5′-TCGAGGCTCA-GAGGAGTTGGAG (3′ end at nt −636), and a reverse primer, 5′-TCCCGCAGCTCTGTGCCTAGT (3′ end at nt −125), followed by subcloning of amplicons into pCRII TOPO vector (Invitrogen) and sequencing.

RESULTS

LD Block Structure of *IMPA2*

We evaluated a total of 26 polymorphisms for genotyping accuracy and heterozygosity, and selected 19 SNPs for subsequent systematic genetic analyses (SNP01 to SNP19 in Figure 1a and Supplementary Table S3). The minor allele frequencies of −241_237InsGGGCT were 1% in both bipolar

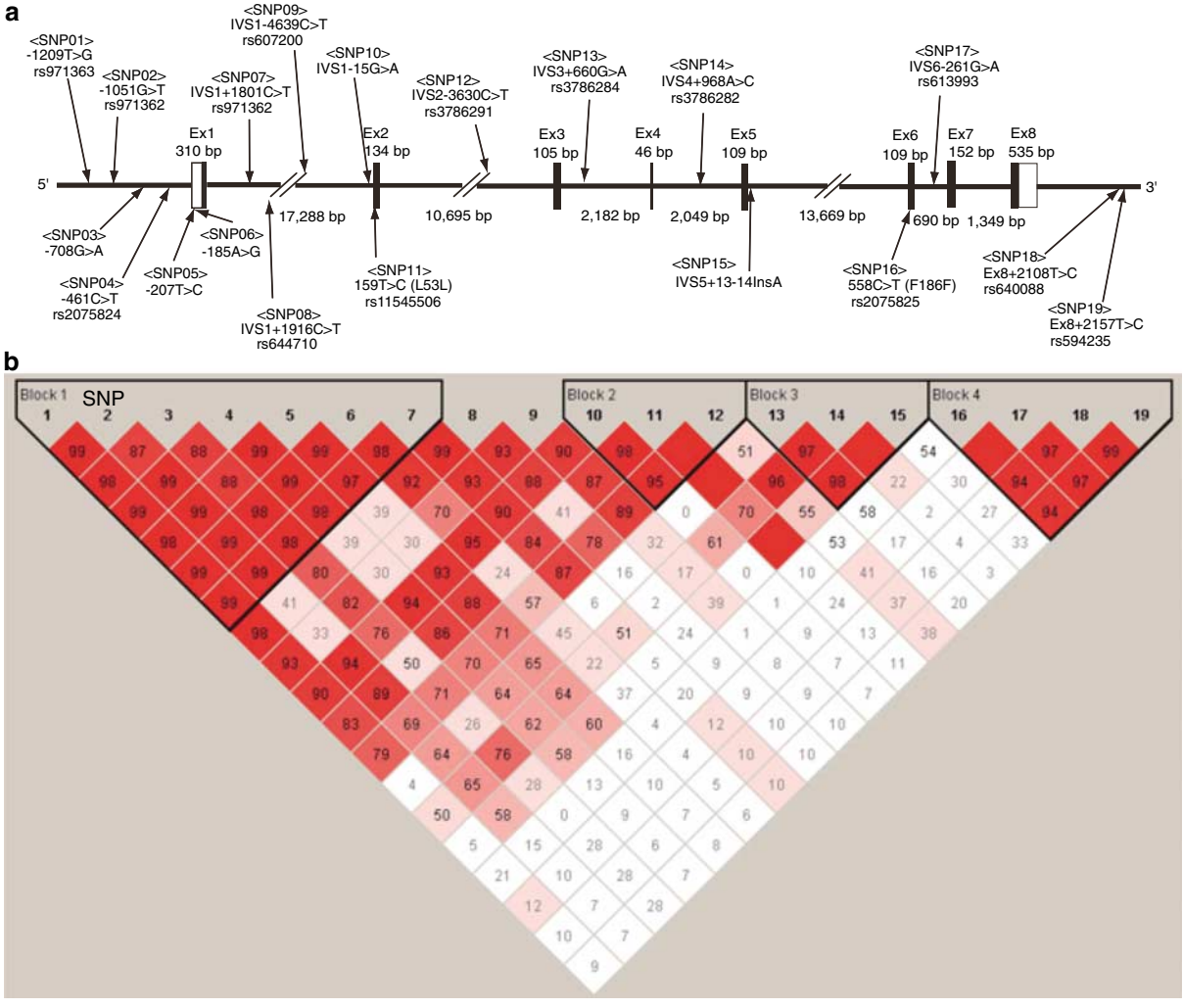

Figure 1 (a) Genomic structure and location of polymorphic sites for *IMPA2*. Exons are denoted by boxes, with untranslated regions in white and translated regions in black. The sizes of exons and introns are also shown. (b) LD block organization of *IMPA2* is in Japanese. The LD block pattern was constructed using Haploview program using the genotype data from both case and control samples (1039 subjects). The number in each cell represents the LD parameter D' (× 100), blank cells mean $D' = 1$. Each cell color is graduated relative to the strength of LD between markers, which is defined by both D' value and confidence bounds on D'.

($n=496$) and control ($n=543$) groups and were not included in the analysis. Sjøholt *et al* (2000) identified possible two tandemly organized inositol/choline responsive element (ICRE)-like sequences separated by 34 bp and approximately 1.5 kb upstream of the ATG codon of *IMPA2*: 5′-ATTTTACGTGG (sense direction) and 5′-ATTTCA TAAGC (antisense direction). However, no polymorphisms were detected in these potentially interesting sequences in 48 subjects.

Pairwise marker LD statistics were determined by using the Haploview program (Barrett *et al*, 2005). LD blocks were generated based on 95% confidence bounds on D', employing the Gabriel *et al* (2002) algorithm. Here, a parameter of LD, D' is D normalized against the maximum value of D possible, given allele frequencies PA (at locus A) and PB (at locus B), $D=\mathrm{PAB}-(\mathrm{PA}\times\mathrm{PB})$ (PAB is the expected haplotype frequency). In the Japanese samples, four LD blocks were evident for *IMPA2* genomic region (Figure 1b).

Genetic Association Analysis between *IMPA2* and Mental Disorders

Allelic and genotypic frequencies of 19 SNPs on *IMPA2* in the bipolar and control groups are summarized in Table 1. Allelic distributions of SNP04 ($-461\mathrm{C}>\mathrm{T}$) and SNP05 ($-207\mathrm{T}>\mathrm{C}$) differed significantly, albeit modest, between bipolar patients and controls, with the modest overrepresentation of -461C (odds ratio = 1.199) and -207T (odds ratio = 1.196) alleles in the disease group. Although *P*-values did not survive correction for multiple tests, these results are consistent with those by Sjøholt *et al* (2004), who reported significantly preferential transmission of only these two alleles from parents to affected offspring (Supplementary Table S3). SNP16 ($558\mathrm{C}>\mathrm{T}$) displayed marginally significant genotypic association with bipolar disorder. However, genotype distributions of this SNP and other SNPs in LD block 4 (Table1 and Figure 1b) showed departure from Hardy–Weinberg equilibrium, thereby necessitating caution in the interpretation of association.

Exploratory sliding haplotype analysis was performed by implementing two- and three-locus windows. Sequential haplotypes from SNP02 through SNP05 within LD block 1, displayed evidence of association with bipolar illness (Table 2), rendering the interval from SNP02 and SNP05 a minimum essential region for further functional inspection (see next section). As the haplotypes in each window are not independent of each other, we performed 10 000 permutations using the data to compute empirical *P*-values for haplotypic associations, where all SNPs and all haplotypes in each window were taken into account. We obtained a significant empirical $P=0.0493$ for the two-locus window (sliding between SNP01 and SNP05), a significant $P=0.0479$ for the three-locus window (sliding between SNP01 and SNP05), and a significant $P=0.0405$ for the four-locus window (SNP02–SNP05).

One of the causes of spurious/unreplicated findings in population-based association studies is sample stratification owing to population admixture. To exclude this possibility, we genotyped 20 SNPs (Supplementary Table S1) randomly located throughout the genome. No evidence for stratification was identified in our bipolar and control samples, with a $Pr\ (K=1)>0.99$. We are currently pursuing this issue, by examining ~1500 SNPs and have so far found no evidence of stratification in our Japanese samples (in preparation).

We have reported previously genetic association of three *IMPA2* SNPs with schizophrenia ($n=302$) (Yoshikawa *et al*, 2001), SNP06, SNP10, and SNP 16 (Supplementary Table S3). In the present study, association was tested on an expanded sample set (570 schizophrenics and 570 age-/gender-matched controls) devoid of population stratification (Yamada *et al*, 2005). Modest nominal genotypic association of SNP10 (IVS1-15G>A) with schizophrenia ($P=0.0376$, Supplementary Table S4) was detected. In addition, association with febrile seizure with *IMPA2* SNPs 11 and 15 has been reported (Nakayama *et al*, 2004). These results leave the possibility of allelic heterogeneity in *IMPA2* among variable diagnostic categories, although the functional significances of these SNPs are unknown.

IMPA2 Promoter Analysis and Haplotype Analysis

Results from our genetic analysis and a prior study (Sjøholt *et al*, 2004) suggest that a bipolar risk-conferring haplotype(s) or SNP(s) may reside within LD block 1 that includes the promoter region. Therefore, we set out to evaluate the functional roles of promoter SNPs, by performing reporter gene assay using NB-1 and HeLa TetOff cells. We generated constructs covering the 1.1 kb 5′-upstream region of *IMPA2*, which contained SNP02–SNP05 sites that displayed haplotypic association in a sliding manner, plus SNP06 site (Figure 2a). SNP06 site was included because it showed marginal association ($P=0.05$) in the study of Sjøholt *et al* (2004). The four haplotypes shown in Table 3 accounted for naturally occurring haplotypes composed of SNP02-03-04-05-06. Four different plasmid constructs reflecting existing haplotypes for *in vitro* transcription assay (Figure 2a) were prepared. In the neuroblastoma NB-1 cell line, the combination of C at SNP04 site and T at SNP05 site, alleles that were more frequent in bipolar samples, elicited a trend of elevated transcriptional activity compared to the reverse T (SNP04)-C (SNP05) constructs (T-A-T-C-G/T-G-T-C-G *vs* G-G-C-T-G), suggesting that the haplotypes constructed by bipolar-associated alleles may result in enhanced promoter activity in the brain (Figure 2b). In the two plasmid constructs carrying C (SNP04)-T (SNP05), the one with A at SNP06 site displayed significantly higher luciferase activity than the construct with the alternative allele G at SNP06. In HeLa TetOff cells, the construct of C-T-A (SNP04-05-06) also showed the highest transcriptional drive (Figure 2c). These *in vitro* data suggested a functional impact of haplotypes with C (SNP04)-T (SNP05) and in particular C (SNP04)-T (SNP05)-A (SNP06).

There were no promoter haplotypes spanning SNP02–SNP06 that were significantly associated with bipolar disorder (Table 3), but the haplotypes containing C (SNP04)-T (SNP05) gave odds ratios slightly greater than 1, suggesting that this combination of alleles constitutes a putative 'basic' risk haplotype. Both alleles at SNP06 were on this putative 'basic' risk haplotype (Table 3), with A giving higher odds ratio than G.

Table 1 Allelic and Genotypic Distributions of *IMPA2* Polymorphisms

Polymorphism	Sample	*n*	Allele counts		*P*-value	Genotype counts			*P*-value	HWE	Minor allele frequency
			T	G		T/T	T/G	G/G			
SNP01	Bipolar	488	664	312	0.479	230	204	54	0.447	0.390	0.320
−1209T>G	Control	534	710	358		233	244	57		0.560	0.335
			G	T		G/G	T/G	T/T			
SNP02	Bipolar	488	531	445	0.143	141	249	98	0.304	0.529	0.456
−1051G>T	Control	534	546	522		133	280	121		0.255	0.489
			G	A		G/G	A/G	A/A			
SNP03	Bipolar	486	829	143	0.462	352	125	9	0.511	0.583	0.147
−708G>A	Control	537	903	171		382	139	16		0.442	0.159
			C	T		C/C	T/C	T/T			
SNP04	Bipolar	487	531	443	**0.042**	141	249	97	0.118	0.494	0.455
−461C>T	Control	536	536	536		130	276	130		0.490	0.500
			T	C		T/T	T/C	C/C			
SNP05	Bipolar	489	538	440	**0.046**	141	256	92	0.114	0.202	0.450
−207T>C	Control	537	543	531		132	279	126		0.363	0.494
			G	A		G/G	G/A	A/A			
SNP06	Bipolar	489	614	364	0.197	190	234	65	0.184	0.596	0.372
−185G>A	Control	538	705	371		238	229	71		0.179	0.345
			C	T		C/C	T/C	T/T			
SNP07	Bipolar	492	675	309	0.423	234	207	51	0.661	0.603	0.314
IVS1+1801C>T	Control	539	721	357		241	239	59		0.982	0.331
			T	C		T/T	T/C	C/C			
SNP08	Bipolar	492	496	488	0.965	126	244	122	0.503	0.858	0.496
IVS1+1916T>C	Control	538	541	535		147	247	144		0.058	0.497
			T	C		T/T	T/C	C/C			
SNP09	Bipolar	494	528	460	0.508	144	240	110	0.756	0.598	0.466
IVS1−4639T>C	Control	540	593	487		169	255	116		0.281	0.451
			G	A		G/G	A/G	A/A			
SNP10	Bipolar	492	682	302	0.157	239	204	49	0.339	0.573	0.307
IVS1−15G>A	Control	538	714	362		237	240	61		0.984	0.336
			T	C		T/T	T/C	C/C			
SNP11	Bipolar	490	753	227	0.713	293	167	30	0.263	0.346	0.232
159T>C	Control	541	839	243		320	199	22		0.192	0.225
			C	T		C/C	T/C	T/T			
SNP12	Bipolar	488	849	127	0.519	368	113	7	0.347	0.614	0.130
IVS2−3630C>T	Control	539	927	151		403	121	15		0.114	0.140
			G	A		G/G	A/G	A/A			
SNP13	Bipolar	485	853	117	0.630	375	103	7	0.600	0.981	0.121
IVS3+660G>A	Control	532	943	121		415	113	4		0.215	0.114
			C	A		C/C	A/C	A/A			
SNP14	Bipolar	486	601	371	0.615	189	223	74	0.865	0.539	0.382
IVS4+968C>A	Control	537	676	398		215	246	76		0.677	0.371
			Del	Ins		Del/Del	Ins/Del	Ins/Ins			
SNP15	Bipolar	486	860	112	0.394	379	102	5	0.407	0.518	0.115
IVS5+13−14InsA	Control	532	954	110		424	106	2		0.085	0.103
			C	T		C/C	T/C	T/T			
SNP16	Bipolar	491	871	111	1.000	393	85	13	**0.049**	0.002	0.113
558C>T	Control	534	947	121		418	111	5		0.424	0.113
			G	A		G/G	A/G	A/A			
SNP17	Bipolar	492	717	267	0.523	262	193	37	0.348	0.860	0.271
IVS6−261G>A	Control	541	774	308		287	200	54		0.032	0.285

Table 1 Continued

Polymorphism	Sample	*n*	Allele counts		*P*-value	Genotype counts			*P*-value	HWE	Minor allele frequency
			T	C		T/T	T/C	C/C			
SNP18	Bipolar	494	710	278	0.627	257	196	41	0.493	0.674	0.281
Ex8+2108T>C	Control	542	768	316		282	204	56		0.039	0.292
			T	C		T/T	T/C	C/C			
SNP19	Bipolar	486	697	275	0.591	252	193	41	0.510	0.639	0.283
Ex8+2157T>C	Control	534	754	314		276	202	56		0.040	0.294

Significant *P*-values are shown in bold type. HWE, *P*-value for Hardy–Weinberg equilibrium.

Table 2 Results of Two and Three SNP-Based Haplotype Analyses

	SNP01	SNP02	SNP03	SNP04	SNP05	SNP06	SNP07	SNP08	SNP09	SNP10	SNP11	SNP12	SNP13	SNP14	SNP15	SNP16	SNP17	SNP18	SNP19
2SNPs	0.206	**0.022**	**0.017**	**0.033**	0.110	0.431	0.473	0.405	0.592	0.321	0.763	0.625	0.570	0.508	0.684	0.795	0.723	0.625	
3SNPs	0.087	**0.030**	**0.015**	0.100		0.132	0.234	0.498	0.332	0.649	0.382	0.748	0.752	0.572	0.606	0.893	0.923	0.890	

Significant global *P*-values are shown in bold type.

Examination of Lithium Effect on *IMPA2* Promoter Activity

Recently, Seelan *et al* (2004) reported that addition of therapeutic concentration of lithium chloride (1 mM) in HeLa cells produced downregulation of the promoter activity of *IMPA2*, and they speculated that this action might be evoked through several 'negative lithium regulatory region homologies' located in the promoter region of *IMPA2* (Figure 2a). A putative lithium responsive region was originally reported by Wang *et al* (2001) in the mouse *Macs* gene encoding the MARCKS (myristoylated alanine-rich C kinase substrate) protein. Therefore, we examined whether the findings of Seelan *et al* (2004) could be replicated in our assay system using *IMPA2* promoter constructs shown in Figure 2a, which represented naturally occurring haplotypes. As shown in Table 4, no suppression of promoter activity was seen in the presence of a therapeutic concentration of lithium ions (1 mM), in any of the constructs tested. In control constructs carrying the SV40 promoter, 5 mM LiCl displayed an inhibitory effect ($P<0.0001$) in HeLa TetOff cells (a derivative of HeLa cells), and 10 mM LiCl suppressed transcription efficiency in both NB-1 and HeLa TetOff cells ($P<0.01$ and $P<0.0001$, respectively). In *IMPA2* promoter constructs, 5 and 10 mM LiCl elicited a trend for decreased transcription in HeLa TetOff cells. When compared to 1 mM LiCl, 10 mM LiCl induced significant suppression of transcription in the two *IMPA2* promoter constructs (T-A-T-C-G and G-G-C-T-A) in HeLa TetOff cells ($P<0.05$). We did not observe any effects with 1 mM LiCl or any '*IMPA2* promoter-specific' transcriptional suppression by higher concentrations of LiCl.

Expression Analysis of *IMPA1* and *IMPA2* in Post-mortem Brains

We quantified *IMPA1* and *IMPA2* mRNA expression levels in the frontal region BA46 (DLPFC) from bipolar and control post-mortem brains, using MGB reactions. For *IMPA1*, transcript levels were not significantly different between bipolars and controls (Supplementary Figure S1). Although *IMPA2* mRNA in bipolar brains was significantly upregulated compared to control brains with respect to all the three internal control probes (Supplementary Figure S1), the expressional differences became nonsignificant when we strictly adjusted sample pH (Supplementary Table S5).

Next, to examine allelic expression differences of *IMPA2*, we chose brain samples whose genotypes were heterozygous at −185A>G (SNP06). Sixteen control brain samples and 18 bipolar samples met this criterion. *IMPA2* transcripts that carried −185A were significantly more abundant than −185G, in the control group ($P<0.0001$) and combined group ($P<0.0001$) (Figure 3). In the bipolar group, the proportion of the −185A allele exceeded 50%, although this was not statistically significant. This indicates that *IMPA2* mRNA displays biallelic expression, and therefore it is unlikely that the *IMPA2* is an imprinted gene. Examination

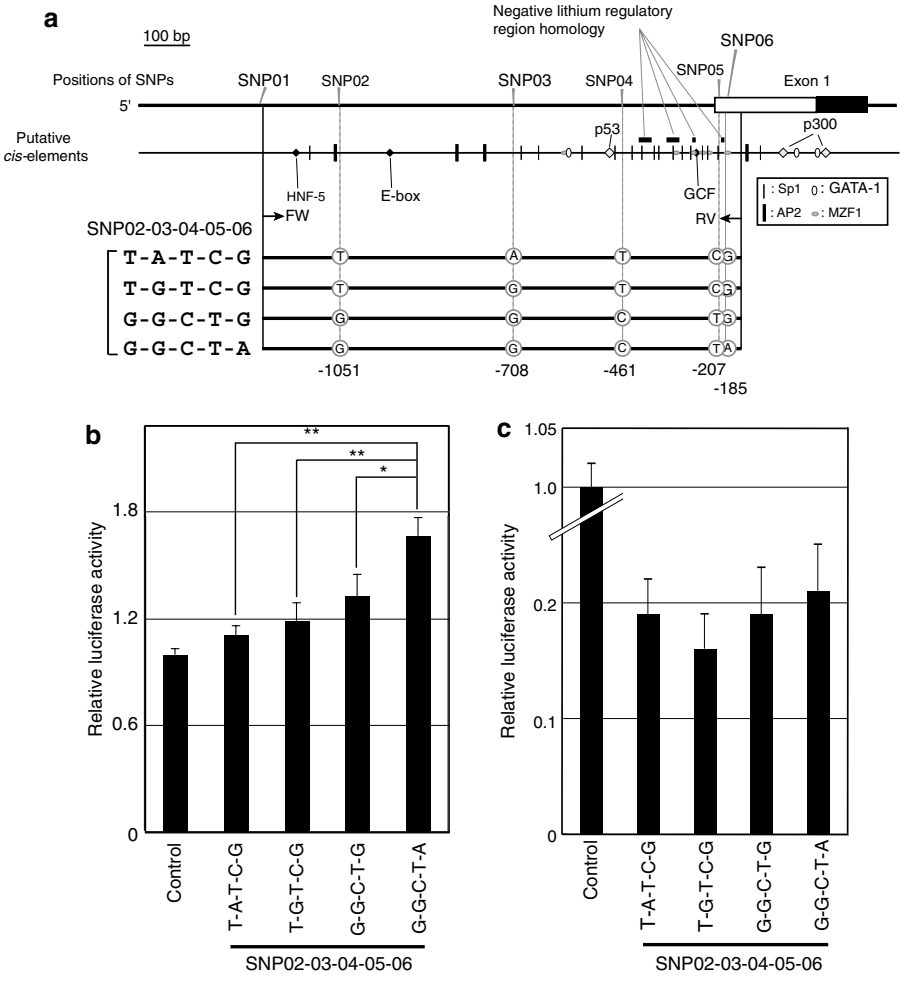

Figure 2 Promoter SNPs of *IMPA2* and *in vitro* transcriptional assay. (a) Location of *IMPA2* promoter SNPs, consensus motifs for transcription factors, and haplotype compositions of promoter constructs used for *in vitro* reporter assays are shown. FW and RV denote the primers used for PCR amplification to prepare constructs. (b) Luciferase assay for transcriptional activities of indicated constructs examined in NB-1 cells. Values represent mean ± SE of at least three independent transfections, each with duplicate determinations. $^{*}P<0.05$ and $^{**}P<0.01$ by Fisher's PSLD test. (c) Luciferase assay examined in HeLa TetOff cells.

Table 3 Distribution of *IMPA2* Promoter Haplotypes

Haplotype (SNP02-03-04-05-06)	Bipolars ($n=496$)	Controls ($n=543$)	χ^2	*P*-value	Odds ratio
T-A-T-C-G	0.136	0.155	1.386	0.239	0.859
T-G-T-C-G	0.320	0.335	0.462	0.497	0.936
G-G-C-T-G	0.165	0.159	0.124	0.725	1.045
G-G-C-T-A	0.380	0.352	1.576	0.209	1.126

Haplotype frequencies were computed by the COCAPHASE program.

of exact haplotypes of genomic DNA from the 34 brain samples heterozygous at −185 position by subcloning showed that −185A, −207T, and −461C were on the same DNA strand (gray circles in Figure 3), except for one control sample whose haplotype could not be determined owing to unsuccessful subcloning/sequencing. In contrast, only four samples out of 33 had (−185G)–(−207T)–(−461C) (black circles in Figure 3), half of which showed higher transcription (>50% in Figure 3). These results show that the bipolar-associated −461C and −207T alleles were related to increased expression of *IMPA2* in the brain.

DISCUSSION

We investigated possible association between *IMPA2* SNPs and bipolar disorder in Japanese cases and controls. Alleles from two promoter SNPs, −461C (SNP04) and −207T

Table 4 Effect of Lithium on *IMPA2* Promoter Activity

Cell line	Plasmid construct (SNP02-03-04-05-06)	LiCl (mM)			
		0	1	5	10
		Relative luciferase activity (mean ± SE)			
NB-1 cells	Control	1.00 ± 0.029	1.03 ± 0.021	1.10 ± 0.041	0.825 ± 0.01*,††
	T-A-T-C-G	1.11 ± 0.053	1.17 ± 0.085	1.08 ± 0.048	0.925 ± 0.032
	T-G-T-C-G	1.19 ± 0.098	1.21 ± 0.118	1.19 ± 0.093	0.952 ± 0.055
	G-G-C-T-G	1.33 ± 0.118	1.39 ± 0.149	1.36 ± 0.093	1.14 ± 0.051
	G-G-C-T-A	1.67 ± 0.104	1.77 ± 0.176	1.67 ± 0.066	1.38 ± 0.069
HeLa TetOff cells	Control	1.00 ± 0.024	1.14 ± 0.098	0.378 ± 0.056**,†††	0.248 ± 0.032**,†††
	T-A-T-C-G	0.187 ± 0.033	0.218 ± 0.043	0.097 ± 0.018	0.080 ± 0.007†
	T-G-T-C-G	0.155 ± 0.031	0.178 ± 0.042	0.082 ± 0.016	0.072 ± 0.005
	G-G-C-T-G	0.188 ± 0.044	0.197 ± 0.044	0.090 ± 0.018	0.073 ± 0.003
	G-G-C-T-A	0.212 ± 0.039	0.223 ± 0.043	0.110 ± 0.020	0.087 ± 0.006†

*$P<0.01$ and **$P<0.0001$ compared to LiCl = 0 mM in each construct and cell line, assessed by Fisher's PSLD test.
†$P<0.05$, ††$P<0.001$ and †††$P<0.0001$ compared to LiCl = 1 mM in each construct and cell line, assessed by Fisher's PSLD test.

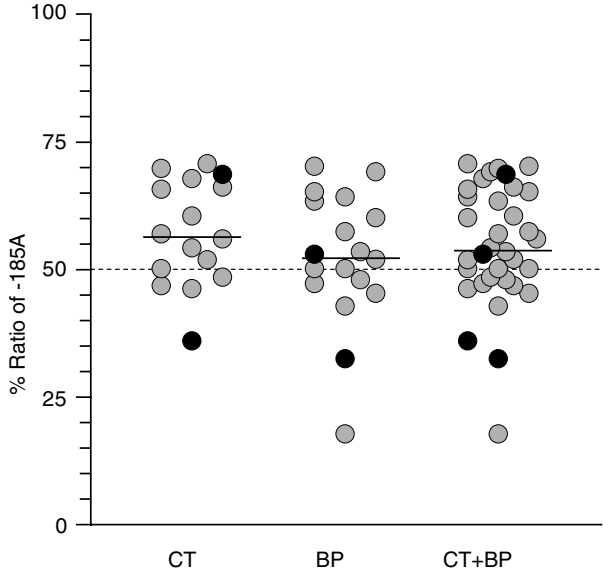

Figure 3 Allelic expression of *IMPA2* in the BA 46 region of control and bipolar brains. Control and bipolar brain samples heterozygous at −185A > G SNP were selected for analysis. Each data point represents % ratio of expressed cDNA clones that had A nucleotide at the −185 site in each brain sample. Grey circles represent samples with (−185A)–(−207T)–(−461C) allele, and black circles with (−185G)–(−207T)–(−461C). Horizontal bars indicate the median value. CT, control, BP, bipolar.

(SNP05) in the LD block 1 of the gene show nominally significant correlation with the increased risk for the disease. Although the effect is modest, it is important that these results are concordant with the findings by Sjøholt *et al* (2004), which showed significant overtransmission of −461C and −207T to offspring with bipolar disorder in Palestinian Arab trios. The detection of the same alleles from the same two 5′-upstream SNPs in the two different ethnic groups suggest that altered transcription of *IMPA2* plays a role in the pathogenesis of bipolar disorder. *In vitro* dual luciferase assay using NB-1 cells unraveled the transcription-enhancing effect of the combination of −461C (SNP04) and −207T (SNP05) alleles. In accordance with these results, transcription of *IMPA2* mRNA from alleles carrying −461C and −207T is upregulated in the frontal cortex of post-mortem brains of bipolar patients. The failure to detect haplotypic association (SNP02-03-04-05-06, Table 3) in the present study may be due to the weak genetic effect of putative risk (or protective) haplotypes as represented by weak odds ratios in Japanese population and/or dampening of statistical significance by increased statistical degree of freedom in haplotype analysis compared to single SNP-based analysis. In either case, the present sample size may not be sufficient to provide robust statistical power. The promoter function assay using both NB-1 and HeLa cells exposes potential impact of −185A allele ($P = 0.05$ in the association study of Sjøholt *et al*, 2004) on transcriptional activation. However, there is no association between the SNP06 and bipolar disorder. Possibly, both alleles of −185A > G SNP are bifurcately linked to the putative 'basic' risk haplotype (−461C (SNP04) and −207T (SNP05)). The genetic and functional data presented here suggest that the *IMPA2* promoter haplotype, −461C and −207T, contributes to elevated risk for bipolar disorder by eliciting increased expression, and −185A allele further augments the transcriptional activity of the risk-conferring (−461C)–(−207T) haplotype. Recently, Dimitrova *et al* (2005) examined eight SNPs including −461C > T of *IMPA2* for association with bipolar disorder, using 121 Bulgarian trios, 116 UK trios, and a panel of 174 cases and 170 controls. None of the SNPs reached statistical significance in any of the sample sets, although trends of overtransmission of −461C in Bulgarian trios and overrepresentation of −461C in unrelated cases were observed. As demonstrated in our Japanese study, the genetic effect of *IMPA2* is modest; most likely much larger sample numbers are needed to derive a definitive conclusion.

We searched for transcription factor binding motifs in the regions spanning SNP04, SNP05, and SNP06 using

the TFSEARCH database (http://mbs.cbrc.jp/research/db/TFSEARCHJ.html) (threshold set at 85.0 point) (Heinemeyer *et al*, 1998), and found that −207C (SNP05) creates a potential Sp1-binding site.

Lin *et al* (2005) assessed the effect of age at onset (AAO) on linkage to bipolar disorder and reported that 18p11.2 may harbor a risk gene(s) for later-onset form (AAO > 21 years) of the disease. Accordingly, we divided our bipolar samples with AAO > / ⩽ 21 years and AAO > / ⩽ 31. But none of these subgroups showed association with the gene, partly suggesting that the *IMPA2* may not have a major effect on AAO-dependent bipolars in Japanese.

It should be noted that the expression of certain genes is affected by sample pH. Indeed, the difference of *IMPA2* mRNA levels in bipolar patients and controls was not significant after strictly adjusting the sample pH. However, allele-specific expression should be less affected by such confounding factors; thus the effect of promoter haplotype of *IMPA2* on its expression in brain is robust to sample pH. The expression and genetic data from the current study as well as prior data (Sjøholt *et al*, 2004) suggest that *IMPA2* exerts a greater role than *IMPA1* in conferring risk to bipolar disorder. The true physiological substrate(s) and activating cofactor(s), if needed, for IMPA2, and distinctive roles of IMPA1 and IMPA2 in brain remain to be demonstrated. Quite recently, lack of lithium-like behavioral and molecular effects in *IMPA2* gene-trapped mice was reported (Cryns *et al*, 2006). Nonetheless, the upregulation of *IMPA2* in bipolar brains may be relevant to 'inositol depletion' hypothesis of lithium action in bipolar disorder (Berridge *et al*, 1989), since in our ongoing experiments, we found that IMPA2 showed IMPase activity, albeit weak compared to that of IMPA1 in a conventional assay condition, and the activity was inhibited by the presence of lithium chloride (Ohnishi *et al*, 2007).

Our data also reveal that therapeutic concentrations of lithium could not suppress the transcription of *IMPA2* mRNA and the transcription-inhibitory effect of higher concentration of lithium was not specific to *IMPA2* promoter, in contrast to the conclusions of Seelan *et al* (2004) where the effects of lithium on control promoter constructs like SV40 was not presented. Our results further suggest that, the mood-stabilizing effect of lithium could be generated via inhibition of enzymatic reaction rather than transcriptional suppression. Therapeutic doses of lithium need to be administered for at least 2 weeks before clinical effects are seen. To examine this issue, it would be necessary to establish cell lines with stable expression of *IMPA2*.

Previously, Yoon *et al* (2001) reported that *IMPA2* showed sex-dependent expressional differences in postmortem temporal cortex. However, we did not replicate the finding in the frontal cortex, nor detected any sex-dependent genetic association between *IMPA2* and bipolar disorder (data not shown).

Another important information obtained from the current allelic expression analysis of *IMPA2* is that the gene is transcribed from both paternal and maternal chromosomes, indicating that *IMPA2* is not under genomic imprinting control. Corradi *et al* (2005) reported that *GNAL*, a gene close to *IMPA2*, and possibly other genes in the region encompassing *GNAL*, is subject to genomic imprinting, as deduced from the detection of methylated and unmethylated DNA in *GNAL* region. In general, imprinting phenomenon involves a cluster of genes in a stretch of genome and imprinted multiple genes in the same locus are coordinately regulated by an imprinting control region (Delaval and Feil, 2004). Our results raise the necessity for further scrutiny on whether genomic imprinting occurs in the *GNAL*/*IMPA2* locus. It is of note that imprinting effect may be tissue-specific (within brain regions), and partial imprinting may also occur in human genes (Buckland, 2004).

In summary, we detected an association between *IMPA2* and bipolar disorder in Japanese cohorts with the same SNPs reported in Palestinian Arabs. Although the *P*-values for allelic association did not survive correction for multiple tests and thus its genetic effect is modest in the Japanese, the present evidence obtained from combined genetic analysis, promoter assay and postmortem brain expression and allelic expression analyses suggest that the (−1051G)–(−708G)–(−461C)–(−207T)–(−185A) haplotype contributes to risk for bipolar disorder by enhancing *IMPA2* transcription. Future large-scale attempts for replication and detailed biochemical characterization of IMPA2 are warranted.

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REFERENCES

Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly PA (2005). Haplotype map of the human genome. *Nature* **437**: 1299–1320.

Aoki-Suzuki M, Yamada K, Meerabux J, Iwayama-Shigeno Y, Ohba H, Iwamoto K *et al* (2005). A family-based association study and gene expression analyses of netrin-G1 and -G2 genes in schizophrenia. *Biol Psychiatry* **57**: 382–393.

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

Belmaker RH, Shapiro J, Vainer E, Nemanov L, Ebstein RP, Agam G (2002). Reduced inositol content in lymphocyte-derived cell lines from bipolar patients. *Bipolar Disord* **4**: 67–69.

Berrettini WH, Ferraro TN, Goldin LR, Weeks DE, Detera-Wadleigh S, , Nurnberger Jr JI *et al* (1994). Chromosome 18 DNA markers and manic-depressive illness: evidence for a susceptibility gene. *Proc Natl Acad Sci USA* **91**: 5918–5921.

Berridge MJ, Downes CP, Hanley MR (1989). Neural and developmental actions of lithium: a unifying hypothesis. *Cell* **59**: 411–419.

Buckland PR (2004). Allele-specific gene expression differences in humans. *Hum Mol Genet* **13**: R255–R260.

Corradi JP, Ravyn V, Robbins AK, Hagan KW, Peters MF, Bostwick R *et al* (2005). Alternative transcripts and evidence of imprinting of *GNAL* on 18p11.2. *Mol Psychiatry* **10**: 1017–1025.

Cryns K, Shamir A, Shapiro J, Daneels G, Goris I, Van Craenendonck H *et al* (2006). Lack of lithium-like behavioral and molecular effects in *IMPA2* knockout mice. *Neuropsychopharmacology* (in press).

Delaval K, Feil R (2004). Epigenetic regulation of mammalian genomic imprinting. *Curr Opin Genet Dev* **14**: 188–195.

Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G *et al* (1999). A high density genome scan detects evidence for a bipolar susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. *Proc Natl Acad Sci USA* **96**: 5604–5609.

Dimitrova A, Milanova V, Krastev S, Nikolov I, Toncheva D, Owen MJ *et al* (2005). Association study of myo-inositol monophosphatase 2 (IMPA2) polymorphisms with bipolar affective disorder and response to lithium treatment. *Pharmacogenomics J* **5**: 35–41.

Dinan TG (2002). Lithium in bipolar mood disorder. *BMJ* **324**: 989–990.

Dudbridge F (2003). Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* **25**: 115–121.

Evans DL, Charney DS, Lewis L, Golden RN, Gorman JM, Krishnan KR *et al* (2005). Mood disorders in the medically ill: scientific review and recommendations. *Biol Psychiatry* **58**: 175–189.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B *et al* (2002). The structure of haplotype blocks in the human genome. *Science* **296**: 2225–2229.

Gould TD, Quiroz JA, Singh J, Zarate Jr CA, Manji HK (2004). Emerging experimental therapeutics for bipolar disorder: insights from the molecular and cellular actions of current mood stabilizers. *Mol Psychiatry* **9**: 734–755.

Gurvich N, Klein PS (2002). Lithium and valproic acid: parellels and contrasts in diverse signaling contexts. *Pharmacol Ther* **96**: 45–66.

Harwood AJ (2005). Lithium and bipolar mood disorder: the inositol-depletion hypothesis revisited. *Mol Psychiatry* **10**: 117–126.

Heinemeyer T, Wingender E, Reuter I, Hermjakob H, Kel AE, Kel OV *et al* (1998). Databases on transcriptional regulation: TRANSFAC, TRRD, and COMPEL. *Nucleic Acids Res* **26**: 364–370.

Kunugi H, Iijima Y, Tatsumi M, Yoshida M, Hashimoto R, Kato T *et al* (2004). No association between the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene and bipolar disorder in Japanese: a multi-center study. *Biol Psychiatry* **56**: 376–378.

Lin P-I, McInnis MG, Potash JB, Willour VL, Mackinnon DF, Miao K *et al* (2005). Assessment of the effect of age at onset on linkage to bipolar disorder: evidence on chromosomes 18p and 21q. *Am J Hum Genet* **77**: 545–555.

Munakata K, Tanaka M, Mori K, Washizuka S, Yoneda M, Tajima O *et al* (2004). Mitochondrial DNA 3644TC mutation associated with bipolar disorder. *Genomics* **84**: 1041–1050.

Nakayama J, Yamamoto N, Hamano K, Iwasaki N, Ohta M, Nakahara S *et al* (2004). Linkage and association of febrile seizures to the *IMPA2* gene on human chromosome 18. *Neurology* **63**: 1803–1807.

Nemanov L, Ebstein RP, Belmaker RH, Osher Y, Agam G (1999). Effect of bipolar disorder on lymphocyte inositol monophosphatase mRNA levels. *Int J Neuropsychopharmacol* **2**: 25–29.

Ohnishi T, Ohba H, Seo K-C, Im J, Sato Y, Iwayama Y *et al* (2007). Spatial expression patterns and biochemical properties distinguish a second *myo*-inositol monophosphatase, IMPA2 from IMPA1. *J Biol Chem* **282**: 637–646.

Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Schneider S, Roessli D, Excoffier L (2000). *Arlequin: A Software for Population Genetics Data Analysis, Ver 2.000*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva.

Seelan RS, Parthasarathy LK, Ranga N, Parthasarathy RN (2004). Lithium modulation of the human inositol monophosphatase 2 (*IMPA2*) promoter. *Biochem Biophys Res Commun* **324**: 1370–1378.

Segurado R, Detera-Wadleigh SD, Levinson DF, Lewis CM, Gill M, Nurnberger Jr JI *et al* (2003). Genome scan meta-analysis of schizophrenia and bipolar disorder part III: bipolar disorder. *Am J Hum Genet* **73**: 49–62.

Shaltiel G, Shamir A, Nemanov L, Yaroslavsky Y, Nemets B, Ebstein RP *et al* (2001). Inositol monophosphatase activity in brain and lymphocyte-derived cell lines of bipolar patients. *World J Biol Psychiatry* **2**: 95–98.

Shamir A, Ebstein RP, Nemanov L, Zohar A, Belmaker RH, Agam G (1998). Inositol monophosphatase in immortalized lymphoblastoid cell lines indicates susceptibility to bipolar disorder and response to lithium therapy. *Mol Psychiatry* **3**: 481–482.

Sjøholt G, Ebstein RP, Lie RT, Berle JO, Mallet J, Deleuze JF *et al* (2004). Examination of *IMPA1* and *IMPA2* genes in manic-depressive patients: association between *IMPA2* promoter polymorphisms and bipolar disorder. *Mol Psychiatry* **9**: 621–629.

Sjøholt G, Gulbrandsen AK, Løvlie R, Berle JØ, Molven A, Steen VM (2000). A human *myo*-inositol monophosphatase gene (*IMPA2*) localized in a putative susceptibility region for bipolar disorder on chromosome 18p11.2: genomic structure and polymorphism screening in manic-depressive patients. *Mol Psychiatry* **5**: 172–180.

Torrey EF, Webster M, Knable M, Johnston N, Yolken RH (2000). The Stanley Foundation brain collection and Neuropathology Consortium. *Schizophr Res* **44**: 151–155.

Wang L, Liu X, Lenox RH (2001). Transcriptional down-regulation of MARCKS gene expression in immortalized hippocampal cells by lithium. *J Neurochem* **79**: 816–825.

Williams RS, Cheng L, Mudge AW, Harwood AJ (2002). A common mechanism of action for three mood-stabilizing drugs. *Nature* **417**: 292–295.

Yamada K, Ohnishi T, Hashimoto K, Ohba H, Iwayama-Shigeno Y, Toyoshima M *et al* (2005). Identification of multiple serine racemase (*SRR*) mRNA isoforms and genetic analyses of *SRR* and *DAO* in schizophrenia and D-serine levels. *Biol Psychiatry* **57**: 1493–1503.

Yoon IS, Li PP, Siu KP, Kennedy JL, Cooke RG, Parikh SV *et al* (2001). Altered IMPA2 gene expression and calcium homeostasis in bipolar disorder. *Mol Psychiatry* **6**: 678–683.

Yoshikawa T, Kikuchi M, Saito K, Watanabe A, Yamada K, Shibuya H *et al* (2001). Evidence for association of the myo-inositol monophosphatase 2 (*IMPA2*) gene with schizophrenia in Japanese samples. *Mol Psychiatry* **6**: 202–210.

Yoshikawa T, Padigaru M, Karkera JD, Sharma M, Berrettini W, Esterling LE *et al* (2000). Genomic structure and novel variants of *myo*-inositol monophosphatase 2 *(IMPA2)*. *Mol Psychiatry* **5**: 165–171.

Yoshikawa T, Turner G, Esterling LS, Sanders AR, Detera-Wadleigh SD (1997). A novel human myo-inositol monophosphatase gene, IMP.18p, maps to a susceptibility region for bipolar disorder. *Mol Psychiatry* **2**: 393–397.

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