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Linkage Disequilibrium Analysis in the LOC93081-KDELC1-BIVM Region on 13q in Bipolar Disorder

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Genome-wide scans in bipolar disorder and a meta analysis on published data have provided evidence for linkage to chromosome 13q, although the reported peaks from various studies have not converged in a narrow region. Recently, single nucleotide polymorphisms (SNPs) at the G72/G30 locus have been shown to be associated with bipolar disorder suggesting its potential role in increasing disease risk. The proposed linkage region on 13q extends over a wide span, and could provide a clue to the existence of other susceptibility variants. In the present study, SNPs in the LOC93081-KDELC1-BIVM, a region proximal to G72, were interrogated in two bipolar family series. KDELC1 has a predicted filamin domain and BIVM contains an immunoglobulin-like motif. The small pedigree series yielded a nominally significant global *P*-value due to undertransmission of a rare haplotype but this finding was not supported by results from the larger series and in the case-control study that compared 278 cases and 277 controls. **© 2005 Wiley-Liss, Inc.**

KEY WORDS: SNP; TDT; linkage; association

INTRODUCTION

Support for linkage to bipolar disorder on chromosome 13q has been detected in several independent studies but the breadth of the highlighted region covers a large physical distance (Fig. 1). Our genome scan on 22 CNG families localized the linkage peak bounded by D13S1271 and D13S779 [Detera-Wadleigh et al., 1999], and around D13S779 and D13S225 [Liu et al., 2001] when additional markers were typed. These two markers are separated by only >42 bp (UCSC Genome Browser, http://www.genome.ucsc.edu/) but when the latter studies were underway, the exact physical distance between these markers was not known. Additional support for linkage at these loci has been derived from a recent genome scan on an independent panel of 40 pedigrees that yielded a LOD score >2 at D13S779 [Liu et al., 2003]. LOD >2 in the region spanning D13S225 and D13S796 was reported recently [Shaw et al., 2003].

Sites proximal to D13S779 and D13S225 have been highlighted in other bipolar disorder datasets (Fig. 1). In the initial scan of the NIMH Genetics Initiative Wave1 collection, LOD >1 was detected with D13S793 [Stine et al., 1997]. Scans by Kelsoe et al. [2001] and Shaw et al. [2003] of two independent sets of families yielded lod score values >2 at D13S154 and D13S793. Loci more proximal to the latter markers have been reported to yield linkage signals: D13S1246 on 13q12.3 in a German collection [Cichon et al., 2002], a peak in an Australian cohort localized at D13S153 on 13q14 [Badenhop et al., 2002], and D13S317 on 13q31 detected linkage signals in pedigrees with psychotic bipolar disorder [Potash et al., 2003].

Portions of the potential bipolar linkage region on 13q have been shown to overlap with linkage peaks for schizophrenia [Lin et al., 1997; Blouin et al., 1998; Brzustowicz et al., 1999; Faraone et al., 2002; Abecasis et al., 2004; Stassen et al., 2004] (Fig. 1), endorsing the speculation that both disorders share some genes involved in susceptibility [Detera-Wadleigh et al., 1999; Wildenauer et al., 1999]. To determine whether a larger sample set could extract significant evidence of linkage meta analysis using the Multiple Scan Probability method was performed on published data from 12 bipolar disorder and 19 schizophrenia genome scans, respectively [Badner and Gershon, 2002]. This analysis uncovered genome-wide significant linkage on 13q and 22q for both disorders. In contrast, results from another meta analysis using the rank-order method on whole-genome genotype data from 18 to 22 genome scans were not consistent with the latter findings [Segurado et al., 2003]. Nonetheless, the multiple reports that highlight 13q in both bipolar disorder and schizophrenia support the possibility that 13q codes for at least one risk-conferring gene for these disorders.

Recently, Chumakov et al. [2002] targeted 13q to screen for association with schizophrenia. The search focused on single nucleotide polymorphisms (SNPs) in a 5 Mb region of 13q32-33 that included D13S174, the marker that displayed the strongest evidence of linkage in a schizophrenia genome scan [Blouin et al., 1998]. Significant association was detected in SNPs at the G72/G30 locus on 13q33, located distally ~3.2 Mb from D13S174, ~8.2 Mb from D13S793 [Brzustowicz et al., 1999] and >7 Mb from D13S122 and D13S128 [Lin et al., 1997] (Fig. 1).

To investigate whether the G72/G30 association would be detectable in bipolar disorder, Hattori et al. [2003] examined families that previously yielded linkage evidence on 13q. The bipolar disorder linkage peak in the CNG families [Detera-Wadleigh et al., 1999; Liu et al., 2001] was ~4.6 Mb proximal to the G72/G30 locus. Hattori et al. [2003] detected excess

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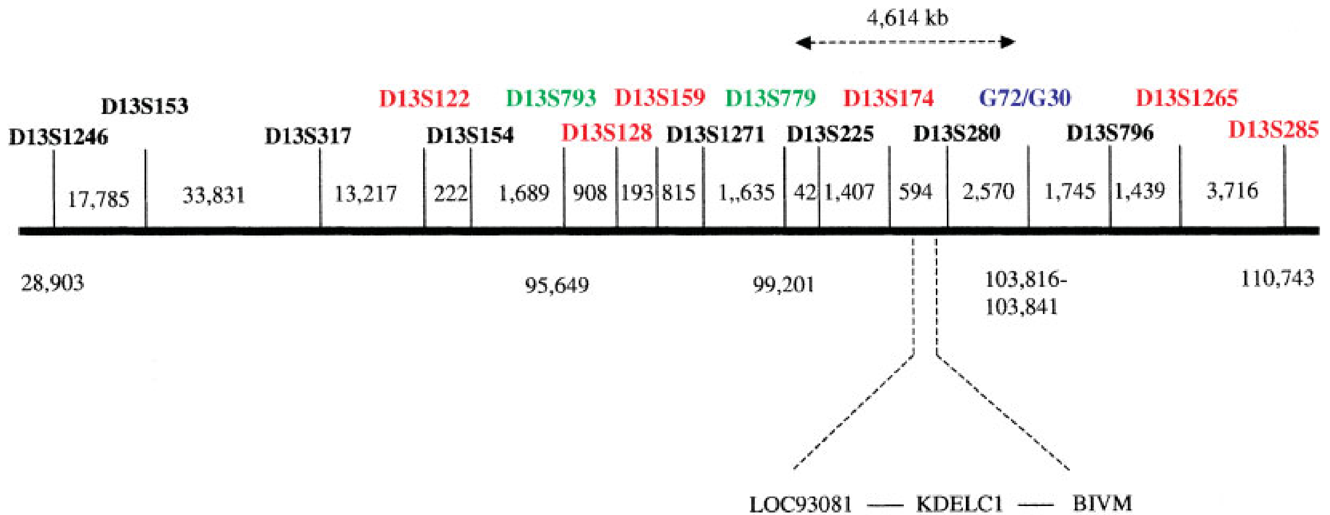


Fig. 1. Loci on 13q that have been highlighted in bipolar disorder and schizophrenia linkage studies. Markers colored black, red, and green were reported in bipolar disorder, schizophrenia and in both bipolar disorder and schizophrenia, respectively, and locus in blue has single nucleotide polymorphisms (SNPs) associated with both bipolar disorder and schizophrenia. Positions were taken from the UCSC Genome Browser July 2003 freeze (http://www.genome.ucsc.edu/) and sizes are in kb. The references for the markers listed are: D13S1246 [Cichon et al., 2002]; D13S153 [Badenhop et al., 2002]; D13S317 [Potash et al., 2003]; D13S122 and D13S128 [Lin et al., 1997]; D13S154 and D13S793 [Kelsoe et al., 2001; Shaw et al., 2003]; D13S793 [Brzustowicz et al., 1999]; D13S159 [Faraone et al., 2002]; D13S1271, D13S779 and D13S225 [Detera-Wadleigh et al., 1999; Liu et al., 2001]; D13S779 [Liu et al., 2003; Stassen et al., 2004]; D13S174 [Blouin et al., 1998]; D13S796 [Shaw et al., 2003]; D13S1265 and D13S285 [Abecasis et al., 2004].

transmission of alleles and of a common haplotype. In a study of bipolar disorder cases and controls results consistent with association with G72/G30 SNPs were generated [Chen et al., 2004]. The convergent findings in both bipolar disorder and schizophrenia suggest a shared risk variant at the G72/G30 locus.

It is conceivable that other distinct susceptibility genes exist on 13q because of the broadness of the highlighted region. In the CNG pedigrees evidence for linkage disequilibrium (LD) has been detected also at D13S280 [Liu et al., 2001], ~2.6 Mb proximal to G72/G30. To explore whether support for LD in D13S280 could be found we screened SNPs within genes that map to this region. The region encodes three positional candidate genes and a predicted gene, *LOC93081*. KDELC1 contains a filamin domain [Kimata et al., 2000], BIVM has immunoglobulin-like variable motif [Yoder et al., 2002], and ERCC5 has been shown to be defective in xeroderma pigmentosum [O'Donovan and Wood, 1993]. Here, we report results from association analysis with SNPs spanning LOC93081, KDELC1, and BIVM on the CNG and NIMH Genetics Initiative for Bipolar Disorder datasets.

METHODS

Pedigrees

The 22 CNG pedigree set [Berrettini et al., 1994] that was subjected to a genome-wide linkage scan [Detera-Wadleigh et al., 1999] was used in the LD study. From these pedigrees 12 "quads" (an affected sib pair and two genotyped parents) and seven trios were extracted for the LD analysis. In addition, 72 quads and 28 trios were derived from the Waves 1 and 2 collection of the NIMH Genetics Initiative for Bipolar pedigrees.

SNPs

SNPs used to genotype the CNG and NIMH 1 and 2 families are presented in Table I. Two SNPs were identified after sequencing exons of KDELC1 (MGC5302) and BIVM (FLJ20159) and the rest were taken from the SNP database and sequence validated. Exons that were sequenced included at least 100 bp of flanking introns. The Primer 3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) was used to design PCR primers. Sequencing was conducted on an ABI 370 automated sequencer using the Big Dye terminator kit (Applied Biosystems, Foster City, CA). Alignment of sequences was done with the aid of the Sequencher software (Gene Codes Corporation, Ann Arbor, MI).

Genotyping

Genotyping was done by pyrosequencing (PSQ MA or PSQ HS96 Pyrosequencer) following PCR amplification and processing of PCR products. PCR was performed either with Taq Gold (Applied Biosystems) or HotStar Taq (Qiagen, Valencia, CA) or HotMaster Taq polymerase (Brinkmann, Eppendorf) under the following conditions: initial denaturation at 96°C for 10 min or 95°C for 15 min or 94°C for 2 min, respectively; then 40 cycles of denaturation at 94°C, 30 sec, annealing at 60°C, 30 sec, extension at 72°C, 1 min; followed by a final extension at 72°C for 7 min and a soak at 4°C. Template DNA was between 10 and 20 ng for each PCR. For pyrosequencing analysis, one of the primers was biotinylated at the 5′ end. The internal sequencing primers (Table I) were designed using the Pyrosequencing software (http://techsupport.pyrosequencing.com/v2/AssayDesign/index.asp). Post-PCR sample processing was done following the manufacturer's recommendations.

Linkage Disequilibrium and Partitioning of Linkage

To calculate the *P*-value for LD between pairs of loci, we used genotypes from one individual in each pedigree. Haplotypes that could be unambiguously inferred from transmitted and non-transmitted genotypes from the parents were estimated using software written by our group (J. A. Badner, unpublished data). LD *P*-values were calculated using CLUMP [Sham and Curtis, 1995].

TDT on individual SNPs was performed using the ASPEX/SibTDT program, which tests for LD in sibships, controlling for evidence of linkage. Empirical probabilities for two

TABLE I. Single Nucleotide Polymorphisms (SNPs) in the LOC93081-KDELC1-BIVM Region Used to Screen for Association

SNP	Position (bp)[a]	SNP	Primers	Internal pyrosequencing primer
rs8054	101116729	A > G	5′-ctcacaatgctgagaggcata 5′-aaaggacggtcgacaaatga	R: 5′-aaagccatgttatcttga
KDELC1 exon 2 (rs1047740)	101147203	T > C	5′-ctgctgttataaaattatattcc 5′-ggtccttcatagtaagatacaga	R: 5′-aaaaatctgaaggtggaa
rs1571069	101171071	A > G	5′-gcttcttgcccaaggatt 5′-tggatgttatccaaaccaatg	F: 5′-atttctgaagttgcaaata
BIVM intron 5	101171498	T > G	5′-ggtactgcataagccgacca 5′-cccagcccatttttatttcac	F: 5′-tggaaagtaagtatgtcaat
rs2274389	101172691	T > C	5′-ttcgtctcaaagactgcctgt 5′-accacggacccaaacaatac	R: 5′-aaaggttagaagaggtaaaa
rs1408047	101182919	C > T	5′-caccacacccttcctgaaa 5′-ggaacccagacaaattccat	R: 5′-tgggacgttggatga
rs2281886	101189173	G > A	5′-aaggcatttcccagctgac 5′-taccagctacttggcccttg	F: 5′-ttagcactctttaggttg
rs2281885	101189393	C > T	5′-caagggccaagtagctggta 5′-gccacaaggccacagttaat	R: 5′-gaaagatgatgatcttac
rs4743	101191826	C > G	5′-gcttgaaatgcaaaggactg 5′-ccagttgagcagaattgaca	F: 5′-ttgtaggttgttgattttg
rs2296147	101196376	T > C	5′-cagggacatcttctggctgt 5′-cgcattaaagcggagagact	F: 5′-gccattctctggacc

[a]Positions are from the UCSC Genome Browser, July 2003 freeze.

statistics were calculated, which accurately reflects association independently of linkage within families. This calculation was done by permuting the parent alleles while fixing the IBD status of sibs within a family. Transmission and frequencies of haplotypes were analyzed by TDTPhase [Dudbridge et al., 2000] using the "tsu" correction for sib pairs, which controls for the presence of linkage. This correction includes only parents transmitting the same haplotype to all sibs. When one or more sibs are ambiguous, only the first sib was included in the analysis. In the calculation of the global tests, haplotypes with counts <5 were dropped. In TDT, the transmission ratio for one randomly chosen individual per sibship was also calculated for each allele and haplotype. The transmission ratio was estimated as the frequency with which an allele or haplotype was transmitted from a heterozygous parent to an affected offspring.

We also tested partitioning evidence of linkage according to genotype, as described elsewhere [Horikawa et al., 2000]. Positive results obtained from this test are regarded as further evidence of association. In the present analysis, 22 and 152 probands were randomly selected from the CNG and NIMH series, respectively, and families were separated into groups according to the genotypes of probands for each SNP. If there was no association between the SNP and the evidence for linkage, the resulting LOD score within each genotype group should be proportional to the number of families within that group. If there is association, the LOD score should be disproportionately high within one or two of the genotype groups. Linkage score for each group was calculated on the basis of the original linkage evidence at 13q32-33 in the CNG series, as demonstrated by the Genehunter Plus [Kong and Cox, 1997] analysis in nuclear families by use of the narrow affection status model [Detera-Wadleigh et al., 1999; Liu et al., 2001], and in the NIMH series (data not shown). Families in which the proband contained the SNP genotype being tested were weighted 1, whereas other families were weighted 0. If there was more than one nuclear family within a pedigree, then all were weighted the same on the basis of the proband's genotype. Empirical *P*-values, for the assessment of the significance of the increase in LOD score for the group defined by the SNP genotype, were obtained by randomly choosing the same number of families included in the group generating the increased LOD score by use of Weight (N. J. Cox, personal communication). Enough replicates were simulated until 20 replicates achieving a LOD score at least as high as that observed in the genotype group were generated.

Case-Control Association Test

Association was evaluated in cases versus controls using CLUMP [Sham and Curtis, 1995]. A total of 278 unrelated cases of Caucasian origin were taken from NIMH Genetics Initiative for Bipolar Disorder collections: Wave1, Wave 2, and Wave 3, designated as NIMH 1, NIMH 2, and NIMH 3, respectively. There were 277 controls of Caucasian origin, of which 93 were from CEPH parents and 184 were from the Caucasian panel (cat #HD200CAU) at the Coriell Cell Repositories. CLUMP [Sham and Curtis, 1995] was used to calculate Chi-squares and *P*-values.

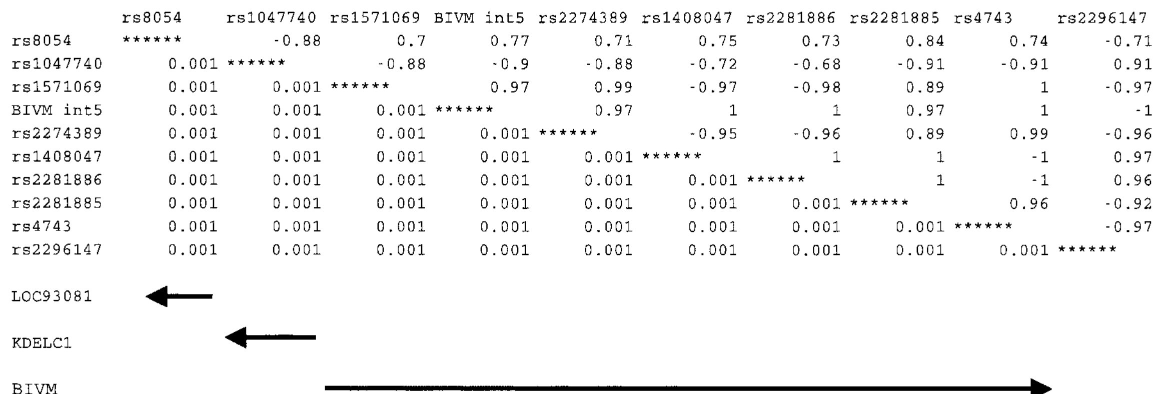
RESULTS

Screening for SNPs on KDELC1 and BIVM

To screen for SNPs on KDELC1 and BIVM, DNA from one affected member of each of the 22 CNG families was used as template for PCR and sequence analysis. On exon 2 of KDELC1 we found a codon change, ATT > GTT, resulting in an amino acid substitution, Ile114Val. This SNP is designated rs1047740 in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). In BIVM, a G > T transversion was detected 16 nucleotides 3′ of the splice junction of exon 5. These two SNPs were added to those taken from the SNP database that were validated by sequencing. A list of the SNPs, SNP position and primers used is shown (Table I).

Marker-to-Marker LD

Pairwise marker LD profile on 10 SNPs was generated by examining one family from each pedigree. The *P*-values revealed significant LD between SNPs forming an LD block that spans >80 kb. Similarly, for the NIMH Genetic Initiative samples, the same set of SNPs showed significant pairwise LD values (Fig. 2). The SNP to SNP LD displayed a continuous range of significance ($P = 0.001$), confirming the results from



	rs8054	rs1047740	rs1571069	BIVM int5	rs2274389	rs1408047	rs2281886	rs2281885	rs4743	rs2296147
rs8054	******	-0.88	0.7	0.77	0.71	0.75	0.73	0.84	0.74	-0.71
rs1047740	0.001	******	-0.88	-0.9	-0.88	-0.72	-0.68	-0.91	-0.91	0.91
rs1571069	0.001	0.001	******	0.97	0.99	-0.97	-0.98	0.89	1	-0.97
BIVM int5	0.001	0.001	0.001	******	0.97	1	1	0.97	1	-1
rs2274389	0.001	0.001	0.001	0.001	******	-0.95	-0.96	0.89	0.99	-0.96
rs1408047	0.001	0.001	0.001	0.001	0.001	******	1	1	-1	0.97
rs2281886	0.001	0.001	0.001	0.001	0.001	0.001	******	1	-1	0.96
rs2281885	0.001	0.001	0.001	0.001	0.001	0.001	0.001	******	0.96	-0.92
rs4743	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	******	-0.97
rs2296147	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	******

Fig. 2. Linkage disequilibrium (LD) in the NIMH 1 and 2 pedigrees between pairs of SNPs. The numbers above the diagonal represent D' while the numbers below the diagonal are P-values. The horizontal bars indicate the approximate location of the gene in relation to the SNPs, and the arrows depict the direction of transcription.

the CNG families for the presence of an LD block encompassing the proximal region of LOC93081 and the 3′ flanking region of BIVM.

TDT and Partitioning of Linkage Evidence

Transmission of alleles from heterozygous parents to affected offspring was evaluated in triads from the CNG and NIMH 1 and 2 series. Analysis of the entire panel of CNG families failed to detect association with any individual SNP. Partitioning of the evidence for linkage can provide further support for association and the families were subdivided based on proband genotype: 1,1, or 1,2, or 2,2. For the subgroup with proband heterozygous genotype 1,2, six SNPs yielded P-values <0.05 (Table II).

We examined haplotypes formed by 10 SNPs. In the CNG sample the global test was nominally significant with the rare haplotype being significantly under-transmitted and the two more common haplotypes being nonsignificantly over-transmitted (Table III). The same SNPs typed on the NIMH 1 and 2 trios gave no evidence of excess transmission of alleles from single SNPs or of SNP haplotypes (Table III).

Case-Control Association Tests

We performed allelic association tests using the three SNPs that yielded the highest evidence of partitioning of linkage in the CNG series. Both cases (n = 278) and controls (n = 277) have Caucasian ancestry. No evidence of association

TABLE II. Locus TDT and Partitioning of Linkage Evidence According to Genotype

		TDT		Partitioning of linkage evidence					
				Genotype					
				1,1		1,2		2,2	
SNPs	Allele 1	P	Transmission ratio	n	LOD	n	LOD (P)	n	LOD
CNG Series									
rs8054	C	0.38	0.27	0	0	7	0.8	20	1.73
KDELC1 ex2	A	0.4	0.71	18	0.32	9	3.27 (0.021)	0	0
rs1571069	A	1	0.44	10	0.89	12	1.92	5	0.01
BIVM int 5	G	0.76	0.38	0	0	9	3.27 (0.021)	18	0.32
rs2274389	A	1	0.4	10	0.89	12	1.92	5	0.01
rs1408047	C	1	0.53	11	1.21	9	1.82	7	0.03
rs2281886	C	1	0.55	11	1.21	9	1.82	6	0.13
rs2281885	A	0.52	0.13	0	0	7	2.35 (0.041)	20	0.76
rs4743	C	0.36	0.36	10	0.89	12	1.92	5	0.01
rs2296147	C	0.9	0.63	5	0.01	12	1.92	10	0.89
NIMH 1 and 2									
rs8054	C	0.7	0.45	13	−0.17	42	−0.11	91	0
KDELC1 ex2	A	0.6	0.55	91	0	39	0	16	−0.78
rs1571069	A	0.2	0.55	53	−0.09	69	−0.04	23	0.01
BIVM int5	G	0.93	0.46	11	−0.48	45	−0.02	89	0
rs2274389	A	0.41	0.54	52	−0.08	68	−0.07	23	0
rs1408047	C	0.15	0.43	58	0	63	−0.77	24	0.35
rs2281886	C	0.26	0.46	59	0	60	−0.8	23	0.62
rs2281885	A	0.66	0.45	8	−0.2	40	0	98	−0.04
rs4743	C	0.43	0.59	51	0	70	−0.26	21	0.06
rs2296147	C	0.44	0.47	22	0.05	73	−0.12	51	−0.04

TABLE III. Haplotype-Based TDT Analysis of the CNG and NIMH Pedigrees

Haplotypes[a]	Frequency	*P*	Transmission ratio
CNG family series			
1211111112	0.14	0.014	0
2121122212	0.26	0.2	0.8
2122211221	0.44	0.2	0.75
Global *P*		0.013	
NIMH genetic initiative pedigrees			
1211111112	0.13	0.7	0.57
2121122212	0.3	0.6	0.54
2122211221	0.39	0.6	0.48
Global P		0.6	

[a]SNPs used are the following: rs8054, rs1047740, rs2181675, rs1571069, rs2274389, rs1408047, rs2281886, rs2281885, rs4743, and rs2296147.

was found as reflected in the CLUMP allelic *P*-values for KDELC1 ex2, BIVM intron 5, rs2281885 of 0.9, 0.6, and 0.7, respectively (data not shown). The haplotype global *P*-value was 0.9.

DISCUSSION

Convergence of linkage peaks generated by various studies in a narrow chromosomal region is still uncommon in complex diseases, and the 13q bipolar disorder linkage is emblematic of this phenomenon. It is not known whether multiple peaks distributed throughout a large segment of a chromosomal arm represent multiple susceptibility genes or a single, indeterminately localized locus. Additionally, some reported linkage signals may be spurious. Nonetheless, if some of the signals on 13q represent valid linkage, it is reasonable to speculate that in addition to potential susceptibility variants at the G72/G30 locus, other disease-predisposing variants mapping to other loci are encoded by 13q.

In the present study, analysis was conducted on SNPs located close to the D13S280 region, upstream of G72/G30 that contains three genes, *KDELC1, ERCC5* and *BIVM*, and a hypothetical gene, *LOC93081*. ERCC5 is a causative gene for xeroderma pigmentosum [O'Donovan and Wood, 1993] and was not analyzed in this study. LOC93081 has a predicted hydrolase motif and may be a true gene because of a predicted promoter and CpG island that flank its 5′ end (UCSC Genome Browser, July 2003 Freeze). KDELC1 and BIVM are arranged in a head-to-head orientation, separated by only 42 bp. Both genes seem to be expressed ubiquitously [Kimata et al., 2000; Yoder et al., 2002]. KDELC1 is a small gene with 10 exons spanning ~15 kb and could be considered an interesting candidate because it contains a filamin domain. The amino acid change, Ile114Val, is in the filamin domain although Ile114 is not evolutionarily conserved because mouse and rat proteins contain the Val residue (Ensembl, http://www.ensembl.org), suggesting that the substitution is functionally neutral. However, mutations in a filamin protein (FILN1) cause failure of neurons to migrate to the cerebral cortex, the major defect in periventricular heterotopia, an X-linked dominant disorder [Fox et al., 1998]. Also, beta-filamin interacts with presenilin 1 and presenilin 2 and has been shown to bind to astrocytes in the normal brain and to neurite plaques, and other regions in brains of AD patients [Zhang et al., 1998].

BIVM contains 11 exons extending >42 kb and its predicted gene product is a basic protein with an immunoglobulin-like variable motif. It is likely that the immunoglobulin-like motif in BIVM has functions similar to an immunoglobulin but this remains to be experimentally confirmed. Although it is not clear whether immunoglobulins contribute to bipolar disorder susceptibility, altered Ig levels have been reported in patients with unipolar depression, acute mania and psychotic disorders [e.g., Legros et al., 1985; Bergquist et al., 1993; Wadee et al., 2002].

Given the intermarker distance and the SNP–SNP LD, it can be stated that a sufficient number of SNPs was used to interrogate the KDELC1 and BIVM region. Although the CNG collection showed significant evidence of LD in the haplotype, this finding was not recapitulated in the larger NIMH sample. If there is a susceptibility variant in this region and it exerts a minor effect, the number of families studied here would have insufficient power to detect association [Risch and Merikangas, 1996]. Data from the case-control analysis with almost 280 samples in each group did not show evidence of association. It is possible that there are disease-predisposing variants in other parts of 13q31-33, outside of the narrow region that we have examined. We are in the process of conducting a more comprehensive association screening of the linked region that includes D13S779 in order to test this hypothesis.

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