article

Stage II follow-up on a linkage scan for bipolar disorder in the Ashkenazim provides suggestive evidence for chromosome 12p and the *GRIN2B* gene

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Purpose: We had previously performed a genome-wide linkage scan for bipolar affective disorder in an Ashkenazi Jewish sample, a population likely to have reduced genetic heterogeneity. This study is a second stage follow-up focusing on regions that showed positive linkage scores in our previous scan but were not fine-mapped at that time. **Methods:** We genotyped an additional 145 highly polymorphic microsatellites and conducted linkage analyses using standard laboratory and analytical methods. **Results:** We saw an improvement of the evidence for linkage in most regions, with the most notable change on chromosome 12p13.1-p12.3, where the evidence of linkage is now suggestive. This region harbors the gene encoding the ionotropic glutamate receptor subunit 2B (*GRIN2B*), a gene that previously yielded evidence for association in a candidate gene study on 323 Ashkenazi Jewish bipolar case-parent trios. We find that the evidence for linkage is significantly correlated with the presence of the putative high-risk allele identified in our candidate gene study. **Conclusions:** Following up weaker signals can significantly improve linkage signals even after relatively small increases in information content. Our results on chromosome 12p support *GRIN2B* as a candidate gene for bipolar disorder that needs further investigation. ***Genet Med* 2007: 9(11):745–751.**

Key Words: *glutamate receptors, linkage disequilibrium, polymorphism (genetics), chromosomes, human, pair 12, chromosome mapping, genes, bipolar disorder*

Bipolar disorder (BP) is a common, disabling psychiatric disease with a lifetime prevalence reaching 2% (American Psychiatric Association, 1994). Several types of BP are recognized in the Diagnostic and Statistical Manual, fourth edition that vary in the nature and duration of symptoms and the extent of disability. One well-established diagnostic classification focuses on the intensity of manic symptoms recognizing two categories, BPI and BPII, with BPII showing episodes of less severe mania described as hypomania. Evidence from family, twin, and adoption studies strongly supports a genetic component of BP, with heritability estimates of 60–80%[1] and risk to first-degree relatives of 5–10%.[2] Although segregation analyses have been inconclusive, they suggest a complex and multigenic susceptibility to BP, involving both genes and environment. Many genome-wide linkage scans and three meta-analyses have been reported,[3–13] identifying multiple candidate genomic regions but with inconsistent replication across studies. One potential reason for this lack of consistency in linkage scan results could be the inconsistency in the definition of the affection status across studies, regarding both the diagnosis and the inclusion of BPI, BPII, and schizoaffective disorder, a condition in which patients present both psychotic and affective symptoms, and which some investigators consider a variant of schizophrenia and others a variant of BP. Another likely reason for the inconsistency in linkage results is the high likelihood of locus heterogeneity, especially if individual genes with small or moderate effects on risk are present or possibly genes that interact are involved. One strategy to reduce locus heterogeneity is to study families from a relatively genetically isolated population that originated from a small number of founders.[14,15] We recently employed this approach in a genome-wide linkage scan of 41 Ashkenazi Jewish (AJ) families with BP.[16] More recently we followed the same strategy in an extended sample of 323 AJ case-parent trios, performing a candidate gene association study.[17]

Typically initial genome scans for linkage utilize a marker density of about one marker for every 10 cM. Guo and Elston[18] proposed this approach as the first part of a two-stage design, where the initial low marker density is followed by more dense marker genotyping in genomic regions that show positive results in the first phase. In our original publication we performed a genome scan using one marker every 8.85 cM with

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follow-up of four regions that showed nonparametric linkage (NPL) scores ≥2.2. Here we report a second phase that involves genotyping an additional 145 polymorphic short tandem repeat markers in nine genomic regions (total length 456 cM on the decode linkage map[19]) that originally showed peak NPL scores between 1 and 2.2.

MATERIALS AND METHODS

The collection of the sample used in this study is described elsewhere.[16] It comprised 41 families (182 subjects with DNA available) ascertained through a proband with BPI disorder, with at least one additional participating relative with BPI or BPII. All recruitment methods and protocols for the collection of blood samples and clinical data were approved by the Johns Hopkins institutional review board, and appropriate informed consent was obtained from all human subjects. Information about each subject was ascertained from multiple sources including a direct evaluation. Two members of our consensus diagnostic committee (psychiatrists or doctoral-level clinical psychologists) independently reviewed all clinical information ascertained and made judgements about diagnoses and clinical dimensions (see Fallin et al. 2004[16]). We excluded families from these analyses if any grandparent of an affected subject was known to be of non-Ashkenazi descent. Among the total set of 41 families, affected individuals included those with a diagnosis of BPI (76.3%) or BPII (23.7%), and this is termed our broad diagnostic model. Twenty-six families had at least two members with BPI. Our narrow diagnostic model included 22 of these families that were informative for linkage when using BPI only as the affected phenotype.

We elected to fine-map all regions that showed a NPL score ≥1.0 in the original ~9 cM scan and that had not been fine-mapped in our original publication.[16] These were nine regions on chromosomes 2, 4, 6, 7, 9, 12, 14, 16, and 19. We obtained genotyping through deCODE Genetics (Reykjavik, Iceland, www.decode.com) with markers selected from a list presented by deCODE that included information on marker expected heterozygosities, call rates, and genetic locations under their recent high resolution linkage map.[19] We selected the most informative set of markers that could be inserted between previously typed markers at even spacing. We genotyped a total of 145 new markers with an average heterozygosity of 0.73. Every interval was evenly populated with about one marker every 2.4 cM increasing the density of the initial phase by 4-fold.

We prepared two 96-well DNA plates (186 DNAs) in our laboratory and sent them to deCODE for genotyping. There, three control individuals from the Centre d'études du Polymorphisme Humain pedigrees were added and the samples were diluted to 15 ng/μL. For each marker the forward primer was fluorescently labeled. Polymerase chain reaction (PCR) amplifications were set up on Zymark ALH 400 (Zymark Corp., Hopkinton, MA), run on MJR Tetrad thermal cycler (Bio-Rad Laboratories, Inc., Waltham, MA) and pooled on Gilson Cyberlab C200 robots (Gilson, Inc., Middleton, WI). Twenty nanograms of genomic DNA were amplified in the presence of 2 pmol of each primer, 0.25 U AmpliTaq Gold, 0.2 mM dinucleotide triphosphate, and 2.5 mM $MgCl_2$ (buffer was supplied by the manufacturer, Applera Corp., Norwalk, CT) in a 5-μL reaction. Thermal cycling conditions were denaturing at 95°C for 10 minutes, followed by 37 cycles of 94°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. The PCR products were supplemented with the internal size standard GS500-LIZ (Applera Corp.), and the pools were separated and detected on ABI-3730 Sequencers (Applera Corp.). Alleles were called automatically using DAC, an allele-calling program developed at deCODE and the program deCODE GT was used to fractionate called genotypes according to quality, and when necessary to edit. Statisticians from deCODE performed quality checks of the genotyping results by examination of plate-panel yield, marker yield, Hardy-Weinberg equilibrium, mismatch error rate, Mendelian error rate, and allele distribution across plates. Genotyping results were delivered to us in a (ASCII) tab-delimited file format including allele calls as binned, but also corresponding calls normalized to Centre d'études du Polymorphisme Humain individual 1347-02 genotypes, a reference sample present in three copies on all plates. An inheritance error report was also provided. Mendelian errors were identified using the PedCheck[20] and GENEHUNTER[21] analysis software, and GENEHUNTER was also used to identify markers showing recombination with both their flanking markers. In the first case, the family data for the marker were deleted, whereas in the latter case, marker data for the corresponding individual (the apparent double recombinant) were deleted. The genetic map used was that published by Kong et al.[19] Allele frequencies were estimated from the parental genotypes and linkage analyses were performed using GENEHUNTER as described in our original genome-wide scan publication.[16] Genotypes for single nucleotide polymorphism (SNP) rs1805539 for members of 30 families had been previously acquired as described,[17] while 25 affected members of the remaining 11 families were genotyped by PCR amplification of genomic DNA using the primers ATCCAGTGGGGAGAGCTAGG and CTGATTCATCCCAGGGCTTC followed by digestion of the 165-bp product with the restriction enzyme MwoI (recognition site: GCNNNNNNNGC) and electrophoresis on a 3% Nusieve—1% agarose gel for detection of the resulting fragments of 82 and 83 bp when the C allele was present in the context GTTTG[G/C]GAAGGGAGC.

RESULTS

We received data on all 145 markers and all 186 DNAs (no DNA failures), and after all error corrections our call rate was 98.9% (26,666 successful genotypes of 26,970 attempted). The new markers showed eight instances of double recombination and one Mendelian inconsistency that were considered likely genotyping errors and treated as described in the methods. The average information content in our nine regions rose from 0.8 to 0.86. Tables 1 and 2 summarize the parametric and nonparametric analysis results for the two diagnostic models. Under the broad model three regions on chromosomes 2, 6, and 19 showed small reductions in NPL scores ranging from −0.07 to −0.11 (total reduction −0.28 NPL points) while the scores

Table 1
Fine-mapping results for 41 multiplex BPI-BPII Ashkenazi pedigrees

	Model-free					Dominant[a]			Recessive[b]		
Chromosome	Loc. (cM)	Peak marker	Info. cont.[c]	NPL	NPL ▲ or ↓	cM	Max HLOD	α^d	cM	Max HLOD	α^d
Chromosome 2											
Before fine-mapping	151	D2S151	0.72	2.16 (0.016)	↓	153	1.54	0.61	139	0.72	0.28
Fine-mapping	155	D2S151	0.86	2.05 (0.021)		145	1.43	0.54	184	0.46	0.18
Chromosome 4											
Before fine-mapping	31	D4S419	0.74	1.89 (0.03)	▲	34	0.71	0.43	39	1.31	0.41
Fine-mapping	40	D4S3013	0.93	2.06 (0.02)		39	0.86	0.39	40	1.07	0.29
Chromosome 6											
Before fine-mapping	117	D6S276	0.74	2.07 (0.020)	↓	113	1.48	0.55	23	0.51	0.21
Fine-mapping	123	D6S262	0.89	1.97 (0.025)		123	1.17	0.45	23	0.51	0.21
Chromosome 7											
Before fine-mapping	4	D7S531	0.85	1.91 (0.028)		137	0.25	0.27	139	0.37	0.24
Fine-mapping	4	D7S531	0.85	1.91 (0.028)		140	0.19	0.2	131	0.53	0.24
Chromosome 9											
Before fine-mapping	9	D9S286	0.85	1.23 (0.11)	▲	6	0.69	0.37	157	1.23	0.39
Fine-mapping	17	D9S269	0.9	1.82 (0.035)		10	0.87	0.37	162	1.23	0.39
Chromosome 12											
Before fine-mapping	32	D12S364	0.87	1.08 (0.139)	▲	35	0.38	0.3	32	0.48	0.22
Fine-mapping	33	D12S373	0.85	1.8 (0.04)		33	0.80	0.39	27	0.84[e]	0.29
Chromosome 14											
Before fine-mapping	40	D14S288	0.82	1.36 (0.088)	▲	40	0.44	0.3	40	0.63	0.23
Fine-mapping	41	D14S288	0.81	1.47 (0.07)		41	0.49	0.31	41	0.68	0.24
Chromosome 16											
Before fine-mapping	42	D16S3068	0.79	1.2 (0.116)	▲	42	0.16	0.19	38	0.72	0.27
Fine-mapping	39	D16S3068	0.79	1.47 (0.07)		39	0.23	0.21	39	0.73	0.26
Chromosome 19											
Before fine-mapping	29	D19S226	0.81	1.34 (0.091)	↓	32	0.60	0.39	22	0.10	0.09
Fine-mapping	26	D19S588	0.84	1.27 (0.1)		34	0.55	0.4	22	0.09	0.09

[a]Dominant parametric HLODs assumed disease allele frequency = 0.005, and penetrances 0.65, 0.65, 0.0096 for homozygotes, heterozygotes, and noncarriers.
[b]Recessive parametric HLODs assumed disease allele frequency = 0.11, and penetrances 0.65, 0.0096, 0.0096.
[c]Information content at the peak marker.
[d]α = estimated proportion of linked families at this location.
[e]This score between D12S391 and D12S1581.
NPL, nonparametric linkage; HLOD, heterogeneity LOD score; ▲, increase in NPL score; ↓, decrease in NPL score.

for chromosome seven remained unchanged. The remaining five regions showed increases in NPL scores ranging from 0.11 to 0.72 (total increase 1.86 NPL points). Similarly, under the narrow model, chromosomes 6, 9, and 19 showed small reductions in NPL scores ranging from −0.04 to −0.08 points (total reduction 0.17 NPL points), while the remaining six showed stronger evidence with increases ranging from 0.05 to 0.68 (total increase 1.61 NPL units). The overall excess and higher magnitude of score increases is encouraging, but the absolute value of the resulting scores remained moderate with the exception of one region on chromosome 12p that improved significantly under both phenotypic models (0.72 and 0.68 NPL units under the broad and narrow model respectively). Most notably under the narrow model (BPI only), the NPL score reached 2.42 and the recessive multipoint heterogeneity LOD score (HLOD) reached 2.05, elevating this region into a "suggestive" result by standard criteria.[22] Interest in this region was further increased by the results of our recent association study of 64 candidate genes for BP and schizophrenia in AJ triads, that had identified the gene glutamate subunit 2B (*GRIN2B*) as being associated with BPI,[17] a finding further supported by an association study reported by Martucci et al.[23] for a SNP lo-

Table 2
Fine-mapping results for 22 multiplex BPI-restricted Ashkenazi pedigrees

	Model-free					Dominant[a]			Recessive[b]		
Chromosome	cM	Peak marker	Info. cont.[c]	NPL (p)	NPL ▲ or ↓	cM	Max HLOD	α^d	cM	Max HLOD	α^d
Chromosome 2											
Before fine-mapping	141	D2S112	0.72	1.85 (0.034)	▲	143	0.89	0.64	137	1.07	0.5
Fine-mapping	132	D2S347	0.87	1.9 (0.03)		133	0.58	0.51	133	0.96	0.46
Chromosome 4											
Before fine-mapping	43	D4S391	0.81	1.42 (0.079)	▲	57	0.23	0.29	36	0.48	0.38
Fine-mapping	37	D4S2620	0.91	1.86 (0.033)		49	0.341	0.32	40	0.375	0.25
Chromosome 6											
Before fine-mapping	164	D6S1581	0.64	1.61 (0.055)	↓	162	0.84	0.53	154	0.7	0.36
Fine-mapping	104	D6S278	0.9	1.57 (0.06)		173	0.83	0.49	164	0.821	0.32
Chromosome 7											
Before fine-mapping	143	D7S530	0.82	1.51 (0.067)	▲	145	0.22	0.33	128	0.82	0.51
Fine-mapping	171	D7S636	0.89	1.81 (0.037)		171	0.45	0.47	131	0.6	0.35
Chromosome 9											
Before fine-mapping	9	D9S286	0.83	1.55 (0.063)	↓	9	0.71	0.48	157	0.88	0.45
Fine-mapping	0	D9S288	0.86	1.5 (0.069)		0	0.52	0.36	162	0.88	0.45
Chromosome 12											
Before fine-mapping	32	D12S364	0.85	1.74 (0.0427)	▲	35	0.57	0.47	32	1.32	0.52
Fine-mapping	33	D12S373	0.83	2.42 (0.009)		33	1.14	0.59	28	2.05[e]	0.64
Chromosome 14											
Before fine-mapping	40	D14S288	0.82	1.54 (0.064)	▲	97	0.5	0.51	40	0.85	0.43
Fine-mapping	40	D14S288	0.81	1.57 (0.06)		95	0.52	0.53	41	0.89	0.44
Chromosome 16											
Before fine-mapping	61	D16S415	0.84	1.85 (0.034)	▲	61	0.39	0.43	61	0.57	0.35
Fine-mapping	57	D16S415	0.83	1.96 (0.03)		39	0.55	0.446	57	0.67	0.37
Chromosome 19											
Before fine-mapping	29	D19S226	0.83	2.06 (0.021)	↓	37	1.2	0.81	22	0.71	0.4
Fine-mapping	26	D19S588	0.86	1.98 (0.025)		37	1.25	0.87	22	0.67	0.38

[a]Dominant parametric HLODs calculated in Genehunter V2.0, assuming disease allele frequency = 0.005, and penetrances 0.65, 0.65, 0.0096 for homozygotes, heterozygotes, and noncarriers.
[b]Recessive parametric HLODs assumed disease allele frequency = 0.11, and penetrances 0.65, 0.0096, 0.0096.
[c]Information Content at the peak marker.
[d]α = estimated proportion of linked families at this location.
[e]This score between D12S391 and D12S1581.
NPL, nonparametric linkage; ▲, increase in NPL score; ↓, decrease in NPL score.

cated ~55 kb from our most significant SNP. *GRIN2B* is located at 13,186,503 Mb on chromosome 12p (UCSC March 2006 assembly) under our current linkage peak (Fig. 1). Furthermore, markers in *GRIN2B* have been associated with schizophrenia by many studies and supported by a recent meta-analysis.[24]

This convergence of evidence led us to examine the hypothesis that *GRIN2B* might be responsible for the observed linkage signal. We first examined whether the recessive genetic model supported by the linkage analysis also improved evidence of association. We did this by analyzing our existing genotype data for the associated SNP (rs1805539) using the Family-Based Association Test method[25] (http://biosun1.harvard.edu/~fbat/fbat.htm) that allows joint tests for association in the presence of linkage under different genetic models in a family based analysis. Consistently, the recessive model gave the most significant evidence, with a *P* value of 0.003 (the high-risk allele for SNP rs1805539 was allele C in the context GTTTG[G/C]GAAGG). The additive *P* value was 0.006, while the dominant model was not significant ($P = 0.13$). We then tested whether linkage was

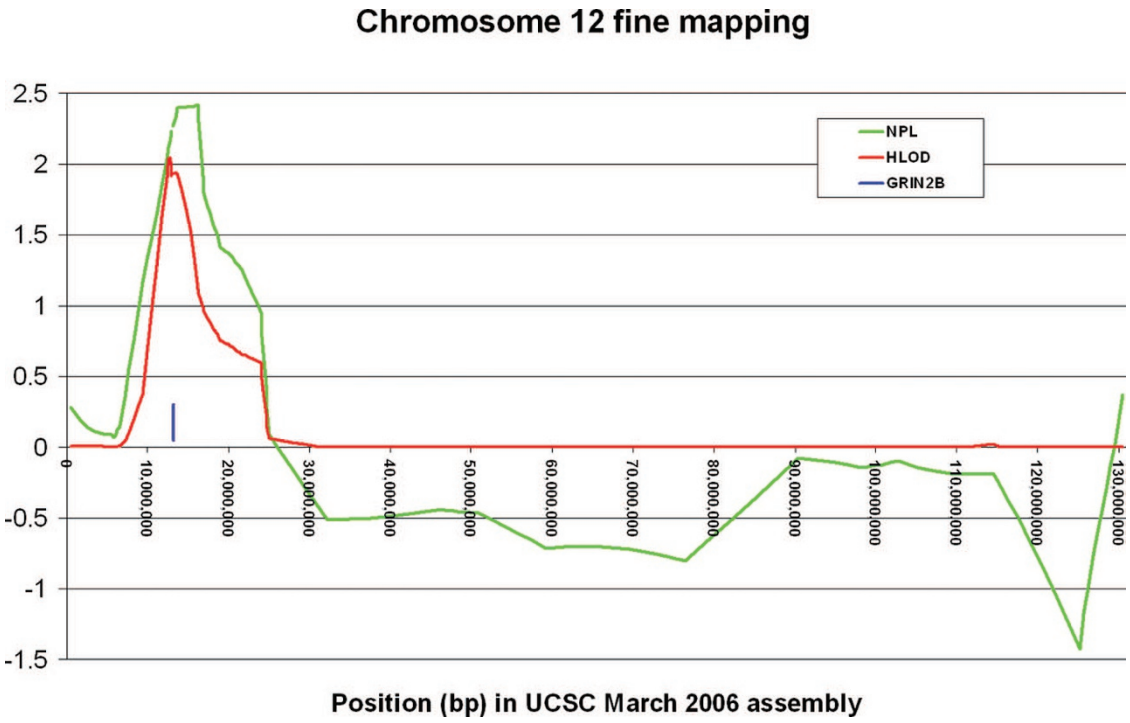

Fig. 1. Nonparametric linkage and heterogeneity LOD scores across chromosome 12. The location of *GRIN2B* is marked by a vertical bar.

Table 3
NPL scores for narrow and broad phenotype model

MODEL	Family genotypes	*N*	NPL
Narrow ($N = 22$)	All CC	8	3.15
	Not all CC	14	0.62
Broad ($N = 41$)	All CC	17	2.14
	Not all CC	24	0.24

MODEL, diagnostic model for the definition of affection status; Family genotypes refers to the risk allele "C" of the SNP rs1805539 among affected family members; *N*, number of families; NPL, nonparametric linkage.

stronger in families with homozygotes for this putative high-risk allele by separating the families in two groups; those in which all patients were homozygous for the C allele (group I; 8 and 17 families for the narrow and broad diagnostic models respectively) and the remainder (group II; 14 and 24 families). Under both phenotypic models the group I families showed increased evidence of linkage (Table 3), highest under the narrow phenotypic model consistently with our overall linkage results. This type of stratification of families creates a potential bias as the selection of homozygotes also selects for increased allele sharing. To avoid this bias, we used an analytical method recently described by Li et al.[26] called Genotype-IBD Sharing Test (GIST). This is a formal method for weighting families according to the members' genotypes and calculating the significance of the correlation between the linkage evidence and a hypothesized high-risk allele. We used the GIST software (http://phg.mc.vanderbilt.edu/content/gist) and a recessive genetic model to examine whether the risk allele correlated with our linkage results, as it would be expected if it accounted for the evidence for linkage according to Li et al.[26] The 41 informative pedigrees based on the broad diagnostic definition showed a significant correlation ($p_{rec} = 0.02$). The narrow phenotype model identifies only 22 informative families although those carrying the CC genotype showed increased evidence of linkage (Table 3), GIST analysis did not achieve statistical significance. This lack of statistical support despite the observed difference is likely to be due to the very small sample size for this model.

DISCUSSION

We present the results of a follow-up, fine-mapping study from our genome-wide short tandem repeat scan for genes controlling risk for BP. The original NPL scores of the regions we examined here ranged from 1.08 to 2.16. Although scores of this magnitude are often neglected, we show here that follow-up with a denser set of markers may yield more significant findings. In fact, most regions showed some increase in linkage scores. Moreover, these increases were in aggregate of greater magnitude than the decreases. The results of the fine-mapping performed for our original linkage study further support this observation. We had then fine-mapped four regions and all regions showed improvements in linkage scores (with the exception of chromosome 3 which under the broad phenotype model showed a slight decrease of 0.05). Although it is difficult to determine the probability that NPL score would increase by chance alone, it is very encouraging because under the null hypothesis of no true linkage, NPL scores should be equally

likely to increase or decrease with higher information content. In addition, the fine-mapping of moderate linkage signals allowed us to focus in a new genomic region and a specific gene (*GRIN2B*) for which we argue that there is now enough converging evidence to warrant further study. In view of these results, this project demonstrates that fine-mapping modest linkage signals might be of value for uncovering remaining linkages from an initial genome-wide screen. The weighing of the cost-benefit of additional fine-mapping, however, is a complicated matter that would need theoretical analyses and require many assumptions.

Our most interesting result was a large increase in NPL and HLOD scores at chromosome 12p12.3, where under the narrow diagnostic model the initial NPL score of 1.7 and the parametric (recessive) HLOD score of 1.32 increased to 2.4 and 2.05, respectively. The parametric linkage scores now reach the threshold for suggestive linkage.[22] Although the linkage evidence alone remains below genome-wide significance, it coincides with the location of a gene that provided one of the strongest association signals in our study of candidate genes in a larger AJ sample of BPI patient-parent trios. That sample of 323 trios included cases from 21 of the families used for this linkage analysis, but removing these overlapping families from the association study sample caused no significant change in the result (the previous $P = 0.006$ changed to 0.007). On the other hand, taking advantage of the partial overlap of these samples and using a method developed for this purpose by Li et al.,[26] we showed the linkage at this locus significantly correlated with the presence of the putative high-risk allele. Li et al.[26] have shown that a correlation between the presence of a high-risk allele defined from an association study with each family's linkage evidence is not expected under the null hypothesis of no true association, even when the same sample is used for linkage and association. Thus, the GIST result provides additional evidence joining together the linkage and association results supporting the involvement of *GRIN2B* in BPI. Further independent support is provided by a recent study reporting an association between *GRIN2B* variants and BP and schizophrenia,[23] a study by Riley et al.[27] in southern African Bantu-speaking families that provided weak linkage evidence and a recent linkage study that applied a variance components method on the age at onset of mania[28] and pointed to marker D12S1292 at 28,992,092 Mb, at the border of our linkage region (Fig. 1).

Despite the converging evidence suggesting that *GRIN2B* may be associated with high risk for BP, it is important to be cautious in the interpretation of our results. The statistical significance we achieve is at best moderate for both the linkage and its correlation with the putative susceptibility allele. This is especially true if one takes in to account the three genetic and two phenotypic models tested that although highly intercorrelated they still constitute multiple testing; thus, the significance of the reported NPL scores should be appropriately adjusted. The possibility of a false-positive result remains, and this possibility can only be resolved by further replication attempts or by experimentally demonstrating the gene's involvement in BP. We have mentioned two studies that support this locus for BP[23,27]; nevertheless, two recently published genome-wide association scans for BP do not show strong significance at this locus.[29,30] A number of reasons could be responsible for this lack of signal not excluding a false positive result. One obvious alternative explanation is the different ancestry of the studied populations. Our signal comes from an AJ population, while others have reported positive results on a Canadian[23] and an African[27] population. The signal from the Canadian sample was relatively weak for BP, and it is hard to assess whether differences of the British and United States samples used in the genome scans might be the reason for not detecting an association above the noise of the many thousands of SNPs examined. When attempting replication for this type of study, it is necessary to minimize differences in study design including the population ancestry. Given the limitations, we mentioned above we believe that attempting such a replication for *GRIN2B* is very important.

GRIN2B is located in a gene-sparse region with intervals larger than 180 kb devoid of known genes on either side. Although linkage disequilibrium is slightly more extended in the Ashkenazim,[31] the nearest gene is beyond the limits of detectable linkage disequilibrium. Thus, although we do not know whether the observed association could be due to variation in *GRIN2B*, neighboring unknown genes or regulatory elements, the functional relevance of *GRIN2B* make it the primary candidate and it should be followed up further. *GRIN2B* encodes a subunit of the NMDA receptor. NMDA receptors are ionotropic glutamate receptors, heteromeric pentameters composed of at least one NR1 subunit and one or more of the four different NR2 subunits: NR2A, NR2B (the product of *GRIN2B*), NR2C, and NR2D.[32] The different isoforms of the NR2 subunit give rise to structurally different glutamate receptors in the brain. In addition to glutamate, the NMDA receptor requires allosteric binding of glycine, to a site on the NR1 subunit. The NR2B subunit also has a binding site for polyamines that modulate the functioning of the receptor.[33] NMDA receptor antagonists, phencyclidine, and ketamine, induce a constellation of behavioral effects in healthy individuals, mimicking the positive, negative, and cognitive symptoms of schizophrenia.[34,35] Such findings have led to the hypothesis that NMDA receptor-mediated decreases in function may increase the risk for schizophrenia. Support for this hypothesis is provided by studies that have shown differences in NMDA receptor subunits expression patterns in schizophrenic brains[36,37] and by the observation that the GRIN2B subunit increases about 1.2-fold after subchronic administration of the antipsychotic drug clozapine.[38] DNA variants in and around the *GRIN2B* gene have been reported to be associated with increased risk of schizophrenia[39–41] or the effect of clozapine treatment.[42,43] Moreover, lithium administration reduces the level of NR2B phosphorylation at Tyr1472 and that this is temporally associated with its neuroprotective effect.[44]

An overlap in genetic susceptibility for schizophrenia and BPI has been argued by many investigators and was recently reviewed by Craddock et al.[45] We agree that there is strong evidence for an overlap of genetic factors contributing to these

two phenotypes, and we recently published a candidate gene association study[17] that investigated 440 SNPs in 64 functional and positional candidates for both disorders, genotyped in two sets of triads of AJ descent ascertained for either BPI or schizophrenia. Although at that time studies had suggested *GRIN2B* only as a candidate for schizophrenia,[40,41] in our study, it was among the best findings for BPI. More recently, a study by Martucci et al.[23] also detected an association between *GRIN2B* DNA variants and both BP and schizophrenia, and a meta-analysis supported the association with schizophrenia.[24] Our current results provide additional support for the involvement of *GRIN2B* in BP and indicate that this gene merits further study.

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