



SHORT COMMUNICATION

Additional support for linkage of schizophrenia and bipolar disorder to chromosome 3q29

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Received 9 August 2006; received in revised form 15 January 2007; accepted 24 January 2007

KEYWORDS

Schizophrenia;
Bipolar disorder;
Linkage;
Chromosome 3q29

Abstract After publishing a genome scan and follow-up fine mapping, suggesting schizophrenia and bipolar disorder linkage to chromosome 3q29, we now genotyped 11 additional SNPs (single nucleotide polymorphisms), in order to narrow down a potential candidate region. Linkage was performed using the GENEHUNTER program version 2.1r3. A NPL score Z_{all} of 3.891 ($p=0.000156$) was observed with SNP rs225. In short, we found significant linkage scores most telomeric on chromosome 3q29, spanning 3.46 Mbp (7 SNPs).

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1. Introduction

Within a recently published genome scan (Bailer et al., 2002), we found suggestive evidence for linkage of schizophrenia and bipolar affective disorder with marker D3S1265 (NPL score $Z_{\text{all}}=3.74$, $p=0.0003$), mapping to chromosome

3q29. Since genome-wide scans typically employ 300–600 markers (average marker-to-marker intervals of 10 cM or 5 cM, respectively), linkage peaks could be situated right between the markers genotyped within genome scans. Consequently, we established a follow-up fine mapping of the 3q29 region by linkage study, genotyping five SNPs (single nucleotide polymorphisms) spanning 4.14 Mbp at the telomere of chromosome 3 (p -values of 0.0321 with SNP rs1835669 and 0.0556 with SNP rs2341399, respectively; Schosser et al., 2004).

Since the space between linkage peaks within this follow-up fine mapping was up to 2.69 Mbp, genotyping further SNPs in between linkage peaks was essential, in order to narrow down a potential candidate region. Within the current work,

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Table 1 SNP-IDs, map position of all SNPs (from centromeric to telomeric) used for statistical analyses and of marker D3S1265 (marker of strongest linkage of our recently published genome scan, Bailer et al. 2002; not included into current statistical analyses), primer pairs and restriction enzymes used are shown (the details of primer cycling conditions, restriction enzyme conditions etc. are available on request to the authors)

SNP-ID	Position	Primer pair	Enzyme
rs2141610	190.78 Mbp	5'-AGGACCTGGAATGCAACAGATT-3' 5'-TCCTGCCTGTCCAATATTCTCA-3'	<i>Stu I</i>
rs3774005	191.60 Mbp	5'-TGTCTTCAACTTCAAAGCTTAGTCCTC-3' 5'-TGCTGTCAGTAGGTGGGATAATTA-3'	<i>Eco RV</i>
rs2293378	192.60 Mbp	5'-TTGGACCTTTTCTGCCACTG-3' 5'-CATTAGCAAACCTCCATTTC-3'	<i>Dde I</i>
rs2292160	193.87 Mbp	5'-GCCTTCTCACGGATCTGTCC-3' 5'-GAAGAGCCTGGGGTGGGTA-3'	<i>Dde I</i>
rs1223989	195.60 Mbp	5'-GCTGAGGGGACTCTACTGTGC-3' 5'-AAACAACGCGCTGTCCGAG-3'	<i>Stu I</i>
rs225	196.06 Mbp	5'-CTACGATAATTAGGTTTGGCAGTGAG-3' 5'-CAGACACCCCTTTCTTGCATATCC-3'	<i>Stu I</i>
D3S1265	197.01 Mbp		
rs3747672	197.10 Mbp	5'-CTCCAGTCGCTTTCCACTGAAC-3' 5'-GACAGACAGCACCAAATGGTCA-3'	<i>BsrI</i>
rs1357289	197.50 Mbp	5'-AGGAGTTTCCAGGGGAAAAA-3' 5'-TTGCTCAGCTTTGCCATCC-3'	<i>Sac II</i>
rs522174	198.20 Mbp	5'-CCTAAGAAGAGACTAAGCAAAGCAGATG-3' 5'-CGTGGAGAATGGTGAACATACTCC-3'	<i>Mlu I</i>
rs538885	199.01 Mbp	5'-GTGGCGGCTGCCTGTAGT-3' 5'-GAACGCTGGAACGCTTTTCTTC-3'	<i>BsrDI</i>
rs2306439	199.06 Mbp	5'-CTGCCCTGCAGTATGAGGAG-3' 5'-ACGGCCCTTTTATGGTTCAC-3'	<i>Dde I</i>

Restriction endonuclease cleavage was used to distinguish between the two alleles of SNPs. SNP = single nucleotide polymorphism, position = map position on chromosome 3, Mbp = mega-base pairs, enzyme = restriction enzyme.

we have analyzed a total of 11 additional SNPs. Statistical analyses have also been conducted including the 5 SNPs of our follow-up fine mapping.

2. Experimental procedures

2.1. Subjects

Within this study, DNA of the same family sample as in the recently published genome scan (Bailer et al., 2002) and follow-up fine mapping (Schosser et al., 2004) was genotyped. Five pedigrees with schizophrenic index patients and three pedigrees with index bipolar disorder patients ($N=86$; 50 of them were available for genotyping) were investigated in Vienna as described (Schosser et al., 2004). Hospitalized and outpatient individuals with a DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association, 1987) diagnosis of schizophrenia (10 patients with schizophrenia and 7 patients with schizoaffective disorder) or bipolar disorder (4 patients with bipolar disorder and 8 patients with major depressive disorder) were identified as index patients as described (Schosser et al., 2004). All participants gave written informed consent. The study was approved by the ethical committee of the Faculty of Medicine at the University of Vienna.

2.2. Diagnostic procedure

The diagnostic process was conducted as described (Schosser et al., 2004) and blind consensus diagnoses were made by at least two independent psychiatrists according to DSM-III-R Axes I and II without knowledge of marker status or family relationship.

2.3. Laboratory work and genotyping

After obtaining written informed consent, 20 ml of venous blood from 50 participating subjects was collected in vacutainers containing EDTA (ethylenediamine-tetraacetic acid). High molecular weight DNA was extracted from blood, using the Nucleon BACC Genomic DNA Extraction Kit (Amersham Biosciences), and was used for genotyping.

SNPs were selected by means of 'NCBI-GENBANK' (<http://www.ncbi.nlm.nih.gov>). The primer pairs and restriction enzymes used are shown in Table 1.

Restriction endonuclease cleavage was used to distinguish between the two alleles of SNPs. Exchange of a single nucleotide within this recognition site generates or abolishes a restriction enzyme recognition site.

2.4. Statistical analysis

Linkage was performed using the GENEHUNTER program version 2.1r3 to compute both the usual parametric logarithm of the likelihood of linkage (LOD) scores (a dominant model was used as described; Schosser et al., 2004), as well as the nonparametric multipoint linkage (NPL) score Z_{all} (for details, see Kruglyak et al., 1996). For further details see Schosser et al. (2004). The map positions of the markers were derived from 'NCBI-GENBANK Build 35.1'. The map positions of all SNPs used for statistical analysis are listed in Table 1. The map positions of all SNPs in relation to D3S1265 (marker of strongest linkage of our recently published genome scan, Bailer et al., 2002; not included into current statistical analyses) are shown in Table 1.

Linkage analyses were performed not only for the 11 SNPs genotyped within this study, but also added the 5 SNPs (located in

Table 2 The results of schizophrenia (SCZ) and bipolar disorder (BP) families combined (SCZ and BP) and subset analyses are shown; the 11 genotyped SNP markers are listed from centromeric to telomeric (position in mega-base pairs, see Table 1)

SNP	SCZ and BP			BP			SCZ		
	LOD	NPL	<i>p</i> -value	LOD	NPL	<i>p</i> -value	LOD	NPL	<i>p</i> -value
SNP 38 (rs2141610)	-0.1212	1.2695	0.106686	0.1282	1.0663	0.132507	-0.2494	0.7798	0.218140
SNP 35 (rs3774005)	-0.0283	1.3073	0.100023	0.1394	1.1043	0.132507	-0.1677	0.7982	0.218140
SNP 29 (rs2293378)	-0.0439	1.3563	0.092019	0.1208	1.0549	0.134949	-0.1648	0.8985	0.181519
SNP 37 (rs2292160)	0.6394	2.2485	0.016285	0.6611	1.9919	0.033020	-0.0217	1.3013	0.102295
SNP 33 (rs1223989)	1.4705	3.8608	0.000176	1.3917	3.9011	0.001038	0.0788	1.8617	0.031433
SNP 31 (rs225)	0.7680	3.8910	0.000156	1.4830	4.0465	0.000549	-0.7150	1.7873	0.042786
SNP 28 (rs3747672)	1.3446	3.8814	0.000157	1.5756	4.0406	0.000549	-0.2310	1.7797	0.042786
SNP 26 (rs1357289)	1.4689	3.8759	0.000167	1.6025	4.0229	0.000671	-0.1336	1.7865	0.042786
SNP 32 (rs522174)	1.8636	3.8016	0.000204	1.6433	3.9850	0.000671	0.2202	1.7219	0.052673
SNP 34 (rs538885)	1.9998	3.8233	0.000189	1.6803	3.9339	0.001038	0.3195	1.7889	0.042786
SNP 30 (rs2306439)	1.9959	3.8083	0.000203	1.6848	3.9269	0.001038	0.3111	1.7754	0.042786

LOD = logarithm of the odds score, NPL = Non-Parametric Lod score.

between SNP rs2292160 and SNP rs538885) of our previous follow-up fine mapping (Schosser et al., 2004), that is to say 16 SNPs all in all (9 SNPs situated centromeric and 7 situated telomeric of D3S1265).

In addition, linkage analyses were conducted for bipolar and schizophrenia families combined on one hand, and separately on the other hand.

3. Results

Within the current study, among the 11 SNPs genotyped (Table 2), a NPL score Z_{all} of 3.891 was observed with SNP rs225, corresponding to $p=0.000156$. In the parametric analysis of SNP rs225, a LOD score of 0.768 resulted. The five SNPs adjoining telomeric and one SNP adjoining centromeric showed NPL scores ≥ 3.802 , corresponding to

p -values ≤ 0.0002 . In short, we found significant linkage scores most telomeric, spanning a region of 3.46 Mbp (Figs. 1 and 2).

Subset analyses of the bipolar disorder and schizophrenia families separately (see Table 2) resulted in NPL scores ≥ 3.901 (corresponding to p -values ≤ 0.001) with SNPs spanning a region of 3.46 Mbp among bipolar disorder families (linkage signals substantially arising from 2 families), and NPL scores ≥ 1.722 (corresponding to p -values ≤ 0.03) with SNPs spanning a region of 3.46 Mbp among schizophrenia families (linkage signals substantially arising from 2 families).

In addition, statistical analyses were conducted adding the 5 SNPs of our recently published follow-up fine mapping

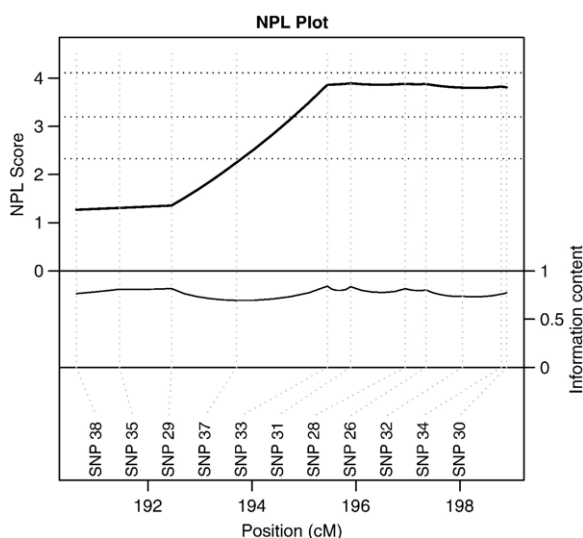


Figure 1 Significant NPL scores (Non-Parametric Lod scores) of schizophrenia and bipolar disorder families combined were found most telomeric at chromosome 3q29, spanning 3.46 Mbp. The line below the zero line shows information content mapping, with maximum information content at the zero line. x-axis: position in Mbp (mega-base pairs) from centromeric to telomeric (derived from 'NCBI-GENBANK Build 35.1'); y-axis: NPL score.

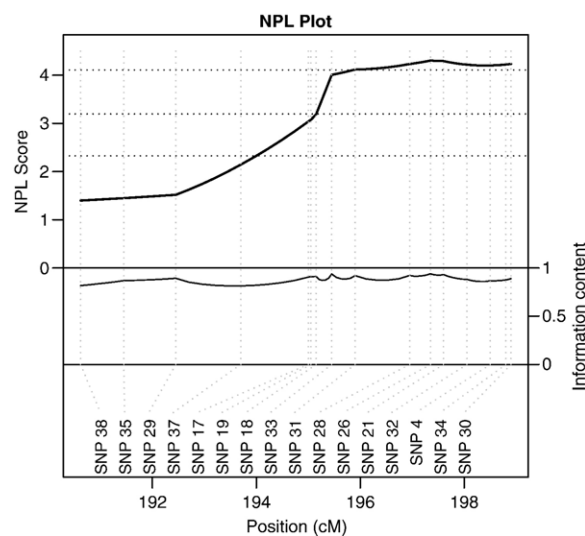


Figure 2 Previous markers (Schosser et al., 2004) are included: SNP 17 (rs1873405), SNP 19 (rs2368036), SNP 18 (rs2368041), SNP 21 (rs2341399), and SNP 4 (rs1835669). Significant NPL scores (Non-Parametric Lod scores) of schizophrenia and bipolar disorder families combined were found most telomeric at chromosome 3q29, spanning 3.46 Mbp. The line below the zero line shows information content mapping, with maximum information content at the zero line. x-axis: position in Mbp (mega-base pairs) from centromeric to telomeric (derived from 'NCBI-GENBANK Build 35.1'); y-axis: NPL score.

(Schosser et al., 2004), located in between SNP rs2292160 and SNP rs538885. That is to say, calculations included 16 SNPs all in all (Fig. 2; results not shown in detail). A NPL score Z_{all} of ≥ 4.006 (corresponding to $p \leq 0.0001$) was observed within the same region of 3.46 Mbp on chromosome 3q29.

4. Discussion

Kelsoe et al. (2001) reported suggestive evidence for linkage to 3q27 in bipolar families; meanwhile the marker of highest linkage is known to be located further telomeric on chromosome 3q, strictly speaking 6.1 Mbp centromeric of marker D3S1265 (Bailer et al., 2002).

Badenshop et al. (2002) found evidence for a bipolar disorder susceptibility locus at 3q25–26 (with a genome-wide maximum score of 2.49 at D3S1279). However, within our schizophrenia and bipolar disorder genome scan (Bailer et al., 2002), we found a NPL score Z_{all} of 0.80 (LOD=1.13; $p=0.21$) with D3S1279. Dick et al. (2003) yielded a maximum LOD score of 2.54 with a marker located on chromosome 3q22.3 among families segregating for bipolar disorder and schizoaffective disorder (bipolar type). Klei et al. (2005) reported on linkage analysis of a completely ascertained population of familial psychosis derived from the oceanic nation of Palau, suggesting that two regions of the genome harbor genetic variants affecting liability in most families, 3q28 (LOD=3.03) and 17q32.2 (LOD=2.80). Camp et al. (2001) published a genome-wide multipoint linkage analysis of seven extended Palauan pedigrees with schizophrenia, observing four regions of interest across the genome, including chromosome 3q24–28 as hint region (LOD=2.0). The Japanese Schizophrenia Sib-Pair Linkage Group (2003) conducted a genome-wide scan for linkage with schizophrenia sib-pair linkage group (JSSLG) families, among other things finding a nominal p -value < 0.05 on chromosome 3q23. A genome scan among schizophrenia kindreds from Daghestan isolates has been conducted by Bulayeva et al. (2005), indicating positive linkage findings on chromosome 3q24, as well as on chromosome 3q13.1–13.3 (the latter were suggested to be most probably related to the genetic polymorphism of the dopamine 3 receptor gene, DRD3). Maziade et al. (2005) conducted a dense genome scan in Eastern Quebec families and found suggestive linkage of both schizophrenia and bipolar disorder with marker D3S2418, which is located 3.2 Mbp centromeric of D3S1265 (Bailer et al., 2002) on chromosome 3q29 (they reported another suggestive linkage peak at 3q21). A number of recent papers (e.g. Berrettini, 2003) have discussed a potential for shared linkage/aetiology for schizophrenia and bipolar disorder. In addition, a 3q29 microdeletion syndrome has been described (Willatt et al., 2005; Baynam et al., 2006). The clinical phenotype is variable and includes a mild-to-moderate mental retardation; autism was noted in at least two of six patients. The deletion (~1.5 Mbp in length) encompasses 22 genes, including *PAK2* and *DLG1* (autosomal homologues of two known X-linked mental retardation genes, *PAK3* and *DLG3*), and is located within our identified schizophrenia and bipolar disorder linkage region.

Within the current study, we found significant linkage scores within the chromosome 3q29 telomere region, spanning 3.46 Mbp, thus supporting our previous 3q29 linkage findings (Bailer et al., 2002; Schosser et al., 2004). Since we

have genotyped the same family sample as in our previous studies, the current results are to be confirmed within an independent sample.

Acknowledgement

This study was supported in part by the European Science Foundation (ESF) Program on Molecular Neurobiology of Mental Illness, "Hochschuljubiläumsstiftung" in Vienna 1993, Austrian National Bank (Grant No. 5777) and "Fonds zur Förderung der wissenschaftlichen Forschung" (FWF) in Vienna (Grant No. 7639).

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