

## Rapid Publication

# Genome-Wide Scan in Portuguese Island Families Implicates Multiple Loci in Bipolar Disorder: Fine Mapping Adds Support on Chromosomes 6 and 11

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As part of an extensive study in the Portuguese Island population of families with multiple patients suffering from bipolar disorder and schizophrenia, we performed an initial genome-wide scan of 16 extended families with bipolar disorder that identified three regions on chromosomes 2, 11, and 19 with genome-wide suggestive linkage and several other regions, including chromosome 6q, also approached suggestive levels of significance. Dick et al. [2003: *Am J Hum Genet* 73:107–114] recently reported in a study of 250 families with bipolar disorder a maxLOD score of 3.61 near marker D6S1021 on chromosome 6q. This study replicates this finding having detected a peak NPL = 2.02 ( $P = 0.025$ ) with the same marker D6S1021 (104.7 Mb). Higher-density mapping provided additional support for loci on chromosome 6 including marker D6S1021 with an NPL = 2.59 ( $P = 0.0068$ ) and peaking at marker D6S1639 (125 Mb) with an NPL = 3.06 ( $P = 0.0019$ ). A similar pattern was detected with higher-density mapping of chromosome 11 with an NPL = 3.15 ( $P = 0.0014$ ) at marker D11S1883 (63.1 Mb). Simulations at the density of our fine mapping data indicate that less than 1 scan out of 10 would find two such scores genome-wide in the same scan by chance. Our findings provide additional support for a susceptibility locus for bipolar disorder on 6q, as well as, suggesting the importance of denser scans. Published 2004 Wiley-Liss, Inc.†

## INTRODUCTION

Bipolar disorder is characterized by periods of mania most often alternating with periods of depression. More than half of the bipolar patients in our studies also suffer psychotic symptoms. Bipolar disorder affects approximately 1% of the world population and has long been noted to demonstrate a strong familial pattern [Tsuang and Faraone, 1990]. Over the last 10 years, we have developed the Portuguese Island Collection to study the genetics of bipolar disorder and schizophrenia in a relatively homogeneous population [Pato et al., 1997]. Geographically and genetically isolated populations have played a key role in disease gene identification [Peltonen, 2000]. Focused on the Azorean and Madeira Islands, this study has benefited from the unique parallel history of these two archipelagoes (reviewed in Sklar et al. [2004]). Despite their relative isolation, the islands are served by modern centralized health systems, and we have established close collaborations with all of the clinicians serving this population. We have recently reported linkages to chromosome 5q and 8p in schizophrenia in this population [Sklar et al., 2004]. The strongest linkage was found on chromosome 5q. This region has recently received strong support from a meta-analysis of 20 genome-wide scans [Lewis et al., 2003].

Our initial studies of bipolar disorder in this population detected several families segregating an expansion mutation at the SCA8 locus on chromosome 13q21 [Pato et al., 2000]. In order to identify additional risk loci, a genome-wide scan of bipolar disorder was performed on families from the Azores, Madeira, and the city of Coimbra on the Portuguese mainland.

## MATERIALS AND METHODS

### Subjects

Families with two or more affected individuals were ascertained from systematic screening of all treating clinicians, treatment facilities, social services, and extensive family interviews. In the Azores, all four psychiatric hospitals and the two general hospitals participated in the study. Similarly in Madeira, both psychiatric hospitals and the general hospital participated. On the mainland, families were identified by our collaborators at the University of Coimbra. Informed consent was obtained in writing from all subjects for participation in the genetic and family studies. Collection of blood and family history information was approved by all of the appropriate

Grant sponsor: National Institute of Mental Health (to C.N.P. and M.T.P.); Grant numbers: MH52618, MH058693; Grant sponsor: Canadian Institutes for Health Research (to J.L.K.); Grant sponsor: NARSAD awards (to C.N.P., M.T.P., and J.L.K.).

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Received 26 November 2003; Accepted 26 November 2003

DOI 10.1002/ajmg.b.30001

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Institutional Review Boards. For our population isolate studies, families were ascertained if both of the proband's parents and all four of the grandparents were native to the islands. Diagnoses were made from data obtained with the Diagnostic Interview for Genetic Studies (DIGS) [Nurnberger et al., 1994]. Interviews were performed at the site of the subjects clinical care, in their homes, or at an alternate site of the subject's choosing. Interviewers were highly trained psychiatrists, psychologists, or social workers. Inter-rater reliability was assured by careful training and evaluation of each interviewer prior to study onset and annually throughout. Periodically, reliability was assessed by having a set of 12 subjects assessed independently by interviewers from different study sites and by a senior supervisor. The chance corrected inter-rater agreement,  $k$ , was high for the diagnoses of schizophrenia, bipolar disorder, and schizoaffective disorder ( $k = 0.9-0.94$ ). Items related to the diagnosis of schizotypy showed slightly less agreement ( $k \sim 0.75$ ). Thorough clinical narratives were completed for all subjects. Best estimate diagnoses according to DSM-IV were made by two independent blinded researchers after review of clinical information, DIGS, Operational Criteria Checklist for Psychotic Illness (OPCRIT) [McGuffin et al., 1991] and written narratives. All cases where there was disagreement were reviewed by a third senior psychiatrist blind to the status of the case (MT Pato, MD).

### Genotyping

Genomic DNA was extracted from whole blood according to standard protocols. A genome-wide screen was performed using a modified set of the Cooperative Human Linkage Centre Screening Set, version 6.0. Fluorescently labeled markers were detected on an ABI-3700 and allele sizes were called using Genotyper v. 3.6 software. The marker map positions were based on the Marshfield map. PEDMANAGER software was used for allele binning and inheritance analysis. For fine mapping on chromosomes 6 and 11, we genotyped an additional 14–16 microsatellite markers in each region with an average inter-marker distance of 4.2 cM.

### Data Analysis

Non-parametric multi-point linkage analysis of the data was performed using GENEHUNTER 2.0 [Markianos et al., 2001]. To establish threshold for suggestive and significant genome-wide linkage, simulation were performed. These simulations were performed using GENSIM (MJD, unpublished), a pedigree genotype simulator that attaches to GENEHUNTER [Rioux et al., 2000; Hirschhorn et al., 2001; Laitinen et al., 2001] that matched our dataset in relevant variables.

## RESULTS

Families were chosen for genotyping based on the presence of at least an affected relative pair appropriate for linkage analysis. All families that had been completely evaluated at the time of this study were included for genotyping. This initial genome scan of bipolar disorder includes 16 families with an average of 2.9 affected: 10 families were from the Azorean Islands, 2 from Madeira, and 4 from Coimbra on the Portuguese mainland (Table I).

A single narrow phenotypic model was chosen that included only bipolar disorder-type 1 and schizoaffective disorder-bipolar type. Genotyping data were available for analysis for 102 family members of which 47 members were affected with either bipolar disorder ( $n = 46$ ) or schizoaffective disorder-bipolar ( $n = 1$ ) for 366 microsatellite markers with an average inter-marker spacing of 9.6 cM.

Non-parametric multi-point linkage analysis of the data was performed using GENEHUNTER 2.0 [Markianos et al., 2001].

TABLE I. Families Segregating for Bipolar Disorder

No. affected/family	Families	Affected
2	6	12
3	6	18
4	3	12
5	1	5
Totals	16	47

Nominally significant  $P$ -values were obtained in seven regions (see Fig. 1 and Table II). The maximum NPL was located at D11S987 (NPL = 2.58,  $P = 0.007$ ). Recent evidence suggests that thresholds established assuming full pedigree and genotyping information are overly conservative under the experimental conditions encountered in a typical primary 10 cM genome scan with respect to marker density, percentage of missing genotypes, and availability of parental information [Wiltshire et al., 2002]. In order to estimate the strength of the observations of linkage in our initial 10 cM genome scan, we performed genome-wide simulations under the model that no genes were present that affected disease risk. We designed simulations that matched our real data in all relevant variables: family structure, individuals available for genotyping, percentage of genotypes achieved in those individuals available for genotyping, marker spacing, and marker heterozygosity. These simulations were performed using GENSIM (MJD, unpublished), a pedigree genotype simulator that attaches to GENEHUNTER [Rioux et al., 2000; Hirschhorn et al., 2001; Laitinen et al., 2001]. One hundred complete genome scans were simulated and these show that the threshold for suggestive linkage (i.e., the level of linkage expected once per genome scan by chance) is an NPL = 2.2 and the threshold for declaring significant linkage (i.e., linkage expected once per 20 genome scans by chance for a genome-wide  $P = 0.05$ ) is NPL = 3.0.

We initiated follow up studies of our original bipolar linkage signals focusing on chromosomes 6 and 11 because these two regions also showed linkage signals in this population with the phenotype of psychosis. We genotyped an additional 14–16 microsatellite markers in each region with an average inter-marker distance of 4.2 cM. The higher-density mapping provided additional support for loci on chromosome 11 with a maximum NPL = 3.15 ( $P = 0.0014$ ) at marker D11S1883 (63.1 Mb) (Fig. 2). A similar pattern was detected with higher-density mapping of chromosomes 6 including marker D6S1021 with an NPL = 2.59 ( $P = 0.0068$ ) and with a maximum NPL = 3.06 ( $P = 0.0019$ ) at marker D6S1639 (125 Mb).

Simulations were performed as described above incorporating the increased density of markers studied in chromosomes 6 and 11 and indicated an NPL of 3.6 represents genome-wide significance, and an NPL of 2.8 would be suggestive of linkage. Simulations at the density of our fine mapping data indicate that less than 1 scan out of 10 would find two such scores genome-wide in the same scan by chance.

## DISCUSSION

No consistently replicated linkage has been found for bipolar disorder. A recently completed meta-analysis by Segurado et al. [2003] failed to identify significant linkage in a combined data set from 18 genome scans for bipolar disorder. An up to date review of both positive and negative findings and the limitations of meta-analytic methods are also summarized in this same report [Segurado et al., 2003]. More recently, Dick et al. [2003] reported, in a study of 250 families with bipolar disorder, a maximum LOD score of 3.61 (genome-wide  $P < 0.05$ ) at 114

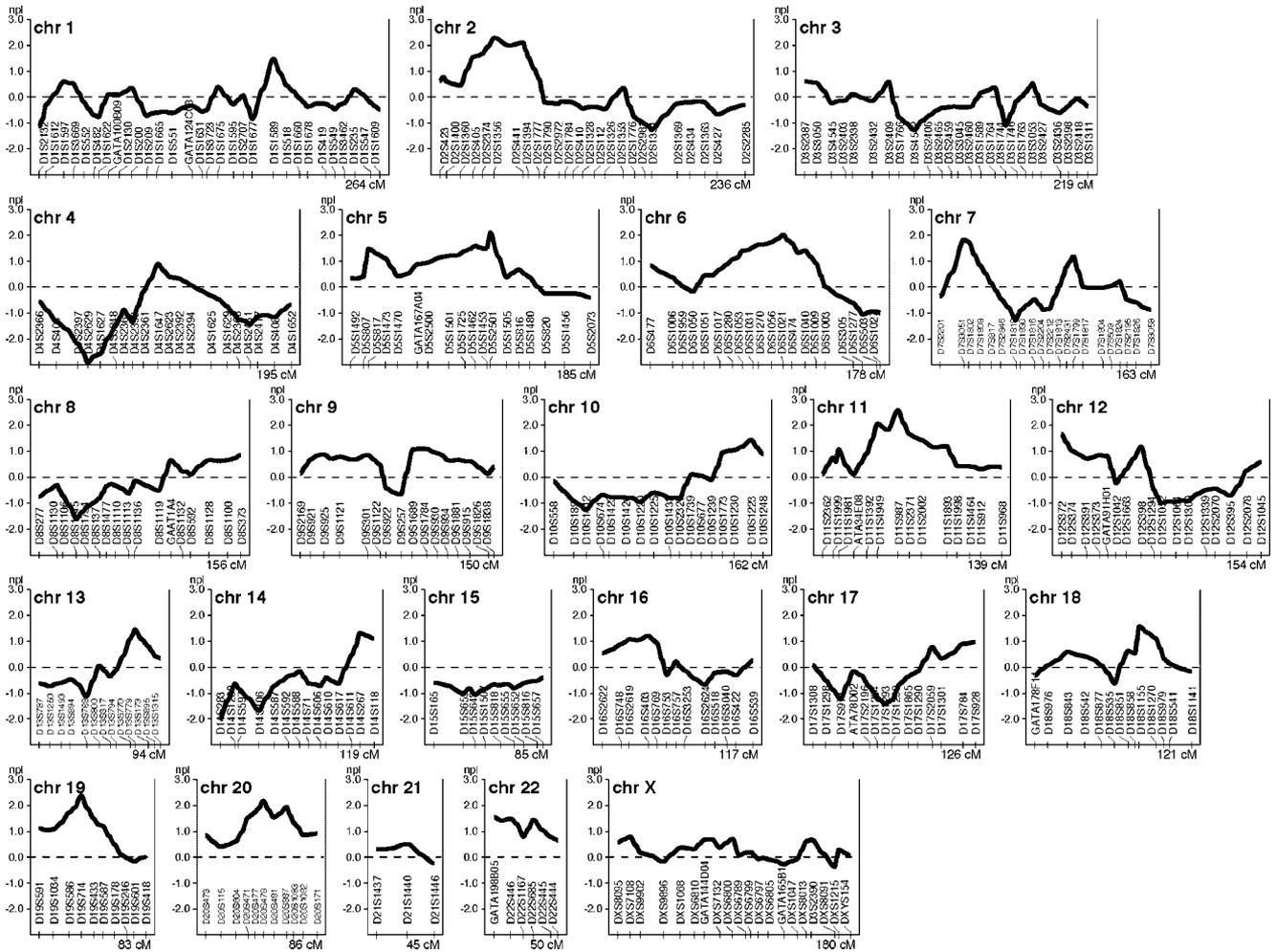


Fig. 1. Non-parametric multi-point linkage analysis by GENEHUNTER 2.0 in bipolar disorder. For each chromosome, the total genetic length is shown below the x-axis. Tick marks indicate the position of the microsatellite markers. The multipoint NPL scores (y-axis) for bipolar disorder.

cM near marker D6S1021 on chromosome 6q. Subsequent corrections for 12 identical twins lowered the LOD score to below genome wide significance. However, the analysis of the combined waves 1–3 of the NIMH collaborative yielded a maxLOD score of 3.8, which again achieves genome wide significance (personal communication, John Nurnberger). A study by Ewald et al. [2002], also identified a linkage signal on 6q with a maximum LOD of 2.59 ( $P=0.00035$ ) at marker D6S1021. Our study supports these findings having detected

an NPL = 2.02 ( $P=0.025$ ) with the same marker D6S1021 (104.73 Mb), in our low-resolution genome-wide scan. Though achieving nominal significance, this region would not have been considered as achieving even suggestive level of significance genome-wide. However, our fine mapping of this region provided additional support for a number of loci on chromosomes 6 including marker D6S1021 with an NPL = 2.59 ( $P=0.0068$ ) and a maximum NPL of 3.06 ( $P=0.0019$ ) at marker D6S1639 (125 Mb). This highlights one of the limitations of meta-analysis using genome scans at 10 cM spacing. The failure to achieve significance on a particular scan might be due to incomplete linkage information at that resolution. This underscores the importance of additional genome scans of bipolar disorder with more complete linkage information derived from higher resolution scans.

Our 10 cM genome-wide scan also revealed two other areas of suggestive linkage. The second highest NPL = 2.37 ( $P=0.01$ ) was obtained on chromosome 19 at marker D19S714 (15.5 Mb), and the third region on chromosome 2 had a maximum NPL = 2.28 ( $P=0.014$ ) at marker D2S1356 (43.35 Mb) between markers D2S2374 (35.6 Mb) and D2S1394 (73 Mb). Interestingly, the Dick et al. [2003] study also revealed suggestive linkage near maker D2S1394 with a maximum LOD score of 2.62 ( $P < 0.1$  genome-wide).

TABLE II. Regions With Peak NPLs Scores With Nominal Significance Obtained With a 10 cM Density Whole Genome Scan

Chromosome	Marker	Position (Mb)	NPL	P-value
2	D2S1356	43.35	2.28	0.014
5	D5S2501	110.2	2.11	0.021
6	D6S1021	104.73	2.02	0.026
7	D7S3051	18.03	1.83	0.038
11	D11S987	67.6	2.58	0.007
19	D19S714	15.5	2.37	0.012
20	D20S478	37.9	2.18	0.018

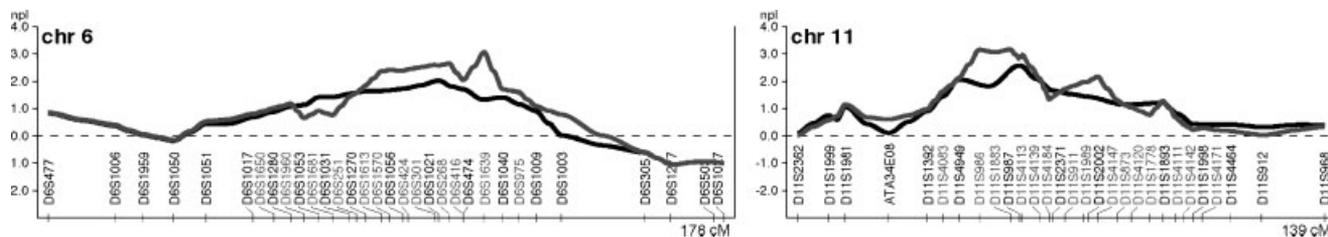


Fig. 2. Fine mapping of chromosomes 6 and 11 in bipolar families. NPL scores (y-axis) for original genome scan markers (black) and for all fine map markers (blue). Tick marks on x-axis represent the position of markers genotyped. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

Given the modest number of pedigrees analyzed in this initial study ( $M=16$ ), the strength of the linkage signal is consistent with a degree of disease homogeneity within this geographically isolated population. The ability to more easily identify areas of linkage in population isolates has been a major foundation of this type of study. The evidence from the Portuguese islands, as well as, studies in other population isolates supports this notion. However, the fact that both our bipolar and our schizophrenia studies have identified linkage in areas identified by other groups supports the generalizability of the population isolate based findings.

In summary, we report strong suggestive evidence for linkage in bipolar disorder to chromosomes 6 and 11 in a population-based sample of Portuguese Island families. The strength of findings from several independent reports on chromosome 6q16.3-21 makes it a solid susceptibility locus for bipolar disorder and suggests that positional cloning approaches are warranted. In addition, our own genome scan using psychosis as a heritable symptom shared between bipolar disorder and schizophrenia not only identifies a potential linkage to the region of chromosome 5 seen in our schizophrenia scan, but also supports the same pattern for the chromosome 6 and 11 regions reported here as potentially linked to bipolar disorder [Sklar et al., 2004]. These results further support the possibility that there may be shared genes for vulnerability to psychosis in bipolar disorder and schizophrenia.

#### ELECTRONIC-DATABASE INFORMATION

URLs for data presented herein are as follows: Marshfield Center for Medical Genetics, <http://research.marshfieldclinic.org/genetics/>

UCSC Genome Bioinformatics, Human Genome Browser, <http://genome.ucsc.edu/>

<http://www-genome.wi.mit.edu/personal/mjdaly/daly-software-page.html>.

#### ACKNOWLEDGMENTS

We thank the families for their participation and Eric Lander for his support for the project. This study was supported in its early stages by NARSAD awards.

#### Lifetime Achievement Award 2003

At its annual WCPG meeting, the ISPG awards the Lifetime Achievement Award to a Scientist or Scientists who made major contributions to the advancement of the field of Psychiatric Genetics. The Lifetime Achievement Award consists of the golden DNA helix specially designed by the internationally recognized sculptor Charles Reina from Long Island, NY. The copyright for the sculpture is owned by ISPG.

Previous awardees were Seymour Kety and George Winokur (1993), Ming Tsuang (1995), Irving I. Gottesman (1997), Ted

Reich (1999), Michael Conneally (2001), and Jules Angst and Nikki Erlemeyer-Kimling (2002).

This year the ISPG award committee, chaired by Peter McGuffin (London, UK), has selected, in consultation with the ISPG Board, C. Robert Cloninger (St. Louis, USA) as the 2003 ISPG Lifetime Achievement Awardee. The Award Ceremony took place during the WCPG Banquet on Tuesday, October 7, 2003. Dr. Cloninger also delivered the Awardee Lecture during the WCPG Plenary session on Wednesday, October 8 to all attending WCPG members and participants.

Dr. C. Robert Cloninger has been a faculty member of Washington University in St. Louis, Missouri since 1973. He is the Wallace Renard Professor of Psychiatry, Genetics and Psychology, and he is the Director of The Center for Psychobiology of Personality. His major research interests have focused on alcoholism, schizophrenia, personality disorders, and somatoform disorders.

In 1966, Dr. Cloninger graduated with a Bachelor of Arts in Philosophy, Anthropology and Psychology from the University of Texas in Austin. He received his medical degree in 1970 at Washington University in St. Louis, and then received his postdoctoral training in Genetics at the University of Hawaii with Newton Morton in 1978.

His publications have included over 300 articles and 5 books on genetics, psychobiology, and classification of alcoholism, personality disorders, and psychopathology. He has been an editor or associate editor on numerous journals, including *Behavior Genetics* and *The American Journal of Human Genetics*, and he has been on the editorial boards for other journals such as *Neuropsychiatric Genetics*.

Some of Dr. Cloninger's past awards include an Honorary Doctorate from the University of Umea, Sweden, The Lifetime Achievement Award from the American Academy of Addiction Medicine in 2000, The Strecker Award from the Institute of Pennsylvania Hospital, The Isaacson Memorial Award, ISBRA, and the Adolf Meyer Award, APA, as well as several distinguished visiting professorships.

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