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The Wellcome trust UK–Irish bipolar affective disorder sibling-pair genome screen: first stage report

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We have completed the first stage of a two-stage genome wide screen designed to identify chromosomal regions that may harbour susceptibility genes for bipolar affective disorder. The first stage screening sample included 509 subjects from 151 nuclear families recruited within the United Kingdom and Republic of Ireland. This sample contained 154 narrowly defined affected sibling pairs (DSM-IV BPI) and 258 broadly defined affected sibling pairs (DSM-IV BPI, SABP, BPII, BPNOS or MDD(R)), approximately two thirds of all families contained at least one other additional typed individual. All individuals were genotyped using 398 highly polymorphic microsatellite markers from Applied Biosystems's Linkage Mapping Set Version 2. The average inter-marker distance was 9.6 cM and the mean heterozygosity was 0.78. Analysis of these data using non-parametric linkage methods (MAPMAKER/SIBS) found no evidence for loci of major effect and no regions reached genome-wide significance for either suggestive or significant linkage. We identified 19 points across the genome where the MLS exceeded a value set for follow up in our second stage screen (MLS≥0.74 (equivalent to a nominal pointwise significance of 5%) under the narrowest diagnostic model). These points were on chromosomes 2, 3, 4, 6, 7, 9, 10, 12, 17, 18 & X. Some of these points overlapped with previous linkage reports both within bipolar affective disorder and other psychiatric illnesses. Under the narrowest diagnostic model, the single most significant multipoint linkage was on chromosome 18 at marker D18S452 (MLS=1.54). Overall the highest MLS was 1.70 on chromosome 2 at marker D2S125, under the broadest diagnostic model.
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Introduction

Bipolar affective disorder (BAD), bipolar disorder or 'manic depression' as it is also widely known (MIM 125480) is a major psychiatric disorder of multifactorial aetiology.[1] It is characterized by recurrent episodes of pathological mood disturbance accompanied by perturbations in thinking and behaviour. Moods may be elated or depressed. The condition affects ~1% of the adult population worldwide at some point during life, and is approximately equally frequent in males and females.[2] It is associated with high morbidity and suicide risk. Between episodes, sufferers generally return to normal mood and are often maintained on prophylactic mood stabilising medication.

The pathophysiology of BAD is poorly understood, but the illness has long been an attractive candidate for molecular genetic analysis, with an elevated familial incidence being noted in some of the earliest descriptions of the disorder.[3] This has since been borne out with most subsequent twin, adoption and family studies indicating a strong genetic basis (reviewed by Craddock and Jones[4]). Estimates of relative recurrence risk in siblings (λ_s) of a proband with narrowly defined bipolar illness are typically in the region of λ_s = 7–8 and estimates of concordance for narrowly defined illness in monozygotic twins is 50–60%.[4,5] Although it is possible that genes of major effect may play a role in a small number of pedigrees, available data suggest that the inheritance of the vast majority of BAD cannot be explained by single major locus models.[5] The inherit-

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ance pattern is consistent with the epistatic action of several genes with modest individual effect sizes (gene-specific $\lambda_s < 2$) or with more complex genetic mechanisms.[5,6] To date no genes demonstrating an unequivocal influence on susceptibility to BAD have been identified, although several chromosomal regions have been repeatedly implicated by different linkage studies (reviewed by Craddock and Jones[4]). These include 4p,[7–11] 4q,[12,13] 12q,[14–17] and 18p+q.[18–26] The fact that support for even the most promising loci is not universal is, of course, to be expected for a complex disorder in which the susceptibility genes exert modest individual effect sizes and interact with both other genes and environmental factors. Due to these factors, the most appropriate molecular genetic approach is one based on allele sharing linkage methods using large samples of nuclear families.[27–29] The efficiency of such an approach can be enhanced by adopting a two-stage strategy with grid tightening and sample splitting in which a relaxed significance level is used in the first stage to identify regions for more intensive follow-up in the second stage.[30] We report here data resulting from the first stage of a two-stage systematic genome-wide screen in which analysis is based solely on allele sharing between affected sibling pairs (ASPs) with bipolar spectrum illness. We have used the narrowest phenotypic classification and a nominal pointwise significance of 5% to define regions of interest for follow-up in the second stage. Our sample, all recruited within the United Kingdom and Republic of Ireland, included 154 narrowly defined ASPs and 258 broadly defined ASPs (Table 1). Consistent with expectations based on previous findings we identified a number of regions warranting further investigation in stage 2. Of these some co-localised with previous reports in BAD, some co-localised with previous reports in other psychiatric illness, while the remainder were novel.

Materials and methods

Subjects

One hundred and fifty-one nuclear families were ascertained in which a proband met the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders version IV[31]) criteria for bipolar I illness (BPI), and at least one other sibling met the criteria for BPI, schizoaffective bipolar illness (SABP), bipolar II illness (BPII), bipolar illness not otherwise specified (BPNOS) or recurrent major depressive disorder (MDD(R)). Families were recruited in the United Kingdom and Republic of Ireland through mental health services, patient support groups and articles in the national media. Subjects from all but three of these families were Caucasian. The mean age of first impairment caused by the illness for all 289 BPI individuals was 25.4 years (SD = 9.1), and the mean age at interview of all affected sibs was 44.4 years (SD = 14.0). Other details relating to the total sample set are shown in Table 1.

Diagnosis

Following receipt of multi-centre research ethics approval, written informed consent was obtained from all participating individuals. Participants were interviewed by trained investigators using a semi-structured research interview (Schedule for Clinical Assessment in Neuropsychiatry; SCAN[32]) and case note information was obtained. Lifetime best estimate psychiatric diagnoses were made according to DSM-IV criteria by two independent raters using all available information. Any disagreements were rated by a third investigator and discussed to reach a consensus. Regular meetings were held between all interviewers and raters in order to maximize clinical consistency and reliability. Inter-rater reliability was assessed using clinical data from 20 cases (chosen to represent a typical cross section of subjects recruited within the study), which were rated by each investigator and compared against consensus to obtain individual kappa coefficients of reliability.[33] Reliability was shown to be excellent with a mean kappa of 0.88.

Markers

A set of 398 highly polymorphic microsatellite markers were used. This was based on Applied Biosystems' Linkage Mapping Set Version 2 (LMS-2, since renamed as the Medium Density 10 cM (MD-10) mapping set). Due to unresolvable amplification problems, a total of seven markers (D2S396, D5S428, D6S1610, D10S192, D11S901, D11S4175, D22S423) were removed from the 400 comprising this set. A further five markers (D2S2354, D5S618, D6S1616, D6S1650, D9S1818) from the Genethon set were added. All markers were dinucleotide repeats, and were supplied as ready to use forward/reverse primer pairs in aqueous solution. The forward primers were fluorescently labelled with FAM, HEX or NED and reverse primers were 'pigtailed' to enhance uniform non-template directed nucleotide addition by Taq DNA polymerase.[34] The marker order and inter-marker distances used were those available from Applied Biosystems at the time of writing. These and any updates are available via Applied Biosystems' website (http://www.appliedbiosystems.com).

Genotyping

Laboratory work was undertaken in two participating centres. Microsatellite markers on chromosomes 1, 3, 4, 7, 8, 12, 13, 14, 15, 16 & X were genotyped by members of the Molecular Psychiatry Group at the University of Birmingham (UK), all those on the remaining chromosomes were genotyped by members of the Neuropsychiatric Genetics Group at Trinity College Dublin (Ireland). The consistency and reliability of cross-centre genotyping strategies were validated by way of joint pilot study using markers on chromosome 21.[35]

DNA was extracted from whole blood using standard protocols. After quantification by UV spectroscopy, working solutions at ~5 ng μl^{-1} were prepared for each participant and stored at 4°C in standard 96-deep well plates. Most microsatellite markers were typically amplified in ~3 fold (range 2–6) multiplex reactions,

Table 1 Summary description of sample set: Diagnostic Models—Narrow (DSM-IV BPI only), intermediate (DSM-IV BPI, SABP or BPII) and broad (DSM-IV BPI, SABP, BPII, BPNOS or MDD(R))

Breakdown of sample set	
By affected sibling pairs	
Number of ASPs—Narrow diagnostic model (all possible)	154
Number of ASPs—Narrow diagnostic model (weighted)	129
Number of ASPs—Intermediate diagnostic model (all possible)	204
Number of ASPs—Intermediate diagnostic model (weighted)	162
Number of ASPs—Broad diagnostic model (all possible)	258
Number of ASPs—Broad diagnostic model (weighted)	198
By family structure	
Sibling pair only	53
Sibling pair plus 1 additional parent or sibling	28
Sibling pair plus ≥2 additional parents or siblings (except both parents)	35
Sibling pair plus at least both parents	35
Total	151
By individuals	
Total individuals with diagnosis of BPI	288 (123 M/165 F)
Total individuals with diagnosis of SABP	12 (7 M/5 F)
Total individuals with diagnosis of BPII	25 (8 M/17 F)
Total individuals with diagnosis of BPNOS	8 (2 M/6 F)
Total individuals with diagnosis of MDD(R)	34 (9 M/25 F)
Total individuals with unknown/other diagnosis	142 (67 M/75 F)
Total individuals	509 (216 M/293 F)

The 'All possible' and 'weighted' pairs figures are calculated using the equations $[n(n-1)/2]$ and $[n-1)]$ respectively, where 'n' is the number of affected individuals in each independent sibship. Data from all independent sibships were then summed to give the overall total for each method. M = male, F = female.

successful primer combinations and concentrations were established empirically. Markers consistently producing unsatisfactory results after three multiplex optimisation attempts were amplified singularly. All PCR reactions were performed on MJ Research Thermal Cyclers using an initial denaturation of 95°C for 12 min followed by 10× (94EC for 15 s/55°C for 15 s/72°C for 30 s), then 25× (89°C for 15 s/55°C for 15 s/72°C for 30 s), with a final incubation at 72°C for 10 min. Each PCR reaction contained 6 μl of 'True allele PCR mix' (Applied Biosystems, Foster City, CA, USA), 2 μl of genomic DNA and primers/water to a final volume of 10 μl. Post PCR, products from individual multiplex reactions were pooled in empirically determined ratios. This permitted up to 20 discrete marker loci to be analysed in a single gel lane, with allele peak fluorescence intensities remaining within optimal limits (typically ~200–4000 units). After pooling, PCR products were separated according to size on 5% denaturing polyacrylamide gels (Long Ranger, FMC Bioproducts, Rockford, ME, USA), using an ABI377 XL DNA sequencer. Gel images were analysed using Genescan version 3.1 and Genotyper version 2.0 software (Applied Biosystems). All genotype and phenotype data were stored in an adapted version of Megabase.[36] This was also used for 'allele binning', basic checks for non-Mendelian inheritance and production of linkage files for subsequent analysis.

Statistical analysis

Before undertaking linkage analysis, genotypic data were assessed for more complex mendelian inconsistencies using the PEDCHECK package.[37] Sibling pairs that were lacking parental genotypic data were also assessed for relatedness using the RELATIVE package.[38] In all, a total of four families generated irreconcilable inconsistencies with one or both of these packages; in two of these, all problems originated from a single individual. Prior to analysis, all relevant information relating to these families/individuals was removed from the data set. MAPMAKER/SIBS, which calculates the maximum LOD score (MLS) at each point along a chromosome by estimating maximum likelihood sharing probabilities (IBD),[39] was used as the primary analysis package in this study. MLS calculations were extended 10 cM 'off end' of the marker map, the 'all pairs' option was used for sibships with more than two affected members, and the information content was calculated using the 'infomap' option. Three different diagnostic models were utilised, narrow (DSM-IV BPI only), intermediate (DSM-IV BPI, SABP or BPII) and broad (DSM-IV BPI, SABP, BPII, BPNOS or MDD(R)). The narrow diagnostic model was the primary model for analysis but additional separate analyses were undertaken for each of the other two diagnostic models. SPLINK and GENEHUNTER were also used to calculate the marker allele frequencies (for

use with MAPMAKER/SIBS) and estimated map lengths respectively.[40,41] No other use was made of these latter two packages in prioritising regions for further study, however a qualitative assessment was made to ensure that no regions demonstrated clear inconsistencies between any of the three packages.

Genome-wide significance levels for our primary method of analysis (MAPMAKER/SIBS using the narrow diagnostic model) were estimated by simulation using software written by Peter Holmans and using a modification of the approach used in a previous genome scan of schizophrenia.[42] Briefly, replicates of the genome were simulated under the hypothesis of there being no disease susceptibility locus for a sample of families identical in structure, affection status and availability of DNA, to those included in the current study. A map of 400 markers, each with four equifrequent alleles (heterozygosity = 0.75) evenly spaced at 10 cM intervals was assumed. The simulated dataset was analysed for the narrow diagnostic model using MAPMAKER/SIBS and the resulting MLS determined. This procedure was repeated on 1000 replicates to estimate the distribution of MLS under the null hypothesis.

In order to obtain an estimate of power, average maximum lod scores (MLS) and probabilities that the MLS exceeded various criteria were estimated by simulating replicate samples containing the relevant number of sib pairs. A 10 cM grid was simulated, in which the markers each contained five equifrequent alleles (heterozygosity = 0.8). Four values of λ_s for the disease locus were used (1.25, 1.5, 2, 3) and in each case, the disease locus was assumed, conservatively, to be midway between two flanking markers (ie the most difficult situation for detection).

Results

We genotyped 398 microsatellite markers in 509 individuals. The mean heterozygosity and polymorphism information content (PIC) of the markers were 0.78 and 0.75 respectively. The average inter marker distance was 9.6 cM (range 2.4–24.1) and the average genome-wide information content was 0.67. Three different diagnostic models were utilised, narrow, intermediate and broad, as defined in the Methods section. The average number of ASPs fully genotyped for each marker was 133.1 (86.4%) for the narrow diagnostic model, 177.5 (87%) for the intermediate diagnostic model and 225.7 (87.5%) for the broad diagnostic model. Genome-wide, published/observed map lengths were in close agreement.

Multipoint ASP analysis identified 19 points on chromosomes 2, 3, 4, 6, 7, 9, 10, 12, 17, 18 and X reaching a nominal pointwise significance of 5% under the narrow (BPI only) diagnostic model. This was equivalent to a maximum lod score (MLS) greater than or equal to 0.74 for the autosomes or 1.18 for the X chromosome.[43] Seven of these same points also achieved nominal significance under the intermediate and/or broad diagnostic models. Further points on chromosomes 1, 4, 8, 11 and 15 achieved equivalent significance under the intermediate and/or broad diagnostic models alone (Figure 1).

Considering the narrow diagnostic model (BPI only) which represents the primary focus of this study, the single most significant multipoint linkage was on chromosome 18 at marker D18S452 (MLS = 1.54), with five additional points (at D7S630, D17S928, D18S474, D18S68 and DXS990) demonstrating a multipoint MLS$\geq$1.0. Consistent with this, marker D7S630 also provided the highest single point LOD score of 1.46. Further details of all 19 points achieving nominal significance under this diagnostic model can be found in Table 2. Considering all diagnostic models the highest overall single (LOD = 1.87) and multipoint (MLS = 1.70) scores were coincident at D2S125 on the telomere of chromosome 2q, under the broadest model of affection status.

Simulation studies suggested that we would have expected to have obtained on average one multipoint MLS of 1.7 per genome scan in the absence of linkage (corresponding to 'suggestive' linkage according to the criteria proposed by Lander and Kruglyak[44]). An MLS $>$3 would have been expected only once in every 20 genome scans in the absence of linkage and thus corresponds to a genome-wide significance of 0.05 (ie 'significant' linkage according to the criteria of Lander and Kruglyak).

Simulation studies demonstrated that for a true locus of effect size $\lambda_s = 3$, our sample is expected to provide an average MLS of 4.0 under the narrow model and has power of 77% to detect MLS $>$3, and power of 88% to detect MLS $>$2 (the corresponding figures under the broad model are: average MLS = 5.8; Power (MLS $>$ 3) = 93%; Power (MLS $>$ 2) = 98%. For a gene with λ_s = 2, the corresponding figures for the narrow model are: expected MLS = 2.7; Power (MLS $>$ 3) = 34%; Power (MLS $>$ 2) = 66%, and for the broad model: expected MLS = 3.5; Power (MLS $>$ 3) = 59%; Power (MLS $>$ 2) = 80%.

Discussion

This report comprises the largest collection of bipolar affected sib pairs yet investigated in a whole genome screen for bipolar disorder—for example, under our narrowest diagnostic model (BPI disorder only) our sample included 154 pairs, as compared with 121 pairs under the narrowest diagnostic model (BPI and SABP) used in the multicentre NIMH Genetics Initiative Study.[8] Despite this large sample, we have found no evidence to support the presence of any loci exerting a major effect on illness susceptibility. This is consistent both with previous genome screens using similar sample sets and with mathematical modelling studies.[5] An important potential cause for failure to detect a locus of major effect is lack of power. However, our power simulations show that this is an unlikely explanation for our findings. The largest MLS found in our primary analysis (ie using the narrow phenotypic model) was MLS = 1.54. Simulations show that we had a power of

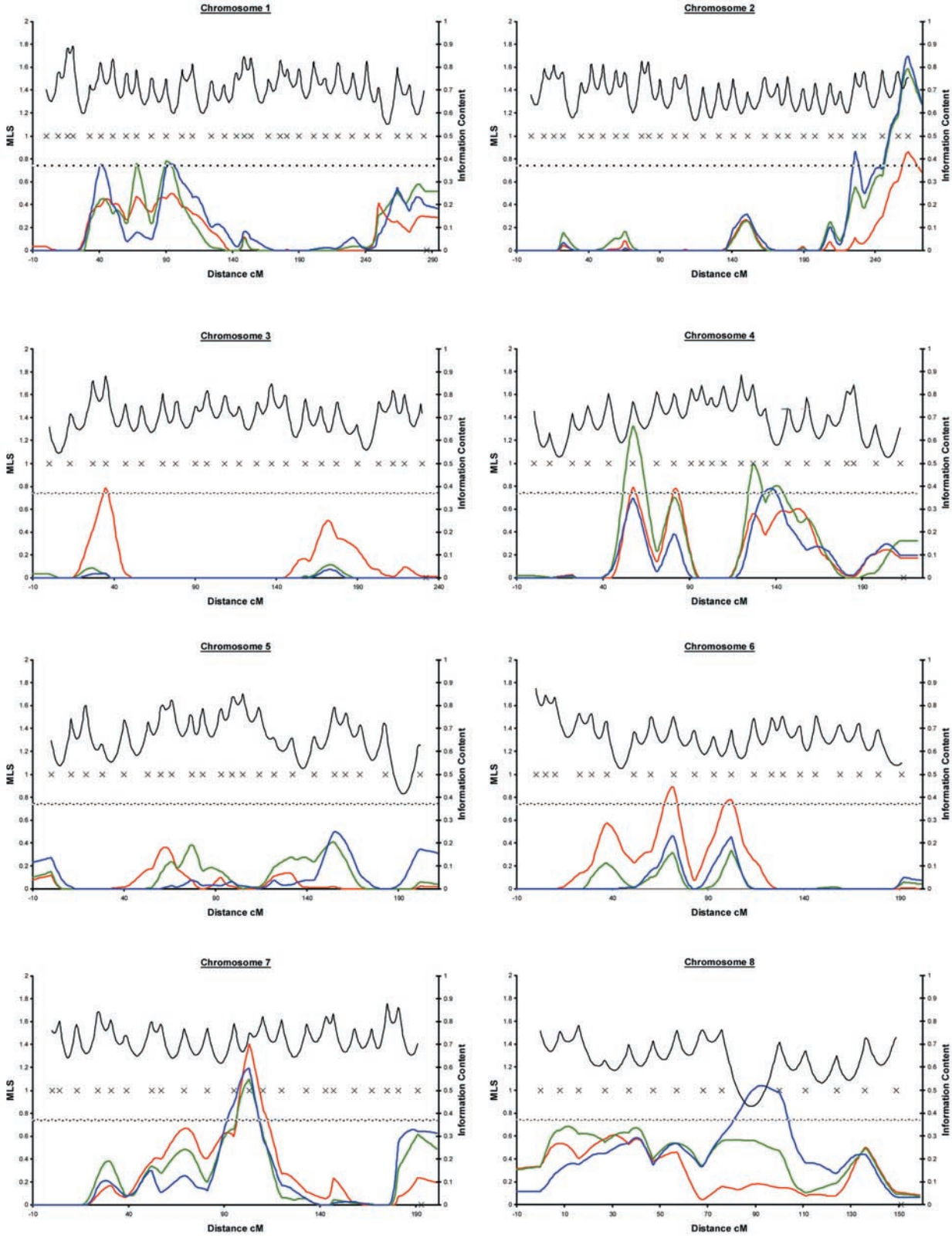

Figure 1 Graphs showing the Maximum Lod Scores (MLS, primary *y* axes) across all chromosomes and under each diagnostic model (see key in figure). Also shown is the marker information content (secondary *y* axes). Distances on *x* axes are in cM with zero representing the position of the most telomeric marker on the 'p' arm. Note, all analyses were extended 10 cM 'off end' of the marker maps.

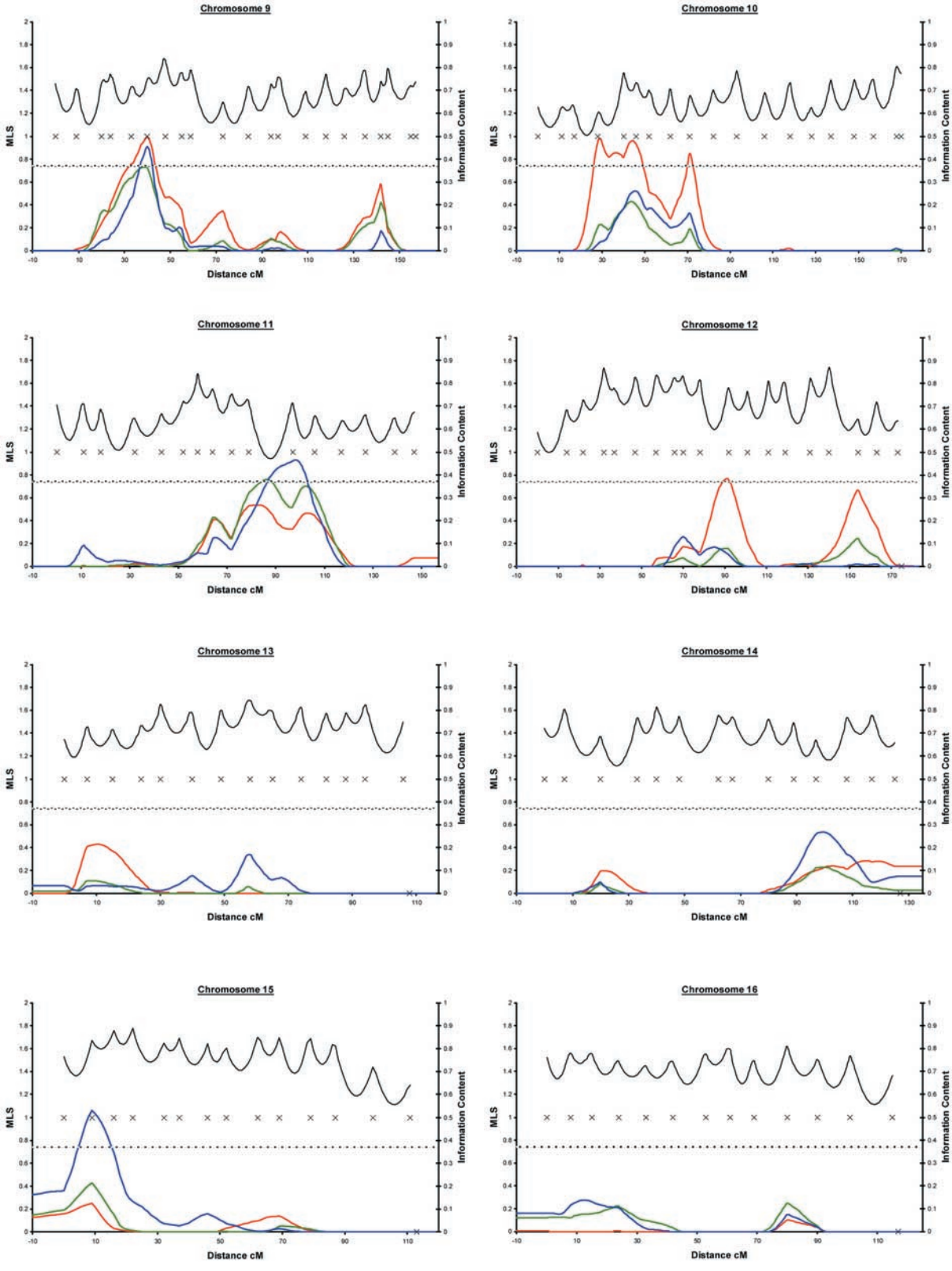

Figure 1 Continued.

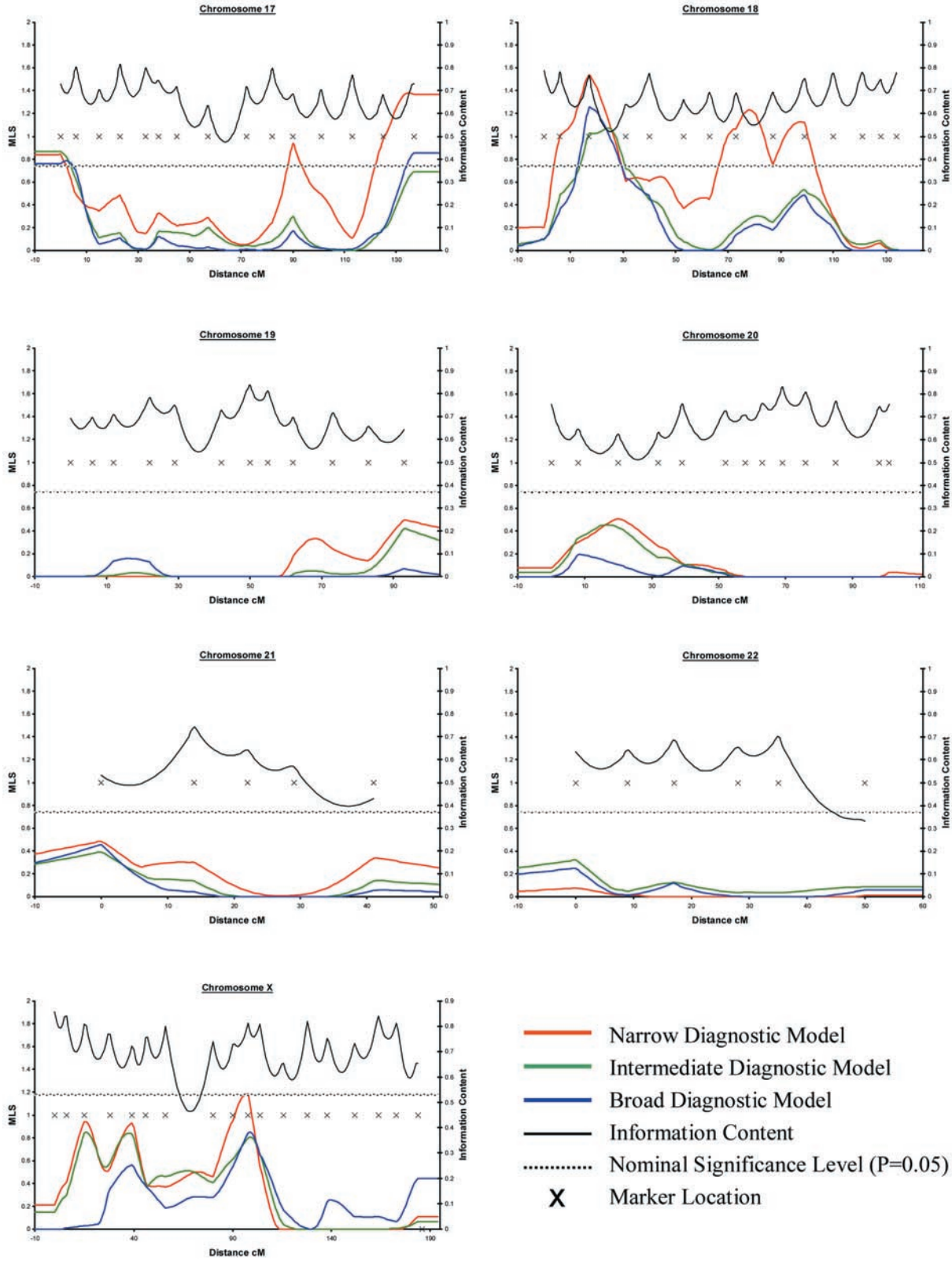

Figure 1 Continued.

Table 2 Details of chromosomal regions reaching nominal significance (MLS≥0.74, under the narrow diagnostic model (BPI only)

Chromosome number	*cM from pter marker*	*Multipoint MLS (narrow model)*	*% IBD sharing*	*Nearest marker(s) (distance cM, single point lod score)*
18p11	17	1.54	59.7	D18S452 17 cM LOD = 1.24
18q21	78	1.23	60.4	D18S474 73 cM LOD = 0.59
18q21–q22	98	1.13	58.5	D18S68 98.8 cM LOD = 0.47
7p15–q21	103	1.40	59.2	D7S630 103.2 cM LOD = 1.46
17q23–q24	135	1.38	59.5	D17S928 137 cM LOD = 0.18
17q11–q23	90	0.94	56.9	D17S944 90 cM LOD = 0.90
17p13	0	0.84	57.3	D17S849 0 cM LOD = 1.36
Xq21.1	98	1.18	63.3 (BB) 64.4 (BS) 25.0 (SS)	DXS986 90.3 cM LOD = 0.24 DXS990 98 cM LOD = 0.39 DXS1106 103.9 cM LOD = 0.57
9p21	40	0.99	57.0	D9S161 40.5 cM LOD = 0.86
10p13–p15	29	0.98	57.4	D10S547 28.4 cM LOD = 0.45
10p12	44	0.96	58.4	D10S548 46 cM LOD = 0.36
10q11–q21	71	0.85	56.4	D10S196 71.3 cM LOD = 1.08
6p21	71	0.89	56.4	D6S257 71.7 cM LOD = 0.70
6q13–q14	102	0.78	56.3	D6S434 102 cM LOD = 0.87
2q37	263	0.86	55.9	D2S125 262.7 cM LOD = 1.15
3p24–p26	35	0.79	55.3	D3S2338 35.1 cM LOD = 0.51
4p14–p15	57	0.79	55.8	D4S405 57.6 cM LOD = 1.03
4q12–q13	82	0.78	55.8	D4S392 81.3 cM LOD = 0.59
12q21	91	0.77	55.7	D12S326 91.7 cM LOD = 0.56

Single and multipoint LOD scores were calculated using SPLINK and MAPMAKER/SIBS respectively. For X chromosome region, BB: brother–brother pairs; BS: brother–sister pairs; SS: sister–sister pairs. Marker distance along chromosome and approximate cytogenetic location are taken from the Location Database (web address: http://cedar.genetics.soton.ac.uk/public_html/).

95% to detect MLS $>$ 1.54 if there were a true locus of effect size $\lambda_s = 3$, and power of 80% for locus of $\lambda_s = 2$. Thus, our results provide important evidence against the existence of common genes of major effect in bipolar disorder. The largest estimated gene-specific effect size of the putative loci identified in our study is $\lambda_s = 1.72$ at D18S452, again consistent with predictions from theoretical studies that suggest the presence of multiple loci with effect sizes below 2. Reports of significant linkage in large multiply affected families raise the possibility that some, relatively rare, alleles of larger effect may exist. However, our findings demonstrate that the majority of cases of bipolar disorder reflect the action of several, or even many, genes of small effect.

We found 19 points throughout the genome meeting our second stage inclusion criteria (namely a nominal pointwise significance of 5%, see above). Although in the first stage of our genome screen, none conformed to the 'Lander and Kruglyak' criteria for either 'significant' (LOD = 3.0) or 'suggestive' (LOD = 1.7) linkage (as determined by our simulation studies), several of our regions of interest have been implicated in previous linkage studies of BAD or schizophrenia and are discussed briefly below. Note that in the following discussion, the positions of all markers used in this study are stated as the genetic distance from the most telomeric marker on the 'p' arm (see Figure 1 and Table 2) and that, where possible, the positions of relevant markers reported by other investigators have been normalised to this to facilitate comparison.

Chromosome 2 Under our narrow diagnostic model, a signal was identified on the q telomere at marker D2S125 (263 cM). There have been no other reports of psychiatric linkage in this area of chromosome 2, although the co-localising ATSV (axonal transport of synaptic vesicles) gene, previously proposed as a candidate for amyotrophic lateral sclerosis-4 may be of interest.[45]

Chromosome 3 A signal coming solely from the BPI/BPI ASPs was seen at marker D3S2338 (35 cM). Few previous reports of potentially interesting psychiatric linkage to chromosome 3 exist. Nevertheless, Ekelund *et al* recently reported a two point lod score of 1.73 in schizophrenia at marker D3S3038 only a few cM away.[46]

Chromosome 4 This has attracted considerable attention in the past with numerous reports of linkage to

BAD within the last 30 cM or so at either end of the chromosome.[7–13] However, these flank our own signals at 57 (D4S405) and 82 cM (D4S392), which overlap the region reported in alcohol dependence (LODs of 2.8 and 2.5 at D4S3242 (~70 cM) and D4S2393 (88 cM) respectively[47,48]).

Chromosome 6 There is little of substantive interest in previous findings for BAD on this chromosome. Two potential schizophrenia loci have been suggested but these essentially flank our region of interest.[49–53]

Chromosome 7 Our signal at D7S630 (103 cM) on 7q21 co-localised almost exactly with those of Blouin *et al*, in schizophrenia and the position of the metabotropic glutamate receptor 3 (GRM3) gene.[54] Regions implicated in other linkage studies of schizophrenia lie ~10–20 cM distally at 7q21–22.[46,55]

Chromosome 9 Morissette *et al*, using the same marker described in this study (D9S161), recently reported a two point LOD score >1 in a large bipolar pedigree from Quebec,[17] although this region has not been implicated in other studies.

Chromosome 10 Previously there has been a clustering of promising linkage reports mainly in schizophrenia but also BAD at the middle of this region near the centromere.[55–58] On a cautionary note however, this region has been implicated in a number of other disorders, and markers at the centre lie very close to D10S211 reported by Paterson and Petronis to show transmission ratio distortion in females.[59]

Chromosome 12 The major focus of interest on chromosome 12 has been 12q23–q24 around the region of the Darier gene.[14,17,60] Our signal at marker D12S326 (92 cM) did not co-localise with any other known psychiatrically relevant linkage findings but a second more telomeric signal at D12S324 (154 cM), although failing to reach nominal significance is within the broad 12q23–q24 region of interest, and co-localised precisely with D12S1939 reported by Ewald *et al*, to have a LOD of 3.37 in two large Danish BAD families.[61]

Chromosome 17 Previously there have been no relevant linkage reports to chromosome 17, although a number of candidate gene studies have been performed at the serotonin transporter gene (SERT).[62–68] SERT however, lies at approximately 50 cM on our map, well away from the regions of interest in our study.

Chromosome 18 Under the narrow diagnostic model, we found our highest multipoint LOD score (MLS = 1.54) on chromosome 18p, with a further region on 18q ranking very highly amongst the other points reaching nominal significance. This was especially interesting as both show overlap with parts of the chromosome that have attracted considerable attention in a number of previous studies. The signal on 18p lay at marker D18S452; this co-localises with D18S62 which lies at the telomeric end of the region first reported for BAD by Berrettini *et al* in 1994.[18] In their study, this marker gave the second best *P* value (0.0016) upon ASP analysis with IBD sharing of 57%. Although most of their other interesting 18p markers lay more centromerically, co-localising with those later reported by Stine *et al* and Detera-Wadleigh *et al*,[19,25] this region also remains above or close to nominal significance in our study. Further, support for this region comes from evidence suggesting potential overlap with schizophrenia; Schwab *et al*, reported a possible schizophrenia locus on 18p11.2, and demonstrated that evidence for linkage in these schizophrenia families increased when individuals with affective diagnoses were also included in the analysis.[69] Returning to our own data it can be calculated that this region on 18p would be expected to confer a gene-specific λ_s of ~1.72. On 18q between D18S1102 (63 cM) and D18S61 (110 cM), the co-localisation of previous findings with ours is more striking. In BAD, Freimer *et al*, and McInnes *et al*, considering BPI individuals only, as in our primary analysis, reported suggestive linkage of markers between D18S64 (87 cM) and D18S1161(121 cM) in a Costa Rican family (CR004).[20,22] Also, Stine *et al*, reported a LOD score of 3.51 at D18S41 (83 cM) in 11 paternal pedigrees (ie father or father's sib affected),[19] later corroborating this finding in 30 new pedigrees.[24] Further support for possible parent-of-origin effects on both 18p and 18q has also been seen in other studies.[21,26] Because our sample is based on sib-pairs in nuclear families we were unable to perform a rigorous analysis according to the mode of parental transmission. Nevertheless, an informal analysis which involved splitting our nuclear families into four subgroups (affected father only, affected mother only, both parents affected, neither parent affected/status unknown) and analysing each one separately, was undertaken. No clear differences were observed between them although there were relatively small numbers in some subgroups. It will however, be important to explore this issue further in our enlarged stage 2 sample. Turning again to schizophrenia, Williams *et al*, identified suggestive linkage on 18q near marker D18S450 (71 cM) in 196 ASPs.[42] There have of course been studies providing less clear support for some of these findings,[70–72] and indeed others which have failed to find any evidence at all of linkage to chromosome 18.[73–77] Nevertheless, chromosome 18 remains very interesting, and could well be one of the first chromosomes to yield a susceptibility gene for a major psychiatric disorder.

Chromosome X Despite three clearly definable signals only one reached the criterion for nominal significance on the X chromosome.[43] This lay at marker DXS990 (98 cM), with much of the signal (MLS = 0.88) coming only from brother/sister pairs. The remainder (MLS = 0.3) came only from brother/brother pairs. Our findings localise near marker DXS8092 (92 cM), reported by Norton *et al*, to show a modest LOD score in schizophrenia ASPs.[78] However, although the subject of numerous previous reports for a range of psychiatric

disorders including schizophrenia and BAD (reviewed by Paterson[79]), collective interpretation of X chromosome data remains difficult due to inconsistencies both with the location and origin (ie brother/brother, brother/sister or sister/sister pairs) of reported linkage signals. Furthermore, a number lie close to the 'Distorter Male-Specific' (DMS-1) locus on Xp, which shows transmission ratio distortion.[80]

To summarise, in a large sib-pair sample we have found no evidence for genes of major effect in BAD and no regions that meet genome-wide significance criteria for suggestive or significant linkage. We have identified numerous chromosomal regions that exceed a nominal point-wise significance level of 5%. Some of these regions have received support in previous linkage studies of BAD or schizophrenia, most notably regions on chromosome 18p and 18q. All the regions that exceeded the 5% nominal significance level will be followed up in the second stage of our genome screen. This will involve genotyping at an increased marker density (~5 cM), and use of an expanded sample set. Our linkage findings, together with those of other groups, will contribute to the localization of susceptibility genes for BAD. However, given the predicted modest effect sizes of these genes, large samples will be necessary and it will be important to use association approaches to complement the linkage paradigm for their fine mapping and identification.[29]

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