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A genome screen of 35 bipolar affective disorder pedigrees provides significant evidence for a susceptibility locus on chromosome 15q25-26

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Bipolar affective disorder is a heritable, relatively common, severe mood disorder with lifetime prevalence up to 4%. We report the results of a genome-wide linkage analysis conducted on a cohort of 35 Australian bipolar disorder families which identified evidence of significant linkage on chromosome 15q25-26 and suggestive evidence of linkage on chromosomes 4q, 6q and 13q. Subsequent fine-mapping of the chromosome 15q markers, using allele frequencies calculated from our cohort, gave significant results with a maximum two-point LOD score of 3.38 and multipoint LOD score of 4.58 for marker D15S130. Haplotype analysis based on pedigree-specific, identical-by-descent allele sharing, supported the location of a bipolar susceptibility gene within the Z_{max-1} linkage confidence interval of 17 cM, or 6.2 Mb, between markers D15S979 and D15S816. Non-parametric and affecteds-only linkage analysis further verified the linkage signal in this region. A maximum NPL score of 3.38 ($P = 0.0008$) obtained at 107.16 cM (near D15S130), and a maximum two-point LOD score of 2.97 obtained at marker D15S1004 (affecteds only), support the original genome-wide findings on chromosome 15q. These results are consistent with four independent positive linkage studies of mood and psychotic disorders, and raise the possibility that a common gene for susceptibility to bipolar disorder, and other psychiatric disorders may lie in this chromosome 15q25–26 region.
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Bipolar (BP) affective disorder (MIM 125480) is a severe mood disorder characterised by alternating periods of mania and depression with reversion to normal behaviour in between these episodes. Bipolar disorder is a relatively common condition with a worldwide prevalence between 0.5 and 1.5%[1] and lifetime prevalence of up to 4%.[2] The disorder has a severe impact on sufferers, being ranked the sixth most debilitating disorder in the World Health Organisation Global Burden of Disease Report,[3] and results in suicide rates 15 times higher than the general population.[4] The aetiology of bipolar disorder remains unknown, with little knowledge of the underlying biological, anatomical or biochemical effects. However, family, twin and adoption studies have provided strong evidence that genetic factors contribute to the disorder with heritability estimates in the range of 60–85%.[5,6] The high heritability, increased relative risks within families,[7] and familial clustering of the disorder provide the opportunity to use genetic approaches to identify predisposing genes. The inheritance pattern of the disorder is complex with non-Mendelian inheritance suggesting the involvement of multiple genes and environmental factors.

Many genetic studies have attempted to map BP susceptibility loci and have provided evidence for linkage to a number of chromosomal regions (reviewed by Baron[8]). One meta-analysis of whole-genome linkage scans did not identify consistent loci across linkage studies,[9] although a separate meta-analysis supported evidence for susceptibility loci on chromosomes 13q and 22q.[10] A third meta-analysis utilising actual genotype data, rather than LOD scores, has identified loci on chromosomes 6q and 8q.[11]

Several genes that confer susceptibility to BP have been proposed in the last decade. The *G72/G30*

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(*DAOA*) genes on chromosome 13q34 were originally implicated as schizophrenia susceptibility genes.[12] Subsequently this locus has been reported to show strong evidence of linkage to bipolar disorder, providing evidence that these same genes might be involved in bipolar disorder susceptibility.[13–15] Similarly, the Disrupted in Schizophrenia 1 locus, originally identified in a large family exhibiting a broad spectrum of psychiatric disorders including schizophrenia, bipolar disorder and unipolar disorder,[16] was shown to be positively associated with bipolar disorder.[17,18] Other putative susceptibility genes that have been implicated in bipolar disorder include *BDNF*,[19,20] *XBP1*[21] and *GRIN1*,[22] which have all shown association in particular ethnic cohorts but have not been replicated when tested for association in independent cohorts of varying ethnic origin.[23–28] The cadherin gene *FAT* on chromosome 4q35 is a recent example of a bipolar susceptibility gene identified through a strategy of genetic linkage and association analysis in several independent cohorts.[29] Considering the number of loci reproducibly linked to bipolar disorder, and the mixed results from association studies among cohorts of various ethnicities, it is clear that precise genetic determinants of BP are yet to be elucidated.

In this present study, we report the results of a genome-wide linkage analysis and subsequent fine mapping in a cohort of 35 Australian BP families that show significant linkage to chromosome 15. This cohort has not previously been the subject of a genome scan.

Materials and methods

Subjects

The families were ascertained as part of an ongoing bipolar genetics study by the Mood Disorders Unit, Prince of Wales Hospital/School of Psychiatry, University of New South Wales, Sydney, Australia. Medium to large multigenerational pedigrees were recruited, plus five nuclear pedigrees. Most pedigrees contained at least three affected individuals, with the exception of two nuclear pedigrees that contained two affected individuals each. Families were almost entirely of British or Irish descent. The families were assessed using the Diagnostic Interview for Genetic Studies (DIGS),[30] with interviews being undertaken by experienced psychiatrists, psychologists and psychiatric nurses trained in this instrument. Best-estimate Research Diagnostic Criteria diagnoses were made by senior psychiatrists after independent evaluation of Diagnostic Interview for Genetic Studies interviews, family informant data and medical records. All spouses were routinely questioned about family history of psychiatric illness to ensure unilateral descent of BP in the pedigrees. All individuals who participated in the study provided appropriate informed written consent. The study was approved by the Ethics Committee of the University of New South Wales and complies with the guidelines of the National Health and Medical Research Council and the Helsinki Declaration.

Blood was collected from 288 available individuals, of whom 130 were affected (according to the broad disease definition described below), and DNA was extracted using standard methods. In total there were 56 individuals diagnosed with BPI (BPI) disorder, 12 with schizoaffective disorder manic type (SZ/MA), 22 with BPII (BPII) disorder and 40 with recurrent unipolar depression. The average number of genotyped individuals in a pedigree was eight and the average number of genotyped affected individuals (BPI, SZ/MA, BPII or unipolar depression) was four per pedigree.

Genotyping

The genome scan was conducted at the Australian Genome Research Facility, Melbourne, Australia. All DNA samples were genotyped with 400 microsatellite markers from the ABI PRISM Linkage Mapping Set Version 2 (Applied Biosystems). The markers in this mapping set had an average heterozygosity of 0.79 and were spaced at approximately 10 cM across the genome.

To further refine the region identified on chromosome 15q25–26, an additional eight markers were selected from the ABI PRISM Linkage Mapping Set and The Genome Database and genotyped in the 35-pedigree cohort. Additional markers comprised D15S655, D15S979, D15S652, D15S1004, all proximal to the peak at D15S130, and D15S816, D15S657, D15S1014 and D15S212, which lay distal to D15S130. The average distance between markers in the 32.5 cM region (between D15S205 and D15S212) was reduced to 3.25 cM by the addition of these markers, with the largest distance between D15S652 and D15S1004 (7.51 cM). These markers had an average heterozygosity of 0.76.

Genome-wide linkage analysis

Two-point linkage analyses were performed using the MLINK computer program of the LINKAGE 5.2 package.[31] Three disease models were used in the analyses. In disease model I (narrow), individuals diagnosed with either BPI or SZ/MA were classified as affected. In disease model II (medium), individuals diagnosed with either BPI, SZ/MA or BPII were classified as affected. In disease model III (broad), individuals diagnosed with BPI, SZ/MA, BPII or unipolar depression were classified as affected. In each model, all other family members were considered unaffected. Four liability classes (class 1, <20 years; class 2, 20–29 years; class 3, 30–39 years; and class 4, >40 years) were used in the analyses with maximum age-specific penetrance levels of either 60 or 90%.[32] In the 90% model, liability classes were defined with penetrances of 0.18, 0.45, 0.68 and 0.9; those in the 60% model were defined with penetrances of 0.12, 0.30, 0.45 and 0.6. The data was analysed under both dominant and recessive inheritance models. The disease-allele frequency was set at

0.035 for the dominant model and 0.2 for the recessive model, and a phenocopy rate of 5% was used for all analyses. Marker allele frequencies were calculated with equal frequencies. Recombination fractions were converted from centimorgan distances provided on the Rutgers Combined Linkage-Physical Map of the Human Genome.[33] The PEDCHECK computer program[34] was used to check for any inheritance inconsistencies prior to performing the linkage analyses.

Chromosome 15q linkage analysis
As allele frequencies in an Australian population may differ from those reported in the databases from European or American populations, we calculated the allele frequencies for each of the 10 markers in the 31 cM region between D15S205 and D15S1014 using the unrelated spouses within our Australian bipolar pedigree cohort. For each marker, genotypes of between 34 and 122 spouses were used to determine Australian allele frequencies. Two-point linkage analysis was performed under the 60% dominant model for the broad disease definition.

Multipoint genetic linkage analysis was performed using the LINKMAP computer program in the LINKAGE 5.2 package[31] on 10 markers in the 31 cM region (D15S205-D15S1014) using allele frequencies from our cohort. This analysis was performed under the 60% age-specific penetrance dominant model for the broad disease definition. The approximate 95% confidence interval for the highest LOD score ($Z_{\max}$) was evaluated by the $Z_{\max-1}$ method.

We employed two other methods of linkage analysis to verify the linkage signal in the region. Firstly, non-parametric multipoint linkage analysis using the program GENEHUNTER (Version 2.0 beta),[35] and secondly, two-point affecteds-only linkage analysis using the MLINK computer program of the LINKAGE 5.2 package,[31] where unaffected individuals were coded as 'unknown'.

Haplotype analysis
We used our previously published approach of haplotype narrowing,[36] which is based on pedigree-specific, identical-by-descent (IBD), allele sharing to define a probable disease region, in the pedigrees that showed evidence of linkage to 15q25–26. Each of the 15 pedigrees that contributed positively to the LOD score, that is, had a LOD score that exceeded the expected LOD score based on simulation analysis using SLINK[37,38] at five or more markers out of the 10 across the locus, was included in the analysis. This approach identifies the percentage sharing of alleles that are IBD, in affected individuals of all linked pedigrees.

Permutation analysis
To determine the statistical significance of our data using our own pedigree structures, we permuted the chromosome 15 genotypes, maintaining pedigree structure and missing data using MERLIN[39] and manually ran 600 permuted pedigree files through GENEHUNTER (Version 2.0 beta).[35] We used the same model parameters and allele frequencies that were used in the two-point linkage analysis.

Sensitivity analysis
To determine the effect of phenotype mis-specification on the linkage signal, the phenotype of each informative unaffected pedigree member was changed, one at a time, to affected. This method described by Hodge and Greenberg,[40] is useful for a trait such as BP where clinical diagnosis requires an individual to meet very strict diagnostic criteria to be defined as affected, and individuals often receive a clinical diagnosis many years after first exhibiting symptoms. In total 95 individuals were identified as informative and had their affection status changed for re-analysis with two-point linkage analysis.

Results

Genome-wide linkage analysis
Applying the genome-wide thresholds for LOD score analysis provided by Lander and Kruglyak[41] (LOD of 3.3 for significant linkage and 1.9 for suggestive linkage), the current genome scan identified a genome-wide significant linkage with an LOD score of 3.87 at marker D15S130 on chromosome 15q. Several other markers gave suggestive evidence for linkage on chromosome 4q, 6q and 13q, in addition to D15S127, a second marker adjacent to D15S130 on 15q (Table 1).

Chromosome 15q linkage analysis
Two-point linkage analysis, under the 60% dominant model for the broad disease definition, and based on allele frequencies from our Australian bipolar disorder cohort, gave a maximum two-point LOD score of 3.38 at marker D15S130. Five markers flanking D15S130, one distal and four proximal, gave suggestive evidence of linkage to BP (Table 2).

The multipoint analysis gave a peak at the marker D15S130 with a multipoint LOD score of 4.58, which was significant at a genome-wide level (Figure 1). The $Z_{\max-1}$ method suggests that the locus lies within a 95% confidence interval of approximately 17 cM (14.5 cM proximal and 2.5 cM distal to D15S130) between the markers D15S979 (86.6 Mb) and D15S816 (92.8 Mb).

The two other methods of analysis, non-parametric multipoint linkage and parametric affecteds-only two-point linkage analysis, also gave positive results across 15q25–26. A maximum NPL score of 3.38 ($P = 0.0008$) was obtained at 107.16 cM (near D15S130). *P*-values of 0.0008 were found across the region from 105.79 to 107.16 cM, between the markers D15S1004 and D15S816 (Figure 2). For the affecteds-only parametric analysis, a maximum two-point LOD score of 2.97 was obtained at marker D15S1004, with markers D15S130 and D15S127 also giving suggestive evidence of linkage to bipolar disorder (Table 2).

Table 1 Two-point LOD scores greater than 1.9 obtained from the genome-wide scan

Marker	*Map distance (cM)*	*Chromosome band*	*Maximum LOD score*	*θ*	*Model*
D4S1534	97.50	4q21.23	2.00[a]	0.00	60% recessive, Model II
D4S415	179.21	4q34.3	2.31[a]	0.02	90% dominant, Model II
D6S1581	171.72	6q25.3	2.65[a]	0.00	60% recessive, Model III
D6S446	192.58	6q27	2.89[a]	0.00	60% recessive, Model III
D13S156	68.66	13q22.1	2.39[a]	0.10	60% dominant, Model III
D13S159	91.53	13q32.2	1.93[a]	0.14	60% dominant, Model III
D15S127	94.64	15q26.1	2.12[a]	0.05	60% dominant, Model III
D15S130	106.15	15q26.2	3.87[b]	0.00	60% dominant, Model III

[a]Indicates suggestive evidence of linkage (LOD score $\geqslant$ 1.9).
[b]Indicates significant evidence of linkage (LOD score $\geqslant$ 3.3).
Model II, affected individuals diagnosed with bipolar I, schizoaffective disorder manic type or bipolar II.
Model III, affected individuals diagnosed with bipolar I, schizoaffective disorder manic type, bipolar II or unipolar depression.

Table 2 Two-point LOD scores for chromosome 15 markers

Marker	*Location (cM)*	*LOD score*	*θ*	*LOD score (affecteds-only)*	*θ*
D15S205[a]	86.09	1.60	0.10	1.59	0.05
D15S655	89.62	0.86	0.10	0.75	0.05
D15S979	91.03	2.49[b]	0.05	1.71	0.05
D15S127[a]	94.64	1.99[b]	0.05	2.13[b]	0.05
D15S652	97.29	2.25[b]	0.01	1.15	0.05
D15S1004	104.80	2.94[b]	0.01	2.97[b]	0.00
D15S130[a]	106.15	3.38[c]	0.00	2.05[b]	0.00
D15S816	108.57	2.17[b]	0.05	1.05	0.05
D15S657	113.82	1.10	0.10	0.31	0.10
D15S1014	116.93	0.04	0.30	−0.02	0.30

[a]Markers from the original 10 cM genome scan.
[b]Indicates suggestive evidence of linkage (LOD Score $\geqslant$ 1.9).
[c]Indicates significant evidence of linkage (LOD Score $\geqslant$ 3.3).
LOD scores were calculated for three markers from original genome scan and for eight additional markers using the calculated allele frequencies from our Australian BP cohort, using the 60% dominant model and the broad disease (Model III) definition. Affecteds-only two-point LOD scores are also shown.

Haplotype analysis

Applying our haplotype narrowing method, the maximal allele sharing was seen at D15S1004, where 96% allele sharing was seen in affected individuals from linked pedigrees. The allele sharing declines gradually to levels around 85% at markers flanking the 30 cM region of interest, showing extensive haplotype sharing across the entire interval (Figure 3a). However, comparison of haplotype sharing in individual pedigrees shows recombinations in two pedigrees, 86 and 125, which places the most commonly shared region between D15S1004 and D15S130 (Figure 3b).

Permutation analysis

We ran 600 permutations for our dataset, and observed LOD scores ranging between −8.26 and 3.05 for marker D15S130 under the null hypothesis. The average permuted LOD was −3.60, with a s.d. of 1.76. The maximum observed LOD of 3.38 at D15S130 lay outside three s.d. from the mean permuted LOD, and all 600 permuted LODs were lower than the observed LOD for D15S130, giving an empirical *P*-value estimate of less than 0.0017.

Sensitivity analysis

Of the 95 individuals identified as unaffected informative pedigree members, seven were in liability class 1 (< 20 years of age), 10 were in liability class 2 (20–29 years of age), 19 were in liability class 3 (30–39 years of age) and 59 were in liability class 4 (> 40 years of age). When the affection status of each of these individuals was changed, one at a time and the analysis re-run, 41 individuals caused the LOD score to be reduced to less than 3.3. However, an increase

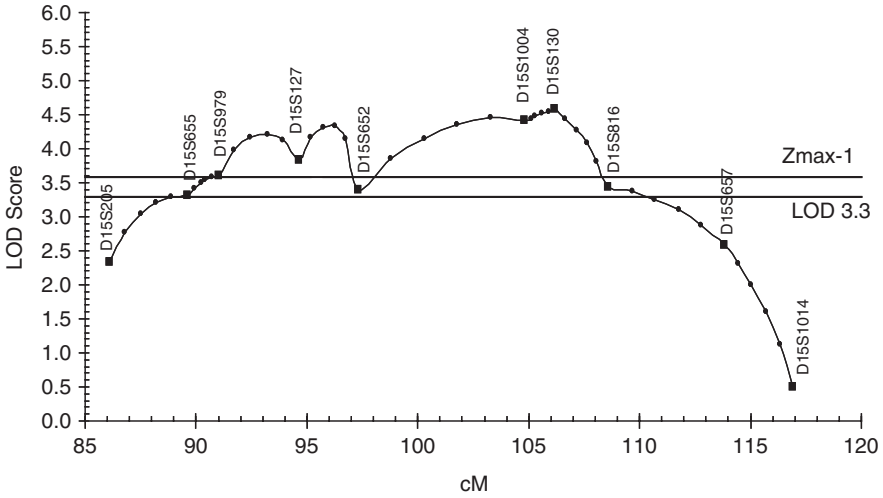

Figure 1 Multipoint linkage analysis across the 30 cM region of chromosome 15q25–26. A maximum LOD score of 4.58 at 106.15 cM was identified. The $Z_{\max-1}$ line is indicated showing the 95% confidence interval between D15S979 and D15S816. The LOD 3.3 line indicates a significance threshold of $P < 0.5$.

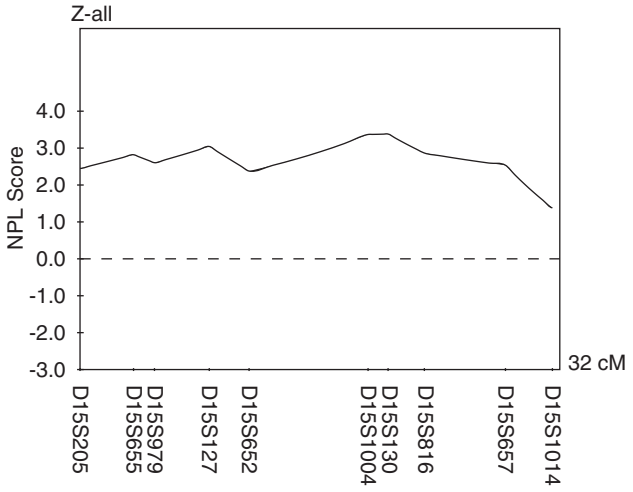

Figure 2 Non-parametric linkage (NPL) plot across the 30 cM region of chromosome 15q25–26. A maximum NPL score of 3.38 ($P = 0.0008$) at 107.16 cM was identified, near marker D15S130 (106.15 cM).

from the observed LOD score of 3.38 occurred in 42 individuals.

The average change across the 95 individuals was a reduction in the total LOD score of 0.14, a decrease of 4%. The greatest individual change showed a reduction in the total LOD score of 28% to 2.43. This individual was in liability class 4, and is in pedigree 113 which contributed the largest effect on the overall LOD.

Discussion

The current genome-wide scan revealed a number of chromosomal regions that showed positive results for linkage to BP. The most notable result was the significant LOD score found at marker D15S130 on chromosome 15q. Other chromosomal regions with suggestive linkage peaks in the genome-wide scan showed consistent results with other studies. The suggestive peaks at 4q and 13q are consistent with significant linkage results previously reported by our group in independent Australian BP cohorts,[32,42] while the 6q locus was reported as one of two confirmed BP loci in a genome-wide meta-analysis of 1067 families.[43]

The supplementary markers were added to confirm the genome-wide linkage to 15q, and to gain greater location estimates for a BP susceptibility gene around the maximal genome scan marker D15S130. Using Australian allele frequencies for the additional markers, a significant two-point LOD score of 3.38 was identified at marker D15S130. This was slightly lower than the original genome-wide scan result using equal allele frequencies, highlighting the importance of using correct population-specific allele frequencies in reporting linkage results. The multipoint analysis revealed a maximal LOD score of 4.58 near D15S130 with five flanking markers, one distal and four proximal, providing suggestive evidence of linkage, supporting the existence of a bipolar susceptibility gene at this locus. These parametric LOD scores for 15q25–26 are among the highest scores previously reported for linkage studies of BP. For example, the highest previously reported two-point LOD scores include 3.5 on chromosome 13q32,[44] 4.06 on chromosome 18q22–23,[45] 3.56 on chromosome 21q22,[46] 3.84 on chromosome 22q11–13[47] and a multipoint LOD score of 4.8 on chromosome 4p16[48] (reviewed by Baron[8]).

The additional statistical methods used to verify the significant genome-wide linkage signal support the original finding of a BP susceptibility locus on chromosome 15q25–26. The multipoint non-parametric analysis revealed an NPL score of 3.38 ($P = 0.0008$) obtained at 107.16 cM, compared to a parametric

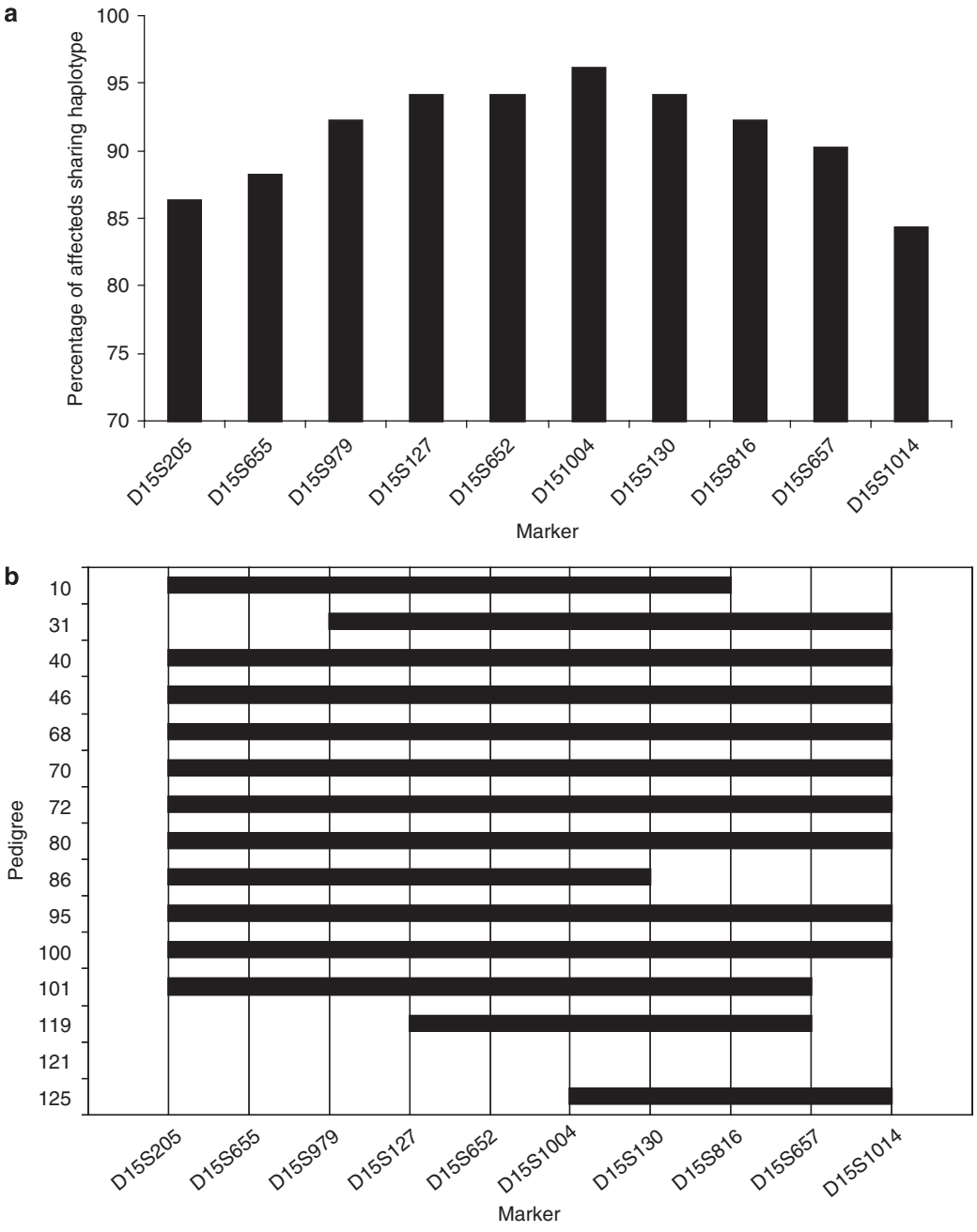

Figure 3 Haplotype narrowing by IBD sharing among affected pedigree members from families showing linkage to chromosome 15q25–26 region. (**a**) Plot of the percentage of affected pedigree members who share an allele of the pedigree-specific transmitted haplotype IBD. (**b**) The extent of the common disease associated haplotype is shown for each pedigree. Pedigree 121 did not share a common allele for any marker across all affected individuals.

multipoint LOD of 4.58, a drop of 26%. Since NPL scores are calculated using only the information from affected individuals, and parametric analysis uses information from all the available members of a pedigree, a loss of power with the NPL statistic is expected, although inaccurate specification of the inheritance model with parametric analysis can result in an increase in power for NPL analysis. To differentiate the effects of the model specification and affecteds-only analysis, a comparison can be made between the two-point parametric affecteds-only analysis (LOD = 2.97) and two-point parametric whole data analysis (LOD = 3.38), showing a drop of 12% in the total LOD score. While multipoint and two-point results are not directly comparable, it is likely that both the specification of the inheritance model, and the pedigree definition contribute to the observed drop in multipoint NPL.

The haplotype analysis based on pedigree-specific, identical-by-descent (IBD), allele sharing supported

the 95% confidence interval defined by the Z_{max-1} method in the parametric multipoint analysis. This haplotype narrowing method greatly aided the narrowing of candidate intervals in our previous study on chromosome 4q35,[36] and since there was no evidence for a common ancestral haplotype across the 15q25–26 linked pedigrees, nor was there a one-to-one correspondence between the disease and putative disease haplotype, it was a suitable method to conduct on this cohort. Two pedigree-specific recombination events prioritize a 2.5 Mb region for future association analysis, however as we are dealing with a complex oligogenic disorder, these recombination events do not guarantee the location of the susceptibility gene as genes at other loci may contribute to susceptibility in the two apparent recombinant families.

The results of the permutation analysis using actual pedigree structures and Australian allele frequencies showed that the linkage peak at D15S130 was statistically significant under the null model. Our observed LOD of 3.38 at D15S130 was outside 3 s.d. of the mean, yielding an empirical *P*-value estimate of less than 0.0017. Our observed LOD at D15S130 was higher than any of 600 permuted LODs; however, as additional replicates would yield a greater distribution of permuted LODs, we accordingly report an estimate of the empirical *P*-value. This analysis indicated that the observed LOD at D15S130 is a rare event and not likely to have occurred in the absence of true genetic linkage.

We performed a sensitivity analysis on our pedigrees, changing 95 informative unaffected individuals to an affected disease status, one at a time. The majority of these unaffected individuals were from liability class 4, where their age was greater than 40 years at the time of diagnosis. As bipolar disorder onset is typically in the late teens or twenties, it is likely that pedigree members would have exhibited symptoms by the age of 40 if they had inherited a susceptibility gene, and hence misdiagnosis is less likely in this age group. The liability threshold for age was accounted for within the penetrance models used with the parametric and non-parametric linkage analyses, so this should allow, to some degree, for phenotypic mis-specifications in younger pedigree members.

Of the 95 targeted informative individuals, the average LOD change was quite low (4% of the total LOD) which indicates the phenotypic stability of our data set. The greatest individual change was an LOD reduction of 28% from an individual in liability class 4 in pedigree 113. Because of the relatively large contribution of this pedigree (35%) to the overall LOD, any individual changes in this pedigree would have a relatively large impact on the total LOD score; however, as this individual and four other informative non-affected family members identified in pedigree 113 were in liability class 4, it is unlikely that phenotypic mis-specification in our pedigrees would be artificially inflating our observed linkage to 15q25–26.

Our original findings of a linkage peak in the chromosome 15q25–26 region have been verified by all three additional analysis methods, NPL analysis, affecteds only analysis and haplotype narrowing. These additional results provide support for a susceptibility gene for BP within the proposed locus on chromosome 15q.

Preliminary investigation of the chromosome 15q, Z_{max-1} confidence interval spanning D15S979 to D15S816, has revealed the 17 cM interval correlates with a physical distance of 6.2 Mb (using Ensembl). The interval contains 11 entries for novel protein-coding genes, and 56 entries for known protein-coding genes that were inspected for evidence of brain expression as well as potential functional roles that would implicate them as candidate genes. Several genes, including *SEMA4B* (semaphorin precursor 4B, which inhibits axonal extension by providing local signals to specify territories inaccessible for growing axons), *SV2B* (synaptic vesicle protein 2B) and *ST8S1A2* (α-2,8-sialyltransferase 2, which regulates normal and pathologic processes, including development, neuronal plasticity and tumour metastasis) are potential candidate genes for screening by association analysis in BP case–control cohorts. Arai *et al*.[49] have recently examined polymorphisms in the promoter region of the *ST8S1A2* gene and shown association with schizophrenia in a Japanese cohort, although association was not seen in an Italian cohort.[50]

Our finding of a BP susceptibility locus on chromosome 15q25–26 is supported by evidence of linkage with other mood and psychotic disorders. In a study of major depressive disorder (MDD), Holmans *et al*.[51] reported a significant multipoint LOD score of 3.73 between the markers D15S652 and D15S816 (90.3–92.8 Mb) in a genome-wide scan. The authors discuss the unclear relationship between major depressive disorder and BP, with BPII being especially difficult to differentiate from major depressive disorder. Maziade *et al*.[52] conducted a genome scan for shared and specific susceptibility loci for schizophrenia and BP. For a common locus phenotype definition combining the schizophrenia and bipolar phenotypes, significant evidence of linkage was found at the marker D15S1014 with a two-point LOD score of 4.55. In their multipoint linkage analysis a multipoint LOD score of 4.55 was observed between the markers D15S1014 and D15S966 (95.8–96.8 Mb). When the phenotypes for schizophrenia and bipolar disorder were analysed alone, a two-point LOD score of 2.31 was observed at D15S657 (94.5 Mb) for BP and a two-point LOD score, also of 2.31, was observed at D15S1014 (95.8 Mb) for the schizophrenia phenotype. Another genome scan by Park *et al*.[53] found suggestive evidence of linkage to chromosome 15q25–26 with bipolar disorder with psychotic features. A maximum two-point LOD score of 1.96 ($\theta = 0.10$) was found at the marker D15S652 under a dominant model. Multipoint affected sib-pair analysis identified a peak with an LOD of 2.62 between the markers D15S652 and

D15S816 (90.3–92.8 Mb). These overlapping linkage findings across the 15q25–26 locus with schizophrenia and major depressive disorder phenotypes suggest that a BP susceptibility gene in this region may also confer susceptibility to other mood and psychosis related disorders. Recently, Vazza *et al.*[50] reported linkage to chromosome 15q26 in a genome scan of an Italian cohort of 16 families with either schizophrenia or bipolar disorder. A maximum non-parametric linkage score of 3.05 was obtained at marker D15S1014. Together, the four linkage studies, combined with our results provide a compelling case that a bipolar disorder/schizophrenia susceptibility gene is located on chromosome 15q26.

It is possible that modest, parametric LOD scores in small samples are false positives, but since the linkage signal was also found in the same area using non-parametric analysis, and it coincides with linkage signals detected in other studies with related phenotypes, this supports our conclusions that the linkage signal we have detected on chromosome 15q is a true positive result.

This work on chromosome 15q25–26 illustrates a common theme across the genome, whereby regions linked to BP overlap those linked to other mood disorders and related phenotypes. Other loci showing evidence of linkage to bipolar disorder as well as to schizophrenia and other mood related disorders have been reported on a number of chromosomes.[10,54] This overlap of loci suggests that there are common molecular mechanisms among these clinically distinct, yet related disorders and may provide important insights to our future understanding of the biological basis of these disorders.

Screening additional pedigrees for linkage to chromosome 15q25–26 should assist with further haplotype narrowing and candidate gene prioritisation as a prelude to association analysis, to identify specific allelic variants that lead to bipolar susceptibility. By understanding the allelic variations, which confer susceptibility to BP, the molecular basis of the disorder can be defined and understood. This will aid in improving our ability to diagnose, treat and prevent the illness, and to reduce the medical, social and financial burden of this severe psychiatric condition.

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Web Resources

Australian Genome Research Facility http://www.agrf.org.au
Ensembl http://www.ensembl.org/
The Genome Database (GDB) http://www.gdb.org

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