American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 133B:18–24 (2005)

Joint Multi-Population Analysis for Genetic Linkage of Bipolar Disorder or "Wellness" to Chromosome 4p

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To test the hypothesis that the same genetic loci confer susceptibility to, or protection from, disease in different populations, and that a combined analysis would improve the map resolution of a common susceptibility locus, we analyzed data from three studies that had reported linkage to bipolar disorder in a small region on chromosome 4p. Data sets comprised phenotypic information and genetic marker data on Scottish, Danish, and USA extended pedigrees. Across the three data sets, 913 individuals appeared in the pedigrees, 462 were classified, either as unaffected (323) or affected (139) with unipolar or bipolar disorder. A consensus linkage map was created from 14 microsatellite markers in a 33 cM region. Phenotypic and genetic data were analyzed using a variance component (VC) and allele sharing method. All previously reported elevated test statistics in the region were confirmed with one or both analysis methods, indicating the presence of one or more susceptibility genes to bipolar disorder in the three populations in the studied chromosome segment. When the results from both the VC and allele sharing method were considered, there was strong evidence for a susceptibility locus in the data from Scotland, some evidence in the data from Denmark and relatively less evidence in the data from the USA. The test statistics from the Scottish data set dominated the test statistics from the other studies, and no improved map resolution for a putative genetic locus underlying susceptibility in all three studies was obtained. Studies reporting linkage to the same region require careful scrutiny and preferably joint or meta analysis on the same basis in order to ensure that the results are truly comparable.

KEY WORDS: allele sharing; variance component; QTL; LOD score

INTRODUCTION

Many genome scans to detect linkage of chromosome regions to common complex diseases have been performed world-wide. Typically, these studies differ in their sample design and method of statistical analysis. For example, study designs using affected relative pairs [Foroud et al., 2000; Bennett et al., 2002] nuclear or multiplex families [Berrettini et al., 1997; Detera-Wadleigh et al., 1999; Friddle et al., 2000; Badenhop et al., 2002; Liu et al., 2003; Zandi et al., 2003] and large extended pedigrees [Blackwood et al., 1996; Ginns et al., 1996; Ginns et al., 1998; Morissette et al., 1999; Ewald et al., 2002] have all been used to detect linkage for bipolar affective disorder (BPAD) [Prathikanti and McMahon, 2001; Segurado et al., 2003]. Depending on the sample design and trait measured, statistical methods such as parametric linkage analysis [Blackwood et al., 1996; Berrettini et al., 1997; Badenhop et al., 2002], non-parametric allele sharing methods [Ginns et al., 1998; Detera-Wadleigh et al., 1999; Foroud et al., 2000; Bennett et al., 2002; McInnis et al., 2003] and variance component (VC) methods [Visscher et al., 1999] have been used.

Generally, genome scans are performed on families ascertained for the disorder under investigation, rather than from a random sample of the population, and data across studies are not pooled for joint analysis. The latter is partly because of practical problems, for example, the problem of combining data or results from different studies which used different designs and different genetic markers, but also of perceived methodological issues. For example, the phenotypes can be defined differently across populations and genetic susceptibility in different populations may be associated with different genetic loci. Nevertheless, multiple reported linkages in the same region across studies or populations are usually taken as supportive evidence of 'true' linkage (rather than a false positive), and this evidence is subsequently used to pursue fine-mapping studies using population-wide association.

This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html.

Henrik Ewald died in January 2004. He will be sadly missed as a colleague, collaborator, and friend.

Grant sponsor: UK Biotechnology and Biological Sciences Research Council (to PMV and CSH); Grant sponsor: Organon (to PMV, DB, and WM); Grant sponsor: UK Medical Research Council; Grant sponsor: The Wellcome Trust; Grant sponsor: The Scottish Executive.

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Received 2 December 2003; Accepted 15 July 2004

DOI 10.1002/ajmg.b.30108

Recently, several examples of meta-analysis have been reported for psychiatric disorders [Badner and Gershon, 2002; Levinson et al., 2003; Segurado et al., 2003]. These studies typically use the published *P* values or test statistics to draw inference about linkage or association from the combined set of studies. These meta-analyses are extremely useful, and are often the only option if it is impossible to do a joint analysis on the original data. However, the number of hypotheses that can be tested are restricted in a meta-analysis and some precision of fine-mapping may be lost if no common markers are used across studies. Therefore, theoretically it is better to use the original data and perform novel analyses on these.

Several reports have shown that the chromosome 4p region shows linkage to bipolar disorder [Blackwood et al., 1996; Detera-Wadleigh et al., 1999; Ginns et al., 1998; Ewald et al., 1998; Als et al., 2004]. The aim of this study was to investigate whether the same underlying loci (quantitative trait loci, QTL) could be responsible, using the original data from studies in Scotland, Denmark, and the USA. These studies reported highly significant test statistics for either linkage with bipolar disorder [Blackwood et al., 1996; Ewald et al., 1998] or "wellness" [Ginns et al., 1998] in a small region of chromosome 4p, with the location of the linkage peak being remarkably consistent.

MATERIALS AND METHODS

Data

Phenotypic and genetic data were available from three populations: (i) data from a large Scottish pedigree [Blackwood et al., 1996], (ii) data from four large Amish pedigrees [Ginns et al., 1998], and (iii) data from two extended Danish pedigrees [Ewald et al., 1998]. Linkage to chromosome 4p has been reported in all three populations. The phenotype of interest in the Scottish and Danish studies was disease, whereas the USA study concentrated on wellness. The exact definitions of the phenotypes were given in the original publications. "Wellness" has been defined the same way by all groups. In all three studies, patients have been interviewed by a psychiatrist using standard schedules with additional diagnostic information gathered from other sources including case note review. All studies used DSM or ICD criteria for psychiatric diseases so a label of "wellness" or "no illnesses detected" should be the same across studies. A summary of the data is given in Table I. Phenotypes were available as binary traits, i.e., presence or absence of unipolar or bipolar disorder. For all analyses, a score of zero was defined as unaffected (well), and a score of one was defined as affected with either unipolar or bipolar disorder.

Marker data from the USA were selected to include markers in common with the Visscher et al. [1999] that used a VC analysis to detect QTL for bipolar disorder. In total, nine 'framework' markers were found which were in common with the 11 markers used by Visscher et al. [1999]. The two additional markers from the Scottish pedigree were not used in subsequent analyses. Although additional markers could have been used, little information is lost because of the small region investigated, ~30 cM. The Danish marker data contained 16 markers, four of which were in common with the Scottish and USA data sets.

TABLE I. Summary of Data

	Population		
	Scotland	USA	Denmark
Families	1	7	2
Individuals	168	637	108
Phenotypic records	143	233	86
Affected	28	93	18
Phenotypic SD[a]	0.40	0.49	0.41
Individuals with marker data	73	165	47
Markers	9	9	16

[a]From the binomial distribution, $\sigma_P = \sqrt{(P(1-P))}$.

Consensus Map Construction

A consensus map was created using all the available marker information. Firstly, the order of the nine markers was determined from separate linkage analysis on the Scottish and USA data sets, and subsequently, these data sets were jointly analyzed, constraining the marker order to that of a known physical map [Evans et al., 2001] if those orders were not significantly worse than the best order. Finally, the Danish data was added to create an overall consensus map. Crimap [Green et al., 1990] was used for these analyses. The consensus map was checked against a published linkage map [Broman et al., 1998].

Identity-by-Descent Coefficients

Given all the marker information, within population pairwise multipoint identity-by-descent (IBD) coefficients were calculated on a chromosome segment of 33 cM using the Markov Chain Monte Carlo (MCMC) software package Loki [Heath, 1997]. The program was run for 10,000 iterations, and the multipoint IBD coefficients were calculated at 1 cM intervals.

Data Analysis

Two methods were used to analyze the data, a VC and an allele sharing method. The VC method was applied before to the Scottish data (see [Visscher et al., 1999] for details). A mixed linear model was fitted to the binary observations, fitting sex and family as a fixed effect, and QTL and polygenes as a random effect. Sex was fitted as a fixed effect to adjust for any possible mean difference in incidence between males or females, which could arise due to real differences in population prevalence or as a result of ascertainment. For each of the three populations, three models were fitted: a residual model, fitting the residual effect as the only random component, a polygenic model, fitting both a polygenic and a residual component, and the full (QTL) model, fitting a residual, polygenic and QTL random effect. The evidence for a QTL was quantified by calculating a likelihood ratio test statistic. The test statistic was calculated as twice the difference in log-likelihood between the full (QTL) model and the reduced (polygenic) model. In the first test, the full model contained a QTL and a residual VC, and the reduced model contained residual variance only, while in the second test both the full and reduced model contained a polygenic VC. Asymptotically, the test statistics are distributed as $1/2\chi^2_{(1)}$ and $1/2\chi^2_{(0)}$ [Almasy and Blangero, 1998]. At each genomic location, the sum of the test statistics from each population was calculated. This sum is distributed proportional to a χ^2 with either 3 or 6 degrees of freedom (three studies and 1 or 2 degrees of freedom per test) under the hypothesis of no linkage. Finally, a joint analysis was performed for all data. Sex and a family-by-population effect were fitted as fixed effects, and the random effects were as in the individual population analyses. To test the hypothesis that variances were equal in the three populations, likelihood-ratio tests were performed. Twice the difference between the sum of the three population specific maximum log-likelihood values and the maximum log-likelihood from the joint analysis is asymptotically distributed as a χ^2 distribution with 2, 4, or 6 degrees of freedom for the residual, polygenic and full model, respectively. For each of the VCs, three components were estimated

from single population analyses, and one component was estimated from the joint analysis. These tests were performed under the assumption of a normal distribution of random effects.

Following the Affected Pedigree Member test presented by Weeks and Lange [1988], and its adaptation to use IBD rather than IBS sharing [Whittemore and Halpern, 1994; Sham, 1998], we implemented a multi-point allele sharing method to test for excess allele sharing among groups of individuals in large pedigrees. The method was performed to compare a non-parametric test for linkage to the VC method. The relevant statistics (observed and expected pairwise IBD coefficients) required to perform the allele sharing test are a natural by-product of the first method, because multi-point IBD coefficients and average relationships are both needed in our implementation of the VC method. Using the IBD coefficients calculated by Loki, an allele sharing test (Z) was calculated per population for each location, as, $Z = \sum(ibd_{ij} - a_{ij})/[var \sum(ibd_{ij})]^{1/2}$, with ibd_{ij} the proportion of alleles IBD for individuals i and j and a_{ij} the average (expected) proportion of alleles IBD. Coefficients a_{ij} were calculated from the numerator relationship matrix [Lynch and Walsh, 1998] and elements ibd_{ij} were calculated from Loki. The summation was taken over all pairs of either affected or unaffected individuals. In addition, an allele sharing statistic was calculated for all discordant pairs in the pedigree. Hence, per population and per location, three allele sharing tests were carried out, one for the affecteds, one for the unaffecteds, and one for discordant pairs. Parent-progeny pairwise comparisons were omitted from the analysis because there is no variation in the proportion of alleles shared IBD between parents and progeny.

The allele sharing test employed assumes that the marker loci are fully informative. The variance of the proportion of alleles shared IBD for a given pair is easy to calculate for fully informative markers in a non-inbred population: $E(ibd_{ij}) = a_{ij} = 1 \times P(2 \text{ alleles IBD}) + 1/2 \times P(1 \text{ allele IBD}) + 0 \times P(0 \text{ alleles IBD}) = P_2 + 1/2P_1$, and, $var(ibd_{ij}) = P_2(1 - P_2) + 1/4P_1(1 - P_1) - P_1P_2$. Except for fullsibs, where $P_2 = 1/4$ and $var(ibd_{ij}) = 1/8$, and double first cousin pairs, the probabilities of sharing two alleles IBD between relatives in a non-inbred population are zero. For those relatives, $P_1 = 2a_{ij}$ and, $var(ibd_{ij}) = 1/2a_{ij}(1 - 2a_{ij})$. Although the variance is relatively easy to derive for such relatives, the covariance of the sharing proportion between two pairs of affected individuals is not [Weeks and Lange, 1988]. With large pedigrees, the contribution of these (positive) covariances can dominate the variance of the overall test statistic, and should not be ignored. For large and complex, possibly inbred, pedigrees, the exact (co)variance of IBD sharing for pairs of relatives is difficult to calculate because it relies on the joint identity of alleles in up to four individuals [Weeks and Lange, 1988; Ward, 1993]. Therefore, a simulation gene-drop approach was used to estimate the variance of the test statistic under the assumption of a fully informative marker. In each simulation, alleles were 'dropped down' the pedigree, and the proportion of alleles IBD (0, 1/2, or 1) between all pairs of affected individuals was recorded. For each population, 100,000 simulations were performed.

The *Z*-test statistic is approximately distributed as a standard normal distribution, and $-\log_{10}(P \text{ value})$ is distributed as $0.22\chi^2(2)$ under the null hypothesis of no excess allele sharing (since $-2\ln(P) \sim \chi^2(2)$). At each genomic location, the sum of the test statistics ($-\log_{10}(P \text{ value})$) from each population was calculated. This sum is approximately distributed proportional to a χ^2 with 6 degrees of freedom (three populations and 2 degrees of freedom per population) under the hypothesis of no linkage.

If a disease allele is segregating in the pedigree, then affected pairs will on average share more of their alleles IBD than expected at and around the disease locus. Unaffected pairs are also expected to show excess sharing, but usually to a lesser extent because of incomplete penetrance. Finally, discordant pairs are expected to show a deficit in allele sharing. These expectations allow for a test of homogeneity of allele sharing statistics. Under the null hypothesis of no linkage, the test statistic $\chi^2 = \sum(Z_i - Z)^2$ with Z_i (i = 1, 3) the three Z statistics, follows a χ^2 distribution with 2 degrees of freedom. This test statistic was calculated for each of the three samples. The use of discordant pairs in an allele sharing framework to utilize all available information on linkage was suggested previously [Ward, 1993].

To investigate which kinds of relatives contribute to evidence for excess allele sharing, separate tests were performed for individuals with $a_{ij} = (1/2)^k$, for k=1,. . .,10.

RESULTS

Consensus Map

The consensus map which retains the order of the nine markers which were in common between the USA and Scottish datasets is given in the supplementary pages (see the online Supplementary Table at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html). Five markers from the Danish data set were ignored in further studies because they were only loosely linked to the region of interest. There is strong statistical support for this map. Individual and joint marker linkage analysis of the Scottish and USA data revealed that the constrained order of the nine markers was not significantly worse than any other order, with a log-likelihood (base 10) difference of 2.0 for the constrained order and the best order for the combined analysis. Adding the markers from the Danish study while constraining the order of the framework markers resulted in a sex-averaged map of 33 cM, from markers D4S2936 to D4S419. Two markers (D4S1551 and D4S391) were closely linked to D4S419 but outside the interval and only scored in the Danish sample. These markers were used for the multi-point IBD calculation in the Danish sample, but test statistics were only estimated in the 33 cM region.

VC Method

Figure 1 shows the result for the relevant statistical tests for QTL variance, from comparing the likelihoods of a model fitting a QTL plus polygenic variance and a model fitting only a polygenic variance. The test indicates that there is strong evidence for linkage in the Scottish families, moderate to strong evidence for linkage in the Danish families, and no evidence for linkage in the USA families. Interestingly, the Scottish data shows a small peak in the LOD curve at marker D4s1582 (22 cM), whereas the Danish families show a small dip at this location. However, this could be due to chance. The sum of the test statistics for each study is not more informative, because it follows the curve for the Scottish data; the curve for the USA data is close to zero, and the curve for the Danish data is fairly flat.

The joint analysis shows a peak at position 15 cM, with a LOD score of 2.7. The maximum likelihood estimator of the polygenic and QTL heritability was 0.10 and 0.24, respectively for this location. The likelihood-ratio test statistic to test for homogeneity of all variances is 29.0 at this position, indicating very strong evidence ($P = 0.0001$, 6 degrees of freedom) for heterogeneous variances. Likelihood-ratio tests for models containing either a residual random effect only or a polygenic and residual effect to test the hypothesis of homogeneous residual variances and homogeneous residual and polygenic variances also indicated strong evidence ($P < 0.0004$, 2 degrees of freedom and $P < 0.00003$, 4 degrees of freedom, respectively) for heterogeneity of variance.

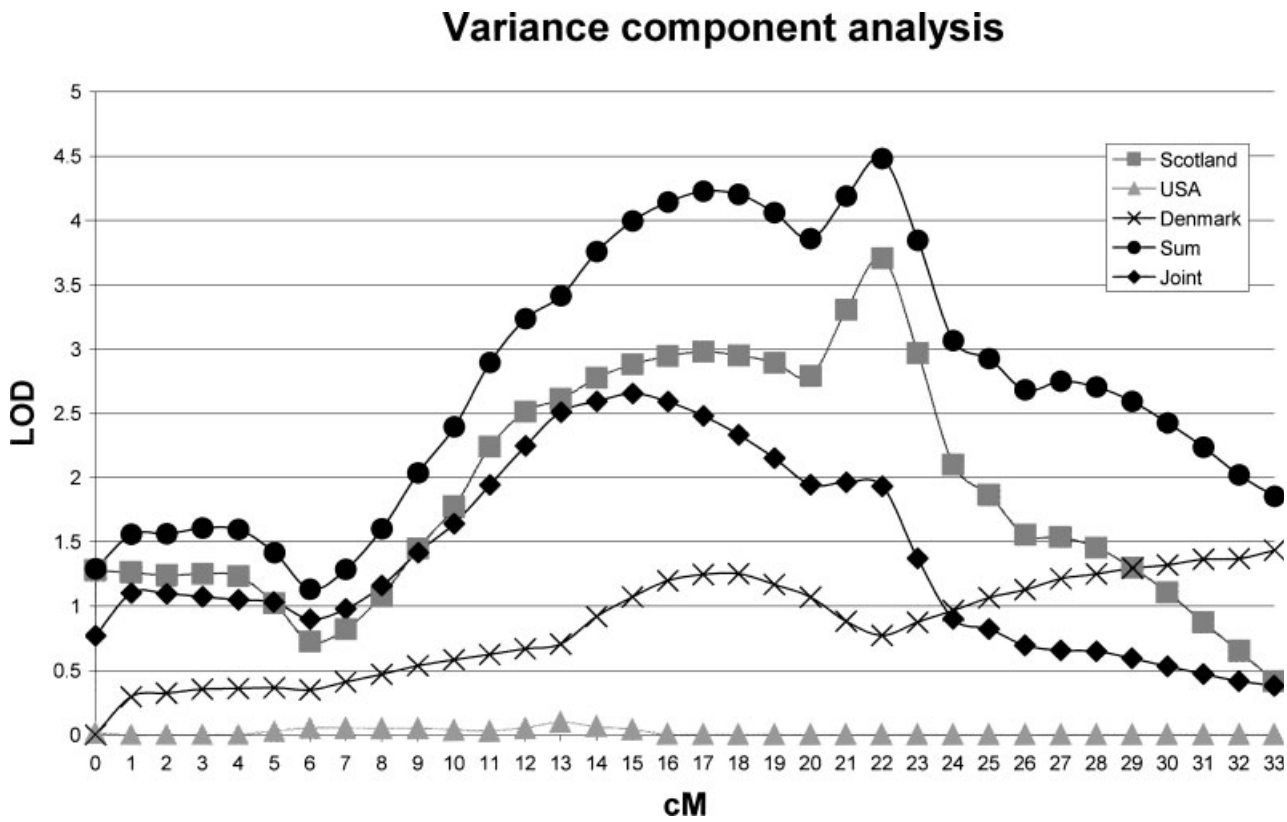

Fig. 1. Test statistics (LOD scores) from variance component (VC) analysis for individuals data sets, the sum of the test statistics and the test statistic from joint analysis.

Allele Sharing Method

Affected individuals. The allele sharing test for affected individuals shows a very strong signal for the Scottish data, with a peak of the Z statistic reaching 4.7 at marker D4s1582 (22 cM), a weaker signal for the USA data (Z statistic of 1.3 at marker D4s394; 13 cM), and no signal for the Danish data. The evidence for the large amount of excess allele sharing in the Scottish data is consistent for different kinds of relatives. For example, the average proportion of excess allele sharing (i.e., $\{ibd_{ij} - a_{ij}\}$ for groups of relatives with $a_{ij} = 0.50$, 0.25, 0.125, and 0.0625 was 0.21, 0.21, 0.22, and 0.24, respectively (results not shown in tables). Hence, for affected relatives that are expected to share only 0.0625 of their alleles IBD (e.g., cousins once removed), the averaged observed proportion of alleles IBD was $0.0625 + 0.24 = 0.3025$. The evidence for allele sharing in the USA data was mainly from two groups with $a_{ij} = 0.25$ (71 pairwise comparisons) and $a_{ij} = 0.015625$ (99 pairwise comparisons). There was no evidence for excess allele sharing among fullsibs (50 comparisons).

Summing up the logarithms of the P values (giving a test which is distributed proportional to a χ^2 with 6 degrees of freedom) again gave no clearer picture for the affecteds because the test statistic is dominated by the Scottish data, and the results are not shown.

Unaffected individuals. When the unaffected individuals were tested for excess allele sharing [Ginns et al., 1998], the USA data showed consistent evidence for allele sharing across the region, with a maximum Z statistic of 2.22, corresponding to a one-sided P value of 0.013 (at marker D4s1605; 20 cM). Excess allele sharing was observed for fullsibs ($a_{ij} = 0.5$, 114 pairwise comparisons) and for individuals and grandparents ($a_{ij} = 0.25$, 73 pairwise comparisons). Both the Scottish and Danish data sets showed random fluctuation around zero, consistent with no excess or deficit allele sharing among unaffected individuals.

Summing up the logarithms of the P values provided no additional information for the unaffecteds because the test statistic is dominated by the USA data, and the results are not shown.

Discordant pairs. The results from the discordant pair analysis are shown in Figure 2c. The USA sample shows a consistent excess sharing among discordant pairs, suggesting (Fig. 2a–c) that there is evidence for excess allele sharing in the entire pedigree. It is not clear why there is apparent excess allele sharing throughout the USA pedigrees. This could point to an unknown problem with one or more of the genetic markers, and we are investigating this issue further. Interestingly, the Danish data shows a consistent deficit of allele sharing among discordant pairs throughout the region, with a largest absolute Z statistic of -1.87, which corresponds to a one-sided test statistic of 0.031.

The P values for the test of homogeneity of the allele sharing statistics is shown in Figure 3. Clearly there is strong evidence for the Scottish data that the three Z statistics are from distributions with different means. The Danish data shows no significant difference between the three Z statistics, presumably because of the sample size. The USA data also shows no significant difference because of the similarity of all three Z statistics.

DISCUSSION

We have applied two statistical analyses to three independent samples which individually had shown strong evidence for linkage to either bipolar disorder or wellness in the same

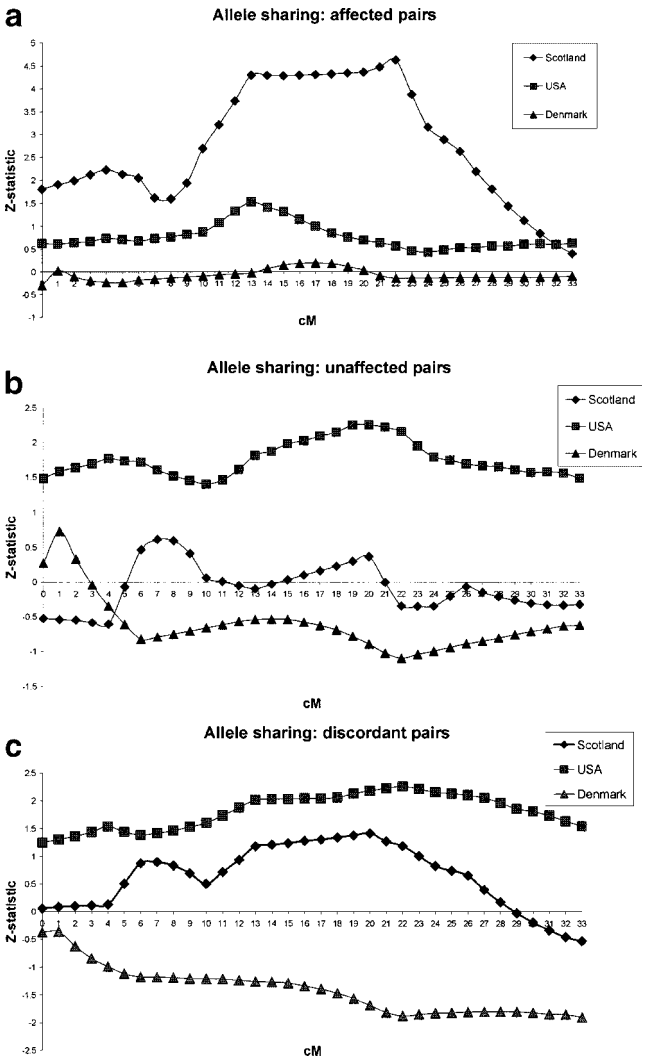

Fig. 2. Test statistics (Z statistics) from the allele sharing analysis for individuals data sets. **a**: Concordant affecteds, (**b**) concordant unaffecteds, (**c**) discordant pairs.

chromosomal region. For each of the three samples studied, the originally published elevated test statistic was confirmed with either one or both analysis methods. For the Scottish data, elevated test statistics were found with both the VC and allele sharing method. For the Danish data, the originally reported significant parametric LOD score was confirmed using the VC method. For the USA data set, evidence for excess allele sharing among the unaffected individuals was confirmed. Once a common subset of markers had been identified between the studies, the data collection and analyses was relatively straightforward. Note that a common set of markers is not strictly needed as long as all markers can be placed on a consensus map. It would be feasible to expand this kind of joint analysis to genome scans of multiple studies. In our opinion, in principle much is to be gained from combining data from multiple studies, because the limiting factor in most linkage studies is the sample size, in particular the number of meioses represented in the samples. Recent meta-analyses [Badner and Gershon, 2002; Levinson et al., 2003; Segurado et al., 2003] have captured on the power of analyses based upon large numbers of observations.

We chose the same subset of nine markers for the USA and Scottish data sets, whereas a larger number of markers were used in the original USA study [Ginns et al., 1998]. For example, we have not used the marker (D4S2949) that previously showed the largest test statistic [Ginns et al., 1998]. However, we still have a relatively dense marker map, nine polymorphic markers across ~30 cM or one marker every ~3–4 cM, which is at least two to three times denser than most genome scans. Both the allele sharing and VC approach that we have taken are multipoint. Hence, we do not analyze the test statistic at a marker location based only on linkage information for that marker, but also use linkage (IBD) information from all linked markers. This means that even though D4S2949 was not used, there is high information content at the same location as the marker so that the test statistics are likely to be very similar as if the marker had been genotyped. The multipoint aspect of our approach can be seen by the relatively smooth test statistic curves.

From our combined study, there is little evidence that the same genetic locus on chromosome 4p is responsible for genetic variation in susceptibility to bipolar disorder in the different data sets. Combining the test statistics across studies did not improve the mapping resolution. This is partially explained by the fact that the signal from the Scottish data is much larger than from the other studies, and that the test statistic for the Danish data is relatively constant across the interval because of the smaller sample size. However, we had hoped that the USA data, which is by far the largest of the three data sets, would have contributed in narrowing down the confidence interval for the putative QTL. Our finding has implications for candidate gene and/or positional cloning studies, because evidence from other studies 'confirming' linkage of loci to bipolar disorder may not be confirmed when analyzing the original data sets using the same statistical method.

The VC method and allele sharing method are consistent for the Scottish data only, i.e., showing evidence for a QTL in the 12–24 cM region. There is no evidence for a QTL in the USA data, but some evidence for excess allele sharing among unaffecteds. The reason that no QTL activity is detected by the VC method in the USA sample is puzzling, and suggests that the evidence from excess allele sharing between unaffected individuals is not consistent with a correlation between the phenotypic scores and IBD status. In the VC method, evidence for variance explained by a QTL partially comes from the difference in IBD coefficients between and within concordant and discordant pairs, and in the USA data this difference is apparently not large enough.

The allele sharing method combines the difference between expected and observed allele sharing for all pairs of relatives. Hence, fullsibs, uncle(aunt)–nephew(niece), cousins, individuals and grandparents and other more distant relatives all contribute to the test statistic. In deep pedigrees, this can be powerful, because for a number of disease models distant relatives provide more information than close relatives. For example, for a fully penetrant dominant disease allele, affected sibpairs will on average share 3/4 of their alleles IBD, and their expected contribution to the test statistic is $(3/4 - 1/2)/\sqrt{(1/8)} = 0.71$. Similarly, for individual-grandparent pairs, the expected contribution is $(1/2 - 1/4)/\sqrt{(1/16)} = 1.0$, i.e., larger contribution because of the smaller variance in allele sharing. The VC analysis also uses all comparisons in the pedigree but implicitly includes the difference in allele sharing of affecteds and unaffecteds analogous to fitting regressions of pairwise affected status on the proportion of alleles shared. Therefore, the VC analysis should be robust to deviations from expected segregation. The heterogeneity of the allele sharing test also combines all available information, but is conservative because it includes a comparison between the sharing statistic for concordant affecteds and concordant unaffecteds. Under a

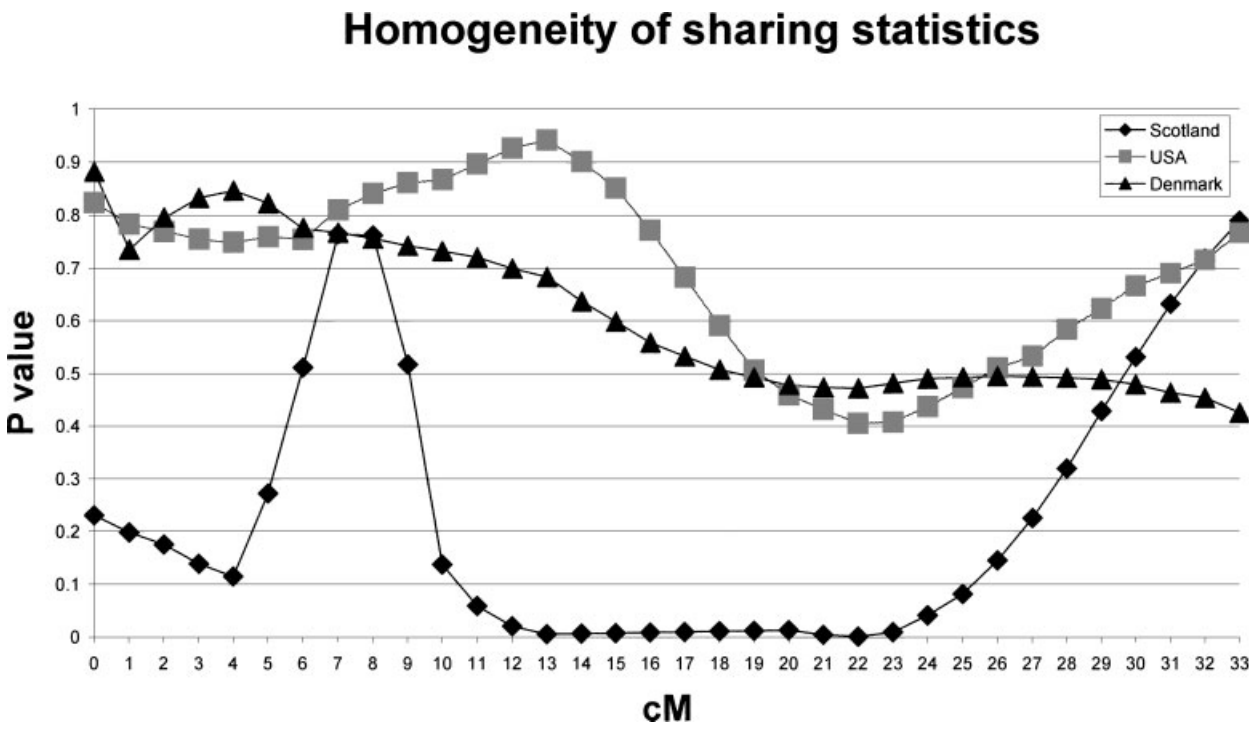

Fig. 3. Test statistics ($\chi^2_{(2)}$ statistics) from the homogeneity of allele sharing analysis for individual data sets.

number of disease models, these sharing statistics should besimilar. In addition, the heterogeneity test is robust to segregation distortion in the whole pedigree (Fig. 3). We illustrate these properties of the tests by plotting the $-\log(P$ values) for the Scottish data for the VC test, the allele sharing test for concordant affecteds and the heterogeneity of allele sharing test statistic (see the online Supplementary Figure at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html). The test statistics are similar in shape and all point to a ~10 cM region with elevated test statistics. The VC analysis appears almost as powerful as the allele sharing analysis. The heterogeneity test appears less powerful because of the small difference in Z statistic between the unaffecteds and the discordant pairs.

For the statistical analyses, we have made a number of assumptions that are strictly speaking not correct. For the VC analyses, we have assumed that the binary trait was normally distributed. However, previous work on QTL mapping in animal populations [e.g., Visscher et al., 1996] and the close correspondence between the LOD curve from multi-point linkage analysis and the VC method [Visscher et al., 1999] suggests that the VC method is robust to violations of assumptions regarding the distribution of the trait [see also Allison et al., 1999]. For the allele sharing method, we have assumed that markers are fully informative in the estimation of the variance of the test statistic. It was shown [Kong and Cox, 1997] that the variance of the test statistic can be severely overestimated (and therefore power decreased) if IBD probabilities are imprecisely estimated. In our study, the relatively dense marker map of approximately one polymorphic microsatellite marker every 3 cM appears to be a reasonable approximation to having fully informative markers, because many of the estimated IBD coefficients had values around 0, 0.50, and 1.0, i.e., the values expected in the case of sharing 0, 1, and 2 alleles identical by descent. Note that in the estimation of the IBD coefficients we do take account of all available pedigree information and hence take account of inbreeding when calculating the numerator of the test statistic. By performing gene-drop simulations to estimate the variance of the test statistic we also take account of inbreeding because simulations are conditional on the observed pedigrees. From the available pedigree information, we calculate that only 12 individuals (in the USA pedigrees) were inbred, with a mean inbreeding coefficient of 0.01.

In the published USA study [Ginns et al., 1998], common allele sharing was tested among people that were well. In this study, these have been termed 'unaffected.' For the VC approach, we have phenotypes that are either 0 ('unaffected') or 1 ('affected'). For the analysis, there is no distinction between an analysis of 'illness' and one of 'wellness,' because the objective of the analysis is to explain variation in the occurrence of illness, which is the same as explaining variation in the occurrence of wellness. In other words, if we had exchanged the codes (0 for affected and 1 for unaffected) we would have obtained the same answer. For the allele sharing approach, we have looked for excess allele sharing among affecteds (the usual approach) but also for excess allele sharing among unaffecteds (as was done in the original study [Ginns et al., 1998]). Depending on the incidence of a complex disease, the gene action underlying the phenotype and the population/pedigrees sampled, an allele sharing analysis of affecteds may or may not be more powerful than an allele sharing analysis of unaffecteds. A protective allele in a population with a high incidence of BPAD may be allelic to a susceptibility allele in a population with a lower incidence.

In conclusion, we have shown that it is feasible to perform joint analyses of independent data sets using different statistical methods based on the original marker and phenotypic data and a consensus linkage map. That the overall results are not as clear-cut as we had hoped is a clear reminder that studies reporting linkage to the same region require careful scrutiny and preferably joint or meta analysis on the same basis in order to ensure that the results are truly comparable.

ACKNOWLEDGMENTS

We thank Robert Elston for helpful comments and R. Filipiak for editorial assistance.

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