Bipolar disorder (BP) is a highly heritable disorder, however attempts to map genetic risk factors are challenging. One possible reason for these difficulties is the genetic heterogeneity of BP. Hence, focusing on clinically homogeneous families to create a genetically more homogeneous sample may increase the power of finding a specific variant. Alcohol abuse (AA) and alcohol dependence (AD) are familial in BP families, and these families may carry a specific risk variant for BP. We tested this hypothesis by performing a genome-wide linkage scan in 638 pedigrees (1,835 individuals) from the National Institute of Mental Health Genetics Initiative for BP, weighting the evidence for linkage according to the family’s frequency of AA or AD. Using AA weighting, we identified a linkage region on 9p22.2 with an NPL score of 3.23. The region had previously been identified in a meta-analysis of linkage in bipolar disorder. We used permutation analysis to assess if weighting by AA increased the linkage signal more than expected by chance and observed a significant $P$-value ($P = 0.048$). Therefore, the genetic risk factor for BP on 9p22.2 has an increased effect in families with high levels of AA. In summary, we present an example of using covariates such as AA and AD to define subtypes of BP, demonstrate how using such subtypes can improve the power of a linkage scan, and demonstrate statistical approaches to validate the suggested interaction.
Here we explore BP in comorbidity with alcohol abuse (AA) or alcohol dependence (AD). AA is defined as continued drinking despite social, legal or interpersonal problems; AD includes physiological tolerance or withdrawal [APA, 2000]. We differentiate between AA and AD because the categories describe different patterns of use: AA can occur when an individual uses alcohol in a manner that seriously interferes with their lives. It may be intermittent, for example in a binge-drinking fashion only during manias or represent a persistent maladaptive pattern of over use, for example, frequent but predictable heavy use with notable personal consequences. Alcohol dependence, on the other hand, describes a level of chronic use that results in physiological consequences (e.g., tolerance) with overwhelming psychological cravings and a repertoire of behaviors centered around using alcohol. Furthermore AA and AD diagnoses describe distinct long-term behavioral patterns; in prospective studies only 3.5% of AA subjects develop AD within 5 years, comparable to 2.5% incidence of AD in subjects with no alcohol-related diagnosis [Schuckit et al., 2001]. Thus, AA is not simply a “first step” to AD, but a different pattern of drinking that persists over time.

AA is comorbid with any BP at a rate of 39.1%; AD at a rate of 23.2% [Merikangas et al., 2007], these rates are twice as high as those in the general population [Hasin et al., 2007]. In a prospective cohort study, risk of AA was increased by pre-existing manic symptoms (OR 2.4 (95% CI 1.2–4.8)), and by a pre-existing diagnosis of BP (OR 9.1 (95% CI 27–31.2)). The risk of AD was also increased by pre-existing manic symptoms (OR 4.4 (95% CI 1.6–12.7)), and by pre-existing BP (OR 21.1 (95% CI 6.6–67.5)) [Merikangas et al., 2008]. Clinically, comorbidity of AA/AD with BP is detrimental to the patient in more ways than the added burden of disease. AA/AD is correlated with higher rates of suicidality [Potash et al., 2000; Baldassano, 2006] and increased number of hospitalizations [Cassidy et al., 2001], a less favorable response to lithium [Goldberg et al., 1999] and rapid-cycling [McKown et al., 2005].

The genetic predisposition to alcoholism has been hypothesized to constitute part of the genetic predisposition to bipolar disorder and vice versa [Winokur et al., 1996, 1998], and this genetic overlap explains the greater propensity for developing both BP and AA/AD. Heritability estimates of AA/AD based on twin studies have been as high as 50–60% [Prescott, 2001]. Furthermore, we and others found AA/AD to be familial in bipolar disorder, indicating that the increased comorbidity may be related to heritable causes [Winokur et al., 1996; Schulze et al., 2006; Nurnberger et al., 2007; Potash et al., 2007; Saunders et al., 2008]. Evidence of heritability for AA/AD and BP, combined with studies of familiality suggest nonlinear interaction of AA/AD and genetic risk variants for BP. In this model such risk variants will be more common in families with large number of members with AA/AD. Thus, we expect stronger signals for linkage in families with high levels of AA or AD. We tested this hypothesis by weighting the evidence for linkage in each family according to the family’s frequency of AA or AD. We consider AA and AD separately because they describe differing, enduring patterns of drinking behavior that may have different genetic bases. In addition to directly considering the resulting linkage signal we also test whether our weighting scheme significantly improves the evidence for linkage.

We obtained phenotype and genotype data on 711 pedigrees (5,364 individuals) from the National Institute of Mental Health Genetics Initiative for BP. This sample was collected over 15 years at 10 sites across the country. Methods for collection of this sample have been described elsewhere [Dick et al., 2003; McInnis et al., 2003]. Diagnostic assessment was done using the Diagnostic Interview for Genetic Studies [Nurnberger et al., 1994], and psychiatric diagnoses including mood and substance use disorder diagnoses were assigned using a best-estimate process [Leckman et al., 1982]. Within that sample, four disorders were of interest: BPI (N = 956), schizoaffective disorder-bipolar type (SAB) (N = 72), BP II (N = 128), and major depressive disorder-recurrent (MDDR) (N = 148). We classified subjects into three affection status models. Model 1 included 635 families with subjects affected by SAB or BPI, model 2 included 637 families affected by BPI, SAB, or BP II, and model 3 included 638 families affected by SAB, BPI, or MDDR.

Information on alcohol use was collected for 1,835 of these persons; 64% of this subsample were female, and the mean age of interview was 43. The mean age of onset of BP was significantly reduced in both the AA (P = 0.006, t-test) and AD (P = 7.2 × 10^-6, t-test) groups. AD in BP families was associated with a smaller likelihood of being currently married (P = 1.6 × 10^-5 χ^2-test, 1 df), lower mean number of school years (P = 3.0 × 10^-11, χ^2-test, 1 df), lower mean number of children (P = 0.009, χ^2-test, 1 df), and more episodes of illness (P = 0.015, χ^2-test, 1 df).

Individuals were typed for 391 microsatellite markers with an average spacing of 9 cM and average heterozygosity of 0.76 from a modification of the Cooperative Human Linkage Center version 9 [Dick et al., 2003; McInnis et al., 2003]. We used GENEHUNTER-PLUS (GHP) [Kruglyak et al., 1996] to perform multipoint non-parametric linkage analyses using the allele-sharing model (ASM) [Kong and Cox, 1997] analysis found in the GHP package calculating an NPL-score for each locus.

To weight for each pedigree by family levels of AA, we counted the number of individuals with AA in a family regardless of affection status and divided this count by the total number of family members with information known regarding alcohol abuse or dependence. We generated weights for family levels of AD analogously. Under this weighting scheme, 510 families based on AA and 355 families based on AD had a weight of zero (Fig. 1) and were thus ignored in the linkage analysis.

Using these weights, we recalculated NPL-scores for each family and each locus in the genome. Note that families with a weight of zero do not contribute to the evidence for linkage. To assess the significance of the observed increase in NPL score, we performed permutation studies. In each replication, we randomly reassigned the AA weights to the 711 families. Using the randomized weight file we repeated the ASM analysis and recorded the maximum NPL score. The process was repeated 1,000 times providing an empirical distribution. Comparing this empirical distribution with NPL scores generated using the original AA weighting, we generated P-values for these observations. We analogously generated P-values for NPL-scores based on AD weights.

Using AA weighting and affection status model 1, we observed a maximum NPL score of 3.23, with a corresponding LOD of 2.77. This linkage peak was located on chromosome 9p22.2, at 38.45 cM.
marker D9S925 (Fig. 2). This NPL score is a substantive improvement over the NPL-score generated using uniform weights (1.74). Nevertheless, the LOD score does not meet genome-wide significance standard of 3.3; however it is higher than the threshold of 1.9 for suggestive linkage [Lander and Kruglyak, 1995]. Permutation analysis indicated that the improved signal for linkage at 9p22.2 is higher than expected by chance ($P = 0.048$) and thus likely a result of our weighting scheme. The second strongest signal generated using weighting by AA was located on chromosome 3p14 at 77.12 cM, marker D3S4542. The NPL of 2.94 and LOD of 2.53 observed at this locus were above the threshold for suggestive linkage as well. Weighting on AD, we did not observe any regions

**FIG. 1.** Number of families with each alcohol abuse (left) or alcohol dependence (right) weight. Weights are a proportion calculated by the number of family members with AA or AD divided by the total number of family members.

**FIG. 2.** A suggestive area of linkage at 9p22 (NPL 3.23) with alcohol abuse (AA) weight. This region showed significant difference from the NPL score without weighting or with alcohol dependence (AD) weight.
with strong linkage. The strongest signal we observed using model 3 affection status was an NPL score of 2.41 found on chromosome 8, p23.2 at 4.30 CM with a corresponding LOD of 2.03. The weighting scheme actually decreased the NPL score; the unweighted score was 2.53.

In summary, we identified a suggestive linkage signal for bipolar disorder on chromosomal region 9p22 after weighting families by frequency of AA. This locus was previously identified as a region of suggestive linkage in bipolar disorder in a recent meta-analysis [Segurado et al., 2003]. This meta-analysis was mostly influenced by two unrelated samples, the Wellcome Trust UK-Irish sample [Bennett et al., 2002], and an unpublished study in Australia. However, further replication is necessary to affirm this finding.

Using permutation, we showed that our weighting scheme significantly enhanced the linkage signal, generating a maximum NPL score that is higher than expected by chance. The statistical significance of the linkage result indicates an increased effect of the detected locus in families with high levels of AA. To further evaluate the nature of this interaction, consider that the weighting scheme included family members with AA regardless of mood disorder. By not restricting the weighting to family members affected by mood disorder, we assumed a risk variant that jointly increases the risk for AA and BP disorder. Such an allele is most likely to segregate with affectation status, and not AD families, it may predispose individuals to impulsive behaviors that then take the form of alcohol abuse during mood episodes. Mood-dependent drinking occurs frequently in BP [reviewed in Goodwin and Jamison, 2007], and is an area of significant concern clinically, as alcohol abuse causes legal and social problems which add to the devastating toll of BP.

AA and AD are both potentially useful markers for defining subtypes of BP and exploring their interaction with genetic risk factors may thus further the understanding of its heterogeneity. Hence, the demonstrated interaction between AA and BP may help identifying subtypes of BP and tailor treatment strategies to the patient.

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