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A Reanalysis of 409 European-Ancestry and African American Schizophrenia Pedigrees Reveals Significant Linkage to 8p23.3 With Evidence of Locus Heterogeneity

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The detection and replication of schizophrenia risk loci can require substantial sample sizes, which has prompted various collaborative efforts for combining multiple samples. However, pooled samples may comprise sub-samples with substantial population genetic differences, including allele frequency differences. We investigated the impact of population differences via linkage reanalysis of Molecular Genetics of Schizophrenia 1 (MGS1) affected sibling-pair data, comprising two samples of distinct ancestral origin: European (EA: 263 pedigrees) and African-American (AA: 146 pedigrees). To exploit the linkage information contained within these distinct continental samples, we performed separate analyses of the individual samples, allowing for within-sample locus heterogeneity, and the pooled sample, allowing for both within-sample and between-sample heterogeneity. Significance levels, corrected for the multiple tests, were determined empirically. For all suggestive peaks, stronger linkage evidence was obtained in either the EA or AA sample than the combined sample, regardless of how heterogeneity was modeled for the latter. Notably, we report genomewide significant linkage of schizophrenia to 8p23.3 and evidence for a second, independent susceptibility locus, reaching suggestive linkage, 29 cM away on 8p21.3. We also detected suggestive linkage on chromosomes 5p13.3 and 7q36.2. Many regions showed pronounced differences in the extent of linkage between the EA and AA samples. This reanalysis highlights the potential impact of population differences upon linkage evidence in pooled data and demonstrates a useful approach for the analysis of samples drawn from distinct continental groups. © 2008 Wiley-Liss, Inc.

KEY WORDS: psychiatry; mental disorders; genetics; epidemiology; genetic susceptibility

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INTRODUCTION

Schizophrenia (SZ) is a severe, debilitating psychiatric disorder, the precise causes of which are unknown. Although family, twin and adoption studies suggest a large genetic component to liability [Kety et al., 1978; Cardno and Gottesman, 2000; Tsuang et al., 2001] and heritability estimates are on the order of 80% [Cannon et al., 1998; Sullivan et al., 2003], inheritance of the disorder is complex and likely involves an oligogenic or polygenic model [Risch, 1990]. To date, the results from genomewide linkage studies indicate an absence of loci of major effect and suggest that susceptibility genes of modest effect may reside in a number of genomic regions, including 1q, 5q, 6p, 6q, 8p, 10p, 10q, and 13q [Owen et al., 2005]. Both the magnitude of the genetic effect and the mode of inheritance (MOI) of individual loci remain unknown. The regions implicated by different studies also vary widely, suggesting that susceptibility genes differ across populations and study samples.

A factor which may reduce the power of SZ linkage analyses is locus heterogeneity, which occurs when only a subset of pedigrees segregate markers linked to a particular risk locus. A statistic which allows for locus heterogeneity is the "admixture" [Smith, 1961] or "heterogeneity" [Ott, 1999] LOD score (HLOD), which involves maximizing the linkage likelihood function with respect to both the recombination fraction (θ) and the probability that a given pedigree is of the "linked" type (α). Under various inheritance models, the HLOD statistic has been shown to provide a robust test for linkage [Durner et al., 1992; Goldin, 1992; Greenberg and Abreu, 2001]. However, the use of HLOD statistics is not entirely straightforward. Their asymptotic null distribution varies with the specified penetrance model [Huang and Vieland, 2001b] and estimates of the heterogeneity parameter (α) are biased when assumptions of the admixture test are violated [Whittemore and Halpern, 2001]. Indeed, α typically does not reflect the proportion of linked pedigrees [Pal and Greenberg,

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2002]. In spite of these caveats, in some circumstances, incorporation of α as a "nuisance" parameter can afford higher power to detect linkage than either homogeneity LODs or "nonparametric" (more appropriately termed "model-(specification)-free") methods [Faraway, 1993; Goldin and Weeks, 1993; Greenberg and Abreu, 2001; Vieland and Logue, 2002]. It may also provide more accurate estimates of θ [Falk, 1997; Finch et al., 2001; Vieland and Logue, 2002]. HLOD statistics may thus be useful when testing for linkage in SZ.

Another issue in genetic analyses of SZ is the requirement for large samples, both for initial detection [Risch and Merikangas, 1996] and replication [Suarez et al., 1994]. Larger samples can readily be achieved by combining the data from multiple, independent datasets; these may be sampled from different populations or from the same population by different groups [e.g. Levinson et al., 2000]. However, if individual samples are derived from populations with different demographic histories, they may exhibit marker and disease allele frequency differences, leading to inter-sample differences in the prevalence of risk loci and the proportion (α) of linked pedigrees. If substantial, such differences can render the power of the pooled sample *less* than that of the constituent dataset/s in which the evidence for linkage is highest [Vieland et al., 2001], due to a reduced proportion of linked pedigrees in the combined sample. For such pooled data, it may be preferable to conduct analyses allowing for inter-sample differences in α. Via simulation, Vieland et al. [2001] demonstrated the utility of adaptations of the HLOD which estimate α separately for constituent datasets. The preferred "compound" HLOD (HLOD-C), is maximized simultaneously over θ and a separate α_i for each dataset, but can be prohibitively inefficient to compute. A simple approximation to HLOD-C which maintains comparable power was denoted the "summed" HLOD (HLOD-S), and is formed by summing the simple HLOD scores obtained in each constituent dataset.

Significant inter-sample differences in locus heterogeneity have been detected for several putative SZ susceptibility loci [Zintzaras and Ioannidis, 2005]. A previous linkage study investigated the impact of such differences via reanalysis of National Institute of Mental Health Genetics Initiative (NIMH-GI) SZ data [Cloninger et al., 1998], which comprised two samples, drawn from the European American (EA) and African American (AA) populations. The original linkage analyses considered the EA and AA samples separately, due to significant founder allele frequency differences. Using the NPL statistic, "suggestive" linkage [Lander and Kruglyak, 1995] to chromosome 10p was detected in the EA sample [Faraone et al., 1998]. A reanalysis of these data incorporating within- and between-sample locus heterogeneity [Holliday et al., 2005] detected genomewide *significant* linkage to 10p in the EA sample, and also in the pooled sample, but only when the heterogeneity parameter was estimated separately for the individual samples. The EA and AA samples showed large differences in linkage evidence to chromosome 10p, suggesting the utility of this approach when combining multiple datasets with known genetic differences in the presence of suspected locus heterogeneity.

The Molecular Genetics of Schizophrenia 1 (MGS1) collaboration recently reported a genomewide linkage scan for SZ, based upon one of the largest affected sib-pair (ASP) cohorts collected to date [Suarez et al., 2006]. The cohort comprised two samples of distinct ancestral origin: European (EA, $n = 263$ pedigrees) and African-American (AA, $n = 146$ pedigrees). The two samples were combined for the primary linkage analysis, which was performed using the exponential LOD score [Kong and Cox, 1997] and the S_{all} scoring function. Exploratory analyses assessed the evidence for linkage within the individual EA and AA samples, also using the exponential LOD score. In the primary analysis, genomewide suggestive linkage was observed in two regions, with the strongest signal obtained at chromosome 8p21 (LOD $= 2.3$, $Z_{lr} = 3.25$). This signal was contributed almost entirely by EA pedigrees.

The EA and AA samples comprising the MGS1 data are of comparable size, if not larger, than other samples in which significant linkage to SZ has previously been obtained [Blouin et al., 1998; Fallin et al., 2003; Abecasis et al., 2004; Arinami et al., 2005]. These individual samples may thus have sufficient power in their own right to detect SZ risk loci. Furthermore, given evidence for genetic differences between these ancestral groups [Bowcock et al., 1994; Jorde et al., 1997; Stephens et al., 2001; Suarez et al., 2006], and previous evidence for locus heterogeneity in SZ, the effect size of risk loci may differ between the two samples. To offer additional insight into the overall evidence for linkage and effect of population differences between the EA and AA samples, we conducted a reanalysis of the MGS1 data, incorporating within- and between-sample locus heterogeneity.

MATERIALS AND METHODS

Ascertainment and Diagnosis

This sample has been previously described in detail [Suarez et al., 2006]. Briefly, ASP pedigrees of predominantly European (EA) or African American (AA) descent were ascertained at nine sites located in the United States and one Australian site. All patients gave informed consent for participation in genetic studies. Patients were interviewed using the Diagnostic Instrument for Genetic Studies (DIGS) [Nurnberger et al., 1994], and diagnoses were made using DSM-IV criteria [American Psychiatric Association, 1994]. Subjects were classed as "affected" if they satisfied a diagnosis of SZ or schizoaffective disorder (SA). The affection status of remaining subjects was considered unknown. All pedigrees contained a minimum of two affected siblings, at least one of whom satisfied a diagnosis of chronic SZ. Subjects' ethnicities were self-reported, with membership of ethnic groups confirmed by cluster analysis of allele frequencies. Fifteen pedigrees (consisting of thirteen not clustering with EA or AA groups (i.e., not attaining a posterior probability of group membership of ≥ 0.85, using STRUCTURE) [Pritchard et al., 2000] and two pedigrees with self-reported Asian ancestry) were excluded from subsequent analyses. A total of 263 EA and 146 AA pedigrees were included in linkage analyses [Suarez et al., 2006].

Genotyping and Marker Characteristics

Subjects were genotyped at 401 STRP markers spanning autosomes and the X chromosome at an average intermarker distance of 9 cM. Genotyping was performed by the Center for Inherited Disease Research (CIDR). For the original linkage analysis, maximum-likelihood allele frequency estimates were obtained separately for the EA and AA families using the USERM13 subroutine of MENDEL [Lange et al., 1988; Boehnke, 1991]. For the current analysis, we used these same allele frequencies and specified the EA and AA allele frequencies separately, due to significant group differences at most markers [Suarez et al., 2006]. In the pooled sample, this was achieved by giving markers distinct designations for each group and treating the two sets as unique markers spaced 0.001 cM apart. Pedigree genotypes were coded null (missing) at map positions corresponding to the alternate ethnicity. Marker genetic positions were obtained from a genetic map [Duffy, 2006] (see Web Resources for genetic map site) integrating Build 35.1 National Center for Biotechnology Information (NCBI) physical map positions and published Rutger's genetic map positions [Kong et al., 2004]. The position

of markers not listed on this map was estimated via linear interpolation using known CIDR map positions (see Web Resources for CIDR genetic map site). Unlikely genotypes were identified and removed using MERLIN [Abecasis and Wigginton, 2005]. Consistency of pedigree and marker data with those from the original analysis was confirmed by replicating the previously reported results using MERLIN [Abecasis and Wigginton, 2005].

Genetic Analyses

Multipoint linkage scores were calculated at 1 cM increments using MERLIN [Abecasis and Wigginton, 2005]. The EA and AA samples were analyzed using parametric heterogeneity LOD scores calculated under simple (i.e., no phenocopies) dominant (HLOD-D) and recessive (HLOD-R) modes of inheritance (MOI) [Greenberg et al., 1998], specifying disease gene frequencies of 0.01 and 0.1, respectively [Pal et al., 2001] and assuming 50% penetrance [Hodge et al., 1997]. The allele-sharing statistic used in original analyses of these data only utilizes the genotypes of affected individuals, while parametric methods use the genotypes of both affected and unaffected individuals. To ensure that differences in our results did not result from the inclusion of genotype-phenotype information from unaffected individuals, an "affecteds-only" parametric analysis was conducted by coding the phenotype of unaffected individuals as "unknown." For the combined sample, HLOD-S statistics were calculated under simple dominant (HLOD-DS) and recessive (HLOD-RS) models by summing the EA and AA HLOD scores obtained at each position for the relevant model [Vieland et al., 2001]. This allowed the heterogeneity parameter α_i to be estimated separately for the EA and AA samples. Six statistics in total were calculated: EA-HLOD-D, EA-HLOD-R, AA-HLOD-D, AA-HLOD-R, HLOD-DS, HLOD-RS. Based on each statistic's empirical distribution, all scores were converted to $-\log_{10}$[*P*-value](see below), to allow their comparison. At each genomic position, the highest of the six scores ($HLOD_{max}$, expressed as $-\log_{10}$[*P*-value]) was recorded. For comparison with the original analysis [Suarez et al., 2006], we also calculated LOD scores in the pooled sample using the Kong and Cox [1997] exponential model and the Whittemore and Halpern [1994] S_{all} statistic (LOD_{exp}).

The HOMOG3 program [Ott, 1991] was used to test the hypothesis of two chromosome 8 peaks. Input for this program comprised familial LODs calculated at 1 cM increments using MERLIN [Abecasis and Wigginton, 2005]. Whereas the MERLIN HLOD analysis specifies two family types [a proportion (α1) of families with linkage between a trait and marker locus, the remaining families without linkage], the HOMOG3 analysis specifies three family types. Log likelihoods are calculated under the assumption that in a proportion (α1) of families a trait is linked to locus 1 and in a proportion (α2) of families the trait is linked to locus 2, where the two loci (or maps of markers) are located on the same chromosome. There may be a third proportion ($\alpha 3 = 1 - \alpha 1 - \alpha 2$) of families without linkage to either locus 1 or 2.

Empirical *P*-Value Determination

Our analytic approach involved calculating multiple (6), correlated, but nonidentically distributed HLOD statistics. To accurately estimate genomewide significance levels, we used an empirical method [similar to Ferreira et al., 2005]. For the EA and AA samples, MERLIN [Abecasis and Wigginton, 2005] was used to generate 5,000 data replicates under the null hypothesis of no linkage, retaining the original marker map, allele frequencies and missing data pattern. Replicates were analyzed for linkage using HLOD-D and HLOD-R. For the combined sample, HLOD-DS and HLOD-RS statistics were formed by summing the empirical EA and AA HLOD-D and HLOD-R statistics, respectively. For each of the six statistics, HLOD scores obtained from the 5,000 replicates were combined with the actual genome scan results to form a single distribution. Based on the relevant distribution, each HLOD score (h) was converted to an empirical pointwise *P*-value, defined as the proportion of scores in the empirical distribution $\geq$h, and expressed as $-\log_{10}P$.

The empirical null distribution of $HLOD_{max}$ was derived by recording, for each replicate, the highest of the six $-\log_{10}P$ values obtained at each position. This generated an empirical distribution of the highest score at each position ($HLOD_{max}$), which naturally accounts for both multiple testing and the correlation between statistics. The genomewide significance level associated with the highest observed $HLOD_{max}$ score was defined as the frequency of equivalent or higher peaks in the empirical distribution. Linkage peaks separated by >40 cM were considered independent [Ott, 1999; Wiltshire et al., 2002].

For LOD_{exp} scores, the genomewide significance of observed peaks was determined by calculating the LOD_{exp} in simulated replicates of the pooled sample and determining the frequency with which peaks equivalent to or higher than the observed peaks occurred in simulated data. In the primary analysis, we did not correct for calculating LOD_{exp} scores, as these were computed only to facilitate comparison with the original results.

Thresholds for genomewide significant and suggestive linkage were defined as scores occurring with probability 0.05 and 1 in a single genome scan by chance [Lander and Kruglyak, 1995]. We also calculated the number of regions showing evidence for linkage at nominal pointwise $P \leq 0.01$ and $P \leq 0.05$, which provide confirmation (replication) of previously reported significant linkages ($P \leq 0.01$), and facilitate comparison with independent studies ($P \leq 0.05$) [Lander and Kruglyak, 1995]. Furthermore, an excess in the number of observed regions exceeding these thresholds (compared with the number expected by chance) can provide evidence for the presence of multiple, distinct susceptibility loci, which may be beneficial if no region achieves genomewide statistical significance [Wiltshire et al., 2002].

RESULTS

The maximum of EA-HLOD-D, EA-HLOD-R, AA-HLOD-D, AA-HLOD-R, HLOD-DS, and HLOD-RS scores ($HLOD_{max}$), expressed as $-\log_{10}P$ and plotted at 1 cM increments across the genome, is shown in Figure 1. Based on the empirical distribution of $HLOD_{max}$ (incorporating correction for the six statistics), the threshold for genomewide significant linkage was 4.542 ($-\log_{10}P$). The threshold for genomewide suggestive linkage was 3.094 ($-\log_{10}P$).

The highest $HLOD_{max}$ was observed at 8p23.3, 10 cM from the p-telomere (p-ter), near D8S262 ($-\log_{10}P = 4.692$: Figs. 1 and 2). This peak surpassed the threshold for genomewide significant linkage, with only 177 peaks $\geq$4.692 observed in 5,000 simulated replicates of $HLOD_{max}$ (corrected genomewide $P = 0.0354$). This result also remained significant after additionally correcting for the LOD_{exp} statistic used in the original analysis ($P = 0.0442$). Linkage to 8p23.3 was detected only in EA pedigrees, in which the strongest evidence was obtained under a recessive model ($-\log_{10}P = 4.692$, EA-HLOD-R = 3.553). Compared to EA-HLOD-R, reduced linkage evidence was observed using HLOD-RS in the combined sample (peak $-\log_{10}P = 4.058$).

In addition to the significant linkage peak at 8p23.3, suggestive linkage was detected for an additional three regions using $HLOD_{max}$: 5p13.3, 7q36.2, and 8p21.3 (Figs. 1 and 2, Table I). After the 8p23.3 peak, the next highest $-\log_{10}P$ was

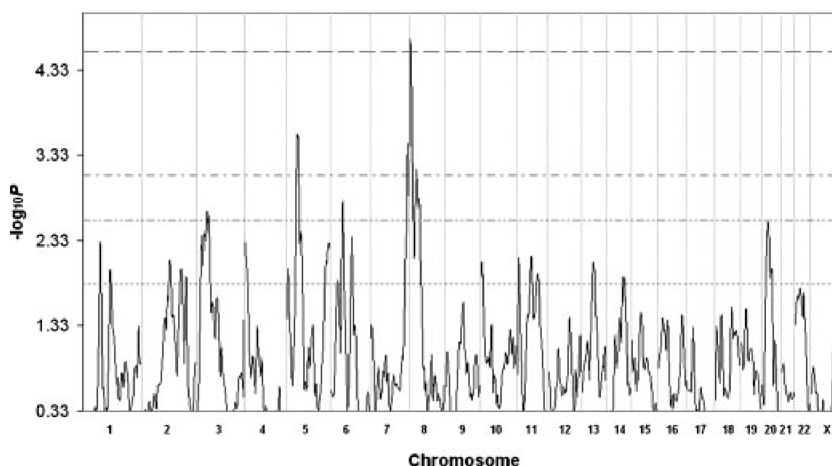

Fig. 1. Genomewide linkage results for EA, AA, and combined samples obtained using $HLOD_{max}$. For each sample, standard HLOD scores (EA and AA samples) or "summed" HLOD scores (HLOD-S; combined sample) were calculated at 1 cM increments under simple dominant and recessive models. Values for each statistic were converted to $-\log_{10}P$ values using 5,000 genome scan replicates generated under the null hypothesis of no linkage. At each position, the highest of the six statistics was taken as the evidence for linkage ($HLOD_{max}$). The significance of individual peaks was determined from an empirical distribution of $HLOD_{max}$, obtained by taking the highest of the six scores at each position for the 5,000 genome scan replicates for each statistic. The minimum value for the Y-axis corresponds to $-\log_{10}(Z)$, where Z is the highest of the pointwise P values corresponding to zero for the six constituent statistics. The figure shows empirical thresholds for genomewide significant linkage (*dashed line*), suggestive linkage (*dash-dot line*), nominal $P = 0.01$ (*dash-dot-dot line*), and nominal $P = 0.05$ (*dotted line*).

observed at 5p13.3, 55 cM from p-ter (D5S1470-D5S1457: $-\log_{10}P = 3.569$, expected number of false positives per genome scan = 0.390). Evidence for linkage to this region was observed almost exclusively in EA pedigrees, with the highest $-\log_{10}P$ value obtained under a recessive model ($-\log_{10}P = 3.569$, EA-HLOD-R = 2.569). Compared to EA pedigrees, the evidence for linkage to 5p was reduced in the combined sample using HLOD-RS (peak $-\log_{10}P = 3.097$). The third highest $HLOD_{max}$ peak was observed at 7q36.2, 174 cM from p-ter, near D7S3058. Evidence for linkage to this region was highest in AA pedigrees under a recessive model ($-\log_{10}P = 3.463$, AA-HLOD-R = 2.523, expected number of false positives per genome scan = 0.486). Compared to the AA pedigrees, the evidence for linkage to 5p was reduced using HLOD-RS in the combined sample (peak $-\log_{10}P = 2.974$). The fourth-highest $HLOD_{max}$ peak was observed at 8p21.3, (D8S1145-D8S560), 29 cM centromeric to the significant 8p23.3 peak. Evidence for linkage to 8p21.3 was detected only in EA pedigrees, in which the highest score was observed under a dominant model ($-\log_{10}P = 3.171$, EA-HLOD-D = 2.202, expected number of false positives per genome scan = 0.863). However, considerable evidence was also present under a recessive MOI ($-\log_{10}P = 2.823$, EA-HLOD-R = 1.888). Reduced linkage evidence was observed using HLOD-DS in the combined sample (peak $-\log_{10}P = 2.648$).

The second chromosome 8 peak appeared (Fig. 2) to be distinct to the higher, more telomeric peak, based on approximate 95% confidence intervals (CI) for the location of each peak. If the asymptotic properties of a linkage statistic are known, the genomic coordinates delineating an approximate 95% CI lie where the linkage trace passes through (peak−drop), where "peak" equals the peak score, and "drop" represents the value of the statistic associated with a one-sided P-value of 0.025 ($95\% = 1 - 2 \times 0.025$). For standard LOD scores, distributed as $0.5\chi_0^2 + 0.5\chi_1^2$, the "drop" equals 0.834, which is commonly (though inaccurately) approximated as a "1-LOD drop." The asymptotic properties of HLOD statistics are complex and variable [Huang and Vieland, 2001b], but properties of their distributions may be empirically derived for given samples and penetrance models. Based on empirical distributions of the EA-HLOD statistics, the "drop" equals 0.827 for EA-HLOD-D and 0.818 for EA-HLOD-R. Using these estimates, the approximate 95% CI's for the two 8p peaks do not overlap, suggesting that they may represent distinct susceptibility loci.

To formally test the hypothesis of one versus two chromosome 8p loci, we used the HOMOG3 program [Ott, 1991]. The maximum log likelihood ($\ln L$) for there being three family types (i.e., 2 linked and 1 unlinked loci) was 10.9835 ($\log_{10}L$ = HLOD = 4.770), whereas the maximum $\ln L$ for there being two family types (i.e., 1 linked and 1 unlinked locus) was 8.179 ($\log_{10}L$ = HLOD = 3.552, analogous to the maximum MERLIN HLOD). Twice the difference between these likelihoods ($2 \times 2.805 = 5.609$) approximately follows a Chi-square distribution on 1 df (two-sided) and provides a test for 2 versus at most 1 locus. These results (i.e., $\chi_1^2 = 5.609$; $P = 0.0179$) thus provide nominal support for the presence of two loci on chromosome 8p. A similar result was obtained using recessive LODs at the 8p23.3 locus and dominant LODs at the 8p21.3 locus ($\chi_1^2 = 4.641$; $P = 0.0312$), which reflect the MOIs providing the strongest evidence for linkage in the genome scan.

For comparison with previous results we have also reported regions showing evidence for linkage at nominal pointwise $P \leq 0.01$ and $P \leq 0.05$ thresholds using $HLOD_{max}$. These thresholds were derived using the empirical distribution of $HLOD_{max}$. A *nominal* $P = 0.01$ corresponds with a $HLOD_{max}$ of 2.593, while a *nominal* $P = 0.05$ is equivalent to a $HLOD_{max}$ of 1.864. Based on these thresholds, six regions achieved nominal $P \leq 0.01$ using $HLOD_{max}$ (see Table I). These comprised the four regions discussed above (5p13.3, 7q36.2, 8p23.3, and 8p21.3) as well as regions in 3p22.3 and 6p21.1. A total of 20 regions achieved nominal $P \leq 0.05$ (Supplementary Table I). Figure 2 shows $-\log_{10}P$ values for the 6 HLOD statistics plotted across the five chromosomes with peaks achieving nominal $P \leq 0.01$.

Compared with $HLOD_{max}$, fewer regions exceeded all significance thresholds using LOD_{exp}. Empirical genomewide significance and suggestive thresholds for LOD_{exp} in the combined sample were 2.89 and 1.57, which are almost identical to those reported in the original scan [Suarez et al., 2006]. Using LOD_{exp}, suggestive linkage was observed for one region, in 8p21.1, near D8S1771 ($LOD_{exp} = 2.23$; empirical type

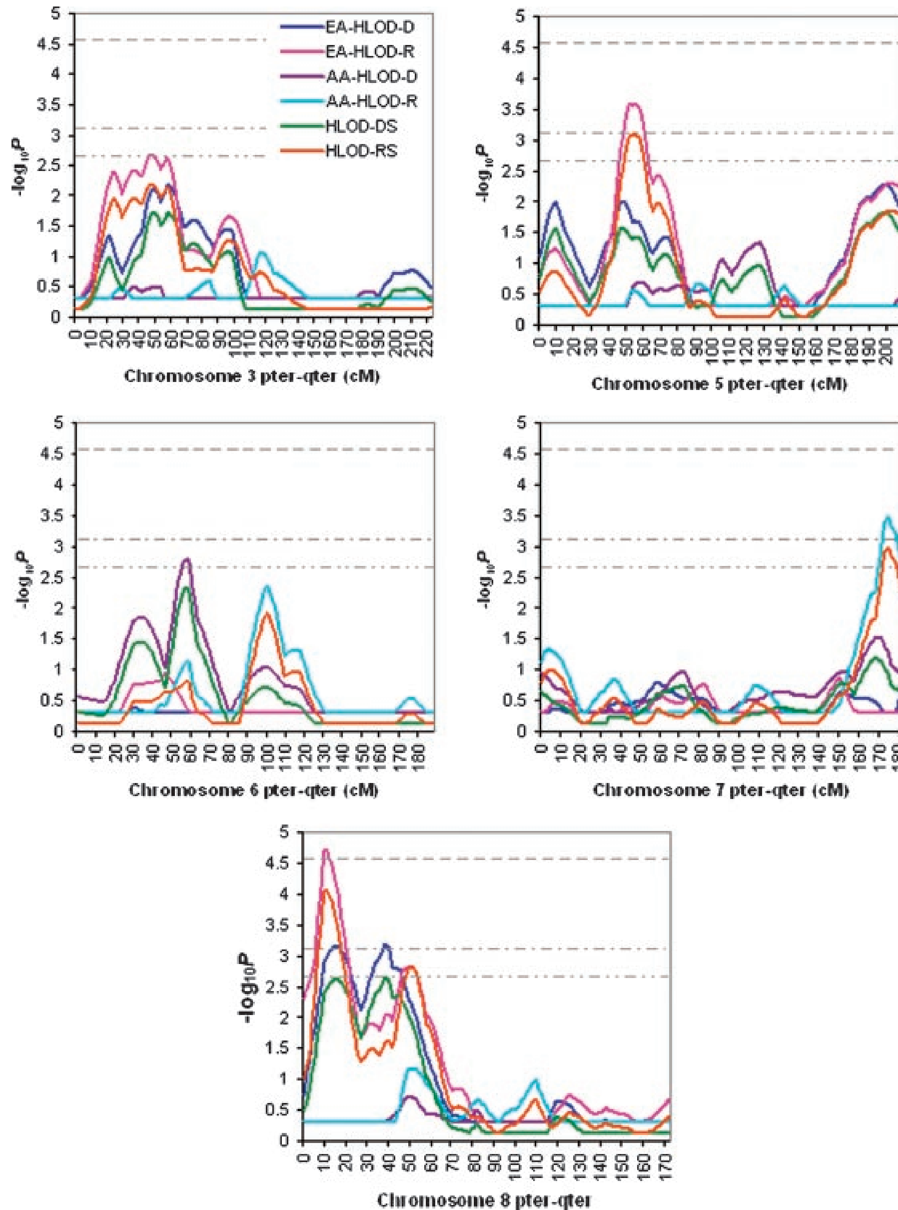

Fig. 2. Chromosome-specific results of linkage analysis performed in individual samples using standard HLOD scores (EA/AA samples) or "summed" HLOD (HLOD-S) scores (combined sample) calculated under simple dominant and recessive models. Figures show empirical thresholds for genomewide significant linkage (*dashed line*), suggestive linkage (*dash-dot line*), and nominal $P = 0.01$ (*dash-dot-dot line*) for $HLOD_{max}$ (the highest of the six statistics at each position).

I error rate = 0.226). A total of three regions achieved nominal $P \leq 0.01$ ($LOD_{exp} \geq 1.18$): 8p21.1, 5p13.3 and 11q13.3, while a total of 15 regions achieved nominal $P \leq 0.05$ ($LOD_{exp} \geq 0.59$).

DISCUSSION

Empirical evidence, combined with theoretical recommendations [Suarez et al., 1994; Risch and Merikangas, 1996] has highlighted the formidable sample sizes required for the detection (and replication) of complex disease risk loci via linkage analysis. Such evidence has contributed to a trend toward pooling multiple independent samples for sample enlargement. However, if constituent samples are drawn from distinct population groups, they may differ substantially in the relative contribution of risk loci. For such data, linkage analyses incorporating within- and between-sample locus heterogeneity may be a useful supplementary approach for identifying disease susceptibility loci [Vieland et al., 2001; Holliday et al., 2005].

In this reanalysis of MGS1 data we used an approach which incorporated analysis of each sub-sample and the combined sample, allowing for within- and between-sample locus heterogeneity. For each of the four regions surpassing the threshold for genomewide suggestive linkage (5p13.3, 7q36.2, 8p23.3, 8p21.3), we observed stronger linkage evidence in the constituent sub-sample in which the evidence for linkage was highest than in the combined sample. Linkage evidence in the pooled sample was reduced in spite of allowing for between-sample locus heterogeneity. Despite the increased multiple testing burden, our $HLOD_{max}$ approach which involved cal-

TABLE I. Genomic Regions With $HLOD_{max}$ of Nominal $P < 0.01$ in the NIMH-Funded Molecular Genetics of Schizophrenia Sample

Chromosome	Map position (cM)[a]	$HLOD_{max}$ ($-\log_{10}P$)	Nearest marker	Marker position (cM)[b]	Sample and statistic with strongest linkage evidence
3	50	2.67208	D3S2432	56.61	EA-HLOD-R
5	55	3.56897[c]	D5S1470	53.80	EA-HLOD-R
6	59	2.78955	D6S2427	59.57	AA-HLOD-D
7	174	3.46292[c]	D7S3058	176.38	AA-HLOD-R
8	10	4.69281[d]	D8S262	10.03	EA-HLOD-R
8	39	3.17094[c]	D8S560	42.13	EA-HLOD-D

[a]Map position of the $HLOD_{max}$ peak.
[b]Map position of marker closest to the $HLOD_{max}$ peak.
[c]Surpasses threshold for genome-wide suggestive linkage.
[d]Surpasses threshold for genome-wide significant linkage.

culating six statistics, detected stronger evidence for linkage to each of these four regions than the single LOD_{exp} statistic calculated in the pooled sample. Notably, we detected genome-wide significant evidence for linkage to 8p23.3.

Compared to LOD_{exp}, $HLOD_{max}$ also identified more regions at the *nominal* $P < 0.01$ (replication) and $P < 0.05$ levels. Detailed comparison of our results with previous reports is beyond the scope of the current paper; however the inclusion of nominal $P = 0.01$ and $P = 0.05$ thresholds should facilitate such comparisons (Fig. 1, Supplementary Table I).

The detection of stronger linkage evidence using $HLOD_{max}$ reflects both the presence of locus heterogeneity within the EA and AA samples, and differences in the extent of heterogeneity between the samples. Such differences may result, in part, from genetic differences between the two ancestral groups. Cluster analyses of marker allele frequencies show that individuals of European/Caucasian or African ancestry represent genetically distinct populations [Bowcock et al., 1991; Tishkoff et al., 1996; Perez-Lezaun et al., 1997] and direct comparisons of marker allele frequencies also indicate significant differences between these groups [Goddard et al., 2000; Grigull et al., 2001]. Accordingly, the original analysis of these data reported significant allele frequency differences between the EA and AA samples for >99% of STRP markers, and via cluster analyses, were able to reproduce the two groups [Suarez et al., 2006]. If the European and African populations differ with respect to the frequency of genetic marker alleles, they may also exhibit different frequencies of disease risk alleles, and/or alleles at interacting loci which influence the penetrance of risk locus genotypes. Such differences could readily produce between-sample heterogeneity of linkage to risk loci such as that observed in the current study. However, other sources of genetic variation may also be present in these data and confounded with the EA and AA groups. These could include differences in the prevalence of clinical SZ subtypes or comorbid drug or alcohol use, which may also influence the linkage evidence observed in each group [Brzustowicz, 2007].

Our results support and extend those obtained in original analyses of these data [Suarez et al., 2006], which yielded a single broad peak on chromosome 8p surpassing the suggestive linkage threshold. The presence of two, perhaps distinct peaks in EA data and a single, smaller peak in AA data were reported [Suarez et al., 2006] and have been statistically confirmed in the current analysis. Our analysis also detected significant linkage to 8p, and suggests a different location of the major risk locus, with the highest peak located 39 cM closer to the p-telomere. This peak was detected exclusively in EA pedigrees; prioritized analysis of which may improve the power of association studies in this region [Fingerlin et al., 2004]. The smaller 8p peak was also detected only in EA pedigrees and spans a 564 kb region containing two implicated SZ susceptibility genes, *PPP3CC* and *EGR3* [Yamada et al., 2007]. While this region was also spanned by the original reported peak, the current analysis provides a smaller CI for the location of the peak and clarifies the population segregating markers in this region. Association analyses of EA pedigrees linked to this region may thus offer high power to replicate previous association findings for *PPP3CC* and *EGR3* and identify risk variants within these genes.

It is noteworthy that numerous studies have reported linkage of SZ to the broad region spanning 8p23-21 [Blouin et al., 1998; Kaufmann et al., 1998; Brzustowicz et al., 1999; Garver et al., 2001; Gurling et al., 2001; Suarez et al., 2006]. Our smaller, 8p21 peak also lies within the ninth-ranked, weighted bin of the SZ genome scan meta-analysis [Lewis et al., 2003]. Our statistical evidence for the presence of two, distinct SZ genes in 8p23 and 8p21 may thus offer valuable information for interpreting a range of previous findings. A previous linkage disequilibrium study also suggested the presence of distinct SZ loci in 8p23.1 and 8p21.3 [Walss-Bass et al., 2006]. However, further investigation will be required to determine whether multiple susceptibility loci exist on 8p.

A potential limitation of our 8p results is the presence of a common polymorphic inversion in the region between our two peaks [Broman et al., 2003], which was also noted in the original analysis of Suarez et al. [2006]. This inversion has a frequency of >20% in Europeans and will prevent recombination in parents heterozygous for the inversion. Indeed, Suarez et al. [2006] reported increased LD in Europeans in this region and noted the potential for uncertainty regarding marker order and location. The impact of the inversion upon our results is unclear. However, Chen et al. [2006] noted that inversion carriers tend to demonstrate spurious recombination events around the inverted region, which can *reduce* the evidence for linkage if not properly accounted for. Hence, it is unlikely that our linkage findings have been inflated by the inversion. Further, one would not expect to observe many spurious recombination events within this specific region in small (two-generational) ASP pedigrees such as these.

Original analyses of these data [Suarez et al., 2006] also detected suggestive linkage to chromosome 11p11.2-11q22.3 ($LOD_{exp} = 1.63$). Due to differences in marker maps, this region did not qualify for suggestive linkage in the current scan (Fig. 1). The original analysis also detected evidence for linkage (nominal $P < 0.01$) to chromosome 5p. Our analysis supports and extends this previous finding, demonstrating suggestive linkage to 5p13.3. Our analysis also detected suggestive linkage to 7q36.2, which did not surpass nominal $P < 0.01$ in the initial scan.

Although we used the HLOD-S statistic to allow for within- and between-sample heterogeneity in the combined sample, other approaches could have been used. Notably, the Bayesian

posterior probability of linkage (PPL) [Vieland, 1998] also aggregates linkage evidence across multiple samples while allowing for intersample differences in specified covariates. The HLOD-S and PPL statistics are both "model-based," requiring explicit specification of genetic models. Alternatively, for analyses of complex disease, "nonparametric" or "model-specification-free" methods could also be helpful for investigating within- and between-sample heterogeneity. Indeed, all "nonparametric" affected pedigree-member (APM) methods reflect the same underlying statistical distribution of particular "parametric" methods [Whittemore, 1996]; for example, the "possible triangle" MLS statistic [Holmans, 1993] is approximately equivalent to a simple, recessive HLOD score (HLOD-R) within a small range of the null hypothesis [Huang and Vieland, 2001a].

In conclusion, this reanalysis detected genomewide significant linkage of SZ to chromosome 8p23.3 and suggestive evidence for the presence of a second SZ susceptibility locus on 8p21.3. Suggestive linkage was also detected for regions on chromosomes 5p13.3 and 7q36.2. Our results demonstrate that when a small number of sub-samples can be identified which have a high probability of genetic differences (like continental ancestral groups) and locus heterogeneity is suspected, it can be more powerful to conduct linkage analysis of the sub-samples individually *and* together, allowing for within- and between-sample locus heterogeneity and use empirical analyses to correct for multiple testing. Whether this holds true sufficiently frequently to make this an optimal primary analytic method, or holds true when there are more than two such groups, remains an area for further research.

WEB RESOURCES

The URLs for the genetic maps referred to herein are as follows:

Integrated physical and genetic map used for linkage analyses, http://www2.qimr.edu.au/davidd/master_map.dat.

CIDR genetic map, http://www.cidr.jhmi.edu/markerset.html.

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