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Genomewide Significant Linkage to Recurrent, Early-Onset Major Depressive Disorder on Chromosome 15q

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A genome scan was performed on the first phase sample of the Genetics of Recurrent Early-Onset Depression (GenRED) project. The sample consisted of 297 informative families containing 415 independent affected sibling pairs (ASPs), or, counting all possible pairs, 685 informative affected relative pairs (555 ASPs and 130 other pair types). Affected cases had recurrent major depressive disorder (MDD) with onset before age 31 years for probands or age 41 years for other affected relatives; the mean age at onset was 18.5 years, and the mean number of depressive episodes was 7.3. The Center for Inherited Disease Research genotyped 389 microsatellite markers (mean spacing of 9.3 cM). The primary linkage analysis considered allele sharing in all possible affected relative pairs with the use of the Z_{lr} statistic computed by the ALLEGRO program. A secondary logistic regression analysis considered the effect of the sex of the pair as a covariate. Genomewide significant linkage was observed on chromosome 15q25.3-26.2 (Z_{lr} = 4.14, equivalent LOD = 3.73, empirical genomewide P = .023). The linkage was not sex specific. No other suggestive or significant results were observed in the primary analysis. The secondary analysis produced three regions of suggestive linkage, but these results should be interpreted cautiously because they depended primarily on the small subsample of 42 male-male pairs. Chromosome 15q25.3-26.2 deserves further study as a candidate region for susceptibility to MDD.

Introduction

We report here on a genome scan of the first phase of a large repository-based study of families multiply affected with recurrent, early-onset major depressive disorder (MDD, unipolar depression) (Levinson et al. 2003). MDD is a multifactorial disorder with moderate heritability. Family and twin studies suggest that the greatest relative risk for MDD, compared with the risk of the general population, probably is observed in families of probands with early age at onset and/or recurrent episodes. The present study is designed to map susceptibility genes in families with these features. Clinical data collection has been completed. For the primary diagnostic model, with consideration of only cases of recurrent, early-onset MDD as defined below, the final sample (prior to genetic analyses to confirm family structures for the last part of the sample) includes 665 informative, predominantly European-ancestry pedigrees containing 921 independent affected sibling pairs (ASPs) and additional affected relatives. This first report considers 297 families containing 415 ASPs that were informative for linkage analysis under this model. The remaining families currently are being genotyped by the Center for Inherited Disease Research (CIDR), and results will be reported later in 2004. DNA and blinded clinical data from this sample will be made available by the National Institute of Mental Health (NIMH) Center for Collaborative Genetics Studies of Mental Disorders (see NIMH CGSMD Web site) during 2004.

MDD is a common disorder associated with increased mortality, particularly because of suicide (reviewed in Schneider et al. [2001]). It is also the leading cause of worldwide disability for individuals 15–44 years of age

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(World Health Organization 1996). The major depressive syndrome is characterized by ⩾2 wks of depressed mood and/or reduced or absent capacity for pleasure, accompanied by additional symptoms, such as disturbed sleep and appetite, reduced concentration and energy, excessive guilt, slowed or agitated movements, and suicidal thoughts or acts (American Psychiatric Association 1994). Estimates of lifetime prevalence range from 2%–19% (Moldin et al. 1991; Tsuang et al. 1994; Weissman et al. 1996). Two recent, large U.S. population surveys (National Comorbidity Survey [NCS] and NCS-Replication) demonstrated similar lifetime prevalences of 17.1% (Kessler et al. 1994) and 16.2% (Kessler et al. 2003). Prevalence is increasing in younger generations (Klerman and Weissman 1989; Lewinsohn et al. 1993; Kessler and Walters 1998; Kessler et al. 2003), apparently owing to increased MDD comorbid with other disorders, rather than "pure" MDD (Kessler et al. 1996). A ratio of MDD in women versus in men of ⩾2:1 generally is observed.

Adoption studies confirm the role for genetic factors (Mendlewicz and Rainer 1977; Cadoret 1978; Wender et al. 1986). The high ratio of correlation in liability between MZ versus DZ twin pairs suggests multigenic inheritance, although evidence for major locus effects has been reported in some studies (Price et al. 1987; Cox et al. 1989; Marazita et al. 1997; Maher et al. 2002). A meta-analysis of twin studies suggested an overall heritability of .37 (Sullivan et al. 2000); estimates have been as high as .70, in community samples assessed twice over time (Kendler et al. 1993*a*), and .79, in adolescent twins (Thapar and McGuffin 1994). One large community-based twin study suggested greater heritability in women (Bierut et al. 1999). A second similar study could not differentiate clearly between the hypotheses of equal or different heritabilities, and it suggested that there was a 70% overlap in the genetic factors contributing to MDD in men and women (Kendler et al. 2001). The sex of an MDD proband has not been reported to determine the ratio of risk of MDD in male and female siblings and offspring.

Some individuals who experience major depressive episodes also have episodes of mania or hypomania (manic syndrome without substantial functional impairment), receiving diagnoses of bipolar-I (BP-I) and BP-II disorder, respectively (American Psychiatric Association 1994). The lifetime prevalence of a manic episode was 1.6% in the NCS (Kessler et al. 1994). The genetic relationships among MDD, BP-I, and BP-II remain unclear. First-degree relatives of BP-I and BP-II probands are at increased risk of both unipolar and bipolar disorders, but the relatives of MDD probands are not at increased risk of BP-I (Gershon et al. 1982; Maier et al. 1993; Tsuang et al. 1994), and males and females are at equal risk of BP-I, suggesting that unipolar and bipolar disorders are at least partially independent genetically. The status of BP-II is less clear. Relatives of BP-II probands are at greater risk of BP-II than of BP-I (Simpson et al. 1993; Heun and Maier 1993; Endicott et al. 1985), and BP-II cases lie between BP-I and MDD in clinical features (Coryell et al. 1985*a*) and in the rates of BP-I and MDD in probands' families (Gershon et al. 1982; Endicott et al. 1985). It has been difficult to differentiate reliably between BP-II and MDD with the use of current methods (Rice et al. 1986; Leboyer et al. 1991), although reliability is better for highly trained interviewers (Simpson et al. 2002).

First-degree relatives of MDD probands are at an ~2–3-fold increased risk of MDD compared with the general population (Gershon et al. 1982; Weissman et al. 1984; Maier et al. 1993; Merikangas et al. 2002). Two clinical features of probands have been reported to predict a greater increase of MDD risk in first-degree relatives: recurrent episodes and early age at onset (AO). A relationship between recurrent episodes and familial risk has been reported in at least six studies (Bland et al. 1986; Gershon et al. 1986; Stancer et al. 1987; Kendler et al. 1994, 1999; McGuffin et al. 1996), but not by Weissman et al. (1986). Increased familial risk for earlier-onset probands has been observed in at least nine studies (Cadoret et al. 1978; Mendlewicz and Baron 1981; Weissman et al. 1986, 1987, 1993; Bland et al. 1986; Stancer et al. 1987; Kupfer et al. 1989; Lyons et al. 1998), but not by Kendler et al. (1994, 1999) or McGuffin et al. (1987, 1996). Only one study reported an analysis of an interaction between earlier AO and recurrence: Bland et al. (1986) observed a morbidity risk for MDD of 17.4% in first-degree relatives of probands with recurrent, early-onset MDD (defined as onset before the median AO of 51.2 years) versus 3.4% in the relatives of probands with single episodes and later onsets, with intermediate risks for relatives of early-onset/single episode and later-onset/recurrent MDD probands.

Epidemiological data are lacking to estimate the population prevalence of recurrent or early-onset recurrent MDD or the recurrence risk in families of MDD probands; neither is there a clear cutoff for "early onset." The most detailed relevant study (Weissman et al. 1984) reported that, for morbid risk of all MDD, first-degree relatives of probands with onset before age 20 years had a recurrence risk of 5.0 compared with relatives of control probands, and this decreased to 3.6 when the proband's onset was before age 30 years, 2.5 when it was before age 40 years, and 1.6 when it was after age 40 years. The risk of MDD in *relatives* of earlier-onset probands clearly rose through their 20s and 30s, and leveled off after age 40 years. Data from this and other family studies (Weissman et al. 1982, 1984, 1993; Bland et al. 1986; Marazita et al. 1997; Maher et al. 2002)

suggest that the recurrence risk to first-degree relatives for recurrent, early-onset MDD is in the range of 3–8.

On the basis of this reading of the literature, we ascertained informative families through probands with recurrent MDD and onset before age 31 years, with one or more additional available siblings with recurrent MDD and onset before age 41 years. We used ages 31 and 41 years as cutoffs, rather than ages 30 and 40 years, because familial risks of MDD decline gradually as a function of the proband's AO, and the slightly older cutoffs increased the number of eligible families without substantially decreasing the reported relative risk of MDD in the families. Cases with a parent or sibling with BP-I were excluded to differentiate this sample clearly from the many bipolar disorder pedigree studies, but relatives (not probands) with BP-II disorder were recruited if they otherwise met age at onset and depression-recurrence criteria, and, subsequently, these cases were entered into secondary analyses.

Material and Methods

Eligibility and Recruitment of Subjects

Subject eligibility and clinical data collection procedures have been described in detail elsewhere (Levinson et al. 2003). In brief, subjects were recruited at six sites (University of Iowa [Iowa], Johns Hopkins University [JHU], New York State Psychiatric Institute [NYSPI], University of Pennsylvania [Penn], University of Pittsburgh [Pitt], and Rush-Presbyterian Medical Center in Chicago [Rush]) where uniform criteria and procedures were used. All subjects gave informed consent following IRB-approved protocols. Ascertainment was opportunistic, with each site seeking eligible families through cooperating mental health facilities (predominantly outpatient clinics and practices), primary care medical settings, and self referrals in response to Web site announcements, media articles, and advertisements. Inclusion/exclusion criteria are summarized in table 1. Prospective probands were screened to identify those who had at least one sibling with an apparent history of depression (whom they were willing to contact about the study), with no sibling or parent with a likely BP-I diagnosis. Families were extended, where possible, through first-degree affected relatives. We included cases with a single episode of MDD that persisted for $\geqslant$3 years. Individuals with no depressive episodes persisting after age 18 years were excluded because of evidence that MDD (often single episode) during adolescence is common and might not be highly familial (Wickramaratne et al. 1998). Eligible cases were required to have significant reduction in major role performance noticeable to others, which has been reported to be more characteristic of familial MDD (Gershon et al. 1986). Cases of MDD with psychotic features (5% of the sample, most with mood-congruent psychotic features) were included because familial risks of various psychiatric disorders have been generally similar in families of psychotic and nonpsychotic depressive probands (see, e.g., Coryell et al. [1985*b*] and Maier et al. [1992]).

In addition to affected cases, we attempted to obtain blood specimens from all available parents, and, when one or both parents were not available, from as many as two additional siblings (unaffected or uninterested in participating in the clinical study).

Clinical Assessment

Probands and relatives with reported histories of depression were interviewed and narrative interview reports were prepared by trained research clinicians (psychologists, psychiatrists, social workers, and nurses with psychiatric research training) with the use of the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al. 1994), modified for mood-disorder studies (version 3.0). DIGS training was provided by the JHU group. Study coordinators participated in regular conference calls and meetings to discuss DIGS items and study criteria. Most interviews were conducted by telephone. Good reliability has been demonstrated for telephone diagnoses of MDD compared with direct interview diagnoses (Simon et al. 1993; Sobin et al. 1993). At least one and usually two family members also were interviewed, with the use of the Family Interview for Genetic Studies (FIGS) to obtain corroborating diagnostic information about participating individuals and to detect BP-I in unavailable parents or siblings. Available parents with no history of mood disorder detected by FIGS were interviewed with a screening instrument (Kendler et al. 1993*b*) and then with DIGS if they reported such a history. Records of previous behavioral or medication treatment were obtained with the subject's consent. All subjects (through interview or blood specimens) were asked to complete the NEO Five-Factor Inventory (Costa and McCrae 1986, 1988, 1992), a personality inventory.

Diagnostic Procedure

Two experienced research clinicians (not the interviewer) reviewed all available data for each case (DIGS, narrative report, FIGS, and available treatment records) and recorded primary DSM-IV Best Estimate Final Diagnoses (Leckman et al. 1982; Klein et al. 1994). They documented any additional diagnoses; the level of certainty and age at onset for each diagnosis; the number of episodes and age at onset of major depression, hypomania, mania, and mood congruent or incongruent psychotic depression; the severity; the course of illness (remitting, chronic/double depression, frequent/brief episodes, or other); and the timing of significant comorbid

Table 1

Inclusion/Exclusion Criteria

Criterion	Probands	Affected Relatives
Diagnosis	DSM-IV	Same
Recurrence	≥2 episodes required	Same
Age at onset	≤30 years	≤40 years
Adult MDD	≥1 episode past age 18	Same
Impairment	Major role impairment required	Same
Psychiatric comorbidity	Exclude schizophrenia, BP-I, ASPD, and somatization disorder	Same
Substance abuse history	MD episode before onset of abuse or ≥2 years after abuse remission	MD episode before onset of abuse or ≥1 year after abuse remission
Bipolar II	Exclude pedigree if proband's consensus diagnosis is BP II	Include BP-II relatives with recurrent MDD (secondary analyses only)
Family psychiatric history	Exclude if positive FH of BP I in sibling or parent	Same

alcohol or substance use (absent, onset after onset of MDD, or, if onset was before MDD, then whether there was at least one MDD episode following 1–2 years of sobriety). Any disagreements between diagnosticians about primary and secondary diagnoses or age at onset were resolved through discussion or, rarely, through review by a third diagnostician.

Collection of Blood Specimens and Creation of Permanent Cell Lines

Blood specimens obtained from participating subjects were sent by overnight courier to the NIMH Cell Repository at Rutgers University, where Epstein-Barr virus-transformed lymphoblastic permanent cell lines were created, frozen, thawed, and regrown to ensure viability; then, DNA was extracted.

Genotyping

Genotyping was performed by CIDR with the use of standard semiautomated genotyping methods for fluorescent microsatellite markers (see CIDR Web site, lab protocols). The genotyping was performed in two phases. A genome map of 389 microsatellite markers (see CIDR Web site, marker information) was genotyped in each phase, with three of the phase 1 markers replaced in phase 2. Average marker heterozygosity was 0.77 and mean intermarker spacing was 9.30 cM. All DNA from a family were genotyped in the same phase for consistency of allele calls. In each phase, a 15-marker "forensic panel" (2 X-chromosome, 2 Y-chromosome, and 11 autosomal markers) was genotyped initially and analyzed for Mendelian inconsistencies, which were then reviewed to identify and correct any sample swap errors and to identify likely false paternities or other family structure problems. The complete genome-scan data sets were reviewed at CIDR to detect binning and Mendelian errors prior to release (see CIDR Web site, quality control). CIDR's procedure is to repeat genotypes only if an entire multiplexed panel appears to have given poor results; otherwise, likely errors are omitted from the data set. CIDR staff rebinned the allele calls for the combined phases 1+2 data set for consistency across the subsets. Data cleaning included analysis of all genotypes with RELCHECK (Boehnke and Cox 1997) to detect errors in specified family relationships, which were then corrected where possible (e.g., where a half-sibling relationship could be established rather than the reported full sibling relationship). On the basis of both the forensic and RELCHECK analyses, 12 individuals were removed (7 because of specimens that proved to be identical to other specimens, either because of MZ twinship or sample errors, and 5 because of other incorrect relationships that could not be resolved). For the complete data set, there were 3.81% missing autosomal and X-chromosome genotypes following QC procedures. A total of 18,089 genotypes of blind duplicate specimens (16 in phase 1 and 12 in phase 2, analyzed with different PCRs but in the same experimental run) produced 13 discordant genotypes (0.072%) for ~0.04% error per analysis. Mendelian inconsistencies were detected for 0.37% of genotypes, primarily from the families with pedigree relationship errors that were corrected as described above. For all remaining Mendelian inconsistencies, the genotypes for the marker were removed for all members of the family. Finally, the data were tested for unlikely genotypes with the use of SIBMED (Douglas et al. 2000), and genotypes inferred to have ≥70% probability of being errors were deleted. All genetic locations are on the deCode map (Kong et al. 2002).

Statistical Analysis

Primary linkage analysis.—The planned primary analysis was a model-free multipoint linkage analysis, including only MDD cases, with the use of ALLEGRO (Gudbjartsson et al. 2000*a*) to compute the *Z* likelihood ratio (Z_{lr}) score, computed for all possible informative

Table 2

Sample Sizes and Pair Types by Site

Sample Group	No. at Site						
	JHU	Rush	Iowa	NYSPI	Penn	Pitt	All
Pedigrees (MDD-RE only)	29	46	61	27	55	79	297
Individuals:							
Affected individuals (MDD-RE)	92	123	165	67	146	216	809
Other genotyped individuals	36	37	44	20	56	37	230
All genotyped individuals	128	160	209	87	202	253	1,039
Possible informative pair types:							
Full-sibling	74	67	128	38	112	117	536
Half-sibling	2	6	3	0	2	6	19
Grandparental	0	2	1	0	0	11	14
Avuncular	13	14	12	2	17	40	98
First cousin	0	0	0	4	2	8	14
Other	0	0	0	0	0	4	4
Total informative pairs	89	89	144	44	133	186	685
Parent-offspring pairs	34	36	23	14	21	62	190

Note.—Number of pedigrees, genotyped affected and other individuals, and genotyped affected pairs (counting all possible pairs) included in the sample. MDD-RE cases are considered affected, and BP-II cases are counted among the other genotyped individuals. Families with no linkage information under the MDD model were excluded from these counts. There were 415 independent affected sibling pairs (full- and half-sib pairs) counting S-1 pairs for S affected siblings in a sibship.

relative pairs under an exponential model (Kong and Cox 1997), and its associated LOD score ($Z_{lr}^2/4.6$). Families were weighted by the square root of the SD of their score function under the null hypothesis of no linkage, which is considered about midway between weighting each pair equally versus weighting each family equally (Gudbjartsson et al. 2000*b*). Genomewide significance levels were estimated by simulating 5,000 replicates of each chromosome, in the absence of linkage, with the use of the actual family structures, allele frequencies, and marker maps. Replicate "genomes" were created by randomly selecting a replicate of each chromosome. The average number of "hits" per genome was recorded (peaks exceeding the observed Z_{lr}). Here, "peaks" were defined as local maxima in the multipoint Z_{lr} statistic, $\geqslant$30 cM apart.

Secondary analyses.—Secondary analyses included recomputation of Z_{lr} scores for European-ancestry families and then for all families with BP-II cases considered affected, and a series of logistic regression analyses of the effects of a series of covariates on allele sharing in affected relative pairs. The MDD+BP-II model yielded very similar results (not presented here). It is beyond the scope of this paper to present the full set of covariate analyses. However, because sex-specific MDD linkage has been reported in other studies (Zubenko et al. 2002, 2003; Abkevich et al. 2003), we present here the results of the analysis of sex as a covariate. In brief, the approach was that of Rice (1997), further developed by Holmans (2002) and extended here to consider all types of affected relative pairs. The multipoint likelihood of the marker data of an affected relative pair at any point in the genome (Risch 1990; Olson 1999) is computed on the basis of the prior and posterior (conditional on the observed marker data) probabilities that pair *i* share *j* alleles identity-by-descent (IBD), obtained with the use of ALLEGRO (Gudbjartsson et al. 2000*a*). The probability P_R that a relative pair of type R share an allele from a common ancestor IBD can then be expressed as a logistic regression on two regression parameters: α (a measure of the divergence of IBD from the null in the sample, without allowing for covariate effects) and β (a factor with three levels corresponding to the status of the pair with respect to the covariate), together with a fixed offset (to ensure that P_R takes its correct value in the absence of linkage or covariate effects). A likelihood can be obtained then as a function of P_R and maximized with respect to the parameters α and β (Holmans 2002). Here, we test for the effect of a binary covariate (male-male, male-female, and female-female pairs) by maximizing the likelihood with respect to α alone, and then with respect to both α and β, to give a LOD score for the effect of the *covariate* (allowing for linkage):

$$\text{LOD} = \log_{10}\left(\frac{L(\hat{\alpha},\hat{\beta})}{L(\hat{\alpha},\beta = 0)}\right) .$$

The maximum value on each chromosome was computed. Empirical *P* values (5,000 replicates) were determined genomewide for the LOD score for *linkage allowing for covariate effects* taking both α and β into

Table 3

Affected Pairs by Sex

Pair Type	No. by Sex			
	Male-Male	Male-Female	Female-Female	All
Informative pair type:				
Full-sibling	35	155	346	536
Half-sibling	1	3	15	19
Grandparental	0	6	8	14
Avuncular	2	38	58	98
First-cousin	4	9	1	14
Other	0	4	0	4
All informative pairs	42	215	428	685
Parent-offspring	8	71	111	190

account (and comparing it to the LOD score with $\alpha = \beta = 0$), and chromosomewide for the *covariate effect* (β). The former were computed by simulating replicate samples in the absence of linkage. The latter were computed by fixing the observed genotypes and randomly permuting covariate values among the affected individuals. Genomewide significance levels cannot be simulated directly for the covariate effect because significance depends not only on the value of the LOD score, but also on the linkage evidence present without allowing for the covariate; in other words, a LOD score for covariate effects on a chromosome with strong evidence for linkage will be more significant than the same LOD score on a chromosome showing no evidence for linkage. For very small chromosomewide *P* values, an approximate genomewide significance level could be obtained by multiplying the chromosomewide *P* by the total length of the genome divided by the length of the chromosome on which the effect was observed.

For chromosome 15, where significant evidence for linkage was observed, heterogeneity in allele sharing in affected pairs across the six clinical sites was tested by introducing a covariate for each site and maximizing the likelihood of the data while allowing the IBD probabilities to differ across sites. The chromosomewide significance of the resulting likelihood-ratio statistic was assessed by randomly permuting the site designations among the pedigrees and repeating the analysis. This procedure allows for the dependence between affected relative pairs from the same pedigree. The analysis was performed for affected sibling pairs and then for all informative affected relative pairs.

Power analysis.—The study was designed to collect a sample that included 900–1,000 independent ASPs, on the basis of power analyses (Levinson et al. 2003) demonstrating that a sample of this size could detect genomewide significant linkage with 80%–90% power for dominant and recessive models predicting a relative risk to probands' siblings (λ_{sibs}) of 1.30, when 5-cM marker spacing was achieved in candidate regions. The results are in good agreement with those of Hauser et al. (1996), who reported that a sample of ~400 ASPs (such as the sample reported here) would have 80% power to detect significant linkage if $\lambda_{sibs} \approx 1.8$. Although λ_{sibs} predicts power to detect linkage for simple ASP families, for families with three or more affected individuals in diverse constellations, power will depend on the (unknown) true parameters of the full transmission model.

Results

Sample Characteristics

There were 1,057 genotyped DNA specimens from 304 eligible families. The sample included 819 individuals with final diagnoses of recurrent, early-onset MDD, as defined above, 14 individuals with final diagnoses of BP-II, and 224 individuals without eligible diagnoses. In the remainder of this paper we consider only the 297 families that were informative for linkage, with only MDD-RE cases considered affected, as shown in table 2. In these families, there were 1,039 genotyped individuals, including 809 MDD cases and 230 individuals without eligible MDD diagnoses (including the BP-II cases in these families). The 809 cases included 297 probands, 388 sibs, 5 half sibs, 56 parents, 27 children, 20 nieces/nephews, 9 aunts/uncles, 2 grandparents, 2 cousins, 2 spouses, and 1 other relative of probands. The 230 other genotyped individuals included 69 sibs (4 of the BP-II cases), 1 half sib, 130 parents, 11 children (3 of them BP-II cases), 7 spouses, 5 nieces/nephews, 1 grandparent, 3 aunts/uncles, and 3 other relatives. There were 415 independent ASPs, counting S-1 pairs from S affected siblings in a sibship, and 685 informative relative pairs, counting all possible pairs (555 ASPs and 130 other pair types), with the breakdown by sex as shown in table 3.

Clinical characteristics have been described elsewhere (Levinson et al. 2003). In brief, the mean age at onset was 18.5 years (SD = 17.3), the mean number of MDD

episodes (lifetime) was 7.3 (SD = 9.5), and the mean longest major depressive episode during the subject's life was 655 d (SD = 1113). The mean age at interview was 41.9 years (SD = 12.3). MDD cases were 79% female and 95% of European ancestry; the remaining cases were of African American, Hispanic, or Asian ancestry. Comorbid diagnoses included: alcohol and substance dependence, not including nicotine (22% of cases); alcohol or substance abuse (19%); panic disorder (28%); social phobia (15%); obsessive-compulsive disorder (9%); and nicotine dependence (18%).

Primary Linkage Analysis Results

The multipoint graph of Z_{lr} across the genome is shown in figure 1. Maximum Z_{lr} scores on each chromosome are shown in table 4, together with their deCODE locations in cM (Kong et al. 2002), the equivalent LOD score, and the number of peaks per genome expected to reach the observed value in the absence of linkage, where peaks are defined as local maxima of the multipoint Z_{lr} score, $\geqslant$30 cM apart. Genomewide levels of significance are defined here by the average number of *P* values exceeding a certain threshold that are expected per genome by chance, as determined by simulation. The peak on chromosome 15q25.3-26.2 (Z_{lr} = 4.14, equivalent LOD = 3.73) is associated with an empirical genomewide *P* = .023 (table 4), a significant result (Lander and Kruglyak 1995). The nominal (uncorrected) significance level reported by ALLEGRO was *P* = .000017. The peak LOD score was observed at 103.4 cM on the deCode map, between markers D15S652 (99.9 cM; 90,247,135 Mb on build 34 of the Human Genome Project [NCBI]) and D15S816 (110.9 cM; 92,749,552 Mb). No other peak was associated with significant or suggestive evidence for linkage. Also listed is a second peak on chromosome 15 near D15S165 (15q13.1, ~22.6 cM; 28,976,675 Mb), which was the second-largest peak in the genome, but more than one peak of this size would be expected by chance in a genome scan. With the exception of the peak on distal 15q, the observed results are consistent with what would be expected in the absence of disease-susceptibility loci (table 5). The multipoint graph of Z_{lr} on chromosome 15 is shown in more detail in figure 2. The peak Z_{lr} score of 4.14 was observed at 103.2 cM, between D15S652 (99.92 cM, 90.1 Mb) and GATA128A02 (108.21 cM, 92.1 Mb). The 1-LOD support interval extends from a point distal to D15S655 (92.08 cM, 85.56 Mb on build 33), to a point proximal to D15S657 (116.48 cM, 94.29 Mb), a region of ~24 cM of genetic distance but <9 Mb of physical distance. When the analysis was restricted to the 286 families of predominantly European ancestry, the peak Z_{lr} on 15q was 4.50 (103.2 cM), with an equivalent LOD of 4.39. There were too few families of non-European ancestry to analyze separately.

Effects of Sex of the Affected Pair

This analysis considered allele sharing in all possible informative affected relative pairs, first with no covariate effect and then with sex as a covariate. Note that, whereas the LOD scores shown in table 4 were computed as described above (see "Primary Linkage Analysis"), the LOD scores shown in table 6 were computed using the likelihood-ratio method described above (see

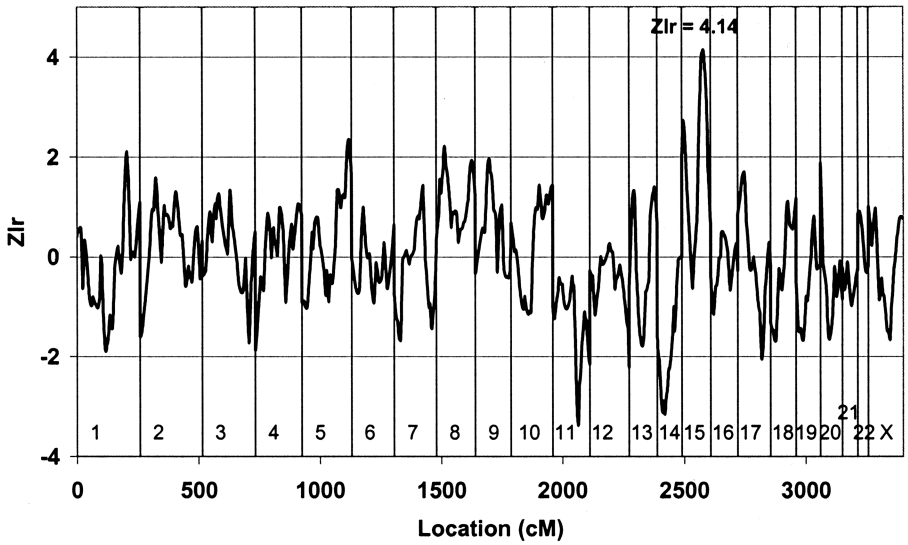

Figure 1 Genome scan results (MDD-RE). The line represents the value of Z_{lr} at each point on the multipoint map across the genome, in cM. Vertical bars separate the chromosomes (chromosome numbers are shown at the bottom of the graph area). Significant linkage was observed on chromosome 15q (103.2 cM).

Table 4

Maximum Z_{lr} Score on Each Chromosome

Chromosome	Max Z_{lr}	Lod Score	Location (cM)	Expected Genomewide Hits
1	2.11	.97	211.2	6.6
2	1.59	.55	67.5	15.6
3	1.33	.38	117.3	22.1
4	1.06	.24	189.4	30.2
5	2.35	1.20	193.6	4.1
6	1.00	.22	63.5	32.1
7	1.43	.44	128.1	19.4
8	2.22	1.07	37.7	5.3
9	1.96	.84	72.1	8.6
10	1.44	.45	121.8	19.1
11	−.39	.03	79.3	77.5
12	.27	.02	87.7	57.9
13	1.41	.43	113.0	19.9
14	.00	.00	110.4	66.8
15	2.73	1.62	22.6	1.8
15	***4.14***	***3.73***	***103.2***	***.023***
16	.51	.06	65.1	49.4
17	1.70	.63	28.0	13.2
18	1.17	.30	110.6	26.7
19	.81	.14	83.4	38.7
20	1.87	.76	2.5	10.0
21	−.10	.00	29.5	69.9
22	.91	.18	8.4	35.2
X	1.01	.22	14.4	31.8

Note.—Maximum Z_{lr} scores on each chromosome from the analysis of MDD, together with locations (cM on deCODE map) and the expected number of peaks in a genome scan at least as large as the observed value in the absence of disease loci ("hits"). Two scores are listed for chromosome 15 because these were the two largest scores in the genome (*largest score in bold italics*).

"Secondary Analyses"). Thus, the peak LOD score on chromosome 15q without covariates was 3.73 in the former and 5.8 in the latter analysis, but the empirical levels of significance are similar.

Table 6 lists the four peaks for which a suggestive (expected once per genome scan) or significant ($P < .05$ per genome scan) effect was observed for the overall LOD score taking sex into account. Note, however, that these P values have not been corrected for the number of secondary analyses, and must be considered hypothesis generating. Shown are the LOD scores, with and without covariates; the peak location and nearest marker; the pair type that produced evidence for linkage; the expected number of *genomewide* scores as large as the observed LOD for linkage, taking the covariate into account; and the empirical *chromosomewide P* value for the covariate effect (in effect, for the increased evidence for linkage when the covariate is taken into account). The covariate effect was nominally significant ($P < .05$) on chromosomes 6, 15, and 17, but not on chromosome 8. The highest IBD proportion was seen in male-male pairs for three of the chromosomes: chromosome 6 (male-male IBD = .66, male-female = .42, female-female = .52), chromosome 8 (male-male = .66, male-female = .54, female-female = .54), and chromosome 17 (male-male = .67, male-female = .58, female-female = .50). These results should be regarded with caution, because of the small number of male-male pairs in the sample. On chromosome 15, the LOD score reached genomewide significance without the covariate effect, and the covariate produced a modest increase in the evidence for linkage (nominal $P = .02$), with the

Table 5

Expected versus Observed Peaks Given Z_{lr} Values

	No. of Peaks[a]	
Z_{lr}	Expected	Observed
0.5	49.7	42
1.0	32.1	26
1.5	17.7	10
2.0	8.1	5
2.5	3.1	2
3.0	1.0	1
3.5	.24	1
4.0	.04	1
4.5	.009	0
5.0	.001	0

[a] Under the assumption of no disease loci in the genome.

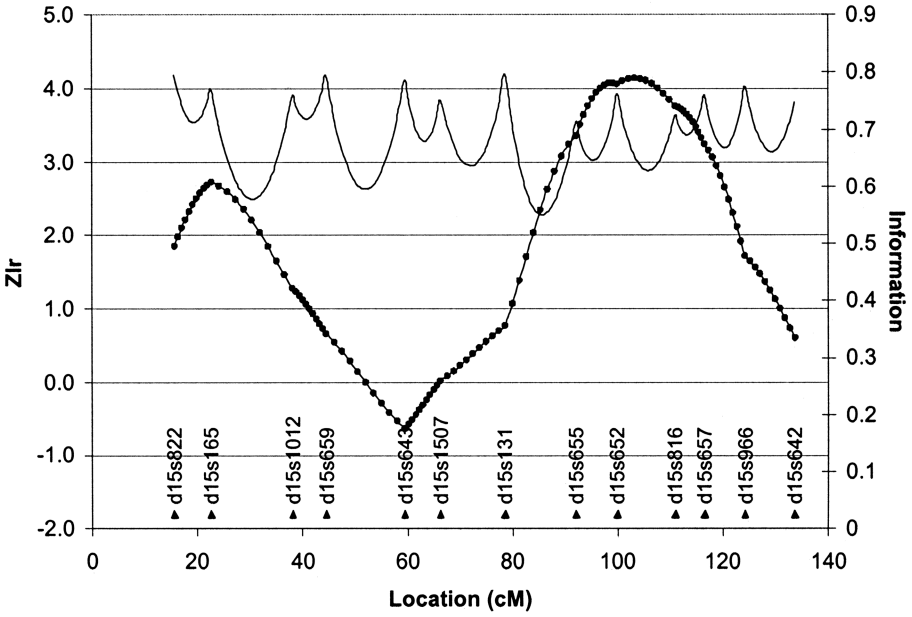

Figure 2 Linkage analysis of chromosome 15 (MDD-RE). The blackened circles and dark black line represent Z_{lr} scores (*left Y-axis*) across the multipoint map of chromosome 15 in cM. The lighter line represents ALLEGRO's information content measure (*right Y-axis*). Marker names are shown at the bottom of the graph area.

highest IBD observed in male-female pairs (.67), compared with female-female (.58) and male-male (.50) pairs.

Cross-Site Heterogeneity Analysis

In tests for cross-site heterogeneity in linkage evidence on chromosome 15, chromosomewide *P* values of .41 and .40 were obtained with the use of all affected relative pairs and ASPs, respectively. Thus, there is no evidence that observed differences in linkage evidence between sites are caused by anything other than stochastic variation.

Discussion

The most striking result observed in this study is the genomewide significant linkage with the MDD-RE phenotype at 103.2 cM on chromosome 15q. Linkage in this region has not been reported elsewhere for MDD or related phenotypes. It would be premature to speculate about the gene or genes in this region that could be producing this linkage signal, because of the imprecise localization of signals in linkage studies of complex disorders (Roberts et al. 1999) and because some of the most promising candidate genes discovered by positional cloning studies of neuropsychiatric disorders, such as schizophrenia, either have been previously unknown, as in the case of G72 (Chumakov et al. 2002), or had not been predicted to be involved in the disorder of interest, as in the case of dysbindin (Straub et al. 2002) and neuregulin 1 (Stefansson et al. 2002). Systematic linkage fine-mapping and linkage disequilibrium-mapping studies of this region will be needed. The analysis of the effect of sex on linkage to MDD was nominally significant, but we are not aware of a biological explanation for the observation that male-female pairs showed the highest IBD sharing, and, therefore, we cannot conclude that there is a sex-specific effect in this region. No significant cross-site heterogeneity was observed in the evidence for linkage to chromosome 15q.

In secondary analyses (not corrected for multiple testing), suggestive evidence for linkage was observed, when sex effects were taken into account, on chromosomes 6q, 8p, and 17p, with male-male pairs showing the highest IBD sharing in each of these regions. The low number of male-male pairs suggests that these results could be spurious. However, it is plausible that, since MDD is more prevalent in females, it could require a greater genetic loading to produce a male-male affected relative pair, and, thus, there could be loci at which it is necessary for a male (but not a female) to possess a particular allele to become affected and at which IBD sharing will be higher in male-male pairs.

There have been two other published genome scans of major depression (Zubenko et al. 2002, 2003; Abkevich et al. 2003), and one of neuroticism (Fullerton et al. 2003), a related trait. Zubenko et al. (2002, 2003) reported a 10-cM genome scan of 81 families ascer-

Table 6

Evidence for Linkage With and Without Accounting for the Effect of Sex of the Pair as a Covariate

	LOD						
Chromosome	No Covariates	+ Sex	Distance (cM)	Nearest Marker	Linked Pairs	Expected No. of Peaks Genomewide (LOD + Sex)	Chromosomewide *P* (Covariate Effect)
6	.26	3.66	80.2	D6S1053	M-M	.25	.002
8	1.76	2.81	38.3	D8S560	M-M	.80	.17
15	5.80	7.57	102.1	D15S652	M-F, F-F	<.01	.02
17	1.04	3.09	27.0	D17S974	M-M, M-F	.54	.015

Note.—Chromosomes and covariates for which LOD + sex (LOD score for linkage including the effect of the sex of the pair as a covariate effect) reached the genomewide "suggestive" level (i.e., a level that was expected to be observed at least one time genomewide in the absence of linkage). The last column shows the chromosomewide *P* value for a direct test of the covariate effect (LOD + sex vs. LOD with no covariates), along with the pair type(s) showing the greatest evidence for linkage. These are secondary analyses and do not include all of the covariate effects that were tested (see main text). *P* values have not been corrected for multiple testing. Note that these LOD scores were computed by a different method than were those shown in table 4 (see main text).

tained through MDD-RE probands. Their ascertainment criteria differed from those of the present study in that (1) probands had nonpsychotic MDD with AO ≤25 years, (2) pedigrees were chosen if two parents and at least one sibling were willing to participate but without regard to the relatives' diagnoses, and (3) diagnoses were based on SADS interviews and RDC criteria (Spitzer and Endicott 1975; Spitzer et al. 1978); note that there was no overlap between this earlierstudy and the present one, that is, no families collected at the University of Pittsburgh were included in both studies. Zubenko et al. (2003) performed logistic regression allele-sharing analyses in affected relative pairs with and without two covariates (sex and observation of LOD >0 at a marker on chromosome 2q with evidence for linkage in the sample) under five phenotypic models ranging from MDD-RE (135 all possible affected relative pairs) to all mood disorders (620 pairs). Positive evidence for linkage was observed near both of the chromosome 15q linkage peaks reported here: the linkage model without covariates giving a LOD = 1.96 for recurrent MDD (240 pairs) at 38 cM on the deCODE map, near our second-largest peak (22.6 cM), whereas inclusion of covariates gave LODs >4.0 for both the MDD-RE and recurrent MDD phenotypes at ~110–125 cM (deCODE map), near our significant peak (103.2 cM). The largest LOD score without covariate effects (4.2) was on chromosome 11pter for recurrent MDD. The most significant evidence for linkage with covariates was observed on chromosome 2q (stronger evidence in females). Several interactions were observed between 2q and other regions.

Abkevich et al. (2003) typed 628 microsatellite markers in 110 Utah families with four or more available affected cases, ascertained through MDD probands. The clinical model was somewhat different from that of the present study: diagnoses were established with the use of a modified CIDI interview, an interview schedule that was designed for epidemiological research (Robins et al. 1988), and BP-I and BP-II cases (15% of the sample) were all considered affected with the "depression" phenotype that produced the significant result. Linkage was analyzed separately for all cases, and then for only male or only female cases considered affected. One genomewide significant result was observed, in male cases only, on chromosome 12q22-q23.2, where evidence for linkage to bipolar disorder has been reported elsewhere (Craddock et al. 1994; Dawson et al. 1995; Morissette et al. 1999; Ewald et al. 2002; Jones et al. 2002). No evidence for linkage was observed on chromosome 15q. In the present study, logistic regression analysis in the 12q linkage region reported by Abkevich et al. demonstrated a LOD score of ~0 that rose to 1.03 when sex was taken into account. This result was not statistically significant, but the pattern of IBD sharing is of interest: .59 in male-male pairs versus .45 for male-female and .50 for female-female pairs. Thus, it could require a much larger sample of male-male pairs to test the Abkevich et al. finding.

Fullerton et al. (2003) performed a genome scan in 559 sibling pairs who were concordant or discordant for neuroticism scores in the top or bottom 2.5% of standardized scores, drawn from ~20,000 sibships screened through primary care medical practices. A substantial overlap has been estimated between the genetic factors underlying MDD and neuroticism (Kendler et al. 1993*a*; Clark et al. 1994; Duggan et al. 1995; Fava et al. 1996; Enns and Cox 1997). Significant evidence for linkage was observed on chromosomes 1q, 4q, 7q, 8p, 11q, 12q, and 13q. The 12q peak was at essentially the same location as the significant result reported by Abkevich et al. (2003), except that the neuroticism linkage was observed primarily in females rather than in males. The 11q peak was within 15 cM of a peak reported by Zubenko et al. (2003); neither result was associated with sex effects.

Only one broad chromosomal region received attention in three different studies: chromosome 8p produced the seventh-strongest result (8 cM) in Fullerton et al. (2003), the fourth-strongest result (37.7 cM) in the present study, and one of the four most positive results for the narrowest diagnostic model (60 cM) in the work of Zubenko et al. (2003). However, the latter two results were not significant, and the first and third results are quite far apart. The inconsistent results for MDD are typical of complex disorders, for which replication of linkage is difficult. It is hoped that additional large samples will produce more convergent results across studies, as has been the case for schizophrenia (Lewis et al. 2003).

In conclusion, we have reported genomewide significant evidence for linkage to recurrent, early-onset MDD on chromosome 15q. This region deserves further study attempting to identify the gene or genes underlying the evidence for linkage. Positional cloning methods have the potential to elucidate the pathophysiology of this common and debilitating disorder.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

CIDR lab protocols, http://www.cidr.jhmi.edu/protocol.html (for description of genotyping protocol)
CIDR marker information, http://www.cidr.jhmi.edu/markerset.html (for list of markers in the screening set)
CIDR quality control, http://www.cidr.jhmi.edu/proj_rdc.html
National Center for Biotechnology Information (NCBI), http://www.ncbi.nih.gov (for physical locations of markers [build 34])
NIMH CGSMD, http://zork.wustl.edu/nimh (for information about sharing biological and clinical material from this study)

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