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Genetic Linkage of Region Containing the *CREB1* Gene to Depressive Disorders in Families With Recurrent, Early-Onset, Major Depression: A Re-Analysis and Confirmation of Sex-Specific Effect

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Abstract

A previously published model-free linkage analysis of chromosome 2q33-35, highlighted by previous case-control studies and supported by within-family analyses employing the transmission disequilibrium test, revealed evidence of sex-specific linkage of the *CREB1*-containing region of 2q to unipolar Mood Disorders among women in 81 Recurrent, Early-Onset, Major Depressive Disorder (RE-MDD) families. Since it has been reported that the LODPAL program from S.A.G.E. v.4.0 used to conduct this previous linkage analysis suffers from an increased type I error rate that is exacerbated by covariates such as sex, we re-analyzed the evidence for this sex-specific linkage result using a simulation approach to estimate the empirical significance of our previous results. The results continue to support sex-specific linkage of the *CREB1* region to Mood Disorders among women from families with RE-MDD. Moreover, these results have been supported by a host of additional published findings that implicate sequence variations in *CREB1* in the sex-dependent development of syndromic Mood Disorders, as well as related clinical features and disorders.

Keywords

Unipolar Mood Disorders; Major Depression; Genetics; Chromosome 2q; *CREB1*; Sex Specificity; Women's Health

Introduction

We previously reported the results of a model-free linkage analysis of six polymorphic markers, located in a 15 cM region of chromosome 2q33-35, and unipolar Mood Disorders in 81 families identified by probands with Recurrent, Early-Onset Major Depressive Disorder (RE-MDD), a severe and familial form of clinical depression (Zubenko et al., 2002a). Our focus on this region of chromosome 2q was based on prior results from a case-control study suggesting an association of the D2S2944 124 bp allele with RE-MDD in

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women (Zubenko et al., 2002c), a sex-specific finding that was confirmed within families by the transmission disequilibrium test (Zubenko et al., 2002b).

Our linkage findings revealed significant evidence of linkage of unipolar Mood Disorders to a 451 Kb region of 2q33-34 flanked by D2S2321 and D2S2208 in these RE-MDD families (Zubenko et al., 2002a). Increasing peak LOD scores were observed in both the single point and multipoint analyses for Mood Disorder phenotypes whose definitions included progressively less stringent severity criteria for inclusion in the affected group. The sex-dependent multipoint linkage analysis of any Mood Disorders produced LOD scores that reached 6.33 and 6.87 at D2S2321 and D2S2208, respectively. Linkage of Mood Disorders to this region was observed exclusively among female affected relative pairs (LOD scores of 5.35 and 6.21 at D2S2321 and D2S2208, respectively); no suggestion of linkage was observed when male affected relative pairs were analyzed alone. These observations implied that a sex-specific susceptibility gene in this region contributes to the vulnerability of women in these families to the development of unipolar Mood Disorders that ranged in severity from minor to severe at the time of clinical assessment. This 451 Kb region includes the *CREB1* gene, which encodes a cAMP-responsive element-binding protein (CREB) that is a member of the bZIP family of transcription factors and is an attractive candidate risk gene for mood and related disorders (Blendy 2006; Nestler et al., 2006; Zubenko et al., 2002a; 2003a,b).

The potential effects of sex were considered *a priori* in our published genetic studies because several lines of epidemiological, clinical, and biological evidence had suggested that there may be important differences in the pathophysiology of MDD in men and women (Zubenko et al., 2002c). To accomplish this, we dichotomized our samples by sex and compared the results of association or linkage studies obtained for men and women. We also performed linkage analyses using the conditional logistic model for affected relative pairs (Olson, 1999), an approach that provided the flexibility to include sex as a covariate in the linkage analysis of pedigrees with arbitrary structures.

While these approaches to evaluating the effect of sex in our genetic analyses have yielded convergent qualitative results, it has been reported that the conditional logistic model for linkage analysis, as implemented in the LODPAL program of the S.A.G.E. version 4.0 software package (S.A.G.E., 1999), has an increased type I error rate that is exacerbated by the inclusion of covariates (Brock et al., 2005; Doan et al., 2005; Hsu et al., 2003). A proposed remedy for this shortcoming has been published and implemented in the LODPAL program of S.A.G.E. version 5.4.1 for datasets containing from 20 to 320 affected relative pairs (ARPs)(Sinha et al., 2006). Using their model, Sinha and coworkers (2006) reported a corrected p value for the peak LOD score observed for one of the models in our published linkage analysis of the *CREB1* region, which remained highly significant. In this report, we describe a re-analysis of our published evidence of sex-specific linkage of unipolar Mood Disorders to the *CREB1* region of chromosome 2q in our sample of 81 RE-MDD families (Zubenko et al., 2002a) using a simulation approach similar to that used by Sinha and colleagues (2006) to directly calculate empirical p values corresponding to our LODPAL LOD scores.

Subjects and Methods

Clinical Characterization of Subjects and Families

Eighty-one families were ascertained through adult probands with unipolar, non-psychotic RE-MDD and included 407 1st-degree relatives and 835 extended relatives, as previously described (Zubenko et al., 2001). Selection criteria for families also included two living parents and at least one sibling who were willing to participate in the study. Families were

ascertained without consideration of the psychiatric diagnoses of any family member other than the proband.

Psychiatric diagnoses for probands with RE-MDD and their family members who provided blood samples were formulated from structured personal interviews, including the SADS-L (Spitzer and Endicott, 1975) or the Kiddie-SADS-Epidemiologic Version (Puig-Antich et al., 1980), structured family history assessments, and available medical records. The remaining family members who participated and those who were deceased were evaluated through the family history method augmented by available medical records (Andreasen et al., 1986; Weissman et al., 1986). The inter-rater reliability of the psychiatric diagnoses established from structured personal interviews, as well as the sensitivity and specificity of the family history method to accurately detect the presence or absence of Mood Disorders in these groups, has been established (Zubenko et al., 2001, 2002c). Prior to conducting the linkage analysis, follow-up assessments were performed for all available family members to further increase the statistical power for detecting susceptibility loci by augmenting the size and informativeness of the sample, and minimizing phenotypic misspecification.

All clinical assessments were performed by experienced mental health clinicians with regular supervision by an ABPN-certified faculty psychiatrist. Best estimate diagnoses were made using a consensus conference approach according to Research Diagnostic Criteria (Endicott et al., 1975; Spitzer et al., 1978) and the DSM-IV criteria (American Psychiatric Association, 1994). For linkage analysis, Mood Disorders were grouped into four categories with decreasing thresholds of severity: RE-MDD, Recurrent-MDD (R-MDD), Major Mood Disorders, and Any Mood Disorder. The numbers of affected relative pairs (ARPs) that met diagnostic criteria for each of these phenotypes is shown in Table 1.

This research project and associated recruitment materials were approved by the Institutional Review Board of the University of Pittsburgh. All subjects provided written informed consent prior to participation.

Statistical Methods

Multipoint identity-by-descent (IBD)-sharing estimates for the marker loci were obtained with the GENIBD program from the S.A.G.E. software package, release 5.4.1 (2008). IBD sharing distributions were calculated at each of the markers using the exact algorithm implemented in GENIBD when the quantity 2 × [(number of non-founders)-(number of founders)] in the pedigree was 18 or less. The exact algorithm is based on the Lander-Green multipoint algorithm (Lander and Green, 1987). Multipoint IBD-sharing estimates in larger pedigrees that did not meet the size restriction were calculated with GENIBD using a modified Markov chain Monte Carlo (MCMC) simulation algorithm (Sobel and Lange, 1996).

We performed linkage analysis for the complete dataset, and for subsets of F-F or M-M ARPS, using the conditional logistic model (Olson, 1999) as implemented in the LODPAL program of the S.A.G.E. v.5.4.1 software package. Multipoint analyses employed the IBD-sharing estimate at each marker position. Multipoint LODPAL LOD scores for these analyses are reported along with empirical p values determined from simulations.

To address the previously described issues with the LODPAL statistic, we performed simulations to generate empirical distributions of the test statistic at each marker. To establish stable estimates of empirical significance, we generated 10,000 simulated replicates for the sex-dependent models that employed the complete dataset and 50,000 replicates for our most significant findings in the Female-Female ARP subset. Sinha and colleagues (2006) reported that permutation of IBD status between affected relative pairs did

not provide the variation needed to adequately construct a proper null distribution. Instead, we used an approach that relies on gene-dropping simulation and re-estimation of simulated null IBD sharing at each iteration (MacCluer et al., 1986; Jung et al., 2006). Such an approach, while computationally demanding, provides the variance in the test statistic needed for proper estimation of a null distribution while holding constant the parameters which influence the null distribution.

We simulated genotype data using the MERLIN package (Abecasis et al., 2002), which uses a gene-dropping algorithm, while maintaining family structure, marker characteristics (allele frequency, map distances, etc.), missing data patterns, and phenotype assignments. Each of the resulting 10,000 (or 50,000 in the analyses of F-F pairs) data iterations was analyzed using the S.AG.E. v5.4.1 software package. As was done in the actual dataset, identity by descent was estimated for all relative pairs, in each simulated dataset, using the GENIBD program. In the analyses including all ARPs, linkage analysis was performed using the LODPAL program with sex included as a covariate. In the sex-specific analyses (F-F and M-M ARP pairs), no covariates were included. The LODPAL LOD score from each iteration, at each marker-phenotype combination, was contributed to the null distribution. Significance was calculated using the formula $(p+1)/(n+1)$, where p is the number of null tests more significant than the actual test and n is the total number of permutations performed.

To assess the influence of the covariate gender in the analyses including all ARPs, we generated a null distribution of the coefficients reflecting the effect of sex, $\beta(sex)$, on the linkage results. The empirical significance of these regression coefficients were assessed using the approach described above. An empirical p value was calculated for the $\beta(sex)$ coefficients estimated in the actual data by comparison to the null distribution of the $\beta(sex)$ values for any given marker-phenotype combination.

Because we analyzed sets of correlated markers and phenotypes, correction for multiple testing across six markers and four phenotypes using broadly applied approaches, such as the Bonferroni correction (Abdi, 2007) or controlling the false discovery rate (Benjamini and Hochberg, 1995), would result in overly conservative estimates of significance. Several investigators have presented approaches to correction in the analysis of correlated phenotypes (Conneely and Boehnke, 2007), but not the instance of nested phenotypes in the complex correlation structure of extended pedigrees. To address this issue, the results of the linkage analyses are presented without adjustments for multiple testing, as well as in the context of overly-conservative adjustments for multiple testing as determined by Bonferroni corrections assuming the linkage analyses employed independent markers and phenotypes (Perneger, 1998). The results were also evaluated in the context of the threshold for genome-wide significance recommended by Lander and Kruglyak that has been widely applied in the literature (1995).

Results

Multipoint LOD scores generated by the LODPAL program from S.A.G.E. v.5.4.1 and the corresponding empirical p values are presented in Table 2 for all ARPs who met criteria for four Mood Disorder phenotypes with decreasing thresholds of severity: RE-MDD, R-MDD, Major Mood Disorders, and any Mood Disorder. The resulting LOD scores were very similar to those that were previously produced by the LODPAL program from S.A.G.E. v. 4.0 (Zubenko et al., 2002a). Consistent with our previous results, a maximum multipoint LOD score occurred at D2S2208 for each phenotype. In addition, increasing peak multipoint LOD scores were observed for Mood Disorder phenotypes whose definitions included progressively less stringent severity criteria for inclusion in the affected group and

progressively greater numbers of ARPs. The last finding implies that the risk locus in this region influences the development of unipolar Mood Disorders with a wide spectrum of severity among women from families identified by probands with RE-MDD.

The uncorrected results for the sex-dependent analysis of the complete dataset (Table 2) provided at least nominal evidence for linkage of the six markers that span this 15cM region of 2q33-35 to all four Mood Disorder phenotypes. The evidence for linkage of D2S2321 and D2S2208 to Major Mood Disorder and any Mood Disorder remained significant even after an overly-conservative Bonferroni correction for six linked markers and four nested phenotypes ($p < 0.05/24 = 2.1\times10^{-3}$). Moreover, the empirical p values for the sex effect (β(sex), Table 3) ranged from marginally significant at markers generating the largest multipoint LOD scores for the narrowest phenotype, RE-MDD, and became increasingly significant as the phenotypic definition broadened to any Mood Disorder. The evidence for an effect of sex on the linkage to Mood Disorders also survived the strict Bonferroni correction for multiple comparisons ($p < 2.1\times10^{-3}$). Since sex was coded as male=1 and female=0 in the models, β(sex) <1 indicates that greater LOD scores were observed for women than for men.

Multipoint LOD scores generated by the LODPAL program of S.A.G.E. v.5.4.1 and the corresponding empirical p values are presented in Table 4 for the subset of F-F ARPs who met criteria for the four Mood Disorder phenotypes. Fifty-thousand simulations (>2 processor-years) were required to produce a single LOD score larger than 6.33, the largest multipoint LOD score obtained from the linkage analysis of the subset of F-F ARPs. As shown in Table 4, the peak multipoint LOD scores for F-F ARPs that fulfilled criteria for each of the Mood Disorder phenotypes were associated with empirical p values that reached at least nominal statistical significance. Even after an overly-conservative adjustment of the p values for multiple comparisons according to the Bonferroni method (six linked markers × four nested phenotypes × two sexes = 48; $p < 0.05/48 = 1.0\times10^{-3}$), the multipoint LOD scores for D2S2321 or D2S2208 and Major Mood Disorder, as well as the multipoint LOD scores for all six markers and any Mood Disorder, remained significant in this subset. Indeed, the threshold for genome-wide statistical significance of linkage results recommended by Lander and Kruglyak (1995), $p = 5\times10^{-5}$, was surpassed by the p value obtained for the maximum multipoint LOD score at D2S2208 for F-F ARPs with any Mood Disorder. Also consistent with our published findings, analysis of the M-M ARPs revealed no evidence for linkage of Mood Disorders in this region of chromosome 2q (multipoint LOD scores = 0.00, p = 1.00, all phenotypes).

Discussion

The results of this re-analysis confirm our published findings of linkage of the *CREB1* region of 2q to Mood Disorders among women from families identified by RE-MDD (Zubenko et al., 2002a). We subsequently completed a genome-wide linkage analysis for loci that influence the development of Depressive Disorders in these families that included both sex and linkage to the *CREB1* region as covariates (Zubenko et al., 2003b). This analysis identified 19 susceptibility loci that often had sex-specific effects on risk, interacted with the *CREB1* region to influence risk, and affected the development of a spectrum of depressive disorders as well as alcoholism and other addictions. The evidence for significant linkage in this genome-wide linkage study relied upon a simulation approach rather than p values generated by LODPAL. In addition, the results of a candidate gene analysis at these loci focused attention on cell signaling pathways, rather than particular neurotransmitters.

Sequence variations in the *CREB1* promoter have been detected that revealed significant evidence of linkage and association (cosegregation) with Mood Disorders among women

from two extended RE-MDD families, providing additional support for *CREB1* as a sex-limited susceptibility gene for unipolar Mood Disorders (Zubenko et al., 2003a). A rare *CREB1* promoter mutation (allele frequency < 0.003 for unaffected women) associated with the development of Mood Disorders in these families produced functional alterations in promoter activity that are both brain cell specific and dependent on gonadal steroid hormones, consistent with sex-specific phenotypic expression (Zubenko and Hughes, 2008a,b). As a result, alleles that predominantly affect the development of Mood Disorders in women may exert the greatest risk at times of large fluctuations in female gonadal hormones (menarche, menses, pregnancy/childbirth, menopause) and diminish after age 35-40, when sex hormone levels begin to fall.

Published genetic linkage studies of RE-MDD (Holmans et al., 2007) and genetic association studies (Muglia et al. 2008; Sullivan et al., 2009) lack adequate power to detect contributions from rare susceptibility alleles, even those that confer large effects (Maher et al., 2008). Morevover, the latter two association studies employed R-MDD and MDD as the affected phenotypes, respectively, with no requirement for a family history of RE-MDD or any Mood Disorder. Since the linkage of Mood Disorders to the *CREB1* region in our sample of 81 RE-MDD families appears to result largely from rare, strongly penetrant promoter variants whose phenotypic effects are sex specific, it is not surprising that these study designs failed to detect linkage of Mood Disorders to this locus.

It is noteworthy that additional work outside of our dataset found evidence for an influence of the *CREB1* region on Mood Disorders or related phenotypes. Nurnberger and colleagues (2001) reported significant evidence for linkage (LOD = 4.12) to comorbid alcoholism and MDD near the marker D2S1371 on 2q in the Collaborative Study of the Genetics of Alcoholism (COGA) replication sample. While there was no evidence of linkage at this locus in their initial sample, the combined sample yielded a suggestive LOD score of 2.16. Venken and colleagues (2005) reported and replicated suggestive evidence of linkage (LOD = 2.26) to the 2q33-35 region containing *CREB1*, which they confirm by dense genotyping (LOD = 2.2) in a study of very large multigenerational kindreds from a Swedish population isolate that were segregating Mood Disorders. Two linkage analyses of neuroticism, a risk endophenotype of depressive disorders, have produced suggestive evidence for linkage on 2q (Kuo et al., 2007; Wray et al., 2008). Kuo and colleagues found suggestive linkage (p=0.022) in the region of 2q distal to *CREB1* and sex-specific suggestive linkage (LOD=1.6) to the neuroticism phenotype among women.

Several investigators have independently evaluated our original report of an association of the D2S2944 124 bp allele with RE-MDD and substance use disorders among women. Philibert and colleagues (2003) confirmed the selective association of the D2S2944 124 bp allele among women with RE-MDD, while Langbehn and coworkers (2006) reported an association of this allele with MDD complicated by alcohol abuse/dependence and/or antisocial personality disorder. Beem and colleagues (2006), following up a finding of suggestive linkage (LOD~1.5) of neuroticism to the region containing *CREB1*, reported a nominally significant (p=0.04) sex-specific association for anxiety in males that did not survive correction for multiple testing.

Several reports have explored the relationship between sequence variation in or near *CREB1* and the clinical features of Mood Disorders. Perlis and colleagues have described associations of non-coding SNPs in the *CREB1* region with expressed anger and treatment-emergent suicidal ideation among men with MDD that are less evident or absent among women with this disorder (Perlis et al., 2007a,b). Whether these genotypes affect the risk of developing syndromic MDD among men or women cannot be determined from these

studies. In addition, Mamdani and coworkers (2008) reported a significant association of two SNPs in the 5′ region *CREB1* and lithium-responsive Bipolar Disorder.

In summary, this reanalysis addresses the statistical treatment of our data in previous publications. After discovery of a problem with the LODPAL program in the S.A.G.E. v4.0 software package used in our initial analyses, we performed a re-analysis of our data using simulation. After re-analysis, the report of linkage of the *CREB1* region to Mood Disorders among women from families with RE-MDD stands. Moreover, our results have been supported by a host of additional published findings that implicate sequence variations in *CREB1* in the sex-dependent development of syndromic Mood Disorders, as well as related clinical features and disorders.

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Table 1

Distributions of Affected Relative Pairs by Phenotype and Sex

		RE-MDD	R-MDD	Major Mood Disorder	Mood Disorder
All Pairs	Sib-Pairs	49	72	152	178
	Other	86	168	368	432
Female-Female	Sib-Pairs	17	32	64	75
	Other	28	68	144	165
Male-Male	Sib-Pairs	5	6	17	19
	Other	10	16	47	56
Female-Male	Sib-Pairs	27	34	71	84
	Other	48	84	177	211

RE-MDD: Recurrent, Early-Onset, Major Depressive Disorder; R-MDD: Recurrent, Major Depressive Disorder.

Table 2

LOD Scores and Empirical P Values for Sex-Dependent Multipoint Linkage Analyses of All Affected Relative Pairs

	RE-MDD			R-MDD			Major Mood Disorder			Mood Disorder		
Marker	**LOD v.4.0**	**LOD v.5.4.1**	**emp p**	**LOD v.4.0**	**LOD v.5.4.1**	**emp p**	**LOD v.4.0**	**LOD v.5.4.1**	**emp p**	**LOD v.4.0**	**LOD v.5.4.1**	**emp p**
D2S1384	2.44	3.20	1.93×10^{-2}	2.53	3.55	7.80×10^{-3}	3.01	3.04	1.01×10^{-2}	4.64	4.79	1.06×10^{-3}
D2S2321	3.11	3.16	1.75×10^{-2}	3.23	3.29	1.15×10^{-2}	4.59	4.50	1.70×10^{-3}	6.33	6.37	8.00×10^{-4}
D2S2208	3.17	3.19	1.73×10^{-2}	3.31	3.35	1.06×10^{-2}	4.75	4.93	1.30×10^{-3}	6.87	7.02	5.00×10^{-4}
D2S1380	1.09	1.10	1.76×10^{-1}	1.45	1.49	9.60×10^{-2}	2.76	3.78	4.00×10^{-3}	4.61	5.76	4.00×10^{-4}
D2S2944	0.88	0.93	2.14×10^{-1}	1.24	1.31	1.18×10^{-1}	2.61	3.03	1.01×10^{-2}	4.44	4.94	1.10×10^{-3}
D2S434	0.65	0.56	3.34×10^{-1}	1.41	1.36	1.14×10^{-1}	2.57	2.92	1.02×10^{-2}	4.72	5.12	1.60×10^{-3}

LOD scores are presented from the previously published multipoint linkage analysis employing LODPAL from S.A.G.E. version 4.0 (Zubenko et al., 2002a) along with the current results obtained using LODPAL from S.A.G.E. version 5.4.1. Empirical p values (emp p) for the current LOD scores (v.5.4.1) were determined from 10,000 gene-dropping simulations. RE-MDD: Recurrent, Early-Onset, Major Depressive Disorder; R-MDD: Recurrent, Major Depressive Disorder.

Table 3

Regression Coefficients for Sex Effect, β(sex), and Corresponding Empirical P Values

	RE-MDD			R-MDD			Major Mood Disorder			Mood Disorder		
Marker	**β(sex) v.4.0**	**β(sex) v.5.4.1**	**emp p**	**β(sex) v.4.0**	**β(sex) v.5.4.1**	**emp p**	**β(sex) v.4.0**	**β(sex) v.5.4.1**	**emp p**	**β(sex) v.4.0**	**β(sex) v.5.4.1**	**emp p**
D2S1384	0.73	1.07	6.60×10^{-3}	0.86	0.92	6.80×10^{-3}	0.31	0.33	6.70×10^{-3}	0.37	0.38	2.70×10^{-3}
D2S2321	0.86	0.88	2.15×10^{-2}	0.85	0.86	8.80×10^{-3}	0.39	0.39	2.70×10^{-3}	0.42	0.42	1.80×10^{-3}
D2S2208	0.86	0.88	2.14×10^{-2}	0.85	0.85	9.60×10^{-3}	0.40	0.40	3.30×10^{-3}	0.44	0.44	2.40×10^{-3}
D2S1380	0.41	0.32	1.66×10^{-1}	0.40	0.32	1.27×10^{-1}	0.32	0.36	3.90×10^{-3}	0.37	0.41	1.80×10^{-3}
D2S2944	0.36	0.29	1.70×10^{-1}	0.37	0.30	1.27×10^{-1}	0.30	0.31	4.30×10^{-3}	0.35	0.36	2.00×10^{-3}
D2S434	0.39	0.22	1.98×10^{-1}	0.50	0.33	1.35×10^{-1}	0.31	0.31	7.50×10^{-3}	0.39	0.39	2.80×10^{-3}

The regression coefficients for sex effect, β(sex), are presented from the previously published multipoint linkage analysis employing LODPAL from S.A.G.E. version 4.0 (Zubenko et al., 2002a) along with the current results obtained using LODPAL from S.A.G.E. version 5.4.1. Since sex was coded as male=1 and female=0 in the models, β(sex) <1 indicates that greater LOD scores were observed for women than men. Empirical p values (emp p) for the current β(sex) values (v.5.4.1) were determined from 10,000 gene-dropping simulations. RE-MDD: Recurrent, Early-Onset, Major Depressive Disorder; R-MDD: Recurrent, Major Depressive Disorder.

Table 4

LOD Scores and Empirical P Values for Multipoint Linkage Analyses of Female-Female Affected Relative Pairs

	RE-MDD			R-MDD			Major Mood Disorder			Mood Disorder		
Marker	**LOD v.4.0**	**LOD v.5.4.1**	**emp p**	**LOD v.4.0**	**LOD v.5.4.1**	**emp p**	**LOD v.4.0**	**LOD v.5.4.1**	**emp p**	**LOD v.4.0**	**LOD v.5.4.1**	**emp p**
D2S1384	0.62	1.48	1.18×10^{-2}	1.20	1.78	6.54×10^{-3}	2.51	3.06	1.42×10^{-3}	3.68	4.30	4.20×10^{-4}
D2S2321	1.20	1.24	1.76×10^{-2}	1.53	1.51	1.10×10^{-2}	4.03	4.06	4.60×10^{-4}	5.35	5.53	1.20×10^{-4}
D2S2208	1.20	1.24	1.72×10^{-2}	1.53	1.55	1.03×10^{-2}	4.03	4.74	1.80×10^{-4}	6.21	6.33	4.00×10^{-5}
D2S1380	0.80	0.69	5.65×10^{-2}	1.12	1.02	2.95×10^{-2}	2.18	2.42	2.95×10^{-2}	3.41	3.77	7.00×10^{-4}
D2S2944	0.68	0.69	5.70×10^{-2}	0.99	1.02	2.91×10^{-2}	2.31	2.45	3.68×10^{-3}	3.54	3.81	9.40×10^{-4}
D2S434	0.17	0.19	1.96×10^{-1}	0.69	0.71	5.34×10^{-2}	2.09	2.17	5.12×10^{-3}	3.65	3.78	7.80×10^{-4}

LOD scores are presented from the previously published multipoint linkage analysis employing LODPAL from S.A.G.E. version 4.0 (Zubenko et al., 2002a) along with the current results obtained using LODPAL from S.A.G.E. version 5.4.1. Empirical p values (emp p) for the current LOD scores (v.5.4.1) were determined from 50,000 gene-dropping simulations. RE-MDD: Recurrent, Early-Onset, Major Depressive Disorder; R-MDD: Recurrent, Major Depressive Disorder.