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Candidate Region Linkage Analysis in Twins Discordant or Concordant for Depression Symptomatology

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Abstract

Genetic risk factors contribute considerably to both clinical affective disorders and subsyndromal mood level. There is moreover evidence to suggest that the genetic basis of bipolar disorder and unipolar depression overlap to some extent, and several linkage analyses have suggested evidence for a common susceptibility locus in affective disorders on chromosome 12q24. In this study we investigated the chromosome 12 candidate region for linkage to the mean level of depression symptomatology, over a 10-year follow-up, using a highly informative sample of concordant and discordant twin pairs selected from 4,731 participants of the Longitudinal Study of Ageing Danish Twins. Our results showed suggestive evidence of linkage to this region with a peak LOD score of 1.91 for marker D12S1634 located at 148 cM, and thus indicates that the previously identified disease locus at 12q24 is also a general vulnerability locus affecting the normal range of mood.

Keywords

depression symptomatology; mood disorders; genetics; linkage analysis

Depressive disorders are among the most common psychiatric diseases, and both clinically manifest depression (i.e., unipolar and bipolar depression) and sub-syndromal depression symptoms are conditions of major importance with impact on capability, morbidity, and mortality [Stek et al., 2004].

It is well established that genetic risk factors contribute considerably to affective disorders [Sullivan et al., 2000]. It has furthermore been suggested that the genetic predispositions to bipolar and unipolar depression may to some extent overlap [McGuffin et al., 2003; Hamet and Tremblay, 2005], although independent genetic risk factors possibly also have a significant impact on disease development. In addition, a recent twin study indicated that genetic factors influence individual levels of depression symptomatology, even when those levels are within the normal (i.e., non-clinical) range of mood and especially when the symptoms are aggregated over multiple assessments. Using a cohort of elderly Danish twins, McGue and Christensen [2003] showed that the average level of depression symptomatology assessed using repeated measures over several years was substantially

heritable (64% in men and 69% in women), even though occasion-specific depression levels were only moderately heritable (22–37%) [McGue and Christensen, 2003].

If hypothesizing that liability to major clinical affective disorders in part constitutes the extreme of a quantitatively measurable depression symptomatology/mood scale, it can be argued that there may be a genetic relationship between general mood regulation and susceptibility to development of depressive disorders.

Genetic linkage studies in psychiatric diseases have identified several candidate regions for affective disorders and especially bipolar disorder. The evidence in favor of a susceptibility locus at chromosome 12q24 is especially strong and has been supported by several independent studies [e.g., Ewald et al., 1998, 2002; Morissette et al., 1999; Curtis et al., 2003; Shink et al., 2005b]. Interestingly, this region generally gives stronger linkage signals when using a broad disease definition combining pedigrees with both bipolar and unipolar depression patients, thus suggesting that this represents a common susceptibility locus in affective disorders [Curtis et al., 2003; Shink et al., 2005b]. Several genes within this chromosomal region have been suggested as risk genes in affective diseases but, as yet, no consensus about the specific genes involved has been reached.

The aim of this study was to investigate the chromosome 12 candidate region for linkage to depression symptomatology using a highly informative sample of concordant and discordant sib pairs, selected from 4,731 participants of the Longitudinal Study of Ageing Danish Twins (LSADT). The LSADT includes all Danish twins aged 70 and older [Skytthe et al., 2002]. The study sample for the current study was drawn from the same population that was used to assess the heritability of depression symptomatology aggregated over multiple times of assessment. The present linkage study thus makes use of the same diagnostic tool and the same population that previously indicated a high heritability of the trait.

In brief, LSADT is an on-going study that was initiated in 1995 and then repeated every second year. Each survey comprises multidimensional face-to-face interviews focusing on health and lifestyle issues, assessment of cognitive and physical abilities, and assessment of depression symptomatology.

Depression symptomatology was assessed using an adaptation of the depression section of the Cambridge Mental Disorders of the Elderly Examination (CAMDEX) as described [McGue and Christensen, 1997]. The 21 items (16 original CAMDEX items and 5 supplementary items) that directly assessed current depression symptomatology were factor analyzed resulting in two factor scales, the first loading on items reflecting affective content and the second loading on items reflecting somatic complaints. The somatic and affective scales derived from the factor analytic results were highly correlated (approximately 0.60), justifying the formation of a total depression score defined as the sum of the two subscale scores.

The total depression scores were adjusted for the effects of age and sex prior to use in the linkage analysis. Specifically, for each wave of data we first log transformed the depression scores and regressed out the effects of age and sex to produce a residual score that was unrelated to age and sex. A mean score (based on from 1 to 5 assessments for each individual) was then computed by averaging for each individual the number of valid residual scores. To place the resulting mean residual scores back on the original log scale, a constant of 3.1 was added to all scores.

From the entire sample of 4731 LSADT participants, 1,152 pairs were intact (both twins in a pair participated), out of which 451 pairs were monozygotic. For the intact dizygotic twin

pair samples both depression symptomatology assessments and DNA was available from a total of 432 twin pairs (179 male and 253 female pairs).

We used a two-stage strategy to select the most informative concordant and discordant twin pairs for linkage analysis. First, we set the criteria for being a discordant pair to the upper 20% percentile for one twin partner and the lower 50% percentiles for the other. The criterion for being a concordant high-scoring pair was set to both twins being in the upper 20% percentile. These criteria were applied to the untransformed mean depression scores and cut-points were calculated for men and women separately. Due to the skewed nature of the trait distribution, with the majority of individuals scoring low, twin pairs where both partners had a low mean score were considered as non-informative. Using this approach 83 twin pairs were selected, the majority of which (64 pairs) were discordant. Next, we estimated the relative informativeness conditional on the trait values of the twin pairs by calculating the expected amount of linkage information in each pair using the MERLIN-regress RANK-Families option implemented in MERLIN [Sham et al., 2002]. Using this approach another 16 discordant pairs were selected based on their very high phenotypic informativeness, which was mainly caused by the extremely high mean depressive phenotype expressed by one twin partner in each pair. The 16 pairs were included in the upper 15% percentile with respect to information when considering the entire sample and the top 5% percentile in the population remaining after exclusion of the concordant and discordant pairs selected in the first stage.

The final study sample for linkage analysis consisted in 99 discordant and high-scoring concordant twin pairs.

DNA was prepared from whole blood using standard procedures [Miller et al., 1988], or from cheek swabs or blood spot cards using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA).

We genotyped 18 markers located on chromosome 12q24.31–12q24.33, which repeatedly have shown linkage signals in unipolar and bipolar disorders [e.g., Curtis et al., 2003; Shink et al., 2005b]. Subsequently, we furthermore added two markers in the 12q23.1 region and three markers in the 12q23.3–12q24.11 region as these previously were found to be linked to unipolar depression [Abkevich et al. 2003; McGuffin et al., 2005]. Primer sequences and PCR conditions were as described in the Human Genome Database (www.gdb.org). PCR products were resolved on the MegaBACE 1000 according to the manufacturer's instructions and analyzed using the Fragment Profiler software (Amersham Biosciences, GE Healthcare, Uppsala, Sweden). Unlikely genotypes were detected using the Error option in MERLIN, and the possibly erroneous genotypes was set to unknown.

Linkage analysis was performed using the combined “squared sums-squared differences” Haseman-Elston regression approach, implemented in MERLIN-REGRESS [Sham et al., 2002]. This method is particularly robust for selected samples drawn from a population with known trait distribution, and additionally takes into account the incomplete IBD information that is a result of using a sample with no parental information.

We specified the sex- and age-adjusted population mean of the depression symptoms trait (1.46), variance (0.62), and heritability (0.6) based on estimates from the entire LSADT sample from which the twins were drawn.

Linkage results are shown in Table I and Figure 1. The 18 markers in the 12q24.31–12q24.33 region span a distance of 26.73 cM with a mean distance of 1.5 cM, and average information of 90%. A peak LOD score of 1.91 ($P=0.0015$) was found at D12S1634 in the 12q24.32 region. There was no evidence of linkage to the additionally included markers in

the 12q23.1 and 12q23.3–12q24.11 regions. We used the Simulate procedure in MERLIN to generate simulated data on the marker map used in our analysis and to estimate the significance level for obtaining randomly an LOD score of 1.91. Based on 1,000 replicates, we obtained an empirical P -value of 0.003, which means that the observed LOD score is still significant when all markers are considered.

Interestingly, when separating the study sample into phenotypically concordant and discordant twin pairs we obtained a slightly stronger linkage signal in the discordant group (D12S1634 peak LOD score 2.12, point wise P -value=0.0009), whereas little or no signal was detected in the group of concordant twins. This could indicate that the genetic contribution to the depression symptom score is of higher importance in discordant pairs than in concordant pairs. Due to the limited sample size in these smaller groups it is also possible, however, that this is a chance observation.

Although the peak LOD score of 1.91 obtained in this study does not reach genome wide significance as recommended by Lander and Kruglyak [1995] we believe that our study adds to the growing body of evidence for linkage to this region.

Thus, in the past years several studies have provided strong evidence for the presence of one or more genes in the 12q region that may be involved in psychiatric disorders, including bipolar disorder [Ewald et al., 2002; Curtis et al., 2003; Shink et al., 2005b], unipolar depression [Abkevich et al., 2003; McGuffin et al., 2005], and neuroticism [Fullerton et al., 2003].

The results of this study indicate the presence of a gene/genes in this region not only conferring susceptibility to clinically manifest mood disorders but also affecting the underlying basic mood level of each individual. This view is furthermore supported by a recent study from Boomsma et al. [2006] showing suggestion of linkage for the exact same region to loneliness, which may be a predictor of psychiatric diseases [Boomsma et al., 2006].

There are several candidate genes located within the chromosome 12q24 linkage region, some of which have been associated to bipolar disorder [e.g., Glaser et al., 2005; Lyons-Warren et al., 2005; Shink et al., 2005a; Kalsi et al., 2006], although these studies still await replication in independent samples.

Recently however, P2RX7, a gene encoding a purinergic ion channel, has been implicated in both bipolar disorder, unipolar depression, and anxiety [Barden et al., 2006; Lucae et al., 2006; Erhardt et al., 2007; McQuillin et al., 2008]. We are currently investigating whether genetic variation in this gene is also associated with underlying mood level.

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FIG. 1. Results of linkage analysis of chromosome 12q23-q24. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I

Tested Markers and LOD Scores in Chromosomal Region 12q23 and 12q24

Marker	Map position (cM)	Info (%)	LOD	Pointwise <i>P</i> -values	Empirical <i>P</i> -values
D12S1300	104.12	83.1	0.063	0.3	0.99
D12S1706	104.13	83.0	0.062	0.3	0.99
D12S1597	114.28	79.1	0.299	0.12	0.89
D12S1613	116.08	78.9	0.547	0.06	0.56
D12S1583	119.55	84.6	0.921	0.02	0.19
D12S395	136.82	72.4	0.895	0.02	0.28
D12S1612	140.17	92.5	1.180	0.01	0.14
D12S1614	144.83	93.7	1.226	0.009	0.10
D12S342	144.84	93.7	1.228	0.009	0.10
D12S340	146.39	94.5	1.393	0.006	0.05
D12S324	147.17	95.6	1.534	0.004	0.03
D12S386	147.18	95.6	1.535	0.004	0.03
D12S1639	148.24	95.4	1.891	0.002	0.003
D12S1634	148.25	95.6	1.914	0.0015	0.003
D12S1658	148.26	95.5	1.905	0.002	0.003
GATA41E12	149.60	91.8	1.504	0.004	0.04
D12S1675	150.70	91.4	1.504	0.004	0.04
D12S1679	153.19	92.6	1.337	0.007	0.05
D12S1609	153.33	91.0	1.301	0.007	0.07
D12S1659	155.94	84.5	0.952	0.02	0.22
D12S367	159.59	88.0	0.640	0.04	0.49
D12S1045	160.68	87.0	0.474	0.07	0.72
D12S63	163.55	75.6	0.263	0.14	0.94

Map position indicates genetic map positions obtained from Marshfield Clinic.
Info indicates information content at each marker position. Empirical *P*-values were obtained from 1,000 simulation replicates.