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Genetic Linkage and Association Analysis for Loneliness in Dutch Twin and Sibling Pairs Points to a Region on Chromosome 12q23–24

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We obtained evidence from a large study in Dutch twins ($N=8387$) and siblings ($N=2295$) that variation in loneliness has a genetic component. The heritability estimate for loneliness, which was assessed as an ordinal trait, was 40% and did not differ between males and females. There were 682 sibling pairs with genotypic (around 400 microsatellite markers) data. We combined phenotypic and genotypic data to carry out a genome scan to localize QTLs for loneliness. One region on chromosome 12q23.3–24.3, showed near suggestive linkage. Genetic association tests within this region revealed significant association (p -value 0.009) with one of the alleles of marker D12S79 and with one of the alleles of neighbouring marker D12S395 (p -value 0.043). We review evidence for linkage in this region for psychiatric disorders and discuss our findings within this context.

KEY WORDS: Association; heritability; linkage; loneliness; ordinal data; QTL; sib-pair.

INTRODUCTION

Loneliness has been described by Weiss (1973) as “a gnawing ... chronic disease without redeeming features” which may occur when one’s intimate and social needs are not adequately met (Baumeister and Leary, 1995). The core experience of loneliness consists of social isolation and the absence of both relational and collective connectedness (Hawkley *et al.*, in press; Russell *et al.*, 1980). There is now substantial evidence that loneliness is at the heart of a constellation of socio-emotional states, which include self-esteem, mood, anxiety, anger, optimism, fear of negative evaluation, shyness, social skills, social support, dysphoria, and sociability (see reviews by

Duck *et al.*, 1994; Ernst and Cacioppo, 1999; Peplau and Perlman, 1982; Berscheid and Reis, 1998; Rook, 1988; Shaver and Brennan, 1991).

Recently, attention has been paid to how genetic and environmental factors influence the development of individual differences in loneliness. McGuire and Clifford (2000) examined the heritability of loneliness in children and found significant genetic ($h^2=55\%$ and 48%, respectively) contributions to individual differences in loneliness. To examine the genetic contribution to variation in loneliness in adults, Boomsma *et al.* (in press) conducted a study based on data of 8387 adult twins from the Netherlands Twin Register. It was found that individual differences in loneliness demonstrated considerable temporal stability and heritability. The estimate of heritability was 48%, and all environmental influences were unique to each individual. The genetic contributions to adult loneliness were similar in males and females and in a younger and an older cohort. Finally, no qualitative sex differences in heritability were found, indicating that the same genes influence loneliness in both sexes.

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59 The heritability of loneliness is comparable to
60 that of other complex traits which have been the
61 subject of genome scans to identify susceptibility loci.
62 As is the case for other complex traits, heritability
63 may be caused by polymorphisms in several genes.
64 Because a large number of genetic and environmental
65 factors may contribute to the liability of loneliness,
66 isolating the genetic component is a daunting
67 undertaking (Stenger *et al.*, 2005). We collected
68 genotypic marker data and phenotypic information
69 in 682 sibling pairs and their parents and aim,
70 through linkage and association analysis, to elucidate
71 candidate regions which may harbor genes influenc-
72 ing variation in loneliness. Loneliness was analyzed
73 as an ordinal phenotype, using a threshold model
74 which assumes a latent normally distributed liability
75 (Falconer, 1989; Lynch and Walsh, 1998). The ordi-
76 nal phenotype and the continuous liability are linked
77 through thresholds and the liability was treated as an
78 unobservable quantitative trait (Yi *et al.* 2004).

79 METHODS

80 Participants and Phenotypic Information

81 In 1991 the Netherlands Twin Register (NTR)
82 started a longitudinal survey study of health and
83 lifestyle in adolescent and adult twins and their family
84 members (Boomsma *et al.*, 2002). Surveys were
85 mailed out in 1991, 1993, 1995, 1997, 2000 and 2002/
86 3. Twins were asked to participate in all waves, sib-
87 lings were included since 1995. Five surveys con-
88 tained items on loneliness from the YASR
89 (Achenbach, 1990), which could be answered on a
90 3-point scale. Based on factor analyses, we selected 2
91 items (item 12: I feel lonely, and item 33: Nobody
92 loves me). Information on loneliness was quantified
93 by averaging over items and over years of observa-
94 tion (Boomsma *et al.*, in press). There were pheno-
95 typic data for 8387 twins, and for 2295 full siblings.

96 DNA Collection and Genotype Data

97 Genotype data came from 2 studies: a cardio-
98 vascular study of unselected DZ twin pairs (Heijmans
99 *et al.*, 2005) and a study of DZ twin and sibling pairs
100 (Boomsma *et al.*, 2000) who were selected for anxious
101 depression. In the cardiovascular study, DNA was
102 collected from blood samples. For 234 individuals,
103 there were phenotypic data on loneliness. Genotyping
104 in these samples was carried out by the Dept of
105 Molecular Epidemiology (Leiden). A 419 marker

genome scan (8.34 cM spacing) was done using a 106
combination of in-house markers (ALFexpress auto- 107
mated sequencer (Amersham Pharmacia Biotech)) as 108
described by Beekman *et al.* (2003), markers from the 109
Weber screening set 8 (ALFexpress) and from the 110
Human Linkage Set v2.5 MD10 and HD5 (ABI Prism 111
DNA Analyzer 3700 (Applied Biosystems)). 112

DNA from buccal swabs was collected as part of 113
the NETSAD project (Boomsma *et al.*, 2000) in sib- 114
ling pairs selected for anxious depression, with addi- 115
tional, non-extreme siblings in the family included in 116
the DNA collection. For 523 siblings (50 of which 117
were also genotyped as part of the cardiovascular 118
study) phenotypic loneliness data were available. 119
Genotyping in the NETSAD samples was performed 120
by the Mammalian genotyping service (Marshfield, 121
USA). A 379 autosomal marker genome scan 122
(9.44 cM spacing) was done using microsatellite 123
screening set 10 (Yuan *et al.* 1997) with few alterna- 124
tive markers. Excessive recombination rates were 125
observed for five markers indicating potential prob- 126
lems. Those markers were not included in the analysis 127
(D1S468; D1S1627; D11S1985; D11S2006 and 128
D20S159-UT1307). Five other markers are currently 129
inconsistently mapped and were also excluded. There 130
were 100 markers typed in both scans. Mendelian 131
errors were detected using PEDSTATS and unlikely 132
double recombinants using MERLIN; both types of 133
error were removed using PEDWIPE (Abecasis *et al.*, 134
2002). Pedigree relationships were checked with GRR 135
(Abecasis *et al.*, 2001). Marker location was taken 136
from an integrated genetic map with interpolated 137
genetic map positions (<http://www2.qimr.edu.au/davidD/>). The position is in Decode cM (Kong *et al.*, 139
2002), estimated via locally weighted linear regression 140
from the Build 34.3 (and 35.1) physical map positions 141
and published Decode and Marshfield genetic map 142
positions. Parents were typed for between 185 and 143
375 markers (mean = 352, SD = 33). For offspring, 144
the number of typed markers ranged from 184 to 678, 145
with an average of 378 (SD = 78). Genotypic data as 146
well as data on loneliness were available for 707 off- 147
spring, from 277 families (for 102 families both par- 148
ents were genotyped and for 40 families one parent 149
was typed)), forming 682 sib pairs. 150

QTL Analysis 151

The phenotypic data were analyzed with a 152
threshold model, with 3 thresholds dividing the lia- 153
bility to loneliness into 4 categories, and different 154
thresholds for males and females (Boomsma *et al.*, in 155

156 press). Because the genotyped pairs are not a random
 157 sample, thresholds were fixed at the estimates from
 158 the model in which data from all twins and siblings
 159 were analyzed. Variation in liability to loneliness was
 160 decomposed into variation due to a QTL (σ_q^2),
 161 additive polygenic influences (σ_a^2), and non-shared
 162 environmental influences (σ_e^2), using structural
 163 equation modelling as implemented in Mx (Neale
 164 *et al.*, 2003). Estimates of the variance component
 165 associated with a putative QTL were obtained by
 166 using a $\hat{\pi}$ approach, in which the covariance due to the
 167 QTL for a sib-pair is modelled as a function of the
 168 estimated proportion of alleles shared identical by
 169 descent. The variance-covariance matrix for pair j,k
 170 of the i -th family (Ψ_{ijk}) is given by:

$$\Omega_{ijk} = \begin{cases} \sigma_a^2 + \sigma_q^2 + \sigma_e^2 & \text{if } j = k \\ \rho\sigma_a^2 + \hat{\pi}_{ijk}\sigma_q^2 & \text{if } j \neq k \end{cases}$$

172 σ_a^2 , σ_q^2 and σ_e^2 denote the background and the QTL
 173 additive genetic and environmental variances. For
 174 DZ twin and sib pairs $\rho = 1/2$ (twice the kinship
 175 coefficient). For DZ and sibling pairs $\hat{\pi}$ depends on
 176 the IBD status of the pair and is obtained
 177 as: $\hat{\pi} = 0.5p_{(IBD=1)} + p_{(IBD=2)}$, where $p_{(IBD=1)}$ denotes
 178 the probability that the pair is IBD=1 and $p_{(IBD=2)}$
 179 denotes the probability that the pair is IBD=2.
 180 Estimates for IBD probabilities were obtained from
 181 Merlin (Abecasis *et al.* 2002). Significance of varia-
 182 tion of the QTL was evaluated by the likelihood ratio
 183 test, from which the LOD score was calculated
 184 (Sham, 1998) by dividing the test statistic χ^2 by
 185 $2 \ln 10$ (~ 4.6).

186 Permutation Tests

187 To obtain empirical estimates of genome-wide
 188 significance levels, 1000 permutations of the dataset
 189 were performed, keeping both the family structure
 190 and the IBD structure intact. These permutations
 191 account for uneven marker spacing and informa-
 192 tiveness (Churchill and Doerge, 1994). Permuted
 193 datasets were obtained as follows: Each row in the
 194 observed dataset represents one family and contains
 195 phenotypes and IBD probabilities across the whole
 196 genome for all pairs within that family. This file is
 197 split into a phenotype file and an IBD file. Families
 198 are labeled with unique numbers one through n . The
 199 phenotypic data are then shuffled by taking a random
 200 permutation of the indices 1, ..., n and matching the
 201 i th phenotypic trait value to the family with index
 202 given by the i th element of permuted indices. This

permutated vector of traits is matched with the original
 (non-permutated) genotypic information for all fami-
 lies. One thousand permuted datasets were generated;
 each permuted dataset was then analyzed. The
 threshold for suggestive linkage was calculated by
 recording the maximum LOD-score for each chro-
 mosome in 1000 runs, and determining what LOD-
 score occurs a 1000 times out of 22,000. This repre-
 sents the average maximum peak size expected once
 per genome scan (Nyholt *et al.*, in press). The
 threshold for suggestive linkage for the ordinal trait
 loneliness was 1.58. The significant linkage threshold
 obtained by determining the maximum LOD-score
 for each scan, and was then defined as the LOD-score
 occurring in 50 of the 1000 permutations corre-
 sponding to a probability of 0.05 in a genome scan
 (Churchill and Doerge, 1994; Lander and Kruglyak,
 1995). The threshold for significant linkage was 2.87.

Genetic Association Testing

Genetic association tests were conducted with
 the extended TDT test proposed by Monks and
 Kaplan (2000) and implemented in the program
 QTDT (Abecasis *et al.*, 2000). This test uses a
 weighing scheme that provides a conservative test of
 association in families with multiple offspring, and
 uses parental genotypes if available. Because the
 program does not handle ordinal data with >2 cat-
 egories, the ordinal variable was dichotomized to
 obtain an affection status by coding all individual
 with ordinal category 0 (i.e. always replied “no” to
 the 2 loneliness items) as unaffected, and all individ-
 uals with ordinal categories >0 as affected.

RESULTS

The previous study of loneliness included data
 from twins only. We repeated the heritability analyses
 of loneliness for twins ($N=8387$) and their siblings. A
 maximum of two brothers and two sisters was added
 to the data set (data from additional brothers
 ($N=33$) and sisters ($N=66$) were not used). This
 added a total of 1019 brothers and 1276 sisters to the
 analyses. The heritability was estimated at 40% (95%
 CI=0.35–0.44). The prevalence for males and
 females in the sample that is available for linkage and
 in the total samples is given in Table I.

Figure 1 shows the results of the whole genome
 scan. One region, 12q23.3–24.3, showed a LOD-score
 (1.38) just below the empirical threshold for sugges-
 tive linkage. The drop 1 LOD-score borders are

Table I. Prevalence of Loneliness Categories in the Sample Available for the Linkage Scan and in the Full Sample

	Loneliness category	Frequency	Percent	% Full sample
Females	0.00	214	52.3	49.7
	1.00	130	31.8	35.6
	2.00	58	14.2	11.9
	3.00	7	1.7	2.8
	Total	409		
Males	0.00	199	66.8	66.2
	1.00	69	23.2	25.3
	2.00	24	8.1	7.2
	3.00	6	2.0	1.3
	Total	298		

251 defined by markers D12S78 and D12S2078, which are
252 37 cM apart (see Figure 2).

253 We performed genetic association tests for
254 loneliness' affection status with microsatellite mark-
255 ers within the 12q23.3–24.3 region. These markers
256 included D12S1300 and D12S2078, and all markers
257 in between, as indicated in Figure 2 ($N=9$ markers;
258 all markers were in Hardy–Weinberg equilibrium).
259 We obtained a significant association between the
260 marker allele with repeat length 169 of marker
261 D12S79 (p -value 0.0094), and suggestive association
262 within the same marker and allele with repeat length
263 157 (p -value 0.0656). For neighbouring marker
264 D12S395 association was seen with the 235 repeat
265 allele (p -value 0.0432), and suggestive association
266 within the same marker and allele with repeat length
267 227 (p -value 0.0662). For the marker allele with
268 repeat length 169 of marker D12S79 we observed that
269 54% of carriers of at least one allele was affected (i.e.
270 scored non-zero on the loneliness trait), whereas 38%
271 of subjects without the 169 allele was affected.

272 DISCUSSION

273 A genome-wide scan for loneliness was con-
274 ducted by treating loneliness as an ordinal phenotype,
275 using a threshold model assuming a latent continuous
276 liability. Considering the moderate heritability of
277 loneliness and the general reduced power of ordinal
278 data versus continuous data the odds of finding sig-
279 nificant linkage seemed rather low at the outset.
280 However, the Lander and Kruglyak (1995) guidelines
281 for suggestive (2.2) and significant (3.6) linkage apply
282 to quantitative traits, and may be too stringent for
283 ordinal traits. Permutation testing confirmed that the
284 thresholds for suggestive and significant linkage were
285 considerably lower, i.e. 1.58 and 2.87. We obtained

286 one near suggestive result from a genome-wide link-
287 age scan for loneliness for a region on chromosome
288 12q23–24. Genetic association tests with markers
289 within this area showed significant association to
290 marker D12S79 and to neighbouring marker
291 D12S395, offering support for the linkage result.

292 This region on chromosome 12 has been impli-
293 cated before in studies of psychiatric disorders.
294 Table II summarizes the evidence for linkage in this
295 region from studies of psychiatric disease, including
296 bipolar disorder, major depression, schizophrenia
297 and indices of anxiety, neuroticism and alcohol abuse
298 (see also Craddock *et al.*, 2005; Hamet and Tremb-
299 lay, 2005). This region on chromosome 12q was first
300 implied at the chromosomes 12 and 16 workshop
301 (Detera-Wadleigh, 1999) as a region with compelling
302 evidence for a bipolar disorder susceptibility locus.
303 This suggestion was further strengthened by the
304 finding that Darier's disease, which maps to this
305 region, co-segregates with bipolar disorder. However,
306 there is strong evidence against the Darier causing
307 mutation itself being the susceptibility risk factor for
308 bipolar disease (Jones *et al.*, 2002). Neither was any
309 evidence found for the DUSP6 gene on chromosome
310 12q23 (Toyota *et al.*, 2000). Shink *et al.*, (2005a, b)
311 investigated the 12q24.31 region by saturating a
312 7.7 Mb genomic region with 20 microsatellite mark-
313 ers and obtained linkage support for bipolar disorder
314 in this area. They next analyzed 32 genes for poly-
315 morphisms in coding sequences and intron/exon
316 junctions. No strong support for any of the genes was
317 found. A positive result was obtained recently with a
318 haplotype in the gene encoding a transcription factor
319 (regulatory factor X4: RFX4) on 12q23 by Glaser
320 *et al.* (2005a). Their haplotype-bipolar association
321 was supported by an association with microsatellite
322 D12S2072. This marker, which was not included in
323 our genome scan, is located between D12S78 and
324 D12S2070/79. The same group (Glaser *et al.*, 2005b)
325 also observed association in this region with Cux2,
326 which is potential regulator of neural cell adhesion
327 molecule expression, and with hypothetical protein
328 FLJ32356; but not with PAH (Green *et al.*, 2003),
329 which we also investigated and for which we found
330 no association.

331 The region on 12q23–q24 may contain a general
332 vulnerability locus for psychiatric disorders. This
333 possibility is reinforced by the finding of linkage for
334 Neuroticism in this region. The 12q23–q24 region
335 was, however, not reported in meta-analyses of
336 bipolar disorder (Segurado *et al.*, 2003) or schizo-
337 phrenia (Lewis *et al.*, 2003). Since the publication of

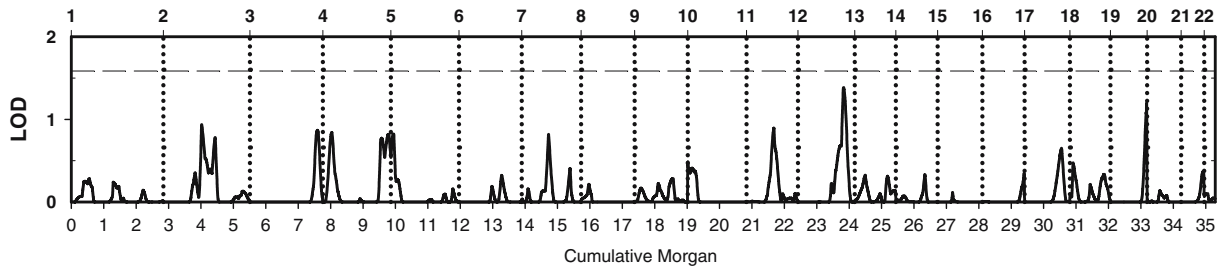


Fig. 1. Multipoint variance-component linkage of the 22 autosomes in 682 sib-pairs for loneliness. The X-axis plots genetic distance (in cM Haldane), and the Y-axis represents the LOD-score.

338 the meta-analyses several further genome scans were
 339 published (see Table II) with at least 2 genome scans
 340 reporting genome wide significance (Ewald *et al.*,
 341 2002; Shink *et al.*, 2005a, b) within the 12q23–24 re-
 342 gion.

343 Understanding the origins of variation in loneli-
 344 ness may be important for clinical as well as scientific
 345 purposes. Loneliness is associated with psychiatric

and behavioral problems, including bipolar disorder, 346
 depression (Eisemann, 1984; 1985; Segrin, 1998), 347
 alcoholism (Akerlind and Hornquist, 1992; Bell, 348
 1956), impaired sleep (Cacioppo *et al.*, 2002a, b), and 349
 suicide (Goldsmith *et al.*, 2002; Wenz, 1977). Patients 350
 with these disorders are socially isolated and have 351
 difficulties in maintaining friendships and relations. 352
 Loneliness may be a consequence as well as a 353

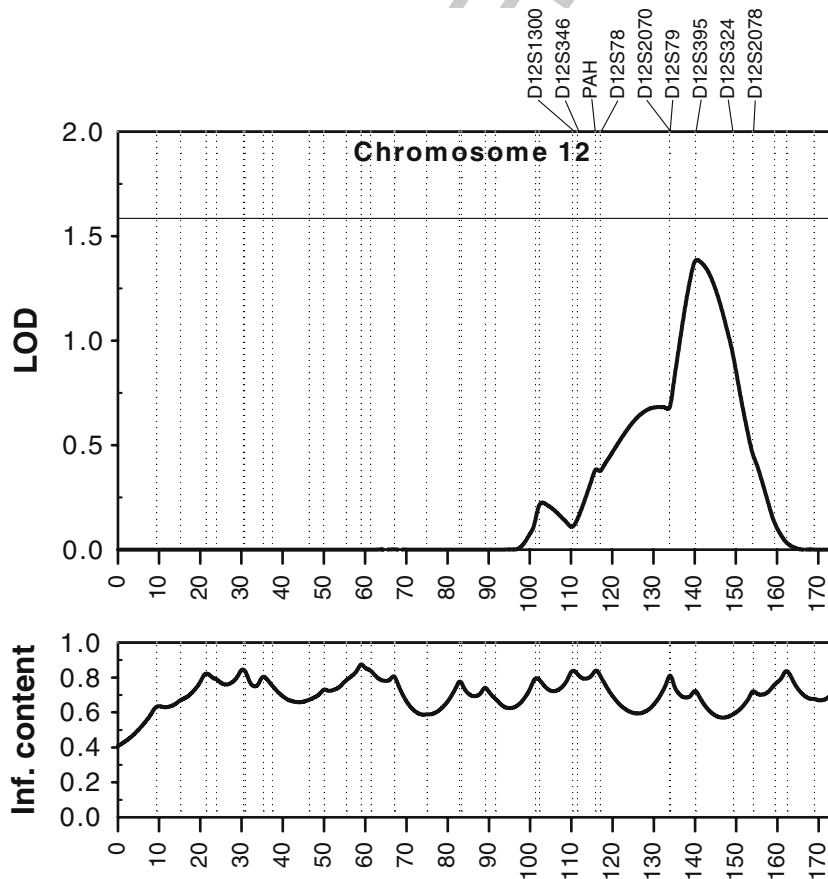


Fig. 2. Best evidence of linkage to loneliness on chromosome 12. The X-axis plots genetic distance (in cM Haldane), and the Y-axis the LOD-score. Markers are arrayed in map order along the top.

Table II. Overview of Linkage Findings for Chromosome 12q23-24

Study	Sample	N/Phenotype	Region
Green <i>et al.</i> , 2005	2 pedigrees UK; 45 markers in region of interest	Co-segregation of BPD and Darter's disease	12q23-24 (LOD = 4.77 between D12S1127 and D12S1646)
Curtis <i>et al.</i> , 2003	146 interviewed Ss from 2 UK and 5 Iceland families; 365 markers	39 Ss bipolar and 29 additional Ss unipolar	LOD = 2.8 at D12S342
Nash <i>et al.</i> , 2004	Selected sibling pairs a sample of 34,371 Ss UK; 408 marker genome-wide scan	711 Ss from 283 sibships selected on composite index G (e.g. anxiety, neuroticism)	LOD = 1.8 in 254 sister pairs (between markers D12S326 and D12S351) for G
Fullerton <i>et al.</i> , 2003	Selected sibling pairs from 20,427 independent sibships UK; 388 marker genome-wide scan	182 discordant, 205 low-low, 174 high-high sib pairs for Neuroticism	At 10 cM ($-\log zP$ value = 4.74 at D12S346)
Shink <i>et al.</i> , 2005a, b	485 (101 new) Ss/41 families from SLSJ area in Quebec; 20 microsat. marker scan in candidate region on chromosome 12	105 BPI / SezBP, 42 BPII, 54 RUMD 57 UMD	12q24.31: association with markers in this region
Shink <i>et al.</i> , 2005a, b	394 Ss/20 families (18 new) from SLSJ area in Quebec; 380 marker genome-wide scan	77 BPI, 28 BPII, 43 RUMD, 45 UMD, 21 AD, 34 ADA	12q21.2-12q24.31 (LOD = 3.35 at D12S378)
Morissette <i>et al.</i> , 1999	114 Ss/1 large pedigree from SLSJ area; 332 markers. replication 34 Ss/1 pedigree; 18 chromosome 12 markers	44 BPI, 3 SezBP, 6 BPII, 11 RUMD, 18 UMD	12q23-q24
Maziade <i>et al.</i> , 2005	480 Ss/21 families Eastern Quebec; 350 marker genome-wide scan; 257 markers for follow-up	81 broad SZ, 72 broad BP	For BP: 12q23.1 (Z = 3.53 at D12S1030)
Camp <i>et al.</i> , 2005	718 affected Ss/87 extended pedigrees Utah; 629 marker genome-wide scan	516 RUMD, 714 RUMD or AD, 99 RUMD and AD	For RUMD and AD at 89.6 cM (LOD = 1.37 at D12S1667)
Abkevich <i>et al.</i> , 2003	1890 Ss from 110 pedigrees Utah; 629 microsat. marker genome-wide scan; 33 markers for follow-up	162 BPD (62 M), 784 RUMD (238 M), 161 UMD (62 Male)	For depression in males 12q23.1 (HLOD = 4.6 at D12S1030; HLOD = 6.1 at D12S1706 at follow-up)
Zubenko <i>et al.</i> , 2003	835 Ss/81 families USA; 520 Ss genotyped for 389 marker genome-wide scan	RUMD, (major) mood disorder, depression spectrum disorder	12q23.1 (LOD = 1.9 at D12S393)

Wilcox <i>et al.</i> , 2002	136 Ss/51 families with SZ/Sez probands USA; 459 marker genome-wide scan	Quantitative assessment of Negative, Positive and disorganized symptoms	At 104 cM (LOD = 2.97 at D12S1300; LOD = 2.12 at 109.5 cM (PAH gene) 12q23 (Zmax = 2.0 at PAH gene at 109.5 cm)
Ekholm <i>et al.</i> , 2003	41 families Finland; 389 markers; additional markers for follow-up	101 BPI and Sez 36 BPI, BPII, Sez, BPD-NOS and RUMD	At 142.2 cM (NPL = 2.43 / LOD = 0.97 at D12S97)
Bailer <i>et al.</i> , 2002	86 Ss/8 families. Austria; 388 markers	5 SZ and 3 BPD families	At 148 cM (LOD = 3.42 at D12S1639)
Ewald <i>et al.</i> , 2002	Two extended pedigrees Denmark; 613 markers; additional markers for follow-up	BPD, mania, Sez	Single marker association at 145 cM (D12S342)
Degn <i>et al.</i> , 2001	14 patients isolate Faroe Islands; 17 markers in candidate region	BPI and BP _a	109 cM (Zmax = 2.60 at PAH gene)
Brzustowicz <i>et al.</i> , 2000	288 Ss/22 families Canada (22 Celtic, 1 German); 381 markers	On average 3.6 Ss per family with SZ and Sez	

SLSJ: Saguenay-Lac-St-Jean area

AD: anxiety disorder

ADA: alcohol/drug abuse

BPD: bipolar disorder

BP_a: bipolar affective disorder

BPD-NOS: Bipolar disorder-not otherwise specified

BPI: bipolar I

BPII: bipolar II

RUMD: recurrent unipolar major depression

Sez: schizoaffective disorder

SezBP: schizoaffective disorder, bipolar type

SZ: schizophrenia

UMD: single unipolar depression

Broad SZ: schizophrenia, schizophreniform and schizotypal personality disorder

Broad BP: BPI, BPII and RUMD

PAH: phenylalaine hydroxylase

HL0D: heterogeneity LOD score

LOD: logarithm of likelihood of linkage

NPL: nonparametric multipoint linkage score

UNCORRECTED PROOF

354 predictor of psychiatric problems. Heikkinen and
 355 Kauppinen (2004) reported that loneliness predicted
 356 changes in depressive symptoms in a 10-year study of
 357 elderly Finns and Cacioppo *et al.* (2005) found that
 358 loneliness predicted subsequent changes in depressive
 359 symptoms and that there was evidence for reciprocal
 360 influences over time. The results of the current study
 361 suggest that alleles in LD with an allele at microsate-
 362 llite marker D12S79 and at neighbouring marker
 363 D12S395 may contribute to individual differences in
 364 loneliness. Although additional research is required to
 365 determine their common phenotypic expression, it is
 366 possible that genes in this region may be involved in
 367 both affective and social regulation and dysregula-
 368 tion.
 369

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