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Combined Linkage and Association Analyses of the 124-bp Allele of Marker D2S2944 with Anxiety, Depression, Neuroticism and Major Depression

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A central issue in psychiatric genetics is whether positive findings replicate. Zubenko *et al.* (2002b, *Mol. Psychiatry* **7**:460–467) reported an association of the 124-bp allele of D2S2944 with recurrent early-onset major depression for females. We tested for association of this allele to continuous measures of anxiety, depression and neuroticism in a Dutch sample of 347 males and 448 females, and to DSM-IV major depression in a subsample of 210 males and 295 females. The association of the 124-bp allele to depression in females was not replicated, but there were significant associations (not significant after correction for multiple testing) with anxiety and anxious depression in males. However, the association occurred in the absence of evidence for linkage in this region on chromosome 2.

KEY WORDS: Anxiety; association analysis; linkage analysis; major depression; neuroticism; sex-specific effects.

Twin and family studies have repeatedly suggested that genetic factors contribute to the development of major depressive disorder (MDD) or to quantitative traits that reflect a liability to this disorder, such as anxiety, depression and neuroticism (Lake *et al.*, 2000; Sullivan *et al.*, 2000). With the advent of relatively affordable and fast methods for large scale genotyping, the next aim has become the identification of the chromosomal regions or loci involved in the development of depression via linkage or allelic association analyses. The results of studies employing such analyses have implicated different loci or regions, which is not uncommon in psychiatric genetics. In addition, results found in one sex do not always replicate in the other.

Sex differences and some ambiguity among results from association and linkage analyses were evident in several studies by Zubenko and colleagues. Zubenko *et al.* (2002c) used DNA pooling for low density genome wide association tests of 386 microsatellite markers to recurrent/early-onset MDD by comparing frequencies of multiple alleles between groups in a case–control design. Their affected sample consisted of 50 males and 50 females (mean age 32.8, minimum age 18) with ≥2 major depression episodes, the first of which occurred ≤25 years. The unaffected sample consisted of an equal number of males and females partially matched for age (mean age 37.6, minimum age 18), sex, race and ethnicity with no individual or family history of mental disorders. For 19 markers across the genome, evidence of association was obtained in the pooling study. After genotyping these markers in all individual

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subjects, the allele frequencies in the affected and unaffected samples were again compared in men and women separately. Statistically significant group differences were obtained for all 19 markers, but those differences were observed for nine markers only in females and for seven markers only in males. The 124-bp allele of the marker D2S2944 (at 210 cM) showed the strongest association with recurrent/early-onset MDD, but this association was female specific ($p = 0.003$).

In a subsequent study, Zubenko *et al.* (2002b) presented a more detailed comparison of the frequencies of each allele of D2S2944 in the same sample of cases and controls. For females, the D2S2944 124-bp allele was significantly more common in cases than controls (odds ratio 4.5, 95% CI 1.9–10.8). These results were supported by evidence from a within-family transmission disequilibrium test of the D2S2944 124-bp allele in another sample of 81 families previously identified through individuals with recurrent early-onset MDD (Zubenko *et al.*, 2001). This finding argues against population stratification as a trivial explanation of the association by linkage disequilibrium. For the same sample of families, Zubenko *et al.* (2003a) presented evidence of cosegregation of MDD in women with *CREB1* (located near D2S2208 at 205 cM). Philibert *et al.* (2003) replicated the association of the 124-bp allele with recurrent early-onset MDD in a comparatively small sample of females (78, 11 affected), although they did not employ some form of transmission disequilibrium testing procedure.

Several studies have used linkage analyses for RE-MDD or related phenotypes. Table I summarizes some of the results, emphasizing recurrent early-onset MDD or MDD and the region containing D2S2944 (Camp and Cannon-Albright, 2005, provide another summary and discussion of these and other findings). Zubenko *et al.* (2002a) reported results of a linkage analysis in the region from D2S1384 (200 cM) to D2S434 (215 cM), which included D2S2944 (210 cM). Evidence for linkage did not reach significance ($p < 0.05$) for recurrent early-onset MDD in females, although significant linkage was established for less severe forms of the illness, which were more prevalent in females. Evidence of linkage was not found for males.

The linkage study of Zubenko *et al.* (2003b) was genome wide. The results in their study were obtained with a model that included one or both of two covariates: sex of affected pairs and a dichotomized variable which encoded whether or not a family LOD score at D2S2208 was >0 in Zubenko *et al.* (2002a). The latter covariate substantially increased the LOD score. The effects were again female specific and occurred for recurrent MDD and less severe disorders, but not for recurrent early-onset MDD. The linkage studies of Holmans *et al.* (2004) and Abkevich *et al.* (2003) were genome wide. The results of Holmans

Table I. Summary of Findings of MDD Linkage Studies

Study	Sample	Phenotype[b]	Max LOD[c]	Chr 2 LODs[d]
Zubenko *et al.* (2002a)[a]	81 Families; 407 first, 835 extended relatives of RE-MDD probands	RE-MDD/F R-MDD/F MMD/F AMD/F	1.2 1.3 4 6.4 D2S2208/205 cM	0.7 0.9 2.4 4.7 D2S2944/210 cM
Zubenko *et al.* (2003b)	Same	R-MDD/F	8.19 D2S2321/205 cM	6.72 D2S2944/210 cM 6.63 D2S427/237 cM
Holmans *et al.* (2004)	297 Families; 415 indep. ASPs	RE-MDD/FM; MDD/FM	3.73 D15S652/103 cM	All LODs <0.55
Abkevich *et al.* (2003)[a]	110 Families; 1890 subjects; 945 (RE)-MDD	(RE)-MDD/M;	6.1 D12S1706/21 cM	All LODs <1.2

[a]LOD scores read from graph.
[b]MDD, major depression disorder; RE, recurrent early-onset; R, recurrent; MMD, major mood disorder; AMD, any mood disorder; F, female; M, male.
[c]Maximum LOD score in the study and its position.
[d]LOD scores on chromosome 2 and their positions.

et al. (2004) were not male specific, while Abkevich *et al*. (2003) reported evidence of linkage for males.

While Zubenko *et al*. (2002b, c) provided evidence for an association of the 124-bp allele of D2S2944 to recurrent/early-onset MDD, their linkage results on chromosome 2 (Zubenko *et al*., 2002a, 2003b) were obtained for disorders that were either less severe or more broadly defined than recurrent early-onset MDD. Furthermore, the best evidence for linkage was obtained at a location which would require linkage disequilibrium to extend a considerable distance from D2S2944. Therefore, the generality of the association of the 124-bp allele to depression and related disorders remains to be delineated. We tested the association of the 124-bp allele of D2S2944 with quantitative measures of anxiety, depression and neuroticism assessed by questionnaires in a Dutch sample of European ancestry [the comorbidity of DSM-IV MDD and neuroticism has been confirmed in our sample by Middeldorp *et al*. (2005)]. Although those tests are the primary focus, association with a clinical diagnosis for MDD (DSM-IV), which was available for a sub sample, was also investigated.

METHOD

Sample and Questionnaires

In 1991, the Netherlands Twin Register (NTR) started a longitudinal survey study of health and lifestyle. Questionnaires were sent out in 1991, 1993, 1995, 1997 and 2000 to adolescent and adult twins and their family members. Twin pairs were asked to participate in all waves, parents were asked to participate in 1991, 1993 and 1995, siblings were included since 1995 and spouses in 2000.

Depression was assessed by the anxious depression symptom scale of the Young Adult Self Report (YASR) (Achenbach, 1990; Verhulst *et al*., 1997) and by the Beck Depression Inventory (BDI) (Beck *et al*., 1961). Neuroticism was assessed by the Amsterdamse Biografische Vragenlijst (ABV) (Wilde, 1970), whose neuroticism scale is very similar to that of the Eysenck Personality Questionnaire (Eysenck *et al*., 1985). The Spielberger State Anxiety Inventory (STAI) (Spielberger *et al*., 1970) was used to measure anxiety. The specific occasions on which those variables were measured and other details of the study are provided by Boomsma *et al*. (2000, 2002).

Based on questionnaire data on anxiety, depression and neuroticism, a genetic factor score for each survey was composed which was used for an extreme discordant and concordant (EDAC) selection for genome wide marker genotyping for a quantitative trait locus (QTL) study of anxious depression (see Boomsma *et al*., 2000, for a detailed description). At each occasion from 1991 to 1997, subjects were classified as scoring in the upper or lower tail of the distributions of the genetic factor scores or as not scoring in one of the tails, the thresholds being defined by preset cumulative probabilities of the empirical distributions. If at least two subjects in a family were classified in one of the tails at any occasion (thus constituting an extreme concordant or discordant pair) the entire family was selected for genotyping. A total of 2724 participants were selected. The resulting distributions at each occasion had heavier tails than the original distributions but also contained a substantial number of subjects towards the centers of the distributions.

The 2724 participants selected for the QTL study were asked to provide a buccal swab for DNA isolation. Of the 1962 participants (72%) who returned a buccal swab, 917 (624 offspring and 293 parents) were selected for genotyping over the entire genome, including D2S2944. These subjects, who were of European ancestry, were mostly selected because they came from larger participating families. An additional 337 twins from DZ pairs who had participated in earlier studies on cardiovascular risk factors (Boomsma *et al*., 1993; Snieder *et al*., 1997) were also genotyped.

D2S2944 was successfully genotyped in 273 parents (one parent in 51 families, two parents in 111 families) and 876 offspring from 374 families. Since the subjects in the studies by Zubenko *et al*. (2002b, c) were $\geqslant$18 years of age, offspring $<$18 years of age at the time of phenotyping were excluded from the association analyses. The remaining sample consisted of 273 parents and 795 offspring (347 males and 448 females). The numbers of offspring with a D2S2944 genotype are slightly different for each quantitative phenotype and are presented in Table IV, which contains results of association analyses.

The 2724 offspring originally selected for genotyping were asked if they agreed to being interviewed on the telephone for administration of the WHO-CIDI (Wittchen, 1994; World Health Organization, 1992). The interview was standardized through a computer program (Peters and Andrews, 1995). An automated scoring procedure was used to obtain diagnoses for a number of lifetime mental disorders, which included a lifetime diagnosis of DSM-IV MDD. Our standardized interview and automated

scoring procedure do not allow for a reliable estimate of the number of recurrences, only for whether the depressive episodes have been single or recurrent. 505 subjects (210 males and 295 females) with a valid genotype for D2S2944 were successfully interviewed to obtain a MDD diagnosis.

Genotyping and Error Checking

Genotyping was conducted by the Mammalian genotyping service (Marshfield Laboratory, USA) for 379 autosomal markers (including D2S2944) from the 10 cM spaced micro satellite screening set 10 (Yuan *et al*., 1997). DZ twin pairs from the cardiovascular studies were genotyped over the entire genome using a partly overlapping set of markers (including D2S2944) consisting of a combination of in-house markers [ALFexpress automated sequencer (Amersham Pharmacia Biotech)] as described by Beekman *et al*. (2003), markers from the Weber screening set 8 (ALFexpress) and from the Human Linkage Set V2.5 MD10 en HD5 [ABI Prism DNA Analyzer 3700 (Applied Biosystems)].

Pedigrees were checked for Mendelian errors with the program Unknown (Schaffer, 1996) and pedigree relationships in the entire sample with the GRR program (Abecasis *et al*., 2001). Mendelian errors were removed by assigning missing values to the marker genotypes if the errors appeared incidental. Likelihoods for recombinations were checked using the program Merlin (Abecasis *et al*., 2002). One unlikely recombination was observed at the marker D2S2944 and the genotype was assigned a missing value.

IBD Estimation

Marker distances were assigned from the Decode map (Kong *et al*., 2002) if available. For markers not mapped by Decode, the original distance provided on the Marshfield website (Broman *et al*., 1998) was transformed by interpolation from adjacent markers with known Decode map values. The program Merlin (Abecasis *et al*., 2002) was used for a multipoint IBD estimation using all available markers. For 27 offspring, two of which came from one family, at least 13 markers but not D2S2944 were successfully genotyped. Offspring for which D2S2944 was not successfully genotyped can still be useful for IBD estimation of offspring pairs with a D2S2944 genotype. If a minimum of 13 genotyped markers were available (giving an average spacing of approximately 20 cM) offspring without a D2S2944 genotype were included in the IBD estimation. These subjects were used only in the IBD estimation, not in the association analyses.

Analyses

The 124-bp allele of D2S2944 was tested for association with anxiety, depression (YASR and Beck depression), neuroticism and the DSM-IV MDD diagnosis. The quantitative scores were transformed by the Van der Waerden transformation (Lehman, 1975). This transformation makes the scores more normally distributed. For each variable two scores were constructed: the average score over all occasions and the score on the last occasion at which the subject participated.

A TDT statistics was used to test the association of D2S2944 with discrete (Abecasis *et al*., 2000b) and quantitative phenotypes (Abecasis *et al*., 2000a). The latter is a generalization of a model proposed by Fulker *et al*. (1999) and produces a TDT for quantitative variables. The quantitative model and the TDT test statistic for discrete variables are implemented in the program QTDT. In the quantitative model the association effect is partitioned into a between and within family contribution. For subject j in family i, g_{ij} is defined as the number of 124-bp alleles minus 1. The between family effect (b) is modeled as the regression parameter β_b associated with b_i, the expected value of g_{ij} in family i. If genotypes for both parents are available, this expectation equals the average of g_{ij} in both parents. If genotypes for both parents are not available, this expectation is estimated as the average of g_{ij} in all the offspring. The within family contribution (w) is modeled as the regression parameter β_w associated with $w_{ij} = g_{ij} - b_i$. Abecasis *et al*. (2000a) show that the value of β_b represents two effects: the additive genetic effect of a locus in linkage disequilibrium with the marker and effects of stratification and admixture, when these latter effects exist. The value of β_w represents the additive genetic effect only. Families whose offspring all have g_{ij} identical to the expected value b_i or its estimate can contribute only to the estimation of β_b and not to the estimation of β_w. For example, families with two homozygous parents or without genotyped parents and only one offspring cannot be informative for association as tested by the null hypothesis $\beta_w = 0$ against the alternative $\beta_w \neq 0$. The within family effect can be dropped from the model if $\beta_b = \beta_w$,

which will usually result in a larger number of subjects that are informative for testing the association.

The phenotypic variance conditional on the general mean and regressors is modeled as $\sigma_a^2+\sigma_p^2+\sigma_e^2$, where *a* refers to the additive effect of a gene linked to the marker but not necessarily in linkage disequilibrium, and *p* and *e* refer to polygenic and residual effects. The within family phenotypic covariances of offspring *j* and *k* in family *i* are modeled as $\pi_{ijk}\sigma_a^2+\delta\sigma_p^2$, where π_{ijk} is the proportion of alleles shared identical by descent (IBD) and δ is 1 for monozygotic twins and 0.5 for other offspring pairs. Previous analyses and analyses of the present data have shown that there is no need to include a common environment variance component. Since our sample is not random, δ may deviate from 0.5, but we have generally observed only minor deviations from 0.5 for our genotyped markers, possibly because both concordant and discordant pairs as well as their family members were selected for genotyping. Moreover, simulations conducted by Abecasis *et al.* (2000a) suggest that the test for a nonzero β_w can be robust against misspecification of the covariances.

RESULTS

A comparison of the allele distributions for parents and offspring from the current study, including only one MZ twin of a pair, to those published in the Zubenko study is presented in Table II. Our sample contains two alleles, the 100-bp and 132-bp allele, not present in the Zubenko study. The frequency of the 124-bp allele in the cases of the Zubenko study is considerably higher than in our sample, while the frequency in the controls is lower.

The null hypotheses of independence of sex and the allele distributions were not rejected for mothers and fathers and for male and female offspring ($\chi^2=8.56$, df = 7, $p=0.28$ for the parents; $\chi^2=11.71$, df = 8, $p=0.16$ for the offspring; the largest deviations from independence occurring for rare alleles). Note, however, that the test for offspring does not take the dependence due to the family structure into account. Since the number of possible genotypes is 45 and some of the alleles are rare, a formal test for Hardy–Weinberg equilibrium was not applied.

Table III contains the distributions of the number of the 124-bp alleles for parents and offspring. The null hypotheses of independence of sex and genotype were not rejected ($\chi^2=2.17$, df = 2, $p=0.34$ for the parents; $\chi^2=4.34$, df = 2, $p=0.11$ for the offspring). Likewise the null hypothesis of HW-equilibrium applied after this dichotomization of the D2S2944 alleles was not rejected ($\chi^2=0.001$, df = 1, $p=0.97$ for the parents; $\chi^2=0.88$, df = 1, $p=0.53$ for the offspring).

Table II. Comparison of Allele Distributions for Parents and Offspring from Current Study to Zubenko Study

Frequencies and percentages of alleles for parents and offspring in the current study										
Alleles	100	104	108	112	116	120	**124**	128	132	Row total
Parents										
Males	0	1	21	53	61	61	**43**	17	3	260
	0.0	0.4	8.1	20.4	23.5	23.5	**16.5**	6.5	1.2	
Females	0	0	17	52	63	78	**61**	15	0	286
	0.0	0.0	5.9	18.2	22.0	27.3	**21.3**	5.2	0.0	
Allele	0	1	38	105	124	139	**104**	32	3	546
Total	0.0	0.2	7.0	19.2	22.7	25.5	**19.0**	5.9	0.5	
Offspring										
Males	1	0	62	153	189	209	**122**	53	3	792
	0.1	0.0	7.8	19.3	23.9	26.4	**15.4**	6.7	0.4	
Females	1	2	64	169	237	245	**180**	50	12	960
	0.1	0.2	6.7	17.6	24.7	25.5	**18.8**	5.2	1.3	
Allele	2	2	126	322	426	454	**302**	103	15	1752
Total	0.1	0.1	7.2	18.4	24.3	25.9	**17.2**	5.9	0.9	
Frequencies and percentages of alleles for females cases and controls in the study of Zubenko *et al.* (2002b)										
Cases	0	1	11	14	20	14	**32**	8	0	100
	0.0	1.0	11.0	14.0	20.0	14.0	**32.0**	8.0	0.0	
Controls	0	0	8	16	35	23	**11**	7	0	100
	0.0	0.0	8.0	16.0	35.0	23.0	**11.0**	7.0	0.0	
Allele	0	1	19	30	55	37	**43**	15	0	200
Total	0.0	1.0	10.0	15.0	28.0	19.0	**22.0**	8.0	0.0	

Table III. Frequencies and Percentages of Number of 124-bp Alleles of Individuals Separately for Male and Female Parents and Offspring

	Parents			Offspring		
Number of 124-bp alleles	0	1	2	0	1	2
Males	91	35	4	287	96	13
	0.70	0.27	0.03	0.73	0.24	0.03
Females	88	49	6	317	146	17
	0.62	0.34	0.04	0.66	0.30	0.04
Total	179	84	10	604	242	30
	0.65	0.31	0.04	0.69	0.28	0.03

Association Analyses

Association analyses were conducted for mean scores over repeated occasions and for scores obtained on the last survey in which subjects participated. Since the results were very similar, we focus on the mean score. The mean ages over occasions for males and females were 33.3 (s.d. 13.0) and 32.3 (s.d. 12.4), respectively.

The Figures 1 and 2 contain plots of the LOD scores from a variance components linkage analysis for chromosome 2 for males and females. The evidence for linkage at the marker was small in both sexes.

Association with the 124-bp allele was tested for mean anxiety, depression (YASR and Beck scales), neuroticism and for MDD. Table IV contains results for the quantitative variables. Age was included as a covariate in all models, but its effect was negligible. The variance σ^2_{a0} of the linked genetic effect(s) was never statistically significant at the 0.05 level. Some evidence for association is apparent for anxiety and YASR anxious depression for males ($p = 0.05$ for YASR on the last occasion), but it occurs in the absence of evidence for linkage. The justification for the approximate validity of the tests in Table IV is based on the applicability of asymptotic theory and its assumptions. QTDT includes permutation tests to calculate exact p-values under models of fewer assumptions. The permuted p-values for males were 0.04 for anxiety and 0.08 for YASR anxious depression (1000 permutations). The Bonferroni adjustment for males and females separately would require a 0.0125 significance level for an overall 0.05 type-I error rate. Since the variables are correlated, use of this critical value is rather conservative and therefore does affect the sensitivity of the tests.

The Marshfield Laboratory determined allele sizes relative to the sizes of CEPH family 1331.

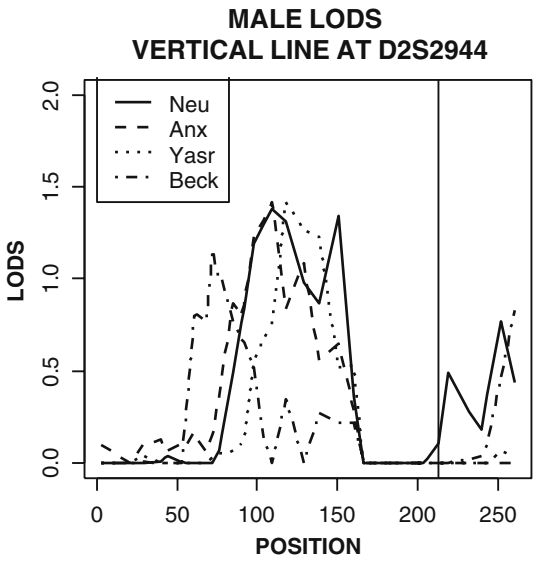

Fig. 1. Male LODs vertical line at D2S2944.

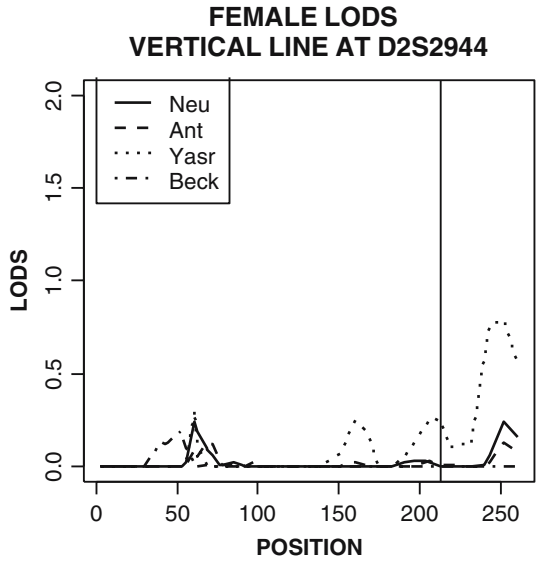

Fig. 2. Female LODs vertical line at D2S2944.

Nevertheless, our 124-bp allele may not correspond to the 124-bp allele assessed in other studies. In that case, effects may occur for the 120-bp or 128-bp alleles. However, if some evidence of an association effect is obtained, the effect of the 124-bp allele in our sample often is also the strongest effect. The 120-bp or 128-bp alleles do not show comparable results.

The association can be estimated from a larger number of subjects if the regression parameters of the between and within effects are equal, implying no stratification effect. For males, the null hypothesis of equality was not rejected for any variable, the smallest *p*-value being 0.64. For females, *p*-values for neuroticism were 0.21. For the other variables the *p*-values were ≥0.35. This test can only be rejected from those observations that are informative under the full model. Therefore accepting the model under the null hypothesis as approximately valid perhaps requires a larger leap of faith than usual. Table IV nevertheless also contains estimates, subscripted by *t*, from the model without within family effect. The results do not change substantially.

MDD was dichotomized as none versus single or recurrent and as none versus recurrent. Both twins from MZ pairs were once included and once excluded in the analysis, and subjects with no MDD but another diagnosis were once included and once excluded in the controls. For males and also for the dichotomization none versus recurrent no sufficient information remained for a sensible within family test. For females and males and females combined the minimum *p*-values from the permutation tests were 0.54 and 0.25, respectively.

The joint distribution of the zygosity of 124-bp alleles and MDD is presented in Table V for males and females. Treating all observations as independent, the *p*-values of the exact test for independence were 0.37 and 0.20 for males and females. The *p*-values were 0.35 and 0.20 after removal of subjects with no MDD but with another DSM-IV diagnosis.

DISCUSSION

In our sample we found only weak evidence of association of the D2S2944 124-bp allele with

Table IV. Results of Association of the 124-bp Allele of D2S2944 with Neuroticism, Anxiety, YASR Depression and Beck Depression

	Males				Females			
	Neu	Anx	YASR	Beck	Neu	Anx	YASR	Beck
σ^2_{e0}	0.61	0.34	0.43	0.31	0.57	0.49	0.43	0.36
σ^2_{p0}	0.19	0.56	0.27	0.35	0.57	0.50	0.31	0.52
σ^2_{a0}	0.19	0.00	0.00	0.00	0.00	0.06	0.23	0.00
σ^2_e	0.63	0.37	0.41	0.32	0.56	0.48	0.42	0.35
σ^2_p	0.16	0.51	0.27	0.34	0.58	0.52	0.3	0.52
σ^2_a	0.19	0.00	0.00	0.00	0.00	0.05	0.22	0.00
β_b	0.18	0.16	0.20	0.03	−0.08	−0.01	0.01	−0.04
$\boldsymbol{\beta_w}$	**0.17**	**0.34**	**0.27**	**0.07**	**0.16**	**0.17**	**0.12**	**0.10**
χ^2	1.0	4.81	3.38	0.22	1.46	1.68	0.83	0.56
Prob	**0.32**	**0.03**	**0.07**	**0.64**	**0.23**	**0.19**	**0.36**	**0.45**
I	124	124	115	112	190	191	186	168
σ^2_{et}	0.63	0.36	0.41	0.31	0.57	0.48	0.42	0.35
σ^2_{pt}	0.16	0.53	0.27	0.35	0.57	0.53	0.33	0.52
σ^2_{at}	0.19	0.00	0.00	0.00	0.00	0.05	0.22	0.00
$\boldsymbol{\beta_t}$	**0.18**	**0.23**	**0.22**	**0.04**	**0.04**	**0.08**	**0.06**	**0.03**
χ^2_t	2.85	5.38	6.01	0.21	0.20	0.76	0.47	0.12
Prob$_t$	**0.09**	**0.02**	**0.01**	**0.65**	**0.65**	**0.38**	**0.49**	**0.73**
N	345	347	319	311	448	448	424	394

σ^2: variance components.

Subscripts: *e*, *p* and *a* refer to error, polygenic and QTL effects; b and w to between and within family effects; 0 and *t* to the model without association effect and to the model without within family effect.

I: number of informative probands for testing the hypothesis $\beta_w \neq 0$; *N*: total number of probands; χ^2 : −2 times the log likelihood ratio for the test $\beta_w = 0$; Prob: the probability associated with the chi-squared approximation to the test statistic; χ^2_t: −2 times the log likelihood ratio for the test $\beta_w = \beta_b$; Prob$_t$: the probability associated with the chi-squared approximation to the test statistic.

Table V. Frequencies and Percentages of Number of 124-bp Alleles of Males and Females with a MDD Diagnosis

	Males			Females		
	Number of alleles			Number of alleles		
	0	1	2	0	1	2
MDD diagnosis						
None	142	47	5	155	66	9
	73.2	24.2	2.6	67.4	28.7	3.9
Single	11	2	0	21	18	1
	84.6	15.4	0.0	52.5	45.0	2.5
Recurrent	1	2	0	19	5	1
	33.3	66.7	0.0	76.0	20.0	4.0

anxiety, depression or neuroticism. Our strongest evidence for association occurs in males for anxiety and anxious depression. Those effects occur in the absence of evidence for linkage, although the association cannot be spurious due to stratification effects because the association is partitioned in between and within family contributions. Zubenko *et al.* (2002b) reported association for recurrent early-onset MDD in females only. Although we did not find any association for females, some evidence for linkage in this region was found for anxious depression as judged by the estimates of the variances.

Our failure to replicate the specific association of the 124-bp allele to female depression is in accordance with the diversity of results from linkage studies involving MDD and related phenotypes across different nonrandom samples (e.g. Abkevich *et al.*, 2003; Holmans *et al.*, 2004; Zubenko *et al.*, 2003b). Because of variation in populations, the criteria for selection of subjects and choice of phenotypes evidently can produce different results, one conclusion could be that this region is not involved in the development of MDD and related quantitative traits. Alternatively, our study may have lacked power to detect the association.

Insufficient power may have arisen through a number of factors, such as too small a sample or too few extreme subjects. The simulations conducted by Abecasis *et al.* (2000a) suggest, that the power for our numbers of informative females is only 60–80% at $\alpha = 0.05$ for a marker accounting for $\sim$5% of the variance.

An indication of the frequency of extreme subjects as compared with those in the studies of Zubenko *et al.* (2002b, c) can be obtained from our sub sample of subjects who were interviewed for a DSM-IV MDD diagnosis. In the studies by Zubenko *et al.* (2002b, c), the subjects were probands or selected through probands. The age at study of the affected subjects is similar to the age of our affected subjects, but their controls are approximately eight years older than our unaffected subjects, which may imply that our presently unaffected subjects are at a higher risk of developing a MDD in the years to come. Other age characteristics of our affected subjects, such as the age of onset, suggest that our selection is somewhat broader, but that the extremes in our selection are probably not too different from those in the studies by Zubenko *et al.* (2002b, c).

In conclusion, we have failed to detect a robust association of the 124-bp allele of marker D2S2944 with MDD or anxiety, depression, and neuroticism. Hence, its reported effects on recurrent early-onset MDD in females (Zubenko *et al.*, 2002b) do not seem to generalize to these quantitative risk factors.

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