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Netherlands twin family study of anxious depression (NETSAD)

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In a longitudinal study of Dutch adolescent and young adult twins, their parents and their siblings, questionnaire data were collected on depression, anxiety and correlated personality traits, such as neuroticism. Data were collected by mailed surveys in 1991, 1993, 1995 and 1997. A total of 13 717 individuals from 3344 families were included in the study. To localise quantitative trait loci (QTLs) involved in anxiety and depression, the survey data were used to select the most informative families for a genome-wide search. For each individual a genetic factor score was computed, based on a genetic multivariate analysis of anxiety, depression, neuroticism and somatic anxiety. A family was selected if at least two siblings (or DZ twins) had extreme factor scores. Both discordant (high-low) and concordant (high-high and low-low) pairs were included in the selected sample. Once an extreme sibling pair was selected, all family members (parents and additional siblings of the selected pair) who had at least once returned a questionnaire booklet were asked to provide a DNA sample. In total, 2724 individuals from 563 families (1007 parents and 1717 offspring) were approached and 1975 individuals from 479 families (643 patients and 1332 offspring) complied by returning a buccal swab for DNA isolation. All offspring from selected families were asked to participate in a psychiatric interview and in a 24-hour ambulatory assessment of cardiovascular parameters and cortisol. The interview consisted of the WHO-Composite International Diagnostic Interview and was administered to 1253 offspring. In this paper we describe the genetic–epidemiological analyses of the survey data on anxiety, somatic anxiety, neuroticism and depression. We detail how these data were used to select families for the QTL study and discuss strategies that may help elucidate the molecular pathways leading from genes to anxious depression. Twin Research (2000) 3, 323–334.

Keywords: anxious depressed, neuroticism, CIDI, twin-sibling study, heritability, genetic factor scores, selection, QTL finding

Introduction

There is extensive evidence that a common gene, or a set of genes, underlies much of the genetic variation in anxiety and depression in humans.[1–4] For an analogous trait in mice – emotionality – several quantitative trait loci (QTLs) have been located on mouse chromosomes 1, 10, 12 and 15[5–10] and mapped to a 0.8 cM region on mouse chromosome 1[11] and a 1.4–3.2 cM region on mouse chromosome 15.[12] These findings in the mouse can be used as a starting point to search for the genes that underlie the genetic susceptibility for anxious depression in humans through linkage and association studies. Genetic association studies can be carried out with positional or functional candidate genes identified in the mouse. Likewise, linkage studies in humans may be carried out with positional markers from syntenic regions in the mouse. Because of the structural and functional homologies between human and animal genomes, and because pathways are often highly conserved, syntenic regions from animal research are primary candidates to be tested in human studies.[13–15]

In this paper we describe a large study of anxious depression in Dutch twin families that has been carried out since 1991. First, a summary is given of the genetic–epidemiological analyses of the twin family data on anxiety, neuroticism, somatic anxiety and depression. Next, we outline the selection strategies that were used to obtain a sub-sample of twin families who are most informative for linkage and association studies to localise the genes that underlie the susceptibility to anxious depression. Information on the number of individuals currently available for gene-finding is provided. Finally, strategies are discussed that may help in elucidating the

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molecular pathways leading from DNA to depression, once a gene has been found.

Linkage approaches that have been developed to map quantitative trait loci for complex traits in humans are often based on identification of marker alleles that are inherited identical-by-descent (IBD) in siblings. These methods suppose that if a DNA marker is cosegregating with a (quantitative) trait, then siblings whose trait values are more similar, are more likely to receive the same alleles identical-by-descent at a closely linked marker locus than siblings who resemble each other less for the trait.[16] However, even with the large numbers of highly polymorphic markers that are currently available, the power to detect loci that influence complex traits in humans is low.[17]

Because of the low power to detect QTLs in humans, we propose to use a combination of strategies for the collection and analysis of data to attain a higher power. Rather than assessing anxious depression as a dichotomy (ie affected/unaffected), indices of anxious depression and associated personality traits such as neuroticism are measured on quantitative scales. These correlated phenotypes are analysed with multivariate genetic models to establish to what extent they are influenced by a common set of genes. Based on these results, the data are summarised into a genetic factor score for each subject, which gives an estimate of an individual's genotypic value for anxious depression. To select families for genotyping, the distribution of genetic factor scores is used to identify sibling pairs with extreme scores from both tails of the distribution. These sibling pairs, their parents and their additional siblings are genotyped to obtain an estimate of IBD status among the offspring.[18] The complete distribution of phenotypic and genotypic data within selected and unselected sibships is then analysed using a structural equation modelling approach.[19–21] When available, longitudinal data are used to carry out the linkage analyses.

Eaves and Meyer[22] and Risch and Zhang[23] have recommended the selection of sib pairs for genotyping who score extreme (high/high, low/low, low/high or high/low) on a quantitative scale of interest. We have carried out extensive simulation studies[24] to derive optimal selection percentages for linkage analysis of a QTL in sib pairs from random samples. From these simulations, the optimal criteria were to select concordant sib pairs whose members both have scores in the top 12% or in the bottom 12% of the phenotypic distribution, and discordant sib pairs whose members have scores in the top 20% and the bottom 20% of the phenotypic distribution. Simulations suggested an 'asymmetrical' criterion for discordant sibling pairs (high scoring siblings from the upper 25%, with low scoring siblings from the lower 20% of the distribution or vice versa). In Figure 1 the selection rules for our study are depicted graphically, based on a hypothetical bi-allelic, co-dominant QTL that explains 10% of the phenotypic variance; the residual background correlation between siblings is 0.25. Numbers in the plot represent the proportion (π) of alleles shared identical-by-descent (IBD/2) by siblings. Very discordant sib pairs have π values between 0.404 and 0.493; highly concordant low scoring sib pairs have π values between 0.511 and 0.538. Figure 1 shows the changes in π as a function of the phenotypic distributions of two siblings. The length of the arrows indicates the gradient of the change in π. The contour lines are lines of equal π. The grey areas represent the areas of phenotypic distribution from which sib pairs were selected.

If the selected sib pairs with extreme scores had additional siblings with complete phenotypic information, these siblings were included in the QTL study. Larger sibships provide dramatic increases in power over size 2 sibships. Simulation studies[25] indicate that, even in unselected families, a size 3 sibship is on average three times, and a size 4 sibship six to seven times, as informative as a size 2 sibship.

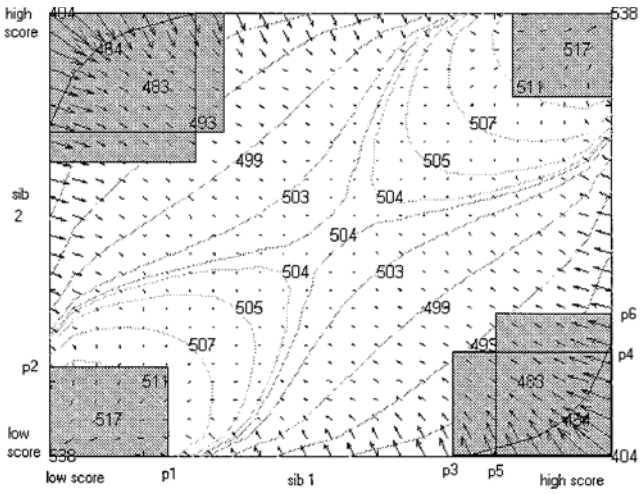

Figure 1 Plot showing the changes in π (the proportion of marker alleles shared IBD) as a function of sib 1 – sib 2 phenotypic scores, based on a codominant bi-allelic QTL that explains 10% of the phenotypic variance. The background correlation in siblings is 0.25 and the QTL allele frequency is 0.5. The length of the arrows indicates the gradient of the change in π. The contour lines, drawn at arbitrary intervals, are lines of equal π. The dark grey areas represent the parts of the distribution from which sibpairs were selected. These areas depend on the selection percentages p1 to p6. In the present study p1 = p2 = 0.12 (concordant low-low), p3 = 0.75, p5 = 0.80, p4 = 0.20, and p6 = 0.25 (discordant low-high). Selection percentages for concordant high-high sibpairs were p1 = p2 = 0.88 (i.e., 1–0.12). Selection percentages for discordant high-low sibpairs were p3 = 0.25, (1–0.75), etc.

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Selection of extreme sib pairs was based on the individual genetic factor scores, because they optimally combine multiple trait information from genetically correlated phenotypes. The procedure to construct these factor scores has been described in detail[26] and the application in linkage analyses has been shown to result in appreciable increases in power.[27–29].

Subjects

Families of adolescent and young adult twins were recruited in 1990/1991 by asking city councils in The Netherlands for addresses of twins aged 13–22 years. There were 252 city councils which supplied 4036 addresses. In 1993 additional addresses were obtained for 1987 twin families. The new addresses included several of the larger cities in The Netherlands. In addition, a number of (mostly adult) twin pairs volunteered throughout the study period for registration with the Netherlands Twin Register (NTR) and were included in the 1997 and 2000 surveys.

Questionnaires on health and lifestyle were sent in 1991 to 2375 families (out of 4036) who had indicated that they were willing to participate in a survey study. Twins and both their parents received a 22-page booklet with personality and psychopathology inventories, and questions about health, demographic background and lifestyle. Completed questionnaires were obtained from 6529 subjects from 1697 families. There were 1471 complete families (father, mother and both twins), 167 families consisting of mother and both twins, 26 families consisting of father and both twins, and 33 families in which only the twin pair returned a questionnaire.

A second booklet (18 pages) was sent in 1993 to 6023 families (including the 1987 new addresses and including all families who did not respond to the first request). Completed questionnaires were obtained from 7592 individuals from 1974 families; 959 families participated for a second time; 877 families came from the new addresses; 138 families had also been contacted before in 1991 but had not responded at the time. The number of complete families was 1727 (both parents and both twins), 200 families consisted of one parent and both children (176 mothers, 24 fathers), in the remaining 47 families there were 14 twin pairs and 33 other combinations of parents and offspring.

For the third wave of data collection, questionnaires (12 pages) were sent by the end of March 1995 to the 2712 families who had participated in the first and/or second waves. This time the data collection included two questionnaires per family for siblings of the twins, if present. Questionnaires were returned by 8175 subjects from 1727 families (3408 twins, 1500 siblings, 1577 fathers and 1690 mothers).

For the fourth wave all families in the NTR with twins aged 12 years or older were initially approached with a request to take part in the 1997 survey, even if they had not complied on previous occasions. Parents were asked how many additional siblings of twins would be willing to fill out a questionnaire and to provide the names and addresses of the siblings. Of the families that were approached, 2773 supplied a form with twin/sib information and these offspring were sent a 20-page questionnaire. A total of 7989 subjects (5546 twins and 2443 additional siblings), but not their parents, was included in the study on this occasion. For the first time, questionnaires were not sent to the parental address for all participants, but to individual twins and siblings. This resulted in a much larger number of twins who returned the questionnaire independently of their co-twin. A completed questionnaire was received from 4585 individuals from 1965 families (3141 twins and 1444 siblings). There were 530 questionnaires from single twins. The number of participating siblings per family varied between 0 and 8. In 785 families one additional sibling, in 199 families two additional sibs and in 69 families three or more sibs sent back the survey.

A fifth (18-page) questionnaire was distributed in May 2000 to 22 374 individuals (13 723 twins, 105 triplets, 2917 sibs and 5629 spouses/partners of twins over the age of 25) from 6914 families.

Selection of extreme sibling pairs for inclusion in the QTL study was based on the survey data collected in 1991, 1993 and 1997. In 1995 the YASR was included only in the survey of twins and not of their siblings. The fifth-wave data collection has just begun. Although the twin data collected in 1995 and 2000 could not be used for selection purposes, they will be used in the proposed linkage and association analyses, as the longitudinal information greatly enhances statistical power for QTL detection.

Instruments

Each survey collected abundant information on lifestyle, including smoking and exercise status, alcohol use and abuse,[30,31] health, demographics, Socio-Economic Status, religion,[32] personality and psychopathology. Table 1 lists the measures of personality and psychopathology that were collected in each of the five surveys.

The 13-item version of the Beck Depression Inventory (BDI)[33] and the anxious/depression symptom scale of the Young Adult Self Report (YASR)[34]

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Table 1 Inventories used to assess personality and psychopathology in twin families in NETSAD

	I	II	III	IV	V
Beck Depression Inventory	–	x	–	x	–
ABV: Neuroticism	x	x	–	x	x
ABV: Extraversion	x	x	–	x	x
ABV: Somatic anxiety	x	x	–	x	x
ABV: Test attitude (lie scale)	x	x	–	x	x
Spielberger Trait Anxiety	x	x	–	x	x
Spielberger Trait Anger	x	x	–	–	–
Zuckerman Sensation Seeking (Boredom Susceptibility, Disinhibition, Experience and Thrill and Adventure Seeking)	x	x	–	x	x
Jenkins Activity Survey (Type-A behaviour)	x	–	–	–	–
Cognitive Failure Questionnaire	x	–	–	–	–
Phobia	–	–	–	x	x
YASR: Young Adult Self Report (Anxious/Depressed, Somatic compaints Withdrawn, Delinquent and Aggressive behaviour, Social-, Thought-, and Attention problems)	x	–	x	x	x
Burn-out	–	–	–	–	x
Traumatic life events	–	–	–	–	x

I = 1991: twins and parents; II = 1993: twins and parents; III = 1995: twins, parents and siblings (YASR only for twins); IV = 1997: twins and siblings; V = 2000: twins, partners and siblings.

were used to assess depression. The YASR consists of seven additional scales, which measure different syndromes. Neuroticism, somatic anxiety, extraversion, and test attitude are part of the Amsterdamse Biografische Vragenlijst (ABV).[35] The item content of the ABV neuroticism and extraversion scales is very similar to that of the Eysenck Personality Questionnaire. Dutch translations of the Cognitive Failures Questionnaire (CFQ),[36] the Jenkins Activity Survey (JAS),[37] the Spielberger State Anxiety Inventory (STAI)[38] and the Trait Anger scale[39] were used to obtain measures of everyday cognitive failures and type-A behaviour, which is thought to reflect coronary-prone behaviour, anxiety and anger/hostility. The four dimensions of Sensation-Seeking behaviour were measured with the Zuckerman[40] Sensation Seeking scales.

Statistical analyses

Principal Components Analysis (PCA) of the combined 1991 and 1993 data on YASR and BDI depression, anxiety, anger, type-A behaviour, neuroticism, extraversion, somatic anxiety, test attitude and the Sensation Seeking scales established three major components in the data, which together explained around 55% of the variance. The first component, which explained 29% of the variance, was characterised by high loadings of anxiety, neuroticism, somatic anxiety and both depression scales. The second component (17% of the variance) consisted mainly of the four Sensation Seeking scales and the third component (9% of the variance) of type-A behaviour, extraversion and trait anger. PCA of the data collected in 1997 almost exactly replicated these results. Three components were found that explained 65% of the variance. Variables loading on the first component (which explained 34% of the variance) again were anxiety, neuroticism, somatic anxiety and YASR and BDI depression. These phenotypes were used to select families for the QTL study and are described in this paper.

Genetic modelling

In order to establish the genetic architecture of the variables that clustered together phenotypically, multivariate genetic models were fitted to the twin data collected in 1991, 1993 and 1997. First, a full model (Choleski decomposition[41]) which specified additive genetic (A), common environmental (C), and unique environmental (E) sources of variation and covariation was evaluated. This model was fitted to the data with and without sex differences in the relative contributions of the genetic and environmental influences. Next, the significance of genetic and common environmental influences in explaining family resemblance was tested, by constraining each of these influences to a zero contribution to family resemblance. Finally, the dimensionality of the genetic and environmental influences was explored by fitting a one-factor structure to genetic and environmental influences. These models test whether a common set of genes (or environments) influence all traits. Model-fitting was carried out in Mx, using maximum likelihood estimation.[42] Testing of submodels was done by likelihood-ratio tests, by

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subtracting the chi-square for the more restricted model from the chi-square for the more general model.

Factor scores

The results from the multivariate genetic analysis were used to compute a genetic factor score for each subject in the study, which represents the individual's value on the common genetic factor and can be interpreted as an estimate of an individual's genotypic value for anxious depression. For each individual a genetic factor score (F) was obtained according to:

$$F = B'P,$$

where ' denotes transpose, B is a ($p \times 1$) vector of weights that is constant across subjects and depends on the loadings of the variables on the genetic factor and on their unique genetic variances. P ($p \times 1$) is the vector of phenotypes of the individual (p is the number of variables measured on each subject). There are several estimators (which are perhaps more accurately described as predictors) of factor scores.[43,44] We used the regression method, which was first recommended by Thurstone.[45] The regression method obtains the weight matrix B by minimising the sum of squares of the difference between estimated and true factor scores. This method is equivalent to finding the linear regression of factor scores on phenotypes. Thompson[46,43] has given a derivation for the weight matrix B. Equivalent estimators of unobserved random effects have been used in other contexts. In animal breeding the estimator is known as the best linear unbiased predictor.[47] In the construction of the genetic factor scores only phenotypic information from the individual subject was used, and not from other family members. The weight matrix then is obtained as:

$$B' = \boldsymbol{\lambda}'\boldsymbol{\Sigma}^{-1}$$

where the ($p \times 1$) vector $\boldsymbol{\lambda}$ contains the factor loadings of the phenotypes on the common genetic factor and $\boldsymbol{\Sigma}$ ($p \times p$) is the population covariance matrix.

All scales were transformed before genetic analyses were carried out, using a natural logarithm (Anxiety (20 items); 10ln(Anx); Neuroticism (30 items): 5ln(Neu); Somatic anxiety (17 items): 9ln(SoA); YASR depression (16 items): 12ln(Ydep); or an arcsine transformation (BDI (13 items): (arcsine(BDI/max score)**0.5)*10). The arcsine transformation does little to render the distribution of the BDI data normal, but substantially reduces kurtosis.

DNA collection

DNA was collected through the mail. A mouth swab procedure was used.[48] Subjects received a test-kit with detailed instructions (photographs). They collected buccal swabs three times a day and sent back their samples in a prepaid envelope. This procedure of DNA collection has been shown to be suitable for large-scale genotyping with markers in the Weber 8/8a set in our laboratory. DNA samples have recently (1999) also been tested successfully in the Marshfield laboratories.

Psychiatric interview data

All offspring from selected families were asked to participate in a telephone interview during which several sections from the WHO Composite International Diagnostic Interview (CIDI) were administered.[49,50] The CIDI is a fully standardised diagnostic interview designed for assessing mental disorders according to the definitions of the Diagnostic Criteria for Research of ICD-10 and DSM-IV. We employed the computer-administered lifetime version (2.1). The following sections were administered: Demographics (section A); Social Phobia, Agoraphobia and Generalised Anxiety Disorder (D33 and further); Depression and Dysthymia (E); Mania Screen and Bipolar Affective Disorder (F) and Obsessive-compulsive disorder (K1-22).

Physiological parameters

Selected offspring were also invited to take part in ambulatory measurement of cortisol and cardiovascular patterns. The VU-AMS device[51,52] is used for continuous 24-hour registration of the electrocardiogram and the impedance cardiogram. These registrations are used to derive indices of vagal tone (RSA: respiratory sinus arrthymia) and cardiac sympathetic drive (PEP: pre-ejection period). This part of the study is still in progress. Subjects are visited at home during a working day and registration of physiological signals begins in the morning. Blood pressure is measured every 30 minutes during the day and salivary cortisol is measured six times during the 24-hour period.

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Table 2 Number of times families and individuals participated in the survey study (note that not all subjects could participate 4 times)

No of times	No of families	No of individuals		
		Parents	Twins	Sibs
A: Longitudinal participation: for entire sample				
One	1259 (38%)	1525 (30%)	2428 (38%)	1456 (66%)
Two	687 (20%)	2110 (41%)	1448 (23%)	744 (34%)
Three	862 (26%)	1456 (29%)	1697 (26%)	–
Four	536 (16%)	–	853 (13%)	–
B: Longitudinal participation for selected families who returned a buccal swab for DNA isolation				
One	120 (24%)	86 (13%)	193 (23%)	246 (52%)
Two	52 (10%)	221 (34%)	83 (10%)	230 (48%)
Three	142 (29%)	336 (52%)	261 (30%)	–
Four	183(37%)	–	319 (37%)	–

Results

Descriptives

There were 13 717 subjects from 3344 families who took part in the study at least once. The subjects consisted of 6426 twins, 2200 siblings of twins and 5091 parents (2453 fathers and 2638 mothers). Table 2A lists the longitudinal response rates for these groups (note that parents could not participate more than three times and siblings not more than once or twice since their participation was requested for the first time in 1995). The results reported below are limited to the anxiety, depression, neuroticism and somatic-anxiety data collected in the twins and their siblings in 1991, 1993 and 1997, except when we report on the longitudinal correlations for these variables and also include the YASR-depression data collected in 1995.

In Table 3 average scores and standard deviations for anxiety, neuroticism, somatic anxiety and depression are presented for males and females who participated in 1991, 1993 and 1997. The data are pooled over zygosities and over twins and siblings, since no effect was found on average scores of either zygosity or of being a twin. Women had higher scores than men for all variables (except age) listed in Table 3. The average age of the total sample in 1993 was almost equal to the average age in 1991, because roughly 50% of the sample in 1993 consisted of new families, with on average somewhat younger twins. In 1997, 36% of the individuals (twins and siblings) participated for the first time in the study; the average age of these who had participated at least once before was 21.9 years.

Cross-sectional and longitudinal correlations between variables are given in Tables 4A, 4B and 4C (the longitudinal correlations for depression in Table 4C also include the 1995 YASR-depression scores). Within each occasion, the correlations between age and the phenotypic scores were low and not statistically significant. Most cross-sectional and longitudinal correlations were somewhat higher in females. Cross-sectionally, somatic anxiety showed the lowest correlation with the other scales. Correlations among the other scales varied between 0.5 and 0.77. The longitudinal correlations were between 0.4 and 0.5 for the longest interval (1991–1997) and around 0.6 for the 2-year intervals.

Genetic modelling

Table 5 gives the twin correlations for the 1991, 1993 and 1997 data for the variables that make up the anxious depression dimension. Correlations for all phenotypes were higher in monozygotic twins than in dizygotic twins. As is suggested by the correlations, the heritability of anxiety, depression, neuroticism and somatic anxiety was around 50% in univariate genetic analyses. The best fitting models for these data indicated that familial resemblance must be attributed entirely to shared genes and not to shared environment. For all traits, a higher heritability in females than in males was found in univariate analyses. This higher heritability was explained by a higher genetic variance in females

Table 3 Means and Standard Deviations for males and females for Anxiety, Neuroticism, Somatic anxiety and Depression (YASR and Beck Depression Inventory)

	1991		1993		1997	
	Males	Females	Males	Females	Males	Females
N	1545	1848	1733	2149	1848	2727
Anx	33.2 (7.7)	34.9 (8.3)	31.9 (7.4)	34.3 (8.7)	30.3 (7.7)	33.6 (9.3)
Neu	52.5 (21.7)	61.9 (23.1)	46.9 (21.6)	55.2 (23.8)	40.5 (21.1)	50.5 (24.4)
SoA	18.3 (5.0)	19.6 (5.7)	17.9 (5.0)	19.1 (5.5)	16.5 (4.5)	18.0 (5.4)
Dep	19.9 (3.7)	21.5 (4.6)	– –	– –	19.5 (3.5)	21.6 (4.7)
BDI	–	–	1.2 (2.1)	1.7 (2.7)	1.3 (2.2)	2.0 (2.9)
Age	17.7 (2.3)	17.7 (2.3)	17.8 (3.1)	17.9 (3.1)	25.8 (10.3)	26.5 (10.1)

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Table 4

	1991					1993					1997				
A: Cross-sectional correlations for Anxiety, Neuroticism, Somatic Anxiety and Depression (measured with YASR depression scale and BDI)															
	Anx	Neu	SoA	Dep	BDI	Anx	Neu	SoA	Dep	BDI	Anx	Neu	SoA	Dep	BDI
Anx	–	0.67	0.47	0.65	–	–	0.64	0.43	–	0.63	–	0.70	0.48	0.69	0.63
Neu	0.72	–	0.56	0.62	–	0.71	–	0.60	–	0.50	0.77	–	0.58	0.65	0.55
SoA	0.48	0.55	–	0.46	–	0.50	0.59	–	–	0.38	0.55	0.60	–	0.45	0.45
Dep	0.73	0.66	0.44	–	–	–	–	–	–	–	0.77	0.74	0.52	–	0.60
BDI	–	–	–	–	–	0.68	0.55	0.47	–	–	0.70	0.59	0.52	0.65	–

B: Longitudinal correlations

	Anxiety			Neuroticism			Somatic Anxiety		
	1991	1993	1997	1991	1993	1997	1991	1993	1997
1991	–	0.60	0.41	–	0.58	0.49	–	0.47	0.44
1993	0.62	–	0.51	0.65	–	0.61	0.61	–	0.40
1997	0.44	0.53	–	0.50	0.58	–	0.43	0.48	–

C: Longitudinal correlations for YASR and BDI Depression scales

	YDe91	BDI93	YDe95	YDe97	BDI97
1991 YASR-Dep	–	0.37	0.45	0.36	0.33
1993 BDI	0.44	–	0.45	0.41	0.50
1995 YASR-Dep	0.56	0.49	–	0.58	0.42
1997 YASR-Dep	0.47	0.38	0.65	–	0.60
1997 BDI	0.19	0.33	0.40	0.65	–

Males upper diagonal, females lower diagonal

Table 5 Twin correlations for Anxiety, Neuroticism, Somatic-Anxiety and Depression (measured with YASR depression scale and BDI)

	1991					1993					1997					
	N	Anx	Neu	SoA	Dep	N	Anx	Neu	SoA	BDI	N	Anx	Neu	SoA	BDI	Dep
MZM	273	0.53	0.45	0.39	0.41	327	0.55	0.48	0.45	0.43	196	0.52	0.51	0.46	0.48	0.57
DZM	259	0.26	0.26	0.15	0.14	284	0.32	0.38	0.33	0.24	126	0.16	0.24	0.17	0.08	0.07
MZF	365	0.54	0.57	0.46	0.51	457	0.56	0.61	0.51	0.55	328	0.51	0.54	0.46	0.36	0.50
DZF	317	0.27	0.27	0.21	0.24	356	0.37	0.38	0.31	0.31	256	0.27	0.27	0.21	0.21	0.32
DOS	483	0.20	0.23	0.20	0.23	543	0.17	0.22	0.15	0.16	288	0.24	0.17	0.09	0.12	0.28

than in males. There was no evidence, however, that different genes influenced anxiety and depression in males and females. These results for the genetic architecture of anxiety, depression and neuroticism are very similar to results obtained in Australian[3] and American twin studies[4,53]

Goodness-of-fit chi-squared statistics for the multivariate genetic analyses of the twin data on anxiety, neuroticism, somatic-anxiety and depression collected in 1991, 1993 and 1997 are summarised in Table 6 (for the 1997 data two series of analyses were carried out: once with YASR-depression and once with BDI-depression scales). These analyses showed very stable results across time: significant sex differences in parameter estimates and no contribution of common family environment to resemblance of family members. Only in the 1993 data set, leaving out the shared environmental variance led to a significant increase in chi-square (33.6, critical value is 31.41 for 20 degrees of freedom). This contribution of shared environment could not be explained by age. We explored if there was a difference between twin pairs who had participated before in 1991 and those who participated for the first time in 1993, but no differences in parameter estimates between the two groups were found. The amount of variance explained by shared environment, however, was very small and it was decided to use the same multivariate model without C in subsequent computation of factor scores for all measurement occasions. The genetic factor model fitted the data well (again with the possible exception of the 1993 data set). All the genetic covariances among measures could be attributed to a common genetic factor. The environmental covariance structure could not be explained by a common environmental factor. Exploration of the environmental covariance matrix showed, that the failure of this model was mainly due to somatic anxiety.

Table 7 gives the percentages of variance for males and females explained by the common genetic factor and the specific genetic factors for each variable. The sum of these two percentages gives the total heritability each trait. Consistent with the univariate

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Table 6 Multivariate model-fitting results for anxiety, neuroticism, somatic anxiety and depression (two series of analyses for 1997 data: with BDI and with YASR depression)

		1991		1993		1997 (B)		1997 (Y)	
	df	χ^2	P	χ^2	P	χ^2	P	χ^2	P
ACE full M≠F	120	136.2	0.15	146.5	0.05	152.7	0.02	154.9	0.02
ACE full M=F	150	244.8	0.00	243.1	0.00	232.7	0.00	272.1	0.00
AE full M≠F	140	148.9	0.29	180.1	0.01	159.9	0.12	162.4	0.09
CE full M≠F	140	246.0	0.00	282.1	0.00	226.2	0.00	228.8	0.00
A factor M≠F	144	157.1	0.21	202.3	0.00	167.7	0.09	166.5	0.10
E factor M≠F	144	165.6	0.10	215.5	0.00	181.0	0.00	175.4	0.04

ACE full is full Choleski decomposition for Additive genetic, Common environmental and unique Environmental sources of variation; M≠F stands for sex differences in parameter estimates; factor models for A or E specify one common factor with specifics. Best model in bold.

Table 7 Standardised heritability estimates (% of variance explained by common genetic factor and genetic specifics) for males and females in 1991, 1993 and 1997; based on multivariate genetic model in which genetic influences are modelled as a common factor and influences specific to each trait. For the 1997 data two series of analyses were carried out, once with BDI depression and once with YASR depression

	G-factor	G-unique	G-factor	G-unique
	Females 1991		Males 1991	
Anx	0.48	0.04	0.41	0.10
Neu	0.44	0.12	0.38	0.07
SoA	0.28	0.15	0.23	0.16
Dep	0.40	0.09	0.32	0.11
	Females 1993		Males 1993	
Anx	0.49	0.06	0.38	0.13
Neu	0.52	0.10	0.41	0.06
SoA	0.35	0.15	0.22	0.21
BDI	0.32	0.19	0.30	0.09
	Females 1997		Males 1997	
Anx	0.48	0.06	0.39	0.05
Neu	0.44	0.12	0.34	0.09
SoA	0.29	0.15	0.21	0.11
BDI	0.34	0.08	0.35	0.10
	Females 1997		Males 1997	
Anx	0.46	0.07	0.42	0.03
Neu	0.45	0.10	0.33	0.10
SoA	0.28	0.17	0.20	0.11
BDI	0.46	0.07	0.35	0.11

genetic analyses, these heritabilities are around 50%. More importantly, the largest part of the genetic variance in all phenotypes can be attributed to the common genetic factor. This is a very good starting point for the computation of genetic factor scores.

Computation of genetic factor scores

Genetic factor scores were calculated as a weighted sum of the observed phenotypes (after transformation) for each individual. Weights were estimated separately across occasions based on the multivariate genetic analyses presented above. Because the genetic factor model did not differ across occasions, the weights were averaged across time points, thus making the factor scores comparable across time. Factor scores were computed separately for males and females and also depended on whether the BDI or YASR depression scale was used in their construction. Using this approach the following formula obtained for males and females:

Males: $F = 0.144 \times Anx + 0.117 \times Neu + 0.039 \times SoA + 0.064 \times YDep$

Males: $F = 0.130 \times Anx + 0.077 \times Neu + 0.040 \times SoA + 0.166 \times BDI$

Females: $F = 0.133 \times Anx + 0.117 \times Neu + 0.066 \times SoA + 0.053 \times YDep$

Females: $F = 0.146 \times Anx + 0.086 \times Neu + 0.077 \times SoA + 0.062 \times BDI$

For males, there is a much larger contribution of BDI to the construction of their genetic factor scores, whereas for females contribution of somatic anxiety is relatively larger than in males. The contributions of anxiety and neuroticism are very similar in both sexes. Twin correlations for factor scores (averaged over all occasions) were 0.61 for MZM, 0.64 for MZF, 0.33 for DZM, 0.40 for DZF, and 0.28 for DOS twins.

Selection

The equations for the computation of genetic factor scores were applied to the twin data collected in 1991, 1993 and 1997 and the sibling data from 1997. The total number of families with at least one sibling pair with extreme genetic factor scores (concordant high-high, low-low or discordant high-low) was 563. These families were asked to participate in a search for QTLs influencing anxious depression.

Selection was initially within each measurement occasion (ie 1991, 1993 and 1997) and additionally also on sib pairs who were extremely concordant or discordant across occasions. Once a family was

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selected, all family members (parents, all twins and siblings who had at least once returned a questionnaire) were asked to supply DNA samples. This request included MZ twin pairs, in which one (or both) of the twins formed an extreme pair with an additional sibling. In total, 2724 subjects (1007 parents and 1717 offspring) were approached and currently 1975 (643 parents and 1332 offspring) have complied by returning a buccal swap for DNA isolation. The average yield from buccal swaps was 25.8 micrograms of DNA (SD = 18.8). The offspring who were approached with the request to take part in the QTL study consisted of 374 MZ twins, 752 DZ twins and 591 sibs. The number of DNA samples returned by these 3 groups was 336, 520 and 476, respectively. The return rate of buccal swap samples did not differ significantly among those scoring in the high tail, the low tail or in the middle of the factor score distribution (67%, 70% and 74%, respectively, P = 0.12).

Figure 2 gives the distribution of genetic factor scores (averaged over time) in the entire offspring sample, in the selected offspring sample, and in the sample that returned a DNA sample. Factor scores in the entire sample showed a normal distribution (mean: 0.03, standard deviation: 0.71, skewness: 0.38 (0.03) and kurtosis: 0.0 (0.056)). There was a large effect of selection on the distribution of factor scores (mean: 0.04, SD: 0.88, skewness: 0.28 (0.06) and kurtosis: –0.81 (0.12) in the selected sample and mean: 0.02, SD: 0.83, skewness: 0.36 (0.06) and kurtosis: –0.61 (0.12) in the sample that returned a buccal swap). The effect of selection is evident in the significant kurtosis of the factor scores: in selected families many more offspring are in the tails of the distribution. The subjects in the middle of the distribution are mainly the additional twins and sibs from larger families, who were invited to participate with the extreme sibling pair in the family, because large sibships provide an increase in power over size 2 sibships.[25] For the families who returned a DNA sample, Table 8 presents an overview of family size (between one and 10 offspring per family; except in the second row MZ twins are counted as 1 genotype) and of the number of families with extreme offspring (between 0 and 6 extremes per family). The extreme individuals per family are grouped according to the concordant criterion (top or bottom 12% of the distribution) or discordant criterion (scores in the top 20% and bottom 25%, or vice versa). For example, in the 43 families in which four offspring returned a DNA sample, there are two, three or four extreme sibs according to the concordant criterion in respectively 18, 12 and two families. According to the discordant criterion the number of families with two, three or four extreme sibs is 12, 22 and seven, respectively. As Table 8 shows, there were 379 families in which at least two siblings, who were not MZ twins, returned material for DNA isolation. The average sibship size in these families was 2.8. Table 2B summarises the longitudinal response pattern of the subjects who returned a mouth swab for DNA isolation.

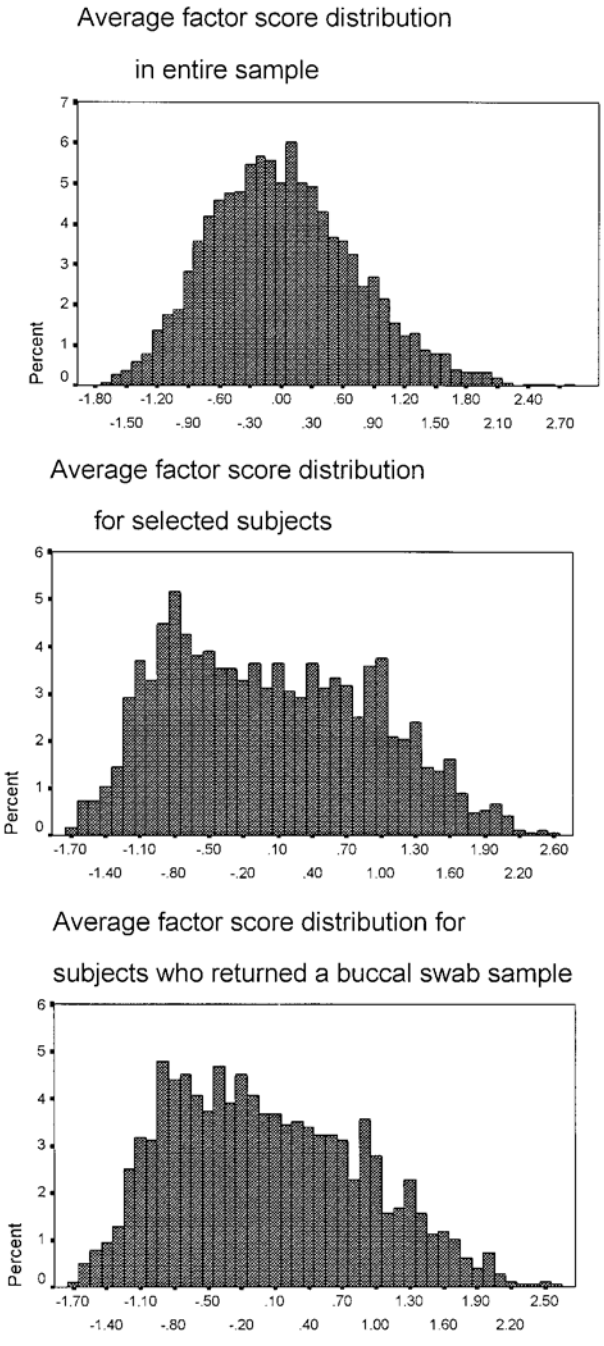

Figure 2 Distribution of genetic factor scores in the entire offspring sample (factor scores averaged over measurement occasions), in the entire selected offspring sample and in the offspring sample that participated in the QTL study

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Table 8 Family composition for offspring who returned a DNA sample. Families are cross-classified by number of siblings (rows) per family and number of extreme siblings (columns) within a family (x/y refer to numbers of families for concordant (x) and discordant (y) criteria)

Family size (sibs)	No of extreme siblings (according to concordant and to discordant criteria) 0	1	2	3	4	>4	No of families	No of subjects	No of sib pairs
1 Subject	28/16	35/47	–	–	–	–	63	63	–
2 Ss (MZ)	4/1	11/8	40/46	–	–	–	55	110	–
2 Ss	16/2	71/34	104/155	–	–	–	191	382	191
3 Ss	6/2	45/15	55/57	20/52	–	–	126	378	378
4 Ss	2/0	9/2	18/12	12/22	2/7	–	43	172	258
5 Ss	–	–	4/2	2/1	5/6	0/2	11	55	110
6 Ss	–	1/0	–	0/1	–	–	1	6	15
7 Ss	–	1/0	1/0	1/1	1/2	1/2	5	35	105
8 Ss	–	–	1/0	0/1	–	–	1	8	28
10 Ss	–	–	–	–	–	1/1	1	10	45
Total							497	1219	1130

Second row: families in which only a MZ pair returned a buccal swab. For another 113 MZ twin pairs with at least one additional sibling, only one twin is included in the Table. The total number of offspring for whom DNA is currently available thus is 1332.

All offspring from selected families were asked if they agreed to being interviewed on the telephone and if they wanted to participate in 24-hour registration of cardiovascular parameters and cortisol. The WHO-CIDI was administered to 1253 offspring. These data were used to obtain DSM-IV (single or recurrent) depression status. Participation in the CIDI interview was related to the factor scores distribution class (73% in the high, 78% in the low, and 69% in the middle of the distribution, P = 0.01). Of those who returned the buccal swap samples, 87% participated in the interview, compared with 28% who did not return the samples. Currently, we have collected 24-hour profiles for cardiovascular parameters in 454 subjects and for cortisol in 328 subjects.

Discussion

The aim of our study is to detect the chromosomal regions harbouring the genetic polymorphisms responsible for variation in anxiety and depression. These traits place a large burden on the individual and on society, both in terms of the loss of quality of life and in health care costs. The genetic perspective does not reflect underestimation of the importance of environmental influences. Such influences are clearly demonstrated by our results: they explain about half of the variance in anxiety and depression at all time points. We found that genetic factors accounted for roughly 50% of the variance in anxiety, somatic anxiety, depression, and neuroticism and that genetic influences accounted for most of the covariance between these traits. The genetic covariance between measures was due to one common genetic factor. The largest part of the heritability in all anxious depression indices could be attributed to this common genetic factor (Table 6). These multivariate analyses confirmed the large overlap in the genes conveying susceptibility to anxiety, neuroticism, somatic anxiety and depression, as previously reported by others.[1,4] This common set of genes appears to lead to a very stable genetic architecture for the traits we studied throughout the period from adolescence to adulthood: identical genetic factor structures were found in 1991, 1993, and 1997, with a substantial part of the subjects participating on all three occasions.

The results from the multivariate genetic analyses were applied to the computation of genetic factor scores for each individual in the study. The genetic factor score obtained by combining the various questionnaires is best described as 'anxious depression' or more appropriately 'genetic susceptibility to anxious depression'. The factor scores are based on questionnaire instruments mostly geared towards a 'normal' non-clinical population. The additional CIDI data strongly suggest that subjects at the extreme high end of the distribution of questionnaire scores are indeed at risk for depression. In short, the genetic factor scores look like a good starting point for linkage analyses to detect QTLs for anxious depression.[27,54]

In order to increase the power of linkage analyses by trying to map the actual genes conveying the genetic susceptibility for anxious depression, offspring with extreme genetic factor scores were selected from both tails of the quantitative distribution. Selection of families was based on the presence of at least two siblings in a family with extreme factor scores. Once an extreme pair was identified within a family, all family members who had participated in the survey (ie parents and additional sibs or twins) were asked to provide a DNA sample. To date we have collected 1975 DNA samples from 643 parents and 1332 offspring. In the offspring generation there are 168 MZ twin pairs (usually part

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of a larger family) who are not informative for traditional linkage analyses. They provide an opportunity, however, to hunt for genes which influence an individual's reactivity to environmental challenges[55] and are extremely valuable in association tests,[56] as are parents and families with larger sibships.[57]

Deviant functioning of the autonomic nervous system, both with regard to its resting tone and its responsiveness to psychological strain, often accompanies anxiety and depression. All three axes of the autonomic system, the sympathetic-medullary axis (SMA), the parasympathetic nervous system and the hypothalamic-pituitary axis (HPA) have been implicated.[58–60] The association may reflect causality; disturbed HPA function in particular has been hypothesised as an aetiological factor in depression.[6] To increase the power of QTL detection further we are collecting extensive data on autonomic nervous system functioning in the selected subjects. Measures include non-invasive assessment of cardiac vagal and sympathetic drive and 24-hour cortisol profiles. Such endophenotypes can increase power for linkage in three ways:

(1) They may be used as phenotypes for linkage instead of the factor scores; by being 'closer' to the actual gene effects some QTLs may explain more variance in the biological markers than in the more complex phenotype of depression.

(2) They may be used in combination with the genetic factor scores to define a new multivariate phenotype that encompasses both questionnaire and psycho-physiological data, thus providing a converging phenotype from multiple measurement levels.

(3) They may be used to refine the questionnaire data, eg a high score in anxious depression with high cortisol may reflect depression, whereas high anxious depression scores with low cortisol may reflect effects of chronic stress or post-traumatic stress disorder.[62] Such distinction may be difficult to make on the basis of self-report only.

The added advantage of endophenotypes, such as cortisol, over increasing power of linkage, is that they can help to elucidate the molecular pathways leading to anxious depression after a gene has been found. This also applies if other candidate genes should derive from parallel research by others or in animal models. In such future follow-up it is particularly important to have access to twin-family samples because the contributions of such candidate genes can be tested against the background of other genetic influences.

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