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The relation between frontal EEG asymmetry and the risk for anxiety and depression

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Abstract

Frontal asymmetry of EEG alpha power (FA) may index the risk for anxiety and depression. Evidence linking FA to the underlying biological mechanisms is scarce. This is unfortunate because FA has potential as a biological marker to support gene finding in anxiety and depression. We examined the heritability of FA in 732 twins and their singleton siblings, and established the genetic and environmental contribution to the relation between FA and the risk for anxiety and depression. Multivariate models showed that FA is heritable only in young adults (males 32% and females 37%) but not in middle-aged adults. A significant relation between FA and the risk for anxiety and depression was only found in young adult females. This relation was explained by shared genes influencing both EEG and disease risk. Future studies on asymmetry of left and right frontal brain activation should carefully consider the effects of sex and age.

Keywords: Twins; Sex differences; Age cohorts; Endophenotype

1. Introduction

Frontal asymmetry of EEG alpha power (FA) has been studied extensively as a correlate of individual differences in emotional responding. These studies assume that alpha power acts as an inverse index of activity: a synchronous state of oscillations reflects inactivity of the underlying neural substrate (Shagass, 1972). This assumption has been supported by fMRI and PET studies that showed a decrease in cortical blood flow with increasing alpha power (Cook et al., 1998; Goldman et al., 2000). Greater left hemispheric activity has been associated with approach related behavioral tendencies, and greater right hemispheric activity with withdrawal related tendencies. In the extant literature, therefore, it is hypothesized that FA acts as an index of the basic emotional dimension of approach versus withdrawal (Coan and Allen, 2004; Harmon-Jones, 2004). On this basis individual differences in asymmetric frontal activity are hypothesized to indicate individual differences in affective style (Davidson, 1992).

Because affective style is related to the liability to develop psychopathology such as depression and anxiety disorders a relation between FA and depression and anxiety can be expected (Coan and Allen, 2004; Davidson, 1992). In adults, many studies provided findings consistent with this view (for an overview, see Coan and Allen). For example, FA has been found to differ between clinically depressed patients and non-depressed controls (Flor-Henry, 1979; Henriques and Davidson, 1991), responders and non-responders to fluoxetine treatment (Bruder et al., 2001), and subjects scoring high and low on a depression scales (Debener et al., 2000; Gotlib et al., 1998; Schaffer et al., 1983). The link between FA and negative affectivity also holds in infants of depressed mothers (Field et al., 1995, 2000) and seasonal affective disorder patients (Allen et al., 1993) or unipolar depression patients currently in remission (Henriques and Davidson, 1990; Gotlib et al., 1998). The latter indicates that FA is a marker for the liability for depression rather than the depressive state itself. Taken together, these studies have established a secure foothold for FA as a biological marker for depression.

As noted by Allen and Kline (2004) much of the research on FA has focused on its relation to psychopathology and other behavioral phenotypes, but evidence linking FA to the underlying biological mechanisms is scarce. This is unfortunate because FA

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has potential significance as a so-called endophenotype to support gene finding in depression. Endophenotypes are psychophysical or psychophysiological phenotypes that are constituents of the causal pathway from gene to phenotype. They represent the expression of a subset of genes from the whole set of genes causing the genetic part of phenotypic variation. As such, they can be useful when the great number of genes involved in the phenotype of interest reduces the statistical power in linkage and association studies. Ideally, endophenotypes possess several features (De Geus, 2002): (i) they are stable, (ii) heritable, (iii) and correlated with the phenotype of interest, and (iv) the relations in (ii) and (iii) share a common genetic source.

Although studies show that the requirements (i) stability and (iii) correlation to the risk for depression have been met with success (Allen et al., 1993; Gotlib et al., 1998; Henriques and Davidson, 1991; Henriques and Davidson, 1990; Schaffer et al., 1983; Tomarken et al., 1992), evidence for genetic contribution to FA and to the association between FA and depression is still scarce. Several conference abstracts reported on the genetic basis of FA. Anokhin and Rohrbaugh (1998) found a mid-parent to offspring correlation of $r = 0.46$ in a family study of alcoholic and depressed patients and controls, providing evidence for familial influences in FA. Allen et al. (1997) used a twin study to further show that these familial influences reflected genetic influences. In 60 pairs of 17-year-old female twins, they found 33% of FA variability to be under genetic control. In a thesis, Coan (2003) reported that genetic influences explained a modest 22% of the variation in mid-frontal FA in 66 female twin pairs, and no significant heritability in males from a normal population. Recently, Anokhin et al. (2005) reported a modest mid-frontal FA heritability of 31% within a young adult female sample.

Here we aim to extend the knowledge base on the genetics of FA by examining FA from resting EEG in large set of twin pairs and their singleton siblings. Additionally we aim to establish the genetic and environmental contribution to the relation between FA and the risk for anxiety and depression. Anxiety disorders have not been studied extensively in relation to FA, and the results are less conclusive than for depression (Baving et al., 2002; Heller et al., 1997; Kentgen et al., 2000; Nitschke et al., 1999; Papousek and Schulter, 2002). However, anxiety disorders are highly comorbid with depression. Moreover, the genetic variance of these phenotypes reflects for the most part a common genetic source (Jardine et al., 1984; Kendler et al., 2003; Middeldorp et al., 2005a,b). We hypothesize that the common genetic factor underlying the risk for anxiety and depression is reflected in individual differences in FA. Since sex differences in the heritability of FA as well as in the relation between FA and depression have been reported, we stratified our sample according to sex (Bruder et al., 2001; Miller et al., 2002).

2. Method

2.1. Subjects

The sample of this study was derived from an ongoing twin family study on mental and physical health in participants of the Netherlands Twin Registry (NTR). Families with adult twins have been receiving surveys on lifestyle and health every 2/3 years since 1991 (Boomsma et al., 2002). Anxiety and depression data were available for 9088 twins and non-twin siblings. These were divided into two age cohorts based on the twins age on 1 January 1999: a young adult cohort (under 35) with 3879 males and 5364 females, and a middle-aged cohort (over 35) with 647 males and 1232 females. On average 2.20 siblings per family participated.

A subset of twins and siblings were invited for detailed psychophysiological study in the laboratory. For the present study, twins were invited who had previously participated in EEG or cardiovascular research. In addition, we invited their non-twin siblings. A total of 760 subjects from 309 twin families accepted the invitation to participate. As with the survey sample, the EEG sample consisted of two age cohorts based on the age of the twins: a younger cohort ($M = 26.2$ years, S.D. = 4.1) and a middle-aged cohort ($M = 49.4$ years, S.D. = 7.2). Participating families consisted of one to seven siblings (including twins). On average, 2.50 participants per family participated. Informed consent was obtained in writing for the EEG study. Both the EEG and the questionnaire studies received approval from the appropriate ethical committees.

2.2. EEG registration

The experimental protocol and background EEG registration has been described in detail elsewhere (Posthuma et al., 2001; Smit et al., 2005), but a brief description will be repeated here. The experimental protocol consisted of two parts. During one part, psychometric intelligence, inspection time, and reaction times were assessed. During the other, EEG was measured at rest and during various reaction time tasks. The order of the two parts of the protocol was randomized across family members. Consequently, half of EEG registration sessions were during morning hours, and half were in the afternoon.

Subjects were seated in a comfortable reclining chair in a dimly lit, sound attenuated, and electromagnetically shielded room. They were instructed to relax and minimize eye and body movement. Resting background EEG was registered for 3 min under both eyes open and eyes closed instructions with 19 Ag/AgCl electrodes mounted in an electrocap. Signal registration was conducted using an AD amplifier developed by Twente Medical Systems (TMS; Enschede, The Netherlands) for 657 subjects (381 young, 380 middle-aged) and NeuroScan SynAmps 5083 amplifier for 103 subjects (24 young, 80 middle-aged). Signals were continuously represented online on a Nec multisync 17 in. computer screen using Poly 5.0 software or Neuroscan Acquire 4.2. Standard 10–20 positions were F7, F3, F1, Fz, F2, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1, and O2 (American Electroencephalographic Society, 1991). For NeuroScan subjects Fp1, Fp2, and Oz were also recorded, but not included in the analysis. The vertical electro-oculogram (EOG) was recorded bipolarly between two Ag/AgCl electrodes, affixed 1 cm below the right eye and 1 cm above the eyebrow of the right eye. The horizontal EOG was recorded bipolarly between two Ag/AgCl electrodes affixed 1 cm left from the left eye and 1 cm right from the right eye. An Ag/AgCl electrode placed on the forehead was used as a ground electrode. Impedances of all EEG electrodes were kept below 3 kΩ, and impedances of the EOG electrodes were kept below 10 kΩ. The EEG was amplified, digitized at 250 Hz and stored for offline processing. Amplifier filter settings for TMS were a single order FIR bandpass filter with cutoff frequencies of 0.05 and 30.0 Hz. NeuroScan filter settings were a lowpass filter at 50.0 Hz.

2.3. Data processing

Computation of FA used the EEG recorded during the eyes closed condition. Signals at leads F3 and F4 were analyzed using NeuroScan software version 4.2. The signals were recalculated with averaged earlobes (A1 and A2) as reference. The 3 min recording was cut into 43 epochs of 1024 data points (4.096 s). Any linear trend was removed from EEG by fitting and subtracting the regression line for each epoch separately. Next, epochs were excluded per lead when EOG channels showed more than 400 μV and EEG more than 175 μV deviation from ground in either direction. EEG traces were then visually inspected per subject for remaining artifact due to muscle activity, swallowing, eye movement, bad recordings, and externally induced artifacts (e.g., experimenter initiated reset pulses, electrical hum). Only epochs with extreme magnitudes of muscle artifacts and eye movements were excluded. Subjects with less than 22 valid epochs after visual inspection were considered unreliable and set to missing (22 epochs ensure at least 1 min and 30 s of data per subject.). The number of

subjects with valid data on both F3 and F4 was 732. Table 1 shows the exact composition of the final sample per age cohort and zygosity of the twins.

For all remaining, artifact-free epochs, power spectra were calculated with a Hamming window for 5% of the epoch duration at the beginning and end of the epochs. Power spectra were averaged, resulting in a single spectrum with a resolution of about 0.25 Hz (1000/4096 Hz). Alpha power was defined as the sum of all data points in the range from 8.0 Hz up to but not including 13.0 Hz. Frontal asymmetry is defined as

$$FA = \ln(\alpha_{F4}) - \ln(\alpha_{F3}),$$

where higher scores reflect lower left alpha power, and consequently higher left cortical activation, relative to the right cortex.

Up to 20% of the population may exhibit low or very low alpha synchronization (Vogel, 2000; Anokhin et al., 2005) yielding denominator values that are close to zero. This can result in an unstable and noisy FA measure. In accordance with Anohkin et al., we repeated our analyses after excluding subjects with the lowest average frontal EEG power. This subject selection aimed to reduce the adverse effects of noise amplification due to the nature of the FA calculation.

2.4. Anxiety and depression surveys

Questionnaires were sent in 1991, 1993, 1995, 1997, 2000, and 2002 to twin families and their siblings who had indicated that they were willing to participate in the survey study. A detailed description of the survey content and response rates at each wave can be found in Boomsma et al. (2000). Data on trait anxiety (Anx), neuroticism (Neu), somatic anxiety (SoA), and anxious depression (Dep) from three waves were analyzed (1997, 2000, and 2002). Trait anxiety and anxious depression were collected using the Dutch versions of the Spielberger Anxiety Inventory (STAI; Spielberger et al., 1970) and the Young Adult Self Report scale (YASR; Achenbach, 1990). Neuroticism and somatic anxiety were assessed with the Amsterdamse Biografische Vragenlijst (ABV; Wilde, 1970). The item content of the ABV neuroticism scale is very similar to that of the Eysenck Personality Questionnaire. From these traits a factor score was calculated after weighing each trait to maximize heritability of the factor score. As depression has repeatedly been shown to differ in genetic makeup between males and females (e.g., Bierut et al., 1999; Kendler et al., 2001, 2003), the subscale weights were calculated separately for males and females:

Risk factor score

$$= 0.144 \times \text{Anx} + 0.117 \times \text{Neu} + 0.039 \times \text{SoA} + 0.064 \times \text{Dep},$$

for males

Risk factor score

$$= 0.133 \times \text{Anx} + 0.117 \times \text{Neu} + 0.066 \times \text{SoA} + 0.053 \times \text{Dep},$$

for females.

after normalization of the Anx, Neu, SoA, and Dep scores. This factor score summarizes the genetic risk for anxiety and depression and has been found to have a heritability of about 60% (Boomsma et al., 2000). Forty-six subjects (seven young adult females) of the subjects with EEG data did not have survey data available on any of these time points.

2.5. Genetic statistical analyses

Prior to genetic model fitting we tested: (1) whether the twin data could be generalized to a singleton population by comparing the means and (co-)variances of twins and singleton siblings, and (2) the equivalence of means and variances across MZ and DZ twins. Significance of these differences were tested by four group omnibus tests, that is, for all four sex by cohort groups simultaneously.

Genetic statistical analysis of the power spectra of the sample was repeated in young males, young females, middle-aged males, and middle-aged females. A linear regression model was employed to include effects of the covariate of age on the observed scores within each group, formally represented as: $\mu_i = \beta_0 + \beta_1 \text{age}_i$, where μ_i is the expected value of individual i, age_i the individual's age in years at time of measurement, β_0 the intercept, and β_1 is the regression estimate of age.

Structural Equation Modeling implemented in the program Mx version 1.57 (Neale, 2004) estimated the contribution of additive genetic variation (σ^2_A), shared environmental variation (σ^2_C), or non-shared environmental variation (σ^2_E) to the observed interindividual variation in power spectra using the full information Maximum Likelihood Estimation (MLE) procedure (Neale and Cardon, 1992). Sources of shared environmental variation by definition include all environmental influences that twins and siblings from the same family share, while sources of non-shared environmental variation refer to the environmental variation that is unique for an individual and that is not shared with other family members. For two members of a DZ twin pair (and sibling pairs) who are raised in the same home and share on average 50% of their segregating genes, the correlation between shared environmental influences (C) was fixed at 1, the correlation between additive genetic influences (A) at 1/2, and the correlation between dominant genetic influences (D) at 1/4. For two members of an MZ twin pair correlations between shared environmental, additive genetic, and dominant genetic influences were all fixed at 1. Correlation between non-shared environmental influences (E), by definition, is set to zero for both MZ and DZ twins. Thus, the expectation for the total variance is $\sigma^2_A + \sigma^2_D + \sigma^2_C + \sigma^2_E$, the expectation for the covariance between MZ twins is $\sigma^2_A + \sigma^2_D + \sigma^2_C$, and the expectation for DZ twins/sibling pairs is $(1/2)\sigma^2_A + (1/4)\sigma^2_D + \sigma^2_C$. Heritability is calculated as the proportional contribution of genetic variation to the total observed variation $((\sigma^2_A + \sigma^2_D)/(\sigma^2_A + \sigma^2_D + \sigma^2_C + \sigma^2_E))$.

The twin design with additional siblings does not allow the simultaneous estimation of dominant genetic and common environmental effects (Neale and Cardon, 1992). If the DZ correlation is larger than half the MZ correlation, this will be taken as evidence of common environmental effect and σ^2_D will be set to zero. If the DZ correlation is less than half the MZ correlation, this will be taken as evidence of dominant genetic effects and σ^2_C will be set to zero. In addition, to

Table 1
Number and composition of families per age cohort and zygosity of the twins

	Young cohort					Middle-aged cohort				
	MZM	MZF	DZM	DZF	OS	MZM	MZF	DZM	DZF	OS
Families with both twins	17	19	9	13	13	16	20	9	15	10
Families with both twins + 1 sibling	9	8	9	13	9	6	14	1	9	8
Families with both twins + 2 or more siblings	2	1	4	2	3	3	3	0	1	3
Families with 1 twin only	1	2	0	1	2	2	3	2	5	5
Families with 1 twin + 1 sibling	1	4	2	2	4	3	1	1	2	1
Families with 1 twin + 2 or more siblings	0	0	0	1	2	0	0	2	0	0
Families with 1 sibling	1	1	0	0	2	0	1	0	2	0
Two or more siblings	1	1	0	0	0	1	0	0	0	0

Note: We based family composition on the participating offspring only. For example, a family with 'both twins only' could potentially consist of more than two children, but these did not participate in the EEG experiment. MZM: MZ male twins; MZF: MZ female twins; DZM: DZ male twins; DZF: DZ female twins; OS: opposite sex twins.

estimate dominant genetic effects sample sizes must be very large (Posthuma and Boomsma, 2000). We will therefore attempt to estimate this effect in the survey data only.

3. Results

3.1. FA descriptives

FA in middle-aged subjects ($M = 0.35$, S.D. = 0.098) was higher than in young adult subjects ($M = 0.54$, S.D. = 0.111). The ANOVA showed the effect of cohort to be significant ($F(1, 728) = 6.84$, MSE = 0.011, $p < 0.01$). Neither the main effect of sex or the sex by cohort interaction were significant.

3.2. FA split-half reliability and temporal stability

The split-half reliability, as an indication of measurement error, can be considered a ceiling for the MZ twin correlation. We selected 10% of the subjects at random to compute FA at odd and even epochs separately. The resulting split-half correlation was 0.87 suggesting that MZ correlations are bound by this upper value.

To compute temporal stability, 32 subjects were invited back after a period varying from 354 to 1322 days. Of those, 27 had valid data available on F3 and F4 on both occasions. Temporal stability calculated as the correlation coefficient between both measurement occasions was 0.44. Jointly these analyses indicate that individual differences in FA are reliable and moderately stable over time.

3.3. Handedness

Generally, studies into hemispheric asymmetry limit their samples to right-handed individuals as handedness may be confounded with the lateralization of brain function. We tested this assumption by comparing FA scores of left against right-handed subjects and with a one-way univariate ANOVA with age and sex as covariates. Nine individuals indicated to be ambidextrous or did not provide an answer. Although the proportion of left-handers was slightly higher in the young adult cohort ($N = 384$, $P = 13.3\%$) than in the middle-aged cohort ($N = 339$, $P = 11.8\%$), this difference was not significant ($\chi^2 < 1$). Although left-handed subjects ($M = 0.032$) showed lower FA scores than right-handed subjects ($M = 0.046$), the effect did not reach significance (MSE = 0.011, $F(1) = 1.41$, ns). Additionally, we tested whether twin pairs discordant for handedness differed from twins concordant for handedness. If left-handedness causes a mirroring of brain function lateralization, twin pairs discordant for handedness should show a negative, or at least a reduced, intrapair correlation compared to concordant twin pairs. Maximum likelihood estimation of the correlations did not show evidence for an effect of concordance for either MZ or DZ twin pairs (both $\chi^2(1) < 1$). From these results, we concluded that handedness is not a confound of FA, and subsequent analyses used all pairs, including left-handed subjects and pairs discordant for handedness.

Table 2
Phenotypic correlations between FA and the risk for anxiety and depression

	Subject group			
	Young adult		Middle-aged	
	Female	Male	Female	Male
All subjects	0.12	0.00	−0.09	0.07
Excluding subjects				
With the 10% lowest frontal EEG power	0.17*	0.02	−0.04	0.06
With the 20% lowest frontal EEG power	0.20**	0.07	−0.03	0.04
With the 30% lowest frontal EEG power	0.25**	0.07	−0.03	0.12

* $p < 0.05$.
** $p < 0.01$.

3.4. Association between FA and the risk for anxiety and depression

Table 2 depicts the correlations between frontal asymmetry and the risk for anxiety and depression for each sex by age cohort group. In addition, it shows the intrapair correlations after removing the subjects with the 10, 20, and 30% lowest average frontal power scores (Anokhin et al., 2005). It is clear that the association was significant only in young females. Hence (bivariate), genetic modeling proceeded in the separate age/sex groups.

3.5. Twin correlations and heritabilities for the risk for anxiety and depression

For all four groups variances and means did not differ significantly between MZ twins, DZ twins, and singleton siblings. Likewise, DZ twin, twin–sibling, and sibling–sibling correlations were not found to differ. Further analyses therefore assumed these parameters to be equal, which increases the degrees of freedom.

Table 3 shows the resulting intrapair correlations obtained for the different sex by zygosity groups. Correlations differed between the cohorts, although the effect was rather small given the large sample size ($\chi^2(5) = 15.00, p = 0.010$). Correlations did not differ between the sexes. The intrapair correlations suggested dominant genetic effects as the DZ/sibling correlations were below half the MZ correlations. These effects were significant for young adult males and middle-aged females. Heritabilities were based on the summed additive and dominant genetic effects for these groups. For young adult females and middle-aged males only additive genetic effects contributed to heritability.

3.6. Twin correlations and heritabilities for FA

As with the risk for anxiety and depression, no differences were found in the means and variances between the zygosity groups (MZ, DZ) and in the means, variances and correlations between DZ twins and siblings.

Table 3 shows the sibling correlations for male MZ twins, female MZ twins, male DZ twins plus all other non-identical male–male sibling relations, female DZ twins plus all other non-identical female–female sibling relations, and opposite sex

Table 3
Twin correlations and heritabilities of FA and the risk for anxiety and depression

	MZM		DZM		MZF		DZF		OS			
	N^a	R	N^a	R	N^a	R	N^a	R	N^a	R	Male h^2 (%)	Female h^2 (%)
Risk factor scores												
All questionnaire subjects												
Young	555	0.52***	1231	0.15***	938	0.60***	1923	0.29***	2912	0.22***	52[b],***	60***
Middle-aged	80	0.62***	287	0.23**	232	0.57***	630	0.14**	733	0.12*	61***	57[b],***
Frontal asymmetry												
All EEG subjects												
Young	28	0.37*	81	−0.02	27	0.51***	78	−0.07	97	−0.05	32*	37**
Middle-aged	25	−0.14	48	0.10	37	0.28	83	−0.10	97	0.03	ns	ns
Excluding subjects												
With the 10% lowest frontal EEG power												
Young	25	0.29	70	0.40	26	0.50***	63	−0.04	85	−0.09	ns	38**
Middle-aged	21	−0.09	35	0.15	36	0.27	64	−0.11	73	0.08	ns	ns
With the 20% lowest frontal EEG power												
Young	24	0.24	61	0.41	22	0.53***	51	0.13	68	−0.06	ns	43**
Middle-aged	19	−0.18	25	0.18	34	0.32	55	−0.16	61	0.22	ns	ns
With the 30% lowest frontal EEG power												
Young	19	0.22	52	0.35	17	0.61***	38	0.19	52	−0.02	ns	44*
Middle-aged	16	−0.46	22	0.25	28	0.27	47	−0.27	47	0.21	ns	ns

Note: MZM: MZ male twins; DZM: DZ male twins and same-sex siblings; MZF: MZ female twins; DZF: DZ female twins and same-sex siblings; OS: opposite sex twins and siblings. DZ and opposite sex correlations entail all fraternal sibling relations and are corrected for intrapair age and sex differences. ns: $p > 0.05$.

[a] N represents the total sum of all possible sibling pairs, e.g., a family with one sister and two MZ brothers has two opposite sex sibling pairs, and one male MZ twin pair. Note that during statistical analyses these sibling relations within a single family were *not* treated as independent.

[b] Heritability (h^2) was modeled including dominant genetic effects (ADE). In all other cases, only additive genetic effects and unique environmental effects were modeled (AE).

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

twin and sibling relations. These intrapair correlations are given for the full EEG sample including those subjects without questionnaire data available on any occasion. In the young cohort, DZ/sibling correlations were less than half the MZ correlations. This pattern of twin correlations suggests the presence of genetic dominance, but in view of the sample size for FA genetic dominance was not explicitly modeled (Posthuma and Boomsma, 2000).

Common environmental variation did not contribute significantly to the observed variation in FA and we proceeded by fitting a model with additive genetic and unique environmental influences only (AE). Under this model, FA heritability was significant only in the younger cohort (32% males, 37% females). These models were refitted after excluding the 10%, 20% and 30% of subjects that scored lowest on the average of F3 and F4 power. Heritability estimates in the middle-aged cohort remained non-significant. In the young males, evidence for a genetic contribution disappeared.

3.7. Genetic and environmental contribution to the association between FA and the risk for anxiety and depression

Bivariate genetic analyses were used to determine whether the observed correlation between FA and the factor score for anxiety and depression is due to genes or environmental factors shared between the two variables. That is, insofar FA and anxiety and depression correlate, how much of that shared variance can be attributed to genetic sources, and how much to environmental sources. Since a significant correlation between FA and the risk for anxiety and depression was only found in young females, we limited the bivariate genetic analysis to this group. As the best fitting models in young females estimated additive genetic and unique environmental effects (AE) on both the risk factor score and FA in the univariate cases, the bivariate models, too, estimated AE effects only.

The results showed that environmental correlations were close to and not significantly different from zero at all selection criteria (all $\chi^2 < 1$, ns). The genetic correlations were not significant in the full sample ($\chi^2(1) = 2.18$, $p = 0.14$), borderline significant after 10% of the subjects with the lowest frontal alpha were excluded ($\chi^2(1) = 3.54$, $p = 0.06$), and significant after 20 and 30% of the subjects with the lowest frontal alpha were excluded ($\chi^2(1) > 5.17$, $p < 0.05$). These results suggest that the observed correlation between FA and the risk for anxiety and depression in young females can be explained by shared genetic sources and not by an overlap in environmental influences.

4. Discussion

FA has been put forward as a biological marker for the risk for anxiety and depression (for reviews: Coan and Allen, 2004; Davidson, 1992). Anxiety and depression are heritable disorders and have found to be influenced by overlapping genes (Jardine et al., 1984; Kendler et al., 1986; Middeldorp et al., 2005b). FA has therefore great potential to be used as a so-called endophenotype in studies searching for genes that influence the shared neurobiological pathways that are affected in these disorders. Two requirements, however, are that FA is heritable and that the genes influencing FA also influence the risk for anxiety and depression. Here we explored this question in male and female twins and their siblings in two different age cohorts.

Our results show that frontal asymmetry was only heritable in young adulthood and was higher in young females (37%) than in young males (32%). FA heritability in young females rose slightly after selecting subjects with sufficient frontal alpha power (Anokhin et al., 2005), whereas in young males heritability disappeared. It may therefore be concluded that heritability is more robust in young adult females. These results are consistent with the previous twin studies on FA in young female adults that reported heritabilities of 33% (Allen et al., 1997) and 31% (Anokhin et al., 2005). The only twin study thusfar to include males (Coan, 2003) found a heritability of 22% in females and no significant heritability in males. With regard to cohort differences, none of these previous studies had included subject groups with mean ages older than 21, so we cannot compare our results in adults to previous work.

The heritability of young adult FA seems rather low, but it must be appreciated that resting FA consists of a mixture of trait and state components (Hagemann, 2004). Using Structural Equation Modeling on data recorded during four recording sessions 4 weeks apart, Hagemann et al. (2002) estimated about 40% of total FA variance to be state and 60% trait variance. Heritability of FA, therefore, was bound by a maximum of 60%. The contribution of state and trait variance may be unequally distributed across gender. Females may be more reactive to the experimental procedures involved in the EEG recordings perhaps in interaction with traits that are known to modulate FA, like defensiveness (Kline et al., 1998, 1999). If the EEG recording environment is more anxiogenic in women than in men, and this reactivity is genetically determined, then trait variance in women will show larger heritability estimates.

To index the risk for anxiety and depression we used a factor score obtained from multiple scales at multiple measurement occasions. As reported previously, this factor score is about 60% heritable in both males and females (Boomsma et al., 2000). The relation between the factor score and FA was not significant in older subjects or in young adult males. As with heritability, young females were the positive exception. Only in this group, the relation between FA and the risk for anxiety and depression became significant after excluding subjects with the lowest average frontal EEG power as suggested by Anokhin et al. (2005).

The finding that the relation between FA and anxiety and depression is restricted to females is in keeping with much of the previous literature. Studies that related FA to psychopathology were often limited to female subjects, or included a majority of females in their samples. This can be observed in the exhaustive summary of studies relating FA to psychopathology by Coan and Allen (2004; Table 3 in their paper), and has been explicitly noted by others (Miller et al., 2002). Of those that included an adult sample, five report exclusively on females (Allen et al., 1993; Field et al., 2000; Gotlib et al., 1998; Reid et al., 1998; Silva et al., 2002). Seven report on samples with a majority of females (Bruder et al., 2001; Davidson et al., 1985; Debener et al., 2000; Henriques and Davidson, 1991; Nitschke et al., 1999; Schaffer et al., 1983; Wiedemann et al., 1999). Four studies included males and females in about equal proportions (Heller et al., 1997; Miller et al., 2002; Minnix et al., 2004; Tomarken et al., 2004). By contrast, only two studies report on a majority of males (Gilbert et al., 1999; Petruzzello and Landers, 1994).

A stronger case for sex differences comes from studies that directly compared results from males and females (Baving et al., 2002; Bruder et al., 2001; Miller et al., 2002; Tomarken et al., 2004). Bruder et al. (2001) found increased right frontal activity only in depressed females not responsive to fluoxetine treatment and no effects in males or responsive females. Miller et al. (2002) found an effect in males opposite of that of females, that is, higher *left* frontal activity for males with family history of depression. Tomarken et al. (2004) found significant interaction effects between depression liability and sex in an ANOVA predicting FA depending on the reference montage: with vertex (Cz) as reference, FA was related to increased liability of depression in females, and not in males. In the field of anxiety, Baving et al. (2002) found greater right frontal activity in anxious 8- and 10-year-old girls and greater left frontal activity in 11-year-old boys. Similar sex differences have been reported in studies investigating defensiveness as measured by the Eysenck *L*-scale (Kline et al., 1998, 1999). These results, plus the results presented here, provide evidence for sex differences in the relation between FA and anxiety or depression.

The summary of studies on FA by Coan and Allen (2004) clearly reveals that while infants, adolescents, and especially young adults have been studied extensively, older adults are underrepresented. Henriques and Davidson (1990), reporting on subjects of 37 years on average, found evidence of group differences between depressed and non-depressed subjects congruent with the FA hypothesis. Baehr et al. (1998) found similar results with a measure related to FA in a sample of 43–57 years. Urry et al. (2004) found that self-reported well-being was related to greater left fronto-central activity in subjects 57–60 years. Kline et al. (1998, 1999) reported similar findings between a young adult and an elderly age group in the relation between FA and defensiveness. Other studies included both younger and older subjects, but did not report their data separately for the age groups (Bruder et al., 2001; Debener et al., 2000; Davidson et al., 2000; Jacobs and Snyder, 1996; Minnix et al., 2004). In contrast to these previous results, the

current results showed no evidence of a relation between FA and the risk for anxiety and depression in a large middle-aged Dutch sample. In view of the genetic analyses, this should not be surprising. In the middle-aged cohort variance in FA only reflected the accumulated effects of environmental factors and life events unique to family members. Because the young cohort showed that FA and the factor score were correlated entirely by underlying genetic factors, the lack of a correlation between FA and the risk for anxiety and depression simply may simply reflect the absence of heritable influences on FA in this age range.

In short, the relation between FA and the risk for anxiety and depression is most robust in young females. This relation was fully explained by shared genes influencing both EEG and disease risk. At least in young females, FA may be a valid endophenotype that can support future gene finding for these disorders (De Geus, 2002), provided subjects are selected who have sufficient resting alpha power on the frontal leads. The most striking conclusion deriving from this study may be that future studies on asymmetry of left and right frontal brain activation should carefully consider the effects of both sex and age.

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