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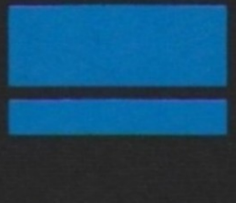

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Genetics and Psychopathology

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In this chapter, we introduce the major principles of genetic research in psychopathology. To illustrate these principles, an overview of genetic studies of depression and anxiety is given. We first introduce the background of genetic epidemiology that focuses on the question to what extent a trait or disorder clusters in families and if this clustering has a genetic basis. The methods to investigate issues going beyond the question of heritability are described, including sex and age differences in the genetic architecture, gene-environment correlation, gene-environment interaction, and multivariate genetic approaches to examine the etiology of comorbidity between traits or disorders. This section is followed by a discussion of the results of these types of studies on anxiety and depression. Next, the methods of gene findings studies are introduced again followed by a discussion of the results of linkage and association studies that aim at localizing and identifying the genes underlying the genetic component in anxiety and depression. Finally, we briefly touch on some issues complicating genetics in psychopathology.

ASSESSMENT OF THE IMPORTANCE OF GENETIC VARIATION IN COMPLEX TRAITS

Individual differences in complex traits (like psychiatric disorders, intelligence, height, or blood pressure) may be due to genetic or environmental factors. These traits are called complex because their genetic architecture most likely is complex. They are influenced by multiple genetic as well as environmental effects and do not show a simple pattern of Mendelian inheritance. The influence of these factors on variation in normal and abnormal human behavior may be additive or may manifest itself through more complex pathways in which the influences of genes and environment interact (Falconer & Mackay, 1996; Lynch & Walsh, 1998; Plomin, DeFries, McClearn, & McGuffin, 2008).

Genetic factors represent the effects of one or many unidentified genes. For quantitative, complex traits these effects are due to a possibly large, but unknown, number of genes (polygenes). Genes can only influence variation if they are polymorphic, that is, occur in two or more variants in the population, called alleles. The effect of alleles can be additive (their effects sum up) or alleles at the same or different loci can interact. Interaction, or genetic nonadditivity, between alleles within the same locus is referred to as *genetic dominance;* interaction between alleles across different loci is referred to as *epistasis.* In data from humans, genetic dominance and epistasis are difficult to distinguish. Moreover, relatives will not show a high degree of resemblance for traits that result from genetic nonadditivity. There is one exception: Identical twins will resemble each other also for traits that show dominance or epistasis. A large difference in the degree of resemblance between identical twins and first degree relatives thus gives an indication that genetic nonadditivity plays a role.

The relative influence of genetic factors on phenotypic variation (where the phenotype stands for any observable trait), the heritability, is commonly defined as the proportion of total phenotypic variance that can be attributed to genetic variance. *Broad-sense* heritability includes all sources of genetic variance (additive and nonadditive), *narrow-sense* heritability only includes additive genetic variance.

All nongenetic influences on phenotypic variation are referred to as environmental influences and include the early influences of prenatal environment, the influence of the (early) home environment, the influence of the neighborhood and many other, usually unidentified nongenetic effects. Environmental influences are often distinguished into two broad classes: common environmental influences that are shared among family members (e.g., siblings) who

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grow up in the same family and that tend to make them alike, and unique environmental influences, that is, environmental influences that are unique to an individual and are not shared among family members. Measurement error and other sources of unreliability in a trait contribute to the unique environmental influences.

To estimate the influences of genotype and environment on phenotypic variation, it is not necessary to collect genetic material (DNA) or to measure the environment. The relative importance of both sources of variation may be estimated by statistically analyzing data that have been collected in groups of individuals who are genetically related or who do not share their genes, but who share their environment (Boomsma, Busjahn, & Peltonen, 2002; Martin, Boomsma, & Machin, 1997). For example, data from adopted children may be compared with data from their biological and adoptive parents. The resemblance between adopted children and their biological parents reinforces the importance of genetic inheritance, the resemblance of adoptive parents and their adopted children relates to the importance of cultural inheritance and shared home environment. There are some famous adoption studies on the inheritance of schizophrenia. For example, Heston (1966) looked at adopted children whose biological parents suffered from schizophrenia versus adopted children whose biological parents did not suffer from schizophrenia. All of the children studied were given up for adoption immediately after birth. Those children with a much higher chance to get the disorder had a biological parent who suffered from schizophrenia. These results clearly indicate a role for genetic factors in the development of schizophrenia.

However, adoptions are relatively rare and often neither the adopted child, nor the adoptive parents are entirely representative of the general population. Therefore, the majority of studies that estimate heritability of complex traits make use of the classical twin design to unravel sources of variance. An introduction to this methodology can be found, for example, in Boomsma et al. (2002), Kendler and Eaves (2005), Plomin et al. (2008), and Posthuma (2003). In the classical twin design, data from monozygotic and dizygotic twins are used to decompose the variation of a trait into genetic and environmental contributions by comparing within pair resemblance for both types of twins. Monozygotic (MZ) twins share their common environment and (nearly always) 100% of their genes. Dizygotic (DZ) twins also share their common environment and on average 50% of their segregating genes (Hall, 2003). If MZ within twin pair resemblance for a certain trait is higher than DZ within twin pair resemblance, this suggests the presence of genetic influences on that trait. A first impression of the narrow-sense heritability (a^2) of a phenotype can be calculated as twice the difference between the MZ and DZ correlations: $a^2 = 2(rMZ - rDZ)$. The expectation of the correlation in MZ twins equals: $rMZ = a^2 + c^2$ (where c^2 represents the proportion of the total variance attributable to common environment, that is, the environment shared by children raised in the same family). The expectation of the correlation in DZ twins equals: $rDZ = ½a^2 + c^2$.

If there is, in addition to additive genetic influences, a contribution of genetic dominance, the expectations for the MZ correlations is: $rMZ = a^2 + d^2 + c^2$ and for the DZ correlation it is: $rDZ = ½a^2 + ¼d^2 + c^2$. This is the situation in which the MZ correlation can be substantially higher than the DZ correlation and where the simple approach of doubling the difference between the two correlations is no longer appropriate. In this situation, the broad-sense heritability (h^2) must be estimated using different approaches, outlined below, and represents the influence of additive and nonadditive genetic factors. Although it is possible that both genetic dominance and common environment are of importance, these effects cannot be simultaneously estimated in the classical twin design if only data from MZ and DZ twins reared together are available.

If MZ within twin pair resemblance for a certain trait is similar to DZ within twin pair resemblance, or if $rMZ < 2rDZ$ this suggests the presence of common environmental influences on that trait. A first impression of the effect of common environmental influences can be calculated as $c^2 = rMZ - a^2$ (or $c^2 = 2rDZ - rMZ$). It may be important to emphasize that it is unknown what the common environment includes. One can think of parenting or socioeconomic status, but this needs to be investigated if a common environmental effect is found. A first impression of the importance of unique environmental influences can be calculated as $e^2 = 1 - rMZ$. Finally, if genetic dominance plays a role, its importance can be estimated as: $d^2 = 4rDZ - rMZ$.

If, for example, the correlation for a certain trait equals 0.60 in MZ twins and 0.45 in DZ twins, then the estimate for $a^2 = 2(0.60–0.45) = 0.30$, for $c^2 = 0.60 - 0.30 = 0.30$ and for $e^2 = 1 - 0.60 = 0.40$. If, on the other hand, the correlation in MZ twins equals 0.8 and the correlation in DZ twin pairs equals 0.3, nonadditive effects are probably present. Then, the estimate for d^2 is $1.2 - 0.8 = 0.4$ and the estimate for a^2 is also 0.4; giving a total heritability for the trait of 0.8 (note that in this case the total heritability is equal to the MZ correlation). The effect of e^2 is 0.20. The effect of c^2, *if it were present, cannot be estimated.*

Structural Equation Modeling in the Classical Twin Design

To test how well the expectations for the resemblance of relatives describe the actual data and to test which model (e.g., AE, ACE, CE, or ADE) describes the data best, parameters can be estimated by maximum likelihood or other

approaches. Structural equation modeling (SEM) or genetic covariance structure modeling (GCSM) provides a general and flexible approach to analyze data gathered in genetically informative samples such as in the classical twin study. In applying GCSM to data from relatives, genotypic and environmental effects are modeled as the contribution of latent (unmeasured) variables to the (possibly multivariate) phenotypic individual differences. These latent factors represent the effects of many unidentified influences, for example, polygenes and environment. For a more detailed overview of GCSM, see Boomsma and Molenaar (1986), Boomsma and Dolan (2000), Neale (2000), and Posthuma et al. (2003). Structural relations between measured variables (phenotypes) and unmeasured variables are often graphically represented in a path diagram, which is a mathematically complete description of a structural equation model. An example of such a model for a single trait in a twin pair is shown in Figure 60.1. The variables in squares are the observed phenotypes in twin 1 and in twin 2. The variables in circles are latent (unobserved). Their influence on the phenotype is given by path coefficients a, c and e.

Identification of structural equation models in genetics is achieved from a design that includes relatives at different degrees of relatedness, for example, by inclusion of monozygotic (MZ) and dizygotic (DZ) twins into the study. Knowledge about Mendelian inheritance patterns defines the correlations among the latent factors in Figure 60.1. The path coefficients in Figure 60.1 define the relative importance of A, C and E on the phenotype (P): P = aA + cC + eE. Expressed in variance components, the phenotypic variance can be written as: var(P) = a^2 var(A) + c^2 var(C) + e^2 var(E), under the assumption that A, C and E are uncorrelated and do not interact. If var(A) = var(C) = var(E) = 1, the expression for the population variance reduces to: var(P) = a^2 + c^2 + e^2. Please note a possible source of confusion: in the above expression a^2, c^2, and e^2 represent variance components; often, as we did ourselves in the introduction above, they represent standardized components (i.e., the variance components divided by the variance of P).

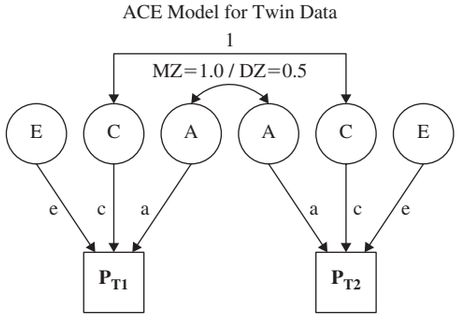

Figure 60.1 Path diagram for a single phenotype (P) assessed in twin 1 and twin 2.

Note: P is influenced by latent factors A (additive genetic influences), C (common environment shared by twins) and E (unique environment). Parameters a, c and e represent the nonstandardized factor loadings. The latent factors are standardized. The correlation between the latent A factors depends on zygosity, and is 1 for MZ (monozygotic) and 0.5 for DZ (dizygotic) twins. The correlation between C factors is independent of zygosity as MZ and DZ twins share the same amount of environment. The correlation between A factors and between C factors may depend on sex of the twins. Especially in the presence of qualitative sex differences, in dizygotic twins of opposite sex the correlation between A factors can be lower than 0.5 or the correlation between C factors can be lower than 1 (indicating that different genes are expressed in men and women; or that different common environmental factors are of importance in the two sexes).

The contributions of the latent variables are estimated as regression coefficients in the linear regression of the observed variables on the latent variables. Given an appropriate design providing sufficient information to identify these regression coefficients actual estimates may be obtained using a number of well disseminated computer programs, such as LISREL (Jöreskog & Sörbom, 1989) or Mx (Neale, Boker, Xie, & Maes, 2006). These programs allow estimation of parameters by means of a number of estimators including normal theory maximum likelihood (ML) and weighted least squares (WLS).

These estimators can also be applied to estimate and analyze correlations among family members for discrete variables or variables that show a nonnormal distribution (Derks, Dolan, & Boomsma, 2004). Data from relatives on categorical traits (e.g., presence or absence of disorder) are analyzed within this framework by making use of a threshold model that assumes there is a continuously and normally distributed liability underlying a disorder in the populations. One or more thresholds divide the continuous distribution into discrete classes, for example, affected and unaffected (Figure 60.2; Falconer & Mackay, 1996). The tetrachoric (for dichotomous traits) or polychoric (for ordered-category data with more than 2 categories) correlations represent the resemblance between relatives on the unobserved liability dimension.

The significance of parameters a, c (or d) in Figure 60.2 is tested with a likelihood ratio test. The test involves constraining the parameter of interest at zero and then testing whether the constraint leads to a significant decrease in goodness-of-fit of the model. Twice the difference between the log-likelihood of two models (e.g., an ACE model and an AE model in which the influence of C is constrained at zero) is distributed asymptotically as χ^2. The degrees of freedom for the test are equal to the difference in parameters being estimated. Utilizing the principle of parsimony, the most restrictive model is accepted as the best fitting one in case the difference between a nested and a more comprehensive model is not significant (Neale & Cardon, 1992). The e parameter cannot be dropped from the model because this also includes the measurement error.

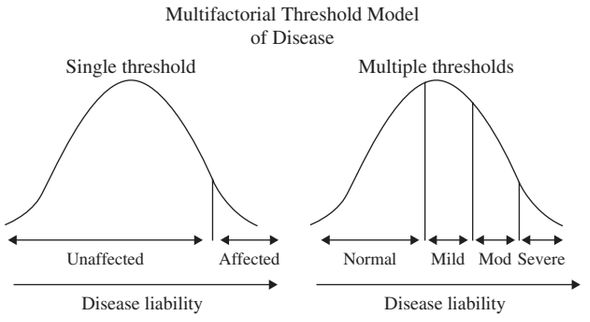

Figure 60.2 A threshold model assumes a normally distributed liability (or vulnerability) underlying a disorder or an ordered-category trait.

Note: The left figure shows a single threshold model with subjects scoring below the threshold on the, unobserved, liability scale being unaffected and subjects scoring above the threshold being affected. The right figure shows a model with multiple thresholds, for example mild, moderate, or severe depression. The tetrachoric correlation (for binary data) and the polychoric correlation (for ordered-category data) estimate what the correlation between family members would be if ratings for these traits were made on a continuous scale.

Beyond the Question of Heritability

Sex and Age Differences in the Genetic Architecture

The contributions of genetic factors to phenotypic variance may differ between men and women, as may the contribution of environmental factors. The expression of the genotype may also change with age. If we again denote the influence of A, C, and E on the phenotype by parameters a, c, and e, and the proportion of variance due to each of these factors as the square of these parameters, then three different models can be examined for quantitative sex differences in genetic architecture:

1. A full model in which estimates for a, c, and e are allowed to differ in magnitude between males and females. The outcome of the model can be, for example, that the genetic variance is the same in both sexes and the environmental variance larger in men than in women.
2. A scalar model in which heritabilities are constrained to be equal across sexes, but in which the total trait variance may differ in men and women. In the scalar model, all variance components for females, for example, are constrained to be equal to a scalar multiple i, of the male variance components, that is, $a_f = ia_m$, $c_f = ic_m$, and $e_f = ie_m$. As a result, the standardized variance components (such as heritabilities) are equal across sexes, even though the unstandardized components differ (Neale et al., 1992).
3. A constrained model in which parameter estimates for a, c, and e are constrained to be equal in magnitude across sexes.

If data from male and female twins are available, these quantitative sex differences models can be evaluated with standard likelihood ratio tests comparing the fit of the different models. If, for example, in males the correlation in MZ twins equals 0.60 and in DZ twins 0.30, while in females the correlation in MZ twins equals 0.70 and in DZ twins 0.40, the estimate for $a^2 = 0.60$ in males and females, but the estimate for $c^2 = 0$ in males and 0.10 in females. The estimate for e^2 is 0.40 in males and 0.30 in females. The likelihood ratio test will show whether these differences are significant. Likewise, if data are available for twins of different ages, for example, adolescent and adult twin pairs, then the significance of age differences in heritability can be tested.

If data are available for dizygotic opposite-sex (DOS) twins, a model for qualitative sex differences can be evaluated. Within this model, the test of interest is whether the same genes are expressed in men and women. This model is tested by estimating the correlation between genetic factors in the DOS pairs instead of fixing it at 0.5. If the correlation is significantly lower than 0.5, this indicates that different genes are expressed in the two sexes. A large difference between the correlation in same-sex DZ twins and DOS twins points to qualitative sex differences.

This qualitative sex differences model can also be applied in the context of an environmental hypothesis: instead of fixing the correlations between C factors at 1 in DOS twins, it can be estimated as a free parameter. If it is significantly lower than 1.0, this indicates that the influence of the shared environment differs in the two sexes. Note, however, because there is only one group of opposite-sex twins (there are no monozygotic twins of opposite sex, except in extremely rare cases) that a choice needs to be made to test either the genetic or the common environment correlation, but they cannot be estimated simultaneously.

Multivariate and Longitudinal Analyses

The decomposition into genetic and environmental variances for a single trait can be generalized to longitudinal and multivariate data where the variation and covariation of traits is decomposed into genetic and nongenetic sources (Boomsma et al., 2002; Boomsma & Molenaar, 1986; Martin & Eaves, 1977). In such data, the cross trait-cross twin correlations indicate how the value of twin 1 for trait A (e.g., depression) predicts the value of twin 2 for trait B (e.g., anxiety), and vice versa. The pattern of cross trait-cross

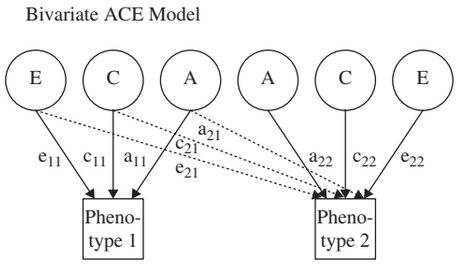

Figure 60.3 Genetic bivariate model, represented for one individual who is measured on two phenotypes.

Note: A, C, and E are the latent additive genetic, common environmental, and unique environmental factors, respectively, that influence the first phenotype (with factor loadings a_{11}, c_{11}, e_{11}), and/or the second phenotype (with factor loadings a_{21}, c_{21}, e_{21}), The second phenotype can also be influenced by a second set of independent latent factors (with factor loadings a_{22}, c_{22}, e_{22}). This model leads to the following equations for the estimates of the value of an individual's phenotype (P), the total variance (V_P), and the heritability (h^2) for phenotype 1 and phenotype 2:

twin correlations for MZ twins and DZ twins indicates (as described previously) to what extent the covariance between traits is influenced by genetic or environmental factors. Thus, if the cross trait-cross twin correlation is larger in MZ twins than in DZ twins, genetic effects are likely to explain the covariance between traits.

Multivariate and longitudinal studies thus offer insight into the etiology of associations between traits, the comorbidity between disorders, and the stability of traits across time. If, for example, the same set of genes influences multiple traits, this constitutes evidence for genetic pleiotropy. If longitudinal stability is due to genetic factors, this indicates that the same set of genes is expressed across the life span. For two variables (for a single individual), the correlation between traits can be decomposed into parts caused by correlated genetic and correlated environmental factors (Figure 60.3).

Genotype Environment Correlation and Interaction

The designs discussed all assumed that genetic factors act independently from environmental factors. However, this assumption might be false. Gene-environment correlation or gene-environment interaction might play a role (Eaves, 1987; Kendler & Eaves, 1986; Rutter & Plomin, 1997). In passive gene-environment correlation the environment of an offspring depends on the genotype of parents (Eaves, 1987). For example, children who inherit the risk for depression may also grow up in a suboptimal environment because of a depressed parent. Gene-environment correlation can also arise because an individual's environment depends on his own genotype, for example, in creating adverse life events. In other words, the exposure to a certain environment is under genetic control (Eaves, 1987; Kendler & Eaves, 1986). Gene-environment interaction reflects genetic control of sensitivity to the environment, that is, the effect of an environmental risk factor depends on the genetic make-up of an individual (Eaves, 1987; Kendler & Eaves, 1986). In the next section, methods used to investigate gene-environment correlation and interaction are described beginning with gene-environment correlation.

The classic twin design can be used to estimate to what extent the variation in exposure to a specific environment, for example, marital status or the experience of life events, is under genetic control. In other words, the twin design can be used to calculate the heritability of the "environmental" trait. Using the classical twin design, Johnson, McGue, Krueger, and Bouchard (2004) and Middeldorp, Cath, Vink, and Boomsma (2005) found that propensity to marry is heritable and McGue and Lykken (1992) found that divorce risk was, to a substantial degree, genetically mediated.

To examine whether the association between a specific environment and a trait is due to gene-environment correlation, the bivariate twin design can be applied (Purcell, 2002). This design investigates whether the genes influencing a behavioral trait also affect the chance of being exposed to a certain environment. Another approach to investigate this issue is the co-twin control design (Cederlof, Friberg, & Lundman, 1977; Kendler et al., 1993). In this design, the relative risk to have a disorder in the presence of a putative risk factor is calculated in a group of monozygotic (MZ) twins discordant for exposure to the risk factor, a group of dizygotic (DZ) twins discordant for exposure to the risk factor, and in a population consisting of unrelated subjects. If the relation between the risk factor and the disorder is causal and gene-environment correlation is absent, the relative risks will be the same in the three groups. If, on the other hand, the correlation between the risk factor and the disorder is due to genes that lead both to a higher risk for the disorder and to a higher risk of exposure to the risk factor, the relative risk will be higher in the total population than in the discordant dizygotic twins, whose relative risk will in turn be higher than the relative risk in the discordant monozygotic twins. Moreover, when gene-environment correlation entirely explains the relation between the risk factor and the disorder, the relative risk in MZ twins will be unity. This is because the unexposed member of MZ twins has the same genetic vulnerability to get the disorder as the twin who is exposed to the risk factor. Since DZ twins share on average half of their genes, the unexposed twin will share some of the genetic

vulnerability to the disorder with the twin exposed to the risk factor. Unrelated subjects will show the highest relative risk.

Kendler et al. (1993) investigated whether the relation between smoking and depression was causal or due to shared genes influencing vulnerability for both traits. Although the relative risk for ever smoking given a lifetime history of depression was 1.48 in the entire sample, it was 1.18 and 0.98, respectively, in DZ and MZ twin pairs discordant for a history of depression. The relative risk for a history of depression given ever smoking was 1.60 in the entire sample, while in DZ and MZ twins discordant for smoking, it was 1.29 and 0.96, respectively. These results suggest that the association between smoking and depression in women is not a causal one but arises largely from familial factors, which are probably genetic, that predispose to both smoking and depression.

An approach to investigate gene-environment interaction is to estimate the relative influences of genotype (heritability) and environment on a trait conditional upon environmental exposure (Boomsma & Martin, 2002; Eaves, 1987; Heath, Eaves, & Martin, 1998; Heath, Jardine, & Martin, 1989; Kendler & Eaves, 1986). When there is no G $\times$ E interaction, the influence of genetic and environmental factors should not differ between subjects with different degrees of exposure. If genetic effects are modified by environmental exposure, such that heritabilities differ significantly between exposure-positive and exposure-negative groups, then this constitutes evidence for gene $\times$ environment interaction. Thus, this type of interaction is detected by testing whether the amount of variance explained by genetic factors differs between exposure-positive and exposure-negative groups.

Purcell (2002) developed a model to investigate gene-environment interaction more extensively, for example, when an environmental risk factor is measured on a continuous instead of a dichotomous scale. The genetic effects are partitioned into a mean part, which is independent of the environmental moderator, and a part that is a linear function of the environmental moderator. The model also allows for a test of gene-environment interaction in the presence of gene-environment correlation.

GENETIC EPIDEMIOLOGY OF ANXIETY AND DEPRESSION

Univariate Analyses

A large number of twin studies have investigated the influence of genetic and environmental factors on depression and anxiety. The review of these studies will be limited to population-based twin studies on depression and anxiety disorders as classified by the *DSM* (American Psychiatric Association, 1980, 1987, 1994), starting with the results of single trait analyses. A meta-analysis showed that major depression is around 37% heritable with the remaining part of the variance explained by individual specific environmental factors (Sullivan, Neale, & Kendler, 2000). Environmental factors shared by family members did not seem to be of major importance. The genetic epidemiology of depression was also investigated in 42,161 twins including 15,493 complete pairs from the national Swedish Twin Registry (Kendler, Gatz, Gardner, & Pedersen, 2006). Due to the large sample, this study detected common environmental influences or differences between men and women in the etiology of depression. The heritability was estimated at 29% in men and 42% in women, which was significantly different. Common environment did not explain any of the familial clustering. The genetic correlation between men and women was 0.69 indicating the existence of sex-specific genetic factors in addition to a set of shared genes.

The amount of measurement error is reflected in the estimate of the influence of the unique environment. One study that assessed lifetime diagnosis of major depression at two occasions was able to parse out the effect of measurement error due to unreliability, resulting in a heritability estimate of 66% (Foley, Neale, & Kendler, 1998). This suggests that genetic factors might be more important for major depression than generally assumed.

Anxiety disorders have been less extensively investigated. Meta-analyses showed that genetic factors explain 43% of the variance in panic disorder and 32% in generalized anxiety disorder (GAD; Hettema, Neale, & Kendler, 2001). For phobias, the heritability estimates varied somewhat around 30% with a maximum of 48% for agoraphobia (Kendler, Jacobson, Myers, & Prescott, 2002). The findings regarding the influence of common environment were inconsistent for social phobia, animal phobia, and GAD in women (Hettema, Prescott, & Kendler, 2001; Kendler et al., 2002; Kendler, Karkowski, & Prescott, 1999b; Kendler, Neale, Kessler, Heath, & Eaves, 1992). Partly depending on the definition of the disorder, a significant influence of the common environment was found. The authors suggested that these findings were due to stochastic factors and that it is most probable that the effect of the common environment is negligible (Hettema, Prescott, et al., 2001; Kendler et al., 2002).

Regarding sex differences in the genetic architecture for anxiety disorders, no major differences were found for GAD (Hettema, Prescott, et al., 2001; Middeldorp, Birley, et al., 2005). For social phobia, one study found quantitative sex differences but another did not (Kendler et al., 2002;

Middeldorp, Birley, et al., 2005). No sex differences were found for panic syndromes (Kendler, Gardner, & Prescott, 2001), while for agoraphobia, results indicated that the genes conveying the risk are probably not entirely the same (Kendler et al., 2002). The latter finding appeared to be supported by another study that investigated panic disorder and/or agoraphobia together (Middeldorp, Birley, et al., 2005). Qualitative sex differences were also found for situational and blood/injury phobia (Kendler et al., 2002).

One study on phobias took measurement error due to unreliable assessment into account (Kendler et al., 1999b). That resulted in heritability estimates around 50% indicating that for the anxiety disorders, as well as for depression, the heritability might be higher than assumed.

Multivariate and Longitudinal Analyses

Multivariate analyses of depression and anxiety disorders address the etiology of the comorbidity between these disorders. The frequent comorbidity within anxiety disorders and between anxiety disorders and depression is an important issue: Does the comorbidity arise because of shared genetic risk factors or are there other explanations? The results of the Epidemiologic Catchment Area (ECA) Study and the National Comorbidity Survey (NCS) have shown that the occurrence of one anxiety disorder increases the risk of having an additional anxiety disorder (odds ratio on average 6.7; Kessler, 1995). The same holds for the combination of affective disorders (including dysthymia and mania) and anxiety disorders (odds ratio 7.0; Kessler, 1995). These increased odds ratios indicate that comorbidity between anxiety and depression is not only due to chance. Moreover, since the ECA and NCS studies are population based, sampling bias is highly unlikely to explain comorbidity rates. The NCS replication study showed similar results (Kessler, Chiu, Demler, Merikangas, & Walters, 2005).

The issue of comorbidity gives rise to questions at a nosological level (Neale & Kendler, 1995). Do anxiety disorders and depression reflect an arbitrary division of a single syndrome? Are the different anxiety disorders and depression distinct entities, possibly influenced by common genetic and environmental etiological factors? Are the comorbid conditions independent of the separate anxiety disorders and depression? A review of twin and family studies investigating comorbidity within anxiety disorders and between anxiety disorders and depression concluded that they are distinct disorders with comorbidity probably partly explained by shared genetic factors (Middeldorp, Cath, van Dyck, & Boomsma, 2005). Possibly, this shared genetic vulnerability is expressed in the personality trait neuroticism (Middeldorp, Cath, et al., 2005). Most studies in this review performed bivariate analyses. Three twin studies carried out more extensive analyses (Hettema, Neale, Myers, Prescott, & Kendler, 2006; Hettema, Prescott, Myers, Neale, & Kendler, 2005; Kendler, Prescott, Myers, & Neale, 2003). Earlier factor analyses of common mental disorders showed that the latent structure of these disorders is best described by a three-factor model (Krueger, 1999; Vollebergh et al., 2001). One factor represents externalizing problems. The other two factors, which are subfactors of a higher-order factor representing internalizing problems, reflect anxious misery and fear. Kendler, Prescott, et al. (2003) aimed to extend these findings into a genetic epidemiological model. They showed that there are two genetic risk factors. One predisposes for internalizing disorders and the other for externalizing disorders. In addition, within the internalizing disorders, two genetics factors are seen that predispose to disorders dominated by anxious misery and fear.

Hettema et al. (2005, 2006) focused in more detail on the internalizing disorders. In their first study, including GAD, panic disorder, agoraphobia, social phobia, animal phobia, and situational phobia, they confirmed that two genetic factors influence these disorders (Hettema et al., 2005). In their second study, they focused on the association between neuroticism on the one hand and depression and anxiety disorders on the other. They showed that the genetic correlation between neuroticism and these disorders are high with estimates varying between 0.58 and 0.82. They also identified a second neuroticism-independent genetic factor significantly increasing the risk for major depression, generalized anxiety and panic disorder in addition to disorder specific genetic factors for the phobias. Comparing their results with the results of the previous studies performed in the same sample, it was hypothesized that a model with a third genetic factor influencing the phobias would provide a better fit. However, that model could not be tested due to computational problems (Hettema et al., 2006).

In all three studies, the influence of individual-specific environmental factors was largely disorder specific. The heritability estimates for depression and anxiety disorders were similar to the estimates from the univariate analyses, varying around 20% and 30%. For major depression and generalized anxiety disorder, these studies did not find an effect of the common environment. However, for panic disorder and social phobia, the common environment might explain 10% of the variance. Estimates vary somewhat for animal and situational phobia, but, in general, seem to be negligible (Hettema et al., 2005, 2006; Kendler, Prescott, et al., 2003).

Longitudinal studies on anxiety and depression are scarce. There has been one longitudinal twin study in adults with

a follow-up over 10 years (Gillespie et al., 2004). Symptoms of anxiety and depression measured with self-report questionnaires were assessed in Australian twins aged 20 to 96 years at three points over a period of 16 years. For male anxiety and depression, there was no genetic innovation after age 20, thus the same genes remained to explain variation in anxiety and depression at ages 30, 40, 50, and 60. Most of the lifetime genetic variation in female anxiety and depression could also be explained by a stable set of genetic factors; however, there were also smaller age-dependent genetic innovations at age 30 for anxiety and at ages 40 and 70 for depression.

In children, a longitudinal study on anxious depression was carried out in the population-based Netherlands Twin Register (Boomsma, van Beijsterveldt, Bartels, & Hudziak, 2008). Maternal and paternal ratings for anxious depression (A/D) were available for twins at ages 3, 5, 7, 10, and 12 with over 9,025 twin pairs at age 3 and over 2,300 pairs at age 12 being assessed. The influence of genetic factors declined with increasing age. The heritability was around 60% at age 3 and declined to 40% at age 12. The decrease in heritability when children grew older was accompanied by an increase in the influence of the common environment shared by twins (8% at age 3 and 23% at age 12). These results argue for shared environmental factors playing an important role in protecting children from or putting them at risk for the expression of A/D. The contribution of nonshared environmental factors ranged between 26% and 36%. This last result indicates that nonshared environment, or environmental influences that contribute to differences between siblings, plays a substantial role across development when considering the expression of A/D. However, when comparing this estimate for e^2 with the estimates from studies in adults, it is clear that the importance of unique environmental factors increases across the life span.

The results showed that the stability of A/D was relatively low between age 3 and later ages (correlations around 0.30), but became higher after age 7 (up to 0.67 between ages 10 and 12). The genetic correlations between A/D assessed at age 3 and other ages were modest, suggesting a small overlap of genes that influence A/D in preschool children and in middle childhood (genetic correlations between 0.24 and 0.35 for A/D at age 3 with other ages). These results raise the possibility of different genetic influences of genes across development, either by variable expression patterns, variable response to environmental mediators and modifiers, or simply, evidence of developmental genetic processes. After the age of 7, the genetic correlations were larger (0.63 to 0.70), indicating that the extent to which the same genes operate across ages 7 to 12 was increased.

Across ages, the same common environmental factors were suggested because a single C factor could explain the covariance pattern across age. Family variables, such as parental conflict, negative familial environments, and separation are likely candidates for these shared environmental influences. Future genetic research should include such environmental variables (e.g., parental divorce) to specify the role of these environmental factors. Nonshared environmental factors operate mainly in a time-specific manner.

A part of the shared environment reflects parental bias. By using the same rater at two or more points, the prediction of A/D could reflect some shared rater bias. If this is the case, the observed stability is not only a reflection of stability of children's problem behavior but also a reflection of the stability of the mother's perception. However, by applying a longitudinal model to both father and mother ratings, it is possible to disentangle the effects due to real environment and the effect due to rater bias (Hewitt, Silberg, Neale, Eaves, & Erickson, 1992). The results indicated that there is still evidence for shared environmental influences on the stability of A/D when data from the father and mother are analyzed simultaneously. However, results indicate also that the rater-specific shared environment contributes to stability of A/D. This could point to possible rater bias that is persistent and affects the stability of A/D.

Conclusions

Depression and anxiety in adults are moderately heritable with genetic influences estimated mostly around 30% and 40%. These might be underestimates because studies taking measurement error into account have found that 50% to 60% of the variance could be explained by genetic factors. A common environment does not seem to play a major role in most internalizing disorders, but might be of importance in panic disorder and social phobia, although accounting for only 10% of the variance. Quantitative and qualitative sex differences in etiological factors appear to be modest. The comorbidity between major depression and anxiety disorders and within anxiety disorders is largely explained by common genetic factors. It seems that one genetic factor, expressed in the personality trait neuroticism, explains the comorbidity between internalizing disorders with two additional genetic factors influencing the risk for anxious misery and fear. Individual specific environmental factors are largely disorder specific. Although the genetic determinants of anxiety and depression appear relatively stable across the adult life span for men and women, there is some evidence to support additional mid-life and late-age gene action in women for depression. In children, the influence of genetic factors decreases with age, while the influence of the common environment becomes more important between age 7 and 12. Correlations between measures of A/D are low

at younger ages, but become higher after age 7. Most of the stability across age is due to genetic stability and it appeared that other genes become important when children become older, In contrast, there is one common environmental factor influencing anxious depression in children through age 7 and 12. Unique environmental influences are largely age-specific. The conclusions regarding the influences of genes and environment across life span are based on one study in adults and one study in children, so confirmation by other studies is required.

Gene-Environment Correlation and Interaction

An important indication that gene-environment correlation exists is given by a review demonstrating that several environmental factors, such as life events, parenting style, peer deviance, and social support, are modestly influenced by genetic factors (Kendler & Baker, 2006). Weighted heritabilities ranged from 7% to 39% with most estimates falling between 15% and 35%. Studies with multiple measurements of life events and social support found that temporal stability in the environment is influenced to a much greater extent by genetic factors than occasion specific events.

Gene-environment correlation has been shown to explain the association between life events and depression in some twin and family studies (Kendler, Karkowski, & Prescott, 1999a; Kendler & Karkowski-Shuman, 1997; McGuffin, Katz, & Bebbington, 1988), but not in others (Farmer et al., 2000; Romanov, Varjonen, Kaprio, & Koskenvuo, 2003). One of the studies that found support for gene-environment correlation suggested that the correlation could be explained by genes that influence personality traits associated with depression (Kendler et al., 1999a). This hypothesis was later confirmed for neuroticism, extraversion, and openness for experience (Kendler, Gardner, & Prescott, 2003; Saudino, Pedersen, Lichtenstein, McClearn, & Plomin, 1997).

One study investigated the relation between the exposure to life events and anxious depression measured with a self-report questionnaire in a longitudinal and a genetic design (Middeldorp, Cath, Beem, Willemsen, & Boomsma, 2008). The results suggested that the relation between life events and anxious depressive symptoms is due to a causal reciprocal relation. Gene-environment correlation did not seem to play a role. The personality traits neuroticism and extraversion were also included in the analyses. The latter was not related to life events at all. In contrast, neuroticism scores were increased, but to a lesser extent than depression scores, after life events. Moreover, higher neuroticism scores increased the chance of the exposure to a life event later in life. Again, gene-environment correlation did not seem to be important.

The results so far have been inconclusive. Some findings are in favor of gene-environment correlation between life events, depression, or neuroticism, but others are not. Investigating gene-environment correlation is important to get more insight into etiological mechanisms. Exclusion of gene-environment correlation is an essential step before investigating gene-environment interaction (Moffitt, Caspi, & Rutter, 2005).

Studies on gene-environment interaction for anxiety and depression with unmeasured genes have been limited to life events and marital status (Heath et al., 1998; Kendler et al., 1995; Silberg, Rutter, Neale, & Eaves, 2001). The risk for depression after a life event is higher if there are indications of a genetic vulnerability for depression, that is, a family history of depression and vice versa the genetic variance increases in the presence of life events (Kendler et al., 1995; Silberg et al., 2001). Regarding marital status, it appears that being married protects against the expression of genetic risk for depression (Heath et al., 1998).

GENE FINDING METHODS

Linkage Studies

Obtaining evidence that a trait is heritable opens up a whole new avenue of research: Where are the genes localized that influence the phenotype and can we identify them? The first question can be addressed in linkage studies, the second question in genetic association studies. Although there is a wide range of dedicated software packages to carry out genetic linkage and association studies (Abecasis, Cardon, & Cookson, 2000; Abecasis, Cherny, Cookson, & Cardon, 2002; Abecasis, Cookson, & Cardon, 2000; Almasy & Blangero, 1998; Gudbjartsson, Thorvaldsson, Kong, Gunnarsson, & Ingolfsdottir, 2005; Kruglyak, Daly, Reeve-Daly, & Lander, 1996; Purcell et al., 2007), we introduce this type of analyses within the context of genetic structural equation modeling (GCSM). GCSM can relatively easy incorporate measured genotypic information into the analysis. Genotypic information derives from polymorphic marker data that are assessed in DNA samples of subjects for whom phenotypic information is also available. If DNA markers are roughly evenly spaced along the genome, if their location is known, and if they are highly polymorphic (e.g., microsatellite markers that have multiple alleles), then they can be used in linkage studies. These studies make use of the fact that when enough DNA markers are measured, a stretch of markers will be close to the gene that influences the trait of interest.

The location of a gene that influences a complex, often quantitative trait, is called a quantitative trait locus (QTL).

A QTL represents a stretch of a chromosome, which includes a segregating gene, or multiple genes, that contributes to individual differences in the phenotype of interest. The segregating gene has a relatively large contribution to the phenotypic variance compared to the contributions of each polygene making up a genetic latent variable. However, compared to the total effects of the polygenetic and environmental effects, the effect of the QTL may be quite small. For instance, the QTL may account for a mere 5% or 10% of the phenotypic variance. The QTL can be treated in the same way as a polygenetic or an environmental factor, that is, as a latent variable and the relationship between the QTL and the phenotypic individual differences is modeled as a linear regression. If a set of markers is close to the QTL, then resemblance on the markers reflects resemblance for the QTL. The effect of the QTL is modeled on the covariance structure: If siblings who share more markers identical by descent (IBD) across a stretch of chromosome are more alike (their correlation for the trait is higher) than siblings who do not share any markers (Sham, 1997; Vink & Boomsma, 2002), this is evidence for linkage.

Figure 60.4 shows a path model for DZ twins or siblings that incorporates the effect of a QTL on a measured phenotype. The correlation between QTL factors of DZ twins or siblings is obtained from measured genotypic (marker) data. IBD status for the marker data determines this correlation. IBD status in sibling pairs can be 0, 1, or 2, depending on whether the two siblings inherit the same marker allele from each parent (in which case, IBD = 2), whether they receive one allele IBD from one, but not from the other parent (in which case, IBD = 1), or whether they receive a different allele from each parent (in which case, IBD = 0). Parents always share one allele with their offspring, therefore their IBD probabilities of sharing 0, 1, or 2 alleles will be IBD0 = 0.0, IBD1 = 1.0, and IBD2 = 0.0. For two siblings, the IBD probabilities depend on their genotypes on the marker position and surrounding markers. When their parents are genotyped, the IBD status of siblings can be derived from the transmission of alleles from parents to offspring. When parents are not genotyped, the allele frequencies of markers are used in the estimation of IBD. If parents have four distinct alleles, for example, the father "A" and "B" and mother "C" and "D," the IBD status of their offspring can easily be determined (see Figure 60.5). If sibling 1 has AC and sibling 2 also has AC, the IBD probabilities will be IBD0 = 0.0, IBD1 = 0.0 and IBD2 = 1.0. If sibling 2 has AD instead, IBD0 = 0.0, IBD1 = 1.0, IBD2 = 0.0, and so on. However, if one of the parents is homozygous (e.g., AA), or if a genotype is missing, the IBD probabilities are calculated by maximum likelihood from all possible combinations of genotypes and their probabilities based on the allele frequencies of the marker, for example, IB0 = 0.0, IBD1 = 0.752, and IBD2 = 0.248. (For details on these procedures, see Haseman & Elston, 1972; Kruglyak & Lander, 1995; or Abecasis et al., 2002). From the IBD probabilities, the correlation for the QTL marker (π) is calculated as $0 \times \text{IBD0} + 0.5 \times \text{IBD1} + 1 \times \text{IBD2}$ for each individual pair in each family (Sham, 1997). Within the context of genetic structural equation modeling

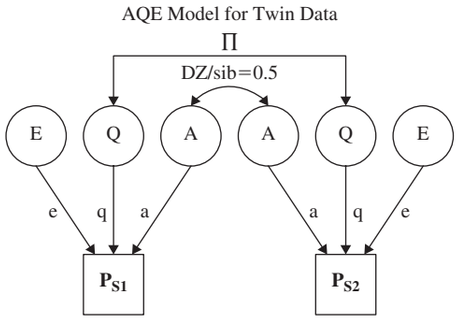

Figure 60.4 Linkage model for DZ twin or sibling data: the phenotype assessed in sibling 1 and sibling 2 (P_{S1} and P_{S2}) is influenced by additive genetic factors (A), environment (E), and a quantitative trait locus (QTL).

Note: The correlation n in two siblings for the QTL depends on the measured DNA marker data and is defined based on their Identity by descent (IBD) status. If path coefficient q is significant, this is evidence for linkage, that is, the trait locus is close to the markers that defined IBD.

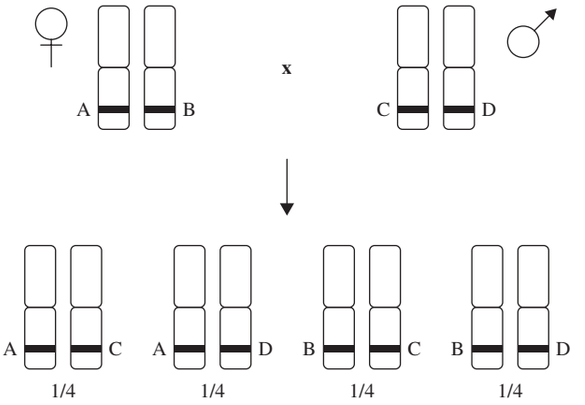

Figure 60.5 (A) Graph showing the possible allele combination for children from a mother carrying alleles A and B and a father with alleles C and D. (B) The table shows the 16 possible combinations of genotypes and the number of alleles identical by descent (IBD) for each combination for two siblings with a mother carrying alleles A and B and a father carrying alleles C and D.

Note: The chance for each combination (AC, AD, BC, and BD) in an offspring is ¼. The probability that two siblings share two parental alleles (IBD=2) is 4/16 = ¼. The probability that they do not share any parental alleles is also 4/16 = ¼, but the probability that they share one parental allele is 8/16 = ½.

(see also Figure 60.4), the test for linkage involves constraining the factor loading (q) of the phenotype on the QTL factor at zero and testing if this constraint leads to a significant decrease in goodness of fit. This test is based on a likelihood-ratio test and is distributed as χ^2. In classical linkage analyses of Mendelian traits (traits that are influenced by a single locus), the commonly used test-statistic is the LOD score. In parametric linkage analysis, it is standard practice to summarize the results of a linkage analysis in the form of an LOD score (Morton, 1955). LOD score stands for the logarithm of the odds that the locus is linked to the trait and indicates the strength of the linkage. Evidence for linkage is present when the maximum LOD-score exceeds a predefined threshold, which depends on the size of the genome and the number of markers (Lander & Kruglyak, 1995). A commonly used threshold is a LOD score of 3. This critical value can be interpreted as stating that the evidence in favor of linkage is 1,000 times more likely than the null hypothesis of no linkage. If an LOD score of 2 was observed, then the null hypothesis of no linkage is 100 times more likely than the alternative. There is a simple correspondence between χ^2 and LOD scores: LOD = $\chi^2/2\ln 10$ (Sham, 1997).

Association Studies

In contrast to linkage studies that model the covariance structure in relatives, genetic association analysis models the effect of a genetic polymorphism on the trait levels. This can be done in cases and controls (e.g., dichotomous traits) in groups of unrelated individuals (for the analysis of quantitative traits) or within families. Association studies in unrelated subjects are similar in design to classic case-control studies in epidemiology. DNA collected from all participants and frequencies of the various allelic variants are compared in subjects with particular phenotypes (e.g., presence or absence of disease) to detect an association between a particular allele and the occurrence of the phenotype. The association test can be carried out for presence or absence of a particular allele, or a particular genotype. For quantitative traits, the trait values are compared across the various allelic or genotypic variants of the DNA marker. The advantage of association over linkage analysis is that association studies can detect the region of a QTL that has only very small effects on the trait (Risch & Merikangas, 1996). This increase in statistical power comes from the fact that the test is carried out on first-order statistics (means or prevalences) whereas linkage tests are carried out on second-order statistics (covariances).

Provided that either the selection of cases does not introduce population stratification or that the analyses properly control for such stratification, association studies provide a good complement to the linkage strategy. However, a potential problem of association studies is the danger that a spurious association is found between the trait of interest and any locus that differs in allele frequency between subpopulations. This situation is illustrated by the chopstick gene story described by Hamer and Sirota (2000). They describe a hypothetical study in which DNA markers were assessed in American and foreign students who often used chopsticks and students who did not. One of the DNA markers showed a correlation to chopstick use. Of course, this gene had nothing to do with chopstick use, but just happened to have different allele frequencies in Asians and Caucasians, who differ in chopstick use for purely cultural rather than biological reasons. Witte, Gauderman, and Thomas (1999) have evaluated the asymptotic bias in relative risk estimates resulting from using population controls when there is confounding due to population stratification. The direction of the bias is what one would expect from the usual principles of confounding in epidemiology: if the allele frequencies and baseline risks are both higher in a population, the bias is positive; if different, the bias is negative. Case-control studies of genetic associations thus can lead to false positive as well as to false negative results.

To prevent significant findings due to population stratification, within-family association designs have been developed because family members are usually well matched on a number of traits that could give rise to stratification effects (Spielman, McGinnis, & Ewens, 1993). Most available family-based tests for association were initially developed for binary traits, such as the Transmission Disequilibrium association Test (TDT) and the Haplotype Relative Risk test (HRR). Those tests are based on a design in which DNA is collected in affected individuals and their biological parents. Affected individuals must have received one or two susceptibility alleles from their parents. These alleles transmitted from parents to the affected individual can be viewed as a group of "case" alleles. The nontransmitted alleles from the parents can be considered as "control" alleles. In other words, those tests only need affected individuals and their parents; no other control group is required. In a different approach, the effects of genotypes on phenotypic means are partitioned into between-family and within-family components, by comparing the association of alleles and trait values across siblings from different families to the association of alleles and trait values across siblings within the same family. Sibling pairs are by definition ethnically and racially homogeneous and any difference in trait scores between siblings of different genotypes at a candidate marker, therefore, reflects true genetic association. By partitioning the mean effect of a locus into a between and a within-sibship component, spurious associations due to population stratification and admixture are controlled (Abecasis, Cookson, et al.,

2000; Fulker, Cherny, Sham, & Hewitt, 1999; Posthuma, de Geus, Boomsma, & Neale, 2004).

One problem with the candidate gene approach for most complex traits is the potentially huge number of genes that can serve as candidates. Several strategies are possible to select an optimal set of candidate genes. First, genes that are part of physiological systems known to influence the trait can be tested as candidates. Second, genes or chromosomal regions that are known to influence the trait in animals can be tested as candidate genes (or regions) in humans. Third, genes lying under a linkage signal can be the focus of research.

GENOME-WIDE ASSOCIATION STUDIES

Linkage is usually genome-wide, although until recently association studies were limited to candidate genes or candidate regions. This has changed with the possibility to assess very large numbers of genetic markers within an individual. So called Genome-Wide Association studies (GWA) assess 300,000 to 600,000 markers along the genome and test if an association between a disorder, or a quantitative trait level, and a specific allele can be detected in groups of unrelated cases (e.g., patients) and controls (e.g., healthy subjects) or within families. Association can be found either with functional genetic variants that have biological consequences, or with other variants that are in linkage disequilibrium with these variants. Linkage disequilibrium occurs when a marker allele (i.e., a single nucleotide polymorphism [SNP]) and the QTL are so close on a chromosome that they co-segregate in the population over many generations of meiotic recombination.

Gene Finding Studies in Anxiety and Depression

Linkage Studies

Seven genome-wide linkage analyses have been performed, aiming to locate genes for MDD on the genome (Camp et al., 2005; Holmans et al., 2004, 2007; McGuffin et al., 2005; Nurnberger et al., 2001; Zubenko et al., 2003). Other studies have focused on quantitative traits associated with a diagnosis of MDD, such as neuroticism (Cloninger et al., 1998; Fullerton et al., 2003; Kuo et al., 2007; Nash et al., 2004; Neale et al., 2005; Wray et al., 2008). Table 60.1 summarizes the most promising results of these studies, excluding the study of Holmans et al. (2004) because this is based on the same sample as used by Holmans et al. (2007). Several regions have shown a linkage signal with a LOD-score <3 in at least one study. The following five regions have reached a LOD-score >3 in one study and a LOD-score >1.5 in a second study: chromosome 1 between 126 and 137 cM (Fullerton et al., 2003; Neale et al., 2005), chromosome 8 between 8 and 38 cM (Cloninger et al., 1998; Fullerton et al., 2003), chromosome 11 between 2 and 35 cM (Camp et al., 2005; Zubenko et al., 2003), chromosome 11 between 85 and 99 cM (Fullerton et al., 2003; Zubenko et al., 2003) and chromosome 12 between 105 and 124 cM (Fullerton et al., 2003; McGuffin et al., 2005).

The most promising results of genome-wide linkage studies on anxiety phenotypes are summarized in Table 60.2 (Crowe et al., 2001; Fyer et al., 2006; Gelernter et al., 2001, 2003; Gelernter, Page, Stein, & Woods, 2004; Hamilton et al., 2003; Kaabi et al., 2006; Knowles et al., 1998; Middeldorp et al., 2008; Smoller et al., 2001; Thorgeirsson et al., 2003; Weissman et al., 2000). Three regions (chromosome 4, 9, and 13) showing significant linkage have not been replicated yet (Kaabi et al., 2006; Thorgeirsson et al., 2003; Weissman et al., 2000). Three other regions (chromosome 1, 7, and 14) have shown evidence for linkage in two studies (Crowe et al., 2001; Gelernter et al., 2001; Kaabi et al., 2006; Knowles et al., 1998; Middeldorp et al., 2008; Smoller et al., 2001). Gelernter et al. (2001, 2003, 2004) found a suggestive linkage signal on chromosome 14 for simple phobia, social phobia, and panic disorder. These three studies were performed on the same sample, thus the studies are not considered to be replications.

Only the region on chromosome 7 has been found in linkage studies on neuroticism and anxiety. No further overlap between Table 60.1 and 60.2 is seen.

Association Studies

Despite the large number of candidate gene studies, efforts to identify QTLs for depression and anxiety through this approach have met with limited success. For an overview of the results, we refer to Levinson (2006) and Stoppel, Albrecht, Pape, and Stork (2006). In this chapter, we limit the discussion of association studies to the widely investigated association between the promoter-based length polymorphism of the serotonin transporter gene (5-HTTLPR) and anxiety and depression.

The 5-HTTLPR polymorphism is located in the promoter of the gene and is defined by a length variation of a repetitive sequence with the short and the long fragment consisting of 484 and 528 base pairs, respectively. These variants are often denoted as "s" and "l." Genes involved in the serotonin system are considered likely candidates, since medication such as selective serotonin reuptake inhibitors (SSRIs), for example Prozac, have been proven to be effective in the treatment of patients with anxiety disorders or depression. 5-HTTLPR seemed an excellent candidate because in vitro analyses showed that the basal activity of

TABLE 60.1 Most promising linkage results in the order of the chromosomes for neuroticism, harm avoidance, MDD, or the subtypes recurrent MDD (R-MDD) and recurrent early-onset MDD (RE-MDD)

Location (Chromosome, cM)/ References	LOD	N Subjects[A]/Families	Phenotype
Chromosome 1, 42 and 90 cM (Camp et al., 2005; Nash et al., 2004).	1.7	426/90	MDD-RE
	1.6	711/283	Neuroticism
Chromosome 1, 126–137cM (Fullerton et al., 2003; Neale et al., 2005)	4.0[b]	1122/561	Neuroticism
	2.5[b]	293/129	Neuroticism
Chromosome 2, 237–248 cM (Nurnberger, Jr. et al., 2001; Zubenko et al., 2003)	2.2	224 possible pairs	MDD comorbid with alcoholism
	2.5[b]	?/81	MDD
Chromosome 4, 176cM (Fullerton et al., 2003)	3.8[b]	1122/561	Neuroticism
Chromosome 7, 42 cM (Fullerton et al., 2003)	3.9[b]	1122/561	Neuroticism
Chromosome 8, 8–38 cM (Cloninger et al., 1998; Fullerton et al., 2003)	3.2	987/105	Harm avoidance
	2.9[b]	1122/561	Neuroticism
Chromosome 10, 5–9 cM (Camp et al., 2005; Wray et al., 2008)	1.6	426/90	RE-MDD
	2.0	2030/564	Neuroticism
Chromosome 10, 76 cM (Zubenko et al., 2003)	3.0	?/81	MDD
Chromosome 11, 2–35 cM (Camp et al., 2005; Zubenko et al., 2003)	1.6	426 / 90	RE-MDD and anxiety
	4.2	?/81	R-MDD
Chromosome 11, 85–99 cM (Fullerton et al., 2003; Zubenko et al., 2003)	3.7[b]	1122/561	Neuroticism
	2.5	?/81	RE-MDD
Chromosome 12, 105–124 cM (Fullerton et al., 2003; McGuffin et al., 2005)	4.7[b]	1122/561	Neuroticism
	1.6	994/497	R-MDD
Chromosome 13, 64cM (Fullerton et al., 2003)	3.8[b]	1122/561	Neuroticism
Chromosome 18, 73cM (Camp et al., 2005)	3.8	96/21	RE-MDD and anxiety
Chromosome 18, 109–117cM (Cloninger zet al., 1998; Wray et al., 2008)	1.6	987/105	Harm avoidance
	1.9	8552/2509	Neuroticism

Note: Regions with a LOD≥3 or with a LOD≥1,5 found at least twice are shown. Sex specific effects are not included.

[a]For the studies of MDD, the number of affected individuals is given.

[b]This is the -logP, not the LOD score.

the long variant was about threefold higher than that of the short variant, indicating that the s-l polymorphism is functional (Heils et al., 1996).

In 1996, Lesch et al. (1996) reported an association between the promoter-based length polymorphism of the serotonin transporter gene (5-HTTLPR) and the anxiety-related personality traits neuroticism and harm avoidance. The association of the short variant with higher neuroticism and harm avoidance scores was not only found in two independent groups of subjects, but also within families. The family population included 459 siblings from 210 families, of which 78 sibling pairs from 61 independent families had discordant 5-HTTLPR genotypes (one or two copies of the short form versus homozygous for the long form). The difference in personality scores between siblings with the long form and siblings with the short form of the 5-HTTLPR genotype was statistically significant. This within-family association effect indicated that the significant associations found in the samples of unrelated individuals were not due to population stratification and could be a genuine effect.

Remarkably, subjects with the short form scored higher than subjects with the long form. This is in contrast to what would be expected considering the effect of the SSRIs on anxiety and depression, which is thought to be due to an increased serotonin concentration in the synapse. The

TABLE 60.2 Promising linkage results for anxiety phenotypes

Location/Reference	LOD	N Subjects/Families	Phenotype	Sample Ascertainment
Chromosome 1, 218–234 cM (Gelernter et al., 2001; Smoller et al., 2001)	2.04	153/20	Panic disorder	Probands with panic disorder
	2.05[a]	99/1	Aniety proneness	Probands with panic disorder
Chromosome 4, 157 cM (Kaabi et al., 2006)	4.5[b]	153/20	Broad anxiety phenotype	Probands with panic disorder
Chromosome 7, 47–57 cM (Crowe et al., 2001; Knowles et al., 1998)	1.71	–/23	Panic disorder	Probands with panic disorder
	2.23	253/23	Panic disorder	Probands with panic disorder
Chromosome 9, 105 cM (Thorgeirsson et al., 2003)	4.18	−/25	Anxiety/panic disorder	Probands with panic attacks, GAD, or phobias
Chromosome 13, 96 cM (Weissman et al., 2000)	4.2	−/34	Panic disorder combined with bladder/kidney problems	Probands with panic disorder
Chromosome 14, 36–45 cM (Gelernter et al., 2001; Gelernter et al., 2003; Gelernter et al., 2004)	3.7	129/14	Simple phobia	Probands with panic disorder
	2.93[c]	163/17	Social phobia	Probands with panic disorder
	2.38[a]	153/20	Panic disorder	Probands with panic disorder
Chromosome 14, 105 cM (Kaabi et al., 2006; Middeldorp et al., 2008)	1.7[b]	153/20	Broad anxiety phenotype	Probands with panic disorder
	3.4	1602/1566	Broad anxiety phenotype	Population based twin-family sample

Note: Regions with a LOD≥3 or with a LOD≥1,5 found at least twice are shown.

[a]This is the NPL score, not the LOD score.

[b]Lod score based on the nominal P-value reported by the authors.

[c]This is the Zlr score, not the LOD score.

short form of 5-HTTLPR is associated with less activity of the transporter and therefore with a higher serotonin concentration in the synapse. As a consequence, it would be expected that these subjects score lower on anxiety-related personality traits instead of higher. The authors could not explain the contradictory effect they found.

The numerous studies that have investigated the association since then showed conflicting results. Even meta-analyses on the association between 5-HTTLPR and personality traits (Munafo, Clark, & Flint, 2005a; Munafo et al., 2003; Schinka, Busch, & Robichaux-Keene, 2004; Sen, Burmeister, & Ghosh, 2004) or affective disorders (Lasky-Su, Faraone, Glatt, & Tsuang, 2005; Lotrich & Pollock, 2004) reached conflicting conclusions. This might be due to methodological differences between the meta-analyses (Munafo, Clark, & Flint, 2005b; Schinka, 2005; Sen, Burmeister, & Ghosh, 2005). Munafo et al. (2005b), therefore, stated that "Very large, well designed primary studies remain the most reliable way of obtaining reproducible results." (p. 896).

Two such studies have been performed since then. Willis-Owen et al. (2005) carried out an association study in three independent samples including, respectively, 564, 1,001, and 5,000 subjects. Subjects were selected from two general population samples based on their extreme high or low scores on neuroticism. The studies retained virtually 100% power to detect a genetic effect accounting for just 0.5% of phenotypic variance at an alpha level of .05. No significant association was found between 5-HTTLPR and neuroticism (measured with the Eysenck Personality Questionnaire; Eysenck & Eysenck, 1975) or major depression (as defined by the *DSM-IV,* American Psychiatric Association, 1994). Middeldorp et al. (2007) performed a family-based association study in a sample consisting of twins, their siblings, and parents from the Netherlands Twin Register (559 parents and 1,245 offspring). Subjects had participated between one and five times in survey studies measuring neuroticism, anxiety, and depression. Within-family and total association was tested for each time point and for the average scores across time points. Only 3 of the 36 tests showed a significant effect of 5-HTTLPR ($p < .05$). These effects were in opposite directions, that is, both negative and positive regression coefficients were found for the s allele. Offspring of these families were also approached to participate in a psychiatric interview diagnosing *DSM-IV* major depression. No additive effect of the s allele was found for *DSM-IV* depression. Three additional association analyses were carried out selecting (1) subjects aged over 30 years whose personality scores are considered to be most stable, (2) subjects scoring in the middle

range at each occasion because it was suggested that the effect of 5-HTTLPR is the largest at that part of the distribution (Sirota, Greenberg, Murphy, & Hamer, 1999), and (3) families with sibling pairs scoring concordant high or low since it is conceivable that the genetic load is highest in these families. These analyses converged with the other analyses in not showing an association with 5-HTTLPR.

Notwithstanding the well designed study of Lesch et al. (1996), there does not seem to be a straightforward association between 5-HTTLPR and neuroticism, anxiety, and depression. Caspi et al. (2003) suggested that 5-HTTLPR may not be directly associated with depression, but could moderate the serotonergic response to stress. They showed in a sample of 847 subjects that individuals with one or two copies of the short allele of 5-HTTLPR exhibited more depressive symptoms, diagnosable depression, and suicidality than individuals homozygous for the long allele in relation to stressful life events experienced in the 5 years before assessment. Uher and McGuffin (2008) reviewed the studies attempting to replicate the gene-environment interaction. They conclude that genetic moderation by 5-HTTLPR of vulnerability to adverse environment appears plausible. Findings are most consistent in young adult samples. Contradictory findings have been reported in adolescent boys and elderly people. This is in agreement with the results in a Dutch sample with a mean age of 39.2 years in which no interaction was found between 5-HTTLPR and the sensitivity to the exposure to life events regarding anxious depression scores measured with the Young Adult Self Report (Achenbach, 1990; Verhulst, Ende, & Koot, 1997; Table 60.3). In a regression analysis, including sex as a covariate, only the main effect of the number of life events was significant ($p = 0.001$). The main effect of 5-HTTLPR and the interaction did not reach significance with p values of 0.75 and 0.18, respectively.

On the whole, the candidate gene approach with the choice of genes based on the mono-amine hypothesis for the etiology of depression has not been very successful.

Table 60.3 Log transformed anxious depression scores (SD) per 5-HTTLPR genotype (ss, sl, and ll)

	N	SS	SL	LL
0 life events	722	18.5 (11.2)	20.1 (10.6)	19.1 (10.4)
1 life event	295	21.6 (10.6)	21.4 (9.6)	20.1 (10.6)
2 or more life events	137	23.9 (9.1)	23.2 (10.7)	21.1 (10.3)

Note: Results for individuals who were (1) not exposed to a negative life event, such as death of a significant other, serious illness, divorce, in the previous year; (2) exposed to one life event; or (3) exposed to two or more life events. There is a significant main effect of the number of life events. The main effect of the 5-HTTLRP or the 5-HTTLPR-life events interaction effect did not reach significance.

Possibly, trying to find genes underlying the linkage peaks might be a more fruitful approach. Two studies successfully followed up on their linkage results and demonstrated a significant association of the apoptosis protease activating factor 1 (apaf-1) gene and the regulator of G-protein signaling 2 (RGS-2) gene with depression and anxiety respectively (Harlan et al., 2006; Leygraf et al., 2006). These findings need replication, but suggest that the genes influencing the vulnerability for anxiety and depression are involved in other biological pathways than previously thought.

Genome-Wide Association Studies

We are awaiting the results of the first genome-wide association study on major depression. From the Netherlands Twin Register (NTR) and the Netherlands Study of Depression and Anxiety (NESDA), 1,862 participants with a diagnosis of depression and 1,857 controls at low liability for depression have been selected for genome-wide genotyping (Boomsma et al., 2008) by the U.S. Foundation for the National Institutes of Health Genetic Association Information Network (FNIH/GAIN; www.fnih.org/GAIN2/home_new.shtml).

Currently, two genome-wide association studies for bipolar disorder and one for neuroticism (Baum et al., 2007; Shifman et al., 2008; Welcome Trust Case Control Consortium, 2007) have been carried out. Two of these studies (Baum et al., 2007; Shifman et al., 2008) used DNA pooling instead of genotyping 500K SNPs in each individual. This approach is more cost-effective, but reduces power. The results confirm the idea that complex diseases are influenced by multiple genes of small effect. Odds ratios for significant associations varied between 1.2 and 1.5. In the two studies on bipolar disorder there is one overlapping finding. Both studies found an association with a SNP in the DFNB31 gene on chromosome 9. The genome-wide association study for neuroticism was followed by a replication study in which the SNPs showing the most significant results were tested in independent samples. Ultimately, one SNP within the phosphodiesterase 4D, cAMP specific (PDE4D) gene showed the most promising result (Shifman et al., 2008).

SUMMARY

There is clear familial clustering for anxiety and depression and the main, or even sole, reason is the genetic relatedness of biological family members. However, no chromosomal region or gene has been unequivocally identified as yet to be involved in anxiety and depression. In the near future, the results of the first genome-wide association study for

depression will be published, no doubt to be followed by several other studies. The exploratory approach seems warranted as other biological pathways than those previously expected might be involved in the etiology of anxiety and depression. The findings from the two genome-wide association studies on bipolar disorder are encouraging, as are the results of the genome-wide association study on neuroticism. To distinguish between false and true-positives, the chromosomal regions identified in linkage studies might be helpful.

This chapter provided the introductory information on commonly used methods in genetic epidemiology and gene hunting studies, some issues have been underexposed. One issue involves the definition of the phenotype in psychiatric genetics. One frequently mentioned hypothesis to explain the divergence in results from gene-finding studies is the definition of the phenotypes according to the *DSM* (American Psychiatric Association, 1980, 1987, 1994). It is possible that *DSM* categories cannot double as phenotypes when trying to discover robust genetic markers (Charney et al., 2002). The effect of a gene can, for instance, be missed, when this gene leads to a different pattern of symptoms than the disorders as defined by the *DSM-IV* (for an illustration of this problem, see Hudziak, 2002).

A multivariate analysis of the entire range of symptoms instead of using a single end-diagnosis is a way to try to find genes related to psychiatric symptoms (Hottenga & Boomsma, 2008). As an alternative, Gottesman and Gould (2003) suggested focusing on endophenotypes defined as traits along the pathway between genotype and disease. Although this seems a useful approach, it has so far not yielded more conclusive results than the genetic research of the psychiatric disorders themselves. Another approach could be to refine the phenotypes in order to diminish heterogeneity. A family study on depression identified four factors: (1) mood symptoms and psychomotor retardation; (2) anxiety; (3) psychomotor agitation, guilt, and suicidality; and (4) appetite gain and hypersomnia (Korszun et al., 2004). The first three factors showed significant sibling correlations and might be interesting phenotypes for future gene finding studies.

Other areas of research go beyond the investigation of genetic polymorphisms. A new development is the use of expression arrays or so called "gene chips." Thousands of individual gene sequences can be bound to tiny chips (glass plates). When a sample of RNA is applied, those genes actively express in the sample, bind to their embedded ligand and the resulting interaction is visualized. This method has also been suggested to investigate depression from an epigenetic perspective (Mill & Petronis, 2007). Epigenetic factors are inherited and acquire modifications of DNA and histones that regulate various genomic functions occurring without a change in nuclear DNA sequence. These could provide a direct mechanistic route via which the environment can interact with the genome.

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