Chapter 10: Summary and General Discussion

This chapter discusses the implications of the results of the studies described in this thesis. First, a summary is given of the results of the previous chapters.

Summary

As a prelude to the subsequent analyses, **Chapter two** addressed the co-morbidity between anxiety and depression, by reviewing empirical evidence regarding mechanisms that might underlie the frequent co-morbidity within anxiety disorders, and between anxiety disorders and depression. The theory of Gray & McNaughton (2000) was taken as a frame of reference. Gray & McNaughton (2000) hypothesized that the anxiety disorders as well as depression are distinct entities with the co-morbidity explained by 1) recursive interconnections linking the brain regions involved in fear, anxiety and panic and 2) heritable personality traits such as neuroticism underlying anxiety as well as depression. Their theory can be translated into the co-morbidity models of Klein and Riso (1993) and Neale and Kendler (1995). These models describe causes for co-morbidity in general, which can be formally tested in longitudinal or twin-family designs. Co-morbidity due to recursive interconnections linking brain regions can be viewed as one disorder being an epiphenomenon of the other, i.e. multiformity. Co-morbidity due to neuroticism being a heritable risk factor for anxiety as well as depression can be interpreted as co-morbidity due to a partly shared genetic etiology. Twin and family studies investigating these models for anxiety, depression and neuroticism, were reviewed. To compare the outcomes systematically, genetic and environmental correlations between disorders were calculated for 23 twin studies and the results of 12 family studies were summarized according to the method of Klein & Riso (1993). Twin studies showed that co-morbidity within anxiety disorders and between anxiety disorders and depression is explained by a shared genetic vulnerability for both disorders. Genetic factors influencing neuroticism seemed to overlap with vulnerability genes for anxiety and depression. Some family studies supported this conclusion, but most family studies suggested that co-morbidity is due to one disorder being an epiphenomenon of the other. These discrepancies between the twin and family studies could be due to differences in methodology. All twin and some of the family studies used biometrical model fitting, whereas most family studies compared prevalence rates of disorders in family members of affected probands. Two simulation studies testing the validity of the Klein and Riso predictions and the Neale and Kendler model fitting approach revealed that the latter method was more valid to discriminate the correct co-morbidity model

(Rhee et al., 2003; Rhee et al., 2004). The predictions of Klein & Riso (1993) did not seem to be valid under some circumstances (Rhee et al., 2003). However, all studies using biometrical model fitting only tested the model of shared etiology. Rhee et al. (2004) demonstrated that this model might also fit when another model, e.g. multiformity, describes the data even better. Nevertheless, considering the fact that the model of shared etiology fits the data in 23 twin and three family studies, the theory of Gray and McNaughton (2000) is supported that anxiety disorders and depression are distinct entities with at least part of the co-morbidity explained by shared genetic risk factors reflected in the personality trait neuroticism. Further research should reveal whether recursive interconnections linking brain regions are also of importance in the frequent co-morbidity.

Chapter three continued with the co-morbidity issue, by describing two studies performed to investigate the association between neuroticism, extraversion and sensation seeking, on the one hand, and anxious and depressive psychopathology, on the other. In both studies, co-morbidity between depression and other psychopathology was explicitly taken into account. In study I, data from 7969 twins and siblings of the Netherlands Twin Register were analyzed. Correlations were estimated within and between self-report measures of the three personality dimensions, anxiety and depression. Furthermore, to take co-morbidity into account, subjects were divided into cases and controls on the measures of anxiety and depression, with the 95th percentile used as a cut-off score. Next, the mean scores of the "pure cases" and the "co-morbid cases" were compared with the means of the control subjects. In study II, analyses were performed on DSM-IV diagnoses of major depression, dysthymia, social phobia, generalized anxiety disorder and panic disorder. These data were obtained for a selected sample of 1240 individuals. Per diagnosis, personality scores were compared between affected and unaffected subjects, correcting for co-morbidity by including all disorders in the model. Additionally, personality scores were compared between subjects with zero, one, two or three or more diagnoses. Study I showed that high neuroticism and low extraversion were related to anxiety as well as depression. Sensation seeking was related to neither of them. These results were replicated when the personality scores of the "pure" and "co-morbid cases" were compared with control subjects. In study II, high neuroticism was related to all disorders, except dysthymia. Low extraversion was related to social phobia and panic. High neuroticism and low extraversion were both related to the number of disorders. No associations were found with sensation seeking. Thus, high neuroticism and low extraversion appear to be related to anxiety and depression, also when co-morbidity is taken into account.

Remarkably, sensation seeking seems an independent personality dimension, which is not associated with anxious and/or depressive psychopathology.

In **chapter four** data from Australian (N=2287) and Dutch (N=1185) dizygotic twins and siblings, who were selected for a linkage study and participated in clinical interviews to obtain lifetime DSM-IV diagnoses, were used to investigate familial influences and their dependence on sex for panic disorder and/or agoraphobia, social phobia, generalized anxiety disorder and major depression. A correction for ascertainment bias was carried out by including the selection variables in the analyses (Little & Rubin, 1987). In a liability model, tetrachoric correlations were estimated in male, female and opposite-sex sibling pairs. For each diagnosis, the sibling correlations could be constrained to be equal across the Australian and Dutch samples. Next, sex differences in the correlations were tested. For each diagnosis, the sibling correlations were similar for brothers and sisters. With the exception of panic disorder and/or agoraphobia, the same sex correlations could be constrained to be equal to the correlations of the opposite-sex sibling pairs. For major depression, social phobia and generalized anxiety disorder, the correlations were estimated to be about 0.20. For panic disorder and/or agoraphobia, the correlation was 0.23 in brother and sister pairs, but absent in opposite-sex sibling pairs. To conclude, upper heritability estimates, based on twice the correlations in the sibling pairs, vary between 36% (major depression) and 50% (social phobia). Furthermore, different genetic risk factors appear to contribute to the vulnerability for panic disorder and/or agoraphobia in men and women. No other sex differences were found.

Chapter five investigated the association between the serotonin transporter gene polymorphism (5-HTTLPR) and quantitative measures of neuroticism, anxiety and depression. Chapter three had clearly shown an association between these measures and DSM-IV anxiety disorders and depression. Genetic epidemiological analyses of the neuroticism, anxiety and depression scores had already indicated heritabilities of 40%-50% (Boomsma et al., 2000). Lesch et al. (1996) were the first to report an association between 5-HTTLPR and anxiety-related personality traits; subjects with the short variant of the gene scored significantly higher on these traits than subjects with only the long variant. Since then, the association between 5-HTTLPR and anxiety-related personality traits or depressive psychopathology has been investigated in numerous studies, but with conflicting results. In a large study retaining 100% power to detect a genetic effect accounting for just 0.5% of

phenotypic variance no significant association between 5-HTTLPR and neuroticism or major depression was found (Willis-Owen et al., 2005). However, population stratification, which can lead to both type I and type II errors, was not considered in their analyses. Therefore, we carried out a family based association analyses of 5-HTTLPR and neuroticism, anxiety and depression. In 466 families from the Netherlands Twin Register, 254 fathers, 305 mothers, 501 male and 744 female offspring were genotyped for 5-HTTLPR. These families were selected from the Netherlands Twin Register to include sibling pairs scoring extremely high or low on a composite score of neuroticism, anxiety and depression. The subjects had participated between one and five times in a survey study measuring neuroticism, anxiety or depression. The association between the *ss, sl* and *ll* variants of 5-HTTLPR, and these traits was investigated, modeling an additive effect of the *s*-allele with sex included as a fixed effect. Both within family association and total association were tested in QTDT for the five measures of the three traits and for the mean scores of the traits for each subject over the five occasions. Only 3 of the 36 association tests showed a significant effect of 5-HTTLPR ($p < 0.05$). Neuroticism and anxiety measured in 1991 showed a significant negative regression coefficient for the *s* allele, whereas neuroticism measured in 2000 showed a significant positive regression coefficient for the *s* allele. The overall results clearly suggest no association between 5-HTTLPR and anxiety-related traits. They also show how associations can be found by coincidence. Had we chosen to report the results of the 1991 survey, we would have drawn the conclusion that there is a significant association between 5HTTLPR and anxiety-related traits.

As a preparation for a study on the association between burnout and employment on the one hand and anxiety and depression on the other, we investigated in **chapter six** whether burnout clusters within families and, if yes, whether this is due to genetic influences or to environmental factors shared by family members. Finally, we tried to identify specific risk factors for burnout. Earlier research on risk factors for burnout had mainly focused on circumstances at work and personal characteristics and had not addressed the issue of familial clustering. In 2707 twins, 736 of their siblings and 575 of their spouses from a population based twin-family sample, burnout was measured using a self-report questionnaire. Correlations in burnout scores were calculated in monozygotic (MZ) and dizygotic (DZ) male and female twin pairs and in brothers, sisters and sibling pairs of the opposite sex. Next, differences in correlations between MZ, DZ and sibling pairs were tested as well as differences in correlations between brothers and sisters and between same-sex and opposite sex pairs. Moreover, correlations between twins and their spouses were derived conditional on

the length of the relationship. In the final model, correlations of the MZ and DZ twin pairs and sibling pairs were significantly different from zero, but not significantly different from each other. No sex differences in the correlations were found either. The sibling correlation was estimated at 0.22. The correlation between spouses was also significant. This was mainly due to the group with a relationship longer than 5 years in which the correlation was 0.24. From the investigated specific risk factors, only a high level of education in the parents was associated with higher burnout scores. Age did not have a considerable effect on burnout. To conclude, there appears to be familial clustering for burnout, which is due to environmental factors shared by family members, explaining 22% of the variance. Genetic factors do not seem to be of importance. The significant correlation between spouses supports the conclusion that common environment plays a role in burnout. A high level of parental education could be one of the familial risk factors.

Chapter seven aimed to find out whether the association between burnout and anxious depression as well as between employment and anxious depression could be caused by shared etiological factors. Both conditions have repeatedly been found to be related to anxiety or depression, but the causes of these relations were still unclear. In a sample of 4309 Dutch twins and 1008 of their siblings, bivariate genetic analyses of employment and anxious depression and of burnout and anxious depression were carried out using structural equation modeling. These analyses revealed that employment and anxious depression were both influenced by genetic and unique environmental factors. The association between employment and anxious depression was small, but significant, estimated at –0.08. Statistical power was too low to decide whether the covariance was explained by genetic or unique environmental factors. In burnout, familial clustering was due to genetic factors in men, while in women genetic and common environmental factors explained familial resemblance. In both sexes, there was a strong correlation of around 0.40 with anxious depression, which was explained by shared genetic and shared unique environmental factors. Thus, the associations between employment and anxious depression as well as between burnout and anxious depression seemed to be due to overlapping genetic and unique environmental factors. Work related circumstances, e.g. financial strain or work-family conflict, might be of importance in burnout and anxious depression. Furthermore, these results could support the notion that a genetic vulnerability for depression can also increase the risk for exposure to high-risk environments such as unemployment, i.e. gene-environment correlation.

Before performing a study on the relation between life events and depression, **Chapter eight** tested two assumptions often made in studies investigating the effect of life events on psychiatric disorders. Twin studies assume that there are no differences in prevalences of the experience of life events between twins and singletons. Violation of this assumption signifies that the results from twin studies might not generalize to singletons. Twin studies as well as other epidemiological designs investigating the association between environmental risk factors and psychiatric disorders also often assume that the exposure to life-events is random and not influenced by familial factors, either genetic or common environmental factors. If this assumption does not hold and the exposure to a life event is, for example, partly genetically influenced, this signifies that the relation between the life event and the disorder might not be causal. Instead, genes influencing the vulnerability for a disorder might also increase the risk for exposure to a life event. To test these assumptions, we first investigated differences in prevalences of experienced life events in a Dutch sample of 2086 monozygotic (MZ) twins, 2090 dizygotic (DZ) twins and 1307 of their siblings. Since siblings of twins are matched regarding parental social economic status and upbringing, they are the perfect control group. Self-reported data on life events (serious illness or injury of self, serious illness or injury of a significant other, being married or involved in a romantic relationship, divorce / break-up of a relationship, death of a significant other, traffic accident, robbery, violent assault, sexual assault) were available from the Netherlands Twin Register survey studies. Second, we investigated whether familial resemblance was present for the exposure to these life events and, if yes, whether this resemblance was due to genetic or common environmental factors. No differences were found in the prevalences of life events between MZ twins, DZ twins and their non-twin siblings. There was evidence for familial aggregation of all life events, except for traffic accidents in women. Results indicated genetic control on the presence of a spouse or involvement in a relationship. Familial resemblance of illness and death of a significant other was mainly due to common environment. For the other life events, it was not possible to decide whether familial clustering was due to genetic and common environmental effects.

Chapter nine investigated the relation between life events and anxious depression. Since chapter three concluded that anxious depression was related to neuroticism and extraversion in, these personality traits were also considered in the analysis. In addition to establishing whether or not an association exists between these measures and life events, the aim was to get more insight into the mechanism underlying a possible association. Is this due to causality or are there genes that influence both depression and increase the risk to experience a life

event, so called gene-environment correlation. Cross-sectional data on life events, anxious depression and personality were collected with self-report questionnaires for 1918 male and 3864 female twins. For 1058 male and 2226 female twins, personality data were also available from the survey previous to the survey in which the exposure to life events was assessed. Life events were measured on two occasions. Inconsistencies in answers did not appear to be due to recall bias. The life events serious illness of self, divorce, traffic accident, robbery, violent assault and sexual assault were investigated. Sexual assault was not included in the analyses of the specific life events, as the prevalence was too low. Paired t-tests of anxious depression scores before and after the exposure to a life event showed that the life events serious illness of self, divorce and traffic accident increase symptoms of anxious depression lead to an increase in symptoms. Robbery and violent assault do not have that effect. Neuroticism and extraversion scores are hardly influenced by life events exposure. Next, to investigate whether depression, neuroticism or extraversion might be causally related to the experience of life events, depression, neuroticism and extraversion scores two years before the life event report were compared between subjects that had reported a life event last year and subjects who had reported no events last year. Higher neuroticism and anxious depression scores preceded the life events serious illness of self, divorce and robbery. Higher scores on anxious depression, but not on neuroticism, also seemed to precede violent assault, but the prevalence of this life event was too low to draw this conclusion. With the co-twin control method, gene-environment correlation was investigated. In the presence of gene-environment correlation, e.g. for life events and depression, MZ twins discordant for life events show no difference in depression scores. DZ twins discordant for life events do show a difference in depression scores, but this difference is smaller than in a population of unrelated subjects. These differences in scores are due to differences in the scores of non-exposed subjects. The non-exposed subjects of the discordant MZ twin pairs score higher than the non-exposed subjects of the discordant DZ twin pairs, who in turn score higher than the non-exposed subjects in the total population. This pattern of results was not seen for anxious depression, neuroticism or extraversion. These results suggest a reciprocal causal relation between most of the life events under study and anxious depression. High neuroticism scores in general preceded life events. There was no association between life events and extraversion. Gene-environment correlation appeared to be absent for the investigated life events and anxious depression, neuroticism and extraversion.

General Discussion

A few small explanations

Kendler (2005b) stated that "What we can best hope for is lots of small explanations, from a variety of explanatory perspectives, each addressing part of the complex etiological processes leading to disorders. It will be particularly challenging to understand how these many different small explanations all fit together." This goal of this thesis was to add some "small explanations" to the current knowledge on anxiety and depression from a genetic perspective. The first part (chapter two to five) aimed to get more insight in the genetic background of anxiety and depression. Using the paradigm of advanced genetic epidemiology (Kendler, 2005a), it was examined whether genetic risk factors are shared for anxiety and depression and if yes, whether a genetic vulnerability for anxiety or depression can be expressed as a personality trait. Furthermore, sex differences in genetic architecture for anxiety and depression were investigated. Finally, employing the paradigm of gene finding methods (Kendler, 2005a) a family association analysis was carried out with 5-HTTLPR and anxiety, depression and neuroticism. Chapter two showed that anxiety and depression are distinct entities, but with a partly shared genetic background, probably expressed in the personality trait neuroticism. Chapter three confirmed that anxiety as well as depression is related to neuroticism. Moreover, extraversion appeared to be negatively related to these symptoms. Sensation seeking was not associated with anxiety and depression at all. Chapter four supported the findings of earlier studies that major depression, generalized anxiety disorder, social phobia and panic disorder are for 30%-40% heritable. Sex differences in genetic architecture of anxiety and depression are probably limited. With the exception of panic disorder, the genes that influence anxiety or depression appeared to be the same for men and women. This signifies for gene finding studies that neuroticism and extraversion might be appropriate endophenotypes for research aiming to identify genes underlying the vulnerability for anxiety and depression and that it is not strictly necessary to take sex differences into account. Next, the association between 5-HTTLPR polymorphism and self-report measures neuroticism, anxiety and depression was investigated in a large sample of parents and siblings. Overall, there did not appear to be a significant association. Together with a recent study in a large sample of unrelated subjects (Willis-Owen et al., 2005), this study strongly suggests that the short form of 5-HTTLPR is not associated with higher scores on neuroticism, anxiety and depression measures which are associated with DSM-IV anxiety and depression .

The second part of the thesis investigated specific risk factors that are mostly considered to be purely environmental. More knowledge on the mechanisms underlying exposure to certain risk factors can enable genetic research to better model the combined effect of genes and other risk factors on anxiety and depression. Again using the paradigm of advanced genetic epidemiology (Kendler, 2005a), we examined whether familial or individual factors can increase the risk for exposure to specific environments. Moreover, the issue of gene-environment correlation was addressed, i.e. does a genetic vulnerability for anxiety or depression also increase the risk for exposure to high-risk environments? Our results indicated that the occurrence of risk factors is fairly random in the population, but familial factors are also of importance. For employment status, genetic factors seem to cause resemblance in family members. For burnout both genetic and common environmental factors might explain familial clustering. In the case of life-events, it was not possible to decide whether genetic or common environmental factors were of importance. Bivariate genetic epidemiological analyses suggested that the associations between employment and depression and between burnout and depression are due to shared genetic and shared unique environmental factors. Regarding the association with life events gene-environment correlation seems to be absent, but high scores on depression or neuroticism lead to a higher risk for life events. Life events, on the other hand, also increase depression scores. These results indicate the complexity that can exist in a relation between a risk factor and a disorder. Further research is needed to unravel more precisely the mechanisms of the associations. Still, these issues need to be considered in the treatment of depression as well. It is necessary to discuss what the circumstances at work are like for a patient, since stressful conditions might play a role in the development of burnout as well as depression. Moreover, as a genetic vulnerability for depression may also increase the risk for unemployment, it is important to know whether the patient can meet the demands of his job. Problems in this area should be a focus of treatment. The same holds for the higher risk to experience life events in depressed subjects, especially since these life events might worsen the depression. If possible, measures should be taken to prevent life events, such as meetings with the spouse of a depressed patient to discuss the risk of a divorce.

The bits of knowledge that are still missing

The conclusions of the previous chapters raise several new research questions and identify bits of knowledge that are still missing. These will be discussed in the following section together with recommendations for future studies.

Chapter six and seven describe, to our knowledge, the first twin-family studies on respectively burnout and the associations between burnout and anxious depression as well between employment and anxious depression. It is obvious that further investigations are necessary to shed more light on these issues. The results were somewhat contradictory regarding the influence of genetic and common environmental factors on burnout. This could be partly due to an increased power to detect small effects in the bivariate analysis. Another reason could be the different order of the steps taken in the model fitting procedure. In chapter six, the correlations between the monozygotic and dizygotic male pairs were first constrained to be equal before this correlation was constrained to the correlation in the brothers. However, first constraining the correlation of the dizygotic male twin pairs to the pairs of brothers, leads to slightly different results as shown in Table five in chapter seven. While the model fitting procedure as described in chapter six did not show any genetic influences, the presence of genetic effects was suggested in the other chapter. In chapter six, the significant spouse correlations support the finding of common environmental factors, but in chapter seven the significantly higher MZ than DZ cross-twin–cross-trait correlations for burnout and anxious depression support the influence of genetic factors. Additional data on burnout in the parents of the twins and siblings might provide the opportunity to resolve this issue by including the effect of cultural transmission from parents to offspring as well as environment shared by twins and siblings in the model (Truett et al., 1994).

The association between employment status and anxious depression also needs to be further investigated. The negative association between employment and depression had already been repeatedly found. Therefore, we decided that, the small, but significant, correlation of –0.08 found in our study fitted into the general picture. An explanation for such a low correlation could be that all unemployed subjects were considered as one group. Possibly, unemployment is more harmful when it is involuntarily than when it is a well-contemplated choice. In the former case, financial consequences might, for example, be more severe. Our group of unemployed subjects was too small to further examine this question (see Appendix A). Another reason for the low correlation might be that, as is also suggested by our results, being employed might protect against depression in the right circumstances, but might provoke a depression in stressful conditions. To summarize, more extensive data collection, especially regarding employment status and work conditions, is necessary. This needs to be done in a longitudinal or twin-family design to be able to investigate the underlying mechanisms of associations.

What do our results mean for future research focusing on the genes underlying the vulnerability for anxiety and depression? Chapter two has shown that the DSM-IV anxiety disorders and major depression are distinct entities with a partly shared genetic background. Therefore, there are probably two strategies that can be followed in gene finding studies. It can either be useful to focus on the differences by defining narrow phenotypes or to focus on what these disorders have in common. An example of the first strategy is a study in which four factors were defined in subjects with a history of at least 2 depressive episodes (Korszun et al., 2004). These factors were "mood symptoms and psychomotor retardation", "anxiety", psychomotor agitation, guilt and suicidality" and "appetite gain and hypersomnia". Sibling correlations were significant for three of the symptom dimensions, suggesting a genetic etiology. These factors might be more appropriate phenotypes than DSM IV depression in a gene finding study, as there are several combinations of symptoms possible to get a diagnosis.

An example of the second strategy is to focus on continuous traits that are related to all these phenotypes, e.g. high neuroticism, low extraversion or high scores on self-report questionnaires for anxiety or depression. As a lot more information is used in analyses of quantitative measures versus dichotomous variables, power to detect a small effect of a gene is increased (Williams & Blangero, 2004).

As it is well known that anxiety and depression are multifactorially determined, multiple risk factors should be considered together. This stresses the continuing importance of research on other risk factors than genetic ones. Focus of these studies needs to shift from finding associations to finding out what mechanisms underlie these associations. Is there a causal relation and, if yes, is this effect additive to or interactive with genetic effects? Or does gene-environment correlation play a role? The study on the interaction between the number of experienced life events and the effect of the short variant of 5-HTTLRP demonstrates that modeling genetic and environmental factors together can be a promising strategy (Caspi et al., 2003), although studies trying to replicate this finding do not all find a significant interaction effect (Eley et al., 2004; Gillespie et al., 2005; Kendler et al., 2005; Surtees et al., 2005). The association between employment status, stressful circumstances at work and depression is interesting for further investigation. The advantage of research on employment status and work related factors is that they might explain more variation in depression on a population level than life events, since it affects more subjects' lives. Relatively rare life events, like sexual assault, can have a large effect on the individual, but explain little variance in depression on a population level.

Future research in the Netherlands Twin-Family Study on Anxious Depression

It is repeatedly mentioned in this thesis that the Netherlands twin-family study on anxious depression was designed to perform a linkage study aimed to find genes underlying the vulnerability for anxiety and depression. Just as this thesis was going to press, additional marker data had become available, giving a grand total of 1541 sibling pairs in which at least 250 markers were genotyped. Genotyping was performed in Marshfield (USA) and Leiden University Medical Center. In the near future, a multivariate genome-wide linkage study will be carried out on these marker data for neuroticism and extraversion. This is another design to investigate multiple risk factors, in this case genetic, simultaneously. Furthermore, genes involved in the serotonin system in the brain will remain a focus of attention. The population analysed in chapter five, will be genotyped for a total of 12 polymorphisms in the genes coding for the serotonin receptors (e.g.1A, 1D, 2A, 2D) and BDNF in the department of molecular epidemiology in the Leiden University Medical Center. Rat studies have shown that BDNF stimulates function and growth of neurons producing serotonin in the brain, while the expression of BDNF is also regulated by serotonin (for a review of the function of BDNF see Angelucci et al. (2005)). A study in adults with childhood onset mood disorder, mostly major depression, found an association with BDNF (Strauss et al., 2004). Another promising candidate is the gene encoding for Tryptophan Hydroxylase type 2, the rate limiting enzyme in the synthesis of serotonin, expressed in the brain only (Zhang et al., 2004). Recently, several studies found evidence for association with major depression (Harvey et al., 2004; Zhang et al., 2005; Zill et al., 2004), but one study was not able to replicate the association found by Zhang et al. (Garriock et al., 2005). We aim to find a method to genotype subjects for TPH2. The relation between these polymorphisms and neuroticism, extraversion and the self-report measures of anxiety and depression will be analysed simultaneously in a family based association analysis.

The effect of environmental risk factors will be considered in these linkage and association analyses. Interaction between genes and risk factors, like the exposure to life events, being single and urbanicity, will be investigated. Gene-environment correlation will be included in these analyses where necessary.

The frequent co-morbidity between anxiety and depression will be taken into account if necessary. Moreover, the co-morbidity models as discussed in chapter two will be tested. These results can provide information regarding which phenotypes can be analyzed simultaneously in a linkage or association analysis.

Finally, if one or more genes have been found to influence, for example, neuroticism, the next step is to investigate whether this gene is associated with a certain cluster of symptoms. Ultimately, the small explanations deriving from this research in combination with the findings from other fields like neuro-imaging and neurophysiology, hopefully lead to a more detailed description of the mechanisms underlying anxiety and depression.

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