Summary and conclusions

The primary aim of this thesis was to study the genetic and environmental contributions to children's problem behaviors at ages 3 and 7. Also the determinants of continuity and change of problem behaviors were examined longitudinally from ages 3 to 7. To match most demands of statistical power required for the genetic analysis of kinship data we used all twin pairs of birth cohorts 1987 - 1991 of the population-based Netherlands Twin Registry (Boomsma *et al.*, 1992; Boomsma, 1998a). Questionnaires filled out by the parents, separately for oldest and youngest twin, were collected for 4016 3-year-old twin pairs (3873 pairs rated by mothers; 2087 pairs rated by fathers (for only half of the sample had fathers been asked to complete a CBCL)) and for 1940 7-year-old twin pairs (1924 pairs rated by mothers; 1545 pairs rated by fathers). Of all 3-year-old twin pairs for whom we had collected data, 54% had reached the age of 7 at the second assessment. Longitudinal data was obtained for 1638 twin pairs rated by mothers and 913 twin pairs rated by fathers.

We focussed on two broad groupings of problem behaviors, reflecting a distinction between anxious, inhibited behavior (Internalizing Problems) on the one hand, and aggressive, antisocial behavior (Externalizing Problems) on the other. In order to measure these problem behaviors parents were asked to fill out the Child Behavior Checklist (CBCL), a questionnaire developed to rate the behavioral and emotional problems of children. The CBCL for 2- and 3-year-old children (CBCL/2-3; Achenbach 1992) was used to rate the 3-year-old twins, while the CBCL for 4- to 18-year-old children (CBCL/4-18; Achenbach, 1991a) was completed for the 7-year-old twins. At each age the Internalizing and Externalizing broad-band scales were computed using the best fitting factor solutions for Dutch populations at that particular age. An advantage of using these broad-band scales as level of analyses, is that they are relatively unsensitive to population and/or age specific questionnaire differences because they are composed of a large number of similar items. Furthermore, several studies have found support for the validity of the internalizing - externalizing distinction (Achenbach, 1991a; Achenbach, 1992; De Groot, 1994; Koot *et al.*, 1997; Verhulst *et al.*, 1996), including the study of Hartman et al. (1999) testing the validity of the CBCL on a general population.

Genetic factors explained most of the variance for both problem behaviors at age 3 (see chapter 2 and 3). For mother's ratings of Internalizing Problems genetic factors explained 69% and for father's ratings genetic factors accounted for 59% of the variance, while for Externalizing Problems this was 52% and 56%, respectively. Four years later (see chapter 4), genetic influences for Externalizing Problems had not changed, suggesting a large continuing genetic influence for Externalizing Problems during childhood. For Internalizing Problems, however, estimates of genetic factors had decreased to explaining 38% of the variance for mother's ratings and 35% of the variance for father's ratings. Possibly this remarkable result was caused by developmental differences between older and younger children. Internalizing Problems of preschool children may predominantly be influenced by the child's genotype, while for schoolage children environmental influences shared between siblings (like style of parenting, socioeconomic level, or religion) may become relatively more important.

For 3-year-old twins, environmental influences shared between twins only had an influence on Externalizing Problems, explaining for mother's ratings 27% and for father's ratings 19% of the variance. Because often it is not family adversity as such but its persistence that predicts chronic problems (Campbell, 1995) finding shared environmental influences for children as young as 3-years of age seemed to be a remarkable finding. To examine whether this shared environmental effect was spuriously caused by siblings influencing each other, we tested for sibling interactions in chapter 2. Especially for behaviors which are easily observable for the other sibling, like aggressive behaviors, one can expect siblings to influence each other. Indeed, for Externalizing Problems, cooperative sibling interactions were found, indicating that twins reinforced each other's behaviors. However, no change in estimates occurred, showing that sibling interactions did not inflate the estimates of the shared environmental factors. Hudziak et al. (2000) proposed that the interaction effects we found could be synonymous with rater effects, meaning that parents compare the children with each other and thereby "distort" the behavioral ratings. Rater biases, i.e. tendencies of an individual rater to overestimate or underestimate scores consistently, may inflate the estimates of the shared environmental factors. To explore whether rater bias possibly inflated the estimates of the shared environmental factors we fitted Rater Bias and Psychometric models to the observed data. Results (see chapter 3)

showed that rater bias as measured in these models only had small effects, accounting for 8% of the variance. Again, the estimates of the shared environmental factors remained almost unchanged. It thus seems that for children as young as 3-years of age, pure shared environmental factors (unbiased by sibling interactions or rater biases) influence the occurrence of Externalizing Problems.

For 7-year-old twin pairs estimates of the shared environmental factors, both for mother and father ratings and both of Internalizing and Externalizing Problems, had increased to explaining 32% of the variance. Fitting Rater Bias and Psychometric models to the data (see chapter 4) showed that also for these older twin pairs possible rater biases were small, accounting at most for 14% of the variance. Thus also for these older children pure shared environmental factors influence both Internalizing and Externalizing Problems. Possibly, however, these shared environmental influences do not only refer to the environment children share within the family, but may also refer to the environment they share in the wider community. This point has also been stressed by Harris (1995) who argues that we should think about environmental effects on development in terms of group processes where peers play an important role. That is, phenomena such as within-group assimilation and between-group contrast that increase the homogeneity of behaviors within groups and widen differences between social groups could show as shared environment in a behavior genetic analysis. Thus, the possible larger shared environmental effects in schoolage versus preschool children could also reflect a developmental shift due to socialization experiences outside the home which become increasingly important as children grow older.

Environmental influences not shared between twins, i.e. idiosyncratic experiences like personal friends or diseases and trauma's, explained 31% of the variance of Internalizing Problems, both for mother and father ratings at ages 3 and 7. For Externalizing Problems these influences decreased a little over time, accounting for mother and father ratings at age 3 for 21% and 25% of the variance, respectively, and at age 7 for 16% and 14% of the variance, respectively. Measurement errors are incorporated in the estimates of the nonshared environment. Thus the assessment of problem behaviors of 7-year-old children with the CBCL/4-18 seems to have been just as well as the assessment of problem behaviors of 3-year-old children with the CBCL/2-3.

Although sex differences did exist for Externalizing Problems at both ages, genetic and environmental estimates for boys and girls were quite alike. For Internalizing Problems no sex differences emerged in genetic and environmental estimates, even though girls tended to get higher scores than boys. Fitting a Psychometric model to the data showed that behaviors similarly rated by both parents were more important to explain the etiology of problem behaviors than behaviors uniquely rated by one parent. No differences were found between the genetic and environmental estimates of mother and father ratings, neither for Internalizing Problems at age 3, nor for Externalizing Problems at ages 3 and 7. Only for Internalizing Problems at age 7 did the genetic and environmental estimates of mother and father ratings differ, although the estimates found were still quite comparable.

Genetic, shared environmental, and nonshared environmental contributions to continuity and change of problem behaviors at ages 3 and 7 were estimated using a two wave behavior genetic model (see chapter 5). The central findings of this study were that genetic influences underlie the stability of problem behaviors over a 4-year period. For Internalizing Problems the phenotypic stability ($r = .38$) was accounted for 66% by genetic factors, while for Externalizing Problems the phenotypic stability ($r = .54$) was explained for 55% by genetic factors. Some inborn vulnerability thus appears to exist for problem behaviors during childhood and even though children experience developmental changes, it seems that these genetic influences persist during development. Shared environmental influences also influenced the stability, accounting for 23% of the covariance for Internalizing Problems and 37% of the covariance of Externalizing Problems. This result is in accordance to results of epidemiological studies showing that, even though factors like family discord and disruption, lack of affection and poor supervision all predispose to problem behaviors (Rutter, 1985), it is often the persistence of these factors that predict chronic problems (Campbell, 1995).

Just as important as the finding of genetic continuity was the finding of genetic change for both problem behaviors during this 4-year period. That is, significant genetic effects were obtained for both problem behaviors at both ages that were independent of the continuing genetic influences. Indeed, about half of the genetic variance for both problem scales at both ages was independent of the continuing genetic variance, and thus influenced change. In other words, if genes are eventually found that account for genetic influences on Internalizing

and/or Externalizing Problems during childhood, these results suggest that different genes may contribute to variation in the problem behaviors from preschool to schoolage. The many developmental transitions, i.e. on physic, cognitive, social, and emotional levels, that children experience between ages 3 and 7 could be a possible explanation for this change in etiology. Schoolage children, in comparison with preschool children, experience many new environmental demands. These new environmental influences, together with the accompanying different interactions between these new environmental influences and the biological make-up of the child, may change the etiology of children's problem behaviors during this period.

Nonshared environmental factors largely had age specific effects, explaining 18% and 30% of Externalizing and Internalizing Problems, respectively. At both ages 3 and 7 these effects remained relatively the same. Although these results do not imply that nonshared environmental experiences, like illnesses or a possible trauma, are unimportant to children, they do suggest that these factors might be of a transient nature and that children appear to "recover" from them.

In order to study the determinants of continuity and change of problem behaviors during adolescence, data from a longitudinal adoption sample was used (see chapter 6). Apart from enabling us to study development in a genetically informative sample at an older age, this also allowed us to compare the results obtained by two different genetic designs. As discussed in chapter 1, each genetic design has its own limitations and finding similar results with different designs strengthens the representativeness of the findings. Parents of 111 pairs of adopted biological siblings, 221 pairs of adopted nonbiological siblings and 1484 adopted singletons completed the CBCL/4-18. At initial assessment all siblings were between 10 and 15 years of age. Three years later, parents completed the CBCL/4-18 for 75 biological pairs, 154 nonbiological pairs, and 1080 singletons, respectively. Remarkably, results of this longitudinal adoption sample were quite similar to the results of the twin sample. For Externalizing Problems genetic influences were most important, explaining 50% of the variance at both assessments. At the second assessment continuing genetic influences were as important as age specific genetic influences. Just as in the twin sample, this implied that apart from the continuing genetic influences, also different genes contributed to the variation in

Externalizing Problems during development. For Internalizing Problems at both assessments small genetic influences (explaining 16% and 8% of the variance, respectively) and larger shared environmental influences (explaining 30% and 33% of the variance, respectively) were found. Obtaining such similar results in both the twin and adoption samples gives a strong suggestion that for Internalizing Problems genetic influences decrease, while shared environmental influences increase over time. Of the shared environmental factor, continuing influences explained most of the variance at the second assessment point. It thus seems that also for this adoption sample the persistence of factors like, family discord and disruption, lack of affection and poor supervision, predict chronic problems (Campbell, 1995). Or possibly socialization experiences outside the home become increasingly important as children grow older (Harris, 1995).

Limitations and methodological issues

Although this study found large genetic influences for both problem behaviors at both ages these results should not lead to a sense of fatalism or genetic determinism for parents or for clinicians. As pointed out by Pike and Plomin (1996), even if genetic differences completely explain differences in problem behaviors - and this is not the case - does this by no means rule out the possibility of effective treatment, because environmental factors not widely represented at present in the population could have a major impact on these problem behaviors.

Quantitative genetic analyses done in this thesis assume an underlying continuous liability for behaviors, meaning that active genes which are not pathological in themselves are still associated with an increased (or decreased) risk for showing these behaviors. In this thesis, individuals showing extreme problem behaviors were thus not assumed to be qualitatively different, but to be variations of this particular behavior on a quantitative continuum. An underlying continuous liability for problem behaviors was found by Van den Oord & Rowe (1997) for a non-clinical population. Whether this assumption holds for clinical populations needs to be examined by further studies. For instance, if clinical depressions are affected by other genes or other environmental factors than "mood" differences between children in the general population, genetic and environmental etiologies

may be quite different for clinical and non-clinical populations.

Zygosity of the twin pairs was determined by blood/DNA tests either administered by the NTR, or (if the NTR had no zygosity data available) by their parents. In each chapter of this thesis we used all data available. For this reason, older chapters based the discriminant function for zygosity determination on fewer twin pairs for whom zygosity by blood/DNA was known than more recent chapters. The discriminant function, depending on the data available, obtained a 93% to 94% correct classification, suggesting that at most 3% to 4% of the twins' zygosity was wrongly classified. Misclassifying monozygotic twins (MZ) as dizygotic twins (DZ) will overestimate the DZ twin similarity, and misclassifying DZ twins as MZ twins will underestimate the MZ twin similarity. Therefore misclassifications always diminish the difference between MZ and DZ twin correlations, thereby underestimating genetic influences and overestimating shared environmental influences. In the appendix, an article is given describing the accuracy of zygosity determination by questionnaire items. In this article, a small preference is found for parents towards labeling a twin as dizygotic. Because 3% to 4% of the twins' zygosity might have been wrongly classified, estimates of shared environmental influences reported in this thesis could have been slightly overestimated. However, the effects of this misclassification will probably be small because most twins' zygosity (at least 96%) was correctly classified.

Future directions

Both at ages 3 and 7 (chapter 3 and 4, respectively), a Psychometric model fitted the data of Internalizing and Externalizing Problems significantly better than a Rater Bias model. This implied that rater differences did not merely reflect biases and/or measurement errors, but were also the result of parents assessing different aspects of the child's behavior. Thus at both ages, each parent provided unique information from his or her own perspective, apart from the behavioral views both parents shared. These results underscore the observation made by Achenbach (1992) "because any reports by any informants may be affected by characteristics of the informants, as well as by their own particular knowledge of the child's behavior, no single informant's reports can provide a complete picture". For future research it thus seems important to ask both parents to respond when collecting information about children's

behaviors, because no single rater may be able to provide a complete picture of the child's behavior.

In this thesis we conducted a prospective, longitudinal study to clarify the mechanisms involved in the etiology of Internalizing and Externalizing Problems. Knowing the relative genetic and environmental influences is important for future gene finding studies. Also, knowing how the etiological influences develop over time can help tremendously for finding genes at different ages. Results showed a major contribution of genetic influences to these childhood problems, especially in 3-year-olds. The most important determinants of stability of problem behaviors were genetic influences, unconfounded by possible sibling interactions, rater biases, and/or measurement errors. At each age also age specific genetic factors were expressed. This implied that if genes are eventually found that account for genetic influences on Internalizing and/or Externalizing Problems during childhood, different genes may contribute to variation in one or both of the problem behaviors from preschool to schoolage.

Further research is needed conducting similar analyses on the lower-order subscales at different ages and over time. Also the comorbidity between subscales, the fact that some subscales tend to occur together, needs to be better understood. Psychometric models should be applied to longitudinal data, studying the genetic and environmental contributions to continuity and change of problem behaviors while correcting for possible rater differences. Most importantly, the sample of twin pairs used in this thesis should be followed during their development. This shall enable future research to examine the genetic and environmental contributions to problem behaviors during development from child to young adult. Knowing the etiology of the problem behaviors will help researchers to locate genes underlying childhood disorders. Now that the human genome sequence is available and new methods of very precise genotyping have been developed, chances are much higher that genes will be located with linkage or association studies. However, because most problem behaviors are the result of a complex interplay of multiple genetic and environmental factors, finding genes and understanding their effects will not be an easy task. More research is needed to explore the ways in which genes interact with each other and with the environmental factors to influence an individual's susceptibility to showing problem behaviors. Essential for this kind of research is that the genetic risk can be measured directly, so molecular genetic findings

with strong effects will help tremendously (Plomin & Rutter, 1998). Further, discriminating measures of the environmental risk factors must be made, appropriate samples must be used, and statistical techniques must be employed that are well adapted to detect and test the postulated variety of genetic sensitivity (Kendler & Eaves, 1986). A more thorough description of the interplay between genes and environment is necessary to target appropriate intervention strategies which may improve or even prevent problem behaviors from occurring.