

Summary

Chapter 2 looked at Internalizing problems (INT) in adolescent twins and analyzed self-report data on anxious depression and withdrawn behavior in a bivariate genetic model. The results show that the impact of Additive genetic influences (A), Common environment shared by children from the same family (C) and unique Environment (E) on INT depend on age, thereby indicating age x genotype interaction. At age 12, the estimates of the explained variance by C for anxious depression and withdrawn behavior are significantly present, however, at age 14 and 16 genetic factors were sufficient to explain familial clustering, shared environmental effects were absent. There were no differences in the estimates of A and E between boys and girls.

Chapter 3 indicates that the teacher, school and/or class environment affects the expression of genetic factors for INT and Externalizing problems (EXT). Twins attending the same class and therefore rated by the same teacher show slightly lower levels of problem behavior and greater resemblance in problem behavior than twins who were rated by different teachers. This greater resemblance cannot be ascribed to the possibility that teachers were confusing the twins or by a bias of the teachers. Rather, the results appear to be due to a higher heritability of problem behavior assessed in children who were rated by the same teacher, which constitutes evidence for teacher/classroom x genotype interaction. In children who are exposed to the same teacher and/or classroom environment, a certain behavior can be more or be less triggered, thus teachers and the atmosphere in a classroom can truly make a difference in how children perform and behave at school.

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In chapter 4, Gene-environment interaction (GxE) is investigated for INT and EXT measured at ages 3, 5 and 7 with SES (SES) and formal child care between birth and age 4. In a large sample, two competing theoretical models were tested regarding the question whether mean levels of problem behavior are influenced by formal child care and whether the influence of genetic and environmental factors on problem behavior is moderated by the attendance of formal child care. One model, the diathesis-stress model, states that higher mean levels of problem behavior are observed in risk environments, accompanied by an increase in the heritability of problem behavior in risk environments. In the other model, the bioecological model, a decrease in heritability and an increase in the mean level of problem behavior are predicted when exposed to the risk factor. Children who went to formal child care scored slightly higher on EXT, especially when these children came from a lower SES family. The impact of environmental factors was higher in the formal child care group, leading to a lower heritability. Children in the lower SES group were also more influenced by environmental effects, thus heritability was lower in those groups. The effects of child care and SES on the influence of genetic and environmental factors were most pronounced at age 7 and for EXT. With regard to the two theoretical models, these findings support the bioecological model above the stress-diathesis model, given the hypothesis that formal child care is a risk environment for problem behavior.

Chapters 5 focused on determinants of triplet birth weight because triplets are often born with a low birth weight, which is associated with disadvantageous childhood development. Gestational age, sex, zygosity and maternal smoking affect birth weight with early born, girls, monozygotic triplets, and children from smoking mothers having a lower birth weight than later born, boys, and di- or trizygotic triplets, and children of non-smoking mothers. No effects of Assisted Reproductive Technologies (ART) or maternal alcohol consumption during pregnancy were found. The resemblance in birth weight was higher in MZ triplets than in DZ triplets indicating that genetic factors are also of importance in birth weight.

Chapter 6 subsequently investigated the effect of chorionicity on birth weight of triplets. Data of 346 triplets from the NTR were successfully linked to chorionicity information in PALGA (a nationwide network and registry of histo- and cytopathology in the Netherlands). Monozygotic, monochorionic triplets had a lower mean birth weight than dizygotic, dichorionic triplets. In dizygotic trios, monozygotic pairs only had a lower mean birth weight than their dizygotic sibling triplet when the pair shared a chorion. In conclusion, sharing a chorion is a more important risk factor for low birth weight than monozygosity.

Discussion

This thesis points to the importance of analyzing multiple phenotypes, different environmental factors, and taking age into account when estimating the heritability of problem behavior. As Kendler and others (Kendler et al., 2008b; Kendler et al., 2008c; Reinke and Herman, 2002) found for INT, the influence of the genome is dynamic during childhood and adolescence. This is also true for environmental factors. The large twin registries that have followed their subjects for, for example, 25 years, such as in the Netherlands Twin Register (NTR) contribute to the disentanglement of the etiology of INT and EXT across the lifespan. This thesis had a focus that went beyond heritability and looked at genotype x environment interactions, where “environment” was broadly defined as age, teacher/classroom, formal child care and parental SES.

The findings of chapter 2 are in line with previous studies on gene x age interaction for INT problems that also showed higher heritability estimates in adolescence than in childhood and a common environmental influence only in childhood (Bergen, Gardner and Kendler, 2007; Gregory and Eley, 2007; Rapee, Schniering and Hudson, 2009; Rice, 2009). These age differences do not necessarily imply that different genes are involved, and longitudinal studies should address this question. In addition to previous research, this thesis shows that the increase in heritability occurs not during but after the onset of puberty as at age 12. Thus, when common environmental influences are still present, most of the children have already entered puberty. Furthermore, the bivariate analysis of anxious-depressive and withdrawn behavior indicated a high (0.85 to 1) genetic correlation between these phenotypes suggesting that in future genome wide association studies (GWAS), genes indicated to affect one of the two phenotypes are also likely to be involved in the other. Moreover, when including subjects in a GWAS analysis, data from individuals with measurements of only one of the two phenotypes could be pooled together to enhance the power of the analysis.

Chapter 3 and 4 investigated the effect of teacher/classroom, SES and formal child care on the influence of genes and environment on INT and EXT. Since their parents or teachers rated the twins at different ages during childhood, gene x age interaction was also taken into account. Overall, the results emphasize that both genetic and environmental effects can vary across ages and different environmental exposures and that it is not possible to draw general conclusions. This is in contrast to the conclusion of Hicks et al. (2009a and 2009b) who studied GxE interaction for several environmental risk factors in INT disorders and EXT disorders and concluded that the way genes and environment interact in a phenotype generalizes over environmental stressors. For EXT, all environmental risk factors increased the genetic variance, and, to a lesser extent, the environmental variance, which could be interpreted as, in the case of EXT phenotypes, evidence for a diathesis-stress model. On the other hand for INT, all environmental risk factors increased the environmental variance, suggesting a bioecological model. The findings in this thesis do not support a phenotype specific pattern. In the analysis of the teacher data the results show the same pattern for INT and EXT, namely a decrease in genetic variance as well as an increase in unique environmental variance when children attend a different class (and thus are exposed to different teachers). In the analysis of SES and formal child care, the environmental variance for INT and EXT was higher when children went to formal child care and when SES was lower, although the effect was more pronounced in EXT. These findings support the bioecological model, with no phenotypic specific pattern, which is thus in contrast to the Hicks et al. (2009b) paper. An important difference between the analyses in current thesis and the Hicks et al. (2009b) paper was that the analyses reported here focus on 3-, 5-, 7-, 10- and 12-year-old children and the Hicks et al. paper analyzed data from adolescents at age 17. Other studies also yielded different results than the Hicks et al (2009a and 2009b) studies. Life events, for example, have also been associated with an increase of the heritability of INT, supporting the diathesis stress model (Eaves, Silberg and Erkanli, 2003; Lau and Eley, 2008; Lau et al., 2007; Silberg et al., 2001), while living in a socio-economically less advantaged neighborhood has been associated with less genetic effects on EXT, supporting a bioecological model (Legrand, Keyes, McGue, Iacono and Krueger, 2008; Tuvblad et al., 2006).

Detecting GxE and theorizing about the mechanisms, which lead to this phenomenon, are important not only for understanding how genes, age and environment interact and ultimately

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result in a phenotypic outcome. There are also more specific implications. The importance of classroom environment for internalizing and externalizing problem behavior has been discussed by (Herman, Reinke, Parkin, Traylor and Agarwal, 2009; Reinke and Herman, 2002). Chapter 3 indicates the effect of a teacher/classroom depends on a child's genetic make-up. As a consequence, the previously reported associations between, for example, positive behavior of a teacher and lower INT and EXT scores (Herman et al., 2009; Reinke and Herman, 2002), might be driven by a specific group of children and different intervention might be more suitable for other groups of children. Future studies should take child characteristics into account that can influence the effect of an intervention.

The importance of SES as a risk factor for problem behavior was already well established. A recent study also showed the enduring effect of low SES in childhood (Ramathan, Balasubramanian and Krishnadas, 2013). Chapter 4 shows that the effect of SES on problem behavior in childhood is due to an increase in environmental variance in line with the bioecological model, which hypothesizes that risk environments mask genetic differences between children. This signifies that in the low SES category, there are more differences in individual circumstances that explain variation in problem behavior, while in the high SES category, there is less variation in the environment. More insight in the differences within the low SES category is necessary to be able to identify the children with the highest risk.

Chapter 5 shows that the same risk factors for low birth weight in singleton and twin pregnancies apply to triplet pregnancies. Since triplet pregnancies are more at risk for a complicated course, these mothers should be even more encouraged to quit smoking. Moreover, given the effect of chorionicity as shown in chapter 6, careful monitoring of mono-chorionic triplets is required.

Birth weight is an important determinant of childhood development and we have found that BW in triplets is 1900 grams (and gestational age is 33.7 weeks). Therefore, the next step in future research with triplets will be to establish associations with BW and the development of behavioral and emotional problems and with cognitive development. To this end, longitudinal data on behavioral and emotional problems are collected with the Child Behavior Check List (CBCL) and the Teacher Report Form (TRF), as described in the Appendix of this thesis. The NTR also collects information on CITO scores (a standardized test on Educational attainment at age 12 years) in twins and triplets (for triplets we currently have 79 scores by self-report and 37 scores via parental report). It is noteworthy that almost 50% of the surveys that are sent to parents of triplets when they have reached the age of 12 years is returned to the NTR with the remark from parents that the children have not reached 'group 8', which is the last group in elementary school in the Netherlands which nearly all children reach at the age of 12 years. When sufficient data are becoming available, we can assess in future projects the influence of BW and genetic factors on behavior in triplets and school performance.

One question to be addressed in future research involves the influence of BW on mean levels of behavioral and emotional problems and cognition. Are triplets at higher risk than singleton and twin children because they have lower GA and BW? NTR studies show that in twins there is very little difference between twins and singletons, especially when birth order within the family is taken into account, but it is not known whether this is also true in triplets (Robbers et al., 2010; de Zeeuw, van Beijsterveldt, de Geus and Boomsma, 2012). An important assumption of all studies on multiples is that the results can be generalized to sin-

gletons, who constitute the largest part of the general population, although currently about 1 in 32 children in the Netherlands are part of a multiple birth (Glasner, Beijsterveldt, Willemsen and Boomsma, 2013). Twins and triplets carry a substantial risk of preterm delivery resulting in 15–20% of all preterm births (Hall, 2003; goldenberg, Culhane, Iams and Romero, 2008). In addition, partly due to premature and partly due to sharing a uterus, multiples have on average a lower birth weight than singletons. In the NTR the average duration of twins pregnancies is 36.3 (2.6) weeks with an average birth weight of 2423 (542) grams in MZ twins and 36.7 (2.4) weeks with an average birth weight of 2533 (550) grams in DZ twins (Gielen et al., 2010). In singletons, prematurity and low birth weight are thought of as indicators of sub-optimal development during the gestation. Both are associated with child and adolescent INT and EXT behavior (Arnoudse-Moens, Weisglas-Kuperus, van Goudoever and Oosterlaan, 2009). In twins, their on average lower birth weight and shorter gestational age does not appear to result in an elevated risk in problem behavior in comparison with singletons. However, statistics on gestational age and birth weight in triplets are worse than in twins. Thus, the question arises how studies on triplets should be interpreted and how birth weight should be handled as possible confounding factors (i.e. do the same standards apply for triplets as they do for singletons when classifying the infants' birth weight (e.g. Low Birth Weight (LBW), Very Low Birth Weight (VLBW))). Before this question can be properly answered, factors affecting birth weight in triplets should be investigated.

A second question involves the interaction of genetic factors and BW on the development of behavioral and emotional problems and cognition in triplets. The models described in this thesis to study GxE interaction in twin data (recognizing that BW cannot be labeled as a purely environmental trait) can be extended to triplet data. Thirdly, it may be that triplet status itself interacts with the genetic influences on behavior. For example, the rearing environment of triplets may differ more from the environment of singleton children than is the case for twins compared to singletons. (Feldman, Eidelman and Rotenberg, 2004) Feldman et al. (2004) have shown that maternal sensitivity is lower for triplets than singletons. Starting from 2012, when parents of triplets received the CBCL they were also sent an additional questionnaire which inquired about triplet well-being and the experience of parents on having and raising triplets (see appendix IV of this thesis). In addition, when triplets and twins in the NTR reach the age of 14 years, they receive surveys for the collection of self-report data (the Dutch Health and behavior questionnaire: DHBQ; Bartels et al., 2007). At that time we also approach the singleton siblings of twins and triplets. The DHBQ includes questions about the rearing environment as perceived by the twins, triplets and their singleton sibs and this, together with the longitudinal data in these groups, will constitute a valuable database for future studies, going beyond heritability studies and also including gene-environment correlation and interaction explorations.