Major Depressive Disorder (MDD) is an important global public health issue, because it is associated with relatively high lifetime prevalence and substantial disability. But even without meeting the criteria for MDD, depressive symptoms can cause serious impairments. Presence of these symptoms in adolescence is a risk factor for depressive episodes later in life. The aim of this dissertation was to examine genetic and environmental risk factors in the development of depressive symptoms in adolescent boys and girls. Understanding the mechanisms through which risk factors are related to depression will help to treat and prevent this disease more effectively in the future. The research described in this thesis is part of the TRacking Adolescents’ Individual Lives Survey (TRAILS), a large prospective study in the general population on determinants of mental health and social development during adolescence and young adulthood.

In Chapter 1, different risk factors involved in the development of depression were discussed. Epidemiological studies show that both genetic and environmental factors are involved in the risk to develop depression. Experience of stressful life events (SLEs) or chronic psychosocial stressors are the most important environmental risk factors. However, not everybody develops depressive symptoms in the face of psychosocial stress. The observed variance in the response to stress between individuals is, in part, influenced by our genetic make-up. Genes that are likely to play a role in the development of depression are those underlying the physiological stress response system and serotonergic neurotransmission in the brain. In this dissertation, functional allelic variants in the following four genes were studied: the serotonin transporter gene (SLC6A4), the glucocorticoid receptor gene (NR3C1), the mineralocorticoid receptor gene (NR3C2), and the brain-derived neurotrophic factor (BDNF) gene. We took account of gender differences because depression is twice as common in women than in men. This gender difference emerges during adolescence.

Chapter 2 described the TRAILS sample (n = 2127) and the Focus sample (n = 715) that participated in the experimental session, which included, among other tasks, a standardised social stress test (Groningen Social Stress Test - GSST). This test consists of public speaking and difficult mental arithmetic and was inspired by the Trier Social Stress Test (TSST) often used in studies of adult populations. The GSST encompasses the three most important triggers of the HPA axis: uncontrollability, threat of failure, and fear of negative social evaluation.

The effect of several factors on adolescents’ depressive symptoms were studied within the total TRAILS sample (n = 2127): the influence of stressful life events (SLEs), gender, parental depressive symptoms (PDS), the val/met polymorphism in the BDNF gene, the polymorphic site in the promoter region of the serotonin
transporter gene, and early life stress. Other factors were studied in the Focus sample \((n = 715)\) selected from the TRAILS population: effects of PDS, gender, menstrual cycle phase, oral contraceptive use and SNPs in the mineralo- and glucocorticoid receptor gene on physiological responses to the GSST.

In Chapter 3 we examined the effect of gender, menstrual cycle phase and oral contraceptive use (OC) on the cortisol response towards awakening and the GSST. This study was the first to examine these associations in a large sample of adolescents from the general population. We did not find differences in the cortisol awakening response (CAR) between boys and girls. Furthermore, no effect of menstrual cycle phase on the CAR and the GSST was found. Boys and girls did show different cortisol responses to the GSST; boys displayed a stronger cortisol response than girls, which is comparable to the gender difference reported in adults. Compared to free-cycling (FC) girls, OC-users displayed a blunted CAR. In addition to this, pre-test cortisol values in the OC-users were high during the morning sessions. This suggests a delayed activation and feedback of the HPA axis after awakening. Although blunted cortisol responses have been reported in adult women, it is remarkable that adolescent girls using OC showed hardly any response to the GSST. It is unlikely that the GSST was not stressful for OC-users, because the subjective experience of the stress test was not different between the two groups of girls. Longitudinal research might elucidate the effects of synthetic hormones on the HPA axis.

The study described in Chapter 4 examined the moderating effect of gender and parental depressive problems on the depressogenic effect of stress. With regard to gender, we showed that the relationship between SLEs and depressive symptoms was stronger in girls than in boys. Secondly, our results indicated that adolescents with parents who had ever experienced depressive symptoms were more sensitive to the depressogenic effect of stressful events (SLEs) than adolescents without depressed parents. This adds to the evidence that familial risk of depression is likely to be expressed in the response to psychosocial stress. The effect of PDS was not mediated by adolescent temperament (fearful or frustrated), family functioning and perceived parenting. An explanation for the absence of mediation might be that our PDS measure has yielded a too heterogeneous group regarding severity and timing of symptoms of the parents. It is also possible that PDS influenced the impact of stressful life events through other family factors that were not included in the study. The effect of PDS was not different for boys and girls.

The study described in Chapter 5 was inspired by the results presented in Chapter 4. We wanted to explore whether adolescents at familial risk for depression would
show different reactivity profiles to the GSST from those not at risk. We showed that PDS was indeed associated with the cortisol response to the GSST, but only in daughters and not in sons. Girls whose parents had ever experienced depressive symptoms displayed blunted cortisol responses. Such responses can be result of down-regulation of the HPA axis, which can be seen as an adaptive response to the damaging effects of expected stressors in the future. However, the effect of PDS on daughters’ cortisol responses was not mediated by overexposure to stressful situations in these girls’ lives. This suggests that the converted risk of parents to daughters is largely the result of transmitted vulnerability genes. Although genetic factors are involved in the aetiology of depression, not much evidence is present for a direct gender-specific effect. It is tempting to speculate that genetic factors indirectly make women more sensitive to the depressogenic effect of psychosocial stress. This hypothesis is supported by findings from animal studies, in which different effects of male and female gonadal hormones on the stress response system were reported.

In **Chapter 6**, we attempted to explore the aforementioned hypothesis further and tested the association between SNPs in the glucocorticoid (Bcl, 9beta) and mineralocorticoid (I180V and -2G/C) receptor genes and physiological responses to the GSST and examined these associations separately for boys, FC-girls and OC-users. We found no associations between any of the four SNPs and heart rate and cortisol in boys. In FC-girls, GR 9beta and MR -2G/C genotype were associated with overall cortisol levels but not with response patterns. In OC-users, MR -2G/C genotype was associated with cortisol levels and heart rate responses. Studies in adult men and OC-using women have reported positive associations between the Bcl, 9beta, I180V and cortisol and heart rate responses. MR -2G/C was never examined in the context of psychosocial stress. The different nature of the associations of this SNP in the MR gene and cortisol in FC-girls and OC-users might be the result of different interactions of female gonadal hormones and genetically based functional differences in the mineralocorticoid receptor. Future studies should explore whether sex hormones can influence the stress response system in humans and, if so, study the underlying mechanism.

In **Chapter 7** we described a study in which we examined whether the polymorphic gene coding for the serotonin transporter (5-HTTLPR) would modulate the depressogenic effect of stressful life events in adolescents who experienced high levels of stress during early childhood (age 0 to 5). We hypothesised that the S allele, resulting in less expression of the transporter protein, would be associated with increased sensitivity to the depressogenic effect of adolescent stress compared to the L allele. Our results indicated that in the high, but not in the low, childhood stress group, boys and girls differed regarding...
the association between genotype and SLEs. When analyses in the ‘high’ group were stratified by gender, an interaction between genotype and adolescent stress was found in girls and not in boys. As opposed to our expectation, the S allele was associated with decreased sensitivity to the depressogenic effect of SLEs. A recent meta-analysis indicated no clear direction of 5-HTTLPR genotype on the depressogenic effect of life stress. Our results show that it is important to account for timing, type and severity of the stressor when examining gene-environment interactions. Moreover, our results support the current idea that gene-environment interactions (GxE) underlying the aetiology of depression are gender-specific.

In Chapter 8 we aimed to replicate the previously reported gene-gene-environment (GxGxE) interaction between the 5-HTTLPR, the val/met polymorphism in the brain-derived neurotrophic factor (BDNF) gene, and several childhood (age 0 to 11) adversity indicators on depression. We could not replicate this GxGxE interaction, possibly due to differences between previous study designs and ours. Alternatively, the positive associations previously found might be chance findings, because positive findings are more likely to be published than so-called ‘null findings’. To conclude, a definite answer to the question of whether 5-HTTLPR, BDNF and childhood adversities interactively predict depressive symptoms can only be answered by replication studies in large samples with valid and reliable adversity indicators.

In Chapter 9 we discussed our findings, formulated conclusions and gave suggestions for further research. The studies presented in this dissertation have several methodological strengths. The large prospective TRAILS study enabled us to adjust for pre-existing problems, use information from multiple informants and contexts and examine the relation between stress experience and depressive problems over time. The use of our standardised social stress test (GSST) allowed us to study the response of the HPA axis and the autonomic nervous system in a large group of adolescents under a controlled condition. We studied gene-environment interactions by associating biologically plausible genes with (endo)phenotypes of depression, instead of using gene hits from whole genome scans that do not always underlie biologically (known) pathways. Finally, while most studies examine gene-environment interactions in mixed samples, we studied these interactions while taking account of gender.

The title of this dissertation refers to the main conclusion of the presented studies: adolescent girls are more sensitive to the depressogenic effect of psychosocial stress than adolescent boys. We showed that boys and girls displayed different stress response patterns to a social stress test in the lab and
that associations between polymorphic gene variants and (endo)phenotypes for depression were dependent on gender, OC use and early life stress.

In the last part of Chapter 9 we suggest that further research should focus on ultimate as well as on proximate questions. Asking ourselves why behaviour has evolved the way it has can bring us further in understanding the how of behaviour. Gender differences in behaviour are deeply embedded in our biology and are a result of a long evolutionary process to serve the gender-specific functions necessary for reproduction and survival. Depressive behaviour seems to be a result of common allelic variants in multiple genes that interact with our psychosocial environment. The presence of genetic variance in our human gene pool enables us to react to our (psychosocial) environment. The challenge for science is to unravel the underlying mechanisms for explaining how environmental stimuli interact with genetic variants in the development of depressive behaviour after stress. On the proximate level, we suggest that future research should focus on the following three areas: 1) the functionality of allelic variants in genes associated with the physiological stress response and depressive behaviour, 2) programming effects of early life stress, and 3) the role of female sex hormones in gender-specific gene-environment interactions.