Gene-environment interactions on the course of Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms
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Chapter 6

General summary and discussion
The purpose of this PhD thesis was to investigate genes, environmental factors, and gene-environment (G×E) interactions in relation to the course of ADHD symptoms across adolescence. Increased understanding of the interplay between genes, environment, and development helps to elucidate underlying mechanisms that are implicated in the course of ADHD symptoms. Therefore, in this PhD thesis, a series of studies was conducted to contribute to the sparse G×E research on the course of ADHD symptoms. In this final chapter, a summary of the findings of this PhD thesis is provided and thereafter the importance and implications of the results will be discussed. The chapter then provides a discussion of the strengths and limitations, clinical implications, and suggestions for future research.

Summary of Research Articles

In Chapter 2, it was examined whether candidate genes (DRD4, 5-HTTLPR, DRD2, COMT, and MAOA), a set of pre- and perinatal factors (index measure of various pregnancy and delivery complications, maternal smoking, maternal drinking, and low birth weight), and their interactions predicted ADHD symptoms across adolescence. While no main effects of the candidate genes were found, there was evidence that pregnancy and delivery complications were associated with a higher level of ADHD symptoms across all three measurement waves (mean ages 11, 14, and 16 years), but with a significantly declining influence over time. Furthermore, significant G×E interactions were found; the simultaneous presence of the 5-HTTLPR LL-allele and respectively pregnancy and delivery complications and maternal smoking; and of low activity MAOA and low birth weight were associated with more ADHD symptoms, particularly during early adolescence; these influences significantly decreased over time. Findings, therefore, suggest an age-dependent role of gene-environment interactions on ADHD symptoms across adolescence.

Chapter 3 focused on the relation of parenting styles (i.e., perceived parental overprotection, rejection, and emotional warmth) to the course of ADHD symptoms across adolescence both independent and dependent of ADHD candidate genes (i.e., DRD4, 5-HTTLPR, and MAOA). Four ADHD symptom trajectories were identified in our database: low stable, moderate stable, high decreasing, and high persistent. Although no G×Parenting style interactions were found, perceived parental rejection predicted class membership in the high persistent trajectory compared to the other classes. Our results suggest a role of perceived parental rejection in the persistence of ADHD symptoms. Therefore, perceived parental rejection should be taken into consideration during prevention and treatment of ADHD in young adolescents.

In Chapter 4, it was investigated whether prospective relations between ADHD symptoms and two types of life stressors (person-related [‘dependent’] vs. environment-related [‘independent’]) were different across the 5-HTTLPR status (SS, LS, and LL). Path
analyses showed that exposure to life stressors did not predict ADHD symptoms. However, the results showed that for the S-allele homozygotes of the 5-HTTLPR gene, ADHD symptoms in middle adolescence predicted exposure to the number of person-related life stressors later in adolescence. There was no relation with environment-related life stressors. The findings of this study suggest that S-allele homozygotes with higher levels of ADHD symptoms in middle adolescence are more vulnerable to becoming exposed to person-related life stressors in late adolescence. Findings emphasize the need to be aware of social-emotional adversities that may occur in genetically vulnerable adolescents with ADHD symptoms in the transition towards adulthood.

Finally, in Chapter 5, it was examined whether bidirectional relations between ADHD symptoms and the family and school climate were influenced by DRD4 and/or 5-HTTLPR genotypes. By using an advanced analytic approach which separates between-person (i.e., stable trait levels) and within-person (i.e., causal processes) levels, no influence of genotype was found. Independent from DRD4 and 5-HTTLPR genotype, results showed significant between-person differences between ADHD symptoms and both the family and school climate. Our findings suggest that higher stable levels of ADHD symptoms (trait levels) are associated with a less favorable family and school climate. Regarding within-person causal processes (state levels), ADHD symptoms predicted a less favorable family climate in early adolescence, while ADHD symptoms predicted a more favorable family climate in the later phase of adolescence. Overall, this study showed that negative associations between ADHD symptoms and both the family and school climate are largely explained by stable between-person differences.

Strengths and Limitations of the Studies Presented in this PhD Thesis

The use of longitudinal data from a large adolescent cohort enabled us to examine the role of various genetic, environmental (both parent and child data), and G×E effects on the course of ADHD symptoms from early into late adolescence. The range of measures and quality of the TRAILS dataset resulted in the use of different analytical approaches. The studies within this PhD thesis had a relatively large sample size compared to other G×E studies. This provided us with higher power compared to existing G×E studies (Dempfle et al., 2008); although future G×E studies should use still larger samples. Concluding, in this PhD thesis different lines of research were combined by investigating a broad variety of environmental contexts across adolescence in relation to the development of ADHD symptoms.

A few considerations and limitations need to be addressed.

First, in the empirical chapters of this PhD thesis I examined individuals beyond the typical onset and peak of ADHD symptoms, which is commonly before the age of 12
years (Ramtekkar, Reiersen, Todorov, & Todd, 2010). One might, therefore, argue that an important life phase in the development of ADHD symptoms was not captured. However, adolescence is an understudied period with specific risks that may influence the course of ADHD symptoms as previously discussed.

Second, by using a pooled sample of a population and clinic-referred cohort there was increased power to detect significant G×E effects. This allowed me to examine the entire spectrum of ADHD symptom levels which included the variation of ratings among unaffected participants. However, this limits generalizability to pure population or clinical samples.

Third, although the use of a CBCL subscale to assess ADHD symptoms showed good discriminative validity (Nakamura et al., 2009), this may not be ideal as it does not capture all 18 DSM ADHD symptoms. Also by only relying on parental assessment of ADHD symptoms over the past six months without taking other informants into account, a suboptimal assessment of ADHD symptoms was used.

Fourth, the observational study design does not allow for firm conclusions regarding causality, even though longitudinal analyses provide stronger evidence for causality than cross-sectional research. Only experimental designs present mechanisms of causality but this is hard to conduct in the area of child- and adolescent psychiatry.

Finally, while one might argue that in a multidisciplinary and longitudinal study such as TRAILS, the range of predictor variables and outcome domains on which data is collected is broad, unfortunately sometimes this is at the expense of depth or detail. Collecting in-depth measures is not always possible due to financial and practical constraints, such as limited time and risk of increased dropout due to placing heavy demands on research participants. A more refined measurement of environmental factors (e.g., age-sensitive and prospectively measured) is likely to be crucial to discover the interplay with genes even in samples of modest size (Rutter, 2003; Wong et al., 2003). Furthermore, in contrast to our studies, future designs should include sufficient time intervals between measurements to capture developmental change.

**General Considerations of G×E Research in Relation to ADHD symptoms**

Existing G×E research has shown that both nature and nurture are involved in psychopathology (e.g., Caspi et al., 2003), including ADHD (e.g., Van der Meer et al., 2014). G×E research has great potential since it underscores the complex interplay between nature and nurture which underlies a multifactorial disorder as ADHD. However, after a strong increase of published candidate G×E studies in the last decade, in recent years fewer candidate G×E articles have been published (Web of Science, 2018). This decline might partly be explained by the increased criticism on candidate G×E research (Duncan & Keller, 2011, 2014). Concerns about candidate G×E studies include a low replication rate, small sample sizes, a high false discovery rate, and typically low explained variation. Also
in the studies in this PhD thesis only partial support for the involvement of candidate genes was found. This underlines the sketchy picture in the literature regarding the role of candidate genes (I will comment on this further below).

In this PhD thesis, in order to make better use of the potential of candidate G×E research in relation to the course of ADHD symptoms, special attention was given to the aspect of development. Our studies point out that ADHD is not only related to genes, environment, and their interaction but also to the timing of their interplay. Integration of this developmental aspect into G×E studies on ADHD symptoms is needed for several reasons. First, ADHD symptoms typically change over time and this should be reflected in the design of studies. Second, study designs should take into account the changing impact of environmental factors across the life span. For example, in adolescence youth strive to gain independence from their families and become more engaged with their peers (Rubin, Bukowski, & Parker, 2006). This age-related change of the relative impact of various environmental factors (here a more important role of peers with increasing age) is something which scientists should be aware of and should be incorporated into G×E studies since the social context might be differently related to ADHD symptoms across development. In this PhD thesis, the specificity of the environment has been incorporated in Chapters 4 and 5. Although there was no genetic moderation of the DRD4 and 5-HTTLPR genotype in Chapter 5 the results showed that ADHD symptoms predicted a positive family climate in early adolescence whereas the direction of the effect was reverse two years later: ADHD symptoms predicted a negative family climate. Lastly, when studying ADHD symptoms across development, attention should be given to the potential impact of these symptoms on an individual’s environment, as shown in Chapter 4 and Chapter 5, a largely neglected aspect in present ADHD research.

To conclude, acknowledging developmental differences in the role of genes and the environment across the lifespan may be a step forward in understanding who is at risk of ADHD symptomatology during the life course.

Considerations Regarding the Measurement of Genes, Environment, and ADHD in Longitudinal G×E Research

Genes
The role of genes must ideally be examined throughout the lifespan since both stable genetic effects and new sources of genetic effects have been found to emerge during adolescent development (Chang et al., 2013). This is also reflected in Chapter 4 of this PhD thesis where only S-allele homozygotes with higher levels of ADHD symptoms were more vulnerable to becoming exposed to person-related life stressors in the later phases of adolescence. Overall, there is support that genetic influences may vary across development.
Genes may also provide a mechanism by which environmental exposures may have effects on gene expression (Weaver et al., 2004). For example, Dadds and colleagues (2016) showed that epigenetic regulation of the \textit{DRD4} gene in the form of increased methylation is associated with the cognitive/attentional deficits in ADHD. Thus, besides main effects of genes, epigenetics may also explain susceptibility to the environment (Van IJzendoorn, Bakermans-Kranenburg, & Ebstein, 2011) in relation to the course of ADHD symptoms.

A new area in G×E research is the upcoming use of polygenic risk scores. A polygenic risk score can be seen as a measure of ‘genetic burden’ associated with a phenotype (Wray, Goddard, & Visscher, 2008). It has been suggested that G×E studies may benefit from using polygenic risk scores (e.g., Salvatore et al., 2015), since it is expected that a polygenic risk score has a larger effect than single genetic variants. This is in line with the notion that ADHD is caused by many common variants with small effects rather than a single gene (Asherson, Kuntsi, & Taylor, 2005). Indeed, a polygenic risk score has proven to be one of the most robust and informative biomarkers of psychiatric diagnosis to date, expressing a risk or liability towards illness (Wray et al., 2014; Wille & McMahon, 2018). In relation to ADHD, a persistent course of ADHD symptoms across childhood and adolescence was associated with a high polygenic risk score (Riglin et al., 2016). However, since a polygenic risk score reflects a heterogeneous genetic construct, information about the role of specific underlying risk genes for the course of ADHD is limited. Nevertheless, polygenic risk scores might help draw distinctions within broad diagnostic categories into genetically (and thus biologically) more homogeneous subsets that reflect differences in underlying genetic causes (Wille & McMahon, 2018). To conclude, it may be expected that novel approaches to study the genetic contribution to the course of ADHD across the lifespan will greatly advance the field.

\textbf{Environment}

Sophisticated, age-appropriate measurements of environmental factors that are most likely related to developmental changes may be pivotal in longitudinal G×E research. A good example of age-appropriate measurements in this PhD thesis is the distinction of person- and environment-related life stressors (see ‘Chapter 4’), which resulted in an age-specific effect of a particular environmental outcome. In short, high levels of ADHD symptoms of S-allele homozygotes of the \textit{5-HTTLPR} genotype predicted person-related life stressors but not environment-related life stressors. The distinction between person- and environmental-related life stressor suggests that fine-grained measures of environmental factors are required in order to obtain significant and meaningful results regarding G×E interactions. Moreover, it is important to collect prospective rather than retrospective data (Moffitt et al., 2006), which, unfortunately, often is not possible due to limited resources. Finding the right balance between the use of appropriate (specific,
time-sensitive) measurements and feasibility is challenging, particularly since examining G×E interactions requires large samples.

**ADHD symptoms**

Difficulties in finding G×E interactions, and also main effects of genes or environment, in relation to neuropsychiatric conditions such as ADHD, may be due to a lack of narrowly defined and valid phenotypes of these disorders. Phenotypic heterogeneity is one of the main reasons for the lack of neurobiological markers in ADHD (Scassellati, Bonvicini, Faraone, & Gennarelli, 2012). Interindividual variability among individuals with ADHD hinders the search for a homogenous group of individuals. Considering subdimensions of ADHD may be a more fruitful approach for future studies, especially as the separate dimensions of inattention and hyperactivity-impulsivity may have a different etiology, particularly with regard to genetic influences (Nikolas & Burt, 2011). This might also be true for the course of inattention and hyperactivity-impulsivity symptoms across adolescence. Although distinguishing between individuals with predominantly hyperactivity-impulsivity or inattention could create more homogenous groups, it has been argued that the distinction of ADHD types may not be stable over time (Coghill & Seth, 2011). Creating groups of individuals with similar ADHD symptoms across adolescent development (see Chapter 3) is an alternative approach to address the heterogeneity of ADHD. That is, creating more homogenous groups of individuals with ADHD (i.e., by type or age of onset) may help in identifying risk factors for the course of ADHD symptoms.

**Considerations Regarding the Impact of ADHD Symptoms on Adolescents’ Lives**

In Chapter 1 of this PhD thesis it was stated that adolescence is a key developmental period for examining ADHD symptoms because of new challenges adolescents are bound to cope with, and associated neurobiological changes and brain development. This may suggest that adolescents are particularly vulnerable to the effects of environmental influences. However, interestingly, with one exception described in Chapter 2, showing that G×Perinatal factors predicted the course of ADHD symptoms, it appears that ADHD symptoms impact on the environment. This was found in Chapter 4, in which adolescents with more severe ADHD symptoms were exposed to more life stressors and in Chapter 5, in which higher ADHD symptom scores influenced family functioning. This implies that adolescents with high levels of ADHD symptoms are at risk for further problems during their development, with possible negative long-term consequences in adulthood. Overall, this highlights the need for awareness and prevention of these age-specific environmental factors that may change in the transition from adolescence into adult life in individuals with ADHD symptoms.
Clinical & Societal Implications of this PhD Thesis

The findings in this PhD thesis provide evidence that determinants of the course of ADHD symptoms depend on the timing of their influence. This informs clinicians and families as it points to changing contributions of risk factors over the course of adolescent development. Moreover, adolescents with ADHD symptoms are vulnerable to be exposed to social-emotional adversities and practitioners should be aware of this, addressing the potential risks of negative parenting styles and an unfavorable home environment. Furthermore, increasing knowledge may eventually lead to the development of more optimal treatments provided to those who need it most.

The findings may also have implications for the wider societal environment which offers opportunities for interventions. One example of the wider context that might influence the course of ADHD symptoms is the ‘10-14’ school (“Basis- en Voortgezet Onderwijs Ontmoeten Elkaar in 10-14”, 2017). This school takes into account that adolescence is a turbulent time where major biological, as well as psychological developmental tasks, take place. It is based upon the idea that by postponing the transition to secondary school to 14 years of age, the ‘10-14’ school serves as a “secure base” for adolescents in getting to know themselves before they go to secondary school. This might be especially relevant for adolescents with the 5-HTTLPR S-allele homozygotes with high levels of ADHD symptoms since they experience more person-related life stressors in the later phases of adolescence (see ‘Chapter 3’). It may be argued that these adolescents are more sensitive to the transition to secondary school. Another example of the influence of the wider social context on the course of ADHD symptoms is that Groningen aims to be the first non-smoking city in the Netherlands (Morssinkhof, 2017). This might be especially relevant for L-allele homozygotes of the 5-HTTLPR genotype since maternal smoking during pregnancy was associated with more ADHD symptoms particularly during early adolescence (see ‘Chapter 2’).

Suggestions for Future Research

The findings in this PhD thesis brought valuable insights into the role of candidate genes, the environment, and their interplay on the course of ADHD symptoms. Since this PhD thesis had a hypothesis-generating approach, as there had been little previous research in this area, there is ample work to be done in the future. First, replication studies are needed to rule out chance findings. Thereafter, more theory-based G×E studies can help to reduce Type I error (i.e., false positives). Second, since the role of genes (see ‘Chapter 3’), environment (see ‘Chapter 5’), and G×E (see ‘Chapter 2’ and ‘Chapter 4’) appears to vary across adolescence, an important recommendation for future longitudinal research is that studies should examine shorter time intervals than used in this PhD thesis to capture the full potential of possible time effects. Third, measurement of predictors (genes and environment) and the outcome (course of ADHD symptoms) in longitudinal G×E
research should have the capacity to reflect possible changes across the lifespan. Clustering individuals into more homogeneous groups (e.g., as per sex or subphenotypes) may be important, both to allow for the development of more targeted treatments and to reduce confounds in studies on genetic and environmental risk factors. Moreover, a broader scope should be taken to measure the contribution of genetic factors across development. As discussed above (see ‘Considerations Regarding the Measurement of Genes, Environment, and ADHD in Longitudinal G×E Research’), epigenetic mechanisms may also contribute to ADHD (Dadds et al., 2016) and should therefore also be considered in relation to the course of ADHD symptoms. Furthermore, first steps have been taken in examining polygenic risk scores versus the course of ADHD symptoms (Riglin et al., 2016).

Final Conclusions
Genetic and environmental influences were traditionally examined in isolation but there is now increasing support that the interplay between genes and the environment is crucial in understanding the course of ADHD symptoms. The search for specific G×E effects, as well as main effects of genes and environment, on the course of ADHD symptoms, however, still represents a complex and unfinished task. Heterogeneity in terms of the definition of variables of interest, research designs, and involved samples have to be taken into account when interpreting results and planning future studies, preferably utilizing advanced statistical and genetic techniques in large-scale samples. Overall, the specificity of G×E effects on the course of ADHD symptoms presented in our studies shows that the precision of timing of the role of genes, environment, and their interaction is a pivotal aspect in discovering determinants of the course of ADHD symptoms across adolescence.

Key Findings
Findings per chapter

• The simultaneous presence of the low activity MAOA genotype and low birth weight as well as the long-version of the 5-HTTLPR genotype with both pregnancy and delivery complications and also maternal smoking were associated with more ADHD symptoms especially during early adolescence, and showed a decreasing influence of these interaction effects over time. This study supports an age-dependent role of the interaction between genes and pre- and perinatal factors on ADHD symptoms across adolescence (see ‘Chapter 2’).

• No G×E interactions were found between ADHD candidate genes (i.e., DRD4, 5-HTTLPR, and MAOA), and perceived parenting styles (i.e., perceived parental overprotection, perceived parental rejection, and perceived parental emotional warmth). However, an important finding of this study was that perceived parental rejection predicted class
membership in the high persistent ADHD symptom trajectory compared to the low stable, moderate stable, high decreasing trajectories. This suggests a role of perceived parental rejection in the persistence of ADHD symptoms (see ‘Chapter 3’).

- S-allele homozygotes of the 5-HTTLPR genotype with higher levels of ADHD symptoms were more vulnerable to becoming exposed to person-related (i.e., dependent) life stressors in the later phases of adolescence, while there were no associations with environment-related (i.e., independent) life stressors; nor did life stressors prospectively predict ADHD symptoms. These findings suggest that genetically vulnerable adolescents with higher levels of ADHD symptoms in middle adolescence are more vulnerable to becoming exposed to person-related life stressors in late adolescence. (see ‘Chapter 4’).

- High stable, trait-like levels of ADHD symptoms were associated with a less favorable family climate and school climate and, high state-like levels of ADHD symptoms (which suggest causal processes) predicted a less favorable family climate in early adolescence, while the opposite pattern was observed in the later phase of adolescence. Overall, negative associations between ADHD symptoms and both the family and school climate seem explained by stable between-person differences. (see ‘Chapter 5’)

**General conclusions**

- Genes, environment and their interaction are important in examining the course of ADHD symptoms across adolescence, but likely only fruitful when adopting age-appropriate measurements.
- In examining genes, environment and G×E interactions in relation to the course of ADHD symptoms it is important to be aware of their varying influence across development.
- Adolescence seems a time where ADHD symptoms may set off further problems for individuals (i.e., exposure to more life stressors or less positive family climate).


REFERENCES


