How do genes get outside the skin? Mechanisms underlying Gene×Environment interactions in child externalizing problems

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Summary and General Discussion

Experimental Testing of Mechanisms Underlying Candidate GenexEnvironment Interactions: Implications for Future Research and Clinical Practice

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Over the past 15 years developmental scientists gained much insight into individual differences in how (strongly) children react to their surroundings. Specifically, we learned much about specific candidate genes that possibly contribute to individual differences in the strength of the relation between environmental risk factors and psychopathology (i.e., candidate gene-by-environment interactions or cG×E). The initial stage of cG×E research has yielded an interesting set of results, detecting many interaction effects between genetic polymorphisms and environmental adversity predicting externalizing behaviors. Although research on cG×E was originally received with great enthusiasm, it is now heavily criticized (for a critical review see Dick et al., 2015). Among the most important points of criticism are: that research on cG×E has not yielded reliable and replicable results; that the methods used to test cG×E are flawed; that so far it has taught us little about *how* genes might contribute to the development of psychopathology; and that it has given us little to no useful knowledge for clinical practice. In this dissertation we aimed to address these critiques by: (1) creating an overview of the literature (Chapter 2) and extending previous findings on cG×E with a randomized controlled trial (the ORCHIDS study; Chapters 3-6); (2) gaining more insight into how these interactions might work by formulating theory-based hypotheses on possible mechanisms underlying cG×E (Chapter 2); and (3) by testing hypothesized underlying mechanisms using two experiments (Chapters 3-7).

Because the *O*bservational *R*andomized controlled trial of *CHI*ldhood *D*ifferential *S*usceptibility (the ORCHIDS study) is central to this dissertation (but not necessarily to all our main research aims), I will first give an overview of this study and findings on the effectiveness of the used intervention. Second, in part 1 of this chapter I will summarize and discuss our overview on previous found cG×E including family adversity and polymorphisms of the *5-HTT*, *MAOA*, *DRD4*, *DRD2*, *COMT* and *DAT1* genes as well as the findings on cG×E tested in the ORCHIDS study. Third, in part 2 and 3 of this chapter I will summarize and discuss the hypotheses we formed on mechanisms underlying cG×E in externalizing behavior problems as well as findings on these mechanisms tested in two experiments (the ORCHIDS study and a focused experiment). Finally, I will discuss the strengths and limitations of this dissertation, as well as my ideas on the implications of our findings for future research and clinical practice.

The ORCHIDS study

Chapters 3 and 4 describe the ORCHIDS study; a three wave (pre-test, post-test and follow-up) randomized controlled trial (RCT) including data on children's genotype, and observed as well as reported parent and child behavior. Although not a primary aim of this dissertation, we did also test cG×E interactions in the process of testing mechanism underlying these interactions. Using an experimental design to test cG×E has important advantages over the correlational designs used in most previous studies. First, in experiments G and E are

uncorrelated because children are randomized into different groups and the environment is manipulated. This manipulation rules out alternative explanations for cG×E, such as gene-by-environment correlations (i.e., *r*GE). For example, certain genetic predispositions in children might evoke certain parenting behaviors. In an experimental design this covariance cannot occur. Second, using standardized experimental manipulation of the environment reduces measurement errors in the assessment of the environment. Third, in experimental research designs power is enhanced due to the increased difference between children in different experimental conditions.

Chapter 3 describes the research protocol and a priori hypotheses. For the purpose of transparency, these were pre-registered in our 2012 publication (Chhangur & Weeland et al., 2012). The trial has been registered in the Netherlands Trial Registry (NTR-TC-3594) and was conducted in 2013 (cohort 1) and 2014 (cohort 2). In total, 5,876 families were screened on child externalizing problems. Families of children scoring at or above the 75th percentile of their respective cohort were invited to participate in the further trial. This resulted in a sample of 387 parent-child dyads, which were randomized in a control and intervention group. At the first measurement wave (i.e., pre-test) children (55.3% boys) were between 4 and 8 years of age (*Mage* = 6.31, *SD* = 1.33). The sample mostly consisted of parents and children who were born in the Netherlands (97.4% of children and 80% of parents were born in The Netherlands). At the first wave buccal swabs were collected in order to genotype children (in this dissertation I focused on the *COMT*, *MAOA*, and *5-HTTLPR* polymorphisms, which are related to serotonin and dopamine functioning). Families that were randomized into the intervention group received the evidence based Behavioral Parent Training (BPT) The Incredible Years program (IY, Webster-Stratton, 2008) between pre- and post-test. Families in the control group did not receive intervention through the trial, but were free to seek out additional (mental) health care (i.e., care as usual). Child and parenting behavior were observed and reported on by parents at all three waves (pre-test, post-test and follow-up).

Chapter 4 shows that using full intention-to-treat analyses, IY was successful in decreasing parent reported child externalizing problems at time of follow-up (small effect, partial η^2 = .05 or Cohen's *d* = .20), increasing parent reported (small to medium effect, partial η^2 = .06 or Cohen's *d* = .49) and observed (small effect, partial η^2 = .05 or Cohen's *d* = .06) positive parenting behavior, and decreasing parent reported negative parenting behavior (small to medium effect, partial η^2 = .08 or Cohen's *d* = .29). No intervention effects were found for observed child externalizing problems, observed negative parenting behavior, and reported and observed child prosocial behavior. Furthermore, as expected, when tested in a multi-variate model, in a large sample, no systematic evidence emerged for previous found demographic or intervention-based moderators of IY effects**.** Overall, our findings indicate that IY was successful in decreasing externalizing problems in children of a wide range of families. In part 1 of this chapter I will summarize and discuss our overview on previous

found cG×E including family adversity and polymorphisms of the *5-HTT*, *MAOA*, *DRD4*, *DRD2*, *COMT* and *DAT1* genes as well as the findings on cG×E tested in the ORCHIDS study.

To gain more insight into how cG×E interactions might work we formulated (Chapter 2) and tested (Chapters 5-7) theory-based hypotheses on possible mechanisms underlying cG×E including polymorphisms of the *5-HTT*, *MAOA*, *DRD4*, *DRD2*, *COMT* and *DAT1* genes. Insight into the mechanisms of cG×E can possibly help us interpret the intriguing, but inconsistent, findings, and enhance their empirical and clinical implications. It can point us in the direction of differential pathways leading to externalizing behaviors. And though not directly implementable, it might enable us to better predict which children are specifically vulnerable and target them using personalized interventions not only in terms of clinical focus (i.e., based on specific mechanisms at work), but also of intensity and duration (i.e., based on differences in susceptibility).

1. Findings on cG×E in Externalizing Behavior Problems: Family Adversity, *5-HTT, MAOA, DRD4, DRD2, COMT* and *DAT1*

Chapter 2 presents the results of our review study of 53 studies published between 2002 – 2015 which shows the findings on cG×E between family adversity and polymorphisms of the *5-HTT*, *MAOA*, *DRD4*, *DRD2*, *COMT* and *DAT1* genes are heterogeneous (Weeland, Overbeek et al., 2015). To illustrate, our literature overview shows that for the *5-HTTLPR* VNTR polymorphism, null findings (4 out of 12 studies in the review paper) as well findings indicating that both the low functioning S-allele (4 out of 12 studies in the review paper) and the high functioning L-allele (4 out of 12 studies in the review paper) are "risk allele" for the development of externalizing problems under adversity have been reported. Despite this apparent heterogeneity of results, the current state of the literature might still be an underestimation of true heterogeneity due to a possible publication bias in this field favoring statistically significant findings over non-significant findings, specifically in case of the *5-HTTLPR* (see Duncan & Keller, 2011; Ficks & Waldman, 2014).

In the ORCHIDS study we tested three previously found cG×E interactions between parenting and polymorphisms of the *5-HTT*, *COMT* and *MAOA* genes overcoming important limitations of previous studies by using a RCT (Chapter 5 and 6). We found no significant interaction between parenting (either improved through IY or "normally developed" in the control condition) and the *COMT* Val/Met polymorphism (Chapter 5). This means that the *COMT* polymorphism did not moderate the relation between parenting and child externalizing problems. For the *5-HTTLPR* different moderation patterns were found for different outcome measures (Chapter 6). On the one hand, this might indicate that this gene-based moderation of intervention effects is not highly robust and replicable. On the other hand, this might show that cG×E is very specific and thus differs using different outcomes,

reporters or instruments. I will discuss these findings in more detail in part 3 of this chapter. Altogether, our own empirical findings on the *COMT* and *5-HTTLPR* genotypes, reported in this dissertation, underline previous found heterogeneity of the cG×E literature (see Chapter 5, 6 and 7).

There are however notable exceptions to the rule that cG×E findings are heterogeneous in nature. Our review study (Chapter 2) shows that at least some of the findings on cG×E seem robust. For example, the literature shows a moderately consistent interaction between the *MAOA* low-activity allele (possibly leading to low levels of monoamine oxidase A; lower degradation and higher availability of dopamine and serotonin) and maltreatment in predicting conduct problems in males (see the meta-analyses of Byrd & Manuck, 2014; Kim-Cohen et al., 2006; Taylor & Kim-Cohen, 2007). In line with this previous literature, in Chapter 5 we also found an interaction between the *MAOA* low-activity and the effect of IY in predicting child externalizing problems. Specifically, we found that boys carrying the low-activity allele benefited more from the intervention and showed a larger decrease in externalizing problems compared to boys without this allele (Chapter 5). On the one hand, this might indicate that these boys are more sensitive to environmental enrichment, and therefore show vantage sensitivity (Pluess & Belsky, 2013). On the other hand, combined with previous findings, our results might be interpreted in line with differential susceptibility thinking (Belsky, 1997): Boys carrying the low-activity allele might not only be more vulnerable to environmental risk (i.e., for worse: for example developing conduct problems when maltreated) but might also be more susceptible to environmental enrichment (i.e., for better: showing more improvement in externalizing problems after intervention). Although the interactions between *MAOA* and parenting seem relatively robust, *how* the *MAOA* is related to both an increased vulnerability to maltreatment as well as an increased susceptibility to the effects of a behavioral parent training remains unclear.

Based on both previous literature (Chapter 2), as well as the empirical findings presented in this dissertation (Chapter 5 and 6), one might conclude that cG×E, with few exceptions, has not yet delivered conclusive evidence for specific cG×E. However, our review of the cG×E literature (Chapter 2) also makes clear that there are large methodological differences between studies, such as sample size and composition, conceptualization, and power. cG×E might be very specific and differ when testing them with outcomes based on different reporters or instruments. The current heterogeneity of findings might therefore partly be explained by such methodological differences between studies (Heininga et al., 2015; Weeland, Overbeek et al., 2015, Chapter 2). One way to create more comparable results is by specifying a priori hypotheses together with a priori specifications of research strategies and power to test specific hypotheses: How do specific family adversity factors interact with specific genetic polymorphisms, in predicting specific behavior in specific samples? By consulting extant literature on genetics, (neuro)biology, and psychology we aimed to construct such hypotheses in Chapter 2.

2. Hypotheses on Possible Mechanisms Underlying cG×E

Our literature review in part 2 of Chapter 2 shows that literature on genetics, neurobiology and psychology provides us with many clues on possible mechanisms underlying cG×E. In this dissertation, we formed three hypotheses on possible mechanisms of cG×E between family adversity and the *5-HTTLPR*, *MAOA*, *DRD4*, *DRD2*, *COMT* and *DAT1* polymorphisms, namely 1) serotonin based emotional reactivity; 2) dopamine based reward sensitivity, and 3) serotonin based punishment sensitivity.

Emotional reactivity

Literature on the *5-HTTLPR* polymorphism shows important parallels with research on emotional reactivity: Individual differences in arousability and behavioral responses to others emotions. Serotonin has been related to arousal in response to emotions (Murphy et al., 2013); regulation of this arousal (Gyurak et al., 2013; Miczek, Fish, Joseph, & De Almeida, 2002); and therefore the effects of others' emotions on affective and behavioral responses (El-Sheikh, 2001; Hankin et al., 2009). Children with higher and less stable serotonin availability (e.g., carrying the *5-HTTLPR* S-allele; but possibly also the *MAOA* low-activity allele) who are exposed to negative family emotional climates (e.g., harsh parenting, marital conflict) might show an increase in neurological arousal by emotional stimuli. In turn, children experiencing such dual risk possibly develop a heightened emotional reactivity. We therefore hypothesize that these children show more symptoms of angry/irritable mood and reactive aggression, compared to children growing up without a genetic predisposition, specifically when they are exposed to negative emotions and/or a negative family emotional climate (i.e., in line with the frustration-aggression model of Berkowitz, 1989). In addition, considering the overlap in symptoms between Oppositional Defiant Disorder (ODD) and child depression, these children might also be at risk for internalizing problems (Copeland et al., 2009; Stringaris et al., 2012).

Reward sensitivity

Literature on the dopaminergic polymorphism shows important parallels with research on (brain) systems to reward processing: Dopamine has been related to salience and valence of rewarding experiences (Buckholtz et al., 2010; Comings & Blum, 2000); arousal in response to rewards (Schultz, 2002); and therefore effects of reward-based learning (Comings & Blum, 2000). Children with low dopamine activity (e.g., carrying the *COMT* Val-allele, *MAOA* high-activity allele, *DRD4* 7-repeat allele, *DRD2* A1-allele, and *DAT* 10-repeat allele) might show a decrease in sensitivity to daily rewards in absence or scarcity of rewarding stimuli in the family environment (e.g., positive reinforcement of positive behavior trough praise or tangible rewards). Children experiencing such dual risk might develop a low sensitivity to typical environmental reinforcers (i.e., experiencing them as less rewarding). We therefore

hypothesize that these children show more noncompliant, risky, and thrill seeking behavior, compared to children growing up without a genetic predisposition, specifically when the current environment does not offer them the necessary rewarding stimuli or behavioral monitoring (i.e., short behavioral monitoring intervals). Also, because of their search for arousal these children might be at risk for addiction later in life (e.g., Bhaskar et al., 2012).

Punishment sensitivity

Literature on the serotonin related polymorphisms shows important parallels with research on punishment sensitivity and callous unemotional traits: Serotonin might act as a motivational opponent to dopamine (Daw, Kakade, & Dayan, 2002) by modulating the impact of punishment-related signals (Cools, Roberts, & Robbins, 2008); and therefore the effects of socialization through punishment (Kochanska, Aksan, & Joy, 2007). Children with low and stable levels of serotonin availability (e.g., carrying the *5-HTTLPR* L-allele or *MAOA* high-activity allele) experiencing maltreatment, harsh punishment or punishment mixed with reward, might develop a blunted reactivity to negative emotional arousal and punishment. In turn, children experiencing such dual risk might be less sensitive to punishment oriented parenting strategies. We therefore hypothesize that these children show more antisocial behavior compared to children growing up without a genetic predisposition, specifically when the current environment relies on harsh, unpredictable punishment for socialization. Eventually, this low reactivity and poor conditionality through punishment might induce proactive, instrumental, and maybe even predatory antisocial behavior later in adulthood, forming a risk factor for antisocial personalities and psychopathic traits. Also, these children are possibly at risk for escalating cycles of punishment, as milder forms of punishment may be less effective and parents might get frustrated.

Important to note is that externalizing behavior is a very heterogeneous behavioral cluster, which has different etiologies in different children and across symptoms (Frick, 2012). Specific externalizing behaviors may be triggered by different mechanisms, underlying different cG×E, in different populations. For example, both the *5-HTTLPR* S-allele and the L-allele might contribute to externalizing behavior under the influence of family adversity: The S-allele might be related to emotional reactivity and therefore be a risk for irritability and reactive aggression in the presence of a negative emotional climate (e.g., Cicchetti et al., 2012). In contrast, the L-allele might be related to emotional hypo-reactivity and punishment insensitivity, and might therefore be a risk for proactive and predatory behavior when socialization is mainly based on punishment (e.g., Sadeh et al., 2010). Therefore, different mechanisms are likely to underlie the development of different types of externalizing behaviors.

3. Experimental Testing of Mechanisms

In this dissertation, we experimentally tested two of the hypotheses described above on mechanisms underlying cG×E, namely emotional reactivity and reward sensitivity. Experimental testing of cG×E and underlying mechanisms is important because it overcomes limitations of earlier studies using correlational designs (Jaffee, Price, & Reyes, 2013). Both RCT as well as more focused experimental designs have important advantages over correlational research, and are complementary approaches. Large scale RCT's enable us to target at risk samples and to manipulate the environment with evidence-based interventions, resulting in superior power to robustly detect cG×E if present, while preventing *r*GE. At the same time RCT's have important limitations regarding feasibility and specificity. Specifically, an intervention effect in an RCT covers many different possible change mechanisms. Therefore, small-scale, randomized experiments using a brief and more focused environmental manipulation, designed to target one specific risk or protective mechanism are an important addition to RCT's (i.e., micro-trials, Howe, Beach, & Brody, 2010). Such designs allow us to take a closer look at possible mechanisms. Therefore, in this dissertation we conducted a randomized controlled trial (the ORCHIDS study), using the evidence based BPT IY (N = 387 parent-child dyads), and a more focused experiment using standardized video vignettes of facial expressions and vocalizations of emotions (N = 521 children). Both experiments were successful in manipulating the environment (see Chapter 4 and Chapter 7).

In Chapter 5, using a RCT, we tested whether the previously identified G×I with the *MAOA* polymorphism might be explained by differences in children's reward sensitivity. Genotypes that upregulate dopamine (i.e., causing higher availability), such as the *MAOA* low-activity allele, might contribute to a heightened reward sensitivity. Children carrying this allele might therefore experience reward oriented parenting strategies, such as parental praise, as particularly strong behavioral reinforcers. Therefore, these children might specifically benefit from IY because they benefit substantially from an increase in such parenting behavior. Our results indeed showed that IY caused a large increase in parents' use of praise (partial η^2 = .19). However, this increase did not explain the intervention effects on child externalizing problems in either children with the *MAOA* low-activity or children with the high-activity allele. Therefore, we did not find support for the dopamine related reward sensitivity hypothesis.

In Chapter 6 we tested whether the effectiveness of IY in decreasing child externalizing problems is due to changes in parental affect and behavior, and whether these mechanisms of change are more important for some children than for others, due to a temperamental (i.e., negative affectivity) and/or genetic (i.e., *5-HTTLPR* genotype) predisposition for heightened emotional reactivity. Our results show that neither parental affect nor parenting

behavior was more important in explaining the effectiveness of IY for some children than others due to their negative affectivity or *5-HTTLPR* genotype. We therefore found no evidence for the emotional reactivity hypothesis.

Although we were unable to explain differential effects of our intervention, our results do suggest that differential mechanisms are at work, both in parents and children, due to children's genotype. For example, children's *5-HTTLPR* genotype did moderate the effects of the intervention on observed and reported negative parenting behavior (moderation effects in opposite direction for the two outcome measures) as well as the effects of observed positive parenting behavior on reported child externalizing problems. These findings can be interpreted in different ways. First, our findings might indicate that some parents are more susceptible to the IY program than others, due to their children's genotype. For example, due to heritability parents and children might share the same susceptibility characteristics. However, different moderation patterns were found for observed and reported parental behavior outcomes. Parents of children homozygous for the L-allele *reported* the largest decrease in negative parenting at post-test. However, the same parents *showed* a lower decrease in negative parenting during observed parent-child interactions, compared to parents of children carrying two S-alleles. This might indicate that parents of children carrying two L-alleles are more prone to experience and report improvements in their own, whereas they show less actual changes in their behavior, compared to parents of children carrying two S-alleles.

Second, observed positive parenting was only a significant predictor of decreased child externalizing problem behavior for SS-genotypes (and seemingly in opposite direction than for LL-genotypes). This might indicate that children carrying the S-allele are more susceptible to changes in -specifically positive- parenting behavior than children homozygous for the L-allele (see also Hankin et al., 2011). However, the effect of positive parenting was very small, and the differential effect of parenting was not replicated using any of the other parenting behavior or affect measures. Third, our findings might indicate that actual changes in parenting behavior after intervention are not merely formed in the intervention session, but are also informed and shaped over time by the reciprocal influences between parent and child behavior (Sameroff, 2000). Children's different reactions to intervention-induced changes in parenting behavior might serve as a feedback mechanism for further changes in parenting behavior. For example, when parents see that the use of certain parenting techniques works really well on their children they might further increase the use of these techniques, whether they might stop using techniques which initially evoke resistance. It might be that, due to a decreased sensitivity to specific parenting strategies, children carrying the L-allele are less responsive to (changes in) their parent's behavior, possibly leading to parental frustration and in turn escalating cycles of negativity and punishment (see also our hypotheses in Chapter 2). Directly after the intervention, families of children

with the LL-genotype seemed to experience the biggest relief in parenting stress. However, at the same time these might be the families who are first to fall back in old patterns of negative parent-child interactions, due to certain child characteristics.

Chapter 7 describes our specific experiment. In this study 521 children (52.5% boys, *M*age = 9.72 years) were randomly assigned to happy, angry or neutral dynamic facial expressions and vocalizations. Motor and affective emotional reactivity were assessed through children's self-reported negative and positive affect (n = 460) and facial electromyography activity (i.e., *f*EMG: the zygomaticus or "smile" muscle and the corrugator or "frown" muscle, n = 403). A buccal swab was collected to genotype children and parents reported on their negative and positive parenting behaviors. Children mimicked and experienced the emotion they were exposed to. As expected, children in the happy condition showed the strongest smile and highest response score for positive affect (i.e., happiness), but lowest for negative affect (i.e., anger, sadness and fear) of all conditions. Children in the angry condition showed the strongest frown and lowest response score for positive affect, but highest for negative affect of all conditions. However, neither motor reactivity (small effect partial η^2 = .05) nor affective reactivity (small effect, partial η^2 = .04) to these emotions depended on children's *5-HTTLPR* genotype: children with SS/SL-genotypes did not manifest any stronger response to emotional stimuli than children with LL-genotypes. This finding did not change when we took into account the broader environment children grow up in through parent-reported parenting behavior. Therefore, we did not find support for the serotonin related emotional reactivity hypothesis.

Overall, our results show that mechanisms underlying cG×E are very complex and difficult to grasp (Chapter 5, 6 and 7). In this dissertation, several factors complicated testing of these mechanisms. First, we assessed mechanisms in children 4 to 12 years of age. However, due to ongoing interactions between genetic dispositions and the environment mechanisms underlying cG×E possibly become increasingly complex over developmental time. Studying these mechanisms at an earlier developmental period might therefore yield different results. Second, the processes underlying the development of externalizing behavior, as well as interventions to stop further development of this behavior, might be less clear-cut than we think. Although we assessed parenting strategies that are strongly grounded in theory, our RCT showed only weak relations between parenting and child behavior. Furthermore, in neither of the studies using the RCT we found actual mediation by parental affect or behavior: Changes herein did not explain the changes in child behavior.

One possible explanation for the lack of mediation effects in our studies is that intervention induced changes in parent and child behavior are explained by a third factor. It has been found that BPT has "beneficial side-effects", for example by decreasing parent and family distress, and increasing parents' quality of life, and feelings of self-efficacy (Feldman & Werner, 2002). This might be specifically the case for interventions such as IY because

they use a collaborative approach aiming to empower parents. The group meetings might cause parents to relabel oppositional behavior as common and less problematic, and to feel empowered by sharing experiences and being handed tools for dealing with this behavior. We are not the first to report a lack of evidence for parenting as mechanism of change in BPT programs. More research is needed to provide evidence-based explanations for how effective and well-studied interventions, such as IY, produce change (Forehand et al., 2014, Kazdin, 2007).

Third, some of the hypothesized paths within the mechanisms (e.g., relations between intervention and parenting behavior; between parenting and child behavior; between genotype and child behavior) were not significant. Although significance of all these paths is no statistical requisite for the full mechanism to occur (Zhao et al., 2010), nonsignificant paths do reduce power to find significant effects of the overall mechanism. In our study, for example, intervention effects of IY were most pronounced at post-test, which reduced power for tests regarding the differential effects of IY on child externalizing problems at follow-up *via* changes in parental behavior at post-test. Moreover, in case of the *5-HTTLPR* we did not find a gene-by-intervention (i.e., G×I): Child genotype did not moderate the intervention effects on child behavior.

In sum, in this dissertation we did not find evidence for the mechanisms of emotional reactivity and reward sensitivity: Although we did find a G×I interaction between IY program and children's *MAOA* genotype, these differential intervention effects were not explained by changes in parents' use of praise. In addition, there were no differential effects of changes in positive or negative parental affect after intervention, due to children's *5-HTTLPR* genotype (Chapter 6). Moreover, we did not find differences in how strongly children responded to others' emotion expression and vocalization, due to their *5-HTTLPR* genotype (Chapter 7). We might therefore conclude that we have falsified the formed hypotheses. However, before drawing such conclusions our findings need to be replicated in independent samples. Moreover, replication attempts of our findings should take into account the specific limitations of the assessment of parenting behavior used in this study (described below).

Strengths and Limitations of This Dissertation

The findings of this dissertation add to the literature in several ways. First, we used extant literature to create an overview of findings on cG×E in externalizing behavior and to form theory-based hypotheses on mechanisms underlying these cG×E. This might help researchers in choosing which specific combination of genes, environments and behaviors to study and to specify their research strategies accordingly. Second, we tested two of these hypotheses using multi-informant experimental studies, overcoming important methodological limitations of previous research. Our studies therefore provide robust

tests of cG×E and how these interactions might (or might not) be explained. Moreover, our multi-informant approach shows that using different instruments to measure behavior and affect is important to get a more complete picture of changes herein. In our RCT we used both parent-reported and observed information on parent and child behavior. In our experiment, we used facial electromyography (*f*EMG) and child self-report to measure emotional reactivity. Although both approaches were successful in measuring change in parenting and child behavior and affect, our results show they do measure different aspects of these changes (see Chapter 5-7).

Nevertheless, the findings presented in this dissertation should also be interpreted in the light of a few limitations. First, although our RCT study (N = 387) is one of the largest studies using the effective BPT program IY, retaining 93% of participants at follow-up, we did not reach our target of 480 parent-child dyads (see Chapter 3, we therefore decided to deviate from the proposed 1:2 intervention/control randomization and evenly distribute families over the two conditions). The same is true for our experiment in Chapter 7, for which the target N was 600. This means that with our eventual samples the probability of detecting an interaction was lower than the a priori calculated 80%. A bigger sample would have provided us with more capacity for the complicated moderation and mediated moderation models and the use of stringent criteria for significance (e.g., correcting for multiple testing in Chapter 4 and using confidence intervals for indirect effects in Chapter 5 and 6) and in turn more power to find significant effects when present. Some of our results indeed hint on power issues (see also Chapter 6).

Second, our recruiting procedures generated specific subsamples of families willing to be enrolled, randomized, to put time and effort in filling out questionnaires and, in case of the RCT, welcoming researchers into their homes, as well as attend 14 weekly intervention sessions. As a result, participants of both studies were predominantly white Dutch and relatively high SES families. Our results may therefore not be generalizable to the general Dutch population or other countries. Third, although our multi-informant approach is a major strength of our studies, some of our instruments showed important limitations. Specifically, our observational instrument was used to code parent and child behavior over a 15-minute time-span and during one setting, namely a play session with parent and child. This resulted in limitations regarding ecological validity of this measure to capture the full range of daily parenting behaviors. Moreover, it limited our data to parenting behavior used during play settings (excluding for example routine and use of a token economy), as well as generalizability of the data to other settings. As a result, we did not always have data on specific parenting behavior related to the tested mechanisms. Moreover, the range of score for most behaviors was limited and we were unable to reliably create scales for positive and negative child behavior (see Chapter 4). Also, our instruments used for positive child behavior possibly measured behavior more closely related to children's pro-social skills

instead of positive behavior and compliance. This might possible explain the discrepancy of our results on positive child behavior and that of a recent meta-analysis (Menting et al., 2013).

Moreover, although child externalizing problems is a very heterogeneous cluster of behaviors, we only measures specific forms of this behavior using the Eyberg Child Behavior Inventory (ECBI, Eyberg & Pincus, 1999) and the Dyadic Parent-Child Interaction Coding System. Because we found no intervention effect on observed child behavior, we mostly relied on the ECBI, which specifically measures child conduct behavior. However, specific candidate genes and underlying mechanisms might be related to specific externalizing behaviors. For example, the *5-HTTLPR* S-allele might be related to increased emotional reactivity and in turn irritability and temper tantrums, rather than non-compliance. It might therefore be that some of our null findings are explained by the limitations of our measurement, and failure to measure the specific targeted behavior.

A fourth limitation of this dissertation is that our genetic data was limited to polymorphisms of candidate genes in children's (lacking data on parents' genotypes). In our RCT parents are the ones exposed to the intervention, theoretically acting as a mediator of the intervention effect on child behavior. It would be very interesting to see whether parents also show genetic differential susceptibility to the behavioral parent training. Our data does suggest there are individual differences in how strongly parents decrease their use of negative parenting strategies (Chapter 6). Unfortunately, we currently do not have the data to test such hypotheses on G×I in parents.

Future Research: Alternatives for Candidate Genes

Over the last five years skepticism about cG×E research has been rising (Aliev, Latendresse, Bacanu, Neale, & Dick, 2014; Dick et al., 2015; Duncan & Keller, 2011; Ficks & Waldman, 2014; Szyf & Bick, 2013; Chabris et al., 2013). In response, scientific attention has been shifting towards alternatives for cG×E. Three alternatives for taking into account human biology in the development of externalizing psychopathology have been dominant in the literature, each having important (dis)advantages over cG×E and each other: 1) GWAS, 2) epigenetics, and 3) non-genetic biomarkers.

GWAS

'Genome wide association' (GWA) studies test whether SNPs occur more frequently in people with psychopathology than in those without throughout the entire human genome (for a review of meta-analyses see Gatt, Burton, Williams, & Schofield, 2015). One of the advantages of GWAS is that it is not limited to a single genetic variation. It is hard to believe that a single gene, which is part of our genome of about twenty thousand genes, has a measurable effect on psychopathology. It has indeed been found that adolescents'

and young adults' externalizing problems is predicted by a polygenic score, rather than a single gene (Salvatore et al., 2015). Moreover, through GWAS we keep discovering more genes that possibly contribute to the development of psychopathology. At the same time, GWAS are typically constructed using a small number of SNPs that have been pre-selected using extremely low p-values derived from regression analyses. This might result in a data-driven, rather than theory driven, approach to psychopathology. The effects of the in GWAS identified genes are often very small and a lack of knowledge about the functions of many of the "new found" genes makes it difficult to interpret the GWAS results (Dick et al., 2011; Pappa et al., 2015). Moreover, the question remains whether GWAS results are indeed more consistent than results from candidate gene studies (Gatt et al., 2015). There are examples where different GWA studies show no overlap between the genes associated with specific psychopathology (see for example the GWA studies on Conduct Disorder by Dick et al. (2011) and Anney et al. (2008)). Time will tell whether GWAS leads to improvements in the prediction or explanation of externalizing behavior.

Epigenetics

Evidence from plant, animal and human studies show that the environment can get "under the skin" and trigger non sequence variation in gene expression by DNA-methylation, histone modification, or non-coding RNA associated gene silencing (for reviews see DeLisi & Vaughn, 2015; Isles, 2015; Roth, 2013). Epigenetic processes are normal and universal processes needed for cellular development and regulation of gene function, enabling identical genotypes to express different phenotypes (Szyf, 2013). These processes can be experience-dependent, as such, that experiences can transmit information on the environment to our genes and set epigenetic processes in motion. Experiences such as sexual abuse, for example, appear to be able to cause a group of small molecules, a methyl group, to stick to the DNA influencing the expression of this DNA (Beach et al., 2013). In turn, such methylation has been associated with aggression and antisocial personality disorder (e.g., Beach, Brody, Todorov, Gunter, & Philibert, 2010; Beach et al., 2013; Wang et al., 2012).

It has been argued that epigenetics is a form of non-genetic inheritance and is therefore the answer to the question why we have found little associations between specific genotypes and highly heritable traits such as externalizing behaviors (i.e., "missing heritability"). Indeed, evidence of epigenetic processes has been found for many of the extensively studied candidate genes (for an overview on epigenetics in the context of externalizing behavior see DeLisi & Vaughn, 2015), including the candidate genes studied in this dissertation: *MAOA* (e.g., Melas et al., 2013), and the *5-HTT* transporter gene (e.g., Beach, Brody, Lei et al., 2010). There is also evidence that suggests these processes might be reversible using medication, or with interventions targeting the environment (for reviews see Wu & Zhang, 2010; Zhang, Fu, Yu, & Wu, 2014). This seems very promising with regard to prevention and

treatment of psychopathology. However, we still know little about the functional expression of the methylated genes and their relationship to bio-psycho-social features like brain activity, the stress response system, and ultimately psychopathology. This makes it difficult to determine where (i.e., in which genes, for which environmental influences) we need to search for these processes, and to draw conclusions about the functional involvement of these epigenetic processes in the development of psychopathology (see Heijmans & Mill, 2012).

Non-genetic biomarkers

Biomarkers are objectively measured individual biological differences in the way we process information about and react to our environment (Colburn, DeGruttola, & DeMets, 2001). Theoretically, such markers lie somewhere between genes and phenotypes (observable characteristics of an individual, such as behavior). Research on biomarkers has a long tradition, but now seems to experience a "renaissance" (see for example the review of electroencephalography (EEG) literature Loo et al., 2015). Markers which are investigated intensively are individual differences in stress response systems and brain activity, measured by means of relatively non-invasive procedures such as (variability in) heart rate, levels of cortisol and (nor)adrenaline, startle reflex, blood pressure, muscle tension (EMG) and EEG. Theoretically, such markers lie somewhere between genes and phenotypes (observable characteristics of an individual, such as behavior). For example, a link has been shown between very low and very high heart rate variability (Respiratory Sinus Arrhythmia, or RSA) and poor emotion regulation, and thus indirectly behavioral control and psychopathology (Beauchaine & Thayer, 2015). Biomarkers are also used together as, for example, a cumulative physiological index of chronic stress (i.e., allostatic load). Recently a study reported an association between children's negative emotionality and allostatic load, in such that children high on negative emotionality showed high allostatic load (higher than children low on negative emotionality) when mothers' responsiveness was low and low allostatic load (lower than children low on negative emotionality) when mothers' responsiveness was high (Dich, Doan, & Evans, 2015).

Because biomarkers are generally easy to measure, and hypothetically are closely related to behavior, research on these markers possibly has important implications for clinical practice. However, is has been argued that at present no single biomarker has been shown to be a reliable and discrete predictor of psychopathology, as well as to account for a clinically relevant amount of variance in psychopathology. Furthermore, some biomarkers might simply be correlates of psychopathology, rather than part of an underlying process, which means they might not teach us anything about the etiology of psychopathology (for a critical review see Pollak, 2015)

The three discussed alternatives for candidate gene study are promising: Compared to candidate gene studies the alternatives epigenetics and GWAS might better address the complexity of the human genome, and biomarkers might be closer related to behavior, which makes them easier to implement in clinical practice. However, the question remains what these alternatives teach us about the development and treatment of psychopathology. Even if these alternatives lead to robust findings on significant relationships between human biology and psychopathology, they do not necessarily lead to better prediction or explanation thereof, or to a direct application of such knowledge that is relevant to clinical practice (Jakobsdottir, Gorin, Conley, Ferrell, & Weeks, 2009; Lo, Chernoff, Zheng, & Lo, 2015). Some of the criticism on candidate gene studies therefore also applies to these three alternatives. One possible danger is that these attractive "tools" make us overlook the fact that we may not always have sufficient knowledge to properly formulate a priori hypotheses or to interpret the results of studies using these tools.

One of the challenges of the next decade may therefore lie in integrating insights obtained with the different strategies, which might help us with the interpretation of new results. We have to build bridges between scientific disciplines (DeLisi & Vaughn, 2015). Within such a strategy, we might still be able to use the insights obtained though candidate gene studies. The function of genes involved in psychopathology (e.g., breaking down neurotransmitters such as dopamine) might hold clues about biological processes (e.g., the amount of dopamine released when one anticipates a reward and reward sensitivity) that may contribute to the development of psychopathology ('a reverse endophenotype' approach, Loo et al., 2015). Such an approach may help us create a new research agenda, and in the long run increase our understanding of the role of our biology in the development of psychopathology.

Future Research: Understanding the Function of Mechanisms Underlying Biology×Environment

The processes of interaction of our biology and environment are mostly addressed as processes underlying maladaptation and psychopathology. For example, exposure to chronic stressful life might alter our physiological reactivity to stress (i.e., allostatic load), specifically in individuals with a genetic predisposition, which in turn might cause an increased sensitivity to future stress and therefore an increased risk for psychopathology (e.g. Dich et al., 2015). Although this process eventually leads to *maladaptation* (i.e., psychopathology), the process illustrates an *adaptation* to our environment nevertheless.
Another important step in gaining understanding of interactions between our biology and environment might therefore be increasing our understanding of the function of the processes triggered by our environment. One overarching framework that could deepen our understanding is an evolutionary-developmental one (evo-devo) (Belsky, 2014; Del

Giudice, 2014, 2016; Ellis & Del Giudice, 2014). Evolutionary developmental theory does not approach gene-by-environment interactions in psychopathology merely in terms of dual risk, genetic vulnerability or susceptibility. It also considers the possibility that it reflects a biological trade-off, in which adaptations in individuals high on plasticity/susceptibility might promote direct fitness and survival but may be at the expense of long-term well-being or adjustment. This approach assumes that child development is shaped by environmental cues pertaining to risk and opportunity in an adaptive way (Belsky, Steinberg, & Draper, 1991; Belsky, 2014). Environmental cues can be used as a forecast of the environment children will grow up in to regulate development (Del Giudice, 2014; Nettle, Frankenhuis, & Rickard, 2014; Rickard, Frankenhuis, & Nettle, 2014). Reactivity to such cues might mediate the relation between family environment and child adjustment. For example, family stress and unpredictability might contribute to a shift towards high risk, fast life history strategies (focusing on short term survival and fast and frequent reproduction) instead of low-risk, slow life history strategies (focusing on long term survival through investment) (e.g., Cabeza de Baca, Barnett, & Ellis, 2016). Although behavior that is related to such fast life history strategies is mostly seen as maladaptive, it might reflect an adaptation of behavior that fits the specific environment children grow up in.

In Chapter 2 we gave the example that children growing up in families with a low socio-economic status or even in poverty might develop a preference for immediate rewards (over long-term rewards) (e.g., Griskevicius et al., 2011), often with disregard of punishment or negative consequences of their behavior (e.g., doing something illegal, putting oneself at risk, doing harm to others). Such a tendency might be beneficial for short-term survival (e.g., by exploiting others they obtain resources), but on the long run predicts maladjustment, and risky, externalizing behaviors. Moreover, because these mechanisms might be very specific to certain environmental risk factors, it shows the importance of carefully selecting the environment we choose in studying biology×environment interactions. An evo-devo approach might help us form hypotheses on which specific environments might contribute to specific psychopathology, in particular externalizing behavior problems (but maybe less appropriate in developmental disabilities such as intellectual disabilities or autism), because they trigger specific adaptive processes.

Conclusions and Implications for Clinical Practice

Overall, cG×E has proven to be a very challenging research field. This dissertation shows it is difficult to provide simple answers to questions regarding the role of candidate genes in child externalizing behavior problems. Specifically, to answer questions on which candidate genes might contribute to this behavior, and how these genes might contribute. In fact, the more we know, the less we seem to understand. The most straightforward finding of this

dissertation, which is also grounded in previous empirical findings, is that the behavioral parent training The Incredible Years proved to be effective in decreasing externalizing problems, and proved more effective in decreasing externalizing problems in boys carrying the *MAOA* low-activity allele than in boys carrying the high-activity allele. Findings on the other genotypes (*5-HTTLPR* and *COMT*) were more complex, but do suggest that different mechanisms are at work in the development of child externalizing behavior in different children and families. In clinical practice it might therefore be important to take into account what works for whom, not only for which children but also for which parents.

However, it is unclear if and how information on genetic moderation should be implemented. Most importantly, in order to implement such findings we need reliable and replicable results; effects that are large enough to have clinical implications; and a better understanding of biopsychosocial pathways leading to differences in susceptibility to intervention (Rutter, 2012). In our attempt to shed light on how such interactions might work, we found no support for the hypothesized putative mechanisms (i.e., genetic predispositions for heightened emotional reactivity or reward sensitivity). Our results add to the view that the mechanisms underlying interactions between our environment and biology are very specific and complex (Moore & Depue, 2016; Reiss et al., 2013). In addition, even if we are able to generate larger, replicable, and interpretable findings on cG×E, implementation of genetics in clinical practice raises ethical concerns (Chhangur, Weeland, Matthys, & Overbeek, 2015). Genetic screening is costly and invasive and might lead to false deterministic claims about behavioral development, which might have negative effects on child development by changing attitudes of parents and professionals. Together, this all makes it difficult to effectively use cG×E findings for tailoring interventions. At the same time, we cannot ignore the abundant evidence that there are important individual differences in intervention effectiveness both at the child, and at the family level. Therefore, when it comes to intervening in the development of externalizing behavior problems, we cannot keep up a “one size fits all” approach. Future research should try to find implementable indicators (other than genetic) of differential risk, as well as mechanisms underlying differential risk sensitivity, that may be used to personalize interventions targeting child externalizing problems.